Table 2
Acute GVHD Characteristics

Characteristic	Total (N = 3436)	MRD-BM/PB (n = 926)	MUD-BM + mm* (n = 1671)	UCB (n = 839)
Onset of acute GVHD				
Day ≤28	2344 (68)	560 (60)	1221 (73)	563 (67)
Day ≥29	994 (29)	351 (38)	434 (26)	209 (25)
Unknown	98 (3)	15 (2)	16 (1)	67 (8)
Grade of acute GVHD				
II	2049 (59)	584 (63)	973 (58)	492 (58)
III	1015 (30)	259 (28)	482 (29)	274 (33)
IV	372 (11)	83 (9)	216 (13)	73 (9)
Organ involvement				
Skin only	1110 (32)	288 (31)	579 (34)	243 (29)
Gut only	310 (9)	125 (13)	129 (8)	55 (7)
Liver only	35 (1)	8 (1)	16 (1)	11 (1)
Skin and gut, no liver	1178 (34)	316 (34)	576 (34)	286 (34)
Skin and liver, no gut	177 (5)	56 (6)	72 (4)	49 (6)
Gut and liver, no skin	87 (3)	26 (3)	42 (3)	19 (2)
Skin, gut, and liver	487 (14)	107 (12)	256 (16)	124 (15)
Unknown	52 (2)	0 (0)	1 (0)	51 (6)

GVHD indicates graft-versus-host disease; MRD-BM/PB, HLA-matched related donor bone marrow and HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood.
Data are presented as n (%).
* mm indicates HLA-mismatched related donor bone marrow, HLA-mismatched related donor peripheral blood stem cells and HLA-mismatched unrelated donor bone marrow.

a significantly lower OS rate than those who achieved improvement ($P < .0001$).

To evaluate the impact of the response to corticosteroid therapy on the OS rate, the Cox proportional hazards model was used with all of the clinical features listed in Tables 1 and 2. On univariate analysis, the OS rate was significantly lower in patients with a stable or progressive response to corticosteroid therapy than in patients with an improved response (hazard ratio, 2.18; 95% confidence interval, 1.97 to 2.40). After adjustment by patient age, disease, preconditioning, grade of acute GVHD, and organ involvement of acute GVHD, which were significant on univariate analysis, the OS rate

Table 3
Factors Associated with Improvement of GVHD by Corticosteroid Therapy

Factor (n)	Univariate Analysis Relative Risk* (95% CI)	*P* Value	Multivariate Analysis Relative Risk* (95% CI)	*P* Value
Patient age				
<18 yr (672)	1		1	
18 to 49 yr (1626)	1.33 (1.10 to 1.60)	.003	1.48 (1.18 to 1.85)	<.002
≥50 yr (1138)	1.06 (.88 to 1.30)	.509	1.11 (.88 to 1.40)	.385
Stem cell source				
MRD-BM (445)	1		1	
MRD-PB (481)	.66 (.50 to .87)	.004	.81 (.59 to 1.12)	.201
MUD-BM (783)	.53 (.41 to .68)	<.001	.57 (.43 to .76)	<.001
UCB (839)	.97 (.75 to 1.26)	.839	1.36 (1.01 to 1.83)	.042
MMRD-BM (155)	.26 (.18 to .39)	<.001	.37 (.24 to .57)	<.001
MMRD-PB (161)	.34 (.23 to .49)	<.001	.41 (.27 to .63)	<.001
MMUD-BM (572)	.47 (.36 to .61)	<.001	.57 (.42 to .77)	<.001
GVHD prophylaxis				
Cyclosporine A-based (1676)	1		1	
Tacrolimus-based (1691)	.80 (.69 to .92)	.002	1.02 (.82 to 1.26)	.851
Other (56)	.38 (.22 to .64)	<.001	.61 (.31 to 1.22)	.164
In vivo T cell depletion				
No (3251)	1		1	
Yes (168)	1.47 (1.08 to 2.01)	.015	1.06 (.68 to 1.65)	.787
Onset of acute GVHD				
Day ≤28 (2344)	1		1	
Day ≥29 (994)	1.20 (1.03 to 1.40)	.023	1.10 (.91 to 1.34)	.336
Grade of acute GVHD				
II (2049)	1		1	
III (1015)	.34 (.29 to .39)	<.001	.45 (.37 to .55)	<.001
IV (372)	.04 (.03 to .06)	<.001	.07 (.05 to .10)	<.001
Organ involvement				
Skin only (1110)	1		1	
Gut only (310)	.69 (.52 to .92)	.011	.91 (.66 to 1.24)	.541
Liver only (35)	.22 (.11 to .43)	<.001	.56 (.25 to 1.25)	.157
Skin and gut, no liver (1178)	.55 (.45 to .66)	<.001	.77 (.62 to .96)	.021
Skin and liver, no gut (177)	.39 (.28 to .54)	<.001	.78 (.53 to 1.15)	.214
Gut and liver, no skin (87)	.17 (.11 to .26)	<.001	.36 (.21 to .59)	<.001
Skin, gut, and liver (487)	.13 (.10 to .17)	<.001	.38 (.28 to .51)	<.001

GVHD indicates graft-versus-host disease; MRD-BM, HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; CI, confidence interval.
* Values >1.0 indicate higher probability of improvement; values <1.0 indicate lower probability.

Table 4
Response to Corticosteroid Therapy in Each Stem Cell Source

Stem Cell Source	No. of Cases	Patients with Improved Response, n (%)
MRD-BM	445	328 (73.7)
MRD-PB	481	312 (64.9)
MUD-BM	783	468 (59.8)
UCB	839	614 (73.2)
MMRD-BM	155	66 (42.9)
MMRD-PB	161	78 (48.4)
MMUD-BM	572	324 (56.6)
Total	3436	2190 (63.7)

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow.

was still significantly lower in patients with a stable or progressive response to corticosteroid therapy than in patients with an improved response (hazard ratio, 1.66; 95% confidence interval, 1.49 to 1.85).

DISCUSSION

The present nationwide study revealed that the response rate of grade II to IV acute GVHD to systemic corticosteroid therapy in Japanese patients was approximately 64%, which is comparable to that in Caucasian patients. In a retrospective analysis of 456 patients who were treated with methylprednisolone 2 mg/kg/day for grade II to IV acute GVHD after allogeneic BM transplantation at the Fred Hutchinson Cancer Research Center, 59% of the patients experienced a complete, partial, or mixed response [10]. In another retrospective analysis of 864 patients who were treated with prednisone 60 mg/m^2/day for grade II to IV acute GVHD after BM, PBSC, or UCB transplantation at the University of Minnesota, 65% of the patients experienced a complete, very good partial, or partial response [16].

The factors associated with poor response to corticosteroid therapy were MUD-BM, HLA-mismatched stem cell sources other than UCB (MMRD-BM, MMRD-PB, and MMUD-BM), more severe acute GVHD, and multiple organ involvement including gut of acute GVHD (Table 3). The previous studies also found these features as risk factors for an increased treatment failure rate [9,10], suggesting that these subgroups may be targets for alternate first-line immunosuppressive therapies.

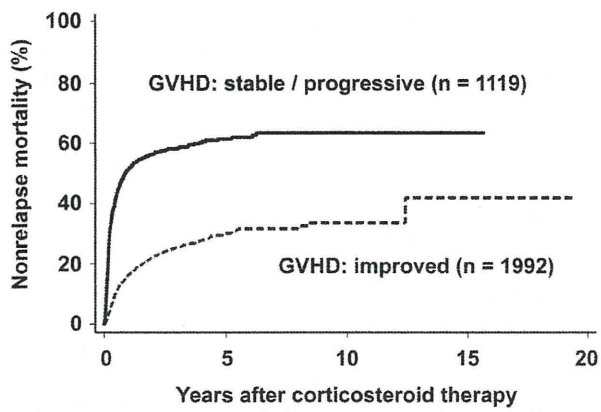

Figure 1. Nonrelapse mortality (NRM) after systemic corticosteroid therapy for patients with grade II to IV acute GVHD. Cumulative incidence rates of NRM after systemic corticosteroid therapy in patients (n = 1992) with an improved response to corticosteroid therapy (dashed line, 22.2% [95% confidence interval, 20.1% to 24.4%] at 2 years, 30.1% [27.1% to 33.0%] at 5 years, 33.5% [29.4% to 37.6%] at 10 years, and 41.8% [26.2% to 56.7%] at 15 years) and patients (n = 1119) with a stable or progressive response to corticosteroid therapy (solid line, 56.3% [53.1% to 59.5%] at 2 years, 61.4% [57.7% to 64.9%] at 5 years, 63.4% [59.2% to 67.3%] at 10 years, and 63.4% [59.2% to 67.3%] at 15 years) are shown ($P < .0001$).

On the other hand, UCB was identified as a factor associated with a higher response to first-line corticosteroid therapy in the present study (Table 3). Although several studies have demonstrated a significantly lower incidence of acute GVHD in UCB transplantation than in unrelated BM transplantation [23-29], no study has compared the response to treatment of acute GVHD between them. The present study demonstrated, for the first time, a higher response of grade II to IV acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation than in those after BM or PBSC transplantation.

Nevertheless, UCB transplantation had no impact on NRM after corticosteroid therapy in the multivariate analysis and, in fact, had higher NRM than MRD-BM transplantation in the univariate analysis (Table 5). Thus, even though there was a higher response of acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation, careful management is required for patients who suffer from grade II to IV acute GVHD after UCB transplantation, as well as those after transplantation with other stem cell sources.

Unexpectedly, adult patient (ages 18 to 49 years) was predictive of a good response to systemic corticosteroid therapy compared with child patient (age <18 years). Additional analysis was performed, and it was found that patients with grade II acute GVHD accounted for 61.4% of adult patient group, whereas 56.1% of child patient group (Fisher exact test, $P = .019$). This difference might affect the above result because severity of acute GVHD was the most significant factor associated with response to corticosteroid therapy (Table 3). Nonetheless, adult patients were likely to have higher NRM than child patients (Table 5). Our data indicate that although adult patients may be more responsive to corticosteroid therapy for acute GVHD, they have a higher risk of transplant-related toxicity than children with acute GVHD.

Despite the fact that multivariate analysis showed a significantly higher response rate to corticosteroid therapy in UCB transplantation than MRD-BM transplantation, the actual percentage was similar between UCB (73.2%) and MRD-BM (73.7%) transplantations (Table 4). Additional analysis found that patients in the age group 18 to 49 years (predictive factor of good response) accounted for only 32.2% of UCB transplantation, but constituted 58.4% of the MRD-BM population (Fisher exact test, $P < .001$) and that patients with grade II acute GVHD (predictive factor of good response) accounted for only 58.6% of UCB transplantation, but constituted 70.1% of the MRD-BM population (Fisher exact test, $P < .001$). These data suggested that the UCB population included fewer patients having predictive factors of good response to corticosteroid therapy compared with the MRD-BM population. This could explain why the actual percentage of patients with an improved response in UCB transplantation was almost the same as the percentage of patients with an improved response in MRD-BM transplantation.

Interestingly, multiorgan involvement that includes the gut was less likely to respond to first-line therapy with corticosteroids (Table 3); however, patients with liver involvement are more likely to have higher NRM (Table 5). Further study is required to elucidate the mechanisms of the difference in the effect of gut and liver GVHD on

Table 5
Factors Associated with Nonrelapse Mortality after Corticosteroid Therapy

Factor (n)	Univariate Analysis Hazard Ratio* (95% CI)	*P* Value	Multivariate Analysis Hazard Ratio* (95% CI)	*P* Value
Patient age				
<18 yr (554)	1		1	
18 to 49 yr (1503)	1.50 (1.21 to 1.85)	<.001	1.72 (1.38 to 2.14)	<.001
≥50 yr (1054)	2.74 (2.22 to 3.38)	<.001	3.34 (2.67 to 4.17)	<.001
Stem cell source				
MRD-BM (402)	1		1	
MRD-PB (447)	1.43 (1.11 to 1.83)	.005	.88 (.68 to 1.15)	.344
MUD-BM (726)	1.40 (1.11 to 1.77)	.004	1.02 (.80 to 1.30)	.866
UCB (720)	1.35 (1.06 to 1.71)	.014	1.15 (.90 to 1.48)	.265
MMRD-BM (141)	1.63 (1.16 to 2.28)	.005	1.15 (.82 to 1.62)	.415
MMRD-PB (153)	1.74 (1.26 to 2.39)	.001	.97 (.69 to 1.37)	.882
MMUD-BM (522)	1.79 (1.41 to 2.27)	<.001	1.25 (.97 to 1.60)	.082
GVHD prophylaxis				
Cyclosporine A-based (1528)	1			
Tacrolimus-based (1520)	1.06 (.94 to 1.21)	.332		
Other (50)	1.28 (.81 to 2.04)	.296		
In vivo T cell depletion				
No (3004)	1			
Yes (91)	.98 (.66 to 1.44)	.919		
Onset of acute GVHD				
Day ≤28 (2212)	1			
Day ≥29 (899)	1.05 (.92 to 1.20)	.476		
Grade of acute GVHD				
II (1864)	1		1	
III (917)	2.21 (1.92 to 2.56)	<.001	1.56 (1.31 to 1.86)	<.001
IV (330)	7.93 (6.67 to 9.43)	<.001	3.53 (2.84 to 4.38)	<.001
Organ involvement				
Skin only (1010)	1		1	
Gut only (266)	1.11 (.84 to 1.47)	.448	.80 (.59 to 1.08)	.139
Liver only (28)	4.11 (2.20 to 7.69)	<.001	2.22 (1.19 to 4.16)	.013
Skin and gut, no liver (1083)	1.27 (1.06 to 1.51)	.008	.97 (.79 to 1.18)	.753
Skin and liver, no gut (160)	2.42 (1.83 to 3.21)	<.001	1.54 (1.13 to 2.08)	.006
Gut and liver, no skin (75)	3.64 (2.57 to 5.16)	<.001	1.88 (1.29 to 2.73)	.001
Skin, gut, and liver (448)	4.82 (4.03 to 5.77)	<.001	2.07 (1.64 to 2.62)	<.001
Response to systemic corticosteroid therapy				
Improved (1992)	1		1	
Stable/progressive (1119)	3.63 (3.20 to 4.12)	<.001	2.45 (2.14 to 2.82)	<.001

MRD-BM indicates HLA-matched related donor bone marrow; MRD-PB, HLA-matched related donor peripheral blood stem cells; MUD-BM, HLA-matched unrelated donor bone marrow; UCB, umbilical cord blood; MMRD-BM, HLA-mismatched related donor bone marrow; MMRD-PB, HLA-mismatched related donor peripheral blood stem cells; MMUD-BM, HLA-mismatched unrelated donor bone marrow; GVHD, graft-versus-host disease; CI, confidence interval.
* Values >1.0 indicate higher probability of non relapse mortality; values <1.0 indicate lower probability.

transplantation outcome. Nevertheless, lack of response to initial therapy is an important risk factor in predicting high NRM in patients with grade II to IV acute GVHD (Table 5).

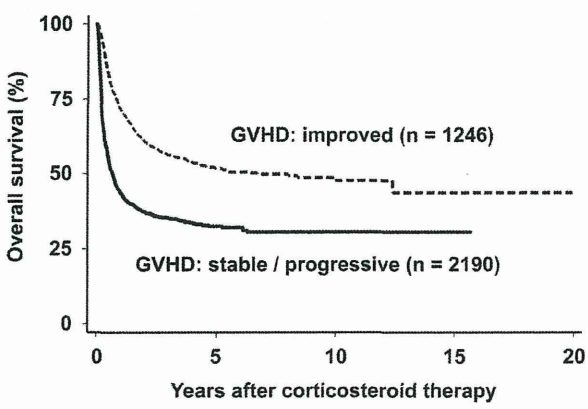

Figure 2. Overall survival (OS) for patients with grade II to IV acute GVHD. OS for patients (n = 2190) with an improved response (dashed line; 61.3% [95% confidence interval, 59.0% to 63.5%] at 2 years, 51.9% [49.2% to 54.5%] at 5 years, 47.8% [44.0% to 51.5%] at 10 years, and 43.8% [35.5% to 51.8%] at 15 years) and OS for patients (n = 1246) with a stable or progressive response (solid line; 37.4% [34.6% to 40.3%] at 2 years, 32.5% [29.5% to 35.6%] at 5 years, 30.6% [27.3% to 34.1%] at 10 years, and 30.6% [27.3% to 34.1%] at 15 years) are shown ($P < .0001$).

The patients who did not achieve improvement of acute GVHD by corticosteroid therapy had approximately 2.5-times higher NRM and approximately .6-times lower OS rates. It is well known that the incidence of acute GVHD in Japanese patients is lower than that in Caucasian patients [30,31]. However, the present data clearly demonstrate that, if the systemic corticosteroid therapy is ineffective, even Japanese patients cannot achieve a satisfactory survival rate. Another important message of this study is that the establishment of second-line treatment for corticosteroid-refractory acute GVHD is required for not only Caucasian, but also for Japanese patients.

This study had several limitations. First, the sort and dose of corticosteroids are not collected in the Japan Society for Hematopoietic Cell Transplantation database. In patients with grade II to IV acute GVHD, initial treatment with prednisone-equivalent steroid doses higher than 2.5 mg/kg has not been shown to provide better outcomes [32], although in patients with grade II acute GVHD, lower-dose initial treatment at 1.0 mg/kg has not been shown to provide worse outcomes [33]. The intensity of corticosteroid therapy may differ by each transplantation team or each patient, as shown by a survey in Europe [34], and this information may give us additional findings. Second, criteria for improvement, or for stable or progressive acute GVHD, had been previously defined in the

database, which did not allow for analysis by outcomes such as complete, partial, or mixed response, as has been performed in previous studies [10,16]. Third, the time of the evaluation of GVHD is not defined in the database. Thus, the response was evaluated using a nonfixed time point, although GVHD sometimes shows a waxing and waning course. This also prevented us from analyzing the speed of the response to therapy. A recent study has reported that the day-28 response to corticosteroid therapy can predict the outcomes for patients with acute GVHD [16]. Fourth, this study was a retrospective analysis, which is challenging given the heterogeneous background. Multivariate analysis was used to attempt to reduce statistical bias, but a prospective study is required to validate the present findings.

The results of this large retrospective study showed a higher response of acute GVHD to systemic corticosteroid therapy in patients after UCB transplantation than for patients after BM and PBSC transplantation, and confirmed the factors previously reported. These results should be considered in the design of future clinical trials of acute GVHD treatment.

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REFERENCES

1. Cahn JY, Bordigoni P, Tiberghien P, et al. Treatment of acute graft-versus-host disease with methylprednisolone and cyclosporine with or without an anti-interleukin-2 receptor monoclonal antibody. A multicenter phase III study. *Transplantation*. 1995;60:939-942.
2. Cragg L, Blazar BR, Defor T, et al. A randomized trial comparing prednisone with antithymocyte globulin/prednisone as an initial systemic therapy for moderately severe acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2000;6:441-447.
3. Graziani F, Van Lint MT, Dominietto A, et al. Treatment of acute graft versus host disease with low dose-alternate day anti-thymocyte globulin. *Haematologica*. 2002;87:973-978.
4. Lee SJ, Zahrieh D, Agura E, et al. Effect of up-front daclizumab when combined with steroids for the treatment of acute graft-versus-host disease: Results of a randomized trial. *Blood*. 2004;104:1559-1564.
5. Patriarca F, Sperotto A, Damiani D, et al. Infliximab treatment for steroid-refractory acute graft-versus-host disease. *Haematologica*. 2004;89:1352-1359.
6. Levine JE, Paczesny S, Mineishi S, et al. Etanercept plus methylprednisolone as initial therapy for acute graft-versus-host disease. *Blood*. 2008;111:2470-2475.
7. Couriel DR, Saliba R, de Lima M, et al. A phase III study of infliximab and corticosteroids for the initial treatment of acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2009;15:1555-1562.
8. Martin PJ, Rizzo JD, Wingard JR, et al. First- and second-line systemic treatment of acute graft-versus-host disease: Recommendations of the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2012;18:1150-1163.
9. Weisdorf D, Haake R, Blazar B, et al. Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: An analysis of clinical risk features and outcome. *Blood*. 1990;75:1024-1030.
10. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: Initial treatment. *Blood*. 1990;76:1464-1472.
11. Weisdorf DJ, Snover DC, Haake R, et al. Acute upper gastrointestinal graft-versus-host disease: Clinical significance and response to immunosuppressive therapy. *Blood*. 1990;76:624-629.
12. Roy J, McGlave PB, Filipovich AH, et al. Acute graft-versus-host disease following unrelated donor marrow transplantation: Failure of conventional therapy. *Bone Marrow Transplant*. 1992;10:77-82.
13. Hings IM, Severson R, Filipovich AH, et al. Treatment of moderate and severe acute GVHD after allogeneic bone marrow transplantation. *Transplantation*. 1994;58:437-442.
14. MacMillan ML, Weisdorf DJ, Wagner JE, et al. Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: Comparison of grading systems. *Biol Blood Marrow Transplant*. 2002;8:387-394.
15. Lee KH, Choi SJ, Lee JH, et al. Prognostic factors identifiable at the time of onset of acute graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Haematologica*. 2005;90:939-948.
16. MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. *Blood*. 2010;115:5412-5417.
17. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol*. 2007;86:269-274.
18. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15:825-828.
19. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: Defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant*. 2009;15:367-369.
20. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: New representations of old estimators. *Stat Med*. 1999;18:695-706.
21. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
22. Cox DR. Regression models and life tables. *J Royal Stat Soc [B]*. 1972;34:187-220.
23. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood*. 2001;97:2962-2971.
24. Rocha V, Labopin M, Sanz G, et al., Acute Leukemia Working Party of European Blood and Marrow Transplant Group. Eurocord-Netcord Registry. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med*. 2004;351:2276-2285.
25. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood*. 2004;104:3813-3820.
26. Barker JN, Hough RE, van Burik JA, et al. Serious infections after unrelated donor transplantation in 136 children: Impact of stem cell source. *Biol Blood Marrow Transplant*. 2005;11:362-370.
27. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: A comparison study. *Lancet*. 2007;369:1947-1954.
28. Atsuta Y, Suzuki R, Nagamura-Inoue T, et al. Japan Cord Blood Bank Network. Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood*. 2009;113:1631-1638.
29. Eapen M, Rocha V, Sanz G, et al., Center for International Blood and Marrow Transplant Research; Acute Leukemia Working Party Eurocord (the European Group for Blood Marrow Transplantation). National Cord Blood Program of the New York Blood Center. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: A retrospective analysis. *Lancet Oncol*. 2010;11:653-660.
30. Morishima Y, Morishita Y, Tanimoto M, et al. Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings; possible role of genetic homogeneity. The Nagoya Bone Marrow Transplantation Group. *Blood*. 1989;74:2252-2256.
31. Morishima Y, Kodera Y, Hirabayashi N, et al. Low incidence of acute GVHD in patients transplanted with marrow from HLA-A, B, DR-compatible unrelated donors among Japanese. *Bone Marrow Transplant*. 1995;15:235-239.
32. Van Lint MT, Uderzo C, Locasciulli A, et al. Early treatment of acute graft-versus-host disease with high- or low-dose 6-methylprednisolone: A multicenter randomized trial from the Italian Group for Bone Marrow Transplantation. *Blood*. 1998;92:2288-2293.
33. Mielcarek M, Storer BE, Boeckh M, et al. Initial therapy of acute graft-versus-host disease with low-dose prednisone does not compromise patient outcomes. *Blood*. 2009;113:2888-2894.
34. Ruutu T, Hermans J, van Biezen A, et al. How should corticosteroids be used in the treatment of acute GVHD? EBMT Chronic Leukemia Working Party. European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 1998;22:614-615.

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ORIGINAL ARTICLE

Recent decrease in non-relapse mortality due to GVHD and infection after allogeneic hematopoietic cell transplantation in non-remission acute leukemia

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Although recent improvements have been indicated in the outcome after allogeneic hematopoietic cell transplantation (allo-HCT), little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We compared the incidence and causes of non-relapse mortality (NRM) after allo-HCT in non-remission among three consecutive four-year periods using a nationwide transplant outcome registry database. A total of 3308 patients with acute leukemia in non-remission were analyzed. The risk of NRM decreased over the three periods, and the hazard ratios (HRs) in 2001–2004 and 2005–2008 compared with 1997–2000 were 0.86 (95% CI, 0.70–1.06; $P=0.16$) and 0.65 (95% CI, 0.53–0.80; $P<0.01$), respectively. A significant decrease in the HR for overall mortality was also observed in 2005–2008 (HR 0.85; 95% CI, 0.75–0.97; $P=0.02$). We found that a decrease in the incidences of death due to GVHD and infection contributed to the reduction in NRM, to which high-resolution donor-recipient HLA matching and other improvements may have contributed. As none of the subgroups showed improved survival without a reduction in NRM, the effective prevention of transplant-related complications appears to be necessary for improving outcomes after allo-HCT in non-remission.

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Keywords: allogeneic hematopoietic cell transplantation; acute leukemia; non-remission; non-relapse mortality; GVHD

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) is recognized as a potentially curative therapy for patients with high-risk hematologic malignancies, which can lower the risk of relapse. However, treatment-related mortality, which may offset the benefit of a reduced risk of relapse, has long been a major problem. Several changes have been made in modalities of allo-HCT, including patient-donor HLA matching, conditioning regimens, immunosuppressive therapy, and the prophylaxis, diagnosis and treatment of GVHD and infection. As a result, the risk of non-relapse mortality (NRM) after allo-HCT has decreased over the past few decades.[1–6]

AML and ALL account for the largest proportion of diseases indicated for allo-HCT. Furthermore, a substantial number of patients with AML or ALL receive allo-HCT in non-remission. Despite the fact that high-risk acute leukemia is definitely indicated for allo-HCT, patients with non-remission leukemia carry various factors that lead to a higher risk of treatment-related toxicity, including comorbidities due to prior chemotherapy and intensified conditioning regimens in need of an antitumor effect,[7–11] and a deteriorated general condition due to refractory disease. Although prior studies have shown improvements in the outcome after allo-HCT,[1–5] little information is available on how changes in transplant modalities have affected the outcomes after allo-HCT in non-remission, based on patient age, donor source and disease type. We recently reported changes in the incidence and causes of NRM after allo-HCT in remission in Japan.[6] Using the same nationwide transplant outcome registry database, we compared the incidence and causes of NRM in patients with AML or ALL in non-remission in three consecutive four-year periods.

SUBJECTS AND METHODS

Data source

Clinical data were extracted from a nationwide transplant outcome registry database provided by the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, to which 267 institutions/departments contributed. The clinical data were consecutively collected through Transplant Registry Unified Management Program as described previously.[12] This study was

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approved by the data management committees of the Japan Society for Hematopoietic Cell Transplantation, the Japan Marrow Donor Program and the Japan Cord Blood Bank Network, and by the Institutional Review Board at the National Cancer Center Hospital.

Patients and definitions

We evaluated data on patients aged between 16 and 70 years who had AML or ALL and who received their first allo-HCT in non-remission between 1997 and 2008. Non-remission status was defined as any percentage of blasts in the peripheral blood, or a BM aspirate containing >5% blasts at the time of transplant. We compared the incidence of NRM after allo-HCT in three consecutive four-year periods (1997–2000, 2001–2004 and 2005–2008) for younger patients (16–49 years), and in the latter two periods for older patients (50–70 years). NRM was defined as death without the detection of recurrent disease after allo-HCT. In 154 patients who died without a confirmed hematological remission within 30 days from allo-HCT, the cause of death was defined as NRM. In 293 patients who died without a confirmed hematological remission after 31 days or later after allo-HCT, the cause of death was defined as refractory disease. A separate analysis that excluded these 447 patients who died without a confirmed remission was performed. We also changed the cutoffs from 30 days to 60 days or 90 days. Analyses were performed on the basis of patient's age (16–49 years and 50–70 years), disease (AML and ALL) and donor source (HLA-matched/1-Ag-mismatched related, unrelated BM and unrelated cord blood (CB)). In this study, matching of unrelated BM between recipient and donor were determined based on serum typing. In 2003, Japan Marrow Donor Program nationally recommended DNA typing of HLA-A and B, as well as HLA-DRB1. Since 2005, Japan Marrow Donor Program required all the candidates of unrelated allo-HCT to examine high-resolution typing of HLA-A, B and DRB1 and also recommended high-resolution typing of C-locus. In the era considered by this study, only BM was used from unrelated volunteer donors in Japan. Conditioning regimens were classified as indicated by Giralt *et al.*[13] The causes of death other than recurrent disease were obtained from the database and the incidences of mortality associated with GVHD, infection or organ failure were compared over the three time periods. In patients who had multiple causes among GVHD, infection and organ failure, information regarding the main cause of death was prioritized. The 447 patients who died without a confirmed hematological remission were excluded from the analyses regarding the causes of death.

Statistical analysis

Data were retrospectively reviewed and analyzed as of March 2012. Among the three time periods, patient characteristics were compared using the χ^2-test. The primary endpoint of the study was NRM after allo-HCT. Probabilities of NRM were estimated with the use of cumulative incidence curves, with relapse viewed as a competing risk of NRM. The Pepe and Mori test was used to evaluate the differences between groups. For the 337 patients (10%) who were known to have relapsed but whose date of relapse was unavailable, midpoint imputation was performed by substituting the midpoint from HCT to date of last contact as the date of relapse. The incidence of NRM was estimated as the probability at 2 years from allo-HCT. Multivariate analyses were performed for NRM and relapse using competing risk regression by the method of Fine and Gray, and for survival using a Cox proportional hazard regression model. The analyses were performed separately among younger patients aged 16–49 years and older patients aged 50–70 years. In the multivariate analyses, we considered the following factors as covariates: the year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008; because of the small number of HCT performed in 1997–2000, we considered 2001–2004 as reference vs 2005–2008 in subgroup analyses among older patients or those who received unrelated CB transplantation (UCBT)), disease type (AML vs ALL), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched-unrelated BM, mismatched-unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduce-intensity conditioning (RIC)). Multivariate analyses were also performed separately for patients who received related allo-HCT, patients who received unrelated BMT (UBMT), and patients who received UCBT. We considered two-sided *P*-values of <0.05 to be statistically significant. Statistical analyses were performed with SAS version 9.1.3 (SAS, Cary, NC, USA) and SPSS software version 11.0.1 (SPSS, Chicago, IL, USA).

Table 1. Patient characteristics according to the time period of transplant

Characteristics	*1997–2000* N(%)	*2001–2004* N(%)	*2005–2008* N(%)	P-*value*
Total number of patients	637	1165	1506	
Gender				*0.064*
Male	355(56)	674(58)	793(53)	
Female	281(44)	489(42)	505(34)	
Age (years)				*<0.001*
16–29	249(39)	277(24)	265(18)	
30–39	139(22)	240(21)	278(18)	
40–49	157(25)	246(21)	304(20)	
50–59	83(13)	296(25)	430(29)	
60–70	9(1)	106(9)	229(15)	
Donor source				*<0.001*
HLA-matched sibling	248(39)	380(33)	365(24)	
Related others	94(15)	165(14)	196(13)	
Matched-unrelated BM	213(33)	288(25)	461(31)	
Mismatched-unrelated BM	34(5)	83(7)	85(6)	
Unrelated CB	23(4)	176(15)	286(19)	
Others	25(4)	73(6)	113(8)	
Disease type				*<0.001*
AML	388(61)	840(72)	1209(80)	
ALL	249(39)	325(28)	297(20)	
Ph-positive ALL	66(10)	75(6)	48(3)	
Conditioning				*<0.001*
Myeloablative	504(79)	668(57)	837(56)	
Reduced-intensity	14(2)	290(25)	426(28)	
Not categorized	119(19)	207(18)	243(16)	
GVHD prophylaxis				*<0.001*
Cyclosporin-based	472(74)	679(58)	618(41)	
Tacrolimus-based	150(24)	423(36)	806(54)	
Disease status at HCT				*<0.001*
No treatment	20(3)	43(4)	115(8)	
Primary induction failure	148(23)	292(25)	576(38)	
First relapse	154(24)	372(32)	485(32)	
⩾Second relapse	55(9)	159(14)	157(10)	
Non-remission/no detailed data	260(41)	299(26)	173(11)	

Abbreviation: CB = cord blood.

RESULTS

Patients

A total of 3308 patients with a median age of 42 years and a median follow-up of 27 months (range, 0–150) was analyzed. The characteristics of the patients and transplantation procedures according to the time period are shown in Table 1. The number of allo-HCT procedures increased over time. The number and proportion of patients aged 50–70 years, allo-HCT from an unrelated CB donor and the use of a RIC regimen increased over the three periods. Most of the myeloablative regimens (96%) consisted of high-dose CY with TBI or BU. Tacrolimus-based GVHD prophylaxis increased, especially in allo-HCT from an unrelated BM and CB donor (BM: 1997–2000, $n=109$, 44%; 2001–2004, $n=231$, 62%; 2005–2008, $n=426$, 78%, CB: $n=5$, 22%; $n=50$, 28%; $n=174$, 61%). The proportion of allo-HCT given for ALL in non-remission decreased over the three periods with decreasing proportions of both Ph-positive ALL and Ph-negative

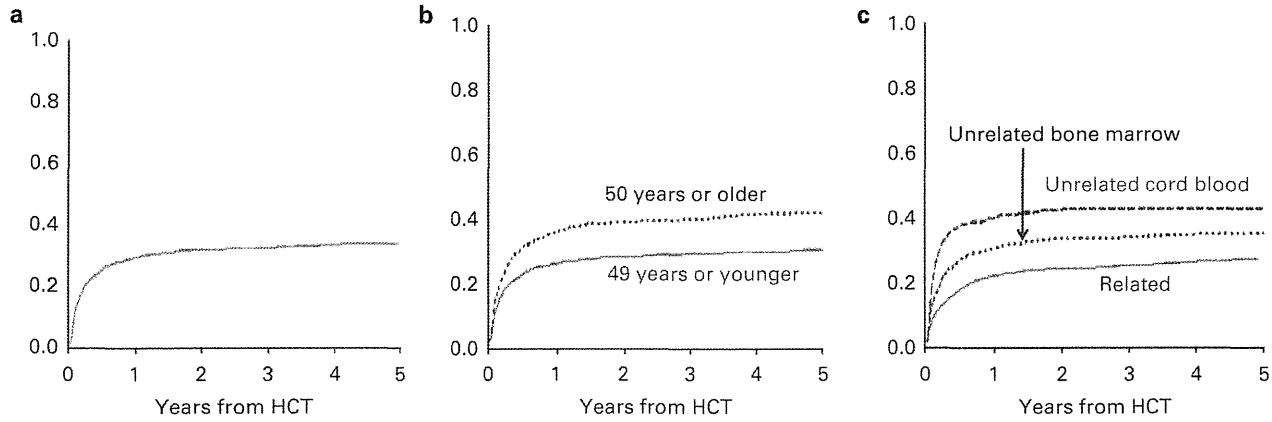

Figure 1. Cumulative incidence curves of NRM over the past 12 years among patients who received allo-HCT in non-remission are shown for the entire population (**a**), and subgroups based on age (**b**) and donor (**c**).

ALL. We categorized patients by detailed disease status; however, about 40% of allo-HCT performed in the earliest time period lacked the necessary information.

Transplant outcomes

Overall, the incidence of NRM was 31% at 2 years after allo-HCT (Figure 1a). Patients who were 50 years or older had a significantly higher incidence of NRM than patients who were 49 years or younger (39% vs 28%, $P<0.001$, Figure 1b). The donor source significantly affected the incidence of NRM, and unrelated CB had the highest risk of NRM (related, 23%; unrelated BM, 33%; unrelated CB, 42%, $P<0.001$, Figure 1c).

Hazard ratios (HRs) for NRM, relapse and overall mortality in 2001–2004 and 2005–2008 compared with 1997–2000, after adjusting for disease type, patient age, patient gender, donor source and conditioning regimens, are shown in Table 2. In the overall 3308 patients, HRs for NRM in 2001–2004 and 2005–2008 were reduced, with a significant decrease in 2005–2008. A significant decrease in the HR for overall mortality was also observed in 2005–2008. The HR for relapse did not change among the three periods. Other factors that were significantly associated with increased NRM were older age (HR 1.43; 95% CI, 1.19–1.71; $P<0.01$), male gender (HR 1.20; 95% CI, 1.04–1.41; $P=0.01$) and donor other than HLA-matched sibling (other family donors, HR 1.55; 95% CI, 1.22–1.97; $P<0.01$; HLA-matched-unrelated BM, HR 1.57; 95% CI, 1.30–1.90; $P<0.01$; HLA-mismatched-unrelated BM, HR 1.82; 95% CI, 1.35–2.47; $P<0.01$; unrelated CB, HR 2.45; 95% CI, 1.96–3.08; $P<0.01$). Younger age and HLA-matched sibling donor were also significantly associated with reduced overall mortality. Although the HR for NRM in the RIC group tended to be higher than that in the myeloablative group (HR 1.20; 95% CI, 0.99–1.47; $P=0.07$), this difference was NS. An analysis according to disease type showed that the HRs for NRM and overall mortality were reduced in AML patients, but not in ALL patients (Table 2). The incidences of NRM and OS are presented as Supplementary Figures 1a–c.

Transplant outcomes based on patient age

As the transplantation modality may vary according to the patient's age, HRs in comparison to those in the reference era were investigated separately for patients aged 49 years or younger (reference era: 1997–2000) and those aged 50 years or older (reference era: 2001–2004). As shown in Table 3, in patients aged 16–49, HRs for NRM and overall mortality in 2005–2008 were significantly reduced. In contrast, in patients aged 50–70, there were no remarkable changes in the HRs for NRM and overall mortality between 2001–2004 and 2005–2008. The incidences of NRM and OS are presented as Supplementary Figures 1d and e. RIC was used in 47% of patients aged 50 years or older (50–59: 36%, 60–70: 72%). There was no remarkable difference in the HR for NRM between the myeloablative and RIC groups (RIC: HR 0.97; 95% CI, 0.74–1.28; $P=0.85$).

Transplant outcomes based on donor

We also performed analyses based on the donor source separately among younger and older patients (Table 3). In related donor transplantation, there were no differences in the HRs for NRM, relapse and OS among the time periods in both younger and older patients. In younger patients who received UBMT, there were significant reductions in the HRs for NRM in 2001–2004 and 2005–2008. The HR for overall mortality was also significantly reduced in 2005–2008. In younger patients who received UCBT, there were significant reductions in the HRs for NRM and overall mortality in 2005–2008. The incidences of NRM and OS are presented as Supplemental Figures 1f–k. The HRs for relapse among younger patients who received UBMT were significantly higher in recent periods. In patients aged 50 years or older, no significant changes in HRs for NRM, relapse or overall mortality were observed among the different time periods in either of the donor subgroups.

Causes of death that accounted for changes in NRM

The causes of death were obtained in 98% of patients who died without recurrent disease. In 17% of patients for whom multiple causes of death were provided, GVHD, infection, or organ failure given as a main cause of death was prioritized. Overall, 151 patients died of acute or chronic GVHD (median OS: 101 days, range: 12–1979), 337 died of infection (median OS: 63 days, range: 1–2700), and 251 died of organ failure (median OS: 88 days, range: 0–2283). In the overall population, no remarkable decrease in the incidences of mortality due to these three causes was observed although the HRs for NRM and overall mortality decreased (Table 2). Meanwhile, significant reductions in the incidences of GVHD-related and infection-related mortality were observed among younger patients who received UBMT (Figure 2a) and UCBT (Figure 2b). In older patients or allo-HCT from a related donor, no remarkable differences were observed in the incidences of mortality due to GVHD, infection or organ failure among the time periods. The incidence of organ failure-related mortality did not decrease in any of the subgroups.

DISCUSSION

We evaluated the changes in NRM after allo-HCT for acute leukemia in non-remission over the last 12 years. Overall, we found higher NRM rates compared with those after allo-HCT in

Table 2. Multivariate analyses for NRM, relapse and overall mortality after allogeneic HCT

	All patients			*AML*			*ALL*		
		N = 3308			*N = 2437*			*N = 871*	
	HR	*95% CI*	P *value*	*HR*	*95% CI*	P *value*	*HR*	*95% CI*	P *value*
NRM									
1997–2000	1.00			1.00			1.00		
2001–2004	0.86	(0.70–1.06)	0.16	0.82	(0.64–1.05)	0.12	0.96	(0.67–1.38)	0.83
2005–2008	0.65	(0.52–0.80)	<0.01	0.59	(0.46–0.75)	<0.01	0.85	(0.58–1.25)	0.42
Relapse									
1997–2000	1.00			1.00			1.00		
2001–2004	1.01	(0.87–1.18)	0.88	1.05	(0.86–1.27)	0.64	0.92	(0.70–1.20)	0.53
2005–2008	1.07	(0.92–1.25)	0.38	1.08	(0.89–1.30)	0.43	1.07	(0.80–1.43)	0.65
Overall mortality									
1997–2000	1.00			1.00			1.00		
2001–2004	0.94	(0.82–1.07)	0.32	0.91	(0.78–1.07)	0.26	0.97	(0.77–1.21)	0.76
2005–2008	0.85	(0.75–0.97)	0.02	0.79	(0.67–0.92)	<0.01	1.07	(0.85–1.36)	0.56

Abbreviations: CI = confidence interval; HCT = hematopoietic cell transplantation; HR = hazard ratio; NRM = non-relapse mortality
Year of allo-HCT (1997–2000 versus 2001–2004 or 2005–2008), disease type (AML versus ALL), patient age (16–29 years versus 30–39, 40–49, 50–59 or 60–70), patient gender (male versus female), donor source (HLA-matched sibling versus other family donors, HLA-matched unrelated bone marrow, mismatched unrelated bone marrow or unrelated cord blood), and conditioning regimens (myeloablative versus reduced-intensity) were considered as covariates. In the analysis for AML and ALL, the 5 covariates were considered other than disease type.

remission (31 vs 22% at 2 years after HCT).[6] The HRs for NRM and overall mortality were lower in more recent time periods. Although several studies have shown changes in outcomes after allo-HCT,[1–6] this is the first analysis restricted to allo-HCT in non-remission, based on the patient age, donor source and disease type. The reduction in the HR for NRM was reflected in the reduced HR for overall mortality, and none of the subgroups showed a reduced risk for overall mortality without an improvement in NRM. This may indicate that lowering the risk of treatment-related mortality is, so far, an absolute requirement for improving outcomes after allo-HCT in non-remission, where a high-risk of relapse has always been an obstacle.

The reductions in the HRs for NRM and overall mortality in the overall population were accounted for by the reductions in HRs in patients with AML, and there was no improvement in NRM or overall mortality in those with ALL in non-remission over the three time periods. We also found that the number and proportion of patients who received allo-HCT for ALL in non-remission decreased over the three time periods despite an increase in the total number of allo-HCT. The proportions of both Ph-positive ALL and Ph-negative ALL decreased and, interestingly, more patients with Ph-positive ALL have received allo-HCT in remission after 2000. The introduction of imatinib may have helped more patients with Ph-positive ALL to receive allo-HCT in a controlled disease status.[14–16] In addition, a lowered expectation for the effect of allo-HCT in ALL in non-remission may have also impacted the indication. In patients who receive allo-HCT in non-remission, strategies that can provide intensified preparative regimens and a GVL effect without increasing toxicity need to be pursued.

No improvement in the HRs for NRM and overall mortality was observed in patients aged 50–70 who received allo-HCT in non-remission. Older patients with acute leukemia have been reported to have a worse prognosis because of more unfavorable disease profiles, deteriorated general conditions and an increased risk of comorbidities.[17] As the eradication of residual disease by provoking GVHD may increase toxicity and become unbearable for elderly patients, it may be necessary to reduce the tumor burden before transplantation. We previously demonstrated a significant reduction in NRM in patients aged 50 years or older who received allo-HCT in remission.[6] The safety and efficacy of modified induction chemotherapy or preparative regimen for elderly patients need to be validated.[18–25]

We found that decreases in GVHD-related and infection-related mortality contributed to the reduced risk of NRM. These findings are consistent with prior reports.[2,3] Based on an analysis of 14 403 patients with leukemia in the first CR who received allo-HCT from a matched sibling donor, Gratwohl *et al.*[3] showed that the rate of mortality due to infection decreased. In a detailed analysis in a single-center study, Gooley *et al.*[2] showed that the rates of severe GVHD and infection were recently reduced. There have been substantial improvements in HLA typing over the period of 1997–2008, with more accuracy in defining HLA haplotypes at high-resolution.[26,27] In addition to high-resolution donor-recipient HLA matching, the more frequent use of tacrolimus,[28–30] the prompt initiation of treatment after a more thorough examination to diagnose GVHD,[31] and supportive care and nutritional management[32] may have contributed to the reduced risk of GVHD-related mortality as did in allo-HCT in remission. Alternatively, the unique HLA epidemiological genetics of Japanese patients may have affected the results.[33,34] As GVHD and infection have been reported to be associated with each other's development and exacerbation,[35–37] an improved control of severe GVHD, along with the introduction of new antifungal drugs, may have led to the reduction of the risk of infection-related mortality. We did not find a reduction in the risk of organ failure-related mortality in any of the subgroups. Although intensified antitumor treatment may be required in allo-HCT in non-remission, continuous effort is needed for monitoring, prevention and intervention with regard to regimen-related toxicity, including late effects.[38–40]

As this analysis is based on a retrospectively collected multicenter database, our results may be susceptible to the disadvantages of any retrospective study using a multicenter registry database. In patients who died without a confirmed hematological remission, we assumed the disease status from the survival time. The impact on transplant outcome of detailed disease status in non-remission patients[41] was not assessed because of the lack of information. In addition, detailed data regarding the incidences of infection or other complications were not available. While we acknowledge these limitations, our data showed that the risks of NRM have decreased after allo-HCT for patients with acute

Table 3. Multivariate analyses for NRM, relapse and overall mortality after allogeneic HCT based on age and donor source

	All patients			*Related HCT*			*UBMT*			*UCBT*		
	N = *3308*			N = *1448*			N = *1164*			N = *485*		
	HR	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*	*HR*	*95% CI*	*P-value*
Patient's age at transplant: 16–49 years												
NRM												
1997–2000	1.00			1.00			1.00					
2001–2004	0.80	(0.63 –1.01)	0.06	0.93	(0.62 –1.40)	0.74	0.64	(0.44–0.92)	0.02	1.00		
2005–2008	0.52	(0.41 –0.68)	<0.01	0.79	(0.51 –1.24)	0.31	0.44	(0.30–0.62)	<0.01	0.60	(0.37 –0.97)	0.04
Relapse												
1997–2000	1.00			1.00			1.00					
2001–2004	1.02	(0.85 –1.21)	0.86	0.83	(0.64 –1.06)	0.14	1.42	(1.07–1.88)	0.02	1.00		
2005–2008	1.13	(0.94 –1.35)	0.19	0.91	(0.70 –1.18)	0.47	1.45	(1.10–1.92)	<0.01	1.28	(0.87 –1.89)	0.22
Overall mortality												
1997–2000	1.00			1.00			1.00					
2001–2004	0.90	(0.77 –1.04)	0.14	0.88	(0.71 –1.08)	0.22	0.92	(0.73–1.15)	0.45	1.00		
2005–2008	0.79	(0.68 –0.92)	<0.01	0.88	(0.71 –1.10)	0.26	0.79	(0.62–0.99)	0.05	0.68	((0.52 –0.90)	<0.0l
Patient's age at transplant: 50–70 years												
NRM												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.87	(0.68 –1.11)	0.27	0.91	(0.59 –1.40)	0.67	0.75	(0.49–1.13)	0.17	0.83	(0.54 –1.29)	0.41
Relapse												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	1.00	(0.80 –1.25)	0.99	0.97	(0.72 –1.30)	0.83	1.04	(0.66–1.64)	0.87	1.39	(0.82 –2.35)	0.22
Overall mortality												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.95	(0.80 –1.13)	0.53	0.96	((0.75 –1.22)	0.72	0.81	(0.60–1.11)	0.18	1.13	(0.75 –1.72)	0.56

Abbreviations: CI = confidence interval; HCT = hematopoietic cell transplantation; HR = hazard ratio; NRM = non-relapse mortality; UBMT = unrelated BMT; UCBT = unrelated CB transplantation.
Year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients; because of the small number of HCT performed in 1997–2000, we considered 2001–2004 as reference vs 2005–2008 in subgroup analyses among older patients or those who received unrelated CB transplantation), disease type (AML vs ALL), patient's age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched-unrelated BM, mismatched-unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. In the analysis for related HCT, HLA-matched sibling vs other family donors were considered as covariates in donor source in addition to the other five factors. In the analysis for unrelated BMT, HLA-matched BM vs mismatched-unrelated BM were considered as covariates in donor source in addition to the other five factors. In the analysis for unrelated CB transplantation, the five covariates were considered other than the donor source.

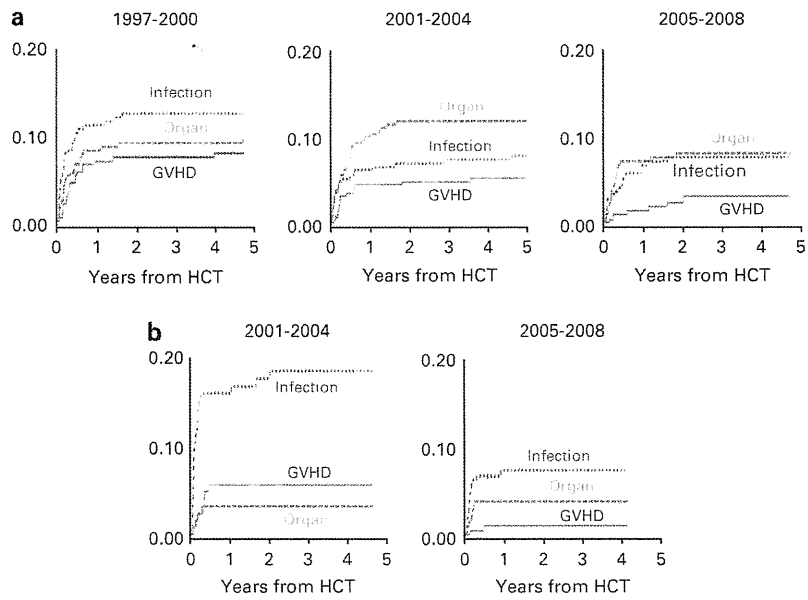

Figure 2. Change in causes of NRM among different time periods in younger patients who received allo-HCT from an unrelated BM donor (**a**), and younger patients who received allo-HCT from an unrelated CB donor (**b**). Because of the small number of transplantation performed in 1997–2000, we considered 2001–2004 as reference in patients who received CB transplantation (Figure 2b). Cumulative incidences of death associated with GVHD (solid line), infection (dotted line) and organ failure (dashed line) are shown in each time period. Significant reductions in the incidences of GVHD-related and infection-related mortality were observed among younger patients who received UBMT (**a**, GVHD: 1997–2000, 8%; 2001–2004, 5%; 2005–2008, 3%; $P = 0.01$, infection: 13%, 7%, 7%; $P = 0.04$, organ failure: 9%, 12%, 8%; $P = 0.58$) and UCBT (**b**, GVHD: 2001–2004, 6%; 2005–2008, 1%; $P = 0.04$, infection: 18%, 7%; $P = 0.02$, organ failure: 3%, 4%; $P = 0.77$).

leukemia in non-remission, using a large nationwide consecutive data. In subgroups that showed a reduced risk of NRM, significant reductions in the incidences of GVHD- and infection-related mortality were observed. We also indicated that there has been no decrease of NRM in older patients or in related donor transplant. In addition, our study showed that non-remission ALL continues to remain a major challenge. As none of the subgroups showed improved survival without a reduction in the HR for NRM, not only the control of refractory disease but also effective prevention, monitoring and treatment of transplant-related complications may be necessary to improve outcomes after allo-HCT in non-remission. Our findings may provide a foundation for future studies to improve outcomes of allo-HCT for acute leukemia in non-remission.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1 Giebel S, Labopin M, Holowiecki J, Labar B, Komarnicki M, Koza V *et al.* Outcome of HLA-matched related allogeneic hematopoietic stem cell transplantation for patients with acute leukemia in first complete remission treated in Eastern European centers. Better results in recent years. *Ann Hematol* 2009; **88**: 1005–1013.
2 Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M *et al.* Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2010; **363**: 2091–2101.
3 Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P *et al.* Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. *Bone Marrow Transplant* 2005; **36**: 757–769.
4 Horan JT, Logan BR, Agovi-Johnson MA, Lazarus HM, Bacigalupo AA, Ballen KK *et al.* Reducing the risk for transplantation-related mortality after allogeneic hematopoietic cell transplantation: how much progress has been made? *J Clin Oncol* 2011; **29**: 805–813.
5 Remberger M, Ackefors M, Berglund S, Blennow O, Dahllof G, Dlugosz A *et al.* Improved survival after allogeneic hematopoietic stem cell transplantation in recent years. A single-center study. *Biol Blood Marrow Transplant* 2011; **17**: 1688–1697.
6 Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H *et al.* Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: an analysis of the Japan Transplant Outcome Registry. *Bone Marrow Transplant* 2013; **48**: 529–536.
7 Barrett AJ. Conditioning regimens for allogeneic stem cell transplants. *Curr Opin Hematol* 2000; **7**: 339–342.
8 Feinstein L, Storb R. Reducing transplant toxicity. *Curr Opin Hematol* 2001; **8**: 342–348.
9 Sayer HG, Kroger M, Beyer J, Kiehl M, Klein SA, Schaefer-Eckart K *et al.* Reduced intensity conditioning for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia: disease status by marrow blasts is the strongest prognostic factor. *Bone Marrow Transplant* 2003; **31**: 1089–1095.
10 Shimoni A, Hardan I, Shem-Tov N, Yeshurun M, Yerushalmi R, Avigdor A *et al.* Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. *Leukemia* 2006; **20**: 322–328.
11 Shimoni A, Nagler A. Optimizing the conditioning regimen for allogeneic stem-cell transplantation in acute myeloid leukemia; dose intensity is still in need. *Best Pract Res Clin Haematol* 2011; **24**: 369–379.
12 Atsuta Y, Suzuki R, Yoshimi A, Gondo H, Tanaka J, Hiraoka A *et al.* Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol* 2007; **86**: 269–274.
13 Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367–369.
14 Mizuta S, Matsuo K, Yagasaki F, Yujiri T, Hatta Y, Kimura Y *et al.* Pre-transplant imatinib-based therapy improves the outcome of allogeneic hematopoietic stem cell transplantation for BCR-ABL-positive acute lymphoblastic leukemia. *Leukemia* 2011; **25**: 41–47.
15 Yanada M, Ohno R, Naoe T. Recent advances in the treatment of Philadelphia chromosome-positive acute lymphoblastic leukemia. *Int J Hematol* 2009; **89**: 3–13.
16 Yanada M, Takeuchi J, Sugiura I, Akiyama H, Usui N, Yagasaki F *et al.* High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol* 2006; **24**: 460–466.
17 Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE *et al.* Age and acute myeloid leukemia. *Blood* 2006; **107**: 3481–3485.
18 Andersson BS, Valdez BC, de Lima M, Wang X, Thall PF, Worth LL *et al.* Clofarabine +/- fludarabine with once daily i.v. busulfan as pretransplant conditioning therapy for advanced myeloid leukemia and MDS. *Biol Blood Marrow Transplant* 2011; **17**: 893–900.
19 Chevallier P, Prebet T, Turlure P, Hunault M, Vigouroux S, Harousseau JL *et al.* Prior treatment with gemtuzumab ozogamicin and the risk of veno-occlusive disease after allogeneic haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2010; **45**: 165–170.
20 Holowiecki J, Grosicki S, Giebel S, Robak T, Kyrcz-Krzemien S, Kuliczkowski K *et al.* Cladribine, but not fludarabine, added to daunorubicin and cytarabine during induction prolongs survival of patients with acute myeloid leukemia: A Multicenter, Randomized Phase III Study. *J Clin Oncol* 2012; **30**: 2441–2448.
21 Iravani M, Evazi MR, Mousavi SA, Shamshiri AR, Tavakoli M, Ashouri A *et al.* Fludarabine and busulfan as a myeloablative conditioning regimen for allogeneic stem cell transplantation in high- and standard-risk leukemic patients. *Bone Marrow Transplant* 2007; **40**: 105–110.
22 Kantarjian HM, Erba HP, Claxton D, Arellano M, Lyons RM, Kovascovics T *et al.* Phase II study of clofarabine monotherapy in previously untreated older adults with acute myeloid leukemia and unfavorable prognostic factors. *J Clin Oncol* 2010; **28**: 549–555.
23 Lowenberg B, Ossenkoppele GJ, van Putten W, Schouten HC, Graux C, Ferrant A *et al.* High-dose daunorubicin in older patients with acute myeloid leukemia. *N Engl J Med* 2009; **361**: 1235–1248.
24 Magenau J, Tobai H, Pawarode A, Braun T, Peres E, Reddy P *et al.* Clofarabine and busulfan conditioning facilitates engraftment and provides significant antitumor activity in nonremission hematologic malignancies. *Blood* 2011; **118**: 4258–4264.
25 Tran H, Yang D. Clofarabine in the treatment of newly diagnosed acute myeloid leukemia in older adults. *Ann Pharmacother* 2012; **46**: 89–96.
26 Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M *et al.* Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood* 2004; **104**: 1923–1930.
27 Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M *et al.* High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007; **110**: 4576–4583.
28 Yanada M, Emi N, Naoe T, Sakamaki H, Takahashi S, Hirabayashi N *et al.* Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: a nationwide survey conducted in Japan. *Bone Marrow Transplant* 2004; **34**: 331–337.
29 Nash RA, Antin JH, Karanes C, Fay JW, Avalos BR, Yeager AM *et al.* Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 2000; **96**: 2062–2068.
30 Hiraoka A, Ohashi Y, Okamoto S, Moriyama Y, Nagao T, Kodera Y *et al.* Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2001; **28**: 181–185.
31 Martin PJ, McDonald GB, Sanders JE, Anasetti C, Appelbaum FR, Deeg HJ *et al.* Increasingly frequent diagnosis of acute gastrointestinal graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2004; **10**: 320–327.
32 Fuji S, Kim SW, Mori S, Fukuda T, Kamiya S, Yamasaki S *et al.* Hyperglycemia during the neutropenic period is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation. *Transplantation* 2007; **84**: 814–820.

33 Hahn T, McCarthy Jr PL, Zhang MJ, Wang D, Arora M, Frangoul H *et al.* Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. *J Clin Oncol* 2008; **26**: 5728–5734.
34 Oh H, Loberiza Jr FR, Zhang MJ, Ringden O, Akiyama H, Asai T *et al.* Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. *Blood* 2005; **105**: 1408–1416.
35 Paulin T, Ringden O, Nilsson B. Immunological recovery after bone marrow transplantation: role of age, graft-versus-host disease, prednisolone treatment and infections. *Bone Marrow Transplant* 1987; **1**: 317–328.
36 Sayer HG, Longton G, Bowden R, Pepe M, Storb R. Increased risk of infection in marrow transplant patients receiving methylprednisolone for graft-versus-host disease prevention. *Blood* 1994; **84**: 1328–1332.
37 Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* 2007; **44**: 531–540.
38 Lee JL, Gooley T, Bensinger W, Schiffman K, McDonald GB. Veno-occlusive disease of the liver after busulfan, melphalan, and thiotepa conditioning therapy: incidence, risk factors, and outcome. *Biol Blood Marrow Transplant* 1999; **5**: 306–315.
39 Baker KS, Gurney JG, Ness KK, Bhatia R, Forman SJ, Francisco L *et al.* Late effects in survivors of chronic myeloid leukemia treated with hematopoietic cell transplantation: results from the Bone Marrow Transplant Survivor Study. *Blood* 2004; **104**: 1898–1906.
40 Khera N, Storer B, Flowers ME, Carpenter PA, Inamoto Y, Sandmaier BM *et al.* Nonmalignant late effects and compromised functional status in survivors of hematopoietic cell transplantation. *J Clin Oncol* 2012; **30**: 71–77.
41 Duval M, Klein JP, He W, Cahn JY, Cairo M, Camitta BM *et al.* Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. *J Clin Oncol* 2010; **28**: 3730–3738.

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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

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.

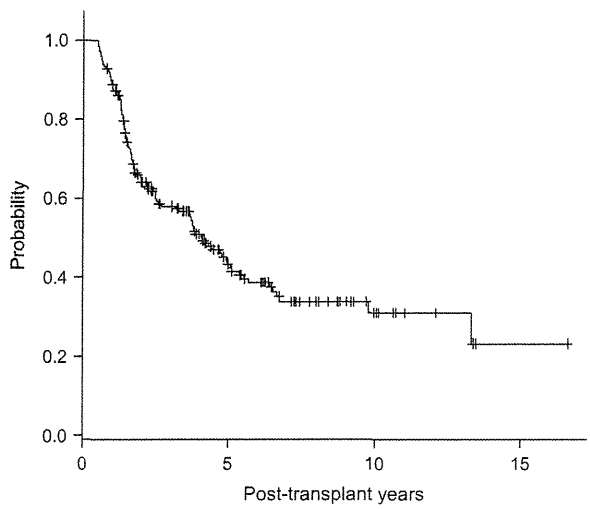

Figure 1 Overall survival of recipients with bronchiolitis obliterans syndrome from time of transplant.

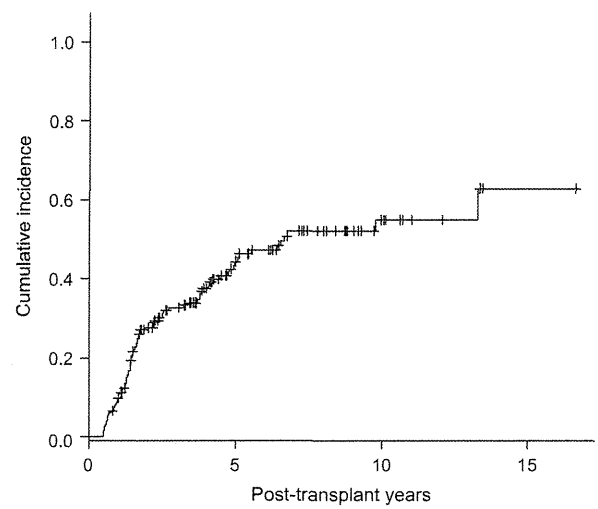

Figure 2 Nonrelapse mortality of recipients with bronchiolitis obliterans syndrome from time of transplant.

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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References

1. Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **28**: 425.
2. Yoshihara S, Yanik G, Cooke KR, Mineishi S. Bronchiolitis obliterans syndrome (BOS), bronchiolitis obliterans organizing pneumonia (BOOP), and other late-onset noninfectious pulmonary complications following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2007; **13**: 749.
3. Chien JW, Duncan S, Williams KM, Pavletic SZ. Bronchiolitis obliterans syndrome after allogeneic hematopoietic stem cell transplantation-an increasingly recognized manifestation of chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 2010; **16**: S106.
4. Soubani AO, Uberti JP. Bronchiolitis obliterans following haematopoietic stem cell transplantation. *Eur Respir J* 2007; **29**: 1007.
5. Williams KM, Chien JW, Gladwin MT, Pavletic SZ. Bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. *JAMA* 2009; **302**: 306.
6. Bacigalupo A, Chien J, Barisione G, Pavletic S. Late pulmonary complications after allogeneic hematopoietic stem cell transplantation: diagnosis, monitoring, prevention, and treatment. *Semin Hematol* 2012; **49**: 15.
7. Filipovich AH, Weisdorf D, Pavletic S, *et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; **11**: 945.
8. Epler GR, Colby TV. The spectrum of bronchiolitis obliterans. *Chest* 1983; **83**: 161.
9. Santo Tomas LH, Loberiza Jr FR, Klein JP, *et al.* Risk factors for bronchiolitis obliterans in allogeneic hematopoietic stem-cell transplantation for leukemia. *Chest* 2005; **128**: 153.
10. Ringden O, Remberger M, Ruutu T, *et al.* Increased risk of chronic graft-versus-host disease, obstructive bronchiolitis, and alopecia with busulfan versus total body irradiation: long-term results of a randomized trial in allogeneic marrow recipients with leukemia. Nordic Bone Marrow Transplantation Group. *Blood* 1999; **93**: 2196.
11. Dudek AZ, Mahaseth H, DeFor TE, Weisdorf DJ. Bronchiolitis obliterans in chronic graft-versus-host disease: analysis of risk factors and treatment outcomes. *Biol Blood Marrow Transplant* 2003; **9**: 657.
12. Yoshihara S, Tateishi U, Ando T, *et al.* Lower incidence of Bronchiolitis obliterans in allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning compared with myeloablative conditioning. *Bone Marrow Transplant* 2005; **35**: 1195.
13. Nakaseko C, Ozawa S, Sakaida E, *et al.* Incidence, risk factors and outcomes of bronchiolitis obliterans after allogeneic stem cell transplantation. *Int J Hematol* 2011; **93**: 375.
14. Atsuta Y, Suzuki R, Yoshimi A, *et al.* Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol* 2007; **86**: 269.
15. Giralt S, Ballen K, Rizzo D, *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367.
16. Przepiorka D, Weisdorf D, Martin P, *et al.* 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant* 1995; **15**: 825.
17. Shulman HM, Sullivan KM, Weiden PL, *et al.* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204.
18. Montgomery RA, Cozzi E, West LJ, Warren DS. Humoral immunity and antibody-mediated rejection in solid organ transplantation. *Semin Immunol* 2011; **23**: 224.
19. Rydberg L. ABO-incompatibility in solid organ transplantation. *Transfus Med* 2001; **11**: 325.
20. Novick RJ, Schafers HJ, Stitt L, *et al.* Recurrence of obliterative bronchiolitis and determinants of outcome in 139 pulmonary retransplant recipients. *J Thorac Cardiovasc Surg* 1995; **110**: 1402; discussion 13-4.
21. Cai J. Intestine and multivisceral transplantation in the United States: a report of 20-year national registry data (1990–2009). *Clin Transpl* 2009; 83.
22. Kocher AA, Schlechta B, Ehrlich M, *et al.* Effect of ABO blood type matching in cardiac transplant recipients. *Transplant Proc* 2001; **33**: 2752.
23. Sjogren J, Ljungdahl-Waller F, Senneby E, Ekmehag B, Koul B, Nilsson J. Heart transplantation with ABO-identical versus ABO-compatible cardiac grafts: influence on long-term survival. *Scand Cardiovasc J* 2010; **44**: 373.
24. Yu NC, Haug MT 3rd, Khan SU, *et al.* Does the donor-recipient ABO blood group compatibility status predict subsequent lung transplantation outcomes? *J Heart Lung Transplant* 1999; **18**: 764.
25. Sano Y, Date H, Nagahiro I, Aoe M, Shimizu N. Relationship between anti-ABO antibody production and hemolytic anemia after minor ABO-mismatched living-donor lobar lung transplantation. *Transplant Proc* 2005; **37**: 1371.
26. Shortt J, Westall GP, Roxby D, *et al.* A 'dangerous' group O donor: severe hemolysis in all recipients of organs from a donor with multiple red cell alloantibodies. *Am J Transplant* 2008; **8**: 711.
27. Kimura F, Sato K, Kobayashi S, *et al.* Impact of AB0-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program. *Haematologica* 2008; **93**: 1686.
28. Worel N, Greinix HT, Keil F, *et al.* Severe immune hemolysis after minor ABO-mismatched allogeneic peripheral

blood progenitor cell transplantation occurs more frequently after nonmyeloablative than myeloablative conditioning. *Transfusion* 2002; **42**: 1293.
29. Yazer MH, Triulzi DJ. Immune hemolysis following ABO-mismatched stem cell or solid organ transplantation. *Curr Opin Hematol* 2007; **14**: 664.
30. Worel N, Greinix HT, Schneider B, *et al.* Regeneration of erythropoiesis after related- and unrelated-donor BMT or peripheral blood HPC transplantation: a major ABO mismatch means problems. *Transfusion* 2000; **40**: 543.
31. Rabitsch W, Knobl P, Prinz E, *et al.* Prolonged red cell aplasia after major ABO-incompatible allogeneic hematopoietic stem cell transplantation: removal of persisting isohemagglutinins with Ig-Therasorb immunoadsorption. *Bone Marrow Transplant* 2003; **32**: 1015.
32. Griffith LM, McCoy JP Jr, Bolan CD, *et al.* Persistence of recipient plasma cells and anti-donor isohaemagglutinins in patients with delayed donor erythropoiesis after major ABO incompatible non-myeloablative haematopoietic cell transplantation. *Br J Haematol* 2005; **128**: 668.
33. Nilsson TK, Sondell KM, Norberg BO, Back O. Plasma levels of endothelial-derived haemostatic factors in autoimmune thrombocytopenia and haemolytic anaemia. *J Intern Med* 1990; **228**: 249.
34. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA* 2005; **293**: 1653.
35. Tanabe M, Kawachi S, Obara H, *et al.* Current progress in ABO-incompatible liver transplantation. *Eur J Clin Invest* 2010; **40**: 943.
36. Marras TK, Chan CK, Lipton JH, Messner HA, Szalai JP, Laupacis A. Long-term pulmonary function abnormalities and survival after allogeneic marrow transplantation. *Bone Marrow Transplant* 2004; **33**: 509.
37. Krivoy N, Hoffer E, Lurie Y, Bentur Y, Rowe JM. Busulfan use in hematopoietic stem cell transplantation: pharmacology, dose adjustment, safety and efficacy in adults and children. *Curr Drug Saf* 2008; **3**: 60.
38. Fischer A, du Bois R. Interstitial lung disease in connective tissue disorders. *Lancet* 2012; **380**: 689.
39. Zhang H, Chen J, Que W. A meta-analysis of unrelated donor umbilical cord blood transplantation versus unrelated donor bone marrow transplantation in acute leukemia patients. *Biol Blood Marrow Transplant* 2012; **18**: 1164.