mucositis, and that engraftment failure might be an extremely strong risk factor for the development of mucositis. Therefore, data for 118 patients were analyzed in this study. The study protocol was approved by the review board of Hokkaido University Graduate School of Medicine.

Conditioning regimens and transplantation procedures
Most of the conventional conditioning regimens consisted of TBI (12 Gy in six fractions) plus CY (60 mg/kg once daily i.v. for 2 days, total dose of 120 mg/kg) ± VP-16 (etoposide) (15 mg/kg once daily i.v. for 2 days, total dose of 30 mg/kg),[13] and most of the reduced-intensity conditioning regimens consisted of fludarabine (30 mg/m^2 once daily i.v. for 6 days, total dose of 180 mg/m^2) plus oral BU (4 mg/kg p.o. in divided doses daily for 2 days, total dose of 8 mg/kg) or i.v. BU (3.2 mg/kg i.v. in divided doses daily for 2 days, total dose of 6.4 mg/kg) plus low-dose TBI (4 Gy in two fractions).[14] CsA (3 mg/kg) or tacrolimus (FK, 0.03 mg/kg) and short-course MTX were used for GVHD prophylaxis. MTX was given at a dose of 15 or 10 mg/m^2 on day 1 and at a dose of 10 or 7 mg/m^2 on day 3 and day 6.

Supportive care and infection prophylaxis
Granulocyte CSF was administered from day 5 until engraftment. Levofloxacin was administered for prevention of bacterial infections until engraftment, and an antifungal (fluconazole, itraconazole or micafungin) was administered for prevention of fungal infections. Oral acyclovir was given from day −7 to day 35 for prevention of HSV infection.

Systemic folinic acid administration and mouthwash
Folinic acid was given i.v. at the same dose as that used for each administration of MTX at 12, 18 and 24 h after administration of MTX on days 1 and 3, and at 24, 30 and 36 h after administration of MTX on day 6. Folinic acid mouthwash (13.0% folinic acid) was given four times a day from day 1 to day 7. Systemic folinic acid administration and folinic acid mouthwash were given according to physicians' discretion. They were given to the patients who were considered by physicians to be at high risk for severe oral mucositis. For example, conventional conditioning regimens, female gender and higher doses of MTX were considered as high risk for severe oral mucositis.

Grading of oral mucositis
Oral mucositis was graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0. The criteria for oral mucositis were as follows: Grade 0, none; Grade 1, erythema of the mucosa; Grade 2, patchy ulcerations of pseudomembranes; Grade 3, confluent ulcerations or pseudomembranes, bleeding with minor trauma; Grade 4, tissue necrosis, significant spontaneous bleeding, life-threatening consequences. Severe oral mucositis was defined as grade 3 or 4 oral mucositis.

The incidence and severity of oral mucositis were evaluated daily by physicians and nurses. Dentists and dental hygienists evaluated oral mucositis at least once per week. The grading of oral mucositis was assigned at the time of evaluation.

Evaluation of GVHD
Acute GVHD was graded according to the consensus criteria.[15]

Statistical analysis
Univariate analyses were performed using the χ^2-test and Fisher's exact test, as appropriate. Factors with a P-value of 0.2 or less in the univariate analyses were included in the multivariate analysis. Stepwise multivariate logistic regression models were used to analyze the influence of selected variables on the risk of severe oral mucositis. Cumulative incidence of acute GVHD was calculated using the Gray method,[16] considering death without acute GVHD or relapse as competing events. Similarly, in the analysis of relapse incidence, death resulting from other causes was considered as a competing risk. In the analysis of non-relapse mortality, relapse was considered as a competing risk. JMP software version 8.0.2 (SAS Institute, Cary, NC, USA) was used for most of the statistical analyses. Analysis of cumulative incidences was carried out with the package 'comprsk' of the R statistical software 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org/). All P-values were two sided, and differences were considered to be statistically significant when $P<0.05$.

Results

The patient characteristics are shown in Table 1. Systemic folinic acid administration was given to 29 patients. The systemic folinic acid administration group had significantly higher proportions of female patients ($P=0.03$), patients who received higher doses of MTX ($P=0.0002$) and patients who received folinic acid mouthwash ($P<0.0001$). The mean duration of neutropenia in all patients was 18.3 days. No significant difference was observed in the duration of neutropenia between patients who received systemic folinic acid administration and those who did not (17.3 days vs 18.6 days, $P=0.53$). There was a difference over time. Systemic folinic acid administration was not given to any patients in 2006–2007. In 2008–2009, 29 (42.0%) of 69 patients received systemic folinic acid administration. Other characteristics in the two groups were the same.

Oral mucositis was observed in 91 (77.1%) of the patients (Table 2), and severe oral mucositis (NCI-CTCAE Grade 3 or Grade 4) was observed in 37 (31.4%) of the patients. The incidence of oral mucositis was significantly lower in patients who received systemic folinic acid administration than in patients who did not receive systemic folinic acid administration (58.6 vs 83.2%, $P=0.0063$), and the incidence of severe oral mucositis was also significantly lower in patients who received systemic folinic acid administration than in patients who did not receive systemic folinic acid administration (10.3 vs 38.2%, $P=0.005$).

Table 1 Patient characteristics

	Total (n = *118*)	*Folinic acid administration*		P-*values*
		Yes (n = *29*)	*No* (n = *89*)	
Age (years)				0.17
Median	47	41	48	
Range	17–68	18–66	17–68	
Gender				0.03
Male	61	10	51	
Female	57	19	38	
Disease				0.48
AML	44	12	32	
ALL	23	9	14	
MDS	9	0	9	
CML	5	0	5	
HL	2	0	2	
NHL	23	5	18	
ATLL	3	1	2	
MM	4	1	3	
AA	4	1	3	
MF	1	0	1	
Disease status at transplantation				0.23
CR	66	18	48	
Non-CR	37	10	27	
Chronic phase/stable disease	15	1	14	
Conditioning				0.07
CST	56	18	38	
VP/CY/TBI	25	7	18	
CY/TBI	21	9	12	
RIST	62	11	51	
Flu/BU/TBI	51	7	44	
GVHD prophylaxis				0.42
CsA + MTX	44	9	35	
FK + MTX	74	20	54	
Doses of MTX				0.0002
15-10-10 (mg/m^2)	72	27	45	
10-10-10 (mg/m^2)	41	1	40	
10-7-7 (mg/m^2)	5	1	4	
Stem cell source				0.21
Related BM	14	4	10	
Related PBSC	13	5	8	
Unrelated BM	82	16	66	
Unrelated CB	9	4	5	
Duration of neutropenia (<500/μL)				0.57
⩾21 days	28	8	20	
<21 days	90	21	69	
Folinic acid mouthwash				<0.0001
Yes	60	25	35	
No	58	4	54	

Abbreviations: AA = aplastic anemia; ATLL = adult T-cell leukemia/lymphoma; CB = cord blood; CST = conventional SCT; FK = tacrolimus; Flu = fludarabine; HL = Hodgkin lymphoma; MDS = myelodysplastic syndrome; MF = myelofibrosis; MM = multiple myeloma; NHL = non-Hodgkin lymphoma; VP16 = etoposide.

Table 3 shows clinical factors and results of univariate analysis of clinical factors associated with the incidence of severe oral mucositis. Severe oral mucositis was significantly associated with VP/CY/TBI ($P = 0.048$) and duration of neutropenia (<500/μL) ($P = 0.0047$). Systemic folinic acid administration and folinic acid mouthwash reduced the incidence of severe oral mucositis ($P = 0.0038$ and $P = 0.0017$, respectively). Age, gender, disease status at transplantation, GVHD prophylaxis, stem cell source and dose of MTX did not correlate with severe oral mucositis. In multivariate analysis, duration of neutropenia was significantly associated with severe oral mucositis (odds ratio (OR) = 4.78, 95% confidence interval (CI) 1.77–13.9, $P = 0.0019$), and systemic folinic acid administration significantly reduced the incidence of severe oral mucositis (OR = 0.13, 95%CI 0.04–0.73, $P = 0.014$) (Table 4). There was a tendency for a higher incidence of severe oral mucositis in patients who received VP/CY/TBI (OR = 2.42, 95%CI 0.86–6.99, $P = 0.095$), and there was a tendency for a lower incidence of severe oral mucositis in patients who received folinic acid mouthwash (OR = 0.39, 95%CI 0.15–1.00, $P = 0.051$).

No significant difference was observed in the incidence of acute GVHD on day 100 after transplantation between patients who received systemic folinic acid administration and those who did not (acute GVHD grade 1–4, 71.3 vs 68.5%, $P = 0.88$; acute GVHD grade 2–4, 49.9 vs 40.4%, $P = 0.36$; acute GVHD grade 3–4, 6.0 vs 11.2%, $P = 0.51$) (Figure 1). There was no difference in the incidence of severe oral mucositis between patients who developed acute GVHD and those who did not (GVHD grade 1–4; 29.6%, grade 0; 31.25%, $P = 0.87$). There was no difference in the incidence of severe oral mucositis between patients who had severe acute GVHD (grade 3–4) and those who did not (GVHD grade 3–4; 16.7%, grade 1–2; 31.9%, $P = 0.47$).

No significant difference was observed in the incidences of relapse and non-relapse mortality after transplantation between patients who received systemic folinic acid administration and those who did not (relapse, 7.4 vs 22.8%, $P = 0.19$; non-relapse mortality, 7.8 vs 12.1%, $P = 0.71$) (Figure 2).

Table 5 shows the effects of systemic folinic acid administration and/or mouthwash. Use of i.v. opioid analgesics and duration of inability to eat were significantly reduced in patients who received systemic folinic acid administration and/or mouthwash compared with those in patients who received neither systemic folinic acid administration nor folinic acid mouthwash. There was no difference in the duration of total parenteral nutrition between patients who received systemic folinic acid administration and/or mouthwash and patients who received neither systemic folinic acid administration nor folinic acid mouthwash.

Discussion

The efficacy and safety of folinic acid administration have been controversial so far. Less than half of the centers surveyed have used folinic acid administration.[11,12] Therefore, we retrospectively analyzed data for 118 patients who had undergone allogeneic hematopoietic SCT and had received MTX for GVHD prophylaxis.

Table 2 Incidence of oral mucositis

Total	*Grades of oral mucositis*				
	0	*1*	*2*	*3*	*4*
	27 (22.9%)	26 (22.0%)	28 (23.7%)	36 (30.5%)	1 (0.85%)
Folinic acid administration					
Yes ($n=29$)	12 (41.4%)	9 (31.0%)	5 (17.2%)	3 (10.3%)	0 (0.0%)
No ($n=89$)	15 (16.9%)	17 (19.1%)	23 (25.8%)	33 (37.1%)	1 (1.1%)

Table 3 Univariate analysis of severe oral mucositis

	n	*Severe oral mucositis (%)*	*OR*	*94%CI*	P-*values*
Age					
⩾50	48	16 (33.3%)	1.17	0.53–2.56	0.70
<50	70	21 (30.0%)	1		
Gender					
Male	61	16 (26.2%)	1		
Female	57	21 (36.8%)	1.64	0.75–3.64	0.22
Disease status at transplantation					
CR	66	24 (36.4%)	1.54	0.65–3.84	0.33
Non-CR	37	10 (27.0%)	1		
Conditioning					
CST	56	18 (32.1%)	1.07	0.49–2.34	0.86
RIST	62	19 (30.6%)	1		
VP/CY/TBI	25	12 (48.0%)	2.51	1.01–6.28	0.048
Non-(VP/CY/TBI)	93	25 (26.9%)	1		
GVHD prophylaxis					
CsA + MTX	44	12 (27.3%)	0.74	0.32–1.65	0.46
FK + MTX	74	25 (33.8%)	1		
Doses of MTX					
15-10-10	72	18 (25.0%)	1.33	0.18–27.0	0.80
10-10-10	41	18 (43.9%)	3.13	0.42–64.1	0.29
10-7-7	5	1 (28.0%)	1		
Stem cell source					
Related BM	14	3 (21.4%)	1		
Related PBSC	13	4 (30.8%)	1.62	0.29–10.2	0.58
Unrelated BM	82	27 (32.9%)	1.80	0.51–8.44	0.38
Unrelated CB	9	3 (33.3%)	1.83	0.27–12.9	0.53
Duration of neutropenia (<500/μL)					
⩾21 days	28	15 (53.6%)	3.57	1.48–8.78	0.0047
<21 days	90	22 (24.4%)	1		
Folinic acid administration					
Yes	29	3 (10.3%)	0.20	0.04–0.62	0.0038
No	89	34 (38.2%)	1		
Folinic acid mouthwash					
Yes	60	11 (18.3%)	0.28	0.12-0.62	0.0017
No	58	26 (44.8%)	1		

Abbreviations: CB = cord blood; CI = confidence interval; CST = conventional SCT; FK = tacrolimus; OR = odds ratio; VP16 = etoposide.

Multivariate analysis showed that systemic folinic acid administration significantly reduced the incidence of severe oral mucositis (OR = 0.13, 95%CI 0.04–0.73, $P=0.014$). Furthermore, use of opioid analgesics and duration of inability to eat were significantly reduced in patients who received systemic folinic acid administration.

Table 4 Multivariate analysis of severe oral mucositis

	OR	*95%CI*	P-*values*
Conditioning			
VP/CY/TBI	2.42	0.86–6.99	0.095
Non-(VP/CY/TBI)	1		
Duration of neutropenia (<500/μL)			
⩾21 days	4.78	1.77–13.9	0.0019
<21 days	1		
Folinic acid administration			
Yes	0.13	0.04–0.73	0.014
No	1		
Folinic acid mouthwash			
Yes	0.39	0.15–1.00	0.051
No	1		

The group of patients who received systemic folinic acid administration had significantly higher proportions of female patients ($P=0.03$) and patients who received higher doses of MTX ($P=0.0002$). Although gender did not correlate with severe oral mucositis in our study, several studies have shown that female gender is one of the risk factors for oral mucositis.[17,18] In our retrospective study, systemic folinic acid administration was performed according to physicians'discretion. Therefore, it is likely that systemic folinic acid administration was used for patients who were considered by physicians to be at high risk for severe oral mucositis.

There are data that provide a rationale for using MTX and folinic acid in combination for GVHD prophylaxis.[19] Gratwohl *et al.*[20] reported that systemic folinic acid administration 6 h after each administration of MTX reduced the toxicity of MTX and maintained the effect of MTX on prevention of GVHD in dogs, and that MTX at concentrations above 10^{-6} M completely abrogated thymidine uptake in lymphocytes with stimulation for 6 h *in vitro*.[21]

In pediatrics, European Group for Blood and Marrow Transplantation Working Party Paediatric Diseases and International BFM Study Group-Subcommittee Bone Marrow Transplantation recommended that folinic acid (15 mg/m^2 per day) should be given 24 h after MTX.[22] However, there are no recommendations or guidelines in adult transplant groups for the use of folinic acid following MTX. Therefore, systemic folinic acid administration was given at various doses and schedules, starting 6–24 h after MTX administration.[11,23] We used folinic acid i.v. at the same dose as that used for each administration of MTX at

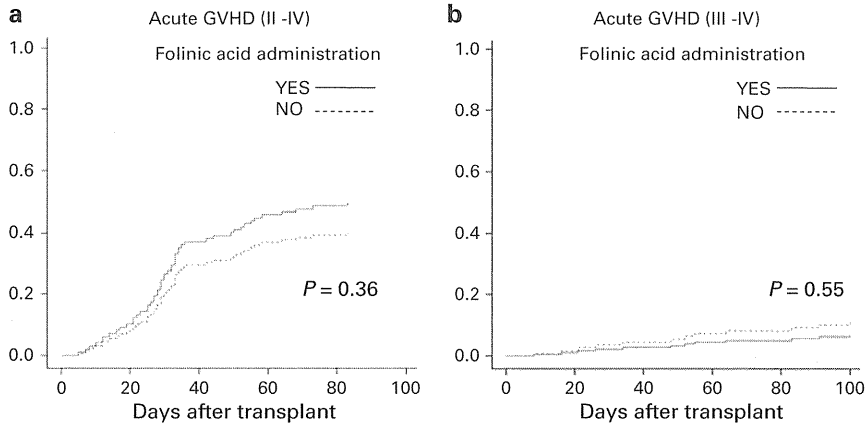

Figure 1 Cumulative incidence of grade II–IV acute GVHD (**a**) and grade III–IV acute GVHD (**b**) grouped according to the use of folinic acid administration.

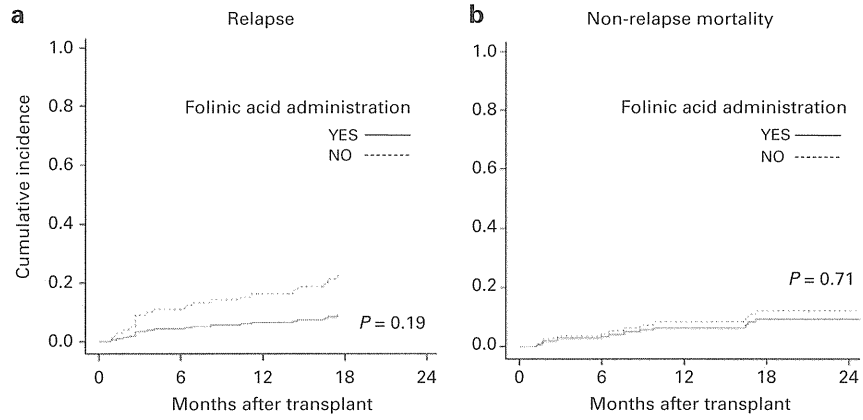

Figure 2 Cumulative incidence of relapse (**a**) and non-relapse mortality (**b**) grouped according to the use of folinic acid administration.

Table 5 Effect of folinic acid administration and mouthwash

	Without folinic acid (n = 54)	*Folinic acid mouthwash without administration (n = 35)*	*Folinic acid administration with or without mouthwash (n = 29)*
Severe oral mucositis (grade 3–4)	25 (46.3%)	9 (25.7%), $P = 0.053$	3 (10.3%), $P = 0.0008$
Use of opioid analgesics	32 (59.3%)	11 (31.4%), $P = 0.010$	10 (34.5%), $P = 0.038$
Duration of inability to eat (days; mean (range))	25.6 (0–53)	12.6 (0–55), $P < 0.0001$	8.5 (0–30), $P < 0.0001$
Duration of total parenteral nutrition (days; mean (range))	41.8 (0–272)	26.0 (0–96), $P = 0.054$	31.6 (0–90), $P = 0.32$

12, 18 and 24 h after administration of MTX on days 1 and 3, and at 24, 30 and 36 h after administration of MTX on day 6. We decided the dose and timing of systemic folinic acid administration according to the dose and timing of systemic folinic acid administration after high-dose MTX. Although there was no significant difference in the incidence and severity of acute GVHD in our study, our dose of folinic acid, which is about three times higher than that of the pediatric recommendation, might be in excess of those required. Further studies are needed to establish the optimal dose and timing of systemic folinic acid administration.

Although there is no evidence to support the use of folinic acid mouthwash for prevention of mucositis, folinic acid mouthwashes have been given to patients who received MTX administration in some centers.[24–26] In our study, multivariate analysis showed that there was a tendency for a lower incidence of severe oral mucositis in patients who received folinic acid mouthwash (OR = 0.39, 95%CI 0.15–1.00, $P = 0.051$). Not only systemic folinic acid administration but also folinic acid mouthwash significantly reduced the use of opioid analgesics and the duration of inability to eat. Therefore, it is likely that folinic acid mouthwash had a positive effect on the prevention of severe oral mucositis.

In multivariate analysis, duration of neutropenia (more than 21 days) was significantly associated with severe oral mucositis (OR = 4.78, 95%CI 1.77–13.9, $P = 0.0019$). The mean duration of neutropenia was 18.3 days in our study. Therefore, the cutoff point of duration of neutropenia appeared to be 3 weeks (21 days). The use of folinic acid did not reduce the duration of neutropenia in our study (17.3 days vs 18.6 days, $P = 0.53$). It is important to reduce the duration of neutropenia to prevent severe oral mucositis.

Hoyt *et al.*[27] reported that etoposide induces more severe mucositis than dose CY when added to TBI. In our study, there was a tendency for a higher incidence of severe oral mucositis in patients who received VP/CY/TBI (OR = 2.42, 95%CI 0.86–6.99, $P = 0.095$). A VP/CY/TBI regimen may increase the incidence of severe oral mucositis compared with the effects of other conditioning regimens. In patients with a high risk of severe oral mucositis, use of MTX for GVHD prophylaxis may cause more severe oral mucositis. Therefore, systemic folinic acid administration may be useful to reduce the incidence of severe oral mucositis in patients who have received a VP/CY/TBI regimen.

Although Takahashi *et al.*[1] reported that the severity of oral mucositis was reduced in reduced intensity stem cell transplantation (RIST) patients compared with that in conventional stem cell transplantation (CST) patients, no significant difference was observed in the incidence of severe oral mucositis between patients who received CST and those who received RIST in our study. Several studies have shown that severe oral mucositis was correlated with TBI.[28] One reason for no significant difference being found in our study might be the use of TBI in most RIST patients.

Although Sonis *et al.*[5] reported that oral mucositis is associated with significantly worse economic outcomes, there was no difference in the duration of total parenteral nutrition in our study. We were not able to show the cost effectiveness of the use of folinic acid. Further studies are needed to clarify the cost effectiveness.

In this retrospective study, systemic folinic acid administration and mouthwash appear to be useful for reducing the incidence of severe oral mucositis in those patients who were considered by physicians to be at high risk for severe oral mucositis. Further prospective controlled studies are needed to assess the efficacy of systemic folinic acid administration and mouthwash.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Professional oral health care reduces oral mucositis and febrile neutropenia in patients treated with allogeneic bone marrow transplantation

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Abstract
Goal of work Little is known about the effects of professional oral health care (POHC) on the outcome of hematopoietic stem cell transplantation (HSCT). We evaluated the effects of POHC given by dentists and dental hygienists on the development of oral mucositis and febrile neutropenia (FN) after allogeneic bone marrow transplantation (BMT).

Patients and methods We retrospectively studied 140 adult patients who had received allogeneic BMT, with or without POHC, in our hospital consecutively between February 2002 and December 2009. Oral mucositis was evaluated according to the World Health Organization scale.
Main results The incidence of oral mucositis was 66.7% (52/78) in the patients who had received POHC, compared to 93.5% (58/62) in the non-POHC group ($P<0.001$). The incidence of FN and the maximal level of CRP were also significantly lower in the POHC group. Multivariate analysis revealed that the POHC was significantly associated with the incidence of oral mucositis (odds ratio, 7.58; 95%CI, 2.45–23.34; $P<0.001$).
Conclusions We concluded that POHC reduced the incidences of oral mucositis and FN by upgrading the overall oral hygiene during HSCT.

Keywords Professional oral health care · Oral mucositis · Bone marrow transplantation · Multivariate analysis · Quality of life

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Introduction

Oral mucositis is a frequent complication of high-dose chemotherapy prior to allogeneic hematopoietic stem cell transplantation (HSCT). It is seen in 75–99% of patients who receive the combination of total body irradiation and chemotherapy [1–3]. And the impairment of oral mucositis leads to an increased risk of infection, need for total parenteral nutrition, intravenous pain control, prolonged hospital stays with increased economic burden, and poor quality of life in patients with HSCT [4–7].

Oral mucositis is a complex biological process involving a series of factors, including cytokine-mediated actions, effects of chemotherapy on the epithelium, generation of reactive oxygen species, and bacterial flora of the oral cavity [8]. As the patients' oral hygiene is thought to modify the incidence and severity of oral mucositis, professional oral health care (POHC) is recommended prior to initiation of conditioning therapy for HSCT [9, 10]. Based on those reports, we set up an interdisciplinary team for oral health care in 2006 and started to provide POHC to all the patients who had undergone allogeneic BMT at our hospital.

Several reports showed that oral hygiene prevented infection [11, 12]; yet other reports showed that there was no significant correlation between dental foci and infections, mucositis, or survival rate in patients with HSCT [13]. Thus, oral hygiene in HSCT has not fully been evaluated, and very few studies have examined the effects of POHC on oral mucositis and febrile neutropenia (FN) accompanying HSCT. Hence, we examined the relationship between POHC and outcomes of oral mucositis and FN in HSCT.

Patients and methods

Patients

A retrospective analysis was performed based on 140 adult patients who had received allogeneic BMT between February 2002 and December 2009 at Hokkaido University Hospital. BMT was performed either by conventional or reduced-intensity stem cell transplantation (RIST). Of the 140 patients, 62 who underwent BMT in 2002–2005 were without POHC. Since we set up an interdisciplinary team for oral health care and started to provide POHC to all BMT patients in 2006, the rest of the 78 patients were with POHC. We compared the incidences of oral mucocitis and FN between the two groups to evaluate the effects of POHC.

Conditioning regimens

Most of the conventional conditioning regimens consisted of total body irradiation (TBI; 12 Gy in six fractions) plus cyclophosphamide (60 mg/kg once daily i.v. for 2 days, total dose of 120 mg/kg)±VP-16 (15 mg/kg once daily i.v. for 2 days, total dose of 30 mg/kg) [14, 15], and most of the reduced-intensity conditioning regimens consisted of fludarabine (30 mg/m^2 once daily i.v. for 6 days, total dose of 180 mg/m^2) plus oral busulfan (4 mg/kg p.o. in divided doses daily for 2 days, total dose of 8 mg/kg), or intravenous busulfan (3.2 mg/kg i.v. in divided doses daily for 2 days, total dose of 6.4 mg/kg) plus low-dose total body irradiation (4 Gy in two fractions). Cyclosporine A (CsA, 3 mg/kg) or tacrolimus (FK, 0.03 mg/kg) and short-course methotrexate (MTX) were used for graft-versus-host disease (GVHD) prophylaxis. MTX was given at a dose of 15 or 10 mg/m^2 on day 1, and 10 or 7 mg/m^2 on day 3 and day 6.

Grading of oral mucositis

Oral mucositis was graded according to the World Health Organisation (WHO) scale. The criteria of oral mucositis were as follows: grade 0, none; grade 1, soreness and erythema; grade 2, erythema, ulcers, ability to eat solids; grade 3, ulcers, requiring liquid diet; and grade 4, alimentation not possible.

The incidence and severity of oral mucositis were evaluated daily by physicians and nurses according to the instruction of the dentist in charge, and the consistency of assessments was double checked by dentists during their rounds at least once per week.

Febrile neutropenia

FN was defined as a single axillar temperature >37.5°C in patients with peripheral neutrophil counts $<0.5\times10^9$/L, as reported previously [16].

Inflammatory response body temperature (BT) was determined daily, and C-reactive protein (CRP) was determined weekly at least. The maximal level of CRP and the highest BT were recorded.

Professional oral health care

POHC consisted mainly of mechanical cleaning given by dentists and dental hygienists (hygiene-based oral health care), namely brushing, flossing, and rubbing the oral mucosa with a sponge brush, which thoroughly cleaned the teeth, the buccal mucosa, the tongue, and the dentures.

The POHC given to all the patients who received HSCT from March 2006 to the present was a part of pre- and post-transplantation management organized by the interdisciplinary team. Namely at least two dentists examined the patients' oral health, including oral hygiene and potential causes of infections in the oral region by radiographic survey and by clinical examination of the hard and soft tissues; and dental problems that might cause infection, such as periapical and marginal periodontitis, dental caries, and semi-impacted or impacted teeth, were treated by surgical procedures as much as possible until HSCT. Furthermore, a dental hygienist gave mechanical cleaning of the mouth and gave instructions on the proper way to brush the teeth until each patient improved his or her

technique, including those how to use toothbrushes, interdental cleaning devices, and tongue brushes. During transplantation period, all patients had to achieve self-management of oral hygiene; tooth blushing after every meal and before going to bed, and oral rinsing with normal saline solution every 3 h during the day. A mouth-wetting agent containing baking soda was applied locally to the xerostomia several times a day. Dentists and hygienists weekly performed a fundamental examination and POHC on the patients and monitored compliance in a clean room. When acute and chronic oral mucositis developed, oral hygiene was restored by using an extra-soft toothbrush and, for the relief of pain, mild or severe, the patient gargled with a physiological saline solution containing anesthetic lidocaine. Opioid analgesics were administered for severe pain.

Data collection

Data were collected from clinical records. Toxicity of oral mucositis was graded from the reviews conducted by nurses or physicians according to the WHO scale, and the consistency of assessments was double-checked by the dentists during their rounds at least once per week. As the markers of inflammation, incidence of FN, the maximal level of CRP and the highest BT were assessed.

Statistical analysis

The incidences of oral mucositis and FN were compared between POHC and non-POHC groups, and the difference was evaluated for coutinuous number by Fisher's exact test and for nominal number by the chi-square test. Univariate analyses were performed using the chi-square test and Fisher's exact test, as appropriate. The factors with a P value of 0.05 or less in the univariate analyses were included in the multivariate analysis. Multivariate logistic regression models were used to analyze the influence of selected variables on the risk for oral mucositis. For most of the statistical analysis, SPSS 14.0 for Windows (SPSS, Chicago, IL, USA) was used. The P value was set to <0.05 as significant.

Results

Characteristics of the patients and BMTs

The characteristics of patients and transplantations are shown in Table 1. Median age, administration of CY/TBI plus VP-16 regimen, and GVHD prophylaxis were significantly different between non-POHC and POHC patients. Other characteristics were equivalent between the two groups.

Incidences of oral mucositis and levels of inflammatory responses

Clinical outcomes of oral mucositis after allogeneic BMT are shown in Table 2. The incidence of oral mucositis was 66.7% (52/78) in the patients who had received POHC, which was significantly lower than the 93.5% (58/62) in the non-POHC group, according to the mucositis/stomatitis category of the WHO scale. The incidence of FN and the maximal level of CRP were also significantly lower in the POHC group. The highest BT was not significantly different between the two groups (median maximal CRP, 2.6 vs. 7.1 mg/dL, $P<0.035$; median highest BT, 38.0°C vs. 38.3°C, $P<0.26$).

Univariate and multivariate analyses for oral mucositis were performed, and results are summarized in Table 3. The results of univariate analysis showed that the conditioning regimen and POHC were significantly associated with the incidence of oral mucositis. Only POHC remained significant in the multivariate analysis (odds ratio, 7.6; 95%CI, 2.45–23.3; $P<0.001$).

Changes in the incidence and severity of oral mucositis

The occurrence and severity of oral mucositis reduced year by year after we introduced POHC intervention in March 2006. The data are shown in Fig. 1. The incidence of oral mucositis was 93.5% before intervention by POHC, whereas it significantly lowered to 72.7% ($p<0.05$) in 2008 and to 45.8% ($p<0.01$) in 2009 (after the intervention). Also grade 3 or severer oral mucositis decreased particularly from 2008 onward, and the incidences of those were significantly lower in the POHC group.

Discussion

Oral mucositis is a frequently described toxicity in patients receiving chemotherapy and/or radiotherapy; it almost invariably develops in patients undergoing high-dose chemotherapy and HSCT. It has been shown that grade 3/4 severe mucositis develops in up to 98% of patients receiving high-dose chemotherapy [17]. The effects of severe mucositis are so adverse, causing serious discomfort, lengthened hospital stays, additional hospital cost, and increased risk for infection and mortality. Several studies have demonstrated an association between the development of severe mucositis and poor survival after transplantation [7, 18].

The pathogenesis of oral mucositis accompanying chemotherapy or radiation is far more complex than it is without those treatments. According to Sonis [8] who reported the biological sequence of mucositis, it results

Table 1 Patients and transplantation characteristics

Characteristics	Non-POHC (n=62)	POHC (n=78)	p Value
Age, median (range)	43 (15–66)	47 (18–77)	0.012
Patient sex, %			
Male	51.6%	56.4%	0.35
Donor type, %			
HLA-matched related donor	29.0%	19.2%	0.17
HLA-matched unrelated donor	58.1%	66.7%	
HLA-mismatched donor	12.9%	14.1%	
CST/RIST			
CST	54.8%	43.6%	0.18
RIST	45.2%	56.4%	
Conditioning regimen, %			
Fludarabine/Busulfan	38.7%	48.7%	0.15
Fludarabine/Melphalan	3.2%	3.8%	0.51
CY/VP-16/TBI	40.3%	24.4%	0.033
CY/TBI	6.5%	16.7%	0.055
Others	11.3%	6.4%	0.34
TBI, %	91.4%	93.5%	0.15
GVHD prophylaxis, %			
Cyclosporine A+methotrexate	58.1%	35.9%	0.006
Tacrolimus+methotrexate	41.9%	64.1%	
Underlying disease, %			
ALL	15.5%	18.3%	
AML	20.7%	35.5%	
MDS	17.2%	9.7%	
CML	13.8%	3.2%	
ML	12.1%	21.5%	
ATLL	6.9%	2.2%	
MM	6.9%	3.2%	
others	6.9%	6.5%	

POHC professional oral health care, *HLA* human leukocyte antigen, *CST* conventional stem cell transplantation, *RIST* reduced-intensity stem cell transplantation, *CY* cyclophosphamide, *VP16* etoposide, *TBI* total body irradiation, *GVHD* graft-versus-host disease, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *MDS* myelodysplastic syndrome, *CML* chronic myelogenous leukemia, *ML* malignant lymphoma, *ATLL* adult T-cell leukemia/lymphoma, *MM* multiple myeloma

from nonspecific direct effects of radiation or chemotherapy on rapidly dividing mucosal basal cells; the initiation phase involves direct damage to DNA and other cellular components that occurs immediately after exposure to radiation or chemotherapy. The ulceration surface can then be colonized by oral bacteria, producing toxins and additional inflammatory cytokines, and angiogenic factors. This may cause bacteremia and sepsis in the presence of granulocytopenia.

In this study, we retrospectively analyzed oral mucositis in patients who received allogeneic BMT with or without POHC intervention. The incidence of oral mucositis in the patients with POHC was significantly lower than in those without POHC. The present data support the findings of an earlier retrospective analysis of patients who had received allogeneic HSCT [19]. Whereas the previous study included patients with several graft sources (bone marrow, peripheral blood, and cord blood), we investigated BMT patients alone to observe the effects of POHC more specifically in BMT cases. The occurrence and severity of oral mucositis decreased year by year after we introduced POHC intervention in March 2006; particularly grade 3 or severer oral mucositis decreased from 2008 onward. We performed multivariate analysis to resolve any bias which might arise from differences in the background since we compared the two groups who had time lapses in their BMT treatments; the analysis revealed that the

Table 2 The effects of POHC on incidence of oral mucositis and inflammatory responses

	Non-POHC (n=62)	POHC (n=78)	p Value
Incidence of oral mucositis	58/62 (93.5%)	52/78 (66.7%)	<0.001
Incidence of febrile neutropenia	51/62 (82.3%)	47/78 (60.3%)	<0.01
Max CRP, median (range)	7.10 (0.20–39.6)	2.64 (0.06–27.3)	0.035
Max BT, median (range)	38.3 (36.5–40.4)	38.0 (36.5–41.2)	0.26

POHC professional oral health care, *CRP* C-reactive protein, *BT* body temperature

Table 3 Univariate and multivariate analysis for oral mucositis

Variables	Incidence of oral mucositis (%)	Univariate	Multivariate	
		P	Odds ratio (95%CI)	*P*
Sex				
Male	75.0	0.23		
Female	81.2			
Age				
–49	78.9	0.53		
50-	78.0			
Donor type				
Related	81.1	0.43		
Unrelated	77.7			
HLA-matched	79.0	0.60		
HLA-mismatched	78.9			
CST/RIST				
CST	83.8	0.10		
RIST	73.6			
Conditioning regimen				
Fludarabine/Busulfan	75.6	0.011	3.3 (0.50–22.3)	0.22
CY/VP-16/TBI	90.9			
CY/TBI	81.8			
Others				
GVHD prophylaxis				
Cyclosporine A+methotrexate	84.1	0.13		
Tacrolimus+methotrexate	75.0			
Professional oral health care				
Without	93.5	<0.001	7.58 (2.45–23.34)	<0.001
With	66.7			
CR				
Without	81.0	0.46		
With	78.7			

CI confidence interval, *CR* complete remission. For other abbreviations, see Table 1

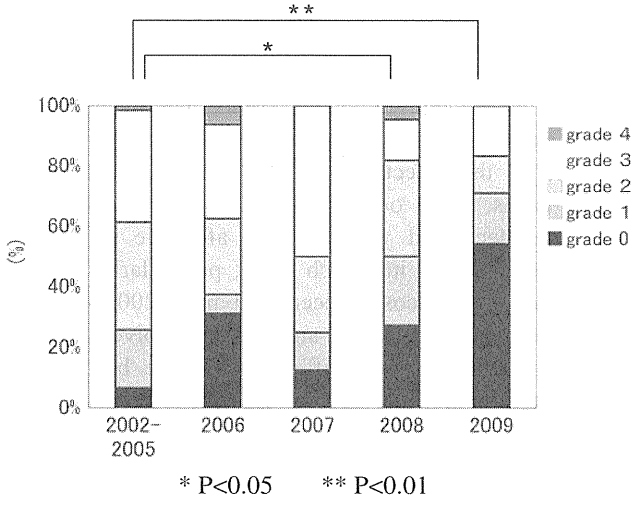

Fig. 1 Changes in the incidence and severity of oral mucositis. The occurrence and severity of oral mucositis reduced year by year after we introduced the POHC in 2006. The incidence of oral mucositis was 93.5% before intervention by POHC, whereas it significantly lowered to 72.7% ($p<0.05$) in 2008 and 45.8% ($p<0.01$) in 2009 after the intervention. Grade 3 or severer oral mucositis decreased particularly from 2008 onward

POHC was significantly associated with reduced incidence of oral mucositis. In our study, median age, CY/TBI plus VP-16 regimen, and GVHD prophylaxis were significantly different between non-POHC and POHC patients. However, either age or GVHD prophylaxis did not affect the incidence of oral mucositis in univariate analysis; CY/TBI plus VP-16 regimen did not affect the incidence either in multivariate analysis. Yet there were a greater number of RIST in the POHC group and this likely contributed to the reduced mucositis seen in this group, even though the difference did not present a statistical significance. We also confirmed that the incidence of febrile neutropenia and the maximal level of CRP were significantly lower in the POHC group than in non-POHC group. This is consistent with the studies that the inflammatory response after conditioning for a HSCT is the result of the chemotherapy-induced mucositis [20, 21].

POHC, i.e., hygiene-based oral health care, is considered the best method for mitigating the colonization of oral bacteria that leads to inflammation, as is more effective than

using disinfectants or antibiotics [22]. Most reports concerning the dental management during HSCT recommend mouth rinses or use of antibiotic pastille for oral decontamination. However, those have been found ineffective for preventing oral mucositis [23–26]. The microorganism in the mouth contains hundreds of species of bacteria as complex, mixed, interdependent colonies in biofilms, and adheres to the teeth [27]. The biofilm protects the adhering bacteria against environmental attacks. Antibiotics or oral rinses are unable to penetrate the plaque to reach the linking film bacteria [28]. However, oral rinses and other detergents may be effective in preventing oral disease when used in addition to the mechanical removal of plaque [29]. Mechanical removal of dental plaque, i.e., tooth brushing, is indispensable to decreasing the biofilm and essential in POHC. Almost all people understand that regular brushing is very important for oral hygiene, but unfortunately few people brush properly. This is why professional and repeated instruction on brushing is critical for controlling plaque.

Plaque control by professionally performed brushing, combined with frequent cleaning of teeth and self-administered oral care, may reduce the total number of microorganisms [30]. A recent study has suggested a relationship between oral health care and aspiration pneumonia in the elderly [31, 32]. POHC has been found to reduce cryptogenic fever [33, 34], aspiration pneumonia, and influenza infection along with reducing oral bacteria or enzymatic activity [34, 35].

The main limitations of our study were that (1) it was a retrospective analysis of 140 consecutive subjects and that (2) the study period was so prolonged to warrant comparability with historical control. Further establishment of a methodology for oral health care in HSCT will be needed, and further research should focus on strategies directed at the prevention and treatment of oral mucositis.

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LETTER TO THE EDITOR

Risks and benefits of ovarian shielding in female patients undergoing TBI: a decision analysis

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In a recent issue of *Bone Marrow Transplantation*, Courbiere *et al.*[1] raised the possibility of leukemia relapse after autologous transplantation of cryopreserved ovarian tissue from leukemic cell contamination in the graft. They detected a small copy number of Bcr–Abl transcripts by RQ-PCR in the ovarian tissue from an 18-year-old woman with CML. We agree that ovarian transplantation could be proposed (once patients are informed of the risk of leukemia relapse), as there were few contaminating leukemic cells and a GVL effect may be protective.

In Japan, cryopreservation of ovarian tissue is not available, but two other strategies are used to preserve fertility in young women undergoing hematopoietic SCT. One is embryo or oocyte cryopreservation for women with or without a partner, respectively. Although the success rate after transfer of thawed fertilized oocytes had been low previously, both post-thaw survival and fertilization rates of frozen oocytes[2,3] have improved. Nonetheless, concern remains regarding the potential for chromosomal aneuploidy or other karyotypic abnormalities in the offspring, as cryopreservation may affect the meiotic spindle of oocytes.[4] In addition, it is generally difficult to obtain good-quality oocytes from patients receiving chemotherapy.[5]

Another strategy is ovarian shielding in women undergoing TBI. Whereas ovarian recovery is observed in only 10–15% of patients receiving standard conditioning with CY and TBI,[6] most patients show ovarian recovery after high-dose CY alone.[6] Ovarian function can therefore be preserved by reducing the radiation dose to the ovaries. We previously reported that ovarian function was recovered in about 80% of patients who underwent ovarian shielding.[7,8] The incidence of leukemia relapse may not increase if this procedure is performed in patients in remission, as the total radiation dose to the ovaries was approximately 3 Gy in this protocol, which is higher than the TBI dose (2 Gy) in the non-myeloablative regimen of the Seattle group associated with a relapse rate similar to that of a myeloablative regimen.[9] However, a large number of patients is required to determine the actual change in the incidence of relapse under ovarian shielding.

To overcome the difficulty for both physicians and patients in deciding whether or not to perform ovarian shielding, we have used a decision analysis approach. We constructed a decision tree using TreeAge Pro 2009 software (Williamstown, MA, USA) (Figure 1). The square at the left represents a decision node. We can decide either to perform ovarian shielding or not. Circles represent chance nodes and each chance node has 2 or 3 possible outcomes with a specific probability, called the transition probability. Every branch finally ends with triangles, called terminal nodes, and each terminal node has an assigned payoff value, called utility, according to different health states. Calculations were performed backward, from right to left in the decision tree. The sum of the products of transition probabilities and the utilities of the branches becomes the expected value for each chance node, and eventually the sum of the expected values in all of the chance nodes following the decision nodes becomes the expected value of each decision. To make a simple decision model, we determined the transition probabilities based on data from patients who underwent allogeneic transplantation for acute leukemia in first remission. The incidences of transplant-related mortality and relapse were assumed to be 0.2 (20%).[10] However, the incidence of relapse may increase with ovarian shielding ('relapse after ovarian shielding' in Figure 1). Therefore, while the cure rate is '1–0.2–0.2 = 0.6 (60%)' after a decision to not perform ovarian shielding, it is '1–0.2—relapse after ovarian shielding' after a decision to perform ovarian shielding. The probability of ovarian recovery was determined to be 10% after a decision to not perform ovarian shielding and 80% after a decision to perform ovarian shielding based on the literature.[6,7] Each patient's view of life can be reflected in the value of 'alive without ovarian recovery'. Under the simple assumption that the payoff values of transplant-related mortality and relapse were both 0 points and the payoff value of cure with ovarian recovery is 100 points, each patient can score the payoff value for 'alive without ovarian recovery' based on her own view of life. Patients for whom ovarian recovery is very important assign a low payoff value for 'alive without ovarian recovery'.

The expected values for the decisions vary according to the values of 'relapse after ovarian shielding' and 'alive without ovarian recovery'. For example, if we fix the value of 'relapse after ovarian shielding' at 30% under the assumption that the incidence of relapse is increased by 10% under ovarian shielding, the expected values for the two decisions vary according to the value of 'alive without ovarian recovery', as shown in Figure 2a (one-way sensitivity analysis). The expected value for a decision to not perform ovarian shielding is higher than that to perform ovarian shielding when a patient scores 'alive without ovarian recovery' higher than 77.3 points. If we fix the value of 'relapse after ovarian shielding' at 40%, the expected value for a decision to not perform ovarian

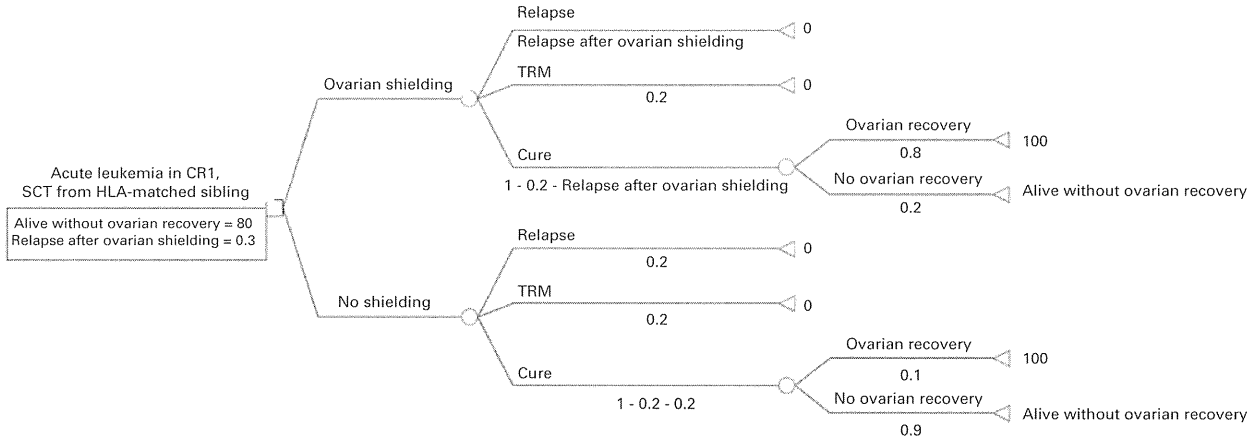

Figure 1 The decision tree used in this decision analysis.

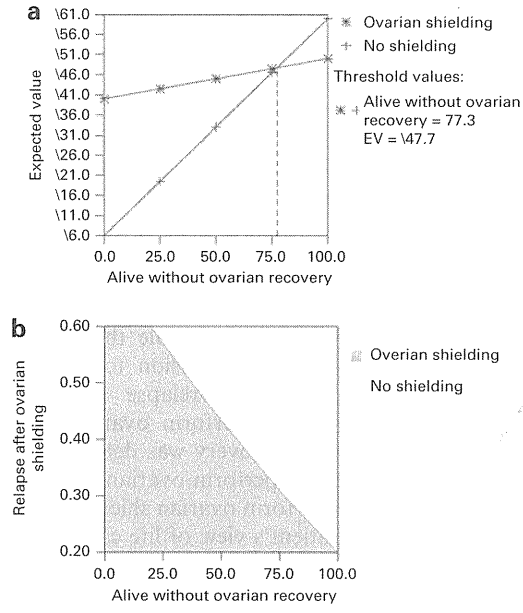

Figure 2 One-way (**a**) and two-way (**b**) sensitivity analyses. In the gray area, the expected value of a decision to perform ovarian shielding is higher than that of a decision to not perform ovarian shielding.

shielding is higher than that to perform ovarian shielding when a patient scores 'alive without ovarian recovery' higher than 56.5 points.

These two values can be changed simultaneously, as shown in Figure 2b (two-way sensitivity analysis). The threshold of the relapse rate, at which there is a change in which decision is made, can be obtained by drawing a vertical line from the 'alive without ovarian recovery' value for each patient. For example, if a patient scores 50 points for the payoff value of 'alive without ovarian recovery', the expected value for a decision to not perform ovarian shielding is higher than that to perform ovarian shielding when 'relapse after ovarian shielding' is higher than 43%, as the vertical line from the X-axis at 'alive without ovarian recovery' of 50 points crosses the borderline of the gray and white areas at 'relapse after ovarian shielding' of 43%.

Although this decision analysis is not definitive, it may be helpful for patients who find it difficult to make a decision when faced with uncertainty. Some young female patients tend to overestimate the value of fertility in their subsequent life, and we should inform patients that they can become pregnant using a donated oocyte even after their ovarian function is lost.

Conflict of interest

The authors declare no conflict of interest.

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Related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction and HLA 8/8-allele-matched unrelated transplantation: a nationwide retrospective study

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Related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction and HLA 8/8-allele-matched unrelated transplantation: A nationwide retrospective study

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Running head: Transplant from HLA 1-AG mismatched RD vs 8/8 MUD

ABSTRACT

To clarify whether we should prefer a related donor with an HLA-1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host direction (RD/1AG-MM-GVH) or an HLA 8/8-allele (HLA-A, HLA-B, HLA-C, and HLA-DRB1) matched unrelated donor (8/8 MUD), we evaluated 779 patients with acute leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome who received a T-cell-replete graft from an RD/1AG-MM-GVH or 8/8 MUD. The use of an RD/1AG-MM-GVH was significantly associated with a higher overall mortality rate than an 8/8 MUD in a multivariate analysis (hazard ratio, 1.49; $P<0.001$), and this impact was statistically significant only in patients with standard-risk diseases ($P=0.001$). Among patients with standard-risk diseases who received transplantation from an RD/1AG-MM-GVH, the presence of an HLA-B-antigen mismatch was significantly associated with a lower overall survival rate than an HLA-DR-antigen mismatch due to an increased risk of treatment-related mortality (TRM). The HLA-C-antigen mismatch or multiple allelic mismatches were frequently observed in the HLA-B-antigen-mismatched group, and were possibly associated with the poor outcome. In conclusion, an 8/8 MUD should be prioritized over an RD/1AG-MM-GVH during donor selection. In particular, an HLA-B-antigen mismatch in the GVH direction has an adverse effect on overall survival and TRM in patients with standard-risk diseases.

Introduction

An HLA-matched unrelated donor (MUD) is considered to be an alternative donor in hematopoietic stem cell transplantation (SCT) for patients who lack an HLA-identical sibling. However, it is difficult to find an MUD for patients with rare HLA haplotypes. SCT from a related donor with a 1-antigen mismatch at HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host (GVH) direction results in a higher but acceptable incidence of acute GVHD and outcomes comparable to that of SCT from a matched related donor (MRD) in patients with high-risk diseases; this is because it reduces the risk of relapse via a graft-versus-leukemia (GVL) effect.[1-3] In previous studies, HLA mismatches in the HVG direction were associated with higher graft failure and lower overall survival.[1,2,4] However, strategies to reduce the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress recipient immune system.[5] Therefore, in current clinical practice in Japan, SCT from a related donor with 1-antigen mismatch in the GVH direction is being performed accepting multiple antigen mismatches in the HVG direction without specific stem cell manipulation,[1,2] although such an approach has not yet been evaluated in a large cohort.

Our previous study showed that SCT from an HLA 1-antigen-mismatched donor in the GVH or host-versus-graft (HVG) direction is comparable to that from an HLA-A, HLA-B, and HLA-DR antigen-matched unrelated donor.[11] However, this cohort was relatively old (1991–2000) and may not reflect our current practice. Furthermore, the analysis was mainly performed based on serological matching, since information on HLA allele matching in unrelated transplantation was insufficient. The importance of allele-matching in HLA-A, HLA-B, and HLA-DRB1 loci in unrelated donor transplantation has been established.[6-8] In addition, the importance of allele-matching in the HLA-C locus has been highlighted in several recent studies regarding unrelated transplantation, although HLA-C matching is, in general, still not considered in related