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once sufficient numbers of mice in other colonies are observed to 1 year and are subjected to similar analyses. If disease progression is unique to mice in our facility, it might suggest that environmental or infectious agents can play a role in the progression to the more severe phenotype in a subset of mice in the context of 12/15-lipoxygenase deficiency. It is important to stress, however, that the defects we reported in the majority of Alox15 mice that are asymptomatic do not in our opinion appear to be unique to the Wistar colony, and these mice remain a valuable tool for defining the role of 12/15-lipoxygenase in hematopoiesis.

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To the editor:

Dasatinib enhances the expansion of CD56$^+$CD3$^-$ NK cells from cord blood

Dasatinib can inhibit T-cell activation through inhibition of the Scr family of tyrosine kinases such as p56 (Lck).[1] It has been reported that some chronic myeloid leukemia (CML) patients who were treated with dasatinib developed chronic large-granular lymphocytosis (LGL) with natural killer (NK) or NK T-cell lineage, and that these patients achieved optimal molecular response.[2] In addition, Mustjoki et al reported clonal expansion of NK T cells during dasatinib therapy.[3] Kreutzman et al reported that mono/oligoclonal T and NK cells were present in CML patients at diagnosis and expanded during dasatinib therapy and that LGL expansion is linked to cytomegalovirus infection.[4,5] Therefore, dasatinib may have a favorable effect on NK-cell proliferation. In this study, we analyzed the effects of dasatinib on the expansion of NK cells from cord blood and transcriptional factors during expansion.

Umbilical cord blood cells (1×10^6/mL; Hokkaido Cord Blood Bank) were cultured with IL-15 (10 ng/mL; PeproTech), IL-2 (5 ng/mL; R&D Systems), and anti-CD3 mAb (OKT3, 10 ng/mL; Janssen Pharmaceutical); with or without dasatinib (10nM; a kind gift from Bristol-Myers Squibb) in culture medium stem cell growth medium (CeeGenix) with 5% human AB serum in 24-well plates, as we reported previously.[6] After a 7-day culture of umbilical cord blood cells (1×10^6/mL), the absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with the culture with cytokines only (before culture $5.3 \pm 1.4 \times 10^4$ in 10^6 cord blood cells, after culture with IL-2 + IL-15 $26.0 \pm 17.8 \times 10^4$, and after culture with IL-2 + IL-15 and dasatinib $66.6 \pm 29.1 \times 10^4$; $P < .05$, means ± SDs, n = 6; Figure 1A). In addition, the proportion of CD56$^+$CD3$^-$ cells, CD56$^+$NKG2D$^+$ cells, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib (Figure 1B).

We analyzed the transcriptional factors Eomesodermin (Eomes) and T-bet using an Applied Biosystems 7300 Real-Time PCR System and GAPDH as an endogenous control. Before stimulation, cord blood of CD56$^+$ cells showed increased expression of Eomes and T-bet compared with the expression in unfractionated whole cord blood cells and CD3$^+$ cells. After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with cells cultured with cytokines only (5.96 ± 3.95 vs 0.81 ± 0.62, $P < .05$; Figure 1C).

At present, there are only a few transcription factors that are known to play an essential role in NK-cell development, especially in humans. T-box proteins, T-bet, and Eomes are involved in NK-cell development.[7-9] T-bet and Eomes are both later required for the differentiation in DX5$^+$(CD49b) CD11b$^+$ NK cells. In addition, Eomes is highly expressed in fully differentiated NK cells. In this study, we showed NK-cell expansion after culture with dasatinb and increased expression of Eomes after 24 hours. Therefore, dasatinib has some role in NK-cell expansion from cord blood under the condition of IL-2 and IL-15 stimulation through increased expression of transcription factors such as Eomes. This observation may have potentially important implication for the treatment of other diseases with dasatinib.[10]

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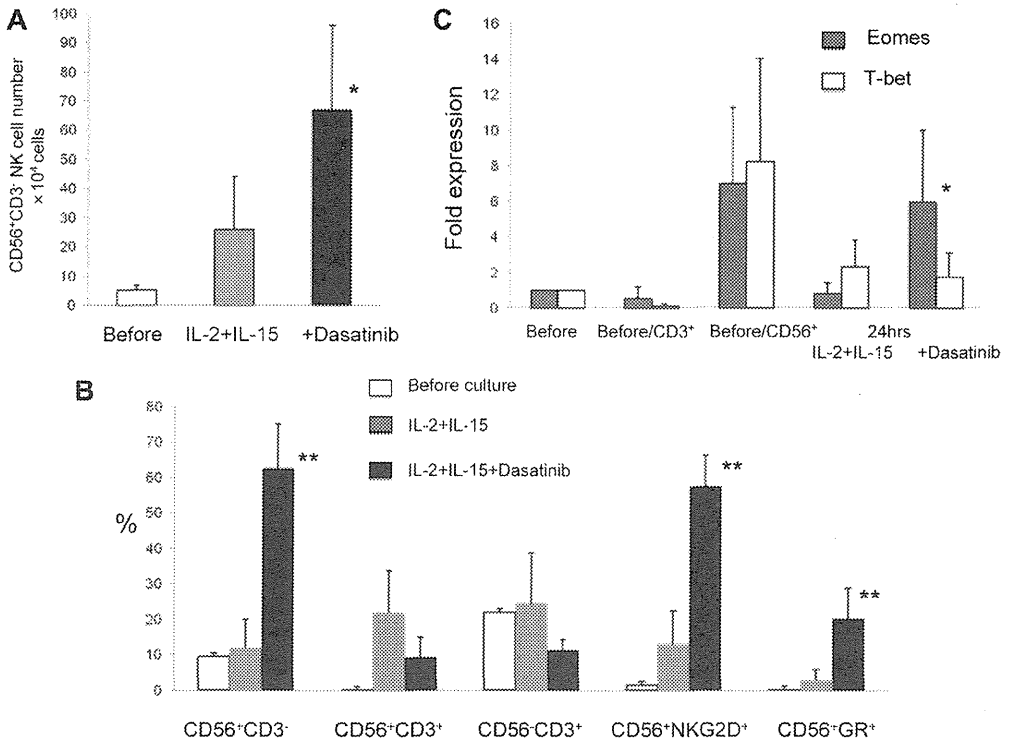

Figure 1. Expansion of CD56$^+$CD3$^-$ NK cells with dasatinib from cord blood cells. (A) The absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with those in the culture with cytokines only. (B) The proportion of CD56$^+$CD3$^-$, CD56$^+$NKG2D$^+$, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib compared with culture without dasatinib. (C) After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with that in cells cultured with cytokines only. T-bet expression was increased after 24 hours culture compared with that before culture, but there was no significant difference between expression level in culture with dasatinib and that without dasatinib (bars indicate means ± SDs, n = 6; $^*P < .05$ and $^{**}P < .01$).

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ORIGINAL ARTICLE

Efficacy of folinic acid in preventing oral mucositis in allogeneic hematopoietic stem cell transplant patients receiving MTX as prophylaxis for GVHD

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As the safety of folinic acid administration and its efficacy for reducing the toxicity of MTX remain controversial, we assessed the effect of folinic acid administration after MTX treatment for GVHD prophylaxis on the incidence of oral mucositis and acute GVHD. We retrospectively analyzed data for 118 patients who had undergone allogeneic hematopoietic SCT and had received MTX for GVHD prophylaxis. Multivariate analysis showed that systemic folinic acid administration significantly reduced the incidence of severe oral mucositis (odds ratio (OR) = 0.13, 95% confidence interval (CI) 0.04–0.73, $P = 0.014$). There was also 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 between patients who received systemic folinic acid administration and those who did not ($P = 0.88$). Systemic folinic acid administration and mouthwash appear to be useful for reducing the incidence of severe oral mucositis in patients who have received allogeneic hematopoietic SCT using MTX as GVHD prophylaxis.

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Introduction

Oral mucositis is one of the most common complications associated with allogeneic hematopoietic SCT, occuring in 60–90% of patients who have received SCT.[1–3] Oral mucositis is associated with severe pain, which can lead to anorexia and dehydration. A large population of patients with severe oral mucositis require total parenteral nutrition and opioid analgesics.[4] Severe oral mucositis is associated with not only severe pain but also poor clinical and economic outcomes.[5]

Oral mucositis is caused mainly by the toxicity associated with chemotherapy and TBI as a conditioning regimen; however, it is also associated with the use of MTX for GVHD prophylaxis.[6,7] Although several studies have shown that folinic acid administration reduced the toxicity of MTX,[8–10] the efficacy and safety of folinic acid administration remain controversial. Ruutu *et al.*[11] reported that folinic acid was administered after MTX in 37 (45.7%) of 81 European Group for Blood and Marrow Transplantation (EBMT) centers, and Bhurani *et al.*[12] reported that folinic acid was administered after MTX in 8 (44.4%) of 8 centers in Australia and New Zealand. More than half of the centers surveyed in those studies did not use systemic folinic acid administration because of the lack of support for its efficacy or because of the risk of acute GVHD being induced by folinic acid.

Therefore, this study was performed to assess the effects of systemic folinic acid administration after MTX for GVHD prophylaxis on the incidence of oral mucositis and acute GVHD.

Patients and methods

We retrospectively analyzed data for 141 consecutive patients who had undergone allogeneic hematopoietic SCT and had received MTX for GVHD prophylaxis between March 2006 and December 2009 in Stem Cell Transplantation Center of Hokkaido University Hospital. We excluded seven patients whose data were insufficient. Furthermore, we excluded 16 patients who failed to achieve engraftment because we hypothesized that duration of neutropenia was a risk factor for the development of

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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

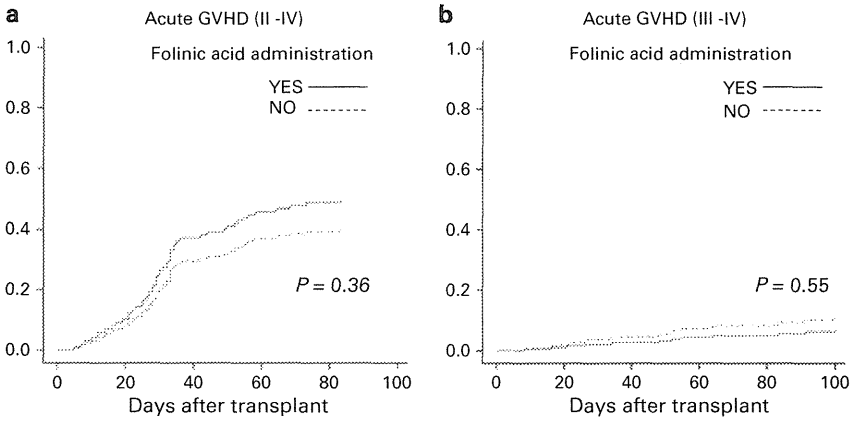

Figure 1 Cumulative incidence of grade II–IV acute GVHD (**a**) and grade III–IV acute GVHD (**b**) grouped according to the use of folinic acid administration.

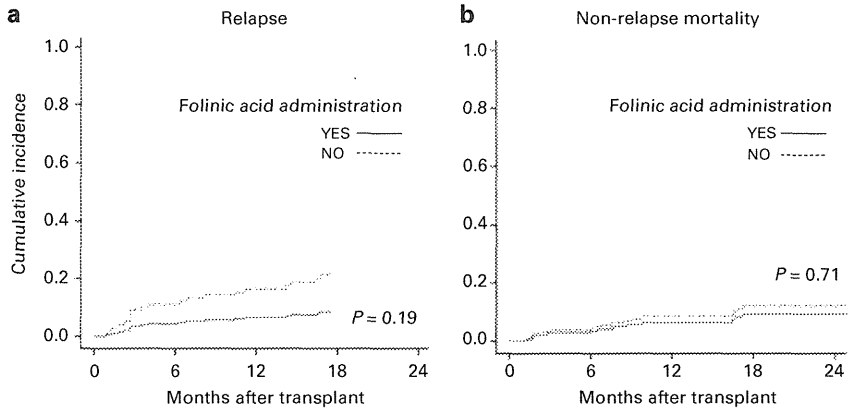

Figure 2 Cumulative incidence of relapse (**a**) and non-relapse mortality (**b**) grouped according to the use of folinic acid administration.

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

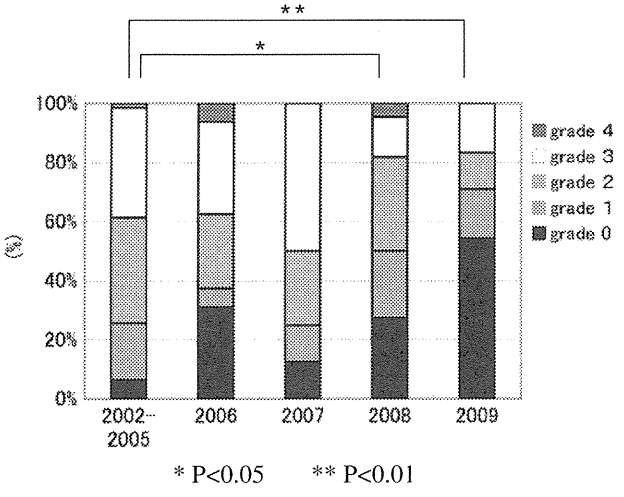

Fig. 1 Changes in the incidence and severity of oral mucositis. The occurrence and severity of oral mucositis reduced year by year after we introduced the POHC in 2006. The incidence of oral mucositis was 93.5% before intervention by POHC, whereas it significantly lowered to 72.7% ($p<0.05$) in 2008 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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ORIGINAL ARTICLE

Differing impacts of pretransplant serum ferritin and C-reactive protein levels on the incidence of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

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Abstract Studies have suggested an association between pretransplant serum levels of ferritin and C-reactive protein (CRP) and complications of allogeneic hematopoietic stem cell transplantation (HSCT). To evaluate the prognostic impact of these biomarkers on the development of acute and chronic graft-versus-host disease (GVHD), we retrospectively studied 211 patients who underwent allogeneic HSCT for hematologic diseases at our institution. The cumulative incidence rate of chronic GVHD at 3 years was 40.7 %. In the multivariate analysis, elevated CRP levels (≥2 mg/L) were significantly associated with a high incidence of chronic GVHD, whereas high ferritin levels (≥880 ng/mL) showed a tendency, though not statistically significant, to association with a low incidence of chronic GVHD. No significant association was observed between the pretransplant serum ferritin or CRP levels and the incidence of acute GVHD. Multivariate analysis indicated that high pretransplant serum ferritin levels were significantly associated with increases in treatment-related mortality and relapse rates. Overall, an elevated pretransplant serum ferritin level, but not an elevated serum CRP level, is a strong risk factor for overall mortality (hazard ratio, 2.16; $P = 0.002$). Our results also indicate that pretransplant serum CRP levels may be a useful biomarker for predicting the risk of chronic GVHD.

Keywords Ferritin · C-reactive protein · Chronic GVHD · Allogeneic hematopoietic stem cell transplantation

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Introduction

Iron overload is frequently observed in patients with hematologic diseases before and after allogeneic hematopoietic stem cell transplantation (HSCT). Many studies have shown that elevated pretransplant serum ferritin levels are associated with lower overall and disease-free survival rates and a higher incidence of treatment-related complications [1–5]. Conversely, several studies have suggested that elevated pretransplant ferritin levels are associated with a lower incidence of chronic graft-versus-host disease (GVHD) [6, 7], and the immunosuppressive effect of iron overload was hypothesized to be an underlying mechanism. However, this association remains controversial because other studies have failed to observe such a relationship [5, 8]. Results are also inconsistent among studies regarding the association between pretransplant serum ferritin levels and the incidence of acute GVHD [4–6, 8].

Serum ferritin is widely used as a surrogate marker of body iron stores. However, ferritin levels are increased by inflammation and iron loading; therefore, it is important to

adjust for the effect of inflammation on ferritin levels. C-reactive protein (CRP), an acute-phase reactant produced by hepatocytes, has been shown to be a reliable biomarker of systemic inflammation [9, 10]. Several studies have suggested that elevated pretransplant or pre-engraftment serum CRP levels are associated with an increased risk of treatment-related mortality (TRM) after allogeneic HSCT [11–14]. Although studies have reported that elevated CRP levels are associated with a higher incidence rate of acute GVHD [11, 12], this finding is not yet fully confirmed. Moreover, the impact of CRP levels on the incidence of chronic GVHD remains unknown.

We previously demonstrated through a multivariate analysis that elevated pretransplant serum ferritin and CRP levels were significantly associated with the development of bacterial infection after allogeneic HSCT [14]. In the present study, we investigated the association between pretransplant serum ferritin and CRP levels and the incidence of acute and chronic GVHD as well as other clinical outcomes of allogeneic HSCT, to determine whether these events could be predicted using these parameters.

Patients and methods

Study population

We retrospectively reviewed the medical records of adult patients who underwent their first allogeneic HSCT for hematologic diseases at the Kyoto University Hospital from January 2000 to December 2010. A total of 211 patients whose pretransplant serum ferritin and CRP profiles were available were included in the analysis. This study was performed in accordance with the Helsinki Declaration and approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine. Written informed consent to undergo the transplantation protocol was obtained from all of the patients.

Serum analysis

The serum ferritin and CRP levels before the start of the conditioning regimen were measured using the standard laboratory technique (reference ranges: ferritin level $\leq$150 ng/mL; CRP level $\leq$2 mg/L) [14].

Statistical analysis

The primary end point was the impact of the pretransplant serum ferritin and CRP levels on the incidence of grade 2–4 acute GVHD and chronic GVHD. In the analysis of chronic GVHD, patients who survived for at least 100 days after allogeneic HSCT were included. The secondary end point was the impact of these 2 markers on the incidence of TRM and relapse, and the overall survival (OS) rate.

The patients were divided into 2 groups depending on their pretransplant serum ferritin or CRP levels. The cutoff point for the CRP levels was 2 mg/dL, the median value. For the ferritin levels, the median value was 470 ng/mL, which was much lower than the cutoff points used in most other studies; therefore, we used 880 ng/mL, which was the higher tertile value, as the cutoff point. The patient and transplant characteristics between the 2 groups were compared using the Mann–Whitney U test or Chi-square analysis, as appropriate. Standard-risk disease was defined as complete remission in cases of acute myeloid leukemia, acute lymphoblastic leukemia, malignant lymphoma, and plasma cell myeloma; as untreated or complete remission in cases of myelodysplastic syndrome and myeloproliferative disorder; as chronic phase in cases of chronic myeloid leukemia; and as nonmalignant disease. High-risk disease was defined as any other hematologic disease status. The conditioning regimen was categorized as either myeloablative or reduced intensity according to the National Marrow Donor Program and the Center for International Blood and Marrow Transplant Research operational definitions [15].

Acute and chronic GVHD were defined and graded according to conventional criteria [16, 17]. Depending on whether it developed before or after day +100, GVHD was classified as acute or chronic, respectively. To eliminate the effect of a competing risk, the cumulative incidence was assessed using methods described elsewhere [18]. The competing event in the cumulative incidence analyses was defined as death without an event of interest. OS was estimated using the Kaplan–Meier methods. The Cox proportional hazards model was applied.

The following items were added as confounders: the age of the recipient (<50 or $\geq$50 years), the sex of the recipient (male or female), diagnosis (myeloid or lymphoid malignancies, or nonmalignant diseases), risk of disease (standard or high risk), source of stem cells (HLA-matched- or HLA-mismatched-related donor graft, unrelated bone marrow, or unrelated cord blood), conditioning regimen (myeloablative or reduced intensity), and prophylaxis against GVHD (tacrolimus or cyclosporine based). Stepwise backward selection procedures were used with a variable retention criterion of $P < 0.05$ to identify important confounders; these confounders, as well as the serum ferritin and CRP levels, were then included in the final model.

$P < 0.05$ was considered to be statistically significant. All the analyses were conducted using the Stata (version 11; StataCorp LP, College Station, TX, USA) and R version 2.13.0 software (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of patients and transplants

The characteristics of the patients and transplants are shown in Table 1 and Supplementary Table S1. The median age of the patients was 48 years (range 17–69 years). The primary diseases were myeloid malignancies, lymphoid malignancies, and nonmalignant diseases in 115, 88, and 8 patients, respectively. A total of 90 patients (43 %) had a high-risk disease. No patient received T cell-depleted grafts. A myeloablative regimen was used in 116 patients (55 %). There was no significant difference in the patient and transplant characteristics between the low- and high-ferritin groups, except in the serum CRP levels ($P = 0.011$). The patients in the high-CRP group were more likely to be male ($P < 0.001$) and have a high-risk disease ($P < 0.001$).

Acute and chronic GVHD

Among all patients, the median follow-up period for the survivors after allogeneic HSCT was 41.2 months (range 1.2–132.6 months). A total of 188 patients survived for 100 days or longer after transplantation.

The cumulative incidence rates of grade 2–4 acute GVHD at 100 days after transplantation were 35.8 % [95 % confidence interval (CI) 27.9–43.7 %] and 32.5 % (95 % CI 22.0–43.5 %) in the low- and high-ferritin groups, respectively, and 34.9 % (95 % CI 26.2–43.7 %) and 34.4 % (95 % CI 25.2–43.8 %) in the low- and high-CRP groups, respectively (Fig. 1, panels a, b). With regard to grade 3 or 4 acute GVHD, the cumulative incidence rates were 7.9 % (95 % CI 4.2–13.1 %) and 14.1 % (95 % CI 7.2–23.3 %) in the low- and high-ferritin groups, respectively, and 10.7 % (95 % CI 5.9–17.3 %) and 9.1 % (95 % CI 4.5–15.8 %) in the low- and high-CRP groups,

Table 1 Characteristics of patients and transplants according to pretransplant serum ferritin and CRP levels

Variables	Low ferritin (<880 ng/mL) $n = 140$	High ferritin (≥880 ng/mL) $n = 71$	P value	Low CRP (<2 mg/L) $n = 112$	High CRP (≥2 mg/L) $n = 99$	P value
Age at transplant						
Median (range), years	47.5 (17–69)	50 (20–66)	0.46	46.5 (17–69)	49 (17–66)	0.26
Sex, *n* (%)			0.15			<0.001
Male	70 (50)	43 (61)		47 (42)	66 (67)	
Female	70 (50)	28 (39)		65 (58)	33 (33)	
Disease, *n* (%)			0.082			0.54
Myeloid malignancies	73 (52)	42 (59)		64 (57)	51 (52)	
Lymphoid malignancies	64 (46)	24 (34)		43 (38)	45 (45)	
Nonmalignant diseases	3 (2)	5 (7)		5 (4)	3 (3)	
Risk of disease, *n* (%)			0.83			<0.001
Standard	81 (58)	40 (56)		80 (71)	41 (41)	
High	59 (42)	31 (44)		32 (29)	58 (59)	
Source of stem cells, *n* (%)			0.72			0.73
HLA[a]-matched related	44 (31)	18 (25)		31 (28)	31 (31)	
HLA[a]-mismatched	14 (10)	10 (14)		11 (10)	13 (13)	
Unrelated bone marrow	62 (44)	33 (46)		54 (48)	41 (41)	
Unrelated cord blood	20 (14)	10 (14)		16 (14)	14 (14)	
Conditioning regimen, *n* (%)			0.76			0.13
Myeloablative intensity	78 (56)	38 (54)		67 (60)	49 (49)	
Reduced intensity	62 (44)	33 (46)		45 (40)	50 (51)	
GVHD prophylaxis, *n* (%)			0.84			0.53
Tacrolimus-based	114 (81)	57 (80)		89 (79)	82 (83)	
Cyclosporine based	26 (19)	14 (20)		23 (21)	17 (17)	
Serum CRP level, *n* (%)			0.011			
<2 mg/L	83 (59)	29 (41)				
≥2 mg/L	57 (41)	42 (59)				

[a] HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, -B, and -DR antigens