［Title］

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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［Running title］

Effect of folinic acid on oral mucositis

［Key words］

oral mucositis, folinic acid, stem cell transplantation

Abstract

Since the safety of folinic acid administration and its efficacy for reducing the toxicity of methotrexate (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 stem cell transplantation and had received MTX for GVHD prophylaxis. 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). 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 stem cell transplantation using MTX as GVHD prophylaxis.

Introduction

Oral mucositis is one of the most common complications associated with allogeneic hematopoietic stem cell transplantation, occuring in 60-90% of patients who have received stem cell transplantation.[1,2,3] 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 total body irradiation as a conditioning regimen; however, it is also associated with the use of methotrexate (MTX) for GVHD prophylaxis.[6,7] Although several studies have shown that folinic acid administration reduced the toxicity of MTX,[8,9,10] the efficacy and safety of folinic acid administration remain controversial. Ruutu et al. reported that folinic acid was administered after MTX in 37 (45.7%) of 81 European Group for Blood and Marrow Transplantation (EBMT) centers,[11] and Bhurani et al. reported that folinic acid was administered after MTX in 8 (44.0%) of 12 centers in Australia and New Zealand.[12] 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 stem cell transplantation and had received MTX for GVHD prophylaxis between March 2006 and December 2009 in Stem Cell Transplantation Center of Hokkaido University Hospital. We excluded 7 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 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 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),[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 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).[14] Cyclosporine A (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 mg/m^2 or 10 mg/m^2 on day 1 and at a dose of 10 mg/m^2 or 7 mg/m^2 on day 3 and day 6.

Supportive care and infection prophylaxis

Granulocyte colony-stimulating factor 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 on day -7 to day 35 for prevention of herpes simplex virus (HSV) infection.

Systemic folinic acid administration and mouthwash

Folinic acid was given intravenously at the same dose as that used for each administration of MTX at 12 hr, 18 hr and 24 hr after administration of MTX on days 1 and 3 and at 24 hr, 30 hr and 36 hr 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 chi-square 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 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 (OR = 4.78, 95%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 intravenous 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 stem cell transplantation and had received MTX for GVHD prophylaxis.

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.

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. reported that systemic folinic acid administration 6 hr after each administration of MTX reduced the toxicity of MTX and maintained the effect of MTX on prevention of GVHD in dogs[20] and that MTX at concentrations above 10^{-6}M completely abrogated thymidine uptake in lymphocytes with stimulation for 6 hours 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/day) should be given 24 hours 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 6-24h after MTX administration.[11, 23] We used folinic acid intravenously at the same dose as that used for each administration of MTX at 12 hr, 18 hr and 24 hr after administration of MTX on days 1 and 3 and at 24 hr, 30 hr and 36 hr 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.[25, 26, 27] 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 three 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. reported that the severity of oral mucositis was reduced in RIST patients compared with that in CST patients,[1] 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.[26,27] 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. reported that oral mucositis is associated with significantly worse economic outcomes,[5] 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.

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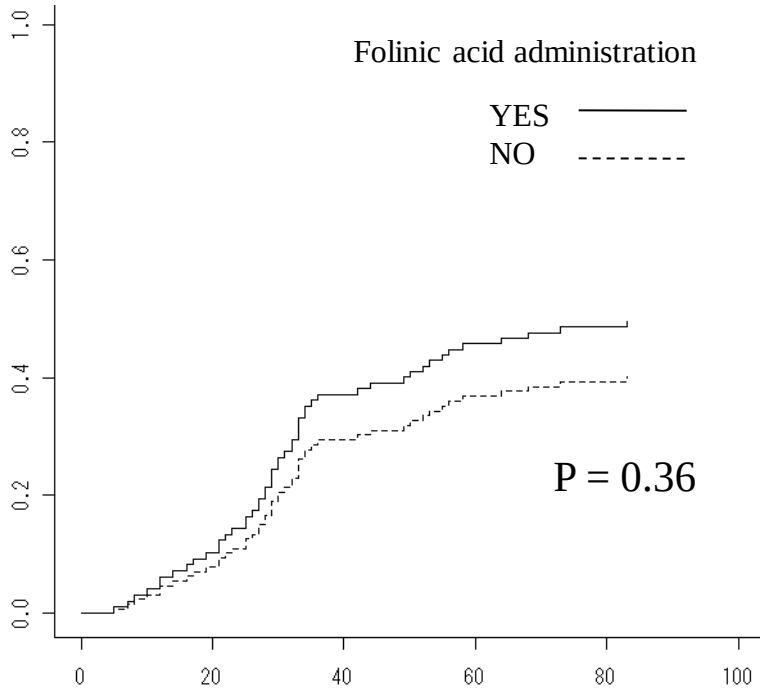
Figure legends

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.

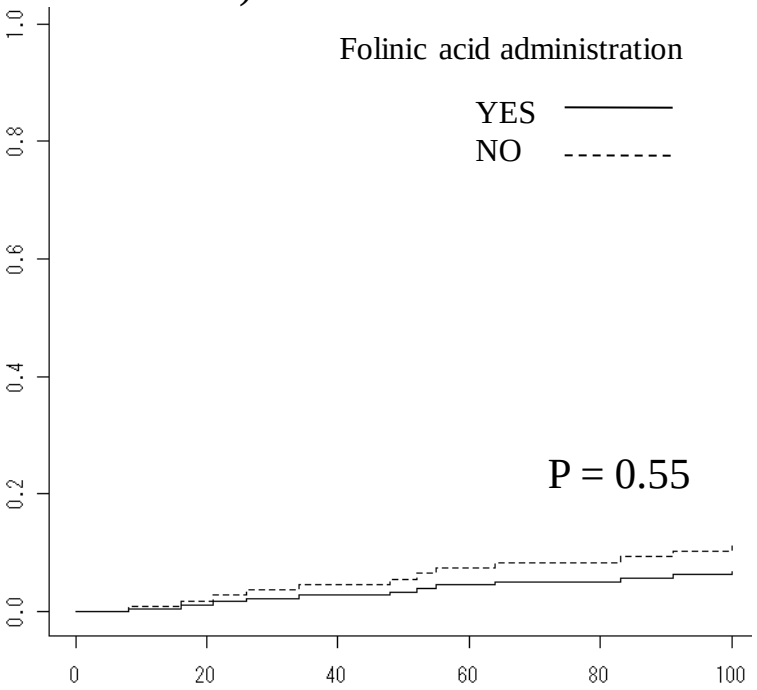
Figure 2. Cumulative incidence of relapse (a) and non-relapse mortality (b) grouped according to the use of folinic acid administration.

a) acute GVHD (II - IV)



Days after transplant

b) acute GVHD (III - IV)



Days after transplant

a) relapse

b) non-relapse mortality

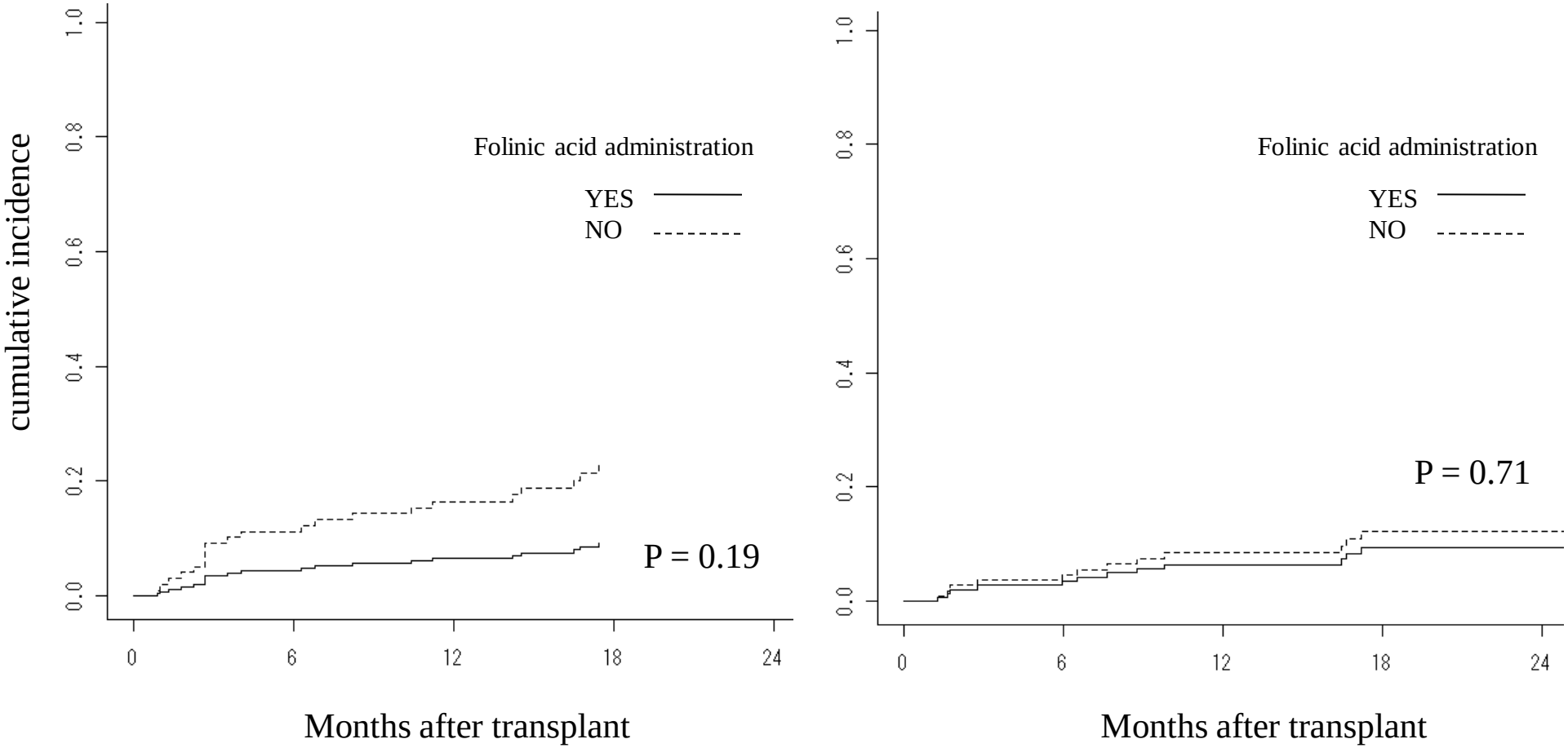


Table 1 Patient characteristics

	Total (n = 118)	Folinic acid administration YES (n = 29)	No (n = 89)	P-values
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 stem cell transplantation; FK = tacrolimus; Flu = fludarabine; HL = Hodgkin lymphoma; MDS = myelodysplastic syndrome; MF = myelofibrosis; MM = multiple myeloma; NHL = non Hodgkin lymphoma; VP16 = etoposide.

Table 2 Incidence of oral mucositis

	Grades of oral mucositis				
	0	1	2	3	4
Total	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	95%CI	P-values
Age					
≥ 50	48	16	1.17	0.53-2.56	0.70
< 50	70	(33.3 %)	1		
Gender		21			
Male	61	(30.0 %)	1		
Female	57		1.64	0.75-3.64	0.22
Disease status at transplantation		16			
CR	66	(26.2 %)	1.54	0.65-3.84	0.33
non CR	37	21	1		
Conditioning		(36.8 %)			
CST	56		1.07	0.49-2.34	0.86
RIST	62	24	1		
VP/CY/TBI	25	(36.4 %)	2.51	1.01-6.28	0.048
non VP/CY/TBI	93	10	1		
GVHD prophylaxis		(27.0 %)			
CsA+MTX	44		0.74	0.32-1.65	0.46
FK+MTX	74	18	1		
Doses of MTX		(32.1 %)			
15-10-10	72	19	1.33	0.18-27.0	0.80
10-10-10	41	(30.6 %)	3.13	0.42-64.1	0.29
10-7-7	5	12	1		
Stem cell source		(48.0 %)			
related BM	14	25	1		
related PBSC	13	(26.9 %)	1.62	0.29-10.2	0.58
unrelated BM	82		1.80	0.51-8.44	0.38
unrelated CB	9	12	1.83	0.27-12.9	0.53
Duration of neutropenia (< 500 /μl)		(27.3 %)			
≥ 21 days	28	25	3.57	1.48-8.78	0.0047
< 21 days	90	(33.8 %)	1		
Folinic acid administration					
Yes	29	18	0.20	0.04-0.62	0.0038
No	89	(25.0 %)	1		
Folinic acid mouthwash		18			
Yes	60	(43.9 %)	0.28	0.12-0.62	0.0017
No	58	1 (28.0 %)	1		

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		

Table 5 Effect of folinic acid administration and mouthwash

	without folinic acid (n = 54)	Folinic acid mouthwash wtihout 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