Article title

Reduced-dose methotrexate in combination with tacrolimus was associated with rapid engraftment and recovery from oral mucositis without affecting the incidence of GVHD

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Abstract

Allogeneic hematopoietic stem cell transplantation is a curable treatment for hematological diseases. Graft-versus-host disease (GVHD) causes morbidity and mortality after HSCT. Methotrexate (MTX) is used for GVHD prophylaxis, but its appropriate dose remains unclear. In the present study, we compared the efficacy and toxicity of 15-10-10 MTX (day +1: 15 mg/m^2; days +3 and +6: 10 mg/m^2) with 10-7-7 MTX (day +1: 10 mg/m^2; day +3 and +6: 7 mg/m^2) in combination with tacrolimus. The cumulative incidences rates of grades Ⅱ−Ⅳ acute GVHD and grades Ⅲ-Ⅳ acute GVHD and chronic GVHD in the 15-10-10 MTX and 10-7-7 MTX groups did not differ to a statistically significant extent. The median time for neutrophil engraftment in the 15-10-10 MTX group was 16 days (range, 11–31 days), while that in the 10-7-7 group was 15 days (range, 12–19 days) (P=0.024). Moreover, the median time for platelet recovery was significantly shorter in the 10-7-7 MTX group (22 days; range, 13–49 days) than that in the 15-10-10 MTX group (27 days; range, 9–405 days) (P=0.027). The duration of oral mucositis was significantly shorter in the patients who received a reduced dose of MTX (median, 4.5 vs 13.0 days; P=0.013). In conclusion, GVHD prophylaxis with a reduced dose of MTX was associated with earlier engraftment and earlier recovery from mucositis in comparison to a standard dose of MTX, without affecting the incidence of GVHD.

Key words

GVHD prophylaxis, methotrexate, oral mucositis, engraftment

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is widely used as curative treatment for various hematologic malignancies, bone marrow failure syndromes, and congenital immunodeficiencies. Graft-versus-host disease (GVHD) is one of the major complications after allogeneic HSCT [1-3]. The standard GVHD prophylaxis regimen is a combination of methotrexate (MTX) and a calcineurin inhibitor, such as cyclosporine A (CSA) or tacrolimus (TAC) [4-6]. Previous randomized controlled trials have suggested that the use of TAC and MTX reduces the incidence of acute GVHD in comparison to CSA and MTX, whereas no differences were observed in the incidence of chronic GVHD or survival [4, 5, 7]. The dose and schedule of MTX in GVHD prophylaxis differs between institutes. The original regimen of short-course MTX for GVHD prophylaxis was 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6 and +11 [8, 9]. MTX can be omitted or the dosage can be reduced on day +11 (10 mg/m^2 on day +1 and 7 mg/m^2 on days +3, +6 and +11) to mitigate the toxicities of MTX, which include mucositis and delayed engraftment [10-14]. However, the impact of modifying the dose of MTX is controversial [10, 11, 15, 16]. Furthermore, fewer studies have investigated the optimal doses of MTX in combination with TAC in comparison to MTX with CSA. In the present study, we retrospectively compared the efficacy and the incidence of toxicities with two different doses of MTX in combination with TAC as a prophylactic treatment against GVHD after allogeneic HSCT.

Patients and Methods

Patients

Among the 208 consecutive patients who received HSCT in our institution between January 2010 and March 2015, the results of 76 patients who underwent HSCT with TAC plus short-course MTX were analyzed. The study was approved by the institutional review board of Hokkaido University Hospital.

GVHD prophylaxis

TAC was intravenously administered by continuous infusion from day -1 to maintain a target blood level of TAC at 10-15 ng/ml. At approximately 1 month after HSCT, it was changed to an oral form to maintain a trough blood level of TAC at 5-10 ng/ml. The blood levels of TAC were measured at least twice a week. The doses of MTX were as followed: 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6 (15-10-10 MTX) or 10 mg/m^2 on day +1 and 7 mg/m^2 on days +3 and +6 (10-7-7 MTX). The dose of MTX was selected according to the discretion of each physician. Patients who received anti-thymocyte globulin for GVHD prophylaxis were excluded from the analysis.

Supportive treatment

Patients received granulocyte colony-stimulating factor (G-CSF) from 5 days after HSCT until the time of neutrophil engraftment. Cytomegalovirus (CMV) reactivation was monitored at least once a week. When CMV reactivation was detected, valganciclovir or ganciclovir was administered as a pre-emptive therapy. Acyclovir and sulfamethoxazole/trimethoprim were administered to prevent herpes simplex virus/varicella zoster virus and *Pneumocystis jirovecii* respectively.

Engraftment

The day of engraftment was defined as the first of 3 successive days after HSCT in which the neutrophil counts was >0.5 × 10^9/L. The day of platelet recovery was defined as the first of 7 successive days in which the platelet count was >20 × 10^9/L without platelet transfusion.

The evaluation of oral mucositis

Oral mucositis was evaluated daily and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0. Briefly, Grade 1 denotes erythema of the mucosa. Grades 2 and 3 indicate patchy and confluent ulcerations or pseudomembranes, respectively. Grade 4 indicates tissue necrosis and significant spontaneous bleeding. Severe oral mucositis was defined as Grade 3 to 4 oral mucositis.

The evaluation of GVHD

Acute and chronic GVHD were graded according to the consensus criteria [17, 18].

Statistical analysis

All statistical analyses were performed using the EZR software program (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [19]. P values of <0.05 were considered to indicate statistical significance. The cumulative incidence rates of GVHD and neutrophil

engraftment and platelet recovery were calculated using the Gray's test and compared using a log-rank test. In these analyses, death or relapse were considered as competing events. Univariate analyses were performed using the χ^2-test and Fisher's exact test. Factors with P values of <0.20 in the univariate analyses were included in a multivariate analysis. Stepwise multivariate logistic regression models were used to analyze the influence of selected variables on the risk of oral mucositis of over 14 days in duration.

Results

Patient characteristics

The patient characteristics are shown in Table 1. Among the 76 patients who underwent HSCT with TAC plus MTX, 60 patients received 15-10-10 MTX and 16 patients received 10-7-7 MTX. With the exception of age, the patients' characteristics (including gender, disease, disease status, intensity of conditioning, graft type, degree of HLA matching, and sex) did not differ to a statistically significant extent between the two groups. The median age of the 10-7-7 MTX group was significantly higher than that of the 15-10-10 MTX group.

GVHD

The cumulative incidences rates of grades Ⅱ–Ⅳ and Ⅲ–Ⅳ acute GVHD in the 15-10-10 MTX and 10-7-7 MTX groups (grades Ⅱ–Ⅳ: 35.0 vs 18.8% [P=0.36]; grades Ⅲ–Ⅳ: 11.7 vs 6.3% [P=1.0], respectively) did not differ to a statistically significant extent (Figure 1 and Table 2). The median onset of acute GVHD was 30 days after transplantation

(range, 11–96 days). The cumulative incidence of chronic GVHD did not differ between the two groups (31.7 vs 25.0%; P=0.76).

Neutrophil engraftment

Graft failure was only documented in three patients (3.9%) in the 15-10-10 MTX group. The median time for neutrophil engraftment was significantly shorter in the 10-7-7 MTX group (15 days; range, 12–19 days) than in the 15-10-10 MTX group (16 days; range, 11–31 days) (P=0.024) (Figure 2 a, b). The median time for neutrophil engraftment after HLA-matched bone marrow transplantation (BMT) was also significantly shorter in the 10-7-7 MTX group (14.5 days; range, 12–16 days) than in the 15-10-10 MTX group (15.5 days; range, 12–27 days) (P=0.0046) (Figure 2 c, d). The median time for neutrophil engraftment in the patients who received HLA-matched HSCT was not significantly different from that of the patients who received HLA-mismatched HSCT (data not shown).

Platelet recovery

The median time for platelet recovery was significantly shorter in the 10-7-7 MTX group (22 days; range, 13–49 days) than that in the 15-10-10 MTX group (27 days; range, 9–405 days) (P=0.027) (Figure 2 e).

Oral mucositis

Severe oral mucositis was observed in 18 patients (23.7%) of the overall population. The incidence of severe oral mucositis in the 10-7-7 MTX group did not differ from that in the

15-10-10 MTX group (12.5 vs 26.7%; P=0.33). Interestingly, the duration of oral mucositis was significantly shorter in the 10-7-7 MTX group (median 4.5 days; range, 0-16 days) than that in the 15-10-10 MTX group (median 13.0 days; range, 0-53 days) (P=0.013) (Table 2).

The univariate and multivariate analyses of the risk factors for prolonged severe mucositis

The duration of oral mucositis was modestly correlated with the time to neutrophil engraftment (Supplementary figure 1). A univariate analysis demonstrated that 15-10-10 MTX and severe oral mucositis were risk factors for prolonged oral mucositis (>14days).

A multivariate analysis confirmed that the existence of severe oral mucositis (OR, 18.0; 95% confidence interval [CI], 3.99-81.05; P<0.001) and 15-10-10 MTX were risks for prolonged oral mucositis (OR, 0.094; 95% CI, 0.0090–0.98; P=0.048) (Table 4).

Discussion

Although it has been well acknowledged that conditioning therapy (such as high-dose chemotherapy and TBI) causes severe mucositis, MTX (which is used for GVHD prophylaxis) also contributes to the progression of mucositis and possibly delayed engraftment [20]. Several strategies have been tested to reduce the incidence of MTX-related toxicities. First, the dose and duration of MTX can be reduced. A meta-analysis comparing the administration of three and four doses of MTX for GVHD prophylaxis showed that administration of MTX on day +11 was associated with an

increased incidence of leukemic relapse, but there was no significant difference in the risk of acute GVHD or overall survival in patients who received three and four doses of MTX after BMT and the incidence of oral mucositis was not compared [15]. Second, MTX can be replaced by mycophenolate mofetil (MMF), which reduces the incidence of mucositis without inhibiting neutrophil engraftment. However, the advantages of MMF were not obvious in HSCT in patients who received a myeloablative regimen [21]. A meta-analysis showed that the incidence of grades Ⅲ-Ⅳ acute GVHD in patients who received a GVHD prophylaxis regimen that contained MMF was higher than that in patients who received a regimen containing MTX. MMF is associated with a decreased risk of oral mucositis [22], , and may lead to reduced narcotics use and a shorter hospital stay [23]. Lastly, the administration of folinic acid may reduce the incidence of oral mucositis [24]. Several studies have reported that the systemic administration of folinic acid, which is routinely used to mitigate MTX toxicities, potentially reduces the incidence of severe oral mucositis after HSCT [25-27]. The incidence of GVHD in Japanese patients is lower than that in Caucasians [28, 29], possibly due to the more homogenous genetic background of the Japanese population [30]. In many Japanese institutes, day +11 MTX is usually omitted in HLA-matched transplantation from a sibling donor [31, 32], whereas four doses are standard in Western countries [15]. To our knowledge, this is the first study to compare 10-7-7 MTX and 15-10-10 MTX in combination with TAC for GVHD prophylaxis. We found that reduced doses of MTX (10-7-7 MTX) resulted in the earlier engraftment of neutrophils and platelets, and the mitigation of oral mucositis, without increasing the incidence of acute or chronic GVHD. The shorter duration of severe mucositis may be

associated with a reduced risk of infection [33]. However, the present study was retrospective in nature and involved a small number of patients. Further study are therefore required to confirm our results in a prospective study. It should be noted that there is great inter-individual variability in MTX metabolism, which may affect the outcome of HSCT using MTX [34, 35]. In conclusion, our analysis indicated the superiority and safety of the 10-7-7 MTX for GVHD prophylaxis in Japanese patients, regardless of HLA disparity.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Figure legends

Figure 1.

(a,b) The cumulative incidence rates of grades Ⅱ-Ⅳacute GVHD (a) and grades Ⅲ-Ⅳ acute GVHD (b) in the 15-10-10 MTX group (solid lines, n=60) and the 10-7-7 MTX group (dashed lines, n=16). (c, d) The cumulative incidence curves of grades Ⅱ-Ⅳ acute GVHD (c) and grades Ⅲ-Ⅳ acute GVHD (d) after HLA-matched HSCT with 15-10-10 MTX (solid lines, n=30) and 10-7-7 MTX (dashed lines, n=11).

Figure 2.

(a-d) The solid lines indicate the cumulative incidence curve of neutrophil engraftment after all HSCT (a: n=60), BMT (b: n=40), HLA-matched HSCT (c: n=30), and HLA-matched

BMT (d: n=22). The dashed lines indicate neutron engraftment after all HSCT (a: n=16), BMT (b: n=11), HLA-matched HSCT (c: n=11), and HLA-matched BMT (d: n=7). (e) The cumulative curves of platelet engraftment in the 15-10-10 MTX group (solid lines, n=60) and the 10-7-7 MTX group (dot lines, n=16) are shown.

Figure 3.

The association between the day of neutrophil engraftment and the duration of oral mucositis (Pearson's correlation coefficient).

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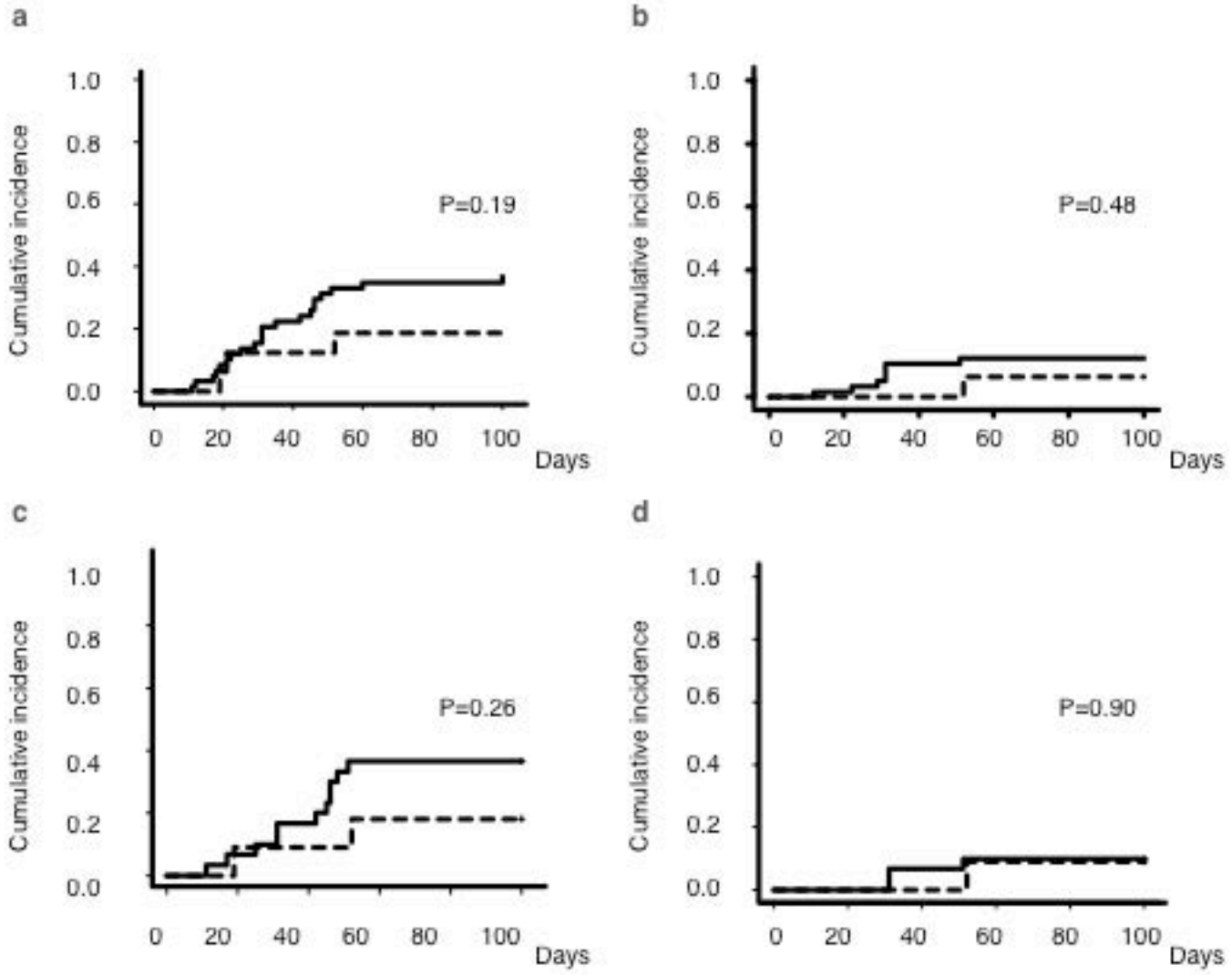
a
b
c
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Cumulative incidence
1.0
0.8
0.6
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P=0.19
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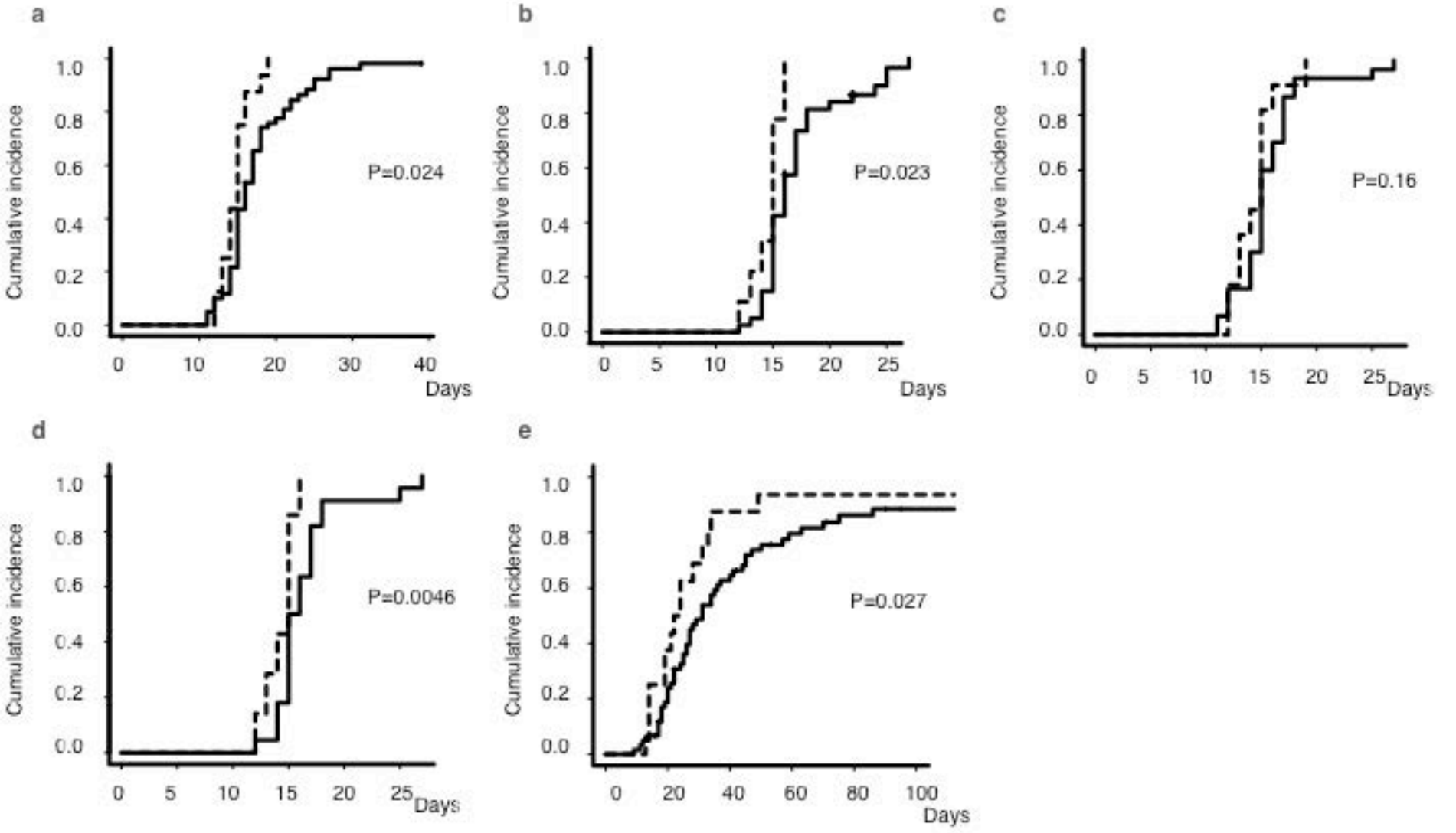
P=0.26
P=0.90

Fig. 1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Cumulative incidence
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Days
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Fig. 2

Table 1 The patient characteristics

	15-10-10 MTX (n=60)	10-7-7 MTX (n=16)	P value
Age (years) median(range)	42 (19-66)	54.5 (24-65)	0.020
Gender			0.58
Male	32 (53.3%)	7 (43.8%)	
Female	28 (46.7%)	9 (56.3%)	
Disease			0.29
AML	19 (31.7%)	6 (37.5%)	
ALL	12 (20.0%)	2 (12.5%)	
CML	1 (1.7%)	1 (6.3%)	
MDS/MPN	4 (6.7%)	4 (25.0%)	
ATL	3 (5.0%)	0	
ML	17 (28.3%)	3 (18.8%)	
AA	4 (6.7%)	0	
Disease status			0.37
CR	33 (55.0%)	8 (50.0%)	
non-CR	23 (38.3%)	5 (31.3%)	
CP/SD	4 (6.7%)	3 (18.8%)	
Conditioning			0.78
MAC	30 (50.0%)	9 (56.3%)	
RIC	30 (50.0%)	7 (43.8%)	
TBI			1
yes	57 (95.0%)	15 (93.8%)	
no	3 (5.0%)	1 (6.3%)	
Graft type			0.70
BM	40 (66.7%)	11 (68.8%)	
PB	9 (15.0%)	3 (18.8%)	
CB	11 (18.3%)	2 (12.5%)	
HLA match			0.38
8/8 or 6/6	30 (50.0%)	11 (68.8%)	
7/8	18 (30.0%)	4 (25.0%)	
6/8	12 (20.0%)	1 (6.3%)	
Donor/Recipient gender			0.94
Male/Male	20 (33.3%)	4 (25.0%)	
Female/Female	10 (16.7%)	3 (18.8%)	
Male/Female	20 (33.3%)	6 (37.5%)	
Female/Male	10 (16.7%)	3 (18.8%)	

AML, acute myeloid leukemia; *ALL,* acute lymphoblastic leukemia; *CML,* chronic myeloid leukemia; *MDS/MPN,* myelodysplastic syndrome/myeloproliferative neoplasms; *ATL,* adult T cell leukemia/lymphoma; *ML,* malignant lymphoma; *AA,* aplastic anemia; *CR,* complete remission; *CP/SD,* chronic phase/stable disease; *MAC,* myeloablative conditioning; *RIC,* reduced intensity conditioning; *TBI,* total body irradiation; *BM,* bone marrow; *PB,* peripheral blood; *CB,* cord blood

Table 2 GVHD and oral mucositis in two different methotrexate dose groups.

	15-10-10 MTX (n=60)	10-7-7 MTX (n=16)	P value
Acute GVHD (Grade Ⅱ- Ⅳ)			0.36
yes	21 (35.0%)	3 (18.8%)	
no	39 (65.0%)	13 (81.3%)	
Acute GVHD (Grade Ⅲ- Ⅳ)			1
yes	7 (11.7%)	1 (6.3%)	
no	53 (88.3%)	15 (93.8%)	
Chronic GVHD			0.76
yes	41 (68.3%)	12 (75.0%)	
no	19 (31.7%)	4 (25.0%)	
Oral mucositis			0.53
yes	14 (23.3%)	5 (31.3%)	
no	46 (76.7%)	11(68.8%)	
Duration of oral mucositis (day)			0.013
median (range)	13.0 (0-53)	4.5 (0-16)	
Severe oral mucositis			0.33
yes	16 (26.7%)	2 (12.5%)	
no	44 (73.3%)	14 (87.5%)	

Table 3 The risk factors for prolonged oral mucositis (univariate analysis)

	n	Oral mucositis persisted over 14 days	OR	95%CI	P value
Age					
≤50	32	7 (21.9%)	1		0.14
>50	44	17 (38.6%)	2.23	0.72-7.47	
Gender					
Male	39	14 (35.9%)	1		0.46
Female	37	10 (27.0%)	0.67	0.22-1.95	
MTX (mg/m^2)					
15-10-10	60	23 (38.3%)	1		0.015
10-7-7	16	1 (6.3%)	0.11	0.0025- 0.81	
Severe oral mucositis					
no	58	10 (17.2%)	1		<0.001
yes	18	14 (77.8%)	15.93	3.99-81.05	
Acute GVHD (Grade Ⅱ- Ⅳ)					
yes	24	9 (37.5%)	1		0.60
no	52	15 (28.8%)	0.68	0.22-2.17	
Acute GVHD (Grade Ⅲ- Ⅳ)					
yes	8	3 (37.5%)	1		0.70
no	68	21 (30.9%)	0.75	0.13-5	
Chronic GVHD					
yes	23	5 (21.7%)	1		0.29
no	53	19 (35.8%)	1.99	0.59-7.99	

OR, odds ratio*; CI,* condidence interval

Table 4 The risk factors for prolonged oral mucositis (multivariate analysis)

	OR	95%CI	P value
Age			
50≤	1		0.47
50>	1.62	0.44-5.90	
MTX (mg/m^2)			
15-10-10	1		0.048
10-7-7	0.0935	0.0090- 0.98	
Severe oral mucositis			
no	1		<0.001
yes	18.0	3.99-81.05	

OR, odds ratio*; CI,* condidence interval