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ORIGINAL ARTICLE

Comparison of calcineurin inhibitors in combination with conventional methotrexate, reduced methotrexate, or mycophenolate mofetil for prophylaxis of graft-versus-host disease after umbilical cord blood transplantation

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Abstract

Umbilical cord blood transplantation (UCBT) is a curative treatment for hematological malignancies. However, appropriate prophylaxis against graft-versus-host disease (GVHD), aimed at obtaining rapid and stable engraftment and avoiding toxicity, remains controversial in UCBT. We retrospectively compared outcomes in 409 patients who received calcineurin inhibitors (CIs) plus conventional-dose methotrexate (conv-MTX/CIs, $n = 77$; methotrexate, 10 mg/m^2 on day 1, 7 mg/m^2 on days 3 and 6) with those who received CIs plus reduced-dose methotrexate (reduced-MTX/CIs, $n = 209$; methotrexate, 5 mg/m^2 or 5 mg/body on days 1, 3, and 6) or CIs with mycophenolate mofetil (MMF/CIs, $n = 123$) for GVHD prophylaxis after UCBT. The cumulative incidence of neutrophil engraftment was significantly higher in the reduced-MTX/CI (82.3%) and MMF/CI (86.6%) groups than the conv-MTX/CI (71.4%) group ($p = 0.014$), although there were no differences in platelet recovery or infectious complications among the three groups. The incidence and severity of GVHD were comparable among the three groups, and there were no significant differences in transplantation-related mortality among the three groups. In conclusion, GVHD prophylaxis with reduced-dose methotrexate and MMF was closely associated with high incidence of neutrophil engraftment without an effect on the incidence and severity of GVHD, which was compared to GVHD prophylaxis with conventional-dose methotrexate.

Keywords GVHD · Prophylaxis · Reduced-dose · Methotrexate · MMF · UCBT

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Introduction

During the past decade, umbilical cord blood (UCB) has been increasingly used as an alternative hematopoietic stem cell source for allogeneic bone marrow (BM) or peripheral blood stem cells (PBSC) because of its potential advantages of rapid availability and lower risk of graft-versus-host disease (GVHD), which has permitted less stringent human leukocyte antigen (HLA) matching [1]. However, UCB transplantation (UCBT) is associated with delayed neutrophil and platelet recovery and a higher incidence of engraftment failure compared with BM or PBSC, which is a major issue that remains to be solved. In addition, GVHD is a major complication after UCBT as well. Thus, appropriate prophylaxis with immunosuppressants is important to obtain rapid engraftment to further improve outcomes in patients undergoing UCBT [2–4].

The globally used standard GVHD prophylaxis regimen comprises calcineurin inhibitors (CIs) such as cyclosporine or tacrolimus in combination with methotrexate at a dose of 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11 [5]. In Japan, a reduced-dose methotrexate regimen comprising 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6, given in combination with CIs, has been generally accepted as a conventional GVHD prophylaxis for allogeneic BM and PBSC transplantation as well as UCBT, based on the very close HLA concordance between the donors and the recipients in the Japanese population [4, 6, 7]. Nevertheless, even reduced-dose methotrexate can cause mucosal damage and delay engraftment, which can be augmented in UCBT [8–11]. Therefore, instead of methotrexate, mycophenolate mofetil (MMF) in combination with CIs has been preferred for GVHD prophylaxis particularly for UCBT at several institutions due to the potential advantages of faster engraftment and less toxicity, especially renal toxicity and mucosal damage [8, 12, 13]. Alternatively, further reduced doses of methotrexate to a variable extent, in combination with CIs, have been administered for GVHD prophylaxis in UCBT in clinical settings, since MMF has not yet been approved by Japanese national health insurance until this year. In this study, we comparatively evaluated the efficacy and toxicity of GVHD prophylaxis using conventional or reduced-dose methotrexate plus CIs with those of MMF plus CIs after UCBT.

Patients and methods

Patients

We retrospectively analyzed 409 patients with hematological malignancies who received their first UCBT between 2010 and 2017 at one of the seven institutions of Fukuoka Blood and Marrow Transplantation Group (FBMTG). The ethics committees of the participating institutions approved this study (Kyushu University Graduate School and Faculty of Medicine; approval nos. 23027-1 and 23027-2).

Conditioning regimens, GVHD prophylaxis, and UCB selection

One of the following myeloablative conditioning (MAC) regimens was employed based on each transplant physician's preference: total body irradiation (TBI, 4 Gy × 3 days) + cyclophosphamide (60 mg/kg × 2 days) (TBI/CY); busulfan (3.2 mg/kg × 4 days) + cyclophosphamide (60 mg/kg × 2 days) (BU4/CY); BU4/CY + fludarabine (30 mg/m^2 × 6 days) (BU4/CY/FLU); and TBI (4 Gy × 3 days) + fludarabine (25–30 mg/m^2 × 5–6 days) + melphalan (60–90 mg/m^2 × 2 days) (TBI/FLU/MEL). For reduced-intensity conditioning (RIC), one of the following regimens were adopted: fludarabine (30 mg/m^2 × 6 days) + busulfan (3.2 mg/kg × 2–4 days) + TBI (2–4 Gy) (FLU/BU/TBI); FLU (25 mg/m^2 × 5 days) + melphalan (40–90 mg/m^2 × 2 days) ± TBI (2–4 Gy × 1 days) (FLU/MEL/TBI); and fludarabine (25–30 mg/m^2 × 5 days) + cyclophosphamide (45 mg/kg × 2 days) + busulfan (2.4 mg/kg/day × 2 days) (FLU/CY/BU).

GVHD prophylaxis in the MMF plus CI (MMF/CI) group comprised cyclosporine (1.5 mg/kg intravenous infusion over 3 h twice daily or 3 mg/kg continuous intravenous infusion for 24 h, aiming for a serum concentration of 300–500 ng/mL) or tacrolimus (0.03 mg/kg 24-h continuous intravenous infusion, aiming for a serum concentration of 9–15 ng/mL) and MMF (15 mg/kg orally, twice daily until day 28, with gradual reduction thereafter, ceasing on day 42 unless active GVHD is observed) [14]. In the methotrexate plus CI (MTX/CI) group, for GVHD prophylaxis for UCBT, cyclosporine and tacrolimus were used as stated above in combination with short-term MTX defined as conventional-dose methotrexate (conv-MTX) (10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6) or reduced-dose methotrexate (reduced-MTX) (5 mg/m^2 methotrexate on days 1, 3, and 6 [low-MTX] or a further reduced minimal-dose methotrexate at 5 mg/body on days 1, 3, and 6 [mini-MTX]).

UCB units, obtained from the Japan Cord Blood Bank Network, were selected to match at least four of six HLA-A and B sero-antigens as well as the DRB1 alleles of the recipient and had a cell dose of at least 1.5 × 10^7 total mononucleated cells per kilogram of recipient body weight, if possible. Anti-HLA antibodies were tested using LABScreen® PRA and Single Antigen (One Lambda, Canoga Park, CA) for class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DR) anti-HLA antibodies [15]. A median fluorescence intensity of > 1,000 was defined as a positive match.

Definitions

Neutrophil engraftment was defined as an absolute neutrophil number of more than 0.5 × 10^9/L for three consecutive days after UCBT. Platelet recovery was defined as a count of 20 × 10^9/L without transfusion support. Human herpesvirus-6 (HHV6)–associated encephalitis was diagnosed based on central nervous system symptoms with amplification of HHV-6 by polymerase chain reaction in spinal fluid [16, 17]. Adenovirus-associated hemorrhagic cystitis was diagnosed based on cystitis symptoms with positivity for adenovirus by a rapid immunochromatography or by adenovirus amplification by polymerase chain reaction in urine [18, 19]. Transplantation-related mortality (TRM) was defined as rate of death within the first hundred days after UCBT without death due to relapse.

Data collection

Patient data were collected from the Transplant Registry Unified Management Program (TRUMP) at the Japanese registry-based data center at each transplantation center [20]. The TRUMP database lacked several critical data such as peri-engraftment reaction, adverse events such as mucosal damage, and chronic GVHD in detail. Therefore, in the present study, there were several missing data necessary to evaluate the efficacy and safety of different approaches for GVHD prophylaxis.

Statistical analysis

Categorical variables between groups were compared with the chi-square or Fisher's exact test, and continuous variables were compared with the Kruskal-Wallis test. Overall survival (OS) and PFS were calculated from the date of stem cell transplantation using the Kaplan-Meier product-limit method, and differences between the groups were assessed using the log-rank test. The probabilities of neutrophil and platelet engraftment; acute GVHD; bacterial, fungal, and viral infections; TRM; and relapse-related death were estimated on the basis of cumulative incidence methods and compared among groups with the Gray test. In hematopoietic recovery and acute GVHD, death without the event was the competing risk. In bacterial, fungal, and viral infection, TRM except from each infection and relapse-related death was considered the competing risk. In TRM, relapse-related death was the competing risk, whereas that for relapse-related death was non-relapse mortality. The post hoc tests with Bonferroni correction were performed to evaluate pairwise differences between GVHD-prophylaxis groups. Variables with a P value < 0.2 for each endpoint were tested in multivariate analysis for temporal events. Multivariate analyses were carried out using Cox proportional hazards for OS and Fine-Gray proportional hazards for neutrophil and platelet engraftment to assess potential risk factors of interest. All statistical analyses were performed using EZR version 1.30 (Saitama Medical Center, Jichii Medical University) [21].

Results

Patient characteristics

The study cohort of 409 patients were divided into three groups based on the GVDH prophylaxis regimen: conv-MTX/CIs (n = 77), reduced-MTX/CIs (n = 209, including low-MTX/CIs [n = 70] and mini-MTX/CIs [n = 139]), and MMF/CIs (n = 123). The background and clinical characteristics of the groups are summarized in Table 1. Briefly, the median patient age was 61 years. The diagnoses requiring UCBT included acute myeloid leukemia (n = 191), myelodysplastic syndrome (n = 47), acute lymphoblastic leukemia (n = 44), malignant lymphoma (n = 58), adult T-cell leukemia lymphoma (ATL) (n = 56), and others (n = 13). The disease statuses were complete remission (CR, n = 166) and non-CR (n = 243, including partial remission and stable disease/progressing disease in 40 and 203 patients, respectively). Conditioning included MAC and RIC in 139 and 270 patients, respectively. The median cell number of infused mononucleated cells (MNC) and $CD34^+$ cells in UCB were 2.62×10^7/kg and 0.87×10^5/kg, respectively. Finally, about 60% of the UCB samples had two or more mismatching loci among the HLA-A/B/DR antigens.

As shown in Table 1, there were no differences among the three groups regarding sex, underlying disease, disease status, performance status (PS), hematopoietic cell transplant-comorbidity index, [22] number of infused MNC of UCB, number of infused $CD34^+$ cells in UCB, HLA compatibility, or antibody positivity for against HLAs. In contrast, conv-MTX/CIs was employed preferentially for younger recipients ($p < 0.0001$) and for those who received a MAC regimen ($p < 0.0001$).

Engraftment

The cumulative incidence of neutrophil recovery in the conv-MTX/CI group (71.4%) was lower than those in the reduced-MTX/CI (82.3%) and the MMF/CI (86.6%) groups (p = 0.014) (Fig. 1a). There were significant differences between conv-MTX/CIs and the reduced-MTX/CIs (p = 0.023) as well as conv-MTX/CIs and MMF (p = 0.011) in the post hoc test by Bonferroni correction, respectively. The median time to neutrophil engraftment of 20 days in the conv-MTX/CI group was not significantly different than that in the reduced-MTX/CI (19 days) or the MMF/CI (19 days) group (p = 0.06). In contrast, the cumulative incidence of platelet recovery in the conv-MTX/CI group (69.5%) was comparable to those in the reduced-MTX/CI (60.1%) and the MMF/CI (71.3%) groups (p = 0.35) (Fig. 1b). The median time to platelet engraftment was 39 days in the conv-MTX/CI group, which was not significantly different than that in the reduced-MTX/CI (37 days) or the MMF/CI (42 days) group (p = 0.06). The cumulative incidence of neutrophil and platelet recovery was equivalent between the MAC versus RIC conditioning regimens (Table 2). Furthermore, there was no significant difference in neutrophil and platelet recovery among each conditioning regimens as described in Table 1 (data not shown).

Acute GVHD

The cumulative incidence of grade II–IV acute GVHD in the conv-MTX/CIs (44.2%) was comparable to those in the reduced-MTX/CI (48.3%) and the MMF/CI (43.3%) groups

Table 1 Patient characteristics

	Total	MMF	Reduced-MTX	Conv-MTX	*p* value
Number of patients	409	123	209	77	
Male/female	235 (57)/174 (43)	74 (60)/49 (40)	115 (55)/94 (45)	46 (60)/31 (40)	0.596
Age, median (range)	61 (18–88)	62 (18–80)	62 (19–88)	53 (18–72)	*< 0.0001*
Diagnosis					
AML	191 (47)	67 (54)	92 (44)	32 (42)	0.111
MDS	47 (11)	10 (8)	30 (14)	7 (9)	0.176
ALL	44 (11)	12 (10)	22 (11)	10 (13)	0.764
ML	58 (14)	15 (12)	30 (14)	13 (17)	0.649
ATL	56 (14)	18 (15)	26 (12)	12 (16)	0.74
other	13 (3)	1 (1)	9 (4)	3 (4)	0.2
Disease status					
CR	166 (41)	45 (37)	87 (42)	34 (44)	0.605
Non-CR	243 (59)	78 (63)	122 (58)	43 (56)	
PR	40 (9.8)	5 (4)	28 (13)	7 (9)	
SD/PD	203 (49.7)	73 (59)	94 (45)	36 (47)	
PS ≥ 2	68 (17)	22 (18)	38 (18)	8 (10)	0.265
HCT-CI ≥ 3	60 (15)	18 (15)	30 (14)	12 (16)	0.967
Median number of MNC in UCB ($\times 10^7$/kg) (range)	2.62 (1.60–6.67)	2.66 (1.67–6.67)	2.58 (1.74–5.39)	2.71 (1.6–5.35)	0.472
Median number of CD34+ cells in UCB ($\times 10^5$/kg) (range)	0.87 (0.07–6.84)	0.90 (0.07–5.19)	0.86 (0.12–3.04)	0.88 (0.41–6.84)	0.689
HLA compatibility					
Full match	48 (12)	14 (11)	23 (11)	11 (14)	0.777
Mismatch in one locus	113 (28)	42 (34)	51 (24)	20 (26)	0.153
Mismatch in two loci	230 (56)	62 (50)	124 (59)	44 (57)	0.244
Mismatch in three or more loci	11 (3)	3 (2)	6 (3)	2 (3)	0.967
Data missing	7 (2)	2 (2)	5 (2)	0	0.383
HLA-Ab positive	73 (18)	24 (20)	36 (17)	13 (17)	0.733
MAC/RIC	139 (34)/270 (66)	15 (12)/108 (88)	79 (38)/130 (62)	45 (58)/32 (42)	*< 0.0001*
Conditioning					
FLU/MEL/TBI	153 (37)	54 (44)	74 (35)	25 (32)	0.186
FLU/BU/TBI	88 (22)	39 (32)	43 (21)	6 (8)	*< 0.0001*
FLU/MEL/BU	19 (5)	11 (9)	7 (5)	1 (1)	*0.0198*
FLU/CY/BU	41 (10)	0	41 (20)	0	
TBI/CY	86 (21)	15 (12)	27 (13)	44 (57)	*< 0.0001*
BU/CY	6 (1)	2 (2)	4(2)	0	0.484
Other	16 (4)	2 (2)	13 (6)	1 (1)	0.2

Values in parentheses are percents, unless otherwise indicated

Abbreviation: *MMF*, mycophenolate mofetil; *MTX*, methotrexate; *conv-MTX*, conventional MTX; *ATL*, adult T cell leukemia lymphoma; *PS*, performance status; *HCT-CI*, hematopoietic cell transplant-comorbidity index; *UCB*, umbilical cord blood; *MNC*, mononucleated cells.

(p = 0.77) (Fig. 2a). The incidence of grade III–IV acute GVHD in the conv-MTX/CI group (8.7%) was also comparable to those in the reduced-MTX/CI (18.2%) and the MMF/CI (11.8%) groups (p = 0.26) (Fig. 2b).

Infectious complications

The incidence of documented ≥ grade 3 bacterial infections in the conv-MTX/CI group (36.4%) was comparable to those in the reduced-MTX/CI (41.1%) and the MMF/CI (43.2%) groups (p = 0.74) (Fig. 3a). The incidence of documented ≥ grade 3 fungal infections in the conv-MTX/CIs (6.5%) was also comparable to those in the reduced-MTX/CI (9.1%) and the MMF/CI (8.9%) groups (p = 0.78) (Fig. 3b). The incidence of HHV6-associated encephalitis in the conv-MTX/CI group (2.6%) was 3.3% in the reduced-MTX/CI group and 4.9% in the MMF/CI group (p = 0.67) (Fig. 3c). The incidence of adenovirus-associated cystitis in the conv-MTX/CI group

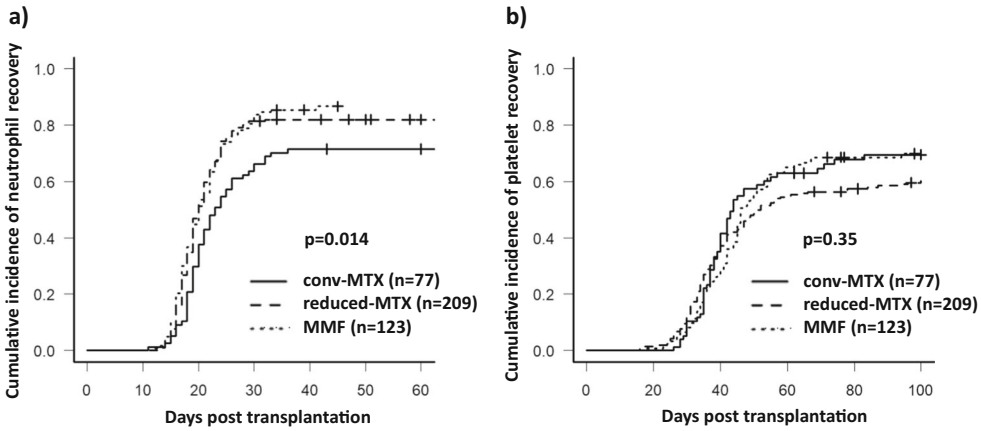

Fig. 1 Cumulative incidence of neutrophil (**a**) and platelet (**b**) engraftment after umbilical cord blood transplantation (UCBT) in conventional-dose methotrexate + calcineurin inhibitor (CI) (conv-MTX/CI), reduced-dose methotrexate + CI (MTX/CI), and mycophenolate mofetil + CI (MMF/CI) groups according to the regimen for graft-versus-host disease (GVHD) prophylaxis

Table 2 Univariate analysis for factors associated with neutrophil and platelet engraftment and overall survival in all recipients

Variable	*n*	Percent (95% CI)	*p* value	Percent (95% CI)	*p* value	Percent (95% CI)	*p* value
Age			0.32		*0.003*		*0.0004*
< 60	176	83.1 (76.6–87.9)		73.4 (65.9–79.6)		36.9 (27.7–46.1)	
≥ 60	233	79.9 (74.2–84.5)		58.9 (52.1–64.9)		25.4 (19.3–32.0)	
PS			*< 0.0001*		*< 0.0001*		*< 0.0001*
< 2	341	85.1 (80.9–88.5)		70.8 (65.5–75.4)		34.9 (29.1–40.7)	
≥ 2	68	61.8 (49.0–72.2)		36.4 (24.8–48.1)		15.1 (7.5–25.1)	
HLA antibody			0.47		0.3		0.84
Yes	93	79.6 (69.8–86.5)		69.4 (58.4–78.0)		24.2 (10.4–41.1)	
No	272	81.7 (76.6–85.8)		66.4 (60.3–71.8)		33.3 (27.1–39.7)	
HCT-CI			0.48		0.71		0.43
< 2	349	81.2 (76.7–84.9)		64.8 (59.4–69.7)		30.5 (24.4–36.7)	
≥ 2	60	81.7 (69.0–89.5)		66.7 (52.9–77.3)		28.2 (16.7–40.8)	
Disease status			*0.015*		*< 0.0001*		*< 0.0001*
CR	167	85.0 (78.7–89.6)		75.1 (67.5–81.2)		44.8 (34.6–54.4)	
Non-CR	242	78.7 (73.0–83.4)		58.2 (51.5–64.2)		20.8 (15.5–26.8)	
GVHD prophylaxis			*0.014*		0.35		0.052
Conv-MTX/CIs	77	71.4 (59.8–80.2)		69.5 (57.2–78.9)		41.7 (29.8–53.1)	
Reduced-MTX/CIs	209	82.3 (76.5–86.9)		60.1 (53.0–66.5)		25.3 (16.2–35.4)	
MMF/CIs	123	86.6 (78.8–91.6)		71.3 (61.8–78.8)		27.7 (18.7–37.5)	
Conditioning			0.15		0.7		0.053
MAC	143	77.0 (69.2–83.1)		63.0 (54.2–70.6)		37.6 (28.9–46.8)	
RIC	266	83.7 (78.6–87.6)		66.1 (60.0–71.6)		25.1 (17.6–33.3)	
HLA compatibility			0.75		0.79		0.128
Full match	48	77.8 (62.0–87.6)		72.7 (48.4–87.6)		31.5 (15.8–48.5)	
Mismatch in one locus	113	80.5 (71.9–86.7)		65.9 (56–74.1)		39.6 (29.2–49.9)	
Mismatch in two or more loci	241	82.2 (76.8–86.5)		63.5 (57.0–69.4)		25.4 (18.6–32.7)	
Number of MNC in UCB			0.92		0.79		0.75
$< 2 \times 10^7$/kg	34	84.3 (63.6–93.8)		62.5 (42.7–77.1)		34.6 (17.4–52.6)	
$\geq 2 \times 10^7$/kg	374	81.1 (71.7–84.7)		65.2 (60.0–69.9)		29.4 (23.5–35.5)	
Number of CD34+ cells in UCB			0.1		*0.003*		0.82
$< 1 \times 10^5$/kg	246	81.5 (76.1–85.8)		61.4 (54.9–67.3)		30.6 (24.0–37.5)	
$\geq 1 \times 10^5$/kg	162	80.9 (73.9–86.1)		70.5 (62.5–77.1)		29.3 (20.4–38.7)	

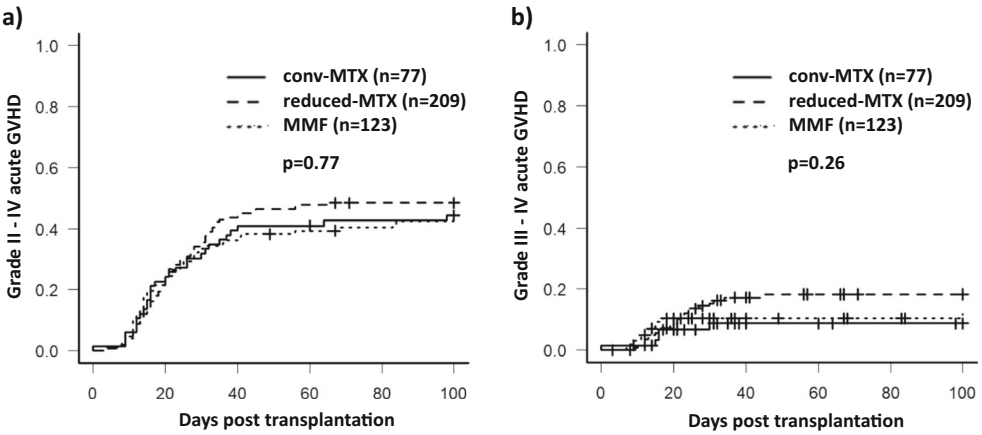

Fig. 2 Cumulative incidence of grade II–IV (**a**) and grade III–IV (**b**) acute GVHD after UCBT in the conv-MTX/CI, reduced-MTX/CI, and MMF/CI groups based on the regimen for GVHD prophylaxis

(3.9%) was not significantly different than that in the reduced-MTX/CI (3.3%) or the MMF/CI (5.7%) group ($p = 0.59$) (Fig. 3d). These results indicated that there were no significant differences in infectious complications such as bacterial or fungal infections, HHV6-associated encephalitis, or adenovirus-associated cystitis among the three groups.

TRM, relapse, OS, PFS

The median follow-up was 926 days (range, 28–2676 days). The cumulative incidence of TRM of 15.6% in the conv-MTX/CI group was comparable to those in the reduced-MTX/CI (25.8%) and the MMF/CI (20.3%) groups ($p = 0.18$) (Fig. 4a). The incidence of relapse was 27.9% in the conv-MTX/CIs, which also was not significantly different than those in the reduced-MTX/CI (22.0%) and the MMF/CI (22.9%) groups ($p = 0.76$) (Fig. 4b). The 5-year OS of the conv-MTX/CI group (47.2%) was also comparable to those of the reduced-MTX/CI (34.3%) and the MMF/CI (32.8%) groups ($p = 0.12$) (Fig. 4c). Finally, the 5-year PFS was 31.9% in the conv-MTX/CI group, which was not significantly different than that in the reduced-MTX/CI (19.7%) or the MMF/CI (26.8%) group ($p = 0.40$) (Fig. 4d).

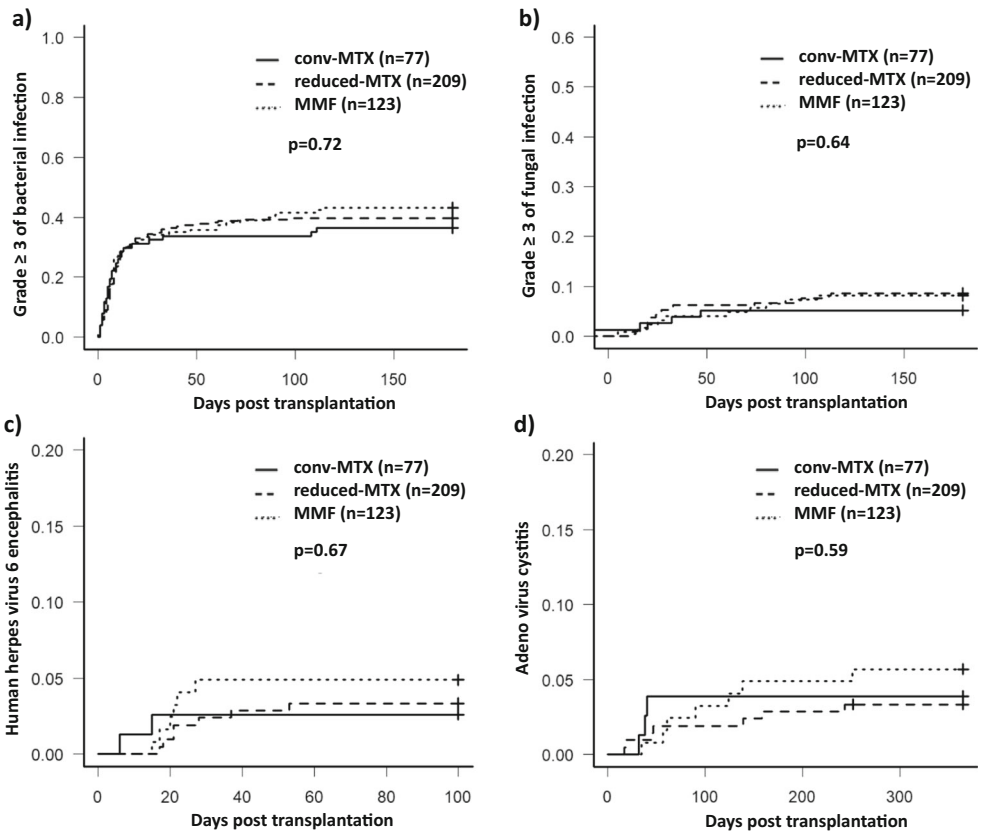

Fig. 3 Cumulative incidence of ≥ grade 3 bacterial infections (**a**), ≥ grade 3 fungal infections (**b**), HHV6-associated encephalitis (**c**), and adenovirus-associated cystitis (**d**) after UCBT in the conv-MTX/CI, reduced-MTX/CI, and MMF/CI groups

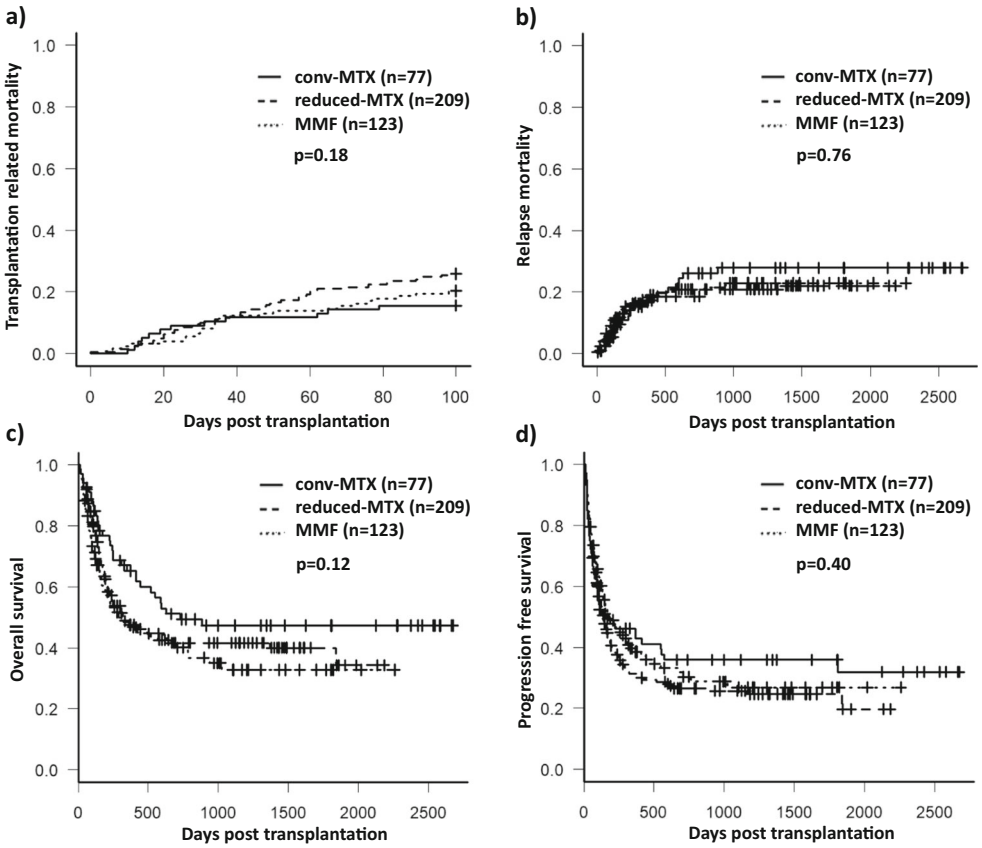

Fig. 4 Cumulative incidence of transplantation-related mortality (TRM) at 100 days (**a**), relapse (**b**), overall survival (**c**), and progression-free survival (PFS) (**d**) after UCBT in the conv-MTX/CI, reduced-MTX/CI, and MMF/CI groups based on the regimen for GVHD prophylaxis

Factors associated with neutrophil and platelet engraftment and OS

In univariate analyses, neutrophil engraftment was inversely associated with poor PS (PS ≥ 2; $p < 0.0001$), advanced disease status (non-CR; $p = 0.015$), and significantly different among three different GVHD prophylaxis groups ($p = 0.014$). Platelet engraftment was associated with younger age (≤ 60 years; $p = 0.003$), good PS ($p < 0.0001$), CR ($p < 0.0001$), and number of $CD34^+$ cells in UCB ($\geq 1 \times 10^5$/kg; $p = 0.003$). OS was associated with younger age ($p = 0.0004$), good PS ($p < 0.0001$), and CR ($p < 0.0001$) (Table 2). There was no association between engraftment and the presence of antibodies against HLA or the conditioning regimen. In addition, we analyzed factors such as conditioning, HLA compatibility, and infused number of MNC associated with neutrophil and platelet engraftment in each three groups. As shown in Table 3, there was no difference in neutrophil and platelet recovery with respect to these three factors in each group.

In multivariate analyses, neutrophil engraftment was associated with good PS (hazard ratio [HR] 0.53, 95% confidence interval (CI) = 0.38–0.73; $p < 0.0001$) and GVHD prophylaxis (reduced MTX; HR 0.67, 95% CI 0.50–0.89; $p = 0.005$, MMF; HR 0.64, 95% CI 0.47–0.86; $p = 0.003$). Platelet engraftment was associated with good PS (HR 0.41, 95% CI 0.27–0.62; $p < 0.0001$), CR (HR 0.69, 95% CI 0.54–0.88; $p = 0.003$), and number of $CD34^+$ cells in UCB (HR 0.74, 95% CI 0.57–0.94; $p = 0.02$). OS was associated with younger age (HR 0.65, 95% CI 0.50–0.85; $p = 0.001$), good PS (HR 0.44, 95% CI 0.33–0.60; $p < 0.0001$), and CR (HR 0.49, 95% CI 0.37–0.65; $p < 0.0001$) (Table 4). Regarding GVHD prophylaxis, conv-MTX/CIs demonstrated a clear disadvantage for neutrophil engraftment compared with reduced-MTX/CIs and MMF/CIs by multivariate analyses. In addition, we analyzed factors associated with neutrophil and platelet engraftment and OS in each three groups. As shown in Table 5, neutrophil recovery was associated with good PS in conv-MTX/CI and reduced-MTX/CI groups, but younger age in MMF/CI group. In contrast, platelet recovery was associated with CR status in all three groups, but not associated with infused number of MNC.

Discussion

In the current study, the cumulative incidence of neutrophil engraftment was significantly higher in the reduced-MTX/CI and the MMF/CI groups than that in the conv-MTX/CI group, although there were no significant differences in platelet engraftment or infectious complications. There were also no significant differences in the incidence and severity of GVHD among the three groups. Reducing methotrexate doses

Table 3 Univariate analysis for conditioning, HLA compatibility, and infused number of MNC associated with neutrophil and platelet engraftment among each three different GVHD prophylaxis groups

	Conv-MTX $n = 77$			Reduced-MTX $n = 209$			MMF $n = 123$		
Variable	n	Percent (95% CI)	p value	n	Percent (95% CI)	p value	n	Percent (95% CI)	p value
Neutrophil engraftment									
Conditioning			0.74			0.17			0.22
MAC	45	71.1 (55.1–82.3)		83	78.5 (67.8–86.0)		15	93.3 (41.5–99.5)	
RIC	32	71.9 (51.3–84.9)		126	84.9 (77.3–90.1)		108	85.7 (77.0–91.3)	
HLA compatibility			0.4			0.83			0.4
Full match	11	54.5 (18.3–80.6)		23	78.3 (53.0–91.0)		14	92.9 (26.8–99.6)	
Mismatch in one locus	20	75.0 (47.5–89.5)		51	82.4 (68.3–90.6)		42	81.0 (64.6–90.3)	
Mismatch in two or more loci	46	73.9 (58.2–84.5)		130	82.3 (74.6–87.9)		65	87.7 (76.3–93.8)	
Number of MNC in UCB			0.54			0.35			0.85
$<2 \times 10^7$/kg	9	66.7 (20.5–90.1)		15	86.7 (50.7–97.0)		10	80.0 (31.1–95.8)	
$\geq 2 \times 10^7$/kg	68	72.1 (59.6–81.3)		194	81.4 (75.3–86.2)		112	87.0 (78.8–92.2)	
Platelet engraftment									
Conditioning			0.71			0.37			0.42
MAC	45	70.1 (52.6–82.2)		83	56.3 (44.5–66.5)		15	80.0 (44.1–94.1)	
RIC	32	68.8 (48.5–82.3)		126	62.7 (53.4–70.6)		108	69.8 (59.5–77.9)	
HLA compatibility			0.65			0.19			0.53
Full match	11	63.6 (10.1–91.5)		23	78.0 (49.8–91.5)		14	57.1 (25.5–79.5)	
Mismatch in one locus	20	73.3 (44.2–88.9)		51	58.8 (43.2–71.4)		42	71.0 (53.7–82.8)	
Mismatch in two or more loci	46	70.3 (53.8–81.8)		130	56.1 (47.0–64.3)		65	74.8 (60.8–84.4)	
Number of MNC in UCB			0.18			0.75			0.81
$< 2 \times 10^7$/kg	9	50.0 (10.9–80.5)		15	64.4 (31.9–84.5)		10	70.0 (25.8–91.0)	
$\geq 2 \times 10^7$/kg	68	72.2 (59.1–81.7)		194	59.9 (52.5–66.5)		112	70.9 (60.9–78.7)	

Table 4 Multivariate analysis for factors associated with neutrophil and platelet engraftment and overall survival in all recipients

Variable	HR	Percent (95% CI)	*p* value
Neutrophil engraftment			
PS < 2	0.53	0.38–0.73	*< 0.0001*
GVHD prophylaxis			
Conv-MTX/CIs	1		
reduced-MTX/CIs	0.67	0.50–0.89	*0.005*
MMF/CIs	0.64	0.47–0.86	*0.003*
Platelet engraftment			
PS < 2	0.41	0.27–0.62	*< 0.0001*
Disease status (CR)	0.69	0.54–0.88	*0.003*
Number of CD34+ cells in UCB (≥ 1 × 105/kg)	0.74	0.57–0.94	*0.02*
OS			
Age < 60 years	0.65	0.50–0.85	*0.001*
PS < 2	0.44	0.33–0.60	*< 0.0001*
Disease status (CR)	0.49	0.37–0.65	*< 0.0001*

Abbreviation: *HR*, hazard ratio; *CI*, confidence interval; *PS*, performance status

is predicted to accelerate neutrophil and platelet engraftment but exaggerate acute GVHD. One possible explanation for our findings might be that the lower dose of methotrexate might relieve severe mucosal damage and cytotoxic injury, leading to attenuated production of inflammatory cytokines from the damaged tissues. Thus, despite reduced-dose methotrexate administration, acute GVHD might not be exacerbated with the reduced-MTX/CI regimen compared with the conv-MTX/CI regimen [8–11, 23–25]. Unfortunately, the current study lacked data related to mucosal damage in the three groups. It is necessary to evaluate the specific relationship between GVHD and mucosal damage by comparing the conv-MTX/CI and the reduced-MTX/CI groups in future.

Table 5 Multivariate analysis for factors associated with neutrophil and platelet engraftment and overall survival among each three groups

Variable	HR	Percent (95% CI)	*p* value
Conv-MTX			
Neutrophil engraftment			
PS < 2	0.09	0.013-0.66	0.018
Platelet engraftment			
Disease status (CR)	0.51	0.3–0.86	0.011
OS			
PS < 2	0.06	0.02-0.17	< 0.0001
Disease status (CR)	0.41	0.2–0.86	0.017
Reduced-MTX			
Neutrophil engraftment			
PS < 2	0.44	0.28-0.67	0.0002
Platelet engraftment			
PS < 2	0.22	0.098–0.53	0.0005
Disease status (CR)	0.65	0.44–0.95	0.027
OS			
PS <2	0.43	0.27–0.67	0.0002
Disease status (CR)	0.45	0.30–0.69	0.0002
MMF			
Neutrophil engraftment			
Age < 60 years	0.62	0.42-0.93	0.02
Platelet engraftment			
Disease status (CR)	0.59	0.38–0.91	0.016
OS			
Age < 60 years	0.58	0.35–0.97	0.037
Disease status (CR)	0.44	0.26–0.74	0.0002

Several clinical studies were conducted to establish optimal GVHD prophylaxis for UCBT as an alternative to methotrexate with the aim to enhance engraftment and avoid mucosal and renal toxicity; however, no apparent consensus has yet been reached [24–30]. Table 6 summarizes representative results on GVHD prophylaxis in UCBT from several publications [4, 12, 14, 27–29, 31, 32]. In several institutions in the USA and Europe, anti-thymocyte globulin has been incorporated into GVHD prophylaxis for UCBT to reduce graft engraftment failure or GVHD [28, 31, 33, 34], although its use remains controversial due to delayed immune reconstitution, risk of graft rejection, or Epstein-Barr virus-associated lymphoproliferative disease [35–38]. Considering the disadvantage of ATG, MMF has also been preferentially used instead of methotrexate to enhance engraftment when used in combination with CIs [12, 14, 25, 39]. However, its utility has not yet been accepted widely as a standard for acute GVHD prophylaxis, because previous studies failed to clearly demonstrate that MMF/CIs provided advantages of faster engraftment and less adverse events with equivalent incidence and severity of acute GVHD compared to methotrexate prophylaxis [4, 12, 14, 32, 39–42]. Thus, instead of MMF, several studies assessed whether reducing the dose of methotrexate could compensate for several disadvantages while preserving

Table 6 Summary of previous reports of GVHD prophylaxis in UCBT

Group	Journal (Reference)	GVHD prophylaxis (*n*)	ATG in conditioning	Neutrophil engraftment, % (day)	Platelet engraftment, % (day)	NRM (%)	Acute GVHD II–IV (%)	Acute GVHD III–IV (%)
CIs only								
Nagoya university group	Bone Marrow Transplant 2007 (4)	CSP only (23) TAC only (12)	None	70 (NA)	40 (NA)	53 (day 100)	28	NA
Toranomon hospital	Transplantation 2011 (12)	TAC only (29)	None	69 (19)	52 (40)	35 (day 100)	50	40
CIs/PSL								
University of Minnesota	Blood 2002 (30)	CSP/PSL (100) CSP/MTX (2)	None	88 (23)	65 (86)	30 (1 year)	39	11
GETH	Bone Marrow Transplant 2012 (32)	CSP/PSL (88)	Rabbit ATG	94 (19)	80 (44)	14 (day 100)	24	13
CIs/MTX								
Nagoya university group	Bone Marrow Transplant 2007 (4)	CIs/MTX (stand-MTX, conv-MTX) (40)	None	72 (NA)	40 (NA)	52 (day 100)	17	NA
JSHCT	Bone Marrow Transplant 2017 (28)	CIs/MTX a (446)	None	66.8 (25)	51.4 (45.5)	NA	24.9	7.3
JSHCT	Bone Marrow Transplant 2017 (40)	CSP/MTX a (824) TAC MTX a (554)	None	79.5 (22) 82.1 (22)	65.1 (47) 67.3 (49)	NA NA	38.1 28.1	8.9 5.8
Showa university	Leuk Res 2016 (33)	TAC/reduced-MTX b (40)	None	92.5 (21)	75 (NA)	12.5 (day 100)	48.6	10.8
Present study		CIs/conv-MTX (77) CIs/reduced-MTX c (209)	None	71.4 (20) 82.3 (19)	69.5 (39) 60.1 (37)	15.6 (day 100) 25.8 (day 100)	44.2 48.3	8.7 18.2
CIs/MMF								
University of Minnesota	Blood 2007 (29)	CSP/MMF (110)	Horse ATG	92 (12)	65 (49)	19 (day 180)	59	22
Toranomon hospital	Transplantation 2011 (12)	TAC/MMF (29)	None	90 (19)	59 (40)	21 (day 100)	66.7	40.7
FBMTG	Int J Hematol 2017 (14)	CSP/MMF (17) TAC/MMF (18)	None None	94 (18) 89 (19)	59 (81) 89 (41)	18 (day 100) 11 (day 100)	53 28	35 11
JSHCT	Bone Marrow Transplant 2017 (28)	CIs/MMF (302)	None	81.7 (21.5)	60.3 (45.5)	NA	36.3	13.4
JSHCT	Bone Marrow Transplant 2017 (40)	TAC/MMF (138)	None	83.3 (20)	61.7 (54)	NA	54.9	16.1
Present study		CIs/MMF (123)	None	86.6 (19)	71.3 (42)	20.3 (day 100)	43.3	11.8

Abbreviations: *ATG*, anti-thymocyte globulin; *NA*, not applicable; *CSP*, cyclosporine; *TAC*, tacrolimus; *GETH*, Grupo Espenol de Trasplante Hematopoyetico Terapia Celulary; *PSL*, prednisolone; *CIs*, calcineurin inhibitors; *JSHCT*, Japanese Society of Hematopoietic Cell Transplantation; *FBMTG*, Fukuoka Blood and Marrow Transplantation Group; *stand-MTX*, standard MTX (15, 10, and 10 mg/m^2 on days 1, 3, and 6); *conv-MTX*, conventional MTX (10, 7, and 7 mg/m^2 on days 1, 3, and 6, respectively)

[a] Schedule of MTX administration was not shown

[b] MTX 10 on day 1 and 7 mg/m^2 on day 3 only

[c] MTX 5 mg/m^2 on days 1,3, and 6 (n = 70), or MTX 5 mg/body on days 1, 3, and 6 (n = 139)

the ability to prevent GVHD [6, 23, 32]. Saito et al. from Showa University reported that lower-dose methotrexate (10 and 7 mg/m^2 on days 1 and 3, respectively) accelerated neutrophil engraftment without highly exaggerating acute GVHD in 40 UCBT recipients, which was consistent with our results [32]. However, these previous studies were also retrospective and might have been affected by biased data.

In the multivariate analyses in three different GVHD prophylaxis groups, MMF group did not show the disadvantage of neutrophil engraftment, platelet engraftment, and OS by worse PS. There might be some reasons to overcome the disadvantage

Because the number of patients in each group of GVHD prophylaxis was limited, we cannot exclude that some of the "non-significant" p values described might be related to insufficient power of the analyses. As this is a retrospective study, the results obtained must be carefully interpreted.

In conclusion, the current retrospective study revealed that lower-dose methotrexate was superior in granulocyte engraftment compared with the conventional-dose methotrexate and was associated with equivalent rates of acute GVHD, OS, and PFS in UCBT recipients. Future, large prospective randomized trials are necessary to further clarify the efficacy of lower-dose methotrexate in comparison with conventional-dose methotrexate and MMF, when used in combination with CIs for GVHD prophylaxis in UCBT.

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Compliance with ethical standards

The ethics committees of the participating institutions approved this study (Kyushu University Graduate School and Faculty of Medicine; approval nos. 23027-1 and 23027-2).

Conflict of interest The authors declare that they have no conflict of interest.

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