

ORIGINAL ARTICLE

Short-term methotrexate could reduce early immune reactions and improve outcomes in umbilical cord blood transplantation for adults

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Post transplant immune disorders are problematic in cord blood transplantation (CBT) for adult patients, and optimal prophylaxis has not been established. We investigated whether intensive graft-versus-host disease (GVHD) prophylaxis using short-term methotrexate (MTX) has a prognostic impact on CBT. Post-CBT immune reactions were classified according to time course as pre-engraftment immune reaction (PIR), engraftment syndrome (ES) or acute GVHD. Between March 2001 and November 2005, a total of 77 patients underwent CBT at eight transplantation centers. Median age was 48 years (range, 18–69 years). Preparative regimens comprised myeloablative ($n=31$) or reduced-intensity ($n=46$). Acute GVHD prophylaxis included cyclosporine alone ($n=23$), tacrolimus alone ($n=12$), cyclosporine plus MTX ($n=17$), tacrolimus plus short-term MTX ($n=23$) or cyclosporine plus methylprednisolone ($n=2$). Cumulative incidences of PIR, ES and grade II–IV GVHD were 36, 12 and 23%, respectively. Short-term MTX exerted significant favorable effects on post-CBT immune reactions (hazard ratio, 0.55; 95% confidence interval (95% CI), 0.31–0.98; $P=0.04$) in multivariate analysis. Overall survival rates for patients with and without short-term MTX at day 180 were 59% (95% CI, 42–73%) and 16% (95% CI, 6.6–30%) ($P=0.0001$), respectively. Short-term MTX could offer one optimal regimen to reduce immune reactions and improve outcomes in CBT.

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Introduction

Cord blood transplantation (CBT) represents an attractive alternative for patients with advanced hematological malignancy who lack matched related or unrelated donors. Adult patients receiving myeloablative or reduced-intensity CBT display a 90% chance of engraftment, but also experience a 50% rate of transplant-related mortality, mostly attributable to infection.^{1–6}

Unique manifestations of immune reactions that differ from those seen in conventional allogeneic stem cell transplantation (allo-SCT) may occur after CBT. The incidence and severity of acute graft-versus-host disease (GVHD) after unrelated CBT are low compared with those after allo-SCT from a matched unrelated or mismatched family donor, despite infusion of human leukocyte antigen (HLA)-mismatched graft.^{7,8} Several groups have reported that adult CBT recipients often develop acute GVHD before engraftment.^{3,9} Recently, a research group from Japan classified post-CBT immune reactions according to time course, as: pre-engraftment immune reactions (PIR); engraftment syndrome (ES); and acute GVHD (post-engraftment immune reaction).¹⁰ PIR is characterized by high-grade fever and weight gain and develops at a median of day 9 in approximately 80% patients.¹⁰

Post transplant immune disorders including early immune reactions and acute GVHD are problematic in CBT for adult patients.^{1,3,10} Such reactions and/or additional immunosuppressive therapy might increase the risk of infection and organ dysfunction, leading to high rates of transplantation-related mortality. However, optimal

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prophylaxis and management for immune reactions have not been established in CBT. Intense immunosuppressive GVHD prophylaxis and/or additional immunosuppressive therapy might reduce the incidence and severity of early immune reactions and acute GVHD. Conversely, donor immune cell function in the graft might be suppressed, increasing the risk of graft failure.¹¹

Methotrexate (MTX) has been demonstrated as an effective GVHD prophylactic drug in combination with calcineurin inhibitor in bone marrow transplantation or peripheral blood stem cell transplantation.¹² However, the effectiveness of MTX in CBT remains unclear, as cyclosporine alone or cyclosporine with corticosteroid have been commonly used for GVHD prophylaxis in CBT for adult patients.^{1,3,5,6,13} The present study therefore investigated whether intense GVHD prophylaxis with short-term MTX has any prognostic impact.

Patients and methods

Study patients

Between March 2001 and November 2005, a total of 77 patients underwent CBT at eight centers of the Nagoya Blood and Marrow Transplantation Group (NBMTG). Transplantation procedures are shown in Table 1. All patients presented with hematological disorders that were

Table 1 CBT patient characteristics

Variables	Number
<i>Age (years)</i>	
Median, range	48 (18–69)
<i>Sex</i>	
Male/female	31/46
<i>Primary disease</i>	
Acute myeloid leukemia	30
Malignant lymphoma	11
Acute lymphoblastic leukemia	8
Chronic myelogenous leukemia	7
Adult T-cell leukemia	7
Myelodysplastic syndrome	6
Aplastic anemia	3
Multiple myeloma	3
Chronic lymphocytic leukemia	1
Chronic active EB-virus infection	1
<i>Risk of underlying disease</i>	
High/low	51/26
Blood-type mismatch (match/mismatch)	21/56
<i>Previous transplant</i>	
Autologous/allogeneic	8/6
<i>Preparative regimen</i>	
<i>Myeloablative regimen</i>	
CY 120 mg/m ² + TBI 8–12 Gy	9
BU 8 mg/kg + L-PAM 120–180 mg/m ² + TBI 10 Gy	7
L-PAM 180 mg/m ² + TBI 8–10 Gy	6
BU 8–16 mg/kg + CY 120 mg/kg + TBI 7.5–10 Gy	5
BU 16 mg/kg + CY 120 mg/kg	3
CA 2 g/m ² + CY 120 mg/kg + TBI 12 Gy	1
<i>Reduced-intensity regimen</i>	
Flud 125–150 mg/m ² + L-PAM 80–140 mg/m ² + TBI 2–4 Gy	15

Table 1 Continued

Variables	Number
Flud 125 mg/m ² + L-PAM 60–180 mg/m ² + BU 4–8 mg/kg	11
Flud 125 mg/m ² + L-PAM 140–180 mg/m ²	8
Flud 125–150 mg/m ² + CY 25–60 mg/kg + TBI 2–4 Gy	6
Flud 180 mg/kg + BU 8 mg/kg	3
Flud 125 mg/m ² + CY 30 mg/kg	1
Flud 180 mg/m ² + thio-tepa 10 mg/kg + ATG 5 mg/kg	1
Flud 125 mg/m ² + VP-16 1800 mg/m ² + L-PAM 140 mg/m ² + CY 120 mg/kg	1
<i>Number of infused nuclear cells</i>	
Median (range), 10 ⁷ /kg	2.50 (1.07–3.89)
<i>Number of infused CD34+ cells</i>	
Median (range), 10 ⁶ /kg	0.11 (0.02–1.36)
<i>HLA matching</i>	
6/6	8
5/6	37
4/6	32
<i>GVHD prophylaxis</i>	
Cyclosporine alone	23
Tacrolimus alone	12
Cyclosporine + short-term MTX	17 ^a
Tacrolimus + short-term MTX	23 ^b
Tacrolimus + mPSL 1 mg/kg	2

Abbreviations: ATG = antithymocyte globulin; BU = busulfan; CA = cytarabine; CY = cyclophosphamide; EB = Epstein–Barr; Flud = fludarabine; GVHD = graft-versus-host disease; HLA = human leukocyte antigen; L-PAM = melphalan; mPSL = methylprednisolone; MTX = methotrexate; TBI = total body irradiation; VP-16 = etoposide.

Acute leukemia in complete remission, chronic myelogenous leukemia in chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anemia (RA), and aplastic anemia were defined as low risk. All other conditions were defined as high risk.

^aPatients received 10, 7 and 7 mg/m² of MTX on days 1, 3 and 6, respectively.

^bMTX was given at doses of 15, 10 and 10 mg/m² on days 1, 3 and 6 ($n=7$), at doses of 10, 7 and 7 mg/m² on days 1, 3 and 7 ($n=15$) and at doses of 6 and 4 mg/m² on days 1 and 3 ($n=1$), respectively.

incurable using conventional treatments, and were considered unsuitable for conventional allo-SCT owing to a lack of HLA-identical siblings or suitable unrelated donor. Patients > 50 years old and/or with organ dysfunction received reduced-intensity cord blood transplant (RI-CBT). Acute leukemia in complete remission, chronic myelogenous leukemia in chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anemia (RA) and aplastic anemia were defined as low risk, whereas all other conditions were considered high risk. All patients provided informed consent to undergo CBT.

Transplantation procedures

Characteristics of cord blood units and preparative regimens are shown in Table 1. Cord blood units were not depleted of T-lymphocytes. Purine analog-based regimens were defined as reduced-intensity preparative regimens.

Acute GVHD were graded according to established criteria.^{14,15} GVHD prophylaxis involved continuous infusion of cyclosporine 3 mg/kg or tacrolimus 0.03 mg/kg from day -1 until the patient tolerated oral administration with or without short-term MTX (Table 1). Dosage and schedule of short-term MTX are shown in Table 1. Patients with grade II–IV acute GVHD were given 0.5–2.0 mg/kg/day of prednisolone or methylprednisolone. Treatment of immune reactions other than GVHD was at the discretion of the physician.

Analysis of chimerism

Chimerism was assessed using fluorescent *in situ* hybridization in sex-mismatched donor–recipient pairs. In sex-matched pairs, polymerase chain reaction for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10%.¹⁶ Whole blood CD3-positive cells or bone marrow cells were assessed for chimerism at the time of granulocyte engraftment.

Definition of engraftment and immune reactions

Neutrophil engraftment was defined as absolute neutrophil count $>0.5 \times 10^9/l$ for 3 consecutive days for neutrophil recovery. Platelet engraftment was defined as $20 \times 10^9/l$ without platelet transfusion. Graft failure was defined as previously described:¹⁷ the combination of peripheral cytopenia and marrow hypoplasia for >60 days of CBT with the existence of donor-type hematopoiesis (mixed or complete donor chimerism) or complete loss of donor-type hematopoiesis occurring anytime after transplantation. Peripheral cytopenia was defined as absolute neutrophil count $<500/\mu l$ and platelets $<20 \times 10^3/ml$. Relapse or progression of underlying disease was evaluated hematologically. Non-relapse mortality (NRM) was defined as any death without progression of underlying disease.

Immune reactions were defined as described previously.¹⁰ When febrile patients (body temperature $\geq 38^\circ C$) with no evidence of infection or adverse effects of medication exhibited skin eruption, diarrhea, jaundice (serum total bilirubin $>2.0 mg/dl$) or body weight gain $>10\%$ of baseline, these changes were defined as immune reactions. Reactions were classified into subtypes of pre-, peri- and post-engraftment according to timing. Immune reactions developing ≥ 6 days before engraftment were defined as PIR, whereas reactions within 5 days of engraftment were defined as ES. Other reactions were defined as post-engraftment syndrome, generally corresponding to acute GVHD. In the treatment of PIR, ES and acute GVHD, response to corticosteroid was evaluated as reported previously.¹⁸

Data collection and categorization of immune reactions

Medical records of the 77 patients were retrospectively reviewed. Immune reactions were retrospectively categorized according to the definitions¹⁰ by experienced physicians based on clinical presentation (such as skin eruptions and weight gain), laboratory results and comorbid events.

End points and statistical analysis

The primary end point of this study was to investigate whether intense GVHD prophylaxis has any prognostic impact on CBT. The secondary end point was to investigate the clinical characteristics of immune reactions.

Overall survival (OS) rate was assessed using the Kaplan–Meier product limit method. Cumulative incidences of engraftment, acute GVHD, NRM, PIR, ES and disease progression were assessed using methods described elsewhere to eliminate the effect of competing risk.¹⁹ The competing event in cumulative incidence analyses was defined as death without event of interest. Relapse or progression of underlying disease was considered as competing risks in analysis of cumulative incidence of NRM. Gray's test was applied to assess the impact of the factor of interest when appropriate. Uni- and multivariate proportional hazard modeling of subdistribution functions in competing risks were applied to assess the impact of potential prognostic factors.²⁰ A multivariate model was built with forward stepwise methods using threshold *P*-values for removal and addition to the model of 0.10. Values of $P < 0.05$ were considered statistically significant. The impact of OS was assessed by uni- and multivariate Cox proportional hazard modeling. All analyses were conducted using STATA version 8.2 software (STATA Corp., College Station, TX, USA) and R version 2.3.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Characteristics of the 77 patients are shown in Table 1. Preparative regimen comprised myeloablative CBT ($n = 31$) or RI-CBT ($n = 46$). All patients received a single cord blood unit. One of the 77 patients received pre-transplant antithymocyte globulin (ATG). Median age of patients was 38 years (range, 18–64 years) for myeloablative CBT and 57 years (range, 22–69 years) for RI-CBT. As of February 2006, median follow-up of surviving patients was 17.1 months (range, 3.5–59.2 months).

Engraftment, chimerism analysis and OS

Primary disease relapsed or progressed before primary neutrophil engraftment in one patient. Of the 76 patients, neutrophil engraftment was achieved in 54 patients. Median time to neutrophil engraftment was 21 days (range, 10–69 days). Cumulative incidence of neutrophil engraftment at day 100 was 70% (95% confidence interval (95% CI), 60–80%). Death occurred before primary engraftment in 14 patients at a median of day 18 (range, day 3–32). Graft failure developed in nine patients at a median of 45 days (range, 26–60 days). Cumulative incidence of platelet engraftment at day 100 was 40% (95% CI, 29–51%). In univariate analysis, short-term MTX did not represent a significant prognostic factor for neutrophil engraftment (hazard ratio, 0.78; 95% CI, 0.45–1.32; $P = 0.35$).

In myeloablative CBT, cumulative incidences of neutrophil engraftment at day 100 in patients with and without short-term MTX were 68% (95% CI, 46–91%) and 70%

Table 2 Clinical impact of short-term MTX

Variables	Patients with MTX	Patients without MTX	P-value
Number of patients	40 ^a	37 ^b	
Age (years): median (range)	38 (18–66)	52 (27–69)	<0.0001
Risk of underlying disease (high/low)	23/17	28/9	0.15
Preparative regimens (myeloablative/reduced-intensity)	21/19	10/27	0.036
<i>HLA matching</i>			
6/6	3	5	0.045
5/6	15	22	
4/6	22	10	
Blood-type mismatch (match/mismatch)	12/28	9/28	0.62
Previous transplant ^c	5	9	0.24
Cumulative incidence of neutrophil engraftment at day 100 (95% CI)	70% (95% CI, 56–85%)	72% (95% CI, 57–87%)	0.33
Cumulative incidence of PIR at day 30	25% (95% CI, 11–39%)	49% (95% CI, 32–65%)	0.015
Cumulative incidence of ES at day 60	10% (95% CI, 1–19%)	14% (95% CI, 2–25%)	0.65
Cumulative incidence of grade II–IV acute GVHD at day 100	17% (95% CI, 4.2–29%)	28% (95% CI, 11–44%)	0.37
Cumulative incidence of post-CBT immune reactions ^d at day 100	48% (95% CI, 32–63%)	65% (95% CI, 49–81%)	0.042
Cumulative incidence of relapse or progression of underlying diseases at day 100	10% (95% CI, 1–19%)	22% (95% CI, 8–35%)	0.20
Cumulative incidence of non-relapse mortality at day 100	52% (95% CI, 32–72%)	53% (95% CI, 36–69%)	0.97
Patients who developed documented infection	19	18	

Abbreviations: CBT = cord blood transplantation; CI = confidence interval; ES = engraftment syndrome; GVHD = graft-versus-host disease; HLA = human leukocyte antigen; MTX = methotrexate; PIR = pre-engraftment immune reaction.

^aThose included cyclosporine + MTX ($n = 17$) and tacrolimus + MTX ($n = 23$).

^bThose included cyclosporine alone ($n = 23$) and tacrolimus alone ($n = 12$) and tacrolimus + methylprednisolone ($n = 2$).

^cPrevious transplant included autologous and allogeneic hematopoietic stem cell transplantation.

^dThose included PIR, ES and grade II–IV acute GVHD.

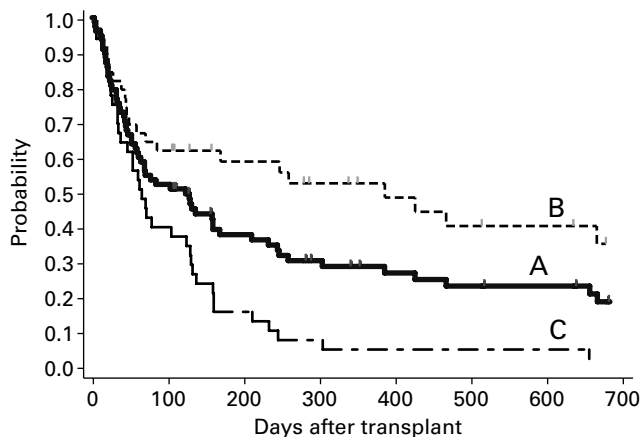


Figure 1 OS rate. OS for the 77 patients was 52% (95% CI, 40–62%) and 38% (95% CI, 27–48%) at day 100 and day 180, respectively (A). OS for patients with short-term MTX (B) and without short-term MTX (C) at day 180 was 59% (95% CI, 42–73%) and 16% (95% CI, 6.6–30%) (log rank, $P = 0.0001$), respectively.

(95% CI, 53–88%) (Gray's test, $P = 0.44$), respectively. In RI-CBT, cumulative incidences of neutrophil engraftment at day 100 in patients with and without short-term MTX were 71% (95% CI, 51–92%) and 70% (95% CI, 38–100%) (Gray's test, $P = 0.50$), respectively.

Chimerism data were obtained from 23 patients. Cumulative incidence of complete donor chimerism at day 60 was 63% (95% CI, 42–83%), and median time to complete donor chimerism was 28 days (range, 14–42 days).

Cumulative incidences of relapse or progression of underlying disease were 9.1% (95% CI, 3.0–16%) at day 50 and 16% (95% CI, 4.5–27%) at day 100. Cumulative

incidences in patients with and without short-term MTX are shown in Table 2. In univariate analysis, short-term MTX was not a significant prognostic factor for relapse or progression of underlying disease (hazard ratio, 0.59; 95% CI, 0.27–2.43; $P = 0.21$).

OS for the 77 patients was 52% (95% CI, 40–62%) and 38% (95% CI, 27–48%) at day 100 and day 180, respectively (Figure 1). OS for patients with and without short-term MTX at day 180 was 59% (95% CI, 42–73%) and 16% (95% CI, 6.6–30%) (log rank, $P = 0.0001$), respectively (Figure 1). Prognostic factors for OS are shown in Table 3. In multivariate analysis, age, blood-type mismatch and short-term MTX were identified as significant prognostic factors for OS.

Post-CBT immune reactions

Cumulative incidence of post-CBT immune reactions, which included PIR, ES and grade II–IV acute GVHD at day 100, was 56% (95% CI, 45–67%). Prognostic factors for post-CBT immune reactions are shown in Table 4. In uni- and multivariate analysis, short-term MTX was a significant prognostic factor (hazard ratio, 0.55; 95% CI, 0.31–0.98; $P = 0.04$) (Table 4). Cumulative incidences of post-CBT immune reactions between patients with and without short-term MTX differed significantly (Gray's test, $P = 0.042$) (Table 2 and Figure 2).

Pre-engraftment immune reaction

PIR developed in 28 patients at a median of 6.5 days (range, 2–18 days). PIR involved the skin ($n = 20$), liver ($n = 6$) and gut ($n = 10$). PIR was observed in eight patients who had never engrafted. Cumulative incidence of PIR at day 30 was 38% (95% CI, 27–49%) (Figure 3). Of these 28

Table 3 Prognostic factors of OS

Univariate factors	HR	95% CI	P-value
Age (years)	1.03	1.01–1.05	0.002
Sex (female vs male)	1.23	0.73–2.08	0.44
Risk of underlying diseases (high vs low)	1.56	0.88–2.76	0.12
Previous transplant ^a	1.21	0.62–2.36	0.58
Blood-type mismatch	0.44	0.25–0.77	0.004
Conditioning regimen (reduced-intensity vs myeloablative)	1.40	0.82–2.40	0.22
GVHD prophylaxis (with short-term MTX vs without short-term MTX)	0.34	0.19–0.58	<0.001
Number of infused nuclear cells ($>2 \times 10^7$ vs $\leq 2 \times 10^7$ /kg)	0.95	0.46–1.94	0.89
HLA matching (4/6 vs 5–6/6)	0.68	0.40–1.17	0.17
<i>Step-wise multivariate factors</i>			
Age (years)	1.02	1.00–1.04	0.038
Blood-type mismatch	0.37	0.21–0.65	0.001
GVHD prophylaxis (with short-term MTX vs without short-term MTX)	0.38	0.21–0.69	0.001

Abbreviations: 95% CI=95% confidence interval; GVHD=graft-versus-host disease; HLA=human leukocyte antigen; HR=hazard ratio; MTX=methotrexate; OS=overall survival; TBI=total body irradiation.

^aPrevious transplant included autologous and allogeneic hematopoietic stem cell transplantation.

Table 4 Prognostic factors of post-CBT immune reactions^a

Univariate factors	HR	95% CI	P-value
Age (years)	1.00	0.98–1.01	0.69
Sex (female vs male)	1.26	0.70–2.26	0.44
Risk of underlying diseases (high vs low)	0.73	0.40–1.35	0.32
Previous transplant ^b	1.13	0.52–2.48	0.75
Blood-type mismatch	1.00	0.50–2.03	0.99
Conditioning regimen (reduced-intensity vs myeloablative)	0.72	0.40–1.28	0.26
GVHD prophylaxis (with short-term MTX vs without short-term MTX)	0.55	0.31–0.98	0.04
Number of infused nuclear cells ($>2 \times 10^7$ vs $\leq 2 \times 10^7$ /kg)	0.89	0.40–1.96	0.77
HLA matching (4/6 vs 5–6/6)	0.87	0.48–1.56	0.63
<i>Step-wise multivariate factor</i>			
GVHD prophylaxis (with short-term MTX vs without short-term MTX)	0.55	0.31–0.98	0.04

Abbreviations: CBT=cord blood transplantation; 95% CI=95% confidence interval; GVHD=graft-versus-host disease; HLA=human leukocyte antigen; HR=hazard ratio; MTX=methotrexate; TBI=total body irradiation.

^aThose included PIR, ES and grade II–IV acute GVHD.

^bPrevious transplant included autologous and allogeneic hematopoietic stem cell transplantation.

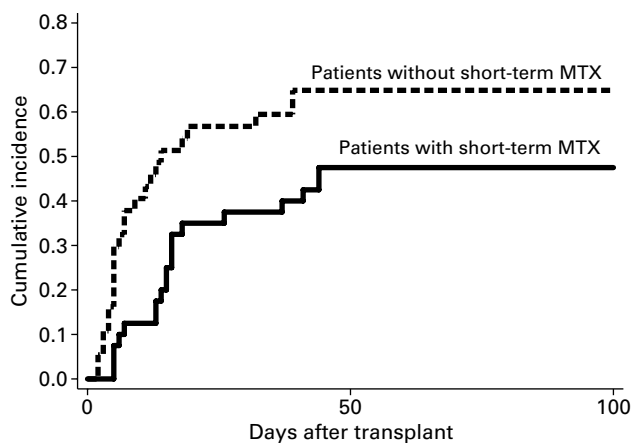


Figure 2 Cumulative incidences of post-CBT immune reactions in patients with or without short-term MTX. Cumulative incidences post-CBT immune reactions in patients with or without short-term MTX were 48% (95% CI, 32–63%) and 65% (95% CI, 49–81%) (Gray's test, $P=0.042$), respectively.

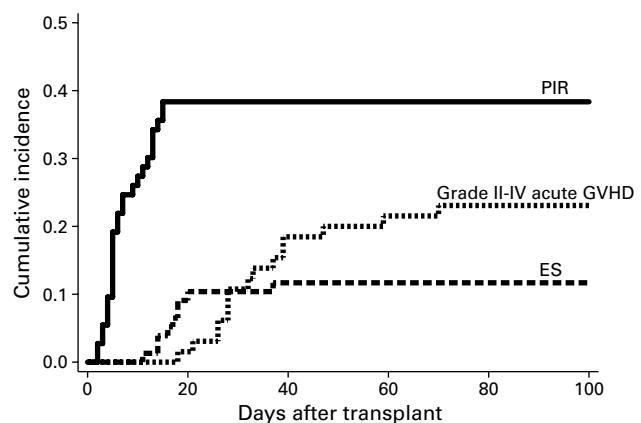


Figure 3 Cumulative incidences of immune reactions. PIR, pre-engraftment immune reaction; ES, engraftment syndrome; GVHD, graft-versus-host disease. Cumulative incidence of PIR at day 30 was 38% (95% CI, 27–49%). Cumulative incidence of ES at day 60 was 12% (95% CI, 4.5–19%). Cumulative incidence of grade II–IV GVHD was 23% (95% CI, 13–33%) at day 100.

Table 5 Prognostic factors for NRM

Univariate factors	HR	95% CI	P-value
Age (years)	1.01	0.99–1.03	0.48
Sex (female vs male)	1.28	0.65–2.53	0.48
Risk of underlying disease (high vs low)	1.16	0.57–2.38	0.69
Previous transplant ^a	0.20	0.05–0.85	0.029
Blood-type mismatch	0.33	0.16–0.69	0.003
Conditioning regimen (reduced-intensity vs myeloablative)	1.02	0.51–2.05	0.96
GVHD prophylaxis (with short-term MTX vs without short-term MTX)	0.99	0.49–1.98	0.97
Number of infused nuclear cells ($>2 \times 10^7$ vs $\leq 2 \times 10^7$ /kg)	1.53	0.67–3.50	0.31
HLA matching (4/6 vs 5–6/6)	1.59	0.80–3.13	0.18
<i>Stepwise multivariate factors</i>			
Previous transplant	0.22	0.05–0.85	0.029
Blood-type mismatch	0.35	0.16–0.76	0.008

Abbreviations: 95% CI=95% confidence interval; GVHD=graft-versus-host disease; HLA=human leukocyte antigen; HR=hazard ratio; MTX=methotrexate; NRM=non-relapse mortality; TBI=total body irradiation.

^aPrevious transplant included autologous and allogeneic hematopoietic stem cell transplantation.

patients, 14 received ≥ 0.5 mg/kg of prednisolone or ≥ 0.4 mg/kg of methylprednisolone, and 13 of the 14 patients responded to corticosteroid therapy. PIR subsided spontaneously in the remaining 14 patients. Cumulative incidences of PIR at day 30 were significantly lower in patients with short-term MTX than in those without short-term MTX (Gray's test, $P=0.015$) (Table 2). Short-term MTX was a significant favorable factor for PIR (hazard ratio, 0.40; 95% CI, 0.19–0.83; $P=0.02$) in multivariate analysis.

Engraftment syndrome

Of the 54 patients who achieved engraftment, ES developed in nine patients. Cumulative incidence of ES at day 60 was 12% (95% CI, 4.5–19%) (Figure 3), ES involved the skin ($n=6$), gut ($n=3$) and liver ($n=2$). Two patients received ≥ 0.5 mg/kg of prednisolone or ≥ 0.4 mg/kg of methylprednisolone, as with treatment for PIR. The remaining seven patients were treated supportively. ES resolved in all nine patients. Short-term MTX was not a significant risk factor for ES in univariate analysis (hazard ratio, 0.74; 95% CI, 0.20–2.71; $P=0.65$). Cumulative incidences of ES at day 60 with and without short-term MTX did not differ significantly (Gray's test, $P=0.65$) (Table 2).

Acute GVHD

Of the 53 patients who survived >6 days after engraftment, 15 patients developed acute grade II–IV GVHD, as grade II ($n=10$), III ($n=4$) or IV ($n=1$). Median time to onset was 33 days (range, 21–84 days). Cumulative incidences of grade II–IV and III–IV acute GVHD at day 100 were 23% (95% CI, 13–33%) and 7.6% (95% CI, 1.1–14%), respectively (Figure 3). Of the 12 patients treated with ≥ 0.5 mg/kg of prednisolone or ≥ 0.4 mg/kg of methylprednisolone, nine patients responded. Of those, three patients were dependent on the corticosteroid. The remaining three patients were refractory to corticosteroid treatment. Grade II–IV acute GVHD involved the skin ($n=13$), liver ($n=6$) and gut ($n=5$). Acute grade II–IV GVHD subsided spontaneously in three patients. Short-term MTX was not

a significant factor for the development of grade II–IV acute GVHD in univariate analysis (hazard ratio, 0.63; 95% CI, 0.23–1.69; $P=0.36$). Cumulative incidences of grade II–IV acute GVHD in patients with or without MTX were not significantly different (Gray's test, $P=0.37$) (Table 2).

Additional corticosteroid therapy

An additional ≥ 0.5 mg/kg of prednisolone or ≥ 0.4 mg/kg of methylprednisolone was administered to 22 patients. Cumulative incidence of additional corticosteroid use at day 100 was 27% (95% CI, 18–38%). Of the 40 patients who received short-term MTX, eight patients received additional corticosteroid therapy (20%; 95% CI, 9.1–36%). Of the 37 patients who did not receive short-term MTX, 14 received additional corticosteroid therapy (38%; 95% CI, 23–55%).

Non-relapse mortality

A total of 39 patients died without disease progression. Cumulative incidences of NRM for the 77 patients were 52% at day 100 (95% CI, 40–65%) and 52% at day 180 (95% CI, 40–65%). NRM of patients with and without short-term MTX at day 100 were not significantly different (Gray's test, $P=0.97$) (Table 2).

Causes of NRM comprised multiorgan failure ($n=9$), pneumonia ($n=9$), thrombotic microangiopathy ($n=5$), hepatic failure ($n=5$), invasive pulmonary aspergillosis ($n=3$), interstitial pneumonia ($n=2$), acute GVHD ($n=1$), sepsis ($n=1$), renal failure ($n=1$), encephalopathy ($n=1$), pulmonary hemorrhage ($n=1$) and intestinal hemorrhage ($n=1$). Prognostic factors of NRM are shown in Table 5.

Discussion

The present results demonstrated that use of short-term MTX was associated with a lower rate of post transplant immune reactions without compromising engraftment, resulting in better OS in adult CBT recipients. The

favorable impact of short-term MTX could be explained by decreased organ damage from post-CBT immune reactions and reduced risk of infectious complications caused by additional corticosteroid use. Nevertheless, short-term MTX exerted no significant effects on NRM in the present study. This finding may be explained by the increased risk of NRM from causes other than immune reaction or leukemia relapse; organ toxicities associated with prior intensive chemo- and/or radiotherapy could have increased the risk of NRM in patients with advanced hematological diseases in this study.

In contrast to our study, few studies in Western countries have described PIR after CBT.^{3–6} Most studies have included pre-transplant ATG and post transplant corticosteroid, which might obscure the manifestation of PIR. PIR was recently characterized in studies from Japan,^{1,10} which used a preparative regimen without ATG and cyclosporine alone as prophylaxis for GVHD. Incidence of PIR was higher in those studies than in the present study,^{1,10} which could be attributable to the addition of MTX in our study. Another possible explanation is that HLA disparities of cord blood units were lower in our study. PIR supposedly develops in conjunction with cytokine storm or homeostasis-driven proliferation of naive T cells.^{10,21} The immunological mechanisms could be ameliorated in CBT with low HLA disparity. However, associations between HLA disparity and risk of immune reactions remain unclear in CBT.^{9,22–25} Further studies are warranted.

Intensive GVHD prophylaxis, such as additional MTX, might suppress donor T cells and proliferative capacity of hematopoietic cells, and thereby induce risk of graft failure.¹¹ Use of MTX is reported to unfavorably affect neutrophil engraftment in related CBT for pediatric patients with hemoglobinopathies.²⁶ In the present study, however, MTX was not identified as a significant adverse factor for neutrophil engraftment both in CBT using myeloablative and reduced-intensity preparative regimen. One possible reason is that MTX may not affect primary engraftment of hematopoietic cells owing to the short half-life. Another is that MTX may alter interactions between donor lymphocytes in cord blood, facilitating engraftment and residual recipient immune cells capable of graft rejection, and thereby modulating cord blood engraftment. Alternatively, the clinical significance of MTX might differ between adult patients with advanced hematological malignancy and pediatric patients with non-malignant diseases.

In the present study, short-term MTX was not significantly associated with greater risk of relapse or progression of underlying diseases. However, physicians have expressed concern that intensive GVHD prophylaxis might increase risk of relapse or progression of underlying diseases owing to suppressed donor immune cells. A research group from MD Anderson Cancer Center reported that all patients with active underlying disease died within 200 days of transplantation in CBT with GVHD prophylaxis using tacrolimus and short-term MTX.²⁷ Intensive GVHD prophylaxis may not be feasible for patients with rapidly progressive disease at transplantation. Further studies are required to identify patients who will benefit from this regimen.

The effectiveness of tacrolimus compared to cyclosporine was unclear in this study. The incidence of immune reactions or additional steroid use was not significantly lower in patients with cyclosporine than in those with tacrolimus (data not shown). In contrast, tacrolimus exerts stronger suppression of acute GVHD than cyclosporine according to several randomized clinical studies.^{28–30} The reason for this contrast is unknown, as studies of CBT using tacrolimus for acute GVHD prophylaxis are highly limited,^{27,31} but unique manifestations of immune reactions in CBT might be involved.

Corticosteroid therapy for PIR was effective in our study, despite being reported as a risk factor for infection in allo-SCT.^{32–35} Little is known about the clinical usefulness of additional steroid use in CBT. A previous study reported that corticosteroid use was not a significant risk factor for infection in RI-CBT.³⁶ Owing to clinical severity, additional steroid for immune reactions might be inevitable. Optimal management for immune reactions in CBT requires further investigation in future.

Despite providing novel information on CBT, several limitations of the present study must be considered. First, this was a small, non-randomized, retrospective study involving patients with heterogeneous backgrounds and preparative regimens. Unrecognized bias and insufficient power to find a difference may alter the conclusion. Second, short-term MTX, which is a post transplant variable, was analyzed together with pre-transplant variables. This might also have affected the results. Third, the diagnostic criteria of PIR based on clinical findings could not exclude infection or toxicity from various drugs, including conditioning regimens. Incidence of PIR may thus have been overestimated. Fourth, we might have underestimated the incidences of ES and GVHD, as additional corticosteroid use for PIR could have suppressed those immune reactions. Finally, infused cell dose and HLA disparity are reportedly major determinants of survival.^{9,37} However, the clinical impact of those factors remains unclear in the present study. This has to be clarified in future studies.

In conclusion, we showed that intensive GVHD prophylaxis using short-term MTX might improve outcomes by reducing the risk of post-CBT immune reactions. Although the addition of MTX did not negatively affect engraftment and relapse in the present study, further prospective studies are required to clarify whether MTX is a key drug as part of prophylaxis regimens in CBT. Diagnostic criteria for early immune reactions are worth establishing. Investigation of both the pathological and clinical characteristics of those reactions in a large clinical study would help to improve our understanding of the mechanisms underlying PIR.

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Appendix

This study was conducted at the following institutions in NBMTG: Japanese Red Cross Nagoya First Hospital, Nagoya; Nagoya Daini Red Cross Hospital, Nagoya; Meitetsu Hospital, Nagoya; National Hospital Organization Nagoya Medical Center, Clinical and Research Center, Nagoya; Fujita Health University Hospital, Toyoake; JA Aichi Showa Hospital, Konan; Nagoya University Hospital, Nagoya; and Anjo Kosei Hospital, Anjo, Japan.