measured from the date of transplantation (day 0) to the date of death from any cause. Event-free survival (EFS) was evaluated in patients who survived in remission for at least 30 days after transplantation and measured from the date of transplantation to the date of relapse or death. Patients who have never achieved CR after transplant were considered to have had a recurrence on day 0. Surviving patients were censored at the time of the last follow-up. RRT was defined as any nonhematologic organ dysfunction from day 0 to day 28 and was graded according to the Bearman's criteria (*19*). Toxicities of foscarnet were evaluated using the Common Terminology Criteria for Adverse Events version 3.0 (*20*). TRM was defined as death without the primary disease progression.

Endpoints and Statistical Analysis

Data were analyzed as of December 2005. The primary end point of this study was to evaluate the feasibility of GVHD prophylaxis using tacrolimus in the setting of RI-CBT. The secondary end points were to assess the incidences of RRT, acute and chronic GVHD, infections, EFS and OS. Cumulative incidences were estimated for engraftment, achievement of donor chimerism, acute GVHD and cytomegalovirus reactivation to take account of a competing event (e.g., death) (*21*). Gray's test was used to compare different cumulative incidence curves (*21*). The probabilities of EFS and OS were estimated from the time of transplantation, according to the Kaplan-Meier product limit method. Log-rank test was used to compare these two outcomes. The following patient or transplant characteristics (baseline factors) were analyzed using a Cox regression model for their prognostic value on EFS and OS: patient age, sex, performance status, disease risk, previous history of autologous stem-cell transplantation (yes/no), disparity of HLA-A, -B, -DR antigen (one/two antigen mismatched), number of infused nuclear cells and number of CD34-positive cell dose. Stat View 5.0, Statistical Analysis System (SAS; SAS Institute Inc., Cary, NC) and S Plus 2000 (Mathsoft, Seattle, WA) were used for all statistical analyses.

RESULTS

Characteristics of the Study Patients

Patient characteristics and cord blood grafts are summarized in Table 1. Median age was 56.5 years (range, 22–68), and median weight was 57 kg (range, 40–75 kg). Twenty-two patients were refractory to cytotoxic chemotherapies. Another nine patients had chemosensitive diseases including acute myeloid leukemia (AML) in second CR (n=4), CML in second chronic phase (n=2), adult T-cell leukemia in partial remission (n=2), and malignant lymphoma in second CR (n=1). Two patients who had AML with prior MDS (n=1) had not received pretransplantation chemotherapy. The remaining patient had transfusion-dependent severe aplastic anemia.

Engraftment

Thirty-one patients achieved neutrophil engraftment at a median of day 20 (range, 12–33). Platelet engraftment was achieved in 27 patients, at a median of day 38 (range, 24–216). All of the three patients without primary engraftment died at a median of day 26 (range, 20–34) due to sepsis (n=2) and intracranial hemorrhage (n=1). Neither primary nor late graft failure was diagnosed in any of the 34 patients.

TABLE 1. Patient characteristics

Variables	N or median (range)
Total patients	34
Sex (male/female)	
Male	11
Female	23
Age, median years (range)	56.5 (22–68)
Weight, mean kg (range)	57 (40–75)
Underlying diseases (n)	
Acute myeloid leukemia	13[a,b]
Myelodysplastic syndrome	3[c,d]
Acute lymphoblastic leukemia	3[e,f]
Adult T-cell leukemia	6[g]
Chronic myeloid leukemia	3[h]
Malignant lymphoma	5[i]
Severe aplastic anemia	1
Risk of underlying diseases	
High	26
Low	8
Previous history of autologous stem-cell transplantation	
Yes	2
No	32
HLA mismatches	
One	40
Two	30
Number of infused nuclear cells/kg, median (range)	2.4 (1.6–4.8)

Patients with hematologic malignancies in complete remission at the time of transplant, in chronic phase of chronic myelogenous leukemia, with refractory anemia or refractory anemia with ringed sideroblasts of myelodysplastic syndrome, and with nonmalignant diseases were defined as being at standard risk. The other patients were defined as being at high risk.

[a] Three patients were in the second complete remission. The primary diseases were refractory to chemotherapy in 8 patients. The remaining 2 patients had not received prior chemotherapy.

[b] Data of Chromosomal abnormalities were available in 10 patients. Those revealed normal karyotype (n=8) and complex karyotype (n=2).

[c] The primary diseases were refractory to chemotherapy in 2 patients. The remaining 1 patient had not received prior chemotherapy.

[d] Data of chromosomal abnormality was available in 1 patient. It revealed 47XY, +21 [1], 46XY [29].

[e] The primary diseases were refractory to chemotherapy in all the 3 patients.

[f] Chromosomal abnormalities reveals t (9; 22) (q34; q11) and 45 X, -Y [4], 69, XXY [1], 46, XY [14] (n=1).

[g] The primary diseases were refractory to chemotherapy in 5 patients. The remaining 1 patient received retransplantation due to graft failure of reduced-intensity cord blood transplantation.

[h] The disease status of those patients were the second chronic phase (n=1), accelerated phase (n=1), and blast crisis (n=1).

[i] One patient was in complete remission. The primary diseases were refractory to chemotherapy in the remaining 4 patients.

TABLE 2. Prognostic factors of neutrophil engraftment and 100% donor chimerism

	N	Percent (95% CI)	*P* value
Neutrophil engraftment			
Variable			
Total cell dose			0.010
≥3×10E7/kg	7	100 (68–100)	
<3×10E7/kg	27	89 (69–100)	
HLA disparities			0.0097
HLA 5/6 match	4	100 (64–100)	
HAL 4/6 match	30	90 (71–100)	
100% donor chimerism			
Total cell dose			0.25
≥3×10E7/kg	7	100 (68–100)	
<3×10E7/kg	27	93 (72–100)	
HLA disparities			0.96
HLA 5/6 match	4	100 (64–100)	
HAL 4/6 match	30	93 (75–100)	

TABLE 3. Regimen-related toxicity according to Bearman's criteria

Grade	0	I	II	III	IV
Central nervous system	32	0	1	0	1
Lung	33	0	0	1	0
Kidney	28	4[a]	2[a]	0	0
Liver	22	10[b]	2[b]	0	0
Heart	34	0	0	0	0
Gut	10	19	5	0	0

[a] Median serum creatinine level: 1.2 mg/dL (range, 0.9–1.8).
[b] Median serum aspartate aminotransferase and alanine aminotransferase levels of those patients were 29 IV/L (range 18–274) and 24 IV/L (range 12–593), respectively.

Chimerism Analysis

Chimerism data were obtained from all the 34 patients. Thirty-two patients (94%) achieved complete donor chimerism at day 60. Median time to complete donor chimerism was 22 days (range, 13–38). One patient who died of TRM within 28 days of RI-CBT had complete donor chimerism before neutrophil engraftment. All the surviving patients were monitored for chimerism every 3 months, showing complete donor chimerism during the follow-up even after the discontinuation of immunosuppressants.

No significant association was identified between complete donor chimerism and either infused cell dose or HLA disparity (Table 2).

Cause of Death

Nine patients died during follow-up. Four patients died due to TRM at a median day of 30 (range, 20–46). The remaining five patients died due to the primary disease progression at a median of day 171 (range, 103–203).

Regimen-Related Toxicity and Transplantation-Related Mortality

RRT was shown in Table 3 Grade III–IV RRT developed in two patients. One patient developed fatal intracranial hemorrhage on day 26. TRM within 100 days of RI-CBT was 12% (95% confidence interval [CI], 1–23%). Primary causes of death were sepsis (n=2), encephalitis (n=1), and intracranial hemorrhage (n=1). Three patients developed creatinine level abnormality associated with tacrolimus: grade 1 in one, grade 2 in one, and grade 3 in one patient. Those patients required dose modifications of tacrolimus for toxicities. No patients developed tacrolimus-associated hypertension, diabetes, neurotoxicity, or microangiopathy.

Pre-Engraftment Immune Reactions

Fifteen of the 34 patients (44%; 95% CI, 27–61%) developed PIR. PIR was treated supportively without corticosteroid in all the patients.

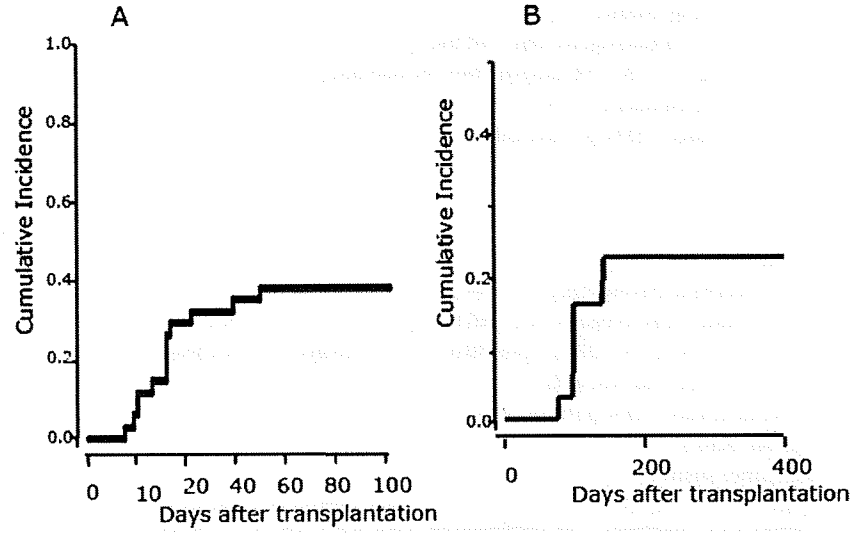

FIGURE 1. (A) Cumulative incidence of grade II–IV acute GVHD. Fourteen of 31 patients (45%; 95% CI, 28%–63%) who achieved primary engraftment developed grade II–IV acute GVHD. (B) Cumulative incidence of chronic GVHD. Seven of the evaluable 26 patients (27%) developed chronic GVHD.

Graft-Versus-Host Disease

Fourteen of 31 patients (45%; 95% CI, 28–63%) who achieved primary engraftment developed grade II–IV acute GVHD: grade II (n=6) and grade III (n=8) (Fig. 1-A). Its median onset was day 26 (range, 12–90). Ten patients with grade II–IV acute GVHD received corticosteroids. The initial response to corticosteroid was CR in seven, partial response (PR) in two, and mixed response in one patient. No patients required second line immunosuppressive therapy for acute GVHD. Seven of the evaluable 26 patients (27%) developed chronic GVHD including extensive-type (n=2) and limited-type (n=5) (Fig. 1B). Neither acute nor chronic GVHD was fatal in any patients.

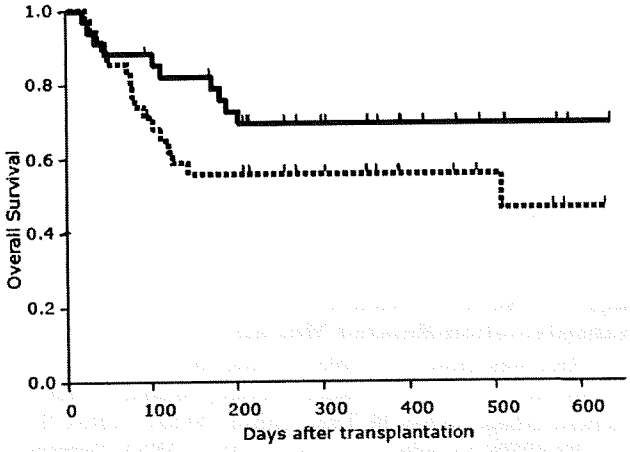

FIGURE 2. Probability of overall survival (OS) and event-free survival (EFS). As of December 2005, the median follow-up after RI-CBT for surviving patients was 12.1 months (range 3.1–21.1). The Kaplan-Meier probability of OS and EFS at 1 year were 70% and 55% (95% confidence interval: 38%–72%), respectively.

Infection

Twelve patients developed bacteremia (n=12). It was fatal in two patents. One patient developed disseminated tuberculosis, which was successfully treated by antitubercular drug (*11*). Reactivation of CMV was documented in 15 patients at a median of day 33 (range, day 17–87). Six patients developed CMV enterocolitis. It was successfully treated by ganciclovir or foscarnet. One developed viral hemorrhagic cystitis, which was successfully treated by vidarabine. Two patients developed encephalitis caused by human herpes virus 6. It was fatal in one patient despite foscarnet use.

Event-Free and Overall Survival

As of October 2005, the median follow-up after RI-CBT for surviving patients was 12.1 months (range 3.1–21.1). Five patients died due to disease progression. The Kaplan-Meier probability of EFS and OS at 1 year were 55% (95% CI, 38%–72%) and 70% (95% CI: 54%–85%), respectively (Fig. 2).

Prognostic Factors

Univariate analysis failed to identify any significant risk factors of OS, and multivariate analysis was therefore not conducted (Table 4).

Univariate analysis showed borderline significances between EFS and either risk of underlying diseases or HLA disparity; however, multivariate analysis failed to identify any prognostic factors of EFS (Table 3).

TABLE 4. Prognostic factors of overall and event-free survival

Univariate factors	Hazard ratio	95% CI	*P* value
Overall survival			
Age (years)	1.01	0.96–1.068	0.63
Sex (female vs. male)	1.99	0.42–9.39	0.38
Performance status (1 vs. 0)	4.15	0.88–19.56	0.072
Risk of underlying diseases (high vs. low)	3.20	0.41–25.31	0.27
Previous history of autologous stem-cell transplantation (yes vs. no)	1.68	0.36–7.93	0.51
Disparity of HLA-A, -B, -DR antigen (two- vs. one-antigen mismatched)	0.56	0.12–2.64	0.46
Number of infused nuclear cells	0.58	0.17–1.97	0.38
Number of infused CD34 positive cells	0.54	0.14–2.14	0.38
Event-free survival			
Age (years)	1.02	0.98–1.06	0.38
Sex (female vs. male)	0.57	0.21–1.55	0.27
Performance status (1 vs. 0)	2.35	0.81–6.79	0.11
Risk of underlying diseases (high vs. low)	2.76	0.63–12.18	0.18
Previous history of autologous stem-cell transplantation (yes vs. no)	1.00	0.23–4.44	1.00
Disparity of HLA-A, -B, -DR antigen (two- vs. one-antigen mismatched)	0.36	0.10–1.31	0.12
Number of infused nuclear cells	1.21	0.58–2.55	0.61
Number of infused CD34 positive cells	1.10	0.56–2.15	0.79
Multivariate factors		0.74–6.37	0.16
Performance status (1 vs. 0)	2.18	0.12–1.55	0.19
Disparity of HLA-A, -B, -DR antigen (one- vs. two-antigen mismatched)	0.43		

TABLE 5. Clinical characteristics of patients treated with cyclosporine and tacrolimus

Variables	Cyclosporine[a]	Tacrolimus	*P* value
N	30	34	
Age, median years (range)	58.5 (20–70)	56.5 (22–68)	0.21
HLA matching (n)			
5/6	6	4	0.37
4/6	24	30	
Pre-engraftment immune reactions (%)	66.7	44.1	0.07
Grade II–IV acute GVHD (%)	37.5	45.2	0.57
Transplant-related mortality before day 100 (%)	26.7	11.8	0.13
Overall survival at 1 year (%)	26.4	69.5	0.02

[a] Patient characteristics were described in reference 7.

Clinical Impact of Cyclosporine and Tacrolimus as GVHD Prophylaxis

We summarized the clinical characteristics of the patients with between cyclosporine and tacrolimus in Table 5. The characteristics of patients with cyclosporine were previously described (*7*).

DISCUSSION

The present study suggests that GVHD prophylaxis using tacrolimus is feasible in adult RI-CBT recipients. Intensification of GVHD prophylaxis can suppress postCBT immune reactions including PIR and GVHD. PIR is an immune reaction before engraftment, which is frequently associated with fever, diarrhea, rash, and weight gain (*9*). We previously reported that 78% of the RI-CBT recipients given cyclosporine developed PIR (*9*), while 15 of the 34 patients (44%; 95% CI, 27–61%) developed it in the present study. PIR was less frequent in the present study than in RI-CBT with cyclosporine (*7*). Interestingly, it was treated supportively without corticosteroid in all the patients, while 66% of the patients with PIR were given corticosteroid than in RI-CBT with cyclosporine (*7*). Severity of PIR was milder in patients given tacrolimus than those given cyclosporine.

GVHD is the most significant concern in allo-SCT. The frequencies of grade II–IV acute GVHD in adult myeloablative CBT using mainly cyclosporine were 25–72% (*22–28*). We previously reported that 66% of adult RI-CBT recipients developed grade II–IV acute GVHD, when cyclosporine was used for GVHD prophylaxis (*9*). In the present study, 14 of 31 patients (45%; 95% CI, 28%–63%) developed grade II–IV acute GVHD. The incidence of acute GVHD was lower in RI-CBT using tacrolimus than that using cyclosporine. Tacrolimus has been shown stronger in its immunosuppressant effects than cyclosporine in randomized controlled studies (*29–31*) and in vitro studies (*32*). It is reasonable to assume that immunosuppression using tacrolimus might suppress acute GVHD in the present study. Alternatively, suppression of PIR with tacrolimus might have contributed to the prevention of acute GVHD following RI-CBT, since PIR can trigger GVHD (*9*).

The present study suggests the possibility that intensification of GVHD prophylaxis decreases TRM after RI-CBT, improving the prognosis. GVHD prophylaxis in adult CBT is mostly cyclosporine, and the TRM ranges 27–52% (*3, 4, 7, 8*). In contrast, TRM in the present study with tacrolimus was 12% (95% CI, 1–23%), which was much lower than those in previous reports (*3, 4, 7, 8*). The major causes of TRM after CBT are infections and GVHD (*3, 4, 7, 8, 10*). As none died of GVHD, intensification of immunosuppression may have reduced TRM. Additional immunosuppression such as steroids for PIR and GVHD may increase the risk for infections. Intense GVHD prophylaxis by tacrolimus probably reduced steroid use, and hence the risk of severe infections.

It remains unknown whether intensification of GVHD prophylaxis using tacrolimus might hamper graft-versus-malignancy (GVM) effects, since they are closely associated with GVHD (*33*). In the present study, the cumulative probability of relapse at 1 year was 37%, and 55% of the RI-CBT recipients survived without disease progression at 1 year after transplantation. Considering the patients' backgrounds in this study, these findings suggest that RI-CBT using tacrolimus carries a considerable GVM effects. Since the impact of GVHD on a GVM effect varies according to disease status and patients' conditions, management of GVHD should be tailored. Further studies are warranted to establish a proper GVHD prophylaxis following RI-CBT.

Adverse effects of tacrolimus were tolerable in RI-CBT recipients in the present study. Its major adverse effects include renal insufficiency, hyperglycemia, and hypertension. Renal insufficiency by tacrolimus has been reported higher in incidence than by cyclosporine (*34*). Despite the advanced age in most of our patients, renal insufficiency by tacrolimus was reversible. While another concern about tacrolimus may be graft failure with intense immunosuppression (*35*), incidence of engraftment in the present study was comparable to those in the previous reports (*3, 4, 7, 8*). This study has demonstrated that tacrolimus is feasible in RI-CBT for patients with advanced age.

While the present study suggested the possible improvement in TRM by intensification of GVHD prophylaxis, there are some problems to be discussed. First, it is a small-sized, retrospective study; unrecognized biases might have affected the results. Large-scale prospective evaluations are required. Second, the follow up was rather short. Little information is available concerning chronic GVHD and GVM effects. Longer follow-up observations are necessary to investigate them. Third, we did not investigate the postCBT immune reconstitutions in the recipients with tacrolimus. Evaluation of immune parameters such as

CD4 and CD8 T cell might be worth investigating. The last optimal strategy should be established in the management of acute GVHD following RI-CBT. An appropriate protocol of tacrolimus use in RI-CBT for patients with advanced age remains unclear and requires further study.

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Invasive Fungal Infection Following Reduced-Intensity Cord Blood Transplantation for Adult Patients with Hematologic Diseases

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ABSTRACT

Invasive fungal infection (IFI) is a significant complication after allogeneic hematopoietic stem cell transplantation (HSCT); however, we have little information on its clinical features after reduced intensity cord blood transplantation (RICBT) for adults. We reviewed medical records of 128 patients who underwent RICBT at Toranomon Hospital between March 2002 and November 2005. Most of the patients received purine-analogbased preparative regimens. Graft-versus-host disease (GVHD) prophylaxis was a continuous infusion of either tacrolimus 0.03 mg/kg or cyclosporine 3 mg/kg. IFI was diagnosed according to the established EORTC/NIH-MSG criteria. IFI was diagnosed in 14 patients. Thirteen of the 14 had probable invasive pulmonary aspergillosis and the other had fungemia resulting from *Trichosporon* spp. Median onset of IFI was day 20 (range: 1-82), and no patients developed IFI after day 100. Three-year cumulative incidence of IA was 10.2%. Four of the 13 patients with invasive aspergillosis (IA) developed grade II-IV acute GVHD, and their IA was diagnosed before the onset of acute GVHD. The mortality rate of IFI was 86%. Multivariate analysis revealed that the use of prednisolone >0.2 mg/kg (relative risk 7.97, 95% confidence interval 2.24-28.4, P = .0014) was a significant risk factor for IA. This study suggests that IFI is an important cause of deaths after RICBT, and effective strategies are warranted to prevent IFI.

KEY WORDS

Invasive aspergillosis • Graft-versus-host disease • Corticosteroid

INTRODUCTION

Cord blood transplantation (CBT) is an attractive alternative for patients with hematologic diseases who lack a matched related or unrelated donor. The usefulness of CBT using myeloablative preparative regimens has been confirmed for pediatric patients [1,2]. Myeloablative CBT for adult patients achieves engraftment in 90% of the patients, but carries 50% risk of transplant-related mortality (TRM), mostly resulting from infection [3,4]. We and other groups have reported the feasibility of CBT using reduced-intensity regimens (RICBT) for adult patients with advanced hematologic diseases [5,6].

Because of delayed immune recovery and graft-versus-host disease (GVHD), infection is the leading cause of TRM after CBT using myeloablative preparative regimens [2-4,7]. However, studies on immune recovery following RICBT gave us hope that RICBT recipients may less frequently experience GVHD and infectious complications. Invasive fungal infection (IFI) has been 1 of the most feared infectious complications in conventional allogeneic marrow or peripheral blood stem cell (PBSC) transplantation [8,9], whereas we have little information on IFI following RICBT. We investigated its incidence and clinical features in patients who underwent RICBT for advanced hematologic diseases.

PATIENTS AND METHODS

Data Collection

We reviewed medical records of 128 recipients who underwent first reduced-intensity allogeneic hematopoietic stem cell transplantation (HSCT) using cord blood (CB) between March 2002 and November 2005 at Toranomon Hospital. Their characteristics are shown in Table 1. Of the 128 patients, 101 had high-risk diseases including acute myelogenous leukemia (AML) in relapse or the second and higher complete remission (CR; n = 42), acute lymphoid leukemia (ALL) except those in the first CR (n = 12), chronic myelogenous leukemia (CML) in blastic phase (n = 4), myelodysplasia except refractory anemia (n = 10), refractory lymphoma (n = 30), idiopathic myelofibrosis (n = 1), plasma cell leukemia in relapse (n = 1), and chronic myelomonocytic leukemia (n = 1).

Table 1. *Patients' Characteristics and Transplantation Procedures*

Variables	Number
Patients Characteristics	
Age, median (range)	56 (17-71)
Sex, male/female	80/48
Primary diseases	
AML/MDS	63
Malignant lymphoma	33
Acute lymphoblastic leukemia	17
Severe aplastic anemia	6
Chronic myelogenous leukemia	6
Chronic myelomonocytic leukemia	1
Plasmacytic leukemia	1
Idiopathic myelofibrosis	1
Risk of underlying diseases,*1 high/low	101/27
Prior autologous stem cell transplant, yes/no	9/119
Transplantation procedures	
Conditioning regimen	
Flu + Mel + TBI 2 Gy or 4 Gy/8 Gy	112/2
Flu + BU + TBI 4 Gy/8 Gy	8/1
Others	5
GVHD prophylaxis, cyclosporine/tacrolimus	64/64
Number of infused nucleated cell, median (range) $\times 10^7$/kg	2.7 (1.6-4.8)
HLA disparity (antigen), 2/1/0	108/17/3
Transplantation outcomes	
Neutrophil engraftment	99/128
Complete donor chimerism*2	90/99
Grade II-IV acute GVHD*2	45/99
Chronic GVHD*3*4	11/40
CMV antigenemia*3	48/93
CMV disease	10
Relapse*2	24/98

*1 We divided the risk of transplantation into two groups. The low-risk group was as follows: acute myelogenous or lymphoid leukemia in first and second remission, chronic myelogenous leukemia in chronic phase, and myelodysplastic syndrome refractory anemia. The other patients were defined as having high-risk diseases.

*2 Percentage was calculated based on 99 patients who achieved primary engraftment.

*3 Percentage was calculated based on the number of patients who achieved engraftment and evaluated.

*4 No patients received systemic corticosteroids for the treatment of chronic GVHD.

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndromes; ULN, upper limit of normal; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

Transplantation Procedures and Supportive Cares

Transplantation procedures were shown in Table 1, and we previously reported details of the procedures [6]. GVHD prophylaxis was either tacrolimus 0.03 mg/kg or cyclosporine 3 mg/kg continuous infusion starting on day −1. Trough blood levels of these drugs were monitored 2-3 times a week and the dosage were modified to maintain the target level of 10-15 ng/mL for tacrolimus and 200-400 ng/mL for cyclosporine [10-12]. Immunosuppressants were tapered off from day 100 until day 150. If grade II-IV acute GVHD (aGVHD) developed, 1-2 mg/kg/day of methylprednisolone was added to cyclosporine or tacrolimus, and tapered from the beginning of clinical response.

The diagnosis and management of preengraftment immune reactions were reported previously [13].

Management of Infections

Patients were managed in reverse isolation laminar airflow-equipped rooms. All patients received tosufloxacin 450 mg/day from the start of conditioning until neutrophil engraftment. Fluconazole 200 mg/day or micafungin 150 mg/day, and acyclovir 600 mg/day were given from the start of conditioning until the discontinuation of GVHD prophylaxis, which were restarted when patients developed GVHD and were treated with steroids and immunosuppressants. They received prophylaxis with trimethoprim-sulfamethoxazole against *Pneumocystis jirovecii* infection from the start of conditioning until the discontinuation of immunosuppressants or disappearance of chronic GVHD (cGVHD). When patients develop neutropenic fever, tosufloxacin was changed to broad-spectrum antibiotics [14]. Intravenous administration of amphotericin B at a dose of 0.5 mg/kg/day was added when the fever persisted for more than 5 to 7 days. If the diagnosis of aspergillus infection was confirmed, the dosage of amphotericin B was increased to 1.0 mg/kg/day. We used blood tests, enzyme-linked immunosorbent assay for galactomannan antigen, (1-3)-beta-D glucan assay, and chest computed tomography for the early diagnosis of invasive aspergillosis (IA), as previously reported [15]. Because most patients had been heavily treated and received multiple transfusions prior to transplantation, anti-CMV antibodies were not examined before transplantation. All patients were monitored for cytomegalovirus pp65 antigenemia once a week. When CMV antigenemia exceeded 10/50,000, patients preemptively received foscarnet 30 mg/kg intravenously twice daily.

Diagnostic Criteria for IFI

Invasive fungal infection was diagnosed according to the established EORTC/NIH-MSG criteria [16]. Briefly, we diagnosed patients as having proved IFI when any 1 of the following examinations was positive: histopathologic or cytopathologic examinations for hyphae or yeasts in needle aspiration or biopsy specimens, fungal cultures obtained from normally sterile sites by sterile procedures, and *Cryptococcus* antigen in cerebrospinal fluid. Probable IFI was diagnosed when a patient satisfied at least 1 host factor, microbiologic criteria, and clinical criteria. Possible IFI was not included in this study. The day of diagnosis of IFI was defined as the day when the first diagnostic test was performed.

Endpoints and Statistical Analysis

The cumulative incidence of IA was evaluated using Gray's method, considering death without IA as a competing risk [17].

Potential confounding factors considered in the analysis of risk factors of IA were age, sex, disease status, previous stem cell transplantation, conditioning regimens, HLA mismatch, stem-cell dose (all nucleated cells, and CD 34-positive cells), GVHD prophylaxis, grade II-IV aGVHD, and use of prednisolone. Proportional hazard modeling was used to evaluate the influence of these factors on the incidences of IA treating the development of aGVHD and the use of prednisolone as time-dependent covariates. Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. P-values of $<.05$ were considered statistically significant. Survival was estimated by the Kaplan-Meier method. Median follow-up of surviving patients was 628 days (range: 26-1347 days).

RESULTS

Clinical Outcomes after RICBT

Ninety-nine (77%) patients achieved primary engraftment at a median of day 20 (range: 9-53 days). Of the remaining 29 patients who failed to achieve primary engraftment, 4 patients received second RICBT, and the other 25 patients died before engraftment. Their causes of death included bacteremia (n = 22), invasive pulmonary aspergillosis (IPA) (n = 1), and progression of primary disease (n = 2). Of the 99 engrafted patients, 45 and 22 patients developed grade II and grade III-IV aGVHD, respectively. The median onset of grade II-IV aGVHD was day 28 (range, 11-92). Eleven of 40 patients (28%) who survived longer than 100 days without disease progression developed cGVHD. Estimated 3-year overall survival was 33% (95% confidence interval (95% CI), 24%-42%). Causes of deaths comprised nonrelapse mortality (n = 31) and disease progression (n = 23). Infection was the leading cause of nonrelapse mortality (n = 20). Autopsy was performed in 5 patients (3.9%) in this series of patients.

Incidence and Clinical Features of IFI

Invasive fungal infection was diagnosed in 14 patients. Their clinical features are shown in Table 2. Thirteen of the 14 patients had probable IA, and the other had fungemia from *Trichosporon* spp. Three-year cumulative incidence of probable IA was 10.2% (Figure 1). Median onset of IFI was day 20 (range: 1-82), and no patients developed IFI after day 100. IFI was diagnosed after day 30 in 1 patient. Prophylactic uses of antifungal agents included fluconazole (n = 12) and micafungin (n = 1) among the 13 patients with IA. Of the 63 patients who survived 100 days or longer, none developed IFI after day 100. Four of the 13 patients with IFI developed grade II-IV aGVHD, and their diagnosis of IFI was before the onset of aGVHD (Table 2).

Seven patients were given prednisolone >0.2 mg/kg/day for the treatment of preengraftment immune reactions, of whom 5 developed grade II-IV aGVHD. Twelve of the 14 patients with IFI died, and the mortality rate was 86%. IFI was the primary cause of deaths in 4 patients.

Risk Factors of IPA

Table 3 shows the results of univariate and multivariate analyses. Reactivation of cytomegalovirus (CMV) is a well-known risk factor of IA [18,19]. However, it was not included in the analysis of this study, because the onset of IFI was earlier than the onset of CMV antigenemia. Use of prednisolone >0.2 mg/kg (relative risk [RR], 7.97; 95% CI, 2.24-28.4; P = .0014) was a significant risk factor in multivariate analysis.

DISCUSSION

The present study demonstrated that IFI early after RICBT is a significant complication. Among IFI, the incidence of IA was high, which was consistent with the studies on reduced intensity stem cell transplantation (RIST) using other stem cell sources [9,19]. Our results contrasted with the previous reports that the incidence of infection because of non-*Candidia albicans* species was high in myeloablative allogeneic stem cell transplantation [20]. The observations may be associated with the milder gastrointestinal mucosal toxicity by conditioning regimens in RIST than in myeloablative transplantation [21] and the less fre-

Table 2. *Clinical Characteristics of Patients with Invasive Aspergillosis*

UPN	Age	Sex	Primary Disease	Disease Status at Transplant	No. of Prior Regimens	Neutrophil Engraftment Day	Grade II-IV Acute GVHD Onset Day	PSL Started	Invasive Fungal Infection (IFI)*1	Onset (Day)	Other Infectious Complication	Overall Survival (Day)	Outcomes of IFI	Causes of Death
286	57	M	AML	PIF	3				Probable IA	4		4	dead	IFI
365	69	M	AML	RLI	3				Probable IA	3		14	dead	IFI
411	56	F	ML	PD	1			11	Probable IA	19	Bacteremia	24	dead	Bacteremia complicated with IA
196	61	M	AML	PIF	2	11	22		*T. cutaneum* fungemia	1		28	dead	IFI
202	62	M	AML	RLI	4	21	21	8	Probable IA	21	Bacteremia	28	dead	Bacteremia complicated with IA
344	55	F	ML	PD	1	19			Probable IA	4	Bacteremia	30	dead	IFI
262	59	M	AML	PIF	1			10	Probable IA	20		31	dead	PD
151	52	F	MDS	RAEB	3	20		7	Probable IA	12		33	improved	GI bleeding
114	52	F	ML	PD	4	13		0	Probable IA	23		39	dead	PD
153	70	M	AML	1st CR	2	14	30		Probable IA	29	Bacteremia	46	dead	IP
197	33	M	MDS	RA	0				Probable IA	28	Bacteremia	47	dead	Bacteremia complicated with IA
160	66	M	ML	PD	2	14	29	9	Probable IA	25		75	dead	MOF
120	70	F	SAA		0	13			Probable IA	82		1308+	improved	
127	20	M	SAA		3	31	55	12	Probable IA	3		1347+	improved	

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndromes; CR, complete remission; GI, gastrointestinal; IA, invasive aspergillosis; IFI, invasive fungal infection; SAA, severe aplastic anemia; RA, refractory anemia; PD, progressive disease; PIF, primary induction failure; RARB, refractory anemia with excess of blasts; MOF, multiple organ failure.

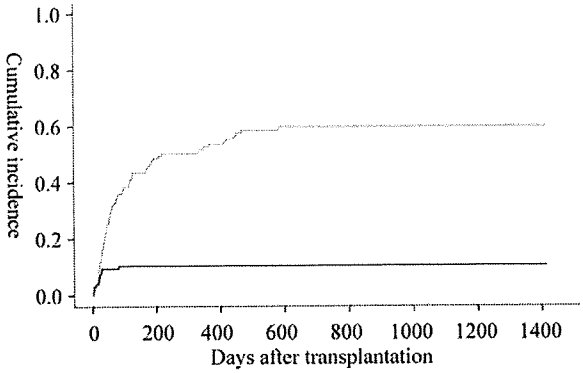

Figure 1. Cumulative incidence of invasive aspergillosis (IA) considering death without IA as a competing risk. Black line indicates incidence of death with IA, and gray line indicates incidence of death without IA.

quent and milder GVHD following CBT. Because gastrointestinal mucosal toxicity is milder in RICBT than in myeloablative transplantation, the incidence of infection from non-*Candida albicans* species as part of the gastrointestinal normal flora might be low, and hence aspergillus infection might become the majority of IFI.

The median onset of IFI was day 20 (range: 1-82) in the present study; the majority developed IFI early after RICBT. Majority of the patients who developed IFI died of causes other than fungal infection (Table 2), as reported previously by Saavedra et al. [7]. Our results were consistent with a previous report on CBT [18], and contrasted to reports on RIST using marrow or peripheral blood [19,22,23], in which IA develops late after transplantation. The low incidence of IA after day 100 would be related to the low incidence of cGVHD. The short duration from RICBT to IFI development suggests aggravation of latent infection, which would have existed before transplantation. These findings were consistent with a recent report by Martino et al. [23]. Given the possibility, several issues need to be addressed in the management of IFI following RICBT. First, selection of RICBT candidates would have to include accurate evaluation for the risk of fungal infection [24] and high-risk patients might need to be excluded from the indication of RICBT. Pretransplant CT scan of the chest and sinus would be useful in the screening of IA following RICBT, and bronchoalveolar lavage should be performed in patients with abnormal findings. Second, the importance needs to be stressed in prophylactic antifungal agents with antiaspergillus activity and attempts for early diagnosis of aspergillosis such as methods using molecular techniques [25], antigen tests [26], and imaging tests [15,22]. Third, the way of steroid use after RICBT requires further investigations. The present study showed that the administration of steroids 0.2 mg/kg and more was a strong risk factor of IA (RR, 7.97; 95% CI, 2.24-28.4; $p = .0014$). Our observation that the use of small-dose steroids was a risk factor of IFI after RICBT supports the previous results of severe immunosuppression early after RICBT [27]. In RICBT using our regimens, immunologic reactions such as a preengraftment immune reaction frequently develops in addition to GVHD, requiring steroid administration early after RICBT [13]. Because steroids suppress phagocyte activities and cellular immunity [28], the risk of fungal infection early after RICBT may be increased.

The incidence of late IFI was not high in the present study. Of 102 patients who survived longer

Table 3. *Univariate and Multivariate Analyses for the Incidence of IA*

	Incidence of IPA (95% CI)	P Value
Univariate analysis		
Pretransplantation factors		
Age		.30
<55	7%	
≥55	13%	
Sex		.94
Female	11%	
Male	10%	
Disease risk		.63
Standard	16%	
High	9%	
Previous ASCT		.55
No	11%	
Yes	0%	
Regimen		.72
FM-based	11%	
FB-based	10%	
HLA mismatch		.076
0 or 1 antigen	0%	
2 antigens	12%	
Cell dose		.42
ANC <2.5 × 10^7/kg	8%	
ANC ≥2.5 × 10^7/kg	12%	
Cell dose		.03
CD34 <0.8 × 10^6/kg	5%	
CD34 ≥0.8 × 10^6/kg	16%	
GVHD prophylaxis		.42
Cyclosporine	13%	
Tacrolimus	8%	
Fungal prophylaxis		.99
Fluconazole	10%	
Micafungin	10%	
Posttransplantation factors (time-dependent covariates)		
Acute GVHD		.96
Grade 0-I	1.00	
Grade II-IV	1.06 (0.12-9.40)	
Prednisolone		.001
<0.2 mg/kg/day	1.00	
≥0.2 mg/kg/day	7.97 (2.24-28.4)	
Multivariate analysis		
Prednisolone		.0014
<0.2 mg/kg/day	1.00	
≥0.2 mg/kg/day	7.97 (2.24-28.4)	

GVHD indicates graft-versus-host disease; ASCT, autologous stem cell transplantation.

than 30 days, 1 patients developed IFI after day 30. None of 63 patients who survived longer than 100 days developed IFI after day 100. Our results contrast with the previous reports on BMT and PBSC transplantation where improvement in fungal management decreased early IFI and late IFI became the majority of IFI [8,19,22,29]. In myeloablative CBT, late infection is considered a significant complication [4], whereas study results focused on fungal infection have not been published. Some hypotheses can explain the low incidence of late IFI after RICBT. First, cGVHD after RICBT is uncommon and mild. There is minimal effect of cGVHD on delay in immune recovery following RICBT. Second, steroids are not frequently administered late after RICBT for the treatment of complications such as GVHD. The incidence of cGVHD was 28% in the present study, and none of them required steroid treatments. Further studies are awaited for the clinical features of late IFI after RICBT.

The present study demonstrated clinical features of fungal infections after RICBT, leaving several issues to be investigated. First, the present study is a small-sized retrospective 1 . Unrecognized bias might affect the study results, and we obtained little information on rare fungal infections such as Fusarium and Zygomycetes. Large-sized prospective studies are awaited. Second, the diagnostic yields of IFI need to be addressed. Most of the diagnoses in our study were made based on EORTC/MSG criteria [16] using clinical, laboratory, and imaging findings. Although the clinical usefulness of the diagnostic criteria has been established, pathologic diagnosis of IFI was not confirmed in many patients and the diagnostic yields remain unclear. Underestimation of IFI incidence also remains possible, because postmortem examinations were not obtained in most patients who died without diagnosis of IFI. Because such limitations cannot be avoided in studying deep fungal infections [30], clinicians need to be aware of the limitations.

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Cytomegalovirus Infections following Umbilical Cord Blood Transplantation Using Reduced Intensity Conditioning Regimens for Adult Patients

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ABSTRACT

Cytomegalovirus (CMV) infection is a major complication after allogeneic hematopoietic stem cell transplantation (Allo-HSCT); however, we have little information on the clinical features of CMV reactivation after cord blood transplantation using reduced-intensity regimens (RI-CBT) for adults. We reviewed medical records of 140 patients who underwent RI-CBT at Toranomon Hospital between January 2002 and March 2005. All the patients were monitored for CMV-antigenemia weekly, and, if turned positive, received preemptive foscarnet or ganciclovir. Seventy-seven patients developed positive antigenemia at a median onset of day 35 (range, 4-92) after transplant. Median of the maximal number of CMV pp65-positive cells per 50,000 cells was 22 (range, 1-1806). CMV disease developed in 22 patients on a median of day 35 (range, 15-106); 21 had enterocolitis and 1 had adrenalitis. CMV antigenemia had not been detected in 2 patients, when CMV disease was diagnosed. CMV disease was successfully treated using ganciclovir or foscarnet in 14 patients. The other 8 patients died without improvement of CMV disease. In multivariate analysis, grade II-IV acute graft-versus-host disease was a risk factor of CMV disease (relative risk 3.48, 95% confidential interval 1.47-8.23). CMV reactivation and disease develop early after RI-CBT. CMV enterocolitis may be a common complication after RI-CBT.

KEY WORDS

Graft-versus-host disease • Ganciclovir • Foscarnet • Cytomegalovirus antigenemia • CD34-positive cells

INTRODUCTION

Cord blood transplantation (CBT) is an attractive alternative for patients with hematologic diseases who lack a matched related or unrelated donor. The value of CBT using myeloablative preparative regimens has been confirmed for pediatric patients [1,2]. Myeloablative CBT for adult patients achieves engraftment in 90% of the patients, but carries a 50% risk of transplant-related mortality (TRM), mostly resulting from infection [3,4]. We and other groups have reported the feasibility of CBT using reduced-intensity regimens (RI-CBT) for adult patients with advanced hematologic diseases [5,6].

Because of delayed immune recovery and graft-versus-host disease (GVHD), infection is the leading cause of TRM after CBT using myeloablative preparative regimens [2-4,7]. However, studies on immune recovery following RI-CBT gave us hope that RI-CBT recipients may less frequently experience GVHD and infectious complications. Cytomegalovirus (CMV) has been 1 of the most feared infectious complications in CBT [8], as well as in conventional allogeneic marrow or peripheral blood stem cell trans-

plantation (PBSCT) [9,10], although we have little information on CMV infection following RI-CBT. We investigated its frequency and clinical features in patients who underwent RI-CBT for advanced hematologic diseases.

PATIENTS AND METHODS

Study Patients and Donors

We reviewed medical records of 140 patients who underwent RI-CBT at Toranomon Hospital between January 2002 and March 2005. All the patients had diseases that were incurable with conventional treatments, and were considered inappropriate for conventional allogeneic stem cell transplantation (allo-SCT) because of the lack of an human leukocyte antigen (HLA)-identical sibling or a suitable related/unrelated donor, aged >50 years old and/or organ dysfunction (generally attributable to previous intense chemotherapy and/or radiotherapy). All the patients provided written informed consent in accordance with the requirements of the institutional review board.

HLA Typing and Donor Matching

An unrelated cord blood donor was searched through the Japan Cord Blood Bank Network [11] for patients without an HLA-identical sibling donor or a suitable related/unrelated donor.

Preparative Regimen

All the patients received purine analog-based preparative regimens (Table 1).

Engraftment

Engraftment was defined as white blood cell counts $>1.0 \times 10^9$/L or absolute neutrophil counts $>0.5 \times 10^9$/L for 2 consecutive days. Granulocyte-colony stimulating factor (G-CSF) was administered i.v. from day 1 until engraftment.

Supportive Care and Management of Preengraftment Fever and GVHD

All the patients were managed in reverse isolation in laminar airflow-equipped rooms, and received trimethoprim/sulfamethoxazole for *Pneumocystis jiroveci* prophylaxis. Fluoroquinolone and fluconazole or itraconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir 600 mg/day was also given [12]. Neutropenic fever was managed according to the guidelines [13].

Diagnosis and management of preengraftment immune reaction were reported previously [14]. GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. Acute (aGVHD) and chronic GVHD (cGVHD) were graded according to the established criteria [15,16]. GVHD prophylaxis was a continuous infusion of cyclosporine 3 mg/kg or tacrolimus 0.03 mg/kg from day −1 until the patients tolerated oral administration. It was tapered off from day 60 until day 150 or depending on the status of GVHD. If grade II-IV aGVHD developed, 0.5-1.0 mg/kg/day of prednisolone was added to cyclosporine or tacrolimus, and tapered from the beginning of clinical response.

Table 1. *Patient Characteristics*

Variable	Number
Age (median [range])	55 (17-79)
Sex (men/women)	81/59
Primary diseases	
Acute lymphoblastic leukemia	19
Acute myeloid leukemia	44
Chronic myelogenous leukemia	5
Adult T cell leukemia	19
Myelodysplastic syndrome	14
Malignant lymphoma	29
Multiple myeloma	4
Aplastic anemia	6
Risk of underlying diseases (high/low)*	99/41
Preparative regimens	
Flud 125 mg/m² + L-PAM (80 mg/m²) + TBI (2-8 Gy)	121
Flud 125 mg/m² + L-PAM (140 mg/m²) + TBI (4-8 Gy)	5
Flud 125 mg/m² + L-PAM (100 mg/m²) + TBI (4-8 Gy)	2
Flud 150 mg/m² + BU 8 mg/kg + TBI (4-8 Gy)	8
Flud 125 mg/m² + L-PAM (80-140 mg/m²)	2
Flud 150 mg/m² + BU 8 mg/kg	1
L-PAM 140 mg/m²	1
Number of infused mononuclear cells ×10E7/kg (median [range])	2.7 (0.4-5.7)
Number of infused CD34⁺ cells ×10E5/kg (median [range])	0.73 (0.01-5.7)
HLA antigen disparity 0/1/2/3	3/21/114/2
GVHD prophylaxis	
Cyclosporine	85
Tacrolimus	55

Flud indicates fludarabine; L-PAM, melphalan; BU, busulfan; TBI, total body irradiation; GVHD, graft-versus-host disease.

*Acute leukemia in complete remission, chronic myelocytic leukemia in the 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 others were considered high risk.

Management of CMV Infection

CMV-specific IgM antibodies in the cord blood units were not examined. Because most patients had been heavily treated and received multiple transfusions, anti-CMV antibodies were not examined before transplantation. Anti-CMV high-titer i.v. immunoglobulin was not regularly administered. All packed

red blood cells and platelets were transfused using leukocyte-depleting filters.

CMV infection was defined as isolation of CMV or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. CMV disease was diagnosed as follows: CMV enterocolitis was diagnosed by gastrointestinal symptoms with histologic demonstration of CMV on biopsy materials obtained by endoscopy; CMV pneumonia was diagnosed when either a bronchoalveolar lavage or a lung biopsy was positive for CMV in a patient with characteristic signs, symptoms, and chest radiographic findings; CMV retinitis was diagnosed by characteristic retinal opacities without other likely explanations for the retinal findings. CMV pp65 antigenemia was monitored weekly after engraftment or when patients died before engraftment. Briefly, 1.5×10^5 peripheral blood leukocytes were attached to slides using a cytocentrifuge and fixed with cold acetone. From 1/3 to 1/2 of the centrifuged cells were fixed on the slides. The cells were incubated with monoclonal antibody HRP-C7 (Teijin, Tokyo, Japan) raised against immediate-early antigen, and stained by the direct immunoperoxidase method. These cells were analyzed under a light microscope and results were presented as the number of positive cells per 50,000 cells [17].

CMV antigenemia was managed according to the report by Kanda et al [17]. If CMV pp65-positive cells exceeded 10/50,000, patients preemptively received either ganciclovir 5 mg/kg once daily or foscarnet 30 mg/kg twice daily. Initiation of ganciclovir or foscarnet with <10 positive cells was optional in the patients who received more than 0.5 mg/kg of prednisolone. The doses were adjusted for renal function [18]. Ganciclovir or foscarnet was discontinued when 2 consecutive results of CMV antigen were negative. When CMV disease was diagnosed during preemptive therapy, we increased the dose of ganciclovir to 5 mg/kg twice a day, or foscarnet to 60 mg/kg twice or 3 times daily.

Endpoints and Statistical Analysis

The aims of this study were (1) to determine the incidence of CMV infection after RI-CBT, (2) to investigate its clinical features, and (3) to identify its risk factors. The cumulative incidences of CMV disease and CMV reactivation defined by the detection of CMV pp65 were evaluated using Gray's method [19], considering death without CMV reactivation as a competing risk. Potential confounding factors considered in the analysis were patient's age, sex, stem cell doses, HLA disparity, GVHD prophylaxis, conditioning regimens, and aGVHD. The influence of these factors on the incidence of CMV disease and CMV reactivation was evaluated with the proportional hazard modeling treating the development of aGVHD and the use of corticosteroids as time-dependent covariates. Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. P-values of <.05 were considered statistically significant.

RESULTS

Patient's Characteristics and Clinical Outcomes

Patient's characteristics are shown in Table 1. Of the 140 RI-CBT recipients, 112 patients (80%) achieved primary engraftment on a median of day 20 (range, 10-57). Sixty (43%) and 8 (6%) patients died of transplant-related causes and disease progression, respectively, within 100 days of RI-CBT. Preengraftment immune reaction [20] was diagnosed in 67 patients. Of the 112 patients who achieved engraftment, 47 (42%) developed grade II-IV aGVHD at a median onset of day 25 (range, 13-94). Sixty-one patients received prednisolone or methylprednisolone >0.5 mg/kg/day within 100 days of RI-CBT because of preengraftment immune reaction (n = 26), engraftment syndrome (n = 3), aGVHD (n = 16), and others (n = 16). As of November 2005, the median follow-up of the surviving patients was 13.0 months (range, 1.0-40.7). Overall survival rates were 85% (95% confidence interval [CI]; 79-91%) and 53% (95% CI; 45-62%) at days 30 and 100, respectively.

Clinical Features of CMV Reactivation and Diseases

Clinical features of CMV reactivation and diseases are summarized in Table 2. CMV antigenemia was found in 77 patients (55%, 95% CI; 51-59%) on a median of day 35 (range, 4 to 92). Twenty-eight of those patients received prednisolone or methylprednisolone >0.5 mg/kg/day before development of

Table 2. *Clinical Features of CMV Reactivation and Disease*

Variable
CMV reactivation
Number of patients
Onset (median [range])
Maximal levels of CMV antigenemia (range)
Preemptive therapy (ganciclovir/foscarnet/none)
CMV disease
Number of patients
Diagnose of CMV disease (median, [range])
Organ involvement
enterocolitis
pneumonia
retinitis
adrenalitis
Use of anti-CMV agents at the onset of CMV disease (ganciclovir/foscarnet/none)

CMV indicates cytomegalovirus.

Table 3. *Univariate and Multivariate Analyses for the Incidence of CMV Reactivation*

Factor	Relative risk (95% CI)	P value
Univariate		
Age	1.005 (0.989-1.021)	.53
Sex	1.10 (0.71-1.72)	.66
Disease status	0.68 (0.44-1.07)	.098
Number of HLA mismatch	1.37 (0.80-2.36)	.25
Number of infused mononuclear cells	0.99 (0.68-1.45)	.95
Number of infused $CD34^+$ cells	1.55 (1.28-1.87)	5.8×10^{-6}
GVHD prophylaxis (cyclosporine vs. tacrolimus)	0.59 (0.37-0.94)	.025
Preengraftment immune reaction	1.14 (0.74-1.78)	.55
Acute GVHD (grade II-IV)	1.21 (0.70-2.10)	.49
Use of steroid*	1.64 (1.02-2.64)	.042
Multivariate		
Number of infused $CD34^+$ cells	1.55 (1.28-1.87)	5.8×10^{-6}

CMV indicates cytomegalovirus; CI, confidence index; GVHD, graft-versus-host disease.
*Use of prednisolone or methyl-prednisolone >0.5 mg/kg/day.

CMV antigenemia. Forty-nine patients received foscarnet (n = 41) or ganciclovir (n = 8) preemptively. Initial dose of ganciclovir was 5 mg/kg once daily. The remaining 28 patients had not received foscarnet or ganciclovir according to our preemptive strategy, because of <10/50,000 of CMV pp65-positive cells.

Diagnosis of CMV disease was established in 22 patients (16%, 95% CI; 13-19%) on a median of day 33 (range 15-106); the diagnosis comprised enterocolitis (n = 21) and adrenalitis (n = 1). Of the 22 patients, 9 patients had received preemptive therapy before developing CMV disease. The remaining 13 patients had not received foscarnet or ganciclovir according to our preemptive strategy, mostly because of <10/50,000 of CMV pp65-positive cells.

Diagnosis of CMV disease was established at postmortem examination in 1 patient. The other 21 patients were treated with either foscarnet or ganciclovir. CMV disease was successfully treated in 14 patients. The remaining 8 patients died without improvement of CMV disease, although CMV disease was not the primary cause of death in any of these 8 patients.

Risk Factors of CMV Antigenemia and CMV Disease

Risk factors of CMV reactivation and CMV disease were shown in Table 3 and Table 4, respectively. CD34-positive cell dose was significantly associated with CMV reactivation on multivariate analysis (relative risk, 1.55; 95% CI 1.28-1.87; $P = 5.8 \times 10^{-6}$). Grade II-IV aGVHD was a risk factor of CMV disease on multivariate analysis (relative risk, 3.48; 95% CI 1.47-8.23; $P = .0045$).

DISCUSSION

The present study demonstrated that CMV infection is a significant complication of RI-CBT. The incidence of CMV reactivation was 55% in our study, which was comparable with previous reports on RIST [21-23] and myeloablative bone marrow transplantation (BMT) and PBSCT [24,25]. In contrast, previously reported incidence of CMV reactivation after CBT (79%) [8] was higher than that of ours, although it is the only previous report on CMV reactivation after CBT. The differences in preparative regimens and patient characteristics between the study [8] and ours may have affected the incidence of CMV reactivation. One of the unique findings in the present study was that the timing of CMV reactivation after RI-CBT was earlier than that after RIST without in vivo or ex vivo T cell depletion [21]. Another unique finding was the high incidence of CMV disease compared with transplantation of other stem cell sources [21,24,25]. Of the 77 patients with CMV antigenemia, 22 developed CMV disease in our study. The risk of progression from CMV reactivation to CMV disease may be high in CBT because of the intense immunosuppression [26].

The present study suggests that CMV infection is more likely to reactivate and to progress in RI-CBT than in transplantation using other stem cell sources. Several reasons can explain this hypothesis. First, the preparative regimens including total body irradiation (TBI) in our study might have damaged recipient-

Table 4. *Univariate and Multivariate Analyses for the Incidence of CMV Disease*

Factor	Relative risk (95% CI)	P value
Univariate		
Age	1.021 (0.991-1.052)	.16
Sex	1.15 (0.50-2.64)	.74
Disease status	1.92 (0.65-5.64)	.24
Number of HLA mismatch	2.54 (0.42-15.22)	.31
Number of infused mononuclear cells	0.59 (0.36-0.98)	.041
Number of infused $CD34^+$ cells	1.34 (0.93-1.93)	.11
GVHD prophylaxis (cyclosporine vs. tacrolimus)	0.85 (0.36-1.99)	.70
Preengraftment immune reaction	0.76 (0.33-1.76)	.52
Acute GVHD (grade II-IV)	3.48 (1.47-8.23)	.0045
Use of steroid*	1.36 (0.53-3.48)	.53
Multivariate		
Acute GVHD (grade II-IV)	3.48 (1.47-8.23)	.0045

CMV indicates cytomegalovirus; CI, confidence index; GVHD, graft-versus-host disease.
*Use of prednisolone or methyl-prednisolone >0.5 mg/kg/day.

derived anti-CMV immune cells. That contrasts with the report that recipient-derived T cells are associated with immune reaction against CMV early after transplantation following preparative regimens without TBI [27]. The issue needs to be considered in determining preparative regimens for RI-CBT. Second, transplanted cord blood stem cells are immunologically naïve. Although anti-CMV cytotoxic T-lymphocytes in transplant grafts are considered to suppress CMV proliferation early after transplantation in CMV seropositive recipients [28], passive immunity via grafts against CMV cannot be expected in CBT, and thus the risk of reactivation may be high. Third, post-transplant immune recovery is delayed in CBT. Little is known about post-CBT immune recovery with only few reports. Although the numbers of T cells, B cells, and NK cells, and their in vitro reactivity after CBT are comparable with those after BMT [29,30], post-CBT incidence of infections including CMV is high [2,7,8,31-35], and immune recovery is probably delayed compared with BMT and PBSCT. Intense reactivation itself can reportedly delay the recovery of cellular immunity [36], which might be associated with the high incidence of CMV disease in the present study. Finally, immunosuppression was intensified to control post-CBT immune reaction. In our study, GVHD prophylaxis was cyclosporine or tacrolimus alone, which was mild compared with conventional transplantation. Immune reaction occasionally occurs before and at engraftment, requiring steroid treatments [6,14,37]. In the present study, 62 patients received steroids within 100 days of CBT. Steroids might have suppressed the recovery of anti-CMV cytotoxic T-lymphocytes [38].

Some challenges remain to improve the management of CMV reactivation after RI-CBT. First, optimal methods need to be established to monitor CMV reactivation. We have introduced preemptive therapy based on the results of CMV antigenemia in our hospital. The efficacy of this method in PBSCT has been reported [17], although it might not be applicable to CBT. Of the 22 patients with CMV disease, 9 progressed from CMV antigenemia to disease despite preemptive therapy. CMV disease developed with less than 10/50,000 pp65-positive cells in 13 patients. These observations suggest that antiviral therapy might be necessary immediately after CMV antigenemia is detected in CBT. More sensitive diagnostic tests such as genetic examinations [39] are also helpful in early detection of CMV reactivation. Second, the optimal preemptive strategy that is applicable to CBT has to be established. Because the disease rate in the untreated CMV positives was 46%, preemptive administration of anti-CMV agents might be required for patients with <10/50,000 pp65-positive cells. Alternatively, universal prophylaxis of CMV might be worth investigating. Optimal dose of preemptive ganciclovir and foscarnet must be also investigated. We reduced doses of preemptive foscarnet and ganciclovir mostly because of concerns of its renal toxicity and myelotoxicity, respectively. However, the failure rate of preemptive ganciclovir or foscarnet was 18%, and it was higher than that in the studies in which those were not reduced [40,41]. Clinical impact of the dose of ganciclovir or foscarnet on preemptive therapy should be investigated in future clinical studies. Finally, identification of high-risk group for CMV reactivation is necessary. The reported risk factors in conventional HSCT include GVHD, steroid administration, CMV serostatus of recipients and donors, and age [22,28,42-44]. The high dose of transfused CD34-positive cells was an independent risk factor for CMV reactivation in our analysis (Table 3). The association between the number of CD34-positive cells and CMV infection has not been reported in previous studies on BMT and PBSCT. It remains unknown and awaits further investigations. aGVHD was an independent risk factor for CMV disease. This is comparable with the report on CMV disease after allo-SCT [25].

Most of the patients with CMV disease had CMV enterocolitis in the present study. None developed CMV pneumonia or retinitis. Although the reason for the high incidence of gastrointestinal CMV disease after RI-CBT remains unclear, the use of TBI and melphalan in the preparative regimens that have significant gastrointestinal mucous toxicity [45] and complications of gut GVHD and thrombotic microangiopathy [14] may be related. Although there are different opinions on the usefulness of antigenemia in diagnosing CMV enterocolitis [46-48], the present study demonstrated that monitoring CMV antigenemia can play a certain role in early diagnosis of CMV enterocolitis after RI-CBT. Further studies are necessary to demonstrate the pathogenesis of gastrointestinal CMV disease after RI-CBT and to develop diagnostic methods for its early detection.

Although the present study provided novel information on CMV infection after RI-CBT, some issues remain to be investigated. First, the present study was retrospective and small sized. Prospective, large-sized studies are awaited. Second, RI-CBT recipients are likely to have potential organ dysfunction because most of them are at advanced ages and have been heavy treated with chemotherapies. Such characteristics of patients may affect the treatment of CMV infection. Pharmacokinetics of antiviral agents in older patients has not been well investigated, requiring further studies. Third, recipient pretransplant CMV serostatus was reported to correlate with mortality after CBT [49]. However, anti-CMV antibodies were not examined before transplantation in this study because most patients had been heavily treated and received multiple transfusions. Pretransplant CMV serostatus needs to be investigated in future studies.

Fourth, day 100 mortality was 49% in the present study. It is higher than that in the previous study reported by the Minnesota group [5]. The exact reason of these differences remains unknown; however, it might be partly from the difference in patient's backgrounds between these studies. This high mortality rate and patient's backgrounds in the present study might have affected the results. Fifth, the management of CMV infection in the present study might have affected the incidence of CMV disease; we used the reduced dose of foscarnet or ganciclovir and anti-CMV high-titer i.v. immunoglobulin was not regularly administered. Finally, late CMV infection remains to be investigated. Because CMV antigenemia-guided pre-emptive strategy has been established [50], the prognosis of CMV infection following BMT and PBSCT improved; however, late CMV disease remains a significant issue [51]. The observation period was short in the present study, and could not provide enough information on late CMV disease.

The present study demonstrated that CMV infection is a significant complication of RI-CBT. Although RI-CBT is an attractive alternative, physicians should be alert to the fact that this transplant procedure is associated with a high risk of CMV infection.

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ORIGINAL ARTICLE

Clinicopathological manifestations and treatment of intestinal transplant-associated microangiopathy

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Intestinal transplant-associated microangiopathy (i-TAM) is an important complication after allogeneic hematopoietic SCT. From 1997 to 2006, 87 of 886 patients with diarrhea after transplantation received colonoscopic biopsy. i-TAM, GVHD and CMV colitis were diagnosed histopathologically. The median duration from transplantation to the onset of diarrhea was 32 days (range: 9–130 days) and that from the onset of diarrhea to biopsy was 12 days (range: 0–74 days). The median maximal amount of diarrhea was 2 l/day (range: 130–5600 ml/day). Histopathological diagnosis included i-TAM ($n=80$), GVHD ($n=26$), CMV colitis ($n=17$) and nonspecific findings ($n=2$) with overlapping. Among 80 patients with i-TAM, abdominal pain was a major symptom, and only 11 patients fulfilled the proposed criteria for systemic TAM. Non-relapse mortality (NRM) among patients without resolution of diarrhea was 72% and i-TAM comprised 57% of NRM. NRM was 25% among patients without intensified immunosuppression, but was 52, 79 and 100% among those with intensified immunosuppression before diarrhea, after diarrhea, and before and after diarrhea, respectively. In conclusion, i-TAM is a major complication presenting massive refractory diarrhea and abdominal pain, which causes NRM. Avoiding intensified immunosuppression that damages vascular endothelium until the resolution of i-TAM may improve transplant outcome.
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Introduction

Transplant-associated microangiopathy (TAM) is an occasional but life-threatening complication after allogeneic hematopoietic SCT (allo-HSCT). [1–3] Diagnostic criteria have been proposed by two large groups. One of which is the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Toxicity Committee Consensus in 2005.[4] In their criteria, TAM was described as a syndrome presenting renal dysfunction, neurological dysfunction and hemolysis with schistocytes and elevated lactate dehydrogenase (LDH). In the criteria proposed by the European Group for Blood and Marrow Transplantation (EBMT) in 2007,[5] TAM was defined as a syndrome presenting more than 4% of schistocytes, thrombocytopenia, elevated LDH, anemia and decreased haptoglobin. Although its etiology and pathogenesis have not been fully clarified, endothelial damage induced by multiple factors, such as conditioning regimens, GVHD, immunosuppressants and infection, contributes to its development.[6–15] Management of TAM is supportive, and withdrawal or decreasing the dose of calcineurin inhibitors is suggested as the first step in the treatment.[4,10]

Severe diarrhea is a serious symptom after allo-HSCT. Acute GVHD and intestinal infection have been the major causes of diarrhea after the resolution of regimen-related intestinal toxicity.[16] Except for infection, severe diarrhea has been treated as intestinal GVHD in many occasions. However, such diarrhea is often refractory to treatment for acute GVHD.[17,18] As one reason for refractoriness to immunosuppressive treatment, we reported for the first time that intestinal tract was also a target organ for TAM.[19] Intestinal TAM (i-TAM) developed as ischemic colitis in some of the patients suffering from severe diarrhea after transplantation. Although clinical symptoms such as abdominal pain and severe and bloody diarrhea resemble those caused by intestinal GVHD, i-TAM can be diagnosed histopathologically by means of colonoscopic biopsies even for patients who do not meet the clinical criteria of systemic