Table 5 Clinical outcome of patients with fasciitis and myositis

Patient no.	*Onset (days from SCT)*	*Onset of chronic GVHD (days from SCT)*	*Treatment for fasciitis or myositis*	*Treatment response*	*Alive or dead*	*Survival (days from onset)*	*Remained disability*
Fasciitis:							
1	212	212	PSL, FK506	Improved with disability	Alive	1464	Joint contracture
2	678	132	PSL, FK506	Improved with disability	Alive	220	Joint contracture
3	728	728	PSL, CsA	Improved with disability	Alive	285	Joint contracture
4	939	164	PSL, CsA, MMF	Improved with disability	Alive	316	Joint contracture
5	1044	146	PSL, FK506	Improved with disability	Alive	1000	Joint contracture
6	1131	165	PSL, FK506, AZP, MMF	Progressive	Dead	337	NA
7	1410	122	PSL, FK506, AZP	Progressive	Dead	529	NA
8	1726	789	PSL, MMF	Resolved	Alive	610	None
Median	991	165					
Myositis:							
6	124	112	PSL, CsA	Resolved	Alive	358	None
5	273	273	CsA	Resolved*	Alive	4064	None
4	380	380	PSL	Resolved	Alive	1598	None
8	432	116	PSL, CsA	Improved with disability	Alive	3083	Muscle atrophy
1	600	110	PSL, CsA	Resolved	Alive	2648	None
3	721	276	PSL, CsA	Resolved	Alive	2010	None
7	789	141	PSL, FK506	Improved with disability	Alive	3348	Spine deformity due to muscle atrophy
2	1110	591	PSL, CsA	Resolved	Alive	968	None
9	1548	100	PSL, FK506	Resolved	Alive	846	None
Median	660	141					

Abbreviations: AZP = azathioprine; MMF = mycophenolate mofetil; PSL = prednisolone.
*Patient 5 with myositis had a recurrence of myositis after tapering off CsA, but it resolved by restarting CsA.[20]

samples showed that the fascia were thickened and loosened, and some showed fibrosis. A patchy lymphocyte infiltration was found on the fibrotic fascia or small aggregates were seen around capillaries (Figure 2a). Immunostaining of CD4 and CD8 antigens revealed that the infiltrating lymphocytes on the fascia were predominantly CD8+ T cells, and the CD8:4 ratio was generally 10:3 (Figure 2b and c). No patient had deep sclerodermatous change but some patients demonstrated mild lymphocyte infiltration in the epidermis and dermis.

The laboratory data at onset showed a slight elevation of C-reactive protein ($\geqslant$0.3 mg/mg/dl) in three patients. An eosinophilia $\geqslant$5% was observed in two patients. The serum creatinine phosphokinase level was normal in all cases. In four cases, antinuclear antibodies were positive. Anti-Jo-1 and anti-Scl-70 antibodies were negative in the tested samples.

Preceding chronic GVHD manifestations occurred in six patients, regardless of the time of onset of fasciitis. The most common organ damage involved lung complications in five patients and sicca syndrome in five patients (Table 2). The lung diseases included BO in four patients, BO organizing pneumonia (BOOP) in one patient, and interstitial pneumonia in one patient.

The immunosuppressive therapies were intensified or restarted for fasciitis in all patients. Six patients responded to the therapy, but five patients had long-term disability such as joint contracture or indurations of skin. Two patients had progressive disease in spite of extensive immunosuppressive treatment and died of disseminated aspergillosis and of multiple organ failure. As of August 2006, six patients were alive and the 5-year survival after developing fasciitis was 50.0%, according to Kaplan–Meier's analysis (Figure 3).

Clinical manifestations and treatment outcome of the patients with myositis

Myositis developed after 124–1548 days (median, 660 days) after SCT. The main clinical manifestations were myalgia in eight patients, muscle weakness in five patients, swelling in five patients and fever in four patients (Table 4). The affected sites of myositis were the upper arms in five patients, forearms in four patients, thighs in seven patients, legs in five patients and the trunk in three patients. The symptoms were symmetrical in all patients.

An MRI scan showed high intensity in muscle in fat-suppressed T2 weighed image (Figures 1c and d). A muscle biopsy, including fascia, was undertaken in six patients. The representative findings were necrosis and reconstruction of muscle cells, irregularity in muscle fibers, interstitial fibrosis and lymphocytic infiltration surrounding the

Table 6 Risk factors for developing fasciitis

Factor	N	*Univariate analysis*		*Multivariate analysis*	
		HR (95% CI)*	*P-value*	*HR (95% CI*)*	*P-value*
GVHD prophylaxis					
CsA-based	1527	1.0			
FK506-based	376	3.54 (0.84–14.94)	0.09		NS
Others	64	0	0.99		
Stem cell source					
BMT	1373	1.0		1.0	
CBT	176	0	0.986	0	0.986
PBSCT	418	7.474 (1.77–31.53)	0.006*	7.474 (1.77–31.53)	0.006*

Abbreviations: CBT = cord blood transplantation; HR = hazard ratio.

muscle fibers and interstitium (Figure 2d). The lymphocytic infiltration was more predominant in the endomysium than in the perimysium. Immunostaining of CD4 and CD8 revealed that the CD8 + T cells were dominant, with a CD8:4 ratio of approximately 2:1 (Figures 2e and f). Electromyography was examined in seven of nine patients and showed myopathic changes. The serum C-reactive protein was elevated in eight of nine patients. Three patients had eosinophilia (≥5%) and eight patients had elevated transaminases. All patients had elevated levels of creatinine phosphokinase (maximum, 4864 U/l). Rheumatoid factor and antinuclear antibodies were present in two cases. Anti-Jo-1 and anti-Scl-70 antibodies were negative.

All patients had preceding chronic GVHD manifestations other than myositis. The main symptoms of chronic GVHD involved the oral mucosa in five patients, the skin in five patients, sicca syndrome in four patients and liver disease in four patients.

All patients responded to immunosuppressive treatment. One patient relapsed after tapering the immunosuppressive treatment, but was successfully treated by increasing the immunosuppression.[20] As of August 2006, all patients are alive. Two patients had residual disabilities, which included muscle atrophy and deformity of vertebrae caused by muscle weakness. The overall survival rate after the onset of myositis was 100% (Figure 3).

Risk factors for developing fasciitis and myositis

Related PBSCT was a significant risk factor for developing fasciitis in comparison with BMT by multivariate analysis (the hazard ratio: 7.474, $P = 0.006$; Table 5). There were no other significant risk factors for developing fasciitis such as a conditioning regimen or acute GVHD, although GVHD prophylaxis with FK506 was a significant factor in univariate analysis (Table 6). For myositis, there were no significant risk factors in univariate analysis (data not shown).

Discussion

In this study, the 5-year cumulative incidence of fasciitis and myositis by chronic GVHD was 0. 55 and 0.54%, respectively, which were lower than those of previous reports. Janin *et al.*[7] reported that 14 out of 475 patients (2.9%) who underwent Allo-BMT from an HLA-identical sibling donor developed fasciitis. Parker *et al.*[10] also showed that the rate of polymyositis among patients who developed chronic GVHD after Allo-BMT from a sibling donor was 3.4%. In this study, only one patient, who underwent R-BMT from an HLA-single locus mismatched donor, developed fasciitis out of 645 R-BMT recipients. No one with R-BMT from an HLA-identical donor developed fasciitis and only two patients of 732 UR-BMT recipients developed fasciitis. We had no fasciitis or myositis in the cord blood transplantation recipients.

For fasciitis, R-PBSCT was a significant risk factor compared to other stem cell sources, and no risk factors were found for myositis. Flowers *et al.*[21] reported that chronic GVHD after PBSCT might be more protracted and more refractory than after BMT. They also reported that skin involvement was more frequent in PBSCT recipients than in BMT recipients. It is interesting that R-PBSCT patients were also associated with the development of fasciitis as well as skin involvement, even though the mechanisms are unknown.

As compared with westerners, the incidence of chronic GVHD in Japanese is lower and its manifestation is slightly different.[4,22] Atsuta *et al.*[22] reported that prognostic scoring systems, which were developed on the basis of clinical findings for western patients, did not produce an effective categorization for Japanese patients. The proportion of a progressive type onset of chronic GVHD in Japanese patients is much lower than that in western countries, and the extent of cutaneous chronic GVHD was also different.[23] This may be explained by the great genetic homogeneity of the Japanese population. However, because of the retrospective nature of this study, there is a possibility that mild form of fasciitis and scleroderma might have missed, thereby , underestimating their incidence.

In fasciitis and myositis, CD8 + T cells were predominantly infiltrated in both fasciitis and myositis. Kojima *et al.*[24] reported the clonal expansion of limited T-cell clonotypes in affected muscle of post-transplant myositis patients. The alloreactive T cells may expand in muscle or fascia through stimulation by limited kinds of antigens. If fasciitis or myositis is suspected, a deep biopsy including skin, subcutaneous fat, intermediate septa, fascia and muscle should be performed for proper diagnosis. However, it might not be mandatory for making a diagnosis of

fasciitis or myositis, because an MRI scan is useful in the context of other symptoms and clinical and laboratory data, and it can effectively confirm the affected lesions.

Almost all patients with fasciitis or myositis were complicated with other manifestations of chronic GVHD, but there were different tendencies regarding the involved organs among the patients with fasciitis and myositis. In patients with fasciitis, lung disease (BO) and sicca syndrome were more frequent than oral and skin involvement. Pulmonary complications will affect the patient's prognosis, because they can cause severe respiratory failure. So the fact that patients with fasciitis tend to have pulmonary complications might be related to their poor prognosis. Some authors reported severe respiratory failure caused by weakness of respiratory muscles in patients with myositis,[25,26] but we had no myositis patients with respiratory failure.

Previous reports have indicated that steroid treatment generally worked well for patients with eosinophilic fasciitis.[8,27] On the other hand, fasciitis caused by chronic GVHD often seemed to result in functional disabilities such as skin tightness, severe joint stiffness and joint contracture, despite continuous steroid therapy and other immunosuppressive treatment for chronic GVHD.[7] In myositis, some reported cases showed a recurrence with either a reduction or cessation of immunosuppressant therapy.[10 12,20] One of our patients also had recurrent acute myositis after CsA was tapered, and achieved a second remission of polymyositis with CsA.[20] We need to recognize that both fasciitis and myositis caused by chronic GVHD can result in disabilities that reduce the patient's QOL. We have to make an early diagnosis and promptly treat fasciitis and myositis to prevent the progression of the complications.

In conclusion, we identified the incidence of fasciitis and myositis as chronic GVHD manifestations according to various stem cell sources and found that R-PBSCT was a significant risk factor for fasciitis. Because the treatment response for myositis was fairly good, an early diagnosis by a biopsy, which includes fascia and muscle or MRI, and prompt treatment are important to prevent impairment of the patient's QOL with persistent disability.

References

1 Armitage JO. Bone marrow transplantation. *N Engl J Med* 1994; **330**: 827–838.
2 Atkinson K, Horowitz MM, Gale RP, van Bekkum DW, Gluckman E, Good RA *et al.* Risk factors for chronic graft-versus-host disease after HLA-identical sibling bone marrow transplantation. *Blood* 1990; **75**: 2459–2464.
3 Farag SS. Chronic graft-versus-host disease: where do we go from here? *Bone Marrow Transplant* 2004; **33**: 569–577.
4 Ozawa S, Nakaseko C, Nishimura M, Maruta A, Cho R, Ohwada C *et al.* Chronic graft-versus-host disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program. *Br J Haematol* 2007; **137**: 142–151.
5 Socie G, Stone JV, Wingard JR, Weisdorf D, Henslee-Downey PJ, Bredeson C *et al.* Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. *N Engl J Med* 1999; **341**: 14–21.
6 Duell T, van Lint MT, Ljungman P, Tichelli A, Socie G, Apperley JF *et al.* Health and functional status of long-term survivors of bone marrow transplantation. EBMT Working Party on Late Effects and EULEP Study Group on Late Effects. European Group for Blood and Marrow Transplantation. *Ann Intern Med* 1997; **126**: 184–192.
7 Janin A, Socie G, Devergie A, Aractingi S, Esperou H, Verola O *et al.* Fasciitis in chronic graft-versus-host disease. A clinicopathologic study of 14 cases. *Ann Intern Med* 1994; **120**: 993–998.
8 Markusse HM, Dijkmans BA, Fibbe WE. Eosinophilic fasciitis after allogeneic bone marrow transplantation. *J Rheumatol* 1990; **17**: 692–694.
9 van den Bergh V, Tricot G, Fonteyn G, Dom R, Bulcke J. Diffuse fasciitis after bone marrow transplantation. *Am J Med* 1987; **83**: 139–143.
10 Parker P, Chao NJ, Ben-Ezra J, Slatkin N, Openshaw H, Niland JC *et al.* Polymyositis as a manifestation of chronic graft-versus-host disease. *Medicine (Baltimore)* 1996; **75**: 279–285.
11 Stevens AM, Sullivan KM, Nelson JL. Polymyositis as a manifestation of chronic graft-versus-host disease. *Rheumatology (Oxford)* 2003; **42**: 34–39.
12 Couriel DR, Beguelin GZ, Giralt S, De Lima M, Hosing C, Kharfan-Dabaja MA *et al.* Chronic graft-versus-host disease manifesting as polymyositis: an uncommon presentation. *Bone Marrow Transplant* 2002; **30**: 543–546.
13 Tse S, Saunders EF, Silverman E, Vajsar J, Becker L, Meaney B. Myasthenia gravis and polymyositis as manifestations of chronic graft-versus-host-disease. *Bone Marrow Transplant* 1999; **23**: 397–399.
14 Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ *et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; **11**: 945–956.
15 Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975; **292**: 344–347.
16 Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). *N Engl J Med* 1975; **292**: 403–407.
17 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 457–481.
18 Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat* 1988; **16**: 1141–1154.
19 Goldstein R, Duvic M, Targoff IN, Reichlin M, McMenemy AM, Reveille JD *et al.* HLA-D region genes associated with autoantibody responses to histidyl-transfer RNA synthetase (Jo-1) and other translation-related factors in myositis. *Arthritis Rheum* 1990; **33**: 1240–1248.
20 Sato N, Okamoto S, Mori T, Watanabe R, Hamano Y, Kawamura J *et al.* Recurrent acute myositis after allogeneic bone marrow transplantation for myelodysplasia. *Hematology* 2002; **7**: 109–112.
21 Flowers ME, Parker PM, Johnston LJ, Matos AV, Storer B, Bensinger WI *et al.* Comparison of chronic graft-versus-host disease after transplantation of peripheral blood stem cells versus bone marrow in allogeneic recipients: long-term follow-up of a randomized trial. *Blood* 2002; **100**: 415–419.
22 Atsuta Y, Suzuki R, Yamamoto K, Terakura S, Iida H, Kohno A *et al.* Risk and prognostic factors for Japanese patients with chronic graft-versus-host disease after bone

marrow transplantation. *Bone Marrow Transplant* 2006; **37**: 289–296.
23 Akpek G, Lee SJ, Flowers ME, Pavletic SZ, Arora M, Lee S *et al*. Performance of a new clinical grading system for chronic graft-versus-host disease: a multicenter study. *Blood* 2003; **102**: 802–809.
24 Kojima K, Kurokawa MS, Tanimoto K, Kojima Y, Hara M, Yoshino T *et al*. Clonal expansion of limited T cell clonotypes in affected muscle from a patient with post-transplant polymyositis. *Bone Marrow Transplant* 2002; **30**: 467–470.
25 Leano AM, Miller K, White AC. Chronic graft-versus-host disease-related polymyositis as a cause of respiratory failure following allogeneic bone marrow transplant. *Bone Marrow Transplant* 2000; **26**: 1117–1120.
26 Stephenson AL, Mackenzie IR, Levy RD, Road J. Myositis associated graft-versus-host-disease presenting as respiratory muscle weakness. *Thorax* 2001; **56**: 82–84.
27 Ustun C, Ho Jr G. Eosinophilic fasciitis after allogeneic stem cell transplantation: a case report and review of the literature. *Leuk Lymphoma* 2004; **45**: 1707–1709.

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LETTER TO THE EDITOR

Development of varicella after allogeneic cord blood transplantation in a varicella zoster virus seropositive patient

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Dear Sir

Cord blood (CB) has been established as 1 of the stem cell sources for both adult and pediatric patients requiring allogeneic hematopoietic stem cell transplantation (HSCT) [1,2]. The most common life-threatening complication after CB transplantation (CBT) is infection, mostly bacterial infection. The high incidence of bacterial infection after CBT can be explained by the delayed neutrophil engraftment particularly in adult patients [3,4]. Furthermore, several investigators and we have reported a notably high incidence of viral infection after CBT as well as cytomegalovirus and human herpes virus type-6 [5,6]. A high incidence of viral infection is considered attributable to the absence of antigen-specific T-lymphocytes in cord blood [5–7]. Because of the exclusive component of naive T-lymphocytes in cord blood, a primary infection of varicella zoster virus (VZV) could occur more frequently after CBT than HSCT using other stem cell sources. However, most of the reported infection of VZV is its reactivation causing herpes zoster, as in other types of HSCT [8]. These observations could be explained by the recipient's residual humoral immunity or the transferred immunity from the donor. Specific IgG is generally used as a useful parameter of the host's defense against primary VZV infection, varicella, even in HSCT recipients [9]. Therefore, the recipients of HSCT at risk for developing varicella are identified according to their VZV serostatus [9]. In contrast to this general practice, we have experienced a patient who developed varicella after CBT despite the fact that the patient possessed a high titer of anti-VZV IgG.

A 33-y-old male with acute myelogenous leukemia (AML) underwent CBT from a human leukocyte antigen (HLA)-mismatched unrelated donor. The conditioning regimen consisted of total body irradiation (12 Gy), cytarabine (8 g/m^2), and cyclophosphamide (120 mg/kg). Before transplantation, he was positive for anti-VZV IgG, with an index of 43.0 by enzyme-linked immunosorbent assay (ELISA; positive index $\geq$2.0). Cyclosporine A (CsA) and short-term methotrexate were used as a prophylaxis for graft-versus-host disease (GVHD). His post-transplant course was complicated with acute GVHD involving the skin and gastrointestinal tract, which was successfully treated by adding prednisolone to CsA. He did not develop chronic GVHD, and CsA was discontinued 7 months after CBT. One y after CBT, a relapse of AML was observed, and 2 courses of high-dose cytarabine were given. 28 d after the second course of high-dose cytarabine, multiple vesicular skin eruptions appeared over the upper and lower extremities, and over the trunk and head without pain or paresthesia. A Tzanck smear from the vesicle showed multinucleated giant cells, the sign of herpes virus infection. Based on these findings, varicella was diagnosed and intravenous acyclovir was given for 14 d in combination with intravenous immunoglobulin. All the eruptions had dried out by the completion of this treatment. At the

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onset of varicella, anti-VZV IgG remained positive (index 17.0 by ELISA described above), and anti-VZV IgM was negative.

In general, serum specific IgG, or serology, is used for the evaluation of anti-VZV immunity [9,10]. However, cell-mediated immunity is considered to play a more important role in the host defense than humoral immunity, possibly because VZV is cell-associated during active infection [10]. Cell-mediated immunity for VZV can be evaluated by some assays such as lymphocyte proliferation in response to VZV antigen, cytotoxicity and enzyme-linked immunosorbent spot forming cell assay, although these assays are only available for use in research laboratories. Since both humoral and cell-mediated immunity are closely related and not discrete, humoral immunity reflects the host's immunity against VZV. Therefore, serology has been widely used as the key to decide whether or not measures should be given at the exposure to VZV, both in healthy individuals and HSCT recipients [9]. The VZV serostatus of donors of HSCT other than CBT is generally positive, since they are usually immunized by primary VZV infection (varicella) or vaccination. However, in the setting of CBT, the donors are exclusively VZV-seronegative, and the graft lacks T-lymphocytes against VZV. Thus, if a recipient still possesses residual humoral immunity, he or she could be a VZV-seropositive patient but not have specific T-lymphocytes responsive against VZV after CBT. This discrepancy between humoral immunity and cell-mediated immunity might mislead physicians, and might explain the mechanisms for the development of varicella in our VZV-seropositive patient after CBT.

Although serology is still an important parameter to evaluate the susceptibility to varicella, transplant physicians should recognize the possibility of the development of varicella after CBT even if the recipients are VZV-seropositive. In the case of exposure to VZV, CBT recipients should be considered as candidates for prophylactic therapy such as intravenous immunoglobulin or acyclovir administration regardless of the recipient's serostatus.

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References

[1] Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, et al. Outcomes among 562 recipients of placental blood transplants from unrelated donors. N Engl J Med 1998;339:1565–77.

[2] Schoemans H, Theunissen K, Maertens J, Boogaerts M, Verfaillie C, Wagner J. Adult umbilical cord blood transplantation: a comprehensive review. Bone Marrow Transplant 2006;8:83–93.

[3] Safdar A, Rodriguez GH, De Lima MJ, Petropoulos D, Chemaly RF, Worth LL, et al. Infections in 100 cord blood transplantations: spectrum of early and late post-transplant infections in adult and pediatric patients1996–2005. Medicine 2007;86:324–33.

[4] Hamza NS, Lisgaris M, Yadavalli G, Nadeau L, Fox R, Fu P, et al. Kinetics of myeloid and lymphocyte recovery and infectious complications after unrelated umbilical cord blood versus HLA-matched unrelated donor allogeneic transplantation in adults. Br J Haematol 2004;124:488–98.

[5] Tomonari A, Iseki T, Ooi J, Takahashi S, Shindo M, Ishii K, et al. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients: a single institute experience in Japan. Br J Haematol 2003;121:304–11.

[6] Yamane A, Mori T, Suzuki S, Mihara A, Yamazaki R, Aisa Y, et al. Risk factors for developing human herpes virus 6 (HHV-6) reactivation after allogeneic hematopoietic stem cell transplantation and its association with central nervous system disorders. Bone Marrow Transplant 2007;13:100–6.

[7] Cohen G, Carter SL, Weinberg KI, Masinsin B, Guinan E, Kurtzberg J, et al. Antigen-specific T-lymphocyte function after cord blood transplantation. Biol Blood Marrow Transplant 2006;12:1335–42.

[8] Varicella zoster virus infection in adult patients after unrelated cord blood transplantation: a single institute experience in Japan. Br J Haematol 2003;122:802–5.

[9] Centers for Disease Control and Prevention; Infectious Diseases Society of America; American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant 2000;6:659–713.

[10] Grose C. The immunology of VZV infection. Herpes 2006; 13(Suppl 1):A9–12.

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Infectious complications in chronic graft-versus-host disease: a retrospective study of 145 recipients of allogeneic hematopoietic stem cell transplantation with reduced- and conventional-intensity conditioning regimens

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Abstract: To assess infectious complications associated with chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) with reduced- and conventional-intensity conditioning regimens (RIC, $n = 91$; CIC, $n = 54$, respectively), we retrospectively analyzed data from 145 consecutive patients with cGVHD after allogeneic HSCT from a human leukocyte antigen-matched related or unrelated donor. In the present retrospective analysis, 57% (83/145) of patients with cGVHD developed infections, with a mortality rate of 27% (22/83). The incidences of bacteremia ($n = 28$), central venous catheter-related infections ($n = 11$), bacterial pneumonia ($n = 4$), invasive aspergillosis ($n = 7$), and adenoviral hemorrhagic cystitis ($n = 8$) were significantly higher in patients with prednisolone dose ≥ 1 mg/kg at the time of diagnosis of cGVHD. The present results suggest that infections associated with cGVHD, especially after high-dose prednisolone, are predictive of poor outcome regardless of whether the patient received RIC or CIC.

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Key words: infectious complication; chronic graft-versus-host disease; allogeneic hematopoietic stem cell transplantation; reduced-intensity conditioning; HLA-matched donor

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Infectious complications contribute to morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT). Well-known factors affecting susceptibility to infections include donor type, conditioning regimen, development of graft-versus-host disease (GVHD), and environmental factors. Reduced-intensity conditioning (RIC) regimens are thought to lower the risk of infections because they involve relatively little damage to vital organs (1). However, our experience indicates that with both RIC and conventional-intensity conditioning (CIC) regimens, the incidence of bacterial infections during neutropenia and *Aspergillus* infections is high after allogeneic HSCT (2, 3). Thus, it appears that RIC alone is not sufficient to improve the safety of allogeneic HSCT.

GVHD and the treatment of GVHD with immunosuppressive drugs are also well-known predominant risk factors for the development of opportunistic infections (4–6). In the case of acute GVHD, inpatients can be given comprehensive prophylaxis, including environmental control, to prevent infections over the short term. In contrast, chronic GVHD (cGVHD) is most often a late complication of allogeneic HSCT, and is usually treated on an outpatient basis. Consequently, the resources that can be used to control infections in patients with cGVHD are limited, and prophylaxis should be considered as a long-term approach, taking into account the safety and emergence of drug-resistant pathogens. In Japanese patients, the incidence of cGVHD after allogeneic HSCT is reportedly as high as 50%, with 20% of those who develop cGVHD contracting concurrent infections (7). At present, more transplantation procedures are being performed with peripheral blood stem cell (PBSC) products, in older patients, and with

unrelated donors. The available evidence suggests that all of these factors would result in greater numbers of patients with cGVHD. Thus, management of cGVHD is one of the greatest challenges to physicians practicing HSCT.

In the present study, we evaluated infectious complications associated with cGVHD in patients who received an RIC or a CIC regimen before undergoing PBSC transplantation (PBSCT) from a human leukocyte antigen (HLA)-matched relative (related PBSCT) or bone marrow transplantation (BMT) from an HLA-matched unrelated volunteer (unrelated BMT).

Patients and methods

Patient characteristics

We retrospectively analyzed data from 145 consecutive adult patients with hematologic malignancies who had received allogeneic HSCT with an RIC ($n = 91$) or CIC ($n = 54$) regimen between January 2000 and December 2004 at our institution. All of these 145 patients had sustained engraftment, had survived for >100 days following transplantation, and had developed cGVHD. The following types of patients were excluded: patients who suffered from disease progression before the development of cGVHD and received donor lymphocyte infusion, and patients with a history of previous allogeneic HSCT. Significant differences were observed between the RIC and CIC groups in terms of the age of the patients and donors, the gender of the patients, diagnosis, disease risk (8), time from diagnosis to transplantation, donor type and source of stem cells, and GVHD prophylaxis. The patient characteristics are summarized in Table 1. Typing for HLA-A, -B, and -DR antigens of the donor and recipient was performed using low-resolution DNA typing. The frequency with which allogeneic PBSCT is performed in Japan has been increasing since it became eligible for reimbursement from health insurance organizations in the year 2000, and our banking system only approves donation of bone marrow. The clinical characteristics of cGVHD, including use of immunosuppressive drugs at diagnosis and initial treatment, are summarized in Table 2. The present study was approved by the Ethics Committee of our institution, and all 145 subjects provided informed consent.

Conditioning regimen and supportive care

The CIC regimen consisted of cyclophosphamide (CY, 120 mg/kg), in combination with either 12 Gy total-body irradiation (TBI, $n = 25$) or busulfan (BU, 16 mg/kg; $n = 29$). The RIC regimen consisted of BU (8 mg/kg) in combination with either fludarabine (Flu, 180 mg/m^2; $n = 70$) or 2-chlorodeoxyadenosine (2-CdA, 0.66 mg/kg; $n = 21$); 14 patients received either anti-thymocyte globulin (ATG, 5–10 mg/kg; $n = 6$) or 4 Gy TBI ($n = 8$). All patients received cyclosporine (CSP, 3 mg/kg/day; $n = 137$) or tacrolimus (TAC, 0.03 mg/kg/day; $n = 8$), with ($n = 78$) or without ($n = 67$) short courses of methotrexate (MTX; related PBSCT, 10 mg/m^2 on day 1, and 7 mg/m^2 on days 3 and 6; unrelated BMT, 10 mg/m^2 on days 3, 6, and 11) as GVHD prophylaxis. All patients received prophylactic ciprofloxacin (200 mg orally 3 times daily) for prevention of infections until neutrophil recovery. Trimethoprim-sulfamethoxazole (80 mg of trimethoprim once daily) was administered for the prevention of *Pneumocystis* pneumonia and encapsulated bacterial infection, from the first day of the conditioning regimen until day 3, and from day +30 until 6 months after transplantation, or for prolonged periods in patients with cGVHD. Patients also received oral or intravenous fluconazole (100 mg once daily) for prevention of infection by *Candida* species, and low-dose acyclovir (600 mg until engraftment, and then 100 mg/day orally), starting at the same time as the conditioning regimens and continuing until cessation of administration of immunosuppressive drugs (9). Cytomegalovirus (CMV) antigenemia was monitored weekly until cessation of the administration of immunosuppressive drugs. Testing for CMV antigenemia consisted of direct immunoperoxidase staining of leukocytes with a peroxidase-labeled monoclonal antibody. Quantitative real-time polymerase chain reaction was not performed.

Definition of outcome

Patients with grades II–IV acute GVHD were treated with prednisolone (PSL) according to a standard regimen (10). Chronic GVHD was assessed and graded according to the standard criteria (11). The diagnosis and staging of cGVHD were also assessed according to the working report published by the National Institutes of Health Consensus Development Project (12). Relapse was defined either by morphologic evidence of the disease in the peripheral blood, marrow, or extramedullary sites, or by recurrence and persistence of pre-transplant chromosomal abnormalities in cytogenetic analysis of the marrow cells.

Infectious complications

A documented infection was defined as signs and symptoms associated with microbiological documentation of a pathogen from the site of infection. Culture-documented bacteremia, fungemia, or viremia was considered to be a definite infection, regardless of symptoms. On the other hand, clinical infection was defined as signs or symptoms consistent with an infection, but without microbiological confirmation. Central venous catheter (CVC)-related

Patient characteristics and transplant outcomes

	RIC (n = 91)	CIC (n = 54)	P
Median age of patients (range)	55 (26–68)	37 (18–53)	< 0.0001
Median age of donors (range)	50 (17–69)	34 (19–54)	< 0.0001
Male/female patient	57[1]/34	22/32	0.015
Female donor for male patient	19	10	0.83
Diagnosis AML (+ MDS)	27 (9)	17 (4)	0.0029
MDS	17	4	
CML	7	12	
ALL	1	8	
ML	36	13	
Others[2]	3	0	
Disease risk group (standard/advanced)[3]	14/77	21/33	0.0023
Median time interval[4] (range), (months)	19 (2–178)	10 (1–100)	0.014
KPS[5] $\leq$ 80%	10	5	0.41
HCT-SCI[6] $\geq$ 2	13	7	0.99
Prior infectious complications	6	3	0.99
Prior autologous transplantation	5	2	0.99
Donor type and source of stem cells			
Related PBSC/Unrelated BM	82/9	34/20	0.0002
GVHD prophylaxis			
CSP orTAC alone/MTX with CSP orTAC	66/25	1/53	< 0.0001
Acute GVHD grade II/III/IV	24/23/3	18/8/2	0.072
Median onset day (range) of grades II–IV acute GVHD[7]	39 (12–97)	32 (14–91)	0.48
Prior use of PSL for acute GVHD			
0.5– < 1.0/1.0– < 2.0/ $\geq$ 2.0 mg of PSL/kg	5/34/18	4/13/9	0.27
Relapse/progressive disease following cGVHD	16	10	0.99
Cause of death	30	20	0.27
Infection	15[8]	7[8]	
Chronic GVHD	9[8]	8[8]	
Lungs/gastrointestinal tract/MOF/Others[9]	3/1/3/2	3/3/2/0	
Others[10]	3	6	
Progression	8	2	
Median follow-up (range), (months)	39 (5–73)	45 (15–79)	0.20

[1]Number of patients, unless indicated otherwise.
[2]Others = myelofibrosis (n = 1), chronic lymphocytic leukemia (n = 1), and multiple myeloma (n = 1).
[3]Patients who were considered standard risk with a diagnosis of AML + MDS or AML, or ALL in first complete remission, CML in first chronic phase, or untreated refractory anemia in MDS. All other conditions were considered to indicate advanced risk.
[4]Time from diagnosis to transplantation.
[5]KPS was evaluated before the start of the conditioning regimen, and was graded according to Karnofsky performance status score.
[6]HCT-SCI was evaluated before the start of the conditioning regimen, and was graded according to hematopoietic cell transplantation-specific comorbidity index (ref. 8).
[7]Time from occurrence of grades II–IV acute GVHD to transplantation.
[8]Total number of patients differs because 8 patients (RIC, 5; CIC, 3) died of both infection and chronic GVHD.
[9]Others = renal (n = 1) and liver (n = 1).
[10]Others = RIC: cerebral infarction (n = 1), secondary hepatocellular carcinoma (n = 1), infection following secondary allogeneic cord blood stem cell transplantation; CIC: acute myocardial infarction (n = 1), cerebral infarction (n = 1), drug-induced interstitial pneumonia (n = 1), infection following chemotherapy (n = 1), and suicide (n = 2).
RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; PBSC, peripheral blood stem cell; BM, bone marrow; CSP, cyclosporine; TAC, tacrolimus; MTX, methotrexate; GVHD, graft-versus-host disease; PSL, prednisolone; cGVHD, chronic graft-versus-host disease; MOF, multiple organ failure.

Table 1

Clinical characteristics of cGVHD

	RIC	CIC	*P*
Median onset day (range)[1]	100 (79–479)	109 (93–348)	0.51
Limited/extensive	5/86	1/53	0.41
De novo/quiescent/progressive	27/38/26	24/9/21	0.16
KPS score 1/2/3	61/16/8	46/2/2	0.045
Skin score 1/2/3	27/33/8	17/12/3	0.15
Mouth score 1/2/3	40/29/3	23/9/1	0.87
Eyes score 1/2/3	30/14/7	15/9/1	0.38
Gastrointestinal tract score 1/2/3	28/4/12	19/1/7	0.84
Liver score 1/2/3	7/23/44	6/13/24	0.89
Lungs score 1/2/3	6/8/4	8/7/1	0.26
Joints and fascia score 1/2/3	13/2/0	8/5/1	0.13
Genital tract score 1/2/3	1/0/0	0/0/0	0.99
Eosinophilia $>0.5 \times 10^9$/L	30	22	0.37
Platelets $<100 \times 10^9$/L	26	20	0.36
Others[2]	5	2	0.39
Immunosuppressive drugs at diagnosis of cGVHD			
CSA/TAC	66/3	37/3	0.76
<0.5/0.5–<1.0/1.0–<2.0/ $\geq$ 2.0 mg of PSL/kg	17/9/11/2	9/2/3/1	0.57
Initial treatment for cGVHD			
Addition or increased dose of CSA/TAC	69/7	41/4	0.99
<0.5/0.5–<1.0/1.0–<2.0/ $\geq$ 2.0 mg of PSL/kg	18/14/15/4	10/7/6/2	0.74
Median follow-up from diagnosis of cGVHD (range) (months)	39 (5–73)	45 (15–79)	0.26

[1]Time from occurrence of cGVHD to transplantation.
[2]Others = pleural effusion ($n = 4$), pericardial effusion ($n = 3$), ascites ($n = 3$), and polymyositis ($n = 1$).
RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; cGVHD, chronic graft-versus-host disease; KPS, Karnofsky performance status; CSP, cyclosporine; TAC, tacrolimus; PSL, prednisolone.

Table 2

infections consisted of exit site infections without bacteremia. Bacterial pneumonia was included in the category of definite infections, and was diagnosed by chest x-ray examination or computed tomography (CT) and identification of a bacterial pathogen on culture of sputum, bronchoalveolar lavage fluid, pleural fluid, or blood specimen. Fungal infections, including proven or probable invasive fungal infections, were diagnosed by identification of a fungal pathogen on culture or *Aspergillus* antigen and CT examination according to consensus criteria (13). Pneumonia of unknown origin was included in the category of undefined pneumoniae, which were diagnosed by chest x-ray and/or CT. There was no significant difference in CMV serostatus between the RIC and CIC groups (data not shown). A polymicrobial infection of 1 organ or several adjacent organs was considered to be a single infection. Death associated with a documented infection was defined as the death of a patient with findings consistent with an infection, or as detection of the pathogen in an autopsy specimen.

Statistical analysis

Comparisons of variables were performed using the 2-tailed Fisher exact test or the χ^2 test. Continuous variables were compared by the Mann–Whitney *U*-test. All *P*-values were 2-sided, and the type I error rate was fixed at $P < 0.05$.

Results

Transplant outcomes

The transplant outcomes are summarized in Table 1. Twenty-two patients (RIC, $n = 15$; CIC, $n = 7$) died of infections, of whom 8 patients (RIC, $n = 5$; CIC, $n = 3$) died of

both infections and chronic GVHD, with cGVHD at a median follow up of 40 months from transplantation (RIC, 39 vs. CIC, 45 months). The median onset of cGVHD was 112 days (RIC, 100 vs. CIC, 109 days), and 47 patients (RIC, $n = 26$; CIC, $n = 21$) developed progressive-type cGVHD at a median follow up of 32 months from diagnosis of cGVHD (RIC, 39 vs. CIC, 45 months). The severity of the Karnofsky performance status (KPS) score was significantly greater in the RIC group ($P = 0.045$).

Infectious complications

A total of 134 infectious episodes occurred in 83 patients (RIC, 51 vs. CIC, 32; $P = 0.73$), as shown in Table 3. Of these, 28 patients (RIC, 18 vs. CIC 10; $P = 0.83$) developed bacteremia, the causative organisms (43 positive cultures) of which are summarized in Table 4. Gram-positive bacteremia (27 positive cultures) was more common than gram-negative bacteremia (16 positive cultures). The bacteremia was caused by 2, 3, and 4 types of organisms in 4, 4, and 1 patient, respectively. The incidence of bacteremia was significantly higher in patients with the following factors: cGVHD including progressive types ($n = 15$, $P = 0.0027$), a KPS score ≥ 2 ($n = 11$, $P = 0.0062$) and a gastrointestinal (GI) score ≥ 2 ($n = 13$, $P < 0.0001$); PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 9$, $P = 0.00090$) and for the initial treatment of cGVHD ($n = 11$, $P = 0.0050$). CVC-related infections ($n = 11$) were caused by *Staphylococcus epidermidis* ($n = 4$), *Staphylococcus* species ($n = 2$), *Stenotrophomonas maltophilia* ($n = 2$), *Acinetobacter iwoffii* ($n = 1$), *Corynebacterium* species ($n = 1$), or methicillin-resistant *Staphylococcus aureus* (MRSA, $n = 1$). The incidence of CVC-related infections was significantly higher in patients with PSL dose ≥ 1 mg/kg at the time of diagnosis of cGVHD ($n = 4$, $P = 0.026$). Bacterial pneumonia was observed in 4 patients, and the isolated organisms were as follows: *Pseudomonas aeruginosa* ($n = 1$), *Hemophilus influenzae* ($n = 1$), *S. epidermidis* ($n = 1$), and *Staphylococcus* species ($n = 1$). The incidence of bacterial pneumonia ($n = 4$) was significantly higher in patients with PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 3$, $P = 0.0051$) and for the initial treatment of cGVHD ($n = 3$, $P = 0.021$). Invasive aspergillosis (IA) and *Candida* infections developed in 7 and 3 patients, respectively. All patients with IA had been given ≥ 0.5 mg of PSL/kg at the time of diagnosis of cGVHD. The incidence

Infectious complications associated with cGVHD

	Total (median onset, range, days)	RIC	CIC	*P*
Bacterial infections				
Bacteremia	28 (175, 104–1629)	18 (5)[1]	10 (2)	0.83
CVC-related	11 (123, 101–1774)	5 (0)	6 (0)	0.33
Pneumonia	4 (311, 101–1045)	3 (2)	1 (1)	0.99
Others[2]	16 (302, 102–1065)	7 (4)	9 (2)	0.11
Fungal infections				
Candida infection	3 (128, 101–358)	1 (0)	2 (0)	0.56
Invasive aspergillosis	7 (181, 112–1232)	6 (0)	1 (0)	0.26
Viral infections				
Adenoviral hemorrhagic cystitis	8 (192, 111–538)	5 (0)	3 (0)	0.99
CMV colitis	1 (343)	0 (0)	1 (0)	0.37
Cutaneous VZV	18 (502, 106–1684)	12 (0)	6 (0)	0.80
Influenza	4 (483, 355–898)	1 (0)	3 (0)	0.15
Others[3]	2 (133, 103–164)	1 (0)	1 (0)	0.99
CMV antigenemia	15 (140, 104–448)	11 (0)	4 (0)	0.42
Pneumoniae of unknown origin	32 (283, 101–1735)	18 (4)	14 (4)	0.41

[1]Number of infectious episodes (number of deaths) is shown.
[2]Others = sepsis of unknown origin (4 episodes), dermatitis (3), hemorrhagic cystitis (2), otitis media (2), meningitis (2), cholecystitis (1), pseudomembranous enterocolitis (1), and urinary tract infection (1).
[3]Others = herpes simplex viral esophagitis (1 episode) and meningitis (1).
cGVHD, chronic graft-versus-host disease; RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; CVC, central venous catheter; CMV, cytomegalovirus; VZV, varicella zoster virus.

Table 3

Bacteremia associated with cGVHD

	RIC ($n = 18$)	CIC ($n = 10$)
Gram-positive organisms	16[1]	11
Staphylococcus epidermidis	7	2
Streptococcus species	2	3
Enterococcus species	3	0
Staphylococcus species	0	3
Bacillus species	0	1
Corynebacterium species	1	0
MRSA	0	1
Gram-positive cocci	3	1
Gram-negative organisms	10	6
Bacteroides species	3	2
Pseudomonas aeruginosa	2	2
Klebsiella species	2	0
Enterobacter species	0	1
Escherichia coli	0	1
Gram-negative rods	3	0

[1]Number of positive cultures.
cGVHD, chronic graft-versus-host disease; RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 4

of IA was significantly higher in patients with cGVHD including a GI score ≥ 2 ($n = 4$, $P = 0.015$), PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 4$, $P = 0.0037$), and for the initial treatment of cGVHD ($n = 7$, $P < 0.0001$). Eighteen patients developed cutaneous varicella zoster virus (VZV); all responded promptly to acyclovir. Eight patients developed adenoviral hemorrhagic cystitis (HC); 2 of these 8 patients developed continuously complicated lethal bacteremia. The incidence of adenoviral HC was significantly higher in patients with cGVHD including a KPS score ≥ 2 ($n = 5$, $P = 0.0071$) and a GI score ≥ 2 ($n = 4$, $P = 0.026$); PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 4$, $P = 0.0069$); and for the initial treatment of cGVHD ($n = 5$, $P = 0.0060$). *De novo* CMV antigenemia before or after development of cGVHD was observed in 62 and 15 patients, respectively. Sixteen and 8 patients, respectively, died of bacterial infections and pneumoniae of unknown origin.

Discussion

In the present retrospective analysis, 57% (83/145) of patients with cGVHD developed infections, with a mortality rate of 27% (22/83). Although the limitations of this study were the retrospective study design and the differences in baseline characteristics in both the RIC and CIC groups, these results illustrate the importance of establishing more effective management of infectious complications associated with cGVHD, which are predictive of poor outcome for both RIC and CIC regimens.

In patients with cGVHD, the major source of bacteremia was heterogeneous, gram-positive organisms such as *S. epidermidis* and *Streptococcus* species, which were more common than gram-negative organisms, and bacteremia caused by *Pseudomonas aeruginosa*, including multidrug-resistant *P. aeruginosa*, occurred only in patients with cGVHD involving a GI tract score ≥ 2. Additionally, *Streptococcus pneumonia* sepsis was a risk factor for non-relapse mortality, as reported previously (4), and pneumococcal vaccination of transplant recipients was found to be relatively ineffective in the presence of cGVHD. In other studies with RIC regimens, the incidence of bacteremia appeared to be significantly lower than in the present study, but this may a result of the shorter follow-up periods in those studies (14, 15). Moreover, 29% (7/24) of the present patients with cGVHD involving a GI tract score ≥ 2 had received ≥ 2 mg of PSL/kg before developing cGVHD, and all 7 of these patients developed bacteremia. Although 50% (14/28) of patients with bacteremia received antibiotic

drugs and all 14 of these patients received intravenous immunoglobulin to maintain IgG levels at >400 mg/dL for prophylaxis of encapsulated bacteria and *Pneumocystis*, these results suggest that patients with cGVHD having a GI tract score ≥ 2, especially after high-dose PSL, are more likely to develop bacteremia than patients with cGVHD not having a GI tract score ≥ 2. This was probably due to colonization of the GI tract resulting from translocation into the bloodstream or disruption of the ecologic GI equilibrium involving GI bacterial overgrowth (e.g., use of antibiotic decontamination), increased permeability of the GI mucosal barrier (e.g., GVHD-induced mucosal damage), or deficiencies in the host immune defenses (e.g., use of immunosuppressive drugs). Thus, a review of strategies for prevention of bacteremia may lead to improvement of patient outcomes after allogeneic HSCT. That is, in patients with cGVHD having a GI tract score ≥ 2, restrictions on the use of broad-spectrum antibiotics may help reduce GI bacterial overgrowth, including overgrowth by antibiotic-resistant organisms, resulting from failure of the GI barrier. In contrast, we recognize the difficulty in identifying bacteremia using culturing blood. Our patients were immunocompromised hosts who presented with undifferentiated fever; therefore, blood culture results were often delayed well into the course of empirical therapy. There is a need to develop suitable strategies for screening of bacteremia associated with cGVHD in patients who receive allogeneic HSCT with either RIC or CIC regimens.

Most of the present patients with cGVHD who developed *Candida* infection or IA received ≥ 0.5 mg of PSL/kg before developing cGVHD and the incidence of IA was significantly higher in patients with cGVHD having a GI score ≥ 2, especially after high-dose PSL. The number of patients with fungal infections was small, but high-dose PSL may be effective for improving the prophylaxis for such infections. Furthermore, the duration of prophylaxis still remains unclear as randomized clinical trials have yet to be conducted.

All the present patients with adenoviral HC developed grades II–IV acute GVHD and received PSL for GVHD therapy, which differs considerably from what has been reported previously(16). The incidence of adenoviral HC was significantly higher in patients with cGVHD having a KPS score ≥ 2, a GI score ≥ 2, and high-dose PSL at the time of diagnosis and for the initial treatment of cGVHD. Although the present study was limited in its ability to detect risk factors for adenoviral HC, because of low patient numbers and lack of prospective investigation of viral infection, the present results suggest that patients who receive high-dose PSL before and after developing cGVHD should be frequently checked for abdominal and urinary symptoms, and that urinary tests should be regularly performed during ongoing use of immunosuppressive drugs. In addition, we identified only 1 patient with cGVHD who suffered from CMV colitis, indicating that it is useful to monitor and treat CMV antigenemia intensively in patients receiving immunosuppressive drugs, especially before development of cGVHD. In contrast, 12% of the present patients with cGVHD developed cutaneous VZV with a median onset of 502 days (range, 106–1684), despite low-dose acyclovir prophylaxis during at least the first year after allogeneic HSCT. Nonetheless, there were no cases of breakthrough VZV infection. This suggests that low-dose acyclovir prophylaxis effectively prevented breakthrough VZV infection, but that reestablishment of antiviral therapy was needed to protect against cutaneous VZV in patients with cGVHD.

In summary, the present data indicate that infections associated with cGVHD, especially after high-dose PSL, are predictive of poor outcome, whether RIC or CIC is used. Accordingly, there is a need for clinical trials to develop new strategies for screening and prevention of infections associated with cGVHD in patients who receive allogeneic HSCT with either RIC or CIC regimens.

References

1. Perez-Simon JA, Diez-Campelo M, Martino R, et al. Influence of the intensity of the conditioning regimen on the characteristics of acute and chronic graft-versus-host disease after allogeneic transplantation. Br J Haematol 2005; 130: 394–403.
2. Hori A, Kami M, Kim SW, et al. Development of early neutropenic fever, with or without bacterial infection, is still a significant complication after reduced-intensity stem cell transplantation. Biol Blood Marrow Transplant 2004; 10: 65–72.
3. Kojima R, Tateishi U, Kami M, et al. Chest computed tomography of late invasive aspergillosis after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005; 11: 506–511.
4. Kulkarni S, Powles R, Treleaven J, et al. Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants. Blood 2000; 95: 3683–3686.
5. Dykewicz CA. Summary of the guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Clin Infect Dis 2001; 33: 139–144.
6. Sepkowitz KA. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. Clin Infect Dis 2002; 34: 1098–1107.
7. Atsuta Y, Suzuki R, Yamamoto K, et al. Risk and prognostic factors for Japanese patients with chronic graft-versus-host disease after bone marrow transplantation. Bone Marrow Transplant 2006; 37: 289–296.
8. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005; 106: 2912–2929.
9. Kanda Y, Mineishi S, Saito T, et al. Long-term low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2001; 28: 689–692.

10. Doney KC, Weiden PL, Storb R, Thomas ED. Treatment of graft-versus-host disease in human allogeneic marrow graft recipients: a randomized trial comparing antithymocyte globulin and corticosteroids. Am J Hematol 1981; 11: 1–9.
11. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980; 69: 204–217.
12. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005; 11: 945–956.
13. Ascioglu S, Rex JH, De Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and haematopoietic stem cell transplants: an international consensus. Clin Infect Dis 2002; 34: 7–14.
14. Junghanss C, Marr KA, Carter RA, et al. Incidence and outcome of bacterial and fungal infections following nonmyeloablative compared with myeloablative allogeneic hematopoietic stem cell transplantation: a matched control study. Biol Blood Marrow Transplant 2002; 8: 512–520.
15. Busca A, Locatelli F, Barbui A, et al. Infectious complications following nonmyeloablative allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis 2003; 5: 132–139.
16. El-Zimaity M, Saliba R, Chan K, et al. Hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation: donor type matters. Blood 2004; 103: 4674–4680.

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ORIGINAL ARTICLE

Reduced-intensity unrelated donor bone marrow transplantation for hematologic malignancies

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Abstract To review a current experience of unrelated bone marrow transplantation (BMT) with reduced-intensity conditioning (RIC) regimens, we conducted a nationwide survey with 77 patients (age, 25–68 years). The backbone RIC regimen was a combination of fludarabine or cladribine, busulfan or melphalan and total body irradiation at 2–4 Gy. Five patients died early, but 71 (92%) achieved initial neutrophil recovery. Thereafter, 36 patients (47%) died of therapy-related complications, 23 (30%) of whom died within day 100. Grades II–IV acute graft-versus-host disease (GVHD) occurred in 34 of the 68 evaluable patients (50%). In a multivariate analysis, a regimen containing antithymocyte globulin (ATG) was significantly associated with a decreased risk of acute GVHD ($P = 0.041$). Thirty-three patients are currently alive with a median follow-up of 439 days (28–2002 days), with an OS of 50% at 1 year. In conclusion, unrelated BMT with RIC regimens can be a curative treatment in a subset of patients.

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1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a possible curative approach for patients with various hematologic malignancies. Recently, the application of reduced-intensity conditioning (RIC) regimens, mostly incorporating fludarabine as a backbone agent, has been explored for patients whose age or concomitant medical conditions contraindicate the use of conventional myeloablative regimens [1–3]. Since only 30–40% of patients have an appropriate family donor available [4], the establishment of an unrelated donor transplantation program with RIC regimens is urgently needed.

Graft rejection, regimen-related toxicities and graft-versus-host disease (GVHD) have been the major problems in unrelated HSCT with RIC [5–13]. In unrelated transplantation, engraftment is influenced by the source of stem cells and superior results have been observed with peripheral blood stem cells (PBSC) compared to bone marrow [9, 14]. Nevertheless, PBSC has not yet been approved as a graft source for unrelated transplantation in Japan [15]. The level of regimen-related toxicities directly depends on the intensity of the regimen, and the incidence of GVHD increases with unrelated donors compared to related donors. Although attempts have been made to overcome these problems, a suitable procedure for unrelated bone marrow transplantation (BMT) with RIC regimens has not yet been established. To accumulate further expertise, we conducted a nationwide survey of Japanese patients with hematologic malignancy who had undergone BMT from an HLA-matched or -mismatched unrelated donor with RIC regimens. Although the present data were obtained from a limited population of patients, these findings may show a current status of unrelated BMT with RIC.

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2 Patients and methods

2.1 Data sources

This survey collected the data of 77 consecutive patients in 17 participating hospitals who received unrelated BMT with RIC for hematologic malignancies between 2000 and 2004. Data were derived from questionnaires distributed to each hospital. Additional questionnaires were sent to confirm the follow-up data, including the occurrence of GVHD. The minimum data required for inclusion of a patient in this study were age, sex, histological diagnosis, status at transplant, donor information, conditioning regimen, date of transplant, donor chimerism status, therapy-related complications, date of last follow-up, disease status at follow-up, date of disease progression (PD)/death and cause of death.

This study was approved by institutional review board of each individual center. All patients provided written informed consent according to the Declaration of Helsinki. Unrelated donors provided consent through the Japan Marrow Donor Program as part of its standard procedures. The indications, conditioning regimens, management of GVHD and supportive care for BMT were left to the discretion of each institution. Patients who had previously received allogeneic HSCT and those younger than 20 years were not included. Patients younger than 50 years who had organ dysfunction and/or have previously received high-dose chemotherapy with autologous HSCT were also included.

2.2 Definitions

RIC regimens were defined as previously reported [6, 9, 10], and conditioning regimens that included either beyond 4 Gy of total body irradiation (TBI), 8 mg/kg of busulfan or 140 mg/m^2 of melphalan were excluded from the study. Alleles at the HLA-A, -B, and -DRB1 loci were identified by middle-resolution DNA typing as described previously [16]. Risk status at transplantation was categorized as either standard risk or high risk. Standard-risk diseases included acute leukemia in first complete remission, chronic myeloid leukemia in first chronic phase, and refractory anemia of myelodysplastic syndrome (MDS). Other diseases were categorized as high-risk disease. Graft failure was analyzed in patients who survived more than 28 days posttransplant according to the criteria reported by Petersdorf et al. [17]. Briefly, the definition included failure of the absolute neutrophil count (ANC) to surpass 500/mm^3 before relapse, death or second transplantation, as well as a decrease in the ANC to less than 100/mm^3 on at least three consecutive determinations with a finding of severe hypoplastic marrow. The degree of donor chimerism among peripheral blood T cells was assessed several times

between day 28 and day 100 after HSCT using fluorescence in situ hybridization (FISH) to detect X and Y chromosomes for recipients of grafts from sex-mismatched donors, and polymerase chain reaction-based analyses of polymorphic microsatellite regions for recipients of sex-matched or sex-mismatched transplants. Mixed chimerism was defined as the detection of 5–90% of donor cells in the peripheral blood. Acute and chronic GVHD were graded according to the consensus criteria [18, 19]. Patients who survived 100 days were evaluable for the assessment of chronic GVHD. Overall survival (OS) was measured as the time from the day of transplantation until death from any cause, and progression-free survival (PFS) was the time from the day of transplantation until PD/relapse or death from any cause. Patients who died from transplantation-related causes were classified as non-relapse mortality (NRM) regardless of their disease status.

2.3 Statistical analysis

The primary endpoint of this study was OS and chimerism. The secondary endpoints were PFS, NRM, PD, and the incidence of acute and chronic GVHD. Descriptive statistical analysis was performed to assess patient baseline information. Patients were divided into two groups: age 60 or above and less than 60. OS and PFS were calculated using the Kaplan–Meier method. The cumulative incidence of acute GVHD was calculated using the method described by Gooley et al. [20] to eliminate the effect of competing risks. The competing event for acute GVHD was defined as death without grades II–IV acute GVHD. For each endpoint, a Cox proportional hazard model was used for univariate and multivariate analyses. The factors included in the analysis were HLA disparity (mismatch vs. identical), recipient age (age 60 or above vs. less than 60), use of TBI (yes vs. no), use of ATG (yes vs. no), diagnosis of AML (yes vs. no), risk status (high vs. standard) and acute GVHD (II–IV vs. 0–I). Acute GVHD in the model was treated as a time-varying covariate. We defined statistical significance as a P value less than 0.05. All statistical analyses were performed using STATA version 8 (College Station, TX).

3 Results

3.1 Patients and diagnoses

The patients' characteristics are listed in Table 1. The median age of the patients was 54 years (range, 25–68 years) as a whole. Twenty-one patients (27%) had acute myelogenous leukemia (AML), 2 (3%) had acute lymphoblastic leukemia, 5 (7%) had chronic myeloid leukemia, 20 (26%) had MDS or myeloproliferative disease (refractory anemia, $n = 8$; refractory anemia with excess blasts, $n = 9$; others, $n = 3$), 19 (25%) had non-Hodgkin lymphoma (follicular lymphoma, $n = 12$; diffuse large B-cell lymphoma, $n = 4$; mantle cell lymphoma, $n = 2$; peripheral T-cell lymphoma, unspecified, $n = 1$), 7 (9%) had adult T-cell leukemia/lymphoma, and 3 (4%) had multiple myeloma. Sixty-three patients (82%) had high-risk disease at the time of allogeneic BMT.

3.2 Conditioning regimens

Conditioning regimens are shown in Table 2. None received ex vivo T-cell depleted transplantation.

3.3 HSCT procedure and supportive care

Forty-seven patients (61%) were transplanted from a matched, 24 (31%) were from a 1 allele-mismatched, and 6 (8%) were from a 2 or 3 allele-mismatched unrelated donor. All patients received bone marrow as a source of stem cells. The prophylaxis of GVHD was either cyclosporine- or tacrolimus-based. Thirty-nine patients (51%) received cyclosporine with methotrexate, including five patients who received an ATG-containing preparative regimen. Nine patients (12%) received cyclosporine alone, including five patients who received ATG. Each patient received cyclosporine with mycophenolate mofetil and cyclosporine with prednisolone, respectively. Twenty-five patients (33%) received tacrolimus with methotrexate, including one patient who received ATG. Two patients (3%) received tacrolimus alone, including one who received ATG. Granulocyte colony-stimulating factor was administered intravenously from day +1 or +6 until neutrophil engraftment in all patients.

3.4 Engraftment and chimerism

Five patients died before the engraftment evaluation, with a median survival time of 15 days (range, 2–17 days). Seventy-one patients (92%) achieved initial neutrophil recovery, but three patients (two AMLs and one MDS) later experienced secondary graft failure; one each with AML and MDS after unrelated BMT from an HLA-1 allele-mismatched donor received a second transplantation when they failed to achieve subsequent complete donor-type chimerism, but both died of infectious complications. The other patient with AML after unrelated BMT from an HLA-6 allele-matched donor achieved initial complete chimerism, but later developed secondary graft failure upon the administration of ganciclovir for cytomegalovirus antigenemia. However, this patient achieved the spontaneous recovery of autologous marrow function and is currently surviving beyond 2,000 days.

Table 1 Patient characteristics

Variable	Younger than 60 years ($n = 60$)	60 years or older ($n = 17$)
Patient age (range, median)	25–59, 52	60–68, 63
Disease		
Acute myelogenous leukemia	16 (27%)	5 (29%)
Acute lymphoblastic leukemia	2 (3%)	0
Chronic myeloid leukemia	5 (8%)	0
Myelodysplastic syndrome or myeloproliferative disease	12 (20%)	8 (47%)
Malignant lymphoma	16 (27%)	3 (18%)
Adult T-cell leukemia/lymphoma	7 (12%)	0
Multiple myeloma	2 (3%)	1 (6%)
Risk status		
Standard	13 (22%)	1 (6%)
High	47 (78%)	16 (94%)
HLA disparity		
Matched	37 (62%)	10 (59%)
One-mismatched	19 (32%)	5 (29%)
Two or more mismatched	4 (7%)	2 (12%)
Donor–recipient sex match		
Male–male	20 (33%)	11 (65%)
Male–female	16 (27%)	2 (12%)
Female–male	9 (15%)	4 (24%)
Female–female	15 (25%)	0
GVHD prophylaxis		
Cyclosporine ± methotrexate	38 (63%)	10 (59%)
Tacrolimus ± methotrexate	21 (35%)	6 (35%)
Others	1 (2%)	1 (6%)
Median nucleated cell dose infused ($\times 10^8$/kg, range)	2.80 (0.39–5.52)[a]	2.92 (0.76–4.30)

HLA Human leukocyte antigen, *GVHD* graft-versus-host disease

[a] The data of two patients were excluded because infused nucleated cell dose was unknown

Chimerism was evaluated in 68 patients (88%), with short tandem repeats analysis ($n = 52$), variable number of tandem repeats analysis ($n = 5$) and FISH analysis in the case of sex mismatch ($n = 11$). Complete donor chimerism was confirmed in 58 (85%) within day 100. Mixed chimerism was confirmed in nine patients (13%), but two later reverted to recipient type. One patient failed to achieve donor-type chimerism due to disease relapse on day 20. The incidence of complete donor chimerism was similar in those younger and older than 60 years (85 and 86%), with a similar incidence of mixed chimerism (15 and 14%). No patients received donor lymphocyte infusion.

3.5 GVHD

Acute GVHD occurred in 41 of the 68 evaluable patients (60%), grades II–IV in 34 (50%) and grades III–IV in 14 patients (21%). Chronic GVHD occurred in 26 of the 42 evaluable patients (62%), with extensive type in 23 (55%). The incidence of grades II–IV acute GVHD was the same in patients younger and older than 60 years (50%). The incidence of grades III–IV acute GVHD (22 and 14%) and extensive chronic GVHD (56 and 50%) was similar. In unrelated BMT, from HLA-6 allele-matched ($n = 40$), HLA-1 allele-mismatched ($n = 23$), and HLA-2 or 3 allele-mismatched ($n = 5$) donors, grades II–IV acute GVHD occurred, respectively, in 18 (45%), 10 (43%) and 3 patients (60%), and chronic GVHD occurred in 15 (38%), 9 (39%) and 2 patients (40%). In univariate and multivariate analyses, an ATG-containing regimen was significantly associated with a decreased risk of the onset of grades II–IV acute GVHD (data not shown).

3.6 Survival

Thirty-three patients are currently alive with a median follow-up of 439 days (28–2,002 days), with an OS of 50% at 1 year and 46% at 2 years. The OS of patients younger than 60 years was 49% at 2 years (95% confidence interval [CI], 34–62%), and this could not be defined in older patients (95% CI, 15–45%). Patients younger than 60 years

Table 2 Conditioning regimens

Conditioning regimens	Younger than 60 years ($n = 60$)	60 years or older ($n = 17$)
TBI-containing		
Fludarabine 180 mg/m^2 (or cladribine 0.66 mg/kg), oral busulfan 8 mg/kg, TBI 4 Gy	30 (50%)	6 (35%)
Fludarabine 125–180 mg/m^2, melphalan 80–140 mg/m^2, TBI 4 Gy	5 (8%)	3 (18%)
Fludarabine 180 mg/m^2 (or cladribine 0.66 mg/kg), oral busulfan 8 mg/kg, TBI 2 Gy	2 (3%)	0 (0%)
Fludarabine 180 mg/m^2, TBI 4 Gy	0 (0%)	1 (6%)
ATG-containing		
Fludarabine 180 mg/m^2 (or cladribine 0.66 mg/kg), oral busulfan 8 mg/kg, ATG	5 (8%)	4 (24%)
Fludarabine 180 mg/m^2, cyclophosphamide 60 mg/kg, ATG	1 (2%)	0 (0%)
Fludarabine 180 mg/m^2, ATG	1 (2%)	0 (0%)
TBI and ATG-containing		
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg, TBI 4 Gy, ATG	1 (2%)	1 (6%)
Non-TBI and non-ATG		
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg	6 (10%)	2 (12%)
Fludarabine 125–180 mg/m^2, melphalan 140 mg/m^2	5 (8%)	0 (0%)
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg, cyclophosphamide 60 mg/kg	2 (3%)	0 (0%)
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg, thiotepa 10 mg/kg	1 (2%)	0 (0%)
Fludarabine 180 mg/m^2, cyclophosphamide 60 mg/kg	1 (2%)	0 (0%)

TBI Total body irradiation, *ATG* antithymocyte globulin (ATG-Fresenius 10 mg/kg or thymoglobulin 5 mg/kg)

tended to show better survival than older patients ($P = 0.124$). The HLA disparity (match vs. mismatch), TBI vs. non-TBI, ATG vs. non-ATG-containing regimen, and disease category (AML vs. MDS or myeloproliferative disease vs. lymphoid malignancies) was not significantly associated with OS (data not shown). Patients with standard risk tended to show better survival than those with high risk ($P = 0.129$). In univariate and multivariate analyses, no variables were significantly associated with OS (data not shown).

3.7 NRM and PD

Thirty-six patients (47%) died of therapy-related complications, with a cumulative incidence of NRM at 1 year of 43% (95% CI, 31–56%). Of the patients who died of therapy-related complications, 23 (30%) died within day 100 of transplantation and 13 (17%) died thereafter. The NRM at 1 year in patients younger and older than 60 years was 38% (95% CI, 25–53%) and 61% (95% CI, 36–85%), respectively, as shown in Fig. 1. The causes of NRM were infection (23%), regimen-related toxicity (14%) and GVHD (9%). GVHD-related mortality was found in 26%. Infection was the major cause of death in patients younger than 60 years. Regimen-related toxicity, mainly pulmonary complications, was the major cause of treatment failure for patients older than 60 years. In univariate and multivariate analyses, no variables were significantly associated with

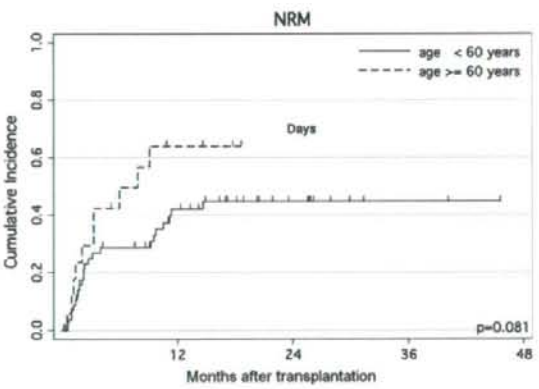

Fig. 1 Non-relapse mortality stratified according to patient age, younger or older than 60 years

NRM (data not shown). Relapse or progression of primary disease after unrelated BMT with RIC regimens was observed in 13 patients (17%; 10 patients younger than 60 years and 3 older than 60 years). There were no relapsed patients after transplantation in standard risk group. The incidence of death due to relapse or progression of primary disease was 14%. In univariate and multivariate analyses, no variables were significantly associated with PD although patients with grades II–IV acute GVHD showed a relatively lower incidence of PD (data not shown).

4 Discussion

This report reviews the current experience of unrelated BMT with RIC regimens in Japan, with particular focus on the risk factors for engraftment, GVHD, NRM, survival and PD. Although the engraftment rate has been reported to be lower when RIC unrelated transplantation was performed with bone marrow compared to peripheral blood cells [9, 10], we observed that sustained engraftment was achieved in 99% of evaluable patients, with complete donor chimerism confirmed in 85%. The incidence of graft failure was not different from that in RIC transplantation from related donors in Japan; 3.7% in recipients with an HLA-matched donor and 5.7% in those with a 1-locus-mismatched donor [21]. Complete donor chimerism in our study was comparable with that reported from the National Marrow Donor Program (85 vs. 84%) [22]. In our study, two-thirds of patients successfully received 2–4 Gy TBI-containing regimens, which were aimed at the enhancement of engraftment, as suggested in a previous report with patients with aplastic anemia [23], while 2 of the 12 patients who received an ATG-containing regimen had late graft failure, similar to a previous report which noted an incidence of 19% [5]. It has been reported that the Japanese population is more homogenous than others in terms of the distribution of HLA. Thus, it would be possible that the impact of minor HLA disparities on engraftment may become prominent after RIC transplantation.

Despite the observed satisfactory engraftment rate, we confirmed a high NRM rate (47%) after unrelated BMT with variable RIC regimens, due mostly to GVHD-related complications, including infections under steroid therapy, as previously designated by Wong et al. [10]. On the other hand, the incidence of death due to relapse or progression of primary disease was low (14%). Hence, successful prophylaxis and treatment of GVHD is particularly important in this procedure, and studies with ATG [5, 24] or alemtuzumab [25–27] have reported encouraging results. Although the number of patients was still small, in our study an ATG-containing regimen resulted in a decreased incidence of acute and chronic GVHD, despite the use of a lower dose (ATG-Fresenius 10 mg/kg or Thymoglobulin 5 mg/kg) than reported elsewhere. This study showed that age older than 60 years tended to be associated with a higher risk of NRM after unrelated HSCT with RIC regimens, though this relation was not statistically significant in a multivariate analysis. This finding, however, is limited by the small sample size. Additional use of ATG may reduce the incidence of GVHD-related NRM even in older patients but ATG should be carefully incorporated since about 20% of patients who received an ATG-containing regimen developed late graft failure in our study.

This study suggested that the onset of grades II–IV acute GVHD was associated with a lower incidence of PD, although this was not statistically significant in a multivariate analysis, possibly due to the small sample size. However, GVHD in turn resulted in a higher incidence of NRM, and a desirable graft-versus-leukemia or lymphoma effect would be offset, particularly in older patients [10, 28]. Hence, our observation echoes the warning that the intentional induction of GVHD should be avoided.

Compared to the long-term follow-up data after unrelated HSCT with RIC from the NMDP reported by Giralt et al. [22], our NRM at 1 year was worse (43 vs. 30%), but OS was likely to be better (50% at 1 year and 46% at 2 years vs. 44% at 1 year, 28% at 3 years and 23% at 5 years). In their report, disease stage, performance status, stem cell source, HLA matching, and timing of transplant were the most important prognostic factors for survival after RIC unrelated donor transplantation. This study suggested that high risk and HLA-mismatched patients were associated with worse OS, although this was not statistically significant in the multivariate analysis. Interpretation of these results, however, should be careful because of relatively short period of follow-up and the small sample size in our study. Although high risk patients was 82%, rate of relapse were unexpectedly low in our study. This might be due to earlier mortality, which precludes estimate of relapse rate. Alternately, more patients (60%) received more intense conditioning composed of 8 mg/kg of busulfan or 80–140 mg/m^2 of melphalan and 4 Gy TBI in our study.

In conclusion, we confirmed that unrelated BMT with RIC regimens can be a curative therapeutic option in a subset of patients with advanced hematologic malignancy, but at the expense of a high risk of severe complications and NRM. The incorporation of low-dose TBI may be advantageous for enhancing engraftment, and a suitable prophylaxis for GVHD still remains a primary target of clinical research. Based on the observed data, a prospective trial is currently underway to determine the value of a lower dose of ATG (ATG-Fresenius 5 mg/kg) to be added to the combination of fludarabine and busulfan.

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References

1. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning