which may lead to inappropriate invasive investigation or over-treatment with antifungal agents. Previous studies have reported various risk factors for the false-positive results, including early childhood,[3] the development of chronic graft-versus-host disease (GVHD),[8] the passage of GM of food origin[9,10] and certain exoantigens from other fungal genera[11] or fungus-derived antibiotics.[12,13] However, little is known about the exact mechanism of false-positive reactions with these factors.

To clarify the cause of false-positive results, we retrospectively analysed the incidence and risk factors for false-positive GM antigenaemia in allogeneic HSCT recipients.

Patients and methods

Study population

GM ELISA became available at the University of Tokyo Hospital as a routine diagnostic test in February 2000. During a 5 year period (February 2000 to May 2005), 163 consecutive adult patients (>16 years old) underwent allogeneic HSCT at the University of Tokyo Hospital. The medical records of 157 patients who had at least two GM ELISA tests after HSCT were available for a retrospective analysis of positive GM antigenaemia. The median follow-up was 519 days (range, 15–2090 days) after HSCT. The patient characteristics are shown in Table 1. Acute leukaemia in first remission, chronic myelogenous leukaemia in first chronic phase, myelodysplastic syndrome with refractory anaemia or refractory anaemia with ringed sideroblasts, and aplastic anaemia were defined as low-risk diseases, whereas others were considered high-risk diseases. Donors other than human leucocyte antigen (HLA)-matched sibling donors were defined as alternative donors.

Transplantation procedure

The conventional preparative regimen for leukaemia/lymphoma was mainly performed with either cyclophosphamide/total body irradiation (TBI)-based regimens or busulfan/cyclophosphamide-based regimens. In cyclophosphamide/TBI-based regimens, the dose of cyclophosphamide was decreased and etoposide was added instead in patients with impaired cardiac function. Fludarabine-based regimens were used as reduced-intensity regimens for elderly or clinically infirm patients.[14] Cyclosporin A or tacrolimus was administered combined with short-term methotrexate for prophylaxis against GVHD. Alemtuzumab was added for patients who received a graft from an HLA-mismatched donor.[15] Methyl-prednisolone or prednisolone at 1 or 2 mg/kg was added for patients who developed grade II–IV acute GVHD, whereas prednisolone at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, herpes simplex virus and *Pneumocystis jirovecii* infections consisted of tosufloxacin, aciclovir and sulfamethoxazole/trimethoprim.

Antigen detection

GM assay was performed at least every other week after HSCT until discharge from the hospital in the majority of patients. In the outpatient setting, the monitoring of GM was continued at each visit in patients who were receiving immunosuppressive therapy, at the discretion of attending physicians. Circulating *Aspergillus* GM was detected using a sandwich immunocapture ELISA (Platelia Aspergillus, Bio-Rad, Marnes-la-Coquette, France) using a rat anti-GM monoclonal antibody.[2] The technique was performed as recommended by the manufacturer. The optical absorbance of specimens and controls was determined with a spectrophotometer set at 450 and 620 nm wavelengths. The optical density (OD) index for each sample was calculated by dividing the optical absorbance of the clinical sample by that of the threshold control. Two consecutive serum samples with an OD index of 0.6 or more were considered positive.[16]

Table 1. Patients' characteristics

Characteristic	Total patients
Sex (male/female)	105/52
Age, median (range)	41 (16–66)
Underlying disease	
acute leukaemia	70
CML	26
MDS	22
SAA	8
other	31
Graft source	
PBSC	69
BM	88
Donor type	
matched sibling	58
mismatched related	15
unrelated	84
Preparative regimen	
Cy (Etp)/TBI-based regimens	105
Bu/Cy-based regimens	15
ATG-based regimens for SAA	5
Flu-based RIC	32
GVHD prophylaxis	
CsA + MTX	115
tacrolimus + MTX	18
alemtuzumab + CsA + MTX	24
Acute GVHD	
grade 0–I	87
grade II–IV	69
Chronic GVHD	
extensive	57
limited	30
none	47

CML, chronic myelogenous leukaemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anaemia; PBSC, peripheral blood stem cell; BM, bone marrow; Cy, cyclophosphamide; Etp, etoposide; TBI, total body irradiation; Bu, busulfan; ATG, antithymocyte globulin; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporin A; MTX, methotrexate.

Antifungal prophylaxis and treatment for IA

As antifungal prophylaxis, fluconazole at 200 mg was principally given daily from day −14 until the end of immunosuppressive therapy. For patients with a history of IA, intravenous micafungin at 150–300 mg or oral itraconazole at 200 mg was administered instead. All patients were isolated in high-efficiency particulate air (HEPA)-filtered rooms from the start of the conditioning regimen to engraftment. Febrile neutropenia was treated with broad-spectrum antibiotics in accordance with

the published guidelines.[17] Antifungal treatment was started when febrile neutropenia persisted for at least 3–4 days or when IA was confirmed or suspected with clinical or radiological signs.

Diagnosis procedures and definitions

Diagnostic procedures included routine cultures of urine and stools, repeated cultures of blood and sputum, weekly chest X-ray, computed tomography (CT) scan of the chest and nasal sinus and, when possible, bronchoscopic examinations and open biopsy. CT scans were principally obtained for patients with (i) clinical signs and/or symptoms suggestive of IA, (ii) persistent or recurrent febrile neutropenia while on broad-spectrum antibiotic treatment, (iii) infiltrates or nodules on chest X-ray or (iv) positive GM antigenaemia. In patients with clinical suspicion of IA, bronchoscopy with bronchoalveolar lavage (BAL) and/or tissue biopsy were also performed whenever feasible. A diagnosis of IA was classified as proven or probable on the basis of the EORTC/MSG definitions.[7] True-positive GM antigenaemia was defined as two consecutive positive results with the established diagnosis of proven or probable IA. Positive GM antigenaemia in episodes that did not fulfil the diagnostic criteria for proven or probable IA was considered as inconclusive-positive if (i) sufficient examinations including chest and/or sinus CT scans were not performed despite the presence of compatible clinical signs and symptoms of IA or (ii) the possibility that the radiological abnormalities on the CT scans were due to IA could not be denied because of the use of empirical antifungal therapy or targeted antifungal therapy for other definite fungal infections at the time of positive antigenaemia. Alternatively, positive antigenaemia without sufficient evidence to diagnose proven or probable IA was considered as false-positive in any of the following: (i) no radiological abnormalities were detected on chest and/or sinus CT scans; (ii) non-specific abnormalities on CT scans improved without any antifungal treatments for IA or culture results for specimens from radiologically abnormal sites including BAL fluid or sinus aspirate were negative; or (iii) CT scans were not performed because of no evidence meeting clinical minor criteria in EORTC/MSG definitions. Positive antigenaemia recurring after the negative conversion at least 3 months apart was considered an independent episode.

Statistical analysis

Sensitivity, specificity and positive predictive value (PPV) of the GM ELISA were calculated on the basis of the clinical diagnosis of proven or probable IA. The cumulative incidences of positive GM antigenaemia and IA were evaluated using Gray's method, considering death without each event as a competing risk.[18] Probabilities in two groups were compared using Fisher's exact test. P values of less than 0.05 were considered statistically significant.

Results

Transplantation outcome

One hundred and fifty-seven allogeneic transplant recipients were included in the study. Neutrophil engraftment was obtained at a median of 17 days (9–43 days) after HSCT in 156 patients. Grade II–IV acute GVHD was observed in 69 and chronic GVHD in 87 of 134 who survived more than 100 days. Seventy patients died, the causes being haematological relapse ($n = 29$), infection ($n = 14$), non-infectious pulmonary complications ($n = 15$), gastrointestinal bleeding ($n = 6$) or other reasons ($n = 6$).

Diagnosis of IA

Twenty-five patients developed proven ($n = 8$) or probable ($n = 17$) IA at a median of 204 days (range 21–1527 days) after HSCT, with a 1 year cumulative incidence of 12.9% (Figure 1). Twenty-two patients (88%) had pulmonary disease, two of whom showed dissemination. The remaining three had tracheobronchitis, sinusitis and gastrointestinal involvement, respectively. IA was the direct cause of death in five patients. Positive GM antigenaemia was observed in 22 patients with proven or probable IA. In a patient-based analysis, the sensitivity and specificity of the test were 88% (22 of 25) and 79% (104 of 132), respectively.

Episodes with positive GM antigenaemia

A total of 3296 serum samples were analysed from 157 patients (mean, 21 samples/patient; range, 2–109 samples/patient). Overall, 50 patients (31.9%) developed positive GM antigenaemia at a median of 107 days (range 12–1193 days) after HSCT, with a 1 year cumulative incidence of 32.2% (Figure 1). Five patients had second positive episodes at a median interval of 358 days (range 119–1103 days) between the first and second episodes. Four positive episodes occurred in one patient.

A total of 58 positive episodes of the 50 patients were therefore analysed (Table 2). Twenty-two episodes were diagnosed true-positive based on the diagnosis of proven or probable IA. In these patients, the microbiological criterion was fulfilled with pathological findings and/or culture results in 10 and GM antigen test in 12. Seven were considered inconclusive-positive. In all the seven episodes, we could not conclude whether the abnormalities on CT scans were attributed to IA or not, because antifungal agents were administered empirically ($n = 5$) or for the treatment of documented candidiasis ($n = 2$) at the time of positive GM antigenaemia.

Twenty-nine episodes were considered false-positive, in all of which piperacillin/tazobactam or amoxicillin/clavulanate was not given at the time of positive GM antigenaemia. *Penicillium* and

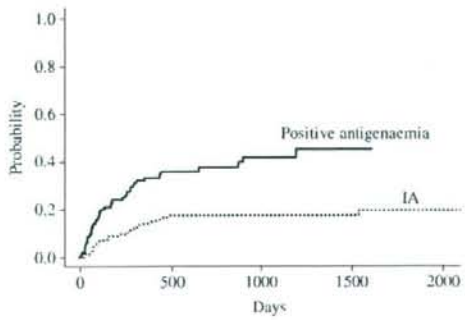

Figure 1. Cumulative incidences of IA and positive GM antigenaemia after HSCT.

Table 2. Incidence of false-positive GM antigenaemia

	Total episodes	Episodes before day 100	Episodes after day 100
True-positive	22	8	14
False-positive	29	15	14
Inconclusive-positive	7	1	6
Total	58	24	34
False-positive rate (%)	50	62.5	41.2

Paecilomyces were not detected in these false-positive episodes. At the time of false-positive antigenaemia, antifungal prophylaxis was given in 23 episodes (fluconazole, 20; itraconazole, 3), and no antifungal agents at all in the remaining 6. Empirical or targeted antifungal therapy was not performed in these episodes. CT scans were performed in 22 episodes, in which no radiological abnormalities were seen in 12, and non-specific abnormalities in the remaining 10 were caused by *P. jirovecii* infections ($n = 2$), bacterial infections ($n = 2$), pulmonary involvement of cancer ($n = 1$), heart failure ($n = 1$), bronchiolitis obliterans organizing pneumonia (BOOP) ($n = 1$) or unknown aetiology ($n = 3$). All three unexplained radiological abnormalities disappeared spontaneously.

Incidence and risk factors for false-positive GM antigenaemia

Of the 58 positive episodes, 29 satisfied the criteria of false-positive antigenaemia, with a false-positive rate of 50% (Table 2). During the first 100 days after HSCT, 15 of 24 positive episodes were considered false-positive, with a false-positive rate of 62.5% (Table 2). PPV was 33.3% or 37.5% when we included the inconclusive episode into the false-positive group or the true-positive group, respectively, in the 24 positive episodes. PPV was 55.6% or 66.7% even in nine with grade II–IV acute GVHD at the time of positive GM antigenaemia. In contrast, 14 of 34 positive episodes beyond 100 days were considered false-positive, with a rate of 41.2%, and PPV was 41.2% or 58.8%. False-positive antigenaemia occurred more frequently and therefore PPV was lower during the first 100 days.

There were no significant parameters that increased the incidence of false-positive GM antigenaemia over the entire period and during the first 100 days (Tables 3 and 4). The incidence was rather decreased in the presence of active GVHD (at any grade) and liver GVHD over the entire period, and grade II–IV GVHD, grade III–IV GVHD and liver GVHD during the first 100 days. In contrast, gastrointestinal chronic GVHD was identified as the only significant risk factor for increased false-positive GM antigenaemia beyond 100 days (Table 5). Twenty of the 30 episodes of positive GM antigenaemia without gastrointestinal chronic GVHD were true-positive, whereas all 4 positive GM antigenaemia episodes in patients with gastrointestinal chronic GVHD were false-positive (PPV 66.7% versus 0%, $P = 0.02$). Gastrointestinal chronic GVHD in these patients was associated with more than 500 mL of diarrhoea at the time of positive GM antigenaemia, the diagnosis of which was pathologically confirmed with colon biopsy.

Table 3. Risk factors for false-positive GM antigenaemia after HSCT

Factors	False-positive	Others	*P* value
Age			
>40 years	18	18	1.00
≤40 years	11	11	
Disease risk			
standard risk	7	5	0.75
high risk	22	24	
Graft source			
bone marrow	16	15	0.79
peripheral blood	13	14	
Donor type			
matched sibling donor	9	9	1.00
alternative donor	20	20	
Neutrophil count			
<500 cells/μL	2	3	1.00
≥500 cells/μL	27	26	
Active GVHD on positive GM			
yes	13	23	0.01
no	16	6	
Gastrointestinal GVHD on positive GM			
yes	6	3	0.47
no	23	26	
Liver GVHD on positive GM			
yes	5	14	0.02
no	24	15	
Skin GVHD on positive GM			
yes	137	20	0.41
no	105	50	
Prednisolone on positive GM (1)			
≥0.5 mg/kg	137	20	0.41
<0.5 mg/kg	105	50	
Prednisolone on positive GM (2)			
≥1.0 mg/kg	137	20	1.00
<1.0 mg/kg	105	50	

In thorough examinations for aspergillosis, no radiological abnormalities were seen in two patients, non-specific abnormalities on CT scan were observed but spontaneously disappeared without clinical symptoms suggestive of IA in one, and radiological findings compatible with BOOP were observed and promptly improved with systemic corticosteroids in one. There was another false-positive episode probably associated with gastrointestinal chronic GVHD, which was included in the 'no gastrointestinal chronic GVHD' group because GVHD was absent at the detection of positive GM antigenaemia, but gastrointestinal chronic GVHD developed soon thereafter. Among these five episodes, the GM levels became normal with the improvement of gastrointestinal chronic GVHD in four, whereas GM antigen monitoring was discontinued because of death from haematological relapse in the remaining one.

Table 4. Risk factors for false-positive GM antigenaemia before day 100

Factors	False-positive	Others	*P* value
Neutrophil count			
<500	1	1	1.00
≥500	14	8	
Active GVHD on positive GM			
yes	4	6	0.09
no	11	3	
Grade II–IV acute GVHD on positive GM			
yes	3	6	0.04
no	12	3	
Grade III–IV acute GVHD on positive GM			
yes	0	3	0.04
no	15	6	
Gastrointestinal GVHD on positive GM			
yes	2	3	0.33
no	13	6	
Liver GVHD on positive GM			
yes	0	5	<0.01
no	15	4	
Skin GVHD on positive GM			
yes	3	4	0.36
no	12	5	
Prednisolone on positive GM (1)			
≥0.5 mg/kg	9	5	1.00
<0.5 mg/kg	6	4	
Prednisolone on positive GM (2)			
≥1.0 mg/kg	5	4	0.68
<1.0 mg/kg	10	5	

Table 5. Risk factors for false-positive GM antigenaemia after day 100

Factors	False-positive	Others	*P* value
Active GVHD on positive GM			
yes	9	17	0.23
no	5	3	
Extensive chronic GVHD on positive GM			
yes	7	10	1.00
no	7	10	
Gastrointestinal GVHD on positive GM			
yes	4	0	0.02
no	10	20	
Liver GVHD on positive GM			
yes	5	9	0.73
no	9	11	
Skin GVHD on positive GM			
yes	5	8	1.00
no	9	12	
Oral GVHD on positive GM			
yes	3	6	0.70
no	11	14	
Prednisolone on positive GM (1)			
≥0.5 mg/kg	3	3	0.67
<0.5 mg/kg	11	17	
Prednisolone on positive GM (2)			
≥1.0 mg/kg	2	2	1.00
<1.0 mg/kg	12	18	

Discussion

This study demonstrated that the sensitivity of the GM ELISA test was 88% in patient-based analysis and PPV was 38% to 50% in episode-based analysis, which were comparable with those in previous reports.[3–6] However, false-positive GM antigenaemia frequently occurred during the first 100 days after HSCT, and PPV was lower even among patients with grade II–IV acute GVHD, in whom the pre-test probability of IA was considered to be much higher than patients without acute GVHD.

A significant correlation between the occurrence of false-positive GM antigenaemia and the presence of gastrointestinal chronic GVHD was observed in this study. GM ELISA results were false-positive in all four episodes with gastrointestinal chronic GVHD at the time of positive GM antigenaemia, and there was another false-positive episode in which GVHD was absent at the detection of positive GM antigenaemia, but gastrointestinal chronic GVHD developed soon thereafter. During these episodes, piperacillin/tazobactam or amoxicillin/clavulanate was not given, and occult infections by some fungi reacting with GM ELISA were not detected, both of which were previously reported as important risk factors for false-positive GM antigenaemia.[11–13] Meanwhile, our results were consistent with the conclusions of other studies that concurrent mucositis in HSCT recipients or immature intestinal mucosa in neonates allows the translocation of GM contained in foods, leading to frequent false-positive GM antigenaemia.[3–5,8–10] These findings suggested the possibility that passage of dietary GM into the blood from the disrupted intestinal mucosal barrier might result in false-positive antigenaemia in patients with gastrointestinal chronic GVHD.

In contrast, the development of gastrointestinal acute GVHD was not significantly associated with the occurrence of false-positive GM antigenaemia in our series. This was probably because the overall false-positive rate during the first 100 days after HSCT was higher than that beyond 100 days. Mucosal damage due to the high-dose chemotherapy or TBI in the conditioning regimen might be the cause of frequent false-positive GM antigenaemia early after HSCT.[5]

Pfeiffer *et al.*[19] recently showed the significant heterogeneity of GM test performance among patients with different prevalences of IA. They demonstrated that GM assay was more useful in immunocompromised high-risk populations such as HSCT recipients or patients with haematological malignancy than in solid-organ transplant recipients. Although emphasizing the utility of GM assay only when there is a high pre-test probability of IA, they also addressed the need for further investigations of the reasons for the heterogeneity. Prior antifungal therapy and false-positive results are possible explanations for the heterogeneity, and our findings may contribute to the effective use of the assay. However, our study is a retrospective evaluation and therefore there are some potential weaknesses. In this study,

regular screening of GM antigen was not rigorously performed, but on an on-demand basis. This is in contrast to the previous studies in which GM antigenaemia was evaluated more intensively.[3–5] This fact might have affected the diagnostic performance of this assay, but the high cost of this test precluded such intensive monitoring in daily practice. In addition, we should mention that this study might lack enough statistical power to detect the other risk factors for false-positive antigenaemia than gastrointestinal chronic GVHD because of the small number of patients with positive antigenaemia. Also, the small number of patients with positive antigenaemia precludes multivariate analysis, which might be another reason for failing to find the possible impact of the other risk factors. The other major limitation is that GM antigenaemia itself was included in the microbiological criteria, which might have precluded the evaluation of true performance of this assay. In this study, however, the number of patients diagnosed with IA falls from 22 to 10, if the GM results are excluded from the criteria, which seemed too small for the statistical analysis. Therefore, we used the original EORTC/MSG definitions that include GM antigenaemia in the microbiological criteria.

In conclusion, frequent false-positive GM antigenaemia was observed in allo-HSCT recipients during the first 100 days after transplantation or in those with gastrointestinal chronic GVHD, leading to a decreased PPV of the GM ELISA test. Therefore, GM antigenaemia results should be considered cautiously in these patients in conjunction with other diagnostic procedures including CT scans.

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Transparency declarations

None to declare.

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ORIGINAL ARTICLE

Clinical features of late cytomegalovirus infection after hematopoietic stem cell transplantation

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Abstract Late cytomegalovirus (CMV) disease beyond day 100 after hematopoietic stem cell transplantation (HSCT) has become an increasing problem after the introduction of preemptive ganciclovir (GCV) administration. To clarify the risk factors and outcome for late CMV reactivation and disease, we retrospectively analyzed the records of 101 Japanese adult patients who underwent allogeneic HSCT between 1998 and 2005 at our hospital. Fifty-one developed late positive CMV antigenemia, with a cumulative incidence of 53%. Recipient CMV seropositivity, the use of alemtuzumab, chronic GVHD, and high-dose steroids were significantly associated with late positive antigenemia. Eight patients developed late CMV disease, with a cumulative incidence of 8%, including retinitis and gastrointestinal disease. None progressed to a fatal disease. The use of alemtuzumab was identified as an independent significant risk factor for late CMV disease, although it was not associated with increased non-relapse mortality. Among the 51 patients with late positive antigenemia, 28 had consistently less than three positive cells, 25 of whom showed negative conversion without antiviral agents. In conclusion, late CMV antigenemia appeared to develop frequently, especially in patients with profound immune suppression; however, a fatal outcome could be prevented by optimal preemptive therapy. Low-level antigenemia may not require antiviral treatments.

Keywords Cytomegalovirus · Antigenemia · Ganciclovir · Preemptive therapy · Hematopoietic stem cell transplantation

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1 Introduction

Despite the widespread use of prophylactic and preemptive ganciclovir (GCV) therapy, cytomegalovirus (CMV) disease remains one of the major causes of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Late occurrence of CMV disease beyond day 100 after HSCT is now increasingly observed, although early CMV disease within the first 100 days has been significantly decreased by the introduction of universal prophylaxis from engraftment or preemptive therapy with monitoring the CMV viral load [1–4]. The delayed CMV-specific immune reconstitution and antiviral drug resistance might have led to an increased incidence of late CMV disease [5, 6]. The main clinical manifestations are pneumonia and gastrointestinal disease [1, 2, 4], whereas retinitis and central nervous system disease are occasionally observed [6, 7]. Late CMV disease has frequently progressed to a fatal outcome with a mortality rate up to 50% [1, 2, 4], probably because most of the recipients are outpatients with less intensive monitoring and therefore, the antiviral agents tend to be administered after CMV-related symptoms are detected.

Although preemptive therapy with CMV monitoring has been successful in preventing early CMV disease [8], the efficacy of such an approach beyond day 100 remains to be evaluated. We have been routinely continuing CMV monitoring beyond day 100 and administered GCV preemptively. In this study, we retrospectively analyzed the incidence, risk factors, and outcome of late CMV reactivation and disease in allogeneic HSCT patients.

2 Patients and methods

2.1 Study population

During a 7-year period (from January, 1998 to September, 2005), 205 adult patients (≥16 years old) underwent allogeneic HSCT for the first time at the University of Tokyo Hospital. Twenty-nine patients died within the first 100 days after HSCT, two of whom developed CMV disease. Among the remaining 176 patients who survived more than day 100 after HSCT, the day of the first negative antigenemia test without any antiviral agents beyond day 100 was defined as the starting point of late CMV antigenemia monitoring. The median period from HSCT to the starting point was 100 days (100–207 days). Eleven patients who developed CMV disease before the starting point and 64 who did not undergo CMV antigenemia assay at least five times after the starting point were excluded. Finally, 101 patients were included in the study.

The median follow-up was 12.6 months (range 4.8–74 months) after HSCT. The patient characteristics are shown in Table 1. Thirty-five, twenty, and forty-six patients received grafts from a HLA-matched related donor, a mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 40 out of 55 related donors provided a peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in the first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, and aplastic anemia were defined as low-risk diseases, while others were considered high-risk diseases. Donors other than HLA-matched related donors were defined as alternative donors.

Table 1 Patients' characteristics

Characteristic	Total patients
Sex (male/female)	65/36
Age, median (range)	41.0 (16–66)
Serostatus before transplant	
Recipient CMV-positive/negative	90/11
Donor CMV-positive/negative	80/21
Underlying disease	
Acute leukemia	46
CML	21
MDS	12
NHL/ATL	11
SAA	5
Other	6
Graft source	
PBSC	40
BM	61
Donor type	
Matched related	35
Mismatched related	20
Unrelated	46
Preparative regimen	
Cy/TBI-based regimen	66
Bu/Cy-based regimen	11
ATG-including regimen	3
Flu-based reduced-intensity regimen	21
GVHD prophylaxis	
CsA + MTX	78
Tacrolimus + MTX	10
Alemtuzumab + CsA + MTX	13
Chronic GVHD	
Extensive	53
Limited	21
None	27

CMV cytomegalovirus, *CML* chronic myelogenous leukemia, *MDS* myelodysplastic syndrome, *NHL* non-Hodgkin lymphoma, *ATL* adult T-cell leukemia/lymphoma, *SAA* severe aplastic anemia, *PBSC* peripheral blood stem cell, *BM* bone marrow, *Cy* cyclophosphamide, *TBI* total body irradiation, *Bu* busulfan, *ATG* anti-thymocyte globulin, *Flu* fludarabine, *GVHD* graft-versus-host disease, *CsA* cyclosporine, *MTX* methotrexate

2.2 Transplantation procedure

The conventional preparative regimen for leukemia/lymphoma was mainly performed with either total body irradiation (TBI) regimen [cyclophosphamide (Cy) at 60 mg/kg/day for 2 days and TBI at 2 Gy twice daily for 3 days] or non-TBI regimen [Cy at the same dose combined with busulfan (Bu) at 4 mg/kg/day for 4 days]. In the TBI regimen, the dose of Cy was decreased to 40 mg/kg for 1 day and etoposide at 20 mg/kg for 2 days was added instead, in patients with impaired cardiac function. Fludarabine (Flu)-based regimens, including FB regimen (Flu at 30 mg/m^2/day for 6 days and Bu at 4 mg/kg/day for 2 days) with or without TBI at 4 Gy, FB16 regimen (Flu at the same dose with Bu at 4 mg/kg/day for 4 days), FM regimen (Flu 30 mg/m^2/day for 5 days and melphalan at 140 mg/m^2/day for 1 day), and FC regimen (Flu at

25 mg/m^2/day for 5 days and Cy at 60 mg/kg/day for 2 days), were used as reduced-intensity regimens for elderly or clinically infirm patients [9]. Gemcitabine at 1,000 mg/m^2/day for 3 days was added to the FB regimen for patients with pancreatic cancer [10]. The conditioning regimen for aplastic anemia was either a rabbit anti-thymocyte globulin (ATG) regimen (Cy at 50 mg/kg/day for 4 days and ATG at 5 mg/kg/day for 5 days with or without TBI at 4 Gy) or an alemtuzumab regimen (Cy at 25 mg/kg/day for 4 days and Flu at 30 mg/kg/day for 4 days combined with alemtuzumab at 0.2 mg/kg/day for 6 days, with or without TBI at 2 Gy).

For prophylaxis against GVHD, cyclosporine A (CsA) at 3 mg/kg/day or tacrolimus at 0.03 mg/kg/day was administered combined with short-term methotrexate (10–15 mg/m^2 on day 1, 7–10 mg/m^2 on days 3 and 6, and optionally on day 11). For patients who received a graft from a haploidentical HLA-mismatched donor, alemtuzumab was added to the TBI regimen or the FB regimen at 0.2 mg/kg for 6 days [11]. Methylprednisolone (mPSL) or prednisolone (PSL) at 1 or 2 mg/kg was added for patients who developed grade 2–4 acute GVHD, whereas PSL at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, fungal, herpes simplex virus, and pneumocystis jirovecii infections consisted of fluconazole, tosufloxacin, acyclovir, and sulfamethoxazole/trimethoprim.

2.3 CMV antigenemia assay

CMV antigenemia assay was performed as described previously [12]. In brief, 1.5×10^5 peripheral blood leukocytes were attached to a slide, using a cytocentrifuge and fixed with formaldehyde. The cells were sequentially immunostained with monoclonal antibody C10/11 (Clonab CMV; Biotest, Dreieich, Germany), which targets CMV pp65 antigen, and reacted with goat alkaline phosphatase-labeled anti-mouse immunoglobulin (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under light microscopy, CMV-positive cells were counted and the results are presented as the sum of the number of positive cells per two slides.

2.4 Preemptive therapy for CMV disease

Preemptive therapy against CMV disease was performed by weekly monitoring of CMV antigenemia after engraftment, as described previously [13]. Until June 2001, intravenous GCV was started at an induction dose of 10 mg/kg/day when ten or more CMV-positive cells were detected in patients who underwent HSCT from a HLA-matched related donor and when positive cells were detected at any level in patients who underwent HSCT from an alternative donor. From July 2001, the induction dose was decreased to 5 mg/kg/day and the threshold of antigenemia to start GCV was changed to twenty and three positive cells for patients who underwent HSCT from a HLA-matched related donor and an alternative donor, respectively [14]. The dose of GCV was increased to 10 mg/kg/day when rising antigenemia was observed. The dose of GCV was adjusted according to renal function [15]. GCV was continued until negative antigenemia was observed. Beyond day 100 after HSCT, CMV monitoring was continued at least every other week in 90 patients and at longer intervals in the remaining 11 patients. GCV was administered in a similar manner as before day 100, at the discretion of attending physicians.

2.5 Definition of late positive CMV antigenemia and CMV disease

Late positive CMV antigenemia was defined as the detection of CMV-positive cells at any level after the starting point. Recurrent CMV antigenemia was defined as the detection of CMV-positive cells after the negative conversion of late positive CMV antigenemia. All patients with symptoms compatible with CMV disease, such as interstitial pneumonia, colitis, or gastritis underwent extensive pathological and microbiological examination of biopsy specimens. Biopsy was performed in all cases of interstitial pneumonia, colitis, and gastritis. The diagnosis of these CMV diseases was made by histopathological examination and immunochemical staining of biopsy specimens, which demonstrated typical CMV inclusion bodies. To establish a definite diagnosis of CMV retinitis, patients received PCR to detect CMV-DNA using aqueous humor and/or ophthalmoscopy, which demonstrated typical findings of CMV retinitis, including a white fluffy retinal infiltrate with several areas of hemorrhage or a granular white area without hemorrhage.

2.6 Statistical analysis

The cumulative incidences of late positive CMV antigenemia and CMV disease, and the impact of possible confounding factors on these events were evaluated using Gray's method, considering death without each event as a competing risk [16]. The cumulative incidence of non-relapse mortality (NRM), and the impact of possible confounding factors on NRM were evaluated, considering relapse as a competing risk. The development of chronic GVHD and the use of systemic corticosteroids at $\geq$0.5 mg/kg/day were treated as time-dependent covariates. Factors associated with at least borderline significance ($P < 0.10$) in univariate analyses were subjected to multivariate

analysis using backward stepwise proportional-hazard modeling. P-values <0.05 were considered significant.

3 Results

3.1 Incidence and risk factors for late positive CMV antigenemia

Overall, 51 of 101 patients developed late positive antigenemia at a median of 29 days (range 1–483 days) after the starting point, with a cumulative incidence of 53% (Fig. 1a). In univariate analyses, recipient CMV seropositivity, the use of alemtuzumab in a conditioning regimen, the preceding detection of CMV antigenemia, prior use of GCV, the development of chronic GVHD, and the use of systemic corticosteroids at ≥0.5 mg/kg/day were significantly associated with the development of late positive antigenemia (Table 2). In multivariate analysis, recipient CMV seropositivity, the use of alemtuzumab in a conditioning regimen, the development of chronic GVHD, and the use of systemic corticosteroids at ≥0.5 mg/kg/day were identified as independent risk factors for late positive antigenemia.

Fifty (98%) of the 51 patients with late positive antigenemia showed negative conversion of antigenemia after a median of 21 days (range 2–430 days). The median peak antigenemia level was only two positive cells per two slides (range 1–268). Twenty-eight patients developed late CMV antigenemia with consistently less than three positive cells (low-level antigenemia), 25 of whom showed negative conversion without GCV administration (Table 3A). Of the remaining 23 patients who developed high-level antigenemia with three or more positive cells, all but one who died of invasive aspergillosis (IA) achieved negative conversion, with ($n = 17$) or without ($n = 5$) GCV administration. Twenty-nine of the 50 patients (58%) had recurrent antigenemia at a median of 14 days (range 3–714 days) after the first negative conversion (Table 3B). The second recurrence was observed in 17 of 26 patients, after a median of 21 days (range 4–323 days) after the second negative conversion.

3.2 Incidence and risk factors for late CMV disease

Eight patients developed late CMV disease at a median of 54 days (range 14–248 days) after the starting point, with a cumulative incidence of 8%, (Fig. 1b). Female sex, the use of alemtuzumab in the conditioning regimen, the preceding detection of CMV antigenemia, prior use of GCV, and the development of chronic GVHD were associated with a higher incidence of late CMV disease with at least borderline significance ($P < 0.10$) (Table 4). Among these, the use of alemtuzumab in the conditioning regimen was the only independent risk factor for late CMV disease.

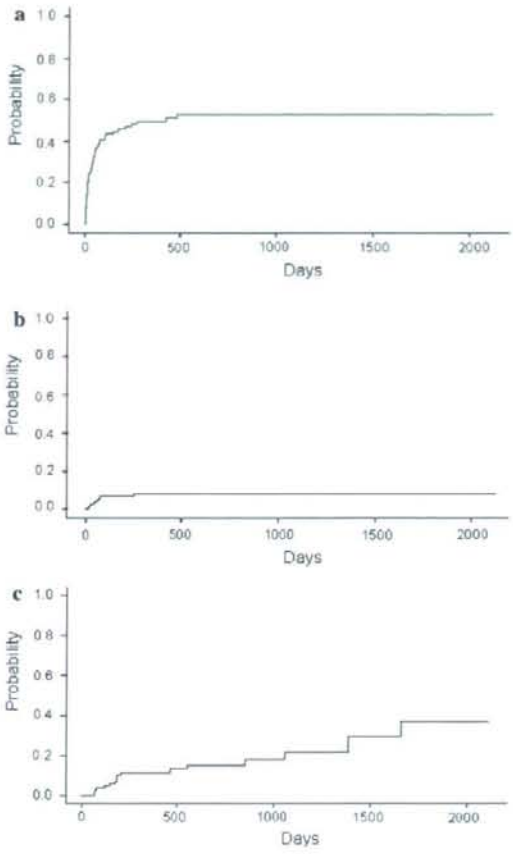

Fig. 1 Cumulative incidences of **a** late positive CMV antigenemia, **b** late CMV disease, and **c** non-relapse mortality. The definition of the starting point is described in the text

Late CMV disease involved retinitis in four, colitis in three, and gastritis in one (Table 5). Three of the four patients with retinitis were asymptomatic. Although all of these CMV diseases were successfully treated with GCV, two patients died of bronchiolitis obliterans and IA, after the resolution of CMV disease. Among the eight patients with late CMV disease, seven developed CMV disease after the development of late positive antigenemia. Five developed CMV disease after the first episode, and the other two after recurrent antigenemia. The median peak antigenemia level was ten positive cells per two slides (range 4–186). The remaining patient developed late CMV disease before the development of late positive antigenemia. All patients achieved negative conversion by the administration of intravenous GCV.

Table 2 Risk factors for late positive CMV antigenemia

Univariate analysis			
Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	63	0.30
≤40 years old	45	44	
Sex			
Male	65	52	0.40
Female	36	54	
Disease risk			
Standard risk	40	51	0.80
High risk	61	54	
Graft source			
Bone marrow	61	46	0.18
Peripheral blood	40	64	
Donor type			
Matched related donor	35	47	0.51
Alternative donor	66	56	
Regimen			
TBI regimen	72	54	0.81
Non-TBI regimen	29	50	
Regimen			
With alemtuzumab	13	69	0.041
Without alemtuzumab	88	47	
Donor CMV			
Seropositive	80	57	0.18
Seronegative	21	33	
Recipient CMV			
Seropositive	90	58	0.0069
Seronegative	11	9	
Prior CMV antigenemia			
Yes	74	61	0.0053
No	27	32	
Prior use of GCV			
Yes	59	62	0.012
No	42	41	
Year of transplant			
Before June 2001	30	50	0.77
After July 2001	71	54	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	3.29 (1.81–5.97)	<0.0001
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	2.77 (1.16–4.45)	0.017
No	45		

Table 2 continued

Multivariate analysis			
Factors	Relative risk	95% CI	*P*-value
Steroid at ≥0.5 mg/kg	2.13	1.04–4.38	0.040
Chronic GVHD	2.87	1.54–5.35	0.00095
With alemtuzumab	2.54	1.20–5.37	0.015
Recipient CMV seropositive	13.0	1.77–95.6	0.012

3.3 Incidence and risk factors for non-relapse mortality

Seventeen patients died of non-relapse causes more than 100 days after HSCT, with a 3-year cumulative incidence of 22% (Fig. 1c). Male sex and the use of systemic corticosteroids at ≥0.5 mg/kg were associated with a higher NRM with at least borderline significance (Table 6). The use of systemic corticosteroids at ≥0.5 mg/kg was identified as independently significant for NRM in multivariate analysis. The direct causes of death included non-infectious pulmonary complications (NIPC) in seven patients, infections other than CMV in five, gastrointestinal bleeding in two, multiple organ failure in two, and acute myocardial infarction in one. Fifteen of the seventeen patients received systemic corticosteroids at ≥0.5 mg/kg after the starting point, for severe chronic GVHD in eight, NIPC in four, respiratory failure caused by infections in two, and hemophagocytic syndrome in one.

4 Discussion

This study demonstrated that the cumulative incidence of late CMV disease was successfully decreased and CMV-related mortality was completely avoided by preemptive therapy with extended CMV antigenemia monitoring beyond day 100, in spite of a high frequency of late CMV reactivation. The use of alemtuzumab was the only significant independent risk factor for late CMV disease, while recipient CMV seropositivity, the use of alemtuzumab, chronic GVHD and high-dose steroids were important determinants for late positive antigenemia. A significant correlation between the development of late positive antigenemia and these risk factors is consistent with the clinical observation that the development of chronic GVHD and the use of alemtuzumab or high-dose steroid resulted in delayed recovery of CMV-specific immune response, leading to an increased incidence of late CMV reactivation [17, 18]. Extended CMV-antigenemia monitoring is strongly recommended in such patients with profound immunosuppression.

Table 3 Time course of late positive CMV antigenemia

A. Time course of CMV-Ag in 51 patients who developed late positive antigenemia

Peak level after late positive Ag	Number of patients (*n*)	Use of GCV	Number of patients (*n*)	Peak value of CMV-Ag	Patients with negative conversion
CMV-Ag < 3	28	(−)	25	1 (1–2)	25
		(+)	3	2 (1–2)	3
CMV-Ag ≥ 3	23	(−)	5	4 (3–8)	5
		(+)	18	12 (3–268)	17

B. Recurrence of late positive CMV antigenemia

Late positive CMV antigenemia	Number of patients (*n*)	Patients with late positive Ag (*n*)	Value of CMV-Ag at late positive Ag	Peak value of CMV-Ag	Use of GCV	Patients with negative conversion
First late positive Ag	101	51	1 (1–268)	2 (1–268)	21	50
Second late positive Ag	50	29	1 (1–18)	1 (1–86)	14	26
Third late positive Ag	26	17	2 (1–126)	3 (1–126)	8	16

Table 4 Risk factors for late CMV disease

Univariate analysis

Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	9	0.66
≤40 years old	45	7	
Sex			
Male	65	5	0.094
Female	36	15	
Disease risk			
Standard risk	40	8	0.90
High risk	61	9	
Graft source			
Bone marrow	61	5	0.18
Peripheral blood	40	13	
Donor type			
Matched related donor	35	3	0.17
Alternative donor	66	11	
Regimen			
TBI regimen	72	10	0.29
Non-TBI regimen	29	3	
Regimen			
With alemtuzumab	13	31	0.00097
Without alemtuzumab	88	5	
Donor CMV			
Seropositive	80	9	0.58
Seronegative	21	5	
Recipient CMV			
Seropositive	90	9	0.31
Seronegative	11	0	

Table 4 continued

Univariate analysis

Factors	*n*	Incidence (%)	*P*-value
Prior CMV antigenemia			
Yes	74	11	0.075
No	27	0	
Prior use of GCV			
Yes	59	12	0.080
No	42	2	
Year of transplant			
Before June 2001	30	3	0.26
After July 2001	71	10	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	9.27 (1.11–77.5)	0.040
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	1.12 (0.21–5.90)	0.90
No	45		

Multivariate analysis

Factors	Relative risk	95% CI	*P*-value
With alemtuzumab	8.20	2.02–33.3	0.0032

The incidence of late CMV disease in our series was lower than that in previous studies, where antiviral agents were not used preemptively beyond day 100 [1–3]. In a recent study by Boeckh et al. [2], 17.8% of the patients

Table 5 Characteristics of patients who developed late CMV disease

UPN	Sex/age	Diagnosis	Preparative regimen	cGVHD grade	Steroid at CMV disease	Starting point to late positive Ag / disease (days)	Treatment	CMV disease	Lymphocyte counts at CMV disease	Peak value of late CMV antigenemia	Negative conversion	Survival months (months)	Cause of death
1	M/38	ALL	Cam/Cy/TBI	Limited	0	18/60	Intravenous GCV	Retinitis (asymptomatic)	1,505	10	+	29.8+	Alive
2	F/43	NHL	Cy/TBI	Extensive	0	1/14	Intravenous GCV / vitrectomy	Retinitis (asymptomatic)	840	6	+	21.4+	Alive
3	M/42	AA	Cam/Flu/Cy/TBI	Extensive	PSL 30 mg	2/48	Intravenous GCV	Colitis	1,125	12	+	12.1+	Alive
4	F/57	AML	Cam/Flu/Bu/TBI	None	0	4/27	Intravenous/ intreavetreal GCV	Retinitis (asymptomatic)	<400	28	+	6.2	IA
5	F/41	ALL	Cam/Cy/TBI	Limited	0	4/77	Intravenous GCV	Retinitis(symptomatic)	1,890	4	+	9.6+	Alive
6	F/25	CML	Cy/TBI	Extensive	mPSL 20 mg	42/17	Intravenous GCV /foscarnet	Gastritis	748	0	+	33.5	BO
7	F/38	AML	Cy/TBI	Extensive	0	7/248	Intravenous GCV	Colitis	704	6	+	48.6+	Alive
8	M/56	NHL	Flu/BU	Extensive	PSL 10 mg	36/69	Intravenous GCV	Colitis	2,465	186	+	38.0+	Alive

UPN unique patient number, *cGVHD* chronic graft-versus-host-disease, *CMV* cytomegalovirus, *GCV* ganiclovir, *AA* aplastic anemia, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *CML* chronic myelogenous leukemia, *NHL* non-Hodgkin lymphoma, *TBI* total body irradiation, *Cam* alemtuzumab, *Cy* cyclophsphamide, *Flu* fludarabine, *Bu* Busulfan, *PSL* predonisolone, *mPSL* methylpredonisolone, *IA* invasive aspergillosis, *BO* bronchiolitis obliterans

Table 6 Risk factors for 3-year non-relapse mortality

Univariate analysis			
Factors	*n*	Incidence (%)	*P*-value
Age			
>40 years old	56	21	0.55
≤40 years old	45	9	
Sex			
Male	65	22	0.092
Female	36	3	
Disease risk			
Standard risk	40	14	0.65
High risk	61	16	
Graft source			
Bone marrow	61	16	0.60
Peripheral blood	40	15	
Donor type			
Matched related donor	35	21	0.10
Alternative donor	66	12	
Regimen			
TBI regimen	72	14	0.26
Non-TBI regimen	29	18	
Regimen			
With alemtuzumab	13	8	0.56
Without alemtuzumab	88	16	
Donor CMV			
Seropositive	80	15	0.60
Seronegative	21	18	
Recipient CMV			
Seropositive	90	15	0.14
Seronegative	11	22	
Prior CMV antigenemia			
Yes	74	15	0.30
No	27	18	
Prior use of GCV			
Yes	59	18	0.65
No	42	12	
Year of transplant			
Before June 2001	30	14	0.78
After July 2001	71	16	

Factors (time-dependent covariates)	*n*	Relative risk (95% CI)	*P*-value
Chronic GVHD			
Yes	74	1.35 (0.35–5.21)	0.67
No	27		
Steroid at ≥0.5 mg/kg			
Yes	56	10.5 (2.34–47.0)	0.0021
No	45		

Multivariate analysis			
Factors	Relative risk	95% CI	*P*-value
Steroid at ≥0.5 mg/kg	1.05	2.34–47.0	0.0021

who did not receive antiviral agents preemptively more than 3 months after HSCT, developed late CMV disease, including pneumonia and gastrointestinal disease predominantly, with a mortality rate of 46%. Other studies have also confirmed that CMV pneumonia was the leading manifestation of late CMV disease with an associated mortality rate ranging from 60 to 80%, in the absence of preemptive therapy [1, 4]. In contrast, we exclusively observed late CMV retinitis and/or gastrointestinal disease, none of which was directly related to death. These findings suggest that preemptive therapy with extended CMV antigenemia monitoring, reduced the incidence of late CMV disease and eradicated fatal CMV disease.

The use of alemtuzumab was the only independent significant risk factor for late CMV disease, although it was not associated with increased non-relapse mortality. This finding is inconsistent with the conclusion of previous studies that the use of alemtuzumab did not result in an increased incidence of CMV disease in two previous studies despite a strong association with the high frequency of CMV reactivation [11, 17]. In this study, four of the 13 patients who received alemtuzumab developed late CMV disease, three of whom had retinitis. Two cases of retinitis were asymptomatic and diagnosed by ophthalmologic screening. We performed routine ophthalmologic screening as a standard practice only in patients who received alemtuzumab, based on the association between a high incidence of CMV reactivation and delayed posttransplant immune reconstitution by alemtuzumab [11, 17]; therefore, asymptomatic retinitis might have been overlooked in patients who did not receive alemtuzumab in this study or in patients who received alemtuzumab in other studies.

More than half of the patients with late positive antigenemia developed low-level antigenemia with consistently less than three positive cells, and 90% showed negative conversion without GCV administration. In all high-level antigenemia patients, except for one who died of IA while on preemptive GCV, negative conversion without progression to fatal disease was obtained by preemptive therapy; therefore, three positive cells per two slides might be an appropriate threshold to start GCV beyond day 100 after transplantation.

In conclusion, late positive antigenemia was frequently observed beyond day 100 after transplantation, especially in profoundly immunosuppressed patients who received alemtuzumab, high-dose steroids, or who developed chronic GVHD. Preemptive therapy with extended CMV antigenemia monitoring beyond day 100, not only reduced the incidence of late CMV disease, but also completely prevented fatal CMV disease; therefore, extended CMV monitoring is recommended at least for patients with such risk factors.

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Long-term ultra-low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation

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To evaluate the efficacy of long-term prophylaxis with ultra-low-dose acyclovir against varicella-zoster virus (VZV) reactivation, we analyzed the records of 242 Japanese adult patients who underwent allogeneic hematopoietic stem cell transplantation for the first time from 1995 to 2006 at our hospital. We started long-term oral acyclovir at 200 mg/day in July 2001. Acyclovir was continued until the end of immunosuppressive therapy and at least 1 year after transplantation. Sixty-six patients developed VZV reactivation at a median of 248 days after HSCT, with a cumulative incidence of 34.7%. Only one breakthrough reactivation occurred during long-term acyclovir, which responded well to therapeutic dose of valacyclovir. The use of long-term acyclovir was the only independent determinant that significantly decreased the overall incidence of VZV reactivation (20% vs. 50%, $P < 0.0001$). With this prophylaxis, visceral dissemination and serious complications other than post-herpetic neuralgia was completely eliminated, and thereby need for hospitalization was significantly reduced (21% vs. 71%, $P = 0.0034$). Fifteen of the 57 patients who discontinued acyclovir developed VZV reactivation, with a cumulative incidence of 32.1%. VZV reactivation following discontinuation tended to occur in patients who were receiving immunosuppressive therapy at the cessation of acyclovir. These findings suggested that long-term prophylaxis of ultra-low-dose acyclovir resulted in a successful prevention of severe VZV-related symptoms and death, with a significantly decreased overall incidence of VZV reactivation. Prolongation of prophylactic acyclovir on profound immunosuppression might be important for thorough suppression of VZV reactivation. Am. J. Hematol. 83:472–476, 2008. © 2008 Wiley-Liss, Inc.

Introduction

Varicella-zoster virus (VZV) infection remains a common complication after hematopoietic stem cell transplantation (HSCT) [1–4]. VZV infection develops as a reactivation of latent virus mainly between the third and twelfth month after transplantation, with a cumulative incidence of more than 30% [1,2]. Localized dermatomal rash is the most common clinical presentation, whereas dissemination or visceral involvement is occasionally observed, leading to a fatal outcome. Although most of VZV infections were successfully treated with antiviral agents, VZV-related complications including post-herpetic neuralgia and secondary infection significantly affect the patient's quality of life [1,5].

The introduction of long-term prophylaxis with low-dose acyclovir against VZV reactivation has therefore been investigated [4,6–10]. Several studies concluded that prophylactic acyclovir at 600–3,200 mg/day continued for a fixed period up to 6 months or 1 year have failed to decrease the overall incidence of VZV reactivation [4,6–8]. Despite that VZV reactivation during prophylaxis was significantly reduced, a substantial number of VZV reactivation occurred following the discontinuation of acyclovir. A most recent randomized placebo-controlled trial showed a predominant occurrence of VZV reactivation after the cessation of acyclovir, which was given at 800 mg/day for 1 year after HSCT, in recipients with prolonged immunosuppression [8]. Moreover, other studies reported that long-term acyclovir at 400 mg/day continued until the end of immunosuppressive therapy could not suppress VZV reactivation after the discontinuation of acyclovir [9,10]. Thus, the appropriate prophylactic dose and duration of acyclovir to decrease the overall incidence of VZV reactivation have not been clarified.

We carried out a novel trial of long-term acyclovir prophylaxis at an ultra-low-dose (200 mg/day) until the end of immunosuppressive therapy and at least 1 year after HSCT, and retrospectively compared the incidence of VZV reactivation with historical control patients who did not receive long-term prophylaxis. With this prophylaxis, lower-cost, less side effects, and better compliance may also be promising.

Results

Incidence and risk factors for VZV reactivation after HSCT

In total of 242 patients, 137 received long-term acyclovir following prophylaxis against HSV infection, whereas the remaining 105 did not receive long-term acyclovir. Overall, 66 out of the 242 patients developed VZV reactivation at a median of 248 days (range 50–1,494 days) after HSCT,

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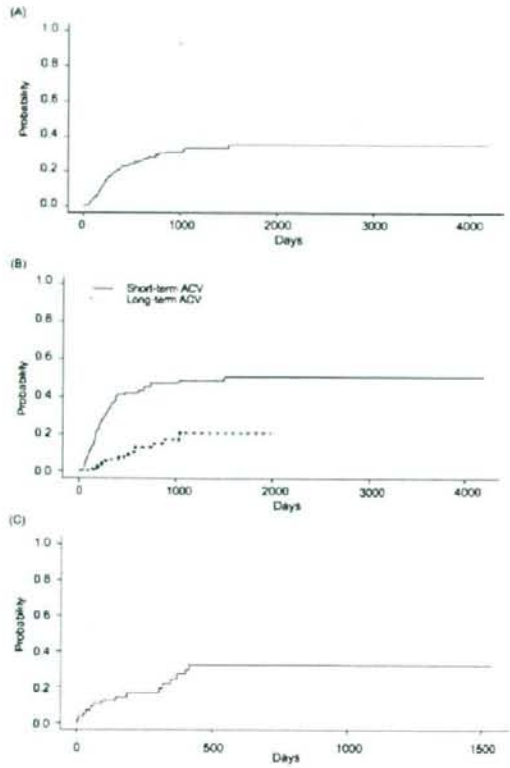

Figure 1. (A) Cumulative incidences of VZV reactivation after HSCT in all 242 patients. (B) Cumulative incidences of VZV reactivation after HSCT in 137 patients who received long-term acyclovir versus 105 patients who did not. (C) Cumulative incidences of VZV reactivation after the cessation of long-term acyclovir in 57 eligible patients for analysis.

TABLE I. Risk Factors for VZV Reactivation After HSCT

Factors	Variables	n	Incidence (%)	*P*-value
Univariate analysis				
Age	≥ 40 years old	117	25	0.005
	<40 years old	125	43	
Sex	Male	154	34	0.71
	Female	88	37	
Disease risk	Standard-risk	96	38	0.63
	High-risk	146	32	
Graft source	Bone marrow	166	40	0.06
	Peripheral blood	73	25	
Donor type	Matched sibling donor	97	40	0.11
	Alternative donor	145	31	
Regimen (1)	Conventional	204	37	0.05
	Reduced-intensity	38	25	
Regimen (2)	TBI regimen	179	36	0.63
	Non-TBI regimen	63	32	
Long-term ACV	Yes	137	20	<0.0001
	No	105	50	

Factors	Variables	*n*	Relative risk 95% CI	*P*-value
Grade II–IV acute GVHD	Yes	97	1.18 (0.72–1.94)	0.51
	No	145		
Chronic GVHD	Yes	131	0.87 (0.51–1.50)	0.62
	No	76		

Factors	Relative risk	95% CI	*P*-value
Multivariate analysis			
Long-term ACV	0.23	0.13–0.39	<0.0001

with a cumulative incidence of 34.7% (Fig. 1A). Only one patient experienced a breakthrough reactivation during long-term acyclovir, which responded promptly to a therapeutic dose of valacyclovir. In univariate analyses, younger age, bone marrow transplantation, conventional regimen, and the use of long-term acyclovir were significantly associated with the low VZV reactivation incidence rate (Table I). In a multivariate analysis, the use of long-term acyclovir was identified as the only independent factor that significantly decreased the incidence of VZV reactivation (20% vs. 50%, $P < 0.0001$, Table I, Fig. 1B).

Clinical features of patients who developed VZV reactivation

Fifty-three of the 66 VZV reactivations (80%) occurred in a localized dermatomal distribution (Table II). Clinically significant complications developed in 17 patients, the most common of which was post-herpetic neuralgia. Among these complications, only post-herpetic neuralgia was seen in three patients with long-term acyclovir, whereas serious complications including CNS involvement, motor neuropathy, and ophthalmic complications were involved in the remaining 14 patients without long-term acyclovir.

Fifty-two of the 66 patients developed VZV reactivation in outpatient setting. Among these patients, hospitalization was required for VZV reactivation in 3 of 14 patients with long-term acyclovir and in 27 of 38 patients without long-term acyclovir (21% vs. 71%, $P = 0.0034$).

Seven of the 66 patients with VZV reactivation (11%) developed recurrent VZV reactivation in the different dermatome, at a median of 95 days (range 55–798 days) after the first episode. All of them never received acyclovir after finishing the treatment for the first episode. At the time of recurrence, five of the seven patients were receiving immunosuppressive therapy and the remaining two showed severe lymphocytopenia less than 300/μl due to chemotherapy for relapse of hematological malignancy. The third episode of VZV reactivation occurred in two patients, at 158 and 240 days after the second reactivation. None was receiving acyclovir at the time of second or third VZV reactivation. All the patients responded well to treatment with antiviral agents, and none of them directly died of VZV reactivation.

Incidence and risk factors for VZV reactivation after the cessation of long-term acyclovir

Of 137 patients who received long-term acyclovir, 73 patients were receiving acyclovir until VZV reactivation, their last follow-up, or death. The other seven died within a week following the discontinuation of acyclovir. Therefore, 80 patients were excluded and only 57 patients were eligible for analysis after the cessation of acyclovir. The median follow-up duration from the discontinuation of acyclovir was 279 days (range 9–1,936 days). They received long-term acyclovir with a median prophylactic period of 358 days (range 49–1,259 days). Fifteen patients developed VZV reactivation at a median of 147 days (range 5–415 days)

TABLE II. Clinical Presentation and Secondary Complications of VZV Reactivation

Low-dose ACV	No	Yes	Total
Total patients	105	137	242
VZV reactivation	50	16	66
Out-patient onset	38	14	52
Hospitalized	27	3	30
Treated as outpatient	11	11	22
Valacyclovir	4	8	12
Acyclovir	7	3	10
Clinical presentations			
Localized	39	14	53
Trigeminal	4	2	6
Cervical	5	1	6
Thoracic	22	5	27
Lumbar	5	4	9
Sacral	3	2	5
Disseminated	11	2	13
Cutaneous	7	2	9
Visceral	4	0	4
Complications	14	3	17
Ophthalmic complications	1	0	1
Motor neuropathy	1	0	1
CNS involvement	3*	0	3
Post-herptic neuralgia	9	3	12

*One patient had both CNS involvement and post-herpetic neuralgia.

TABLE III. Risk Factors for VZV Reactivation After the Cessation of Long-Term ACV

Factors	Variables	*n*	Incidence(%)	*P*-value
Univariate analysis				
Age	≥40 years old	31	34	0.56
	<40 years old	26	29	
Sex	Male	36	21	0.14
	Female	21	54	
Disease risk	Standard-risk	26	30	0.39
	High-risk	31	34	
Graft source	Bone marrow	28	34	0.96
	Peripheral blood	28	41	
Donor type	Matched sibling donor	23	29	0.61
	Alternative donor	34	34	
Regimen (1)	Conventional	43	32	0.94
	Reduced-intensity	14	31	
Regimen (2)	TBI regimen	42	30	0.57
	Non-TBI regimen	15	38	
Duration of long-term ACV	<1 year	33	30	0.85
	≥1 year	24	33	
Immunosuppressive therapy at the cessation of ACV	Yes	25	44	0.12
	No	32	20	

Factors	Variables	*n*	Relative risk 95% CI	*P*-value
Chronic GVHD	Yes	37	1.68	0.47
	No	17	(0.40–6.99)	

after the discontinuation of acyclovir, with a cumulative incidence of 32.1% (Fig. 1C). Although statistically significant risk factors were not identified to affect the incidence of VZV reactivation after discontinuation, ongoing immunosuppressive therapy at the cessation of acyclovir tended to increase the incidence of VZV reactivation (Table III).

Discussion

This study demonstrated that the long-term prophylactic acyclovir at 200 mg/day was highly effective to reduce VZV reactivation, dissemination and serious complications, as well as VZV-related mortality in HSCT recipients. There was only one breakthrough of localized reactivation that responded well to the therapeutic dose of valacyclovir. A once-a-day dosing of 200 mg until the cessation of immunosuppressive therapy and at least 1 year after HSCT significantly decreased the overall incidence of VZV reactivation from 50 to 20%, in contrast with the previous studies in which various doses of 600 mg/day or more were given for a fixed period up to 6 months or 1 year after HSCT without significant reduction of the overall incidence of VZV reactivation [4,6–8]. Although an optimal prophylactic dose and duration of acyclovir administration has not been clarified, this extended prophylactic approach to continue acyclovir until the end of immunosuppressive therapy and at least 1 year after HSCT may be more appropriate than the shorter prophylaxis or fixed-duration prophylaxis. Also, this is the first report that the ultra-low-dose of acyclovir at only 200 mg/day was sufficient to prevent VZV reactivation during prophylaxis.

In this study, however, VZV reactivation was not uncommon after the discontinuation of long-term acyclovir, as previously observed in the other two studies in which acyclovir at 400 mg/day was given until the end of immunosuppressive therapy [9,10]. Nevertheless, the severity of clinical symptoms was ameliorated and thereby need for hospitalization was markedly reduced by the long-term acyclovir. Among the 15 patients who developed VZV reactivation after the cessation of acyclovir, none showed visceral dissemination or serious complications. The less severe symptoms in patients with long-term acyclovir may reflect the contribution of VZV-specific immune recovery, which might have been accelerated by subclinical VZV reactivation. It has been shown that *in vivo* re-exposure to VZV antigens without clinical symptoms may boost immunity and thereby prevent subsequent symptomatic VZV reactivation [11]. Lower daily dosing of 200 mg might have permitted subclinical VZV reactivation to establish the reconstitution of VZV-specific immunity. There is another possibility that need for hospitalization in patients with long-term acyclovir might have been reduced by the use of valacyclovir, which became available from October 2000 in Japan. However, mild cases of VZV reactivation had been treated with oral acyclovir, and actually 7 of 11 patients who developed VZV reactivation without long-term acyclovir were successfully treated with oral acyclovir without hospitalization. Therefore, we suppose that a decreased hospitalization rate in patients with long-term acyclovir was due to less severe symptoms rather than the availability of valacyclovir.

In some patients, long-term acyclovir was discontinued within a year at the physician's discretion or at the request of the patients. This is a limitation of this study, but it revealed that ongoing immunosuppressive therapy at the cessation of acyclovir tended to be more frequently associated with VZV reactivation following discontinuation, which agreed with the conclusion of Boeckh's study that VZV reactivation predominantly occurred in patients with continued systemic immunosuppression [8]. They did not find any significant difference in the reconstitution of VZV-specific immunity between the acyclovir and placebo groups following the 1-year prophylaxis at 800 mg/day. In addition, the study with long-term acyclovir at 400 mg/day also showed that VZV reactivation after the cessation of acyclovir was observed only in patients who were receiving resumed immunosuppressants [10]. In this study, three patients with long-term acyclovir experienced dissemination and/or post-

herpetic neuralgia, all of whom were receiving prolonged immunosuppressive therapy for chronic GVHD both at the cessation of acyclovir and at the time of VZV reactivation. These findings suggest that VZV reactivation as well as the severity of symptoms is strongly related to the decline in VZV-specific immunity as a result of HSCT and/or immunosuppressive therapy. Therefore, continuing acyclovir in patients with profound immunosuppression is recommended for further prevention of VZV reactivation. Another possible approach is to administer inactivated VZV vaccine at the discontinuation of acyclovir [12].

In conclusion, this study showed that the long-term prophylaxis with ultra-low-dose acyclovir might be an effective strategy for the suppression of VZV reactivation during prophylaxis and minimizing the long-term risks of VZV-related complications and mortality. Further investigation is necessary to evaluate the validity of resuming acyclovir for patients with resumed immunosuppressive therapy.

Patients and Methods

Study population

A total of 271 consecutive adult patients (≥16 years old) underwent allogeneic HSCT for the first time at the University of Tokyo Hospital between June 1995 and November 2006. Five patients who died within 35 days after HSCT were excluded, and clinical data for this study were available for 242 of the remaining 266 patients. A median follow-up was 486 days (range, 37–4,209 days) from HSCT for the entire cohort of 242 patients. Thirty-eight patients who received reduced-intensity conditioning were included. The patient characteristics are summarized in TABLE IV. Ninety-seven, 42 and 103 patients received graft from an HLA-matched sibling donor, a mismatched related donor, and a matched unrelated donor, respectively. Unrelated HSCT was performed exclusively using bone marrow, whereas 73 out of 139 related donors chose to donate peripheral blood stem cell graft. Acute leukemia in first remission, chronic myelogenous leukemia in first chronic phase, myelodysplastic syndrome with refractory anemia or refractory anemia with ringed sideroblasts, and aplastic anemia were defined as low-risk diseases, while others were considered high-risk diseases. Donors other than HLA-matched sibling donors were defined as alternative donors.

Transplantation procedure

The conventional preparative regimen for leukemia/lymphoma was mainly performed with either total body irradiation (TBI) regimen (cyclophosphamide (Cy) at 60 mg/kg for 2 days and TBI at 2 Gy twice daily for 3 days) or non-TBI regimen (Cy at the same dose combined with busulfan (Bu) at 4 mg/kg for 4 days). In TBI regimen, the dose of Cy was decreased to 40 mg/kg for 1 day and etoposide at 20 mg/kg for 2 days was added instead in patients with impaired cardiac function. Fludarabine (Flu)-based regimens included FB regimen (Flu at 30 mg/m^2 for 6 days and Bu at 4 mg/kg for 2 days) with or without TBI at 4 Gy, FB16 regimen (Flu at the same dose with Bu at 4 mg/kg for 4 days), FM regimen (Flu 30 mg/m^2 for 5 days and melphalan at 140 mg/m^2 for 1 day), and FC regimen (Flu at 25 mg/m^2 for 5 days and Cy at 60 mg/kg for 2 days) were used as reduced-intensity regimens for elderly or clinically infirm patients [13]. Gemcitabine at 1,000 mg/kg/m^2 for 3 days was added to the FB regimen for patients with pancreatic cancer [14]. The conditioning regimen for aplastic anemia was either a rabbit antithymocyte globulin (ATG) regimen (Cy at 50 mg/kg for 4 days and ATG at 5 mg/kg for 5 days with or without TBI at 4 Gy) or an alemtuzumab regimen (Cy at 25 mg/kg for 4 days and Flu at 30 mg/kg for 4 days combined with alemtuzumab at 0.2 mg/kg for 6 days, with or without TBI at 2 Gy).

For prophylaxis against GVHD, cyclosporine A (CsA) at 3 mg/kg/day or tacrolimus at 0.03 mg/kg/day was combined with short-term methotrexate (10–15 mg/m^2 on Day 1, 7–10 mg/m^2 on Days 3 and 6, and optionally on Day 11). For patients who received graft from an HLA-mismatched donor, alemtuzumab was added to the TBI regimen or the FB regimen at 0.2 mg/kg for 6 days [15]. Methyl-prednisolone (mPSL) or prednisolone (PSL) at 1 or 2 mg/kg was added for patients who developed grade II-IV acute GVHD, whereas PSL at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, fungal, and *Pneumocystis jirovecii* infections consisted of fluconazole, tosufloxacin, and sulfamethoxazole/trimethoprim. Antigenemia-guided pre-emptive therapy against CMV infection was performed as described previously [16].

TABLE IV. Patients' Characteristics

Characteristic		Total patients
Sex (male/female)		154/88
Age, median (range)		39 (16–66)
Underlying disease	Acute leukemia	121
	CML	50
	MDS	26
	NHL/ATL	25
	SAA	10
	Other	10
Graft source	PBSC	73
	BM	166
	CB	3
Donor type	Matched sibling	97
	Mismatched related	42
	Unrelated	103
VZV seropositivity	Positive	231
	Negative	3
	Not examined	8
Preparative regimen	Cy (Etp)/TBI-based regimens	167
	Bu/Cy-based regimens	37
	ATG-based regimens for SAA	7
	Flu-based RIC	31
GVHD prophylaxis	CsA + MTX	200
	Tacrolimus + MTX	18
	Alemtuzumab + CsA + MTX	24
Acute GVHD	Grade 0–I	145
	Grade II–IV	97
Chronic GVHD	Extensive	86
	Limited	45
	None	76

VZV indicates varicella zoster virus; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; ATL, adult T-cell leukemia/lymphoma; SAA, severe aplastic anemia; PBSC, peripheral blood stem cell; BM, bone marrow; CB, cord blood; Cy, cyclophosphamide; Etp, etoposide; TBI, total body irradiation; Bu, busulfan; ATG, antihymocyte globulin; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporine; MTX, methotrexate.

Diagnosis and treatment of VZV reactivation

The diagnosis of VZV reactivation was established by the presence of characteristic vesicular skin lesion on an erythematous base within dermatome or a generalized cutaneous distribution. Microbiological and/or pathological confirmation was performed only in equivocal cases. Post-herpetic neuralgia was defined as dermatomal pain persisting beyond 1 month after initial presentation of VZV reactivation. VZV reactivation was treated with intravenous acyclovir at 15–30 mg/kg/day in the majority of patients, and followed, in some patients, by oral acyclovir at 1–4 g/day or oral valacyclovir at 3 g/day, for a total treatment period of 5–42 days. A proportion of patients received outpatient treatment only, with valacyclovir 3 g/day orally for 5–10 days. The doses and dosing interval of these drugs were adjusted according to the creatinine clearance in patients with renal impairment.

Prophylactic administration of acyclovir

As prophylaxis against herpes simplex virus infection (HSV), acyclovir was given at 750 mg/day intravenously or at 1,000 mg/day orally from Day 7 to 35. We started long-term oral administration of acyclovir at an ultra-low-dose (200 mg/day) as prophylaxis against VZV reactivation (hereinafter described as "long-term acyclovir") in July 2001, and it was applied for all allogeneic transplantation recipients thereafter. Long-term acyclovir was principally given from Day 36 until the end of immunosuppressive therapy and at least 1 year after HSCT. When intravenous ganciclovir was required for the treatment of CMV infection, acyclovir was discontinued during the course of intravenous ganciclovir

and resumed afterward. In some patients, acyclovir was discontinued within a year or before the cessation of immunosuppressive therapy at the physician's discretion or at the request of patients themselves.

Statistical analysis

The cumulative incidence of VZV reactivation and the impact of possible confounding factors on VZV reactivation were evaluated using Gray's method, considering death without VZV reactivation as a competing risk [17]. The development of acute and chronic GVHD was treated as time-dependent covariates. The influence of chronic GVHD was evaluated only in patients who survived longer than 100 days. Factors associated with at least borderline significance ($P < 0.10$) in univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. *P*-values of less than 0.05 were considered statistically significant.

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Decreased incidence of acute graft-versus-host disease by continuous infusion of cyclosporine with a higher target blood level

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Cyclosporine A (CsA) is the mainstay of pharmacologic prevention of acute graft-versus-host disease (GVHD). We previously reported that continuous infusion of CsA with a target blood level between 250 and 400 ng/ml significantly increased the incidence of acute GVHD compared to twice-daily infusion with a target trough level between 150 and 300 ng/ml. Thus, we raised the target level of CsA continuous infusion to 450–550 ng/ml. We treated 33 patients with the higher target level (CsA500) and compared the efficacy and toxicity with those in the 33 historical control patients (CsA300 group). Other transplantation procedures were not changed. The patients' characteristics were equivalent. The average CsA concentration was adjusted around 500 ng/ml and the actual daily dose was maintained at the initial dose (CsA 3mg/kg/day). Toxicities were equivalently observed among the two groups. The incidence of grades II–IV acute GVHD was significantly lower in the CsA500 group (27 vs. 52%, $P = 0.033$). The target level of CsA was identified as an independent significant risk factor for grades II–IV acute GVHD ($P = 0.039$), adjusted for the presence of HLA mismatch. The incidence of chronic GVHD was also decreased in the CsA500 group (47 vs. 73%, $P = 0.016$). We conclude that the toxicity of the continuous CsA infusion with a target level of 450–550 ng/ml is acceptable and the efficacy to prevent acute GVHD is significant. A larger comparative study is warranted to confirm these findings. Am. J. Hematol. 83:226–232, 2008. © 2007 Wiley-Liss, Inc.

Introduction

Cyclosporine A (CsA) is one of the most commonly used immunosuppressive agents for the prevention of acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). However, the dose, target blood level, and schedule of infusion vary among protocols and have not been optimized [1]. On the other hand, the importance of blood CsA concentration as well as administered dose has been shown in several reports [2–5]. We previously compared continuous infusion of CsA with a target blood level between 250 and 400 ng/ml and twice-daily infusion targeted to a trough level between 150 and 300 ng/ml in the early period after transplantation in a retrospective study [6]. The incidence of grades II–IV acute GVHD was significantly higher in patients who received the continuous CsA infusion, adjusted for the other significant factors. The actual daily dose of CsA in the continuous infusion group was decreased from the starting dose of 3–1.9 mg/kg/day on average at 4 weeks after transplantation, which might have adversely affected the incidence of acute GVHD. However, the incidences of renal dysfunction and relapse were significantly lower in these patients. The lower incidence of relapse in the continuous infusion group resulted in better disease-free survival in patients with high-risk diseases (43 vs. 16% at 2 years, $P = 0.039$), but not in standard-risk patients (72 vs. 80%, $P = 0.45$). We thus considered that the target CsA level of 250–400 ng/ml in the continuous infusion group was appropriate in high-risk patients, but too low in standard-risk patients. Therefore, we raised the target level of CsA to 450–550 ng/ml when we continuously infuse CsA in standard-risk patients [7]. In this report, we evaluated the safety and efficacy of the continuous infusion of CsA with this high target blood concentration at 500 ng/ml.

Results

Patient characteristics

We performed allogeneic HSCT for 33 standard-risk patients with the higher target CsA level at 450–550 ng/ml (CsA500 group). The historical control group treated with the original target CsA level at 250–400 ng/ml (CsA300 group) also included 33 patients [6]. The characteristics of the patients were equivalent between the two groups, except for the underlying disease (Table I). The number of patients with chronic myelogenous leukemia (CML) was only 2 in the CsA500 group, including one with chronic neutrophilic leukemia in uncontrollable leukocytosis, due to the introduction of imatinib in the treatment of such patients.

Blood concentration and actual daily dose of CsA

The dose of CsA was adjusted to maintain the blood CsA concentration between 450 and 550 ng/ml in the CsA500 group. All patients required repeated dose adjustments of CsA to maintain the targeted blood level. This adjustment was successful and the mean CsA concentration was 488 ±

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