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

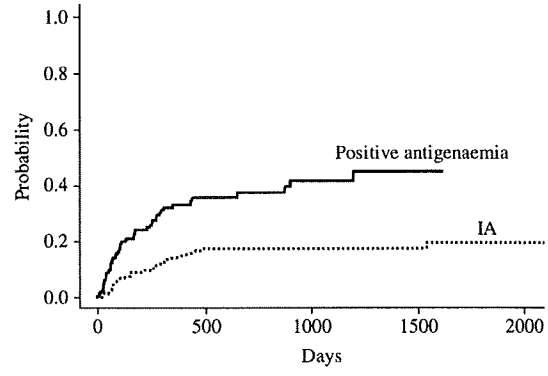

Figure 1. Cumulative incidences of IA and positive GM antigenaemia after HSCT.

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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Presumptive treatment strategy for aspergillosis in allogeneic haematopoietic stem cell transplant recipients

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***Background*: The onset of invasive aspergillosis (IA) after allogeneic haematopoietic stem cell transplantation (HSCT) is bimodal. However, IA early after HSCT has become less frequent due to the shortened neutropenic period, and the clinical significance of empirical treatment for aspergillosis based on persistent febrile neutropenia (FN) became less clear. Therefore, we started a presumptive treatment strategy, in which anti-*Aspergillus* agents were started when patients developed positive serum test and/or infiltrates or nodules on X-ray or CT-scan associated with persistent FN, in 2002.**

***Methods*: We retrospectively reviewed the records of 114 adult patients who underwent allogeneic HSCT between September 2002 and December 2005 in high-efficiency particulate air-filtered clean rooms. Fluconazole was given as anti-*Candida* prophylaxis. The primary endpoint was the development of early IA, which was defined as probable or proven IA according to the EORTC/MSG criteria that developed between the day of HSCT and 7 days after engraftment.**

***Results*: Among 73 patients who experienced persistent FN for 7 days or longer, anti-*Aspergillus* agents were empirically started in 13 patients at the discretion of attending physicians, whereas 60 patients actually followed presumptive treatment strategy. Only 4 of 60 patients received anti-*Aspergillus* agents. Two patients in the presumptive group developed early IA, but were successfully treated with anti-*Aspergillus* agents started after the diagnosis of IA.**

***Conclusions*: These findings suggested the feasibility of a presumptive treatment strategy for aspergillosis in HSCT recipients. A randomized controlled trial is warranted to compare empirical and presumptive anti-*Aspergillus* strategy in allogeneic HSCT recipients.**

Keywords: empirical treatment, febrile neutropenia, invasive aspergillosis

Introduction

Invasive fungal infection (IFI) is one of the leading causes of transplant-related mortality and its incidence in allogeneic haematopoietic stem cell transplantation (HSCT) recipients ranges from 8 to 15%.[1–3] Invasive aspergillosis (IA) is the most common IFI after allogeneic HSCT.[1–4] The development of IA after allogeneic HSCT shows bimodal distribution, one in the neutropenic period early after HSCT and the other 2–3 months after HSCT when patients are taking glucocorticosteroid for acute graft-versus-host disease (GVHD).[1,3,5,6] IA early after HSCT, however, has become less frequent because of the shortened neutropenic period due to the use of peripheral blood stem cells (PBSC), granulocyte colony-stimulating factor (G-CSF) and high-efficiency particulate air (HEPA) filtration and/or laminar air flow.[5–12] Therefore, the clinical significance of empirical treatment for aspergillosis based on persistent febrile neutropenia (FN) has become less clear, although it is supported by old evidence and recent guidelines.[11–16]

Our transplantation unit moved to a new building in September 2002. At the same time, we changed the strategy against aspergillosis during the neutropenic period from

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empirical strategy to presumptive strategy, in which anti-*Aspergillus* agents were started based on positive serum test and/or infiltrates or nodules on X-ray or CT-scan associated with persistent FN.[17,18] In this report, we reviewed the outcomes of 114 patients who underwent allogeneic HSCT in the new transplant unit and evaluated the feasibility of the presumptive strategy during the early neutropenic period after allogeneic HSCT.

Materials and methods

Study patients

Medical records of 124 consecutive adult patients who underwent allogeneic HSCT at the University of Tokyo Hospital between September 2002 and December 2005 were reviewed. All patients received prophylactic antifungal agents. Of the 124 patients, 114 who received fluconazole at 200 mg/day as anti-*Candida* prophylaxis were included in this study.[19,20] The remaining 10 patients were excluded from this study, because they had recent IA and prophylactically received anti-*Aspergillus* agents including micafungin and itraconazole. Characteristics of the 114 patients are summarized in Table 1. The median age was 43 years (range, 20–66 years). Patients' underlying diseases included acute myeloblastic leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myelogenous leukaemia (CML), myelodysplastic syndrome (MDS), non-Hodgkin lymphoma (NHL), aplastic anaemia (AA) and so on. Standard-risk diseases were defined as AML/ALL in first complete remission (CR1) or CR2, CML in first chronic phase (CP1) or CP2, chemosensitive NHL, MDS in refractory anaemia or refractory anaemia with ringed sideroblasts and non-malignant haematological disorders. All other diseases were classified as high-risk diseases. Eight patients had received previous autologous or allogeneic stem cell transplantation. Two patients had a previous history of probable IA prior to HSCT.

Table 1. Characteristics of the 114 patients who were included in this study

Characteristic	
Median recipient age, years (range)	43 (20–66)
Male/female	66/48
Underlying diagnosis, *n* (%)	
AML	30 (26.3)
ALL	20 (17.5)
AUL	1 (0.9)
CML	13 (11.4)
MDS	13 (11.4)
NHL	16 (14.0)
ATL	4 (3.5)
AA	7 (6.1)
Others	10 (8.8)
IA before HSCT, *n* (%)	2 (1.8)
Disease status, *n* (%)	
Standard-risk	65 (57.0)
High-risk	49 (43.0)
Donor, *n* (%)	
Related	67 (58.8)
Unrelated	47 (41.2)
Stem cell source, *n* (%)	
BM	55 (48.2)
PBSC	59 (51.8)
Number of transplantation, *n* (%)	
1	106 (93.0)
2	7 (6.1)
3	1 (0.9)
HLA mismatches at serological level, *n* (%)	30 (26.3)
HLA mismatches at genetic level, *n* (%)	36 (31.6)
Conditioning regimen, *n* (%)	
Myeloablative conditioning	74 (64.9)
Reduced-intensity conditioning	40 (35.1)
GVHD prophylaxis, *n* (%)	
Cyclosporine alone	4 (3.5)
Cyclosporine and short-term MTX	80 (70.2)
Tacrolimus and short-term MTX	3 (2.6)
In vivo T cell depleted	27 (23.7)
Engraftment, *n* (%)	112 (98.1)
Days of engraftment, median (range)	16.5 (9–43)
Antibacterial prophylaxis, *n* (%)	
Tosufloxacin	110 (96.5)
Ciprofloxacin	4 (3.5)
Use of G-CSF, *n* (%)	68 (59.6)

MTX, methotrexate; G-CSF, granulocyte colony-stimulating factor; AUL, acute unclassified leukaemia; ATL, adult T-cell leukaemia/lymphoma.

Transplantation procedure

The stem cell source was bone marrow (BM) from a related donor in 8, BM from an unrelated donor in 47 and PBSC from a related donor in 59. Myeloablative conditioning regimens were used in 74 patients, mainly with total body irradiation plus cyclophosphamide or busulfan plus cyclophosphamide. Fludarabine-based reduced-intensity conditioning regimens were conducted in 40 patients. In these regimens, fludarabine was combined with either busulfan at 8–16 mg/kg in total or melphalan at 140 mg/m^2 in total. In some patients, total body irradiation of 4 Gy in total was added. Therefore, the intensities of regimens were close to the myeloablative conventional regimens. Prophylaxis against GVHD was performed with calcineurin inhibitors (cyclosporine or tacrolimus) with or without short-term methotrexate in the majority of patients. *In vivo* T cell depletion using alemtuzumab or anti-thymocyte globulin was performed in 27 patients, concomitant with cyclosporine and short-term methotrexate.

Neutrophil engraftment was defined as an absolute neutrophil count >500 cells/mm^3 for 3 consecutive days. All patients were housed in double-door HEPA-filtered laminar air flow rooms and provided with low microbial diets until neutrophil engraftment. New quinolones were given prophylactically in all patients. Recombinant G-CSF was routinely administered for patients with non-malignant disease and those with lymphoid malignancies after HSCT. Chest X-ray and non-invasive screening serum tests for IA including galactomannan antigen test (Platelia Aspergillus, Bio-Rad Laboratories, Marnes-la-Coquette, France) and β-D-glucan (BDG) test (β-glucan Test Wako, Wako Pure Chemical Industries, Tokyo, Japan) were performed weekly. Initial empirical antibacterial treatment for FN was started with fourth-generation cephalosporins or carbapenems.[11] For patients with persistent or recurrent FN for 7 days or longer, we did not start anti-*Aspergillus* agents as an early presumptive treatment for aspergillosis until patients developed positive serum test and/or infiltrates or nodules on X-ray or CT-scan (presumptive group). Thirteen patients, however, received

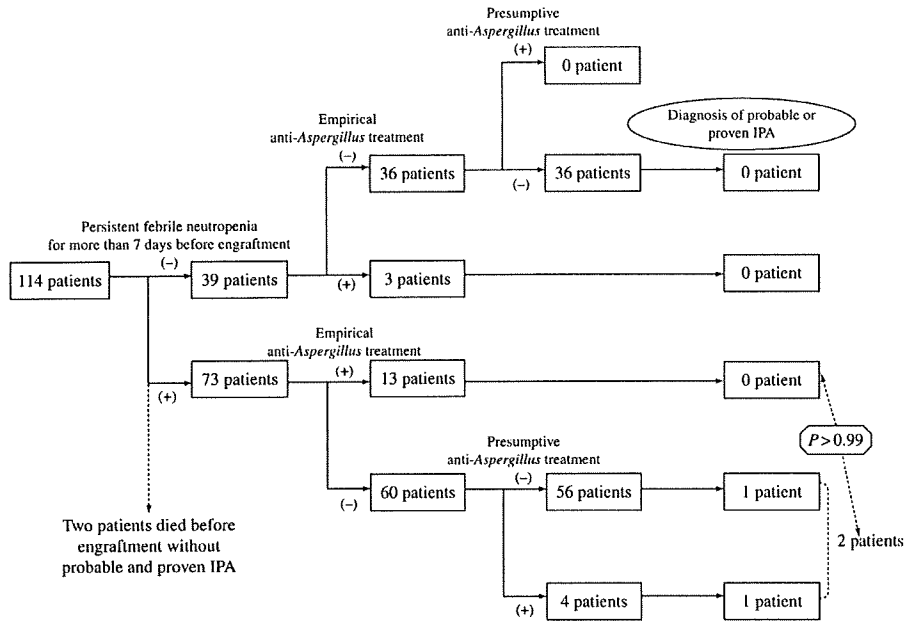

Figure 1. Treatment and outcome of patients included in the study. One hundred and fourteen patients were included in the study. One hundred and nine patients experienced FN. Seventy-three patients experienced persistent or recurrent FN for 7 days or longer. Thirteen patients in the empirical group received anti-*Aspergillus* treatment and the remaining 60 patients were included in the presumptive group. Four patients actually received anti-*Aspergillus* treatment presumptively. In total, early IA was observed in two patients in the presumptive group and none in the empirical group (3.3% versus 0%, $P > 0.99$). IPA, invasive pulmonary aspergillosis.

anti-*Aspergillus* agents empirically at the discretion of attending physicians (empirical group, Figure 1).

Definition of IA

The primary endpoint of this study was the development of probable or proven IA, that was diagnosed according to the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).[21] Microbiological criteria included two consecutive positive galactomannan tests using a reduced cutoff of 0.6 optical density index (O.D.I.).[22] IA that occurred between the day of HSCT and 7 days after engraftment was defined as early IA. The cumulative incidences of IA were calculated using Gray's method considering death without IA as a competing risk.[23]

Results

Clinical outcomes after allogeneic HSCT

Engraftment was observed in all patients at a median of 16.5 days (9–43 days) after HSCT, except for two who experienced early death before engraftment. Forty-two patients, 37.5% of those who achieved engraftment, developed grade II–IV acute GVHD at a median of 21 days after HSCT. Fifty-eight patients, 58.6% of those who survived more than 100 days after HSCT, developed chronic GVHD. Thirty-six patients relapsed at a median of 123.5 days after HSCT. Two-year overall survival of all subjects was 52.4% with a median follow-up duration of surviving patients of 822 (range 107–1603) days after HSCT.

Incidence of IA

Sixteen patients developed probable or proven IFI with a cumulative incidence of 15.1%, including 13 IA, 2 mucormycosis and 1 candidiasis (Table 2). The cumulative incidence of IA was 11.6% (Figure 2) and the median onset was 169.5 days (range, 12–531 days) after HSCT. Twelve out of 13 IA patients suffered from invasive pulmonary aspergillosis (IPA), whereas one developed gastrointestinal aspergillosis. No statistically significant risk factor was identified for the incidence of IA except for male sex (17.8% for male patients versus 2.1% for female patients, $P = 0.018$).

FN and early IA

One hundred and nine patients experienced FN with median duration of 12 days (range, 1–39 days). A median of three (range 1–5) antibiotics per patient were used for empirical antibacterial treatment during FN. Seventy-three patients experienced persistent or recurrent FN for 7 days or longer. The median duration of neutropenia was 21 and 20 days in the empirical group and presumptive group, respectively ($P = 0.91$). Thirteen patients in the empirical group received anti-*Aspergillus* treatment at a median of 9 days (range, 3–21 days) after the onset of FN (Figure 1). Amphotericin B was administered empirically in three patients, which was terminated within 2 days because of renal dysfunction. Micafungin was given to the other 10 patients for a median of 16.5 days (range, 3–76 days). Sixty patients followed the presumptive treatment strategy. Of the 60 patients in the presumptive group, 4 patients actually received anti-*Aspergillus* treatment presumptively, triggered by an elevation of BDG in 1 and infiltrates or nodules on chest

Table 2. Incidence of probable or proven IFI after allogeneic HSCT

	No.
Diagnosis of IFI after HSCT	
Proven diagnosis	4
Probable diagnosis	12
Onset of IFI after HSCT	
Early IFI	2
Late IFI	14
Incidence of late IFI	
Patients without FN	6
Empirical group	0
Presumptive group	8
Organisms that caused IFI	
Aspergillus spp.	13
Candida glabrata	1
Mucor spp.	2
Treatment for IFI	
Amphotericin B	5
Itraconazole	2
Micafungin	4
Voriconazole	3
None	2
Outcome	
Improved	8
No change or progression	6

X-ray or CT-scan in 3. One of them was subsequently diagnosed to have probable IA within a week, because galactomannan test became positive. We changed the anti-*Aspergillus* agent from micafungin to voriconazole and IPA was successfully treated (patient no. 4 in Table 3). Another patient in the presumptive group, who did not receive empirical or presumptive anti-*Aspergillus* agents, developed positive galactomannan test and nodules on CT-scan simultaneously, and was diagnosed to have probable IA (patient no. 5 in Table 3). This patient was also successfully treated with micafungin.

In total, early IA was observed in two patients in the presumptive group and none in the empirical group (3.3% versus 0%, $P > 0.99$). There was no significant difference in the duration of FN between the two groups (15.6 days versus 17.7 days, $P = 0.26$). There was no death that was directly associated with early IA in the whole population.

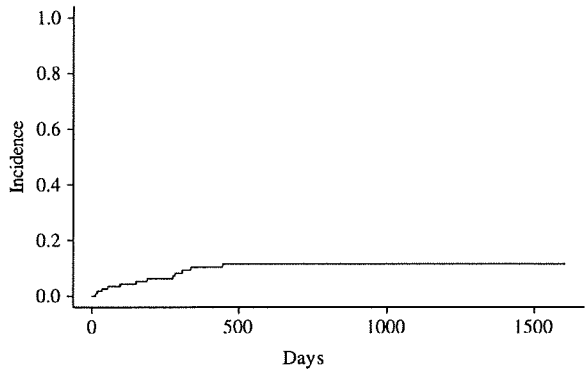

Figure 2. Cumulative incidence of IA after allogeneic HSCT. The cumulative incidence of IA was 11.6% in this study.

Discussion

In this study, the incidences of probable or proven IFI and IA were 15.1% and 11.6%, respectively, which were compatible with other recent studies.[1–3,5,7,8] Only three patients developed IFI other than IA, probably due to the prophylactic use of fluconazole.[5,7,8] Among the 13 patients with IA, only 2 developed IA early after HSCT. Both patients were successfully treated with anti-*Aspergillus* agents and therefore there was no death that was directly related to early IA.

Empirical anti-*Aspergillus* treatment has been recommended for patients with persistent FN.[11,24] However, this strategy is based on two old randomized controlled trials published in the 1980s, before the era of fluconazole prophylaxis.[12,25] Until recently, the standard antifungal agent in this setting has been amphotericin B deoxycholate.[12,25] This approach is limited by the substantial infusion-related toxicity and nephrotoxicity caused by this agent. Recently, lipid formulations of amphotericin B and intravenous itraconazole appeared to have equivalent efficacy compared with conventional amphotericin B with less toxicity.[13,16] Voriconazole and caspofungin were also reported to have similar efficacy.[14,15] However, these alternative agents are very expensive and still more toxic than fluconazole.

A presumptive strategy has been expected to decrease the use of these anti-*Aspergillus* agents by postponing anti-*Aspergillus* treatment until more specific findings are detected in patients with persistent FN. Several findings have been considered specific for IA, such as halo sign on CT-scan in neutropenic patients.[21] In addition, blood tests to detect *Aspergillus* constituents have been investigated, including galactomannan antigen test, BDG test and PCR to detect *Aspergillus* DNA.[22,26,27] Their clinical roles, however, have not been clarified.[26] Previously, we prospectively compared the sensitivity and specificity of these tests and found that the galactomannan test was the most suitable test for the diagnosis of IA with the best cutoff of 0.6 O.D.I.[22] In this study, we included not only blood galactomannan test with this cutoff index and halo sign on CT-scan but also blood BDG test and infiltrates or nodules on X-ray or CT-scan as triggers to start anti-*Aspergillus* treatment to increase sensitivity rather than specificity. By this presumptive strategy, only 2 of the 60 patients with persistent FN developed early IA, both of whom were successfully treated with anti-*Aspergillus* agents after the diagnosis of probable IA. This enabled us to decrease the use of anti-*Aspergillus* agents that are expensive and potentially toxic (4 of 60 in the presumptive group versus 13 of 13 in the empirical group). Considering the low incidence of early IA in the presumptive group, most patients in the empirical group might have been unnecessarily exposed to anti-*Aspergillus* agents. This is a retrospective study and therefore there are several limitations. Especially, we could not exclude the possibility of selection bias that high-risk patients tended to be treated empirically at the discretion of the attending physicians. However, there was no difference in the duration of neutropenia between the two groups. Both patients with a previous history of IA were included in the presumptive group.

Maertens *et al.*[28] recently showed the feasibility of preemptive therapy against IA. They started liposomal amphotericin B

Table 3. Characteristics of patients who received anti-*Aspergillus* agents presumptively (nos. 1–4) and patients who developed early IA (nos. 4 and 5)

No.	Age	Sex	Diagnosis	Prior IA	Triggers to start anti-*Aspergillus* agents	Anti-*Aspergillus* agents	Diagnosis of early IA	Outcome
1	57	Male	AML	—	Elevation of BDG	MCFG	No	Death due to AML progression
2	56	Male	AML	—	XP findings (consolidation)	MCFG	No	Alive
3	35	Male	CAEBV	—	CT findings (small multiple nodules with halo)	MCFG → ITC	No	Alive
4	56	Female	ALL	—	CT findings (nodules with halo) (positive galactomannan test after a week)	MCFG → VRC	Yes	Alive
5	54	Male	MDS	Probable IPA	CT findings (nodules with halo) and positive galactomannan test	MCFG → ITC	Yes	Alive

CAEBV, chronic active Epstein–Barr virus infection; IPA, invasive pulmonary aspergillosis; MCFG, micafungin; ITC, itraconazole; VRC, voriconazole; XP, X-ray photograph.

for patients with two consecutive positive galactomannan tests or with CT findings suggestive of IFI, regardless of the presence or absence of FN. They successfully reduced the use of anti-*Aspergillus* agents and no undetected cases of IA were identified. This approach may be more sensitive than our presumptive strategy to add anti-*Aspergillus* agents only for patients with persistent FN associated with positive serum test and/or radiological evidence. However, frequent galactomannan testing (thrice weekly) is required for this preemptive approach and thus it can be performed in only a limited number of centres.

Recently, prophylactic use of itraconazole, an anti-*Aspergillus* agent, has been evaluated in allogeneic HSCT recipients in two randomized controlled trials.[29,30] The incidence of IA was lower in the itraconazole group than the fluconazole group in both trials. The difference in the incidence of IA appeared 2 or 3 months after HSCT, not in the neutropenic period early after HSCT. Therefore, the prophylactic use of anti-*Aspergillus* agents should be considered for patients at higher-risk for IA, including patients receiving steroid for GVHD or neutropenic patients with a recent history of IA. However, for patients who are receiving anti-*Aspergillus* prophylaxis, another approach other than empirical or presumptive therapy, may be required.

In conclusion, these findings suggested the feasibility of a presumptive strategy for IA in HSCT recipients, provided that they were treated in a HEPA-filtered laminar air flow room. A randomized controlled trial is warranted to compare the efficacy and safety of presumptive and empirical strategy early after HSCT.

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Transparency declarations

None to declare.

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ORIGINAL ARTICLE

Oral valganciclovir as preemptive therapy is effective for cytomegalovirus infection in allogeneic hematopoietic stem cell transplant recipients

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Abstract Between March 2007 and January 2008, the safety and efficacy of oral valganciclovir (VGC) preemptive therapy for cytomegalovirus (CMV) infection was evaluated in ten consecutive patients who received allogeneic hematopoietic stem cell transplantation (HSCT). Patients were screened once or twice per week after engraftment using CMV pp65 antigenemia assay. When more than 2 CMV antigen-positive cells per 50,000 leukocytes were detected, preemptive therapy with oral VGC was initiated at a dose of 900 mg twice daily for 3 weeks. Nine patients (90%) completed the 3-week VGC treatment except for one patient who developed febrile neutropenia. There was no other significant toxicity. CMV antigen-positive cells were rapidly decreased in all nine patients and became undetectable by the end of the VGC treatment. None of the patients developed CMV disease. CMV infection relapsed in four of the ten patients (40%) after the VGC treatment. These observations suggest that preemptive therapy with VGC is effective for preventing CMV disease in allogeneic HSCT patients. Further studies with a large number of patients will be necessary to determine the optimal initial- and maintenance-dose of VGC.

Keywords Allogeneic hematopoietic stem cell transplantation · Cytomegalovirus infection · Preemptive therapy · Valganciclovir

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1 Introduction

Despite improvement in the treatment of cytomegalovirus (CMV) infection and CMV disease with ganciclovir (GCV) and/or foscarnet, CMV disease is still a major cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT) [1–4]. Major risk factors for CMV disease include CMV seropositivity before transplantation, development of graft-versus-host disease (GVHD), unrelated donor transplantation, and T cell depleted transplantation [3, 5–7]. In addition, new transplantation modalities such as nonmyeloablative conditioning regimens consisting of intensive immunosuppression increase the risk of late-onset CMV infection and CMV disease [2, 8]. Therefore, extended prevention of CMV disease may be required, especially for high-risk recipients, not only those within 100 days after HSCT but also those in the later period after HSCT [8–10]. Currently, the prevention of CMV disease involves general prophylaxis and preemptive therapy. Preemptive therapy is based on the early detection of CMV infection by virus surveillance, by monitoring with either CMV antigenemia assay or PCR techniques and followed by immediate treatment with anti-CMV drugs

[4, 11–13]. Intravenous GCV (IV-GCV) and/or foscarnet are commonly used for preemptive therapy and are effective for decreasing the incidence of early CMV disease [11, 13, 14]. However, these antiviral treatments are given intravenously and often require hospitalization, as well as high costs and IV-related complications.

Valganciclovir hydrochloride (VGC) is an oral valine-ester GCV prodrug with a tenfold higher bioavailability than oral GCV, and it is rapidly hydrolyzed to GCV after oral administration. VGC and IV-GCV have similar efficacy in the treatment of CMV retinitis in HIV-infected patients and in preemptive CMV treatment in solid organ (heart, renal, and renal-pancreas) transplant patients [15–19]. Recently, several studies have shown the efficacy of VGC for preemptive therapy in allogeneic HSCT patients [20–23]. We evaluated the safety and efficacy of oral VGC as preemptive therapy for CMV reactivation in ten allogeneic HSCT patients.

2 Patients and methods

2.1 Patients

This was a prospective multicenter study with VGC. The study patients were adults who had received an allogeneic bone marrow or peripheral blood stem cell transplant. Patients were eligible when they screened for CMV infection using CMV pp65 antigenemia assay and more than two CMV antigen-positive cells were detected. Patients unable to take oral medication, and those who impaired renal function (serum creatinine level >2.0 mg/dL) were ineligible. Patients, who developed CMV disease, had received antiviral agents other than acyclovir and who developed more than stage 2 gastrointestinal GVHD were also ineligible. Ten consecutive patients who received allogeneic HSCT at Kyushu University Hospital and Hamanomachi General Hospital between March 2007 and January 2008 were included in the study (Table 1). This study was approved by Institutional Review Board of each institute and a written informed consent was obtained from each participating patient.

Eight patients had acute myeloid leukemia, one had myelodysplastic syndrome, and one had non-Hodgkin's lymphoma. The median age of the patients at the time of transplantation was 56 years (range 33–63). They received bone marrow grafts from an HLA-matched sibling donor ($n = 1$), a matched unrelated donor ($n = 8$), or an HLA-1 locus mismatched unrelated donor ($n = 1$). All of the patients were CMV seropositive before transplantation. Nine patients received myeloablative preparative regimens including total body irradiation/cyclophosphamide (Cy) in five patients and busulfan (BU)/Cy in four patients.

Table 1 Patient characteristics

Number of patients	10
Median age, years (range)	56 (33–65)
Diagnosis	
Acute myeloid leukemia	8
Myelodysplastic syndrome	1
Non-Hodgkin's lymphoma	1
Stem cell source	
HLA-identical sibling bone marrow	1
HLA-matched unrelated bone marrow	8
HLA-mismatched unrelated bone marrow	1
CMV serologic status	
Donor + /Recipient +	9
Donor −/Recipient +	1
Preparative regimens	
TBI/Cy	5
Bu/Cy	4
Flu/Bu/TBI	1
GVHD prophylaxis	
Taclorimus + MTX	9
CSP + MTX	1
Acute GVHD prior to CMV reactivation	
Grade I	1
Grade II	7
Grade III	2
PSL treatment at the time of starting VCG	8

Bu busulfan, *CMV* cytomegalovirus, *CSP* cyclosporine, *Cy* cyclophosphamide, *Flu* fludarabine, *GVHD* graft-versus-host disease, *TBI* total body irradiation, *MTX* methotrexate, *PSL* prednisolone, *VGC* valganciclovir

The remaining patient received a fludarabine-based reduced-intensity conditioning regimen. GVHD prophylaxis consisted of taclorimus/short-term methotrexate (MTX) ($n = 9$) or cyclosporine/short-term MTX ($n = 1$). Patients who developed grade II–IV acute GVHD were given methylprednisolone (mPSL) or prednisolone (PSL) at a dose of 1 or 2 mg/kg. Acyclovir was administered orally (1,000 mg/day) or intravenously (500 mg/day) from days −7 to 35 as a prophylaxis against herpes simplex infection.

2.2 CMV antigenemia assay

CMV antigenemia assay was determined as previously described [7, 24]. In brief, peripheral blood leukocytes isolated from 3 mL of EDTA-treated blood were applied to slides by centrifugation and fixed with cold acetone. The slides were stained using a direct immunoperoxidase technique that employed the peroxidase-conjugated monoclonal antibody HRP-C7 (Teijin, Tokyo, Japan) against the CMV pp65 antigen. CMV antigen-positive cells were counted under a light microscope and the results were

expressed as the number of CMV antigen-positive cells per 50,000 leukocytes.

2.3 Definition of CMV infection and CMV disease

A positive test for CMV antigenemia was defined as the presence of one or more CMV antigen-positive cells per 50,000 leukocytes. CMV infection was considered in patients with a positive test for CMV antigenemia. CMV disease was diagnosed according to published recommendations [25]. Patients with clinical manifestations of CMV disease, such as interstitial pneumonia and gastroenteritis in the presence of CMV infection, were examined histopathologically and immunochemically from biopsy specimens.

2.4 Preemptive therapy with VGC for CMV infection

Monitoring with CMV antigenemia assay was performed at least once per week after engraftment until day 100 after HSCT and once every other week thereafter. Preemptive therapy with VGC for CMV infection was initiated at the time of the first detection of more than two CMV antigen-positive cells per 50,000 leukocytes. VGC was administered orally at a dose of 900 mg twice daily for 3 weeks. The dose was adjusted for patients with impaired renal function according to the manufacturer's recommendation. Acyclovir for the prophylaxis against herpes simplex infection was discontinued when VGC treatment was started. Supplemental immunoglobulin was administered only when a total IgG level was less than 400 mg/dL.

2.5 Endpoints and definitions

The primary endpoint was the rate of complete response of the VGC preemptive therapy to the CMV infection. The efficacy of VGC was monitored weekly using a CMV antigenemia assay. A complete response was defined as the conversion from positive to negative CMV antigenemia test results at the completion of the treatment. Patients who persistently showed positive test results for CMV antigenemia after 3 weeks of preemptive therapy or developed CMV disease during the period of preemptive therapy were considered a treatment failure.

The secondary endpoints included the safety of preemptive therapy, the incidence of CMV disease during VGC treatment, and the incidence of a recurrent CMV reactivation after the completion of VGC treatment. The patients were monitored with the CMV antigenemia assay for 5 weeks after the completion of the VGC treatment. At least once per week, a safety analysis was conducted. The analysis included the monitoring of blood counts, liver and renal function tests, and documenting other unexpected side effects. The incidence of CMV disease was evaluated for the entire period of the study. The incidence of recurrent reactivation of CMV infection after the VGC preemptive therapy was based on the conversion from negative CMV antigenemia to positive CMV antigenemia test results with more than two CMV antigen-positive cells per 50,000 leukocytes during the 5-week follow-up period.

3 Results

3.1 CMV infection and VGC preemptive therapy

Forty-seven patients received allogeneic bone marrow/peripheral blood stem cell transplants at these two institutes during the study period. Thirty-one patients showed positive CMV antigenemia test results after transplantation. Ten patients were enrolled into this study, but the remaining 21 patients were not enrolled mostly by their inability to take oral medication. Ten enrolled patients were given preemptive therapy with VGC for CMV infection (Table 1). All patients were CMV seropositive before transplantation, and nine donors were also CMV seropositive. In these patients, more than 2 CMV antigen-positive cells per 50,000 leukocytes were detected after a median of 69 days (range 22–252) following transplantation. The median number of CMV antigen-positive cells at the initiation of VGC therapy was 5 per 50,000 leukocytes (range 3–59). All of the patients developed acute GVHD prior to CMV infection after a median of 23 days (range 11–135). The severity of acute GVHD was grade I in one patient, grade II in seven, and grade III in two. Eight patients received mPSL or PSL for the treatment of acute GVHD. Preemptive therapy with VGC was started within five days after the detection of CMV antigen-positive cells. Nine patients completed 21 days of VGC treatment, whereas one patient failed to complete the therapy because of the development of grade 4 neutropenia and subsequent febrile neutropenia. Patients were followed at least 5 weeks after the completion of VGC preemptive therapy. The median follow-up was day 122 (range 41–355).

3.2 Response to VGC preemptive therapy

All patients showed negative test results for CMV antigenemia within 3 weeks after the initiation of the VGC treatment. In nine patients, CMV antigen-positive cells became negative within 2 weeks (Fig. 1). The remaining patient, who had 60/50,000 CMV antigen-positive cells at the time of initiation of VGC treatment, took 3 weeks to clear CMV antigen-positive cells. None of the patients required other anti-CMV agents. None of the patients developed CMV disease during the preemptive therapy or

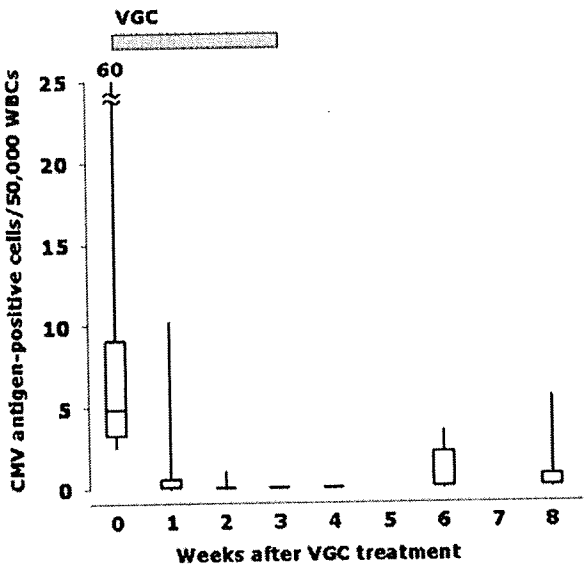

Fig. 1 Time course of the number of cytomegalovirus (CMV) antigen-positive cells after valganciclovir treatment. CMV antigenemia was reduced during treatment with valganciclovir. The *box plots* display the median, the 25th and 75th percentiles (*box*), and the smallest and largest values (*longitudinal line*). One patient discontinued valganciclovir on day 18 due to grade 4 neutropenia

in the subsequent 5 weeks after the completion of the VGC treatment.

CMV infection relapsed in four of the ten patients within 3–5 weeks after the completion of the preemptive VGC therapy. These four patients were successfully treated with IV-GCV.

3.3 Toxicity

Nine patients completed a 21-day course of VGC treatment, but one patient discontinued VGC due to grade 4 neutropenia. Due to impaired renal function (serum creatinine level, 1.68 mg/dL), this patient received a reduced VGC dose of 450 mg once per day for the first week. Renal function improved with the reduced dose, and the VGC dosage was increased to 450 mg twice per day in the second week of treatment. However, this patient developed grade 4 neutropenia (absolute neutrophil counts 0.17 × 10^9/L) after 17 days of treatment and then developed febrile neutropenia. The VGC was discontinued, and the patient immediately received granulocyte-colony stimulating factor (G-CSF) and antibiotic therapy. Neutrophil counts recovered to more than 1.0 × 10^9/L, and neutropenia resolved after five days. Recurrent CMV reactivation was not observed in this patient during the follow-up period. None of the patients developed thrombocytopenia (platelet count <30 × 10^9/L)(Fig. 2).

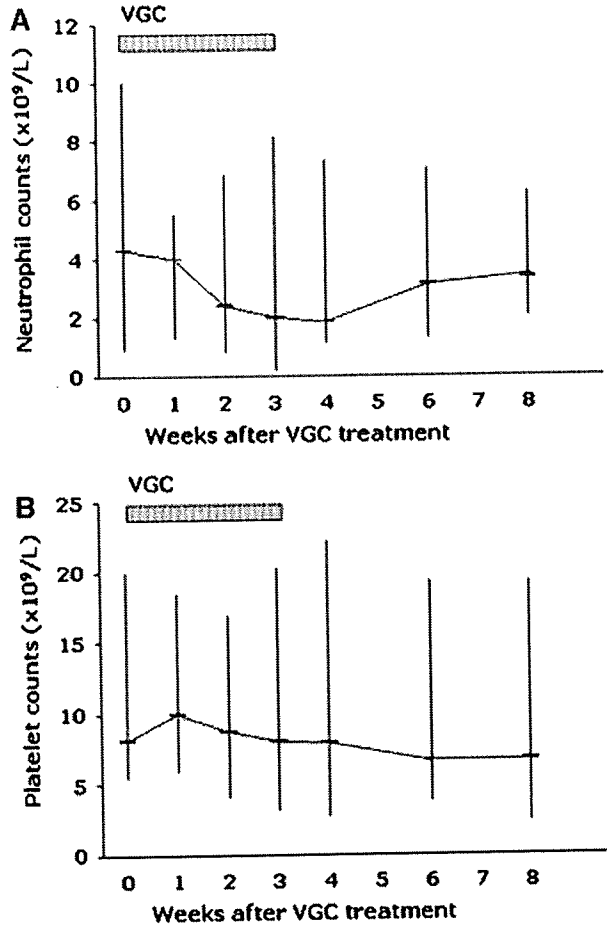

Fig. 2 Time course of neutrophils and platelets during valganciclovir treatment. Time course of neutrophil (**a**) and platelet numbers (**b**) during treatment with valganciclovir. The bar graph displays the median (*horizontal line*), and the smallest and largest values (*longitudinal line*). One patient discontinued valganciclovir on day 18 due to grade 4 neutropenia

Table 2 Adverse events other than hematological toxicities related to valganciclovir

Adverse events		No. of cases
Gastrointestinal		
Diarrhea	Grade 1	1/10
Hepatic		
AST/ALT	Grade 1	3/10

None of the patients experienced renal toxicity during the VGC treatment. Three patients developed grade 1 liver dysfunction, and one patient had grade 1 diarrhea (Table 2). However, none of these complications required discontinuation of the VGC.

4 Discussion

Effective preemptive therapy with IV-GCV reduced the incidence of early CMV disease to 5–10%; however, the risk of late CMV disease beyond day 100 after transplantation has increased over the past few years. Therefore, extended CMV monitoring beyond day 100 is currently recommended, especially in high-risk patients [2, 8]. There is a need for an effective oral anti-CMV drug that can be used for outpatient care. Oral VGC could be a useful alternative to IV-GCV in patients who require preemptive therapy for CMV infection. This study demonstrated the efficacy and safety of preemptive VGC therapy for CMV infection after allogeneic HSCT. There are four published studies that have shown the safety and the efficacy of VGC as preemptive therapy after allogeneic HSCT [20–23]. Although dosage and duration of the drug varied between studies, VGC therapy resulted in a rapid decrease of the viral load in all of the patients. In this study, we administered a dose of 900 mg twice daily for 3 weeks, and corroborated the efficacy and the tolerability of preemptive VGC therapy.

We demonstrated that VGC at a dose of 900 mg twice per day was effective and resulted in a rapid clearance of CMV antigen-positive cells in all patients. No CMV disease developed during the preemptive therapy or the subsequent 5 weeks after the completion of treatment. VGC was well tolerated as 90% of the patients completed the entire treatment course. However, four of the ten patients developed a recurrent CMV reactivation after the discontinuation of VGC treatment, and they were all successfully treated with IV-GCV. Because a guideline for preemptive VGC therapy has not been established for patients that have received allogeneic HSCT, further studies will be necessary to determine the optimal initial- and maintenance-dose of VGC.

We, and four other groups, have obtained good results with VGC starting-doses of 900 mg twice per day [20–23]. This dose was based on observations from previous pharmacokinetics studies in HIV-infected patients and liver transplant recipients. A VGC dose of 900 mg results in an area under the concentration-time curve for GCV similar to that of 5 mg/kg IV-GCV [26, 27], which is the recommended standard dose for preemptive CMV therapy [28, 29]. One of the concerns of using VGC after allogeneic HSCT is the absorption of oral VGC in patients suffering from severe gastrointestinal GVHD. Recently, Einsele et al. [30] conducted a randomized crossover clinical trial of IV-GCV and VGC in patients with or without intestinal GVHD. The results showed that patients without intestinal GVHD who took VGC were exposed to more GCV when compared to those administered IV-GCV. This was also true in patients with grade I and II intestinal GVHD. Thus, VGC may be as effective even in patients developing a mild form of intestinal GVHD as in patients without intestinal GVHD. However, a higher exposure of VGC may increase the toxicity of the drug, and the absorption of VGC was not evaluated in patients with severe intestinal GVHD. Recently, Candoni et al. [22] examined the efficacy of a lower dose of VGC. Preemptive therapy with 900 mg/day VGC was as effective for clearing CMV antigen-positive cells and preventing CMV disease as the standard dose of 1800 mg/day. These findings suggest that the initial dose of VGC could be reduced to 900 mg/day as preemptive therapy in low-risk patients.

The effective duration for preemptive VGC therapy is currently unclear. In the previous studies, patients received VGC for 2 weeks and then it was either discontinued or continued at a maintenance dose of variable duration dependant upon a negative CMV test result. Different from previous studies, we continued an initial dose of VGC for 3 weeks. The dosage and duration of VGC therapy likely affects the incidence of hematological toxicity such as neutropenia. In a study by Busca et al. [21], in which VGC was administered at a dose of 1,800 mg/day for 2 weeks, followed by 900 mg/day for an additional 2 weeks, 4 of the 15 patients failed to complete the 3-week scheduled therapy due to neutropenia and/or thrombocytopenia. In our study, only one of the ten patients failed to complete treatment. Thus, hematologic toxicity may be a significant problem after a 3 week treatment with VGC.

In our study, four of the ten patients treated with VGC developed recurrent CMV reactivation 3–5 weeks after the discontinuation of VGC. This was somewhat similar to the 10–53% recurrence rates in previous studies [20–23]. Thus, careful monitoring after the completion of VGC therapy is recommended. We continued an initial dose of VGC for 3 weeks. However, when considering hematological toxicity and frequent recurrence of CMV antigenemia, the duration of treatment and/or maintenance should be decided by monitoring CMV.

As previously reported [20–23], we found neutropenia to be the main toxic effect of VGC. One patient, who had impaired renal function before the preemptive therapy that required a dose reduction, discontinued the drug on day 17 due to grade 4 neutropenia. In high-risk patients, especially outpatient should be closely monitored, although any other toxicity profile different from IV-GCV was not observed in this study.

Our study demonstrated that the oral VGC preemptive therapy at a dose of 900 mg daily seemed to be as effective as conventional IV-GCV at a dose of 10 mg/kg daily to clear CMV antigen-positive cells. However, as shown in Fig. 1, CMV antigen-positive cells seem to decrease in numbers much faster after VGC treatment than those observed after standard dose of IV-GCV treatment.

Furthermore, hematological toxicities were considerable. Although pharmacokinetic data was not available in this study, these observations coincide with the previous pharmacokinetic study in HSCT recipients that showed the exposure of GCV after administration of 1800 mg daily VGC was significantly higher compared with 10 mg/kg IV-GCV even in patients without gastrointestinal GVHD [30]. Careful monitoring of neutrophil counts will be useful to improve the safety of VGC in HSCT recipients, especially with reduced renal function. Kanda et al. [14] showed the efficacy of response-oriented preemptive therapy using a low initial dose of IV-GCV that resulted in a successful reduction of the total dose of IV-GCV and decreased hematological toxicities. A lower dose of VGC could be also used as preemptive therapy by close CMV monitoring. Similar studies with a large number of patients will be required to define the optimal treatment schedule for preemptive VGC therapy.

Despite a limited number of patients, our results suggest that oral VGC is an effective alternative to IV-GCV for preemptive therapy to prevent CMV disease in allogeneic HSCT patients. Studies with a larger number of patients will be necessary to assess the efficacy and long-term effect of this preemptive therapy.

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Infectious complications in patients receiving autologous CD34-selected hematopoietic stem cell transplantation for severe autoimmune diseases

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Abstract: Long-term analysis of infectious complication after high-dose immunosuppressive therapy with CD34-selected autologous hematopoietic stem cell transplantation for patients with severe autoimmune diseases (AD) was performed. Theoretically, CD34 selection can reduce the risk of reinfusion of autoreactive lymphocytes. However, it is also associated with a significant reduction in T cells, natural killer cells, and monocytes, which in turn may compromise immune reconstitution, thereby increasing the risk of infection. Moreover, AD compromises host immunity and causes organ damage resulting in dysfunction of the cutaneous or mucosal barrier. In this study, the incidence rate of infections is reported in 14 patients who underwent high-dose (200 mg/kg) cyclophosphamide therapy followed by reinfusion of CD34-selected autologous peripheral blood stem cells. Bacterial complication occurred in 3 of 14 (21%) patients. Cytomegalovirus reactivation and adenovirus hemorrhagic cystitis were observed in 9 (64%) and 2 (14%) patients, respectively. As for late infectious complications, 7 patients (50%) developed dermatomal varicella zoster virus infection. No infection-related mortality was seen in this case series. Because the risk for infections approaches that seen in allogeneic transplant recipients, infection surveillance, diagnostic workup, and prophylactic strategies similar to those applicable to allogeneic recipients are warranted.

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Key words: Infection; stem cell transplantation; autologous; hematopoietic; CD34-selected; peripheral blood stem cells; autoimmune disease; cytomegalovirus; varicella zoster virus; adenovirus; bacteremia

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Pilot studies comprising high-dose immunosuppressive therapy followed by transplantation of autologous hematopoietic stem cells (HSC) were conducted to obtain safety and preliminary efficacy data in patients with severe autoimmune diseases (AD) (1–5).

Both unselected and CD34-selected peripheral blood stem cells (PBSC) have been used as sources of HSC (1–5). Theoretically, CD34 + cell selection of PBSC can reduce the possibility of reinfusion of autoreactive lymphocytes. However, the superiority of CD34-selected PBSC over unmanipulated PBSC has not been established. The safety and efficacy of CD34-selected autologous PBSC transplantation (PBSCT) for refractory AD have been investigated at our institute (6).

PBSCT-related complications include regimen-related toxicities and various infections. For the treatment of AD, PBSCT is a more toxic treatment modality than the conventional immunosuppressive therapies. Organ damage due to AD puts the patients at risk of regimen-related toxicities. Thus, careful selection of refractory AD patients for PBSCT is essential to minimize transplant-related mortality.

Infections are major contributors to morbidity and mortality in PBSCT. In hematological malignancies, CD34-selected autologous PBSCT has been reported to increase incidences of opportunistic infections compared with non-CD34-selected autologous PBSCT (7–11). Most AD patients had undergone immunosuppressive therapy, including cyclosporine and corticosteroids, before transplantation. AD

itself compromises host immunity to various infections. Furthermore, AD causes organ damage such as skin ulcers, esophageal dysmotility, and interstitial pneumonia (IP) resulting in dysfunction of the cutaneous or mucosal barrier. Thus, the understanding of infectious complications is important in increasing the safety of CD34-selected autologous PBSCT for AD.

Here, we retrospectively analyze infectious complications during the course of CD34-selected autologous PBSCT for severe AD.

Materials and methods

Protocol

The protocol of this phase I/II clinical trial (6) was approved by the ethics committee of Kyushu University Hospital. Written informed consent was obtained from all patients.

Patients and eligibility

Patients between 16 and 65 years of age were eligible at the time of pre-transplant evaluation. Patient eligibility depended on the diagnosis of AD, as previously described (6).

PBSC mobilization and CD34 + cell selection

PBSC were mobilized during hematological recovery after administration of cyclophosphamide (CY) (2 g/m^2/day) for 2 days, followed by a recombinant human granulocyte-colony stimulating factor (G-CSF, filgrastim; Kirin Brewery, Tokyo, Japan) at a dosage of 2 μg/kg/day. After collecting PBSC to obtain 2 × 10^6 CD34 + cells/kg or more by apheresis, CD34 + cells were positively selected using immunomagnetic beads with an anti-CD34 monoclonal antibody (CliniMACS, Miltenyi Biotec, Cologne, Germany).

Autologous PBSCT and supportive care

Patients were kept in HEPA-filtered rooms until engraftment. For pre-transplant conditioning, high-dose CY (50 mg/kg/day) was administered for 4 days, from days −5 to −2. Frozen-thawed CD34-selected PBSC were infused on day 0. All immunosuppressive and disease-modifying agents were discontinued upon HSC procurement, except systemic corticosteroids, which were tapered to a relatively low dose (5–15 mg of prednisolone/day) over 2–6 months after PBSCT. Acyclovir (intravenous 250 mg/day, from days 1 to 18), ciprofloxacin (by mouth [PO] 600 mg/day, from days −7 to 14), fluconazole (PO 200 mg/day, from days −7 to 30), and trimethoprim-sulfamethoxazole (TMP-SMX) (each 1920 mg/day; from days −14 to −2, and twice a week from days 30 to 180, respectively) were prophylactically administered, as previously described (6). Neutropenic fever was treated with intravenous administration of broad-spectrum cephalosporins according to the guidelines for the use of antimicrobial agents in neutropenic patients (12). After engraftment, weekly monitoring of cytomegalovirus (CMV) pp65 antigenemia was conducted until day 100 after transplant (13). If CMV antigenemia was detected, preemptive therapy was initiated with ganciclovir.

Diagnosis and definition of infections

The day of onset of infection was defined as the day the diagnostic test was performed.

Bacterial infections were categorized as bacteremias and site-specific infections (14). Varicella zoster virus (VZV) infections were defined as typical cutaneous vesicular lesions. CMV infection and disease were defined as previously described (13, 15). In brief, CMV infection was defined as isolation of the CMV virus or detection of the viral proteins or nucleic acids in body fluid or tissue specimen. CMV disease is defined by the presence of organ-specific signs and/or symptoms with the detection of CMV in test specimens (e.g., bronchoalveolar lavage in the lungs or biopsy samples in other organs). CMV infection with unexplained fever for at least 2 days within a 4-day period and the presence of neutropenia or thrombocytopenia is considered CMV syndrome. Hemorrhagic cystitis (HC) due to adenoviruses (AdV) was diagnosed when AdV were detected by either viral culture or polymerase chain reaction in macroscopic hematuria with clinical signs of cystitis. To exclude regimen-related HC, patients with *de novo* hematuria at least 10 days after HSC transplantation (HSCT) and no tendency toward generalized bleeding or bacteriuria were considered to have AdV HC (16). Fungal infection was defined by proven or probable invasive fungal infection (17) and clinical or radiological manifestation along with positive microbiological tests.

Results

Patients

Fourteen patients (4 males, 10 females) with a median age of 54 years (range 21–63 years) were examined (Table 1). Patients No. 1–11 were diagnosed as diffuse systemic sclerosis (SSc). Patient No. 1 was suffering from systemic lupus er-

Clinical characteristics of the autoimmune patients receiving CD34-selected transplant

Patient number	Disease	Age (years)	Sex	Complication	Previous therapies	Follow up (months)[1]
1	SSc/SLE	54	F	IP, digital ulcer	DEX, IVCY	72
2	SSc	55	M	IP, digital ulcer	PSL, IVCY	65
3	SSc	58	M	IP	PSL, IVCY	61
4	SSc	54	F	IP	PSL, IVCY	58
5	SSc	53	F	IP	PSL	56
6	SSc	49	F	IP	m-PSL, CsA, IVCY	52
7	SSc	33	F	IP	–	21
8	SSc	63	F	IP	PSL	36
9	SSc	61	F	IP	PSL, CsA	31
10	SSc	44	F	IP	PSL, IVCY	27
11	SSc	52	M	IP, digital ulcer	PSL, CsA, IVCY	23
12	DM	54	F	IP	PSL pulse, CsA, IVCY	70
13	DM	44	F	IP, skin ulcer	PSL pulse, CsA	12
14	WG	21	M	Exophthalmos	PSL pulse, IVCY	55

[1]After transplantation.
SSc, systemic sclerosis; SLE, systemic lupus erythematosus; IP, interstitial pneumonia; DEX, dexamethasone; IVCY, intravenous cyclophosphamide; PSL, prednisolone; m-PSL, methyl prednisolone; CsA, cyclosporine; DM, dermatomyositis; WG, Wegener's granulomatosis.

Table 1

ythematosus (SLE) for 22 years and SSc for 2 years. Although SLE was inactive, she had progressive IP and severe digital ulcers due to SSc. Patients No. 2–11 (SSc) and 12 and 13 (dermatomyositis) developed IP, which did not respond to immunosuppressive agents. Patients No. 3–6, 8, 9, and 11 showed severe skin sclerosis. Patient No. 3 had been in complete remission from non-Hodgkin's lymphoma for 1 year and was considered eligible. Patient No. 14 (Wegener's granulomatosis) presented with severe exophthalmos due to granuloma formation (18 mm in diameter) in the upper lateral region of the left orbit affecting the superior rectus muscle. He needed monthly steroid pulse therapy to prevent further growth of the granuloma. The Eastern Cooperative Oncology Group performance status (18) was <3 in all patients. CY and cyclosporine were administered to 9 patients and 5 patients, respectively. All patients, except Patient No. 7, were treated with corticosteroids. The median follow-up duration was 53.5 months after transplant (range 8–72 months).

Results are reported as of March 2008.

Infections

Bacterial

Nine of 14 patients developed febrile neutropenia at a median of 6 (0–9) days after PBSCT (Table 2). Among these, Patients No. 6 and 11 revealed *Streptococcus mitis* bacteremia on days 8 and 9 after PBSCT, respectively. Both were empirically treated with broad-spectrum cephalosporins. Vancomycin was added when the blood culture was reported positive. Patient No. 12 developed high-grade fever without signs of local infection on day 119 post PBSCT, and a blood culture turned out to be positive for *Listeria monocytogenes*. Empirical therapy was initiated with broad-spectrum cephalosporin but switched to penicillin/β-lactamase inhibitor after detection of the microbe. All patients responded to the therapy, and no fatal complications occurred.

Patient No. 14 developed *Mycobacterium gordonae* pneumonia 1343 days after PBSCT. However, he was on anti-tumor necrosis factor (TNF) antibody therapy because of the relapse at that time; thus the case is omitted from this study.

Viral

CMV. Nine of 14 patients developed CMV antigenemia at a median of 28 days (range 10–60 days) after PBSCT. All patients who developed CMV infection were seropositive for CMV antibody before PBSCT, and this was considered as reactivation. Patients were preemptively treated with ganciclovir (5 mg/kg twice a day) and none developed CMV disease. High levels of antigenemia were detected in Patients No. 1 and 6. With their clinical symptoms, these patients were considered to have CMV syndrome. Foscarnet