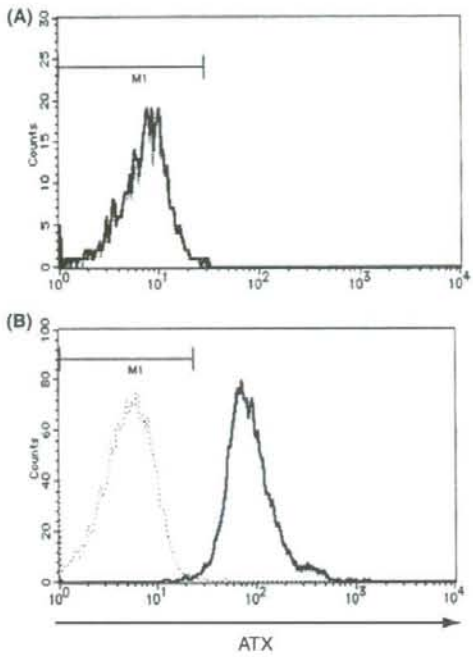

Fig 5. Analysis of the surface expression of ATX in leukaemic tumour cells from patients with FL. We examined the surface expression of ATX in peripheral blood cells using flow cytometry. (A) Normal B-lymphocytes from healthy subjects. (B) Leukaemic tumour cells from patients with FL. The solid line histogram represents staining with anti-ATX antibody, while the dotted line represents the control.

subjects. Furthermore, leukaemic tumour cells from patients with FL were shown to express ATX. These results suggest that the shedding of ATX from lymphoma cells leads to an elevation of serum ATX antigen levels. As described above, the serum ATX activity and ATX antigen level are elevated in patients with chronic liver disease (Watanabe *et al*, 2007a; Nakamura *et al*, 2008a). A recent study using hepatectomized rats suggested that elevated ATX activity in rats with liver injury was caused by a decrease in ATX clearance (Watanabe *et al*, 2007b). Accordingly, the mechanism of elevated ATX antigen levels in FL is opposite to that in chronic liver disease. Although the mechanism by which the plasma/serum ATX level is regulated remains to be solved, both ATX production and clearance should probably be considered.

As yet, little is known about the association between ATX and haematological malignancies. One recent study has shown that the induction of ATX by EBV promoted the growth and survival of HL cells and that ATX expression in lymphoid tissues was mainly restricted to EBV-positive Hodgkin and Reed-Sternberg cells and CD30-positive anaplastic large cell lymphoma (Baumforth *et al*, 2005). In the present study, however, no major difference in the serum ATX antigen levels was found between HL patients and healthy subjects. Although the levels in the total patients (males plus females) with HL were significantly higher than those in the healthy subjects, the difference was marginal. In HL lesions, the major cell constituents are normal or reactive lymphocytes, not tumour cells like Hodgkin and Reed-Sternberg cells; this difference may explain why no difference in ATX antigen levels was observed between HL patients and healthy subjects.

LPA has also been suggested to act as a survival factor in B-cell neoplasms (Rosskopf *et al*, 1998; Hu *et al*, 2005; Satoh *et al*, 2007). As expected from the results of studies on chronic liver disease (Watanabe *et al*, 2007a; Nakamura *et al*, 2008a), the plasma LPA levels in patients with FL are elevated, in parallel with the serum ATX antigen levels. These results indicate that the autocrine or paracrine production of LPA contributes to tumour progression, although direct evidence of this mechanism has not been reported. Considering the specificity of ATX expression in lymphoma cells, ATX could be used as a pharmacological target; the blockage of LPA production via ATX inhibition might be a useful anticancer therapy.

In conclusion, the serum ATX antigen level is a promising marker for FL. Considering the specificity of ATX and the action of LPA as a tumour growth factor for B-cell neoplasms, therapeutical applications may be an important goal of future studies.

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Conventional allogeneic hematopoietic stem cell transplantation for lymphoma may overcome the poor prognosis associated with a positive FDG-PET scan before transplantation

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A positive scan in pretransplantation fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has been shown to be associated with a poor prognosis in patients with lymphoma undergoing high-dose chemotherapy followed by autologous stem cell transplantation (ASCT). For those with a positive FDG-PET scan, treatment that includes allogeneic stem cell transplantation (allo-SCT) may be an alternative. However, it is uncertain whether allo-SCT can overcome a poor prognosis. Therefore, we conducted a retrospective analysis of 14 patients with lymphoma who had undergone FDG-PET scan within one month before allo-SCT at our institution. Eleven patients were FDG-PET-positive and three were negative. With a median follow-up of 17 months (range: 6–44) after allo-SCT, the cumulative incidence of progression was 29.3% in FDG-PET-positive patients and 0% in the FDG-PET-negative patients. Four of the 11 patients who had post-transplantation FDG-PET showed FDG-avid lesions on the first post-transplantation scan. In two of the four, regression of the lesions was observed during the scheduled reduction of immunosuppressant without donor lymphocyte infusion and remained without progression at the last follow-up (34 and 8 months). Durable responses after allo-SCT, at least with conventional conditioning regimens, can be expected in patients with FDG-PET-positive lesions before transplantation. Thus, conventional allo-SCT could be an attractive modality compared to ASCT for patients with positive FDG-PET after the completion of conventional salvage chemotherapy, and particularly for patients with T and NK-cell lymphomas. Am. J. Hematol. 83:477–481, 2008. © 2008 Wiley-Liss, Inc.

Introduction

Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has become a standard imaging test in the management of lymphoma in both initial staging [1,2] and response assessment [3–6]. Response criteria that incorporate FDG-PET can better predict the prognosis than the original International Workshop Criteria [7,8], and revised response criteria for lymphoma using FDG-PET have been published [9]. In addition, the results of FDG-PET before and/or after hematopoietic stem cell transplantation for lymphoma have been reported to have prognostic significance. Several groups have reported that the probability of durable responses with high-dose chemotherapy followed by ASCT for lymphoma is lower in patients with positive FDG-PET before ASCT than in patients with negative FDG-PET [6,10–15]. For those with positive FDG-PET before ASCT, allogeneic hematopoietic stem cell transplantation (allo-SCT) can be an attractive alternative treatment, since the use of uncontaminated graft and a potential graft-versus-lymphoma (GVL) effect are expected to reduce the probability of relapse. To date, however, there has been no report on whether such an approach could overcome the poor prognosis associated with positive pretransplantation FDG-PET. Moreover, the significance of pretransplantation FDG-PET in predicting the clinical outcome of allo-SCT has not yet been reported. Thus, to assess whether patients with positive pretransplantation FDG-PET could achieve a durable response after allo-SCT, we conducted a retrospective analysis of such patients.

Results

Patient population

We identified 14 patients who had undergone FDG-PET scan before allo-SCT for lymphoma. The patient characteristics are shown in Table I. The median follow-up for surviving patients was 17.0 months after allo-SCT (range: 6–44). There were no transplant-related deaths before day 100. Acute GVHD was observed in nine patients (grade I, 2; grade II, 7; grade III–IV, 0). The median time from transplantation to the onset of acute GVHD was 30 days (range: 13–60). Chronic GVHD occurred in eight patients (5 extensive, 3 limited). We did not find any relationship between GVHD occurrence and tumor regression.

Pretransplantation FDG-PET and the outcome of allo-SCT

Eleven patients (79%) had FDG-PET-positive lesions before allo-SCT. All of these lesions had been detected by CT scan. In three patients (21%), there were no FDG-PET-positive lesions, although FDG-avidity had been confirmed before chemotherapy was started in each patient. Of the 14 patients, 12 were alive at the last follow-up. Of the 11 patients with positive pretransplantation FDG-PET, nine were alive without progression of lymphoma. Two patients (patients 7 and 8) suffered progression on days 61 and

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TABLE I. Patient Characteristics

Patient	Sex	Age at diagnosis	Donor source	Conditioning	Diagnosis	Disease status	SUVmax	Diameter max (mm)	Status at the last follow up	Follow up (months)
01	M	37	uBMT	CY/TBI	LBL	PR1	7.3	52	Alive: remission	42.9
02	M	56	rPBSCT	FLU/CY	FL grade 2	PR2	5.7	37	Alive: remission	34.4
03	F	47	uBMT	CY/TBI	FL grade 1	PR2	4.9	16	Alive: remission	31.3
04	M	53	rPBSCT	CY/TBI	MCL	CR1	Negative	–	Alive: remission	30.1
05	F	42	rPBSCT	CY/TBI	Nasal NK/T	PR2	3.6	23	Alive: remission	26.2
06	F	28	uBMT	CY/TBI	t-CTCL	Primary R.	7.1	23	Alive: remission	14.2
07	M	45	rPBSCT	CY/TBI	ALCL	Primary R.	6.0	30	Dead: progression	6.3
08	F	25	CBT	FLU/MEL/TBI	HL	Relapse 2	11.5	40	Alive: progression	7.2
09	F	46	uBMT	CY/TBI	ATLL	CR1	Negative	–	Alive: remission	8.1
10	F	32	rPBSCT	CY/TBI	t-CTCL	Primary R.	6.9	28	Alive: remission	7.5
11	F	48	uBMT	CY/TBI	FL grade 3	Primary R.	10.2	38	Alive: remission	6.2
12	M	47	rPBSCT	CY/TBI	ATLL	PR1	7.8	31	Alive: remission	19.9
13	F	36	uBMT	BU/MEL	ANKL	CR1	Negative	–	Dead: GVHD	6.6
14	M	42	rBMT	CY/TBI	FL grade 1	PR2	6.3	59	Alive: regression	10.4

uBMT, unrelated bone marrow transplantation; rPBSCT, related peripheral blood stem cell transplantation; CBT, cord blood transplantation; rBMT, related bone marrow transplantation; CY/TBI, Cyclophosphamide 60 mg/kg × 2 days + Total Body Irradiation 2 Gy × 2 × 3 days; FLU/CY, Fludarabine 25 mg/m^2 × 5 days + Cyclophosphamide 60 mg/kg × 2 days; FLU/MEL/TBI, Fludarabine 30 mg/m^2 × 5 days + Melphalan 140 mg/m^2 × 1 day + Total Body Irradiation 2 Gy × 2 × 1 day; BU/MEL, Busulfan 4 mg/kg × 4 days + Melphalan 140 mg/m^2 × 1 day; LBL, lymphoblastic lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; Nasal NK/T, Extranodal NK/T-cell lymphoma, nasal type; ATLL, adult T-cell leukemia/lymphoma; t-CTCL, tumor-stage Cutaneous T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ANKL, aggressive NK cell leukemia; disease status, status at transplantation; CR, complete response; PR, partial response, Primary R., primary refractory; SUVmax, maximum standard uptake value in pretransplantation FDG-PET scan; diameter max, maximum diameter (long axis) of the largest lymphoma lesion in pretransplantation CT scan; GVHD, graft-versus-host disease.

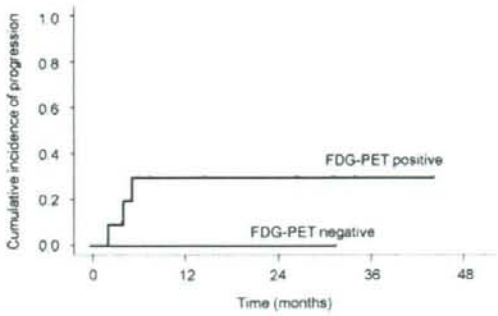

Figure 1. Cumulative incidence of progression in 11 patients with a positive pre-transplantation FDG-PET scan compared to that in 3 patients with a negative pre-transplantation FDG-PET scan.

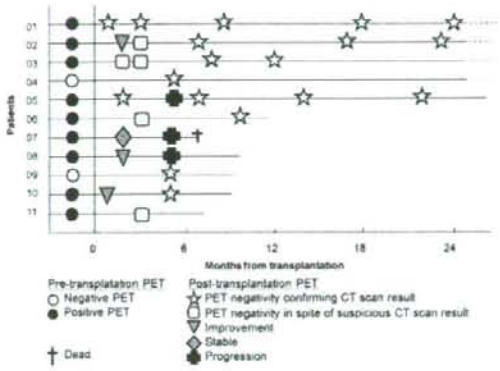

Figure 2. Patient timelines. Results of pretransplantation and post-transplantation FDG-PET scans were performed in 11 patients. Four patients showed FDG-PET-positive lesions on the first post-transplantation PET scan (patient 2, 7, 8, 10). All of them were positive for pretransplantation FDG-PET. Improvement of FDG-PET uptake was observed in patients 2 and 10 during the scheduled reduction of immunosuppressant and remained without progression at the last follow-up (34 and 8 months).

111, respectively and patient 7 died of lymphoma progression on day 190. Among the three patients who had a negative scan in pre-transplantation FDG-PET, one died of severe GVHD with no evidence of disease progression on day 198 (patient 13), while the two other patients remained without progression at the last follow-up. The cumulative incidence of progression at a median follow-up of 17.0 months from allo-SCT was 29.3% in patients with positive pretransplantation FDG-PET and 0% in patients with negative pretransplantation FDG-PET (see Fig. 1). OS at the median follow-up was 90.9% in patients with a positive scan in pretransplantation FDG-PET.

Follow-up FDG-PET

A total of 30 post-transplantation FDG-PET scans were performed in 11 patients (patients 1–11, the median number of follow-up scans per patient: 2, range 1–5) to evaluate the response and to monitor disease progression (see Fig. 2). The median time to the first FDG-PET scan after transplantation was 72 days (range: 28–142). In seven patients (patients 1, 3, 4, 5, 6, 9, 11), the first post-transplantation FDG-PET was negative, including three patients who had residual disease in CT scans (patients 3, 6, 11). Among these seven patients, five had positive pretransplantation FDG-PET scans (patients 1, 3, 5, 6, 11). All of these seven patients but one (patient 5) remained free from progression. Patient 5 achieved a CR defined by CT scan after allo-SCT on day 57 and was negative in the first post-transplantation FDG-PET scan on day 72. However, the second FDG-PET scan and CT scan on day 140 revealed disease recurrence. Cyclosporine was rapidly reduced within one month from the recurrence and this patient remained in remission (26-month follow-up). Including this patient, none of the patients received DLI for residual disease or recurrent disease.

In contrast, the other four showed FDG-PET-positive lesions at the first post-transplantation PET scan (days 38, 77, 64, 30 in patients 2, 7, 8, and 10, respectively). In all of these patients, pre-transplantation FDG-PET was positive, and the same sites remained FDG-positive at the first post-transplantation FDG-PET scan. Two (patients 2, 8) of the four patients received RIC. Regression of lesions detected by CT scans that was associated with an improvement of FDG-PET findings in terms of reduced levels of SUVmax was observed in patients 2 and 10 during the scheduled reduction of cyclosporine, and these patients remained free from progression at the last follow-up (34 months and 8 months, respectively). In patients 7 and 8, the first FDG-PET showed stable disease (day 64) and improvement (day 77), respectively, compared to the pretransplantation FDG-PET. However, follow-up CT scans showed disease progression soon after the first FDG-PET scans in both patients. In patient 7, the second FDG-PET on day 112 showed disease progression and the patient died of septic shock on day 190. In patient 8, rapid tapering of cyclosporine provided transient regression of the disease. However, the second FDG-PET on day 111 revealed disease progression and the patient was alive with disease at the last follow-up (day 216).

Outcome of patients with T and NK-cell lymphomas

The present study included seven patients (patients 5, 6, 7, 9, 10, 12, 13) with T and NK-cell lymphomas. Of these seven, four had chemorefractory disease and five had positive pretransplantation FDG-PET scans. Five patients were alive in CR at the last follow-up (median follow-up: 8 months). In patients 5 and 10, tumor regression was observed in the follow-up FDG-PET scan findings in terms of reduced levels of SUVmax along with decrease in the transverse diameter of tumors in the CT scan findings by only the rapid or scheduled reduction of cyclosporine, respectively, which suggested a potential GVL effect. They remained in remission (26- and 8-month follow-up, respectively).

Discussion

Functional imaging with FDG-PET is now considered to play an important role in staging and response evaluation of lymphoma. Moreover, FDG-PET has also been studied with regard to its ability to predict the outcome of a specific therapy for lymphoma such as ASCT [6,10–15]. To the best of our knowledge, however, only one previous study has addressed the utility of FDG-PET after allogeneic transplantation [16] and there has been no report on the outcome of allo-SCT for lymphoma in patients with positive pretransplantation FDG-PET lesions.

In the present study, the cumulative incidence of progression was 29.3% at 17.0 months from allo-SCT in patients with positive pretransplantation FDG-PET. This supports the idea that allo-SCT could be an attractive modality for the treatment of lymphoma with positive pretransplantation

FDG-PET, since a dismal outcome has been reported in patients undergoing ASCT for aggressive lymphomas with positive pretransplantation FDG-PET lesions [6,10–15]. Although the duration of follow-up in the present study was shorter than in studies on ASCT, several studies have shown that progression after allo-SCT for lymphoma was usually seen within 1 year [17–19]. Therefore, we consider that the duration of follow-up in the current study was long enough for us to discuss the prognosis after allo-SCT for lymphoma.

In this study, six of the seven patients with negative post-transplantation FDG-PET remained progression-free, which supports the previous report by Hart et al., who analyzed patients received allo-SCT with RIC for lymphoma [16]. In that study, the authors proposed that post-transplantation FDG-PET may be useful for guiding DLI after allo-SCT with RIC. In contrast, in the current study, two patients with FDG-avid lesions at the first post-transplantation FDG-PET showed regression along with previously scheduled tapering of cyclosporine, without undergoing DLI. Post-transplantation FDG-positivity may reflect inflammatory responses to a conditioning regimen or even an immune reaction corresponding to the GVL effect. Thus, the clinical significance of FDG-avid lesions that remain after allo-SCT for lymphoma may require further evaluation. In this regard, the optimal timing for post-transplantation FDG-PET should be determined.

The present study has several limitations. First, histological subtypes included in the present study were more heterogeneous than in the previous studies on FDG-PET before ASCT [6,10–15], although most patients in our study had aggressive histological subtypes. During the period of this retrospective analysis, no patient underwent allo-SCT for DLBCL, which reflects the preferred use of ASCT for this subtype. On the other hand, a half of our patients had T and NK-cell lymphomas. Our approach reflected the dismal outcome with conventional chemotherapy or ASCT and the promising data with allo-SCT for these subtypes such as adult T-cell leukemia/lymphoma, advanced stage NK-cell lymphoma, and advanced stage cutaneous T-cell lymphoma [20–22]. The histological subtypes included in the present study, including T and NK-cell lymphomas, have been shown to be FDG-avid [23,24], and FDG-avidity had been confirmed in each patient at the initial staging. Thus, therapy-monitoring with FDG-PET may be supported for these subtypes. Nevertheless, the prognostic significance of pretransplantation FDG-PET should be confirmed in major subtypes such as DLBCL and HL, preferably in future prospective studies. Second, most of the patients received a conventional conditioning regimen and only two received RIC. Thus, the conclusion of the present study should be restricted to cases of allo-SCT who received conventional pretransplantation conditioning, and it remains to be determined whether patients with positive pretransplantation FDG-PET can achieve a durable response after allo-SCT with RIC. Third, this retrospective study may have an inherent patient-selection bias. Although we did not find any patient in whom allo-SCT was canceled based solely on the results of FDG-PET, it could be possible that the result somehow affected the decision as to whether allo-SCT should be recommended. For example, patients with very extensive FDG-PET uptake before allo-SCT may not have been referred to allo-SCT. Although this study did not address the relationship between the intensity of FDG-uptake and the prognosis, this point might be worth further evaluation. Fourth, there might be a false-positive lesion in FDG-PET which reflects an inflammatory process after radiation or infection. We did not routinely perform biopsy for residual lesions before allo-SCT, and we defined PET-positive lesions as in the study by Spaeson et al. [15], both of which were consistent with the recent consensus report on FDG-PET in response assessment [25].

In conclusion, this study suggested that patients with positive pretransplantation FDG-PET could achieve a durable response after allo-SCT as long as a conventional conditioning regimen is used. Therefore, conventional allo-SCT could be an attractive modality compared to ASCT for patients with positive FDG-PET after the completion of conventional salvage chemotherapy, and particularly for patients with T and NK-cell lymphomas. Prospective studies of allo-SCT with conventional pretransplantation conditioning regimens for such patients could be justifiable. However, the role of pretransplantation FDG-PET may be different in allo-SCT with RIC and this point requires further evaluation.

Methods

Patient population

We reviewed a database of adult patients who underwent allo-SCT for Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) at the University of Tokyo Hospital, Japan, between November 2003 and December 2006. We identified 14 patients who had undergone FDG-PET scan within one month before transplantation. Allo-SCT was performed for chemoresistant disease in five patients, whereas nine patients had chemosensitive disease. Some patients were transplanted in the first complete response (CR) or chemosensitive relapse. These patients had a subtype for which conventional chemotherapy or ASCT was unlikely to confer a long-term survival according to the literature that had been published at the time [20–22]. Conditioning regimens were high-dose cyclophosphamide with total body irradiation (CY/TBI; CY 120 mg/kg, TBI 12 Gy) in 11 patients, busulfan and melphalan (BU/MEL; BU 16 mg/kg, MEL 140 mg/m^2) in one patient (patient 13), and reduced-intensity conditioning (RIC) regimens in two patients (FLU/CY; fludarabine 125 mg/m^2, CY 120 mg/kg in patient 2, FLU/MEL/TBI; fludarabine 150 mg/m^2, melphalan 140 mg/m^2, TBI 4 Gy in patient 8). RIC regimens were selected because of advanced age or comorbidity. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and a short course of methotrexate in all patients. T-cell depletion of the graft was not performed. None of the patients had allo-SCT after failure with ASCT in this analysis. We did not use the results of FDG-PET as the sole criterion to exclude allo-SCT or to introduce donor lymphocyte infusion (DLI) after allo-SCT.

Fluorine-18 FDG-PET

FDG-PET was performed as previously described [24]. FDG-PET scans were performed with a whole-body PET camera, ADVANCE (GE Healthcare). Emission data were acquired 60 min after the intravenous administration of 296 MBq of F-18 FDG. PET images were reconstructed using Ge-68 for attenuation correction with the ordered-subsets expectation maximization. Two experienced radiologists scrutinized all scans. Interpretation of the PET images followed the approach in a previous study [15] and was consistent with the recommendations in the recent publication from the International Harmonization Project [25]. Briefly, focal or diffuse FDG uptake above background in a location that is incompatible with normal anatomy or physiology was interpreted as abnormal and was considered to be indicative of a lymphoma lesion. In areas with abnormal FDG accumulation, the standard uptake value (SUV) was calculated according to the standard formula. We did not use SUV as a specific cutoff value for positivity but rather considered the result of FDG-PET to be positive if FDG-PET detected a lymphoma lesion in at least one site that was also detected by physical examination, CT, or BM examination on the basis of the definition in a previous report [23]. In CT scan, a nodule with a long-axis length of more than 1.5 cm at any site was considered to be a lymphoma lesion. In patients with no FDG-PET scan after allo-SCT, disease status was determined by the original CT criteria [7].

Statistical analysis

Time to progression was measured from the day of stem cell infusion (day 0) until the time of disease relapse or progression, or disease-related death, with censoring at the last follow up. The cumulative incidences of progression were evaluated using Gray's method, while considering death before progression as a competing risk [26]. Overall

survival (OS) was measured from day 0 until the date of death, with censoring at the time of the last follow up. OS was calculated according to the Kaplan–Meier method.

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False-positive *Aspergillus* galactomannan antigenaemia after haematopoietic stem cell transplantation

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***Objectives*: Although *Aspergillus* galactomannan (GM) antigen detection is widely applied in the diagnosis of invasive aspergillosis (IA), false-positive reactions with fungus-derived antibiotics, other fungal genera or the passage of dietary GM through injured mucosa are a matter of concern. The aim of this study was to investigate the cumulative incidence and risk factors for false-positive GM antigenaemia.**

***Patients and methods*: The records of 157 adult allogeneic haematopoietic stem cell transplantation (HSCT) recipients were retrospectively analysed. Episodes of positive GM antigenaemia, defined as two consecutive GM results with an optical density index above 0.6, were classified into true, false and inconclusive GM antigenaemia by reviewing the clinical course.**

***Results*: Twenty-five patients developed proven or probable IA with a 1 year cumulative incidence of 12.9%, whereas 50 experienced positive GM antigenaemia with an incidence of 32.2%. Among the total 58 positive episodes of the 50 patients, 29 were considered false-positive. The positive predictive value (PPV) was lower during the first 100 days than beyond 100 days after HSCT (37.5% versus 58.8%). Gastrointestinal chronic graft-versus-host disease (GVHD) was identified as the only independent significant factor for the increased incidence of false-positive GM antigenaemia (PPV 0% versus 66.7%, $P = 0.02$).**

***Conclusions*: GM antigen results must be considered cautiously in conjunction with other diagnostic procedures including computed tomography scans, especially during the first 100 days after HSCT and in patients with gastrointestinal chronic GVHD.**

Keywords: fungal infections, invasive aspergillosis, chronic GVHD, gastrointestinal tract, mucosal damage

Introduction

Invasive aspergillosis (IA) remains one of the leading infectious causes of death after allogeneic haematopoietic stem cell transplantation (HSCT), despite new antifungal agents that have become available in recent years.[1] The high mortality rate of IA was mainly attributed to the difficulty of diagnosis at the early stage of the disease, because histopathological examinations require invasive procedures and fungal cultures have low specificity and sensitivity in detecting IA.

Monitoring of the circulating *Aspergillus* galactomannan (GM) antigen by the sandwich enzyme-linked immunosorbent assay (ELISA) is a feasible non-invasive biological method for early diagnosis of IA.[2] The GM ELISA test has sensitivity of 67% to 100% and specificity of 81% to 99% in neutropenic patients and allogeneic transplant recipients,[3–6] and was introduced as microbiological evidence in the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria for opportunistic invasive fungal infection.[7] However, a concern is the false-positive reactions,

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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

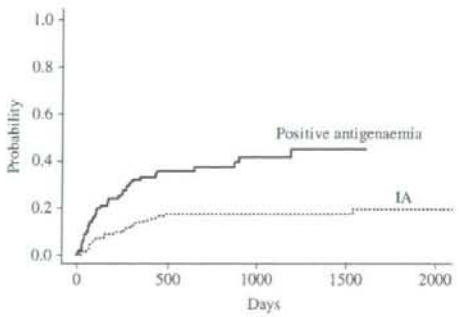

Figure 1. Cumulative incidences of IA and positive GM antigenaemia after HSCT.

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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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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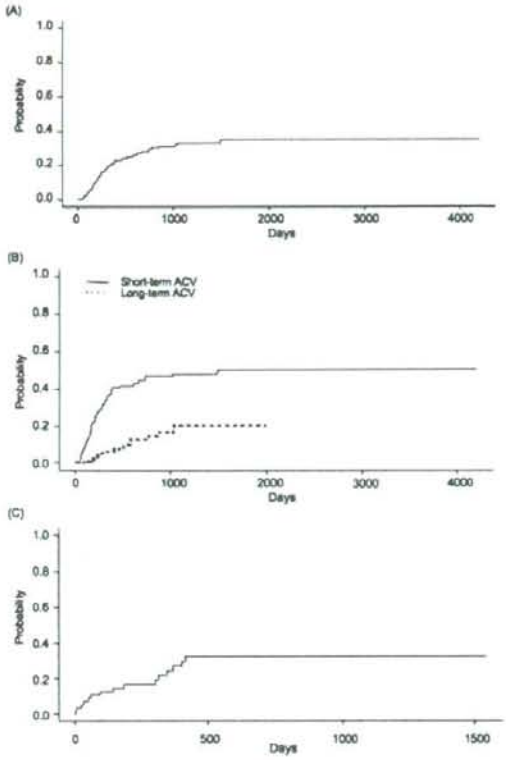

Figure 1. (A) Cumulative incidences of VZV reactivation after HSCT in all 242 patients. (B) Cumulative incidences of VZV reactivation after HSCT in 137 patients who received long-term acyclovir versus 105 patients who did not. (C) Cumulative incidences of VZV reactivation after the cessation of long-term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

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.

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].

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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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.