研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Fuji S, Kim SW, Mori S, Fukuda T, Kamiya S, Yamasaki S, Morita-Hoshi Y, Ohara F, Honda O, Kuwahara S, Tanosaki R, Heike Y, Tobinai K, Takaue Y.	Hyperglycemia during the neutropenic period following conditioning is associated with a poor outcome in patients undergoing myeloablative allogeneic hematopoietic stem cell transplantation.	Transplantation	84	814-820	2007
Onishi Y, Mori S, Kusumoto S, Sugimoto K, Akahane D, Morita Y, Kim SW, Fukuda T, Heike Y, Tanosaki R, Tobinai K, Takaue Y.	Unrelated donor bone marrow transplantation with a conditioning regimen including fludarabine, busulfan and 4 Gy total body irradiation.	International Journal of Hematology	85	256-263	2007
Ozawa S, Nakaseko C, Nishimura M, Maruta A, Cho R, Ohwada C, Sakamaki H, Sao H, Mori S, Okamoto S, Miyamura K, Kato S, Kawase T, Morishima Y and Kodera Y.	Chronic graft-versus-host disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program.	British Journal of Haematology	137	142-151	2007

Ⅳ. 研究成果の刊行物・別刷

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

Kumi Oshima,[1,2] Yoshinobu Kanda,[1,2] Hideki Nakasone,[1] Shunya Arai,[1] Nahoko Nishimoto,[1] Hiroyuki Sato,[1] Takuro Watanabe,[1] Noriko Hosoya,[2] Koji Izutsu,[1] Takashi Asai,[1] Akira Hangaishi,[1] Toru Motokura,[1] Shigeru Chiba,[2] and Mineo Kurokawa[1]

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

Introduction

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

Results

Patient characteristics

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

Blood concentration and actual daily dose of CsA

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

[1]Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan; [2]Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan

Contract grant sponsors: Ministry of Health, Labor, and Welfare.

*Correspondence to: Mineo Kurokawa, M.D., Ph.D., Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: kurokawa-tky@umin.ac.jp

Received for publication 1 May 2007; Revised 14 August 2007; Accepted 30 August 2007

Am. J. Hematol. 83:226–232, 2008.

Published online 4 October 2007 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.21087

TABLE I. Characteristics of the Patients

	CsA500 group (n = 33)	CsA300 group (n = 33)	P-value
Sex			
Male	20	26	0.18
Female	13	7	
Age			
<40	16	17	>0.99
≥40	17	16	
Underlying disease			
AL	24	13	0.017
CML	2	12	
MDS	2	1	
NHL	3	6	
Others	2	1	
Donor			
Related	12	16	0.46
Unrelated	21	17	
HLA			
Match	28	25	0.54
Mismatch	5	8	
Stem cell source			
BM	25	26	>0.99
PB	8	7	
Regimen			
Non-TBI	4	9	0.21
TBI	29	24	
MTX dose			
<31mg/m^2	16	11	0.32
≥31mg/m^2	17	22	

BM, bone marrow; PB, peripheral blood; TBI, total body irradiation; AL, acute leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma.

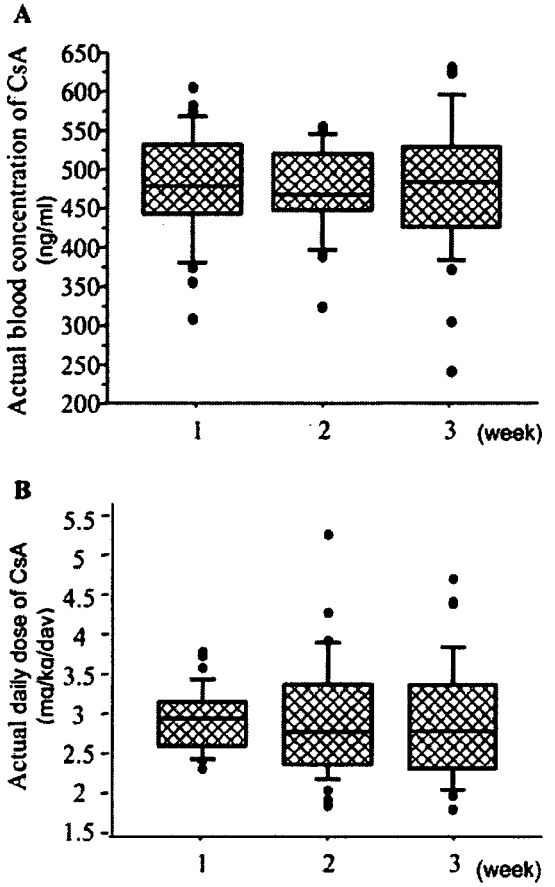

Figure 1. Actual blood concentration (A) and daily dose (B) of cyclosporine. The mean CsA concentration was 488 ± 89, 475 ± 41, and 482 ± 69 ng/ml and the actual dose was 2.9 ± 0.4, 2.8 ± 0.8, and 2.7 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after HSCT, respectively.

89, 475 ± 41, and 482 ± 69 ng/ml at the 1st, 2nd, and 3rd week after HSCT, respectively (Fig. 1A). The actual dose was 2.9 ± 0.4, 2.8 ± 0.8, and 2.7 ± 0.7 mg/kg at the 1st, 2nd, and 3rd week after HSCT, respectively (Fig. 1B). The median duration of intravenous cyclosporine was 41 days (range 16–74 days) after transplantation.

Toxicity

The incidence of renal dysfunction defined as elevation of the serum creatinine level above ×1.5 and ×2.0 the baseline value was equivalent between the CsA500 group and the CsA300 group (Table II, 24 vs. 24%, P = 0.96 and 15 vs. 13%, P = 0.71, respectively). Liver dysfunction defined as elevation of the total bilirubin level above 2 mg/dl was also similar (30 vs. 24%, P = 0.78). Thrombotic microangiopathy was not observed in any patients. No central nerves system toxicities were observed. In the CsA500 group, we decreased the target level of CsA to 300 ng/ml due to hyperbilirubinemia 9 days after HSCT in one patient and substituted prednisolone for CsA in another patient due to hyperbilirubinemia and renal dysfunction at day 21 after HSCT. The latter patient had already had liver cirrhosis classified to Child-Pugh A due to hepatitis C virus infection before HSCT.

TABLE II. Incidences of Adverse Events Due to Cyclosporine

	(−)	(+)	P-value
Incidence of serum creatinine > 1.5 × baseline value			
CsA500	25	8 (24%)	>0.99
CsA300	25	8 (24%)	
Incidence of serum creatinine > 2.0 × baseline value			
CsA500	28	5 (15%)	0.71
CsA300	30	3 (13%)	
Incidence of bilirubin > 2.0 mg/dl			
CsA500	23	10 (30%)	0.78
CsA300	25	8 (24%)	
Incidence of TMA			
CsA500	33	0 (0%)	>0.99
CsA300	33	0 (0%)	

TMA: thromobtic microangiopathy.

Incidences of acute and chronic GVHD

We performed a univariate analysis to evaluate the impact of potential confounding factors on the incidence of grades II–IV acute GVHD and identified two significant factors; the presence of HLA-mismatch including allele-mismatch and the target level of CsA (Table IIIA). As shown in Fig. 2A, the incidence of grades II–IV acute GVHD in the CsA300 group was significantly higher than that in the CsA500 group (52 vs. 27%, P = 0.033). Corticosteroids therapy for acute GVHD was more frequently required in the CsA300 group (39 vs. 15%, P = 0.051). The percentage of patients who received corticosteroids to treat GVHD was lower than the incidence of grades II–IV acute GVHD, because we did not use systemic corticosteroids for grades II acute GVHD with skin involvement only. The difference in the incidence of

TABLE III. Factors Associated the Incidences of Grades II–IV Acute GVHD and Nonrelapse Mortality

A. Univariate analyses

Factor	Acute GVHD	*P*-value	Nonrelapse mortality	*P*-value
Sex				
Male	20 (44%)	0.31	12 (30%)	0.020
Female	6 (30%)		0 (0%)	
Age				
<40 years	15 (46%)	0.30	4 (14%)	0.21
≥40 years	11 (33%)		8 (30%)	
Underlying disease				
CML	7 (50%)	0.25	2 (14%)	0.49
Non-CML	19 (37%)		10 (25%)	
Donor				
Related	11 (39%)	0.97	8 (36%)	0.052
Unrelated	15 (40%)		4 (13%)	
HLA				
Match	17 (32%)	0.0037	10 (23%)	0.78
Mismatch	9 (69%)		2 (18%)	
Stem cell source				
BM	19 (37%)	0.46	9 (21%)	0.68
PBSC	7 (47%)		3 (24%)	
Regimen				
Non-TBI	4 (31%)	0.56	5 (49%)	0.035
TBI	22 (42%)		7 (15%)	
MTX dose				
<31mg/m^2	12 (44%)	0.32	7 (19%)	0.87
≥31mg/m^2	14 (36%)		5 (24%)	
Target levels of CsA				
CsA500	9 (27%)	0.033	2 (8%)	0.051
CsA300	17 (52%)		10 (27%)	

B. Multivariate analyses

Factor	RR of acute GVHD	*P*-value	RR of nonrelapse mortality	*P*-value
Target levels of CsA				
CsA300	1.00	0.039	1.00	0.064
CsA500	0.43 (0.19–0.96)		0.24 (0.053–1.09)	
HLA				
Match	1.00	0.0062		
Mismatch	3.14 (1.39–7.14)			

grades II–IV acute GVHD between the two groups was more prominent in unrelated HSCT (Fig. 2B, 44 vs. 33% in related HSCT and 59 vs. 24% in unrelated HSCT).

Next, we performed a multivariate analysis to identify independent risk factors for the development of Grades II–IV acute GVHD. Two factors were independently significant with a relative risk (RR) of 3.14 (95% confidence interval [CI] 1.39–7.14, $P = 0.0062$) for the presence of HLA-mismatch and RR of 0.43 (95% CI 0.19–0.96, $P = 0.039$) for the CsA500 group, respectively (Table IIIB). The cumulative incidence of Grades III, IV acute GVHD was only 11%. The target level of cyclosporine (CsA500 vs. CsA300: 3 vs. 18%, $P = 0.045$) was identified as the only significant risk factor for the development of Grades III, IV acute GVHD.

The number of patients who developed limited and extensive chronic GVHD was 5 and 18, respectively, in the CsA300 group and 4 and 11, respectively, in the CsA500 group. The incidence of chronic GVHD was also significantly decreased in the CsA500 group (Table IV and Fig. 3, 47 vs. 73%, $P = 0.016$).

Transplantation outcome

The lower incidence of acute GVHD in the CsA500 group translated into the lower incidence nonrelapse mortality (Table III, 8 vs. 27%, $P = 0.051$). On the other hand, the incidence of relapse tended to be higher in the CsA500 group (Table V, 20 vs. 6%, $P = 0.065$), although this difference became smaller when we excluded patients with CML (19 vs. 10%, $P = 0.29$). Finally, there was no significant difference in disease-free survival between the CsA500 group and the CsA300 group (Fig. 4, 72 vs. 63%, $P = 0.68$).

Discussion

We successfully maintained the blood CsA concentration at around 500 ng/ml and the actual dose at around 3 mg/kg/day by twice a week monitoring for the first 3 weeks after transplantation. The preliminary data in these 33 patients suggested the feasibility and efficacy of the continuous infusion of CsA at this higher target level.

Several studies have reported the relationship between the blood concentration of CsA and the efficacy to prevent GVHD after allogeneic HSCT [2–5]. Especially, the area under the concentration–time curve (AUC) has been believed to be the most important pharmacokinetic parameter for the efficacy of calcineurin inhibitors [8,9]. The monitoring of AUC, however, requires frequent blood sampling and is not suitable for daily practice. Therefore, the trough concentration (C_{TL}) has been measured as a surrogate for AUC in twice-daily infusion of CsA, although recent reports

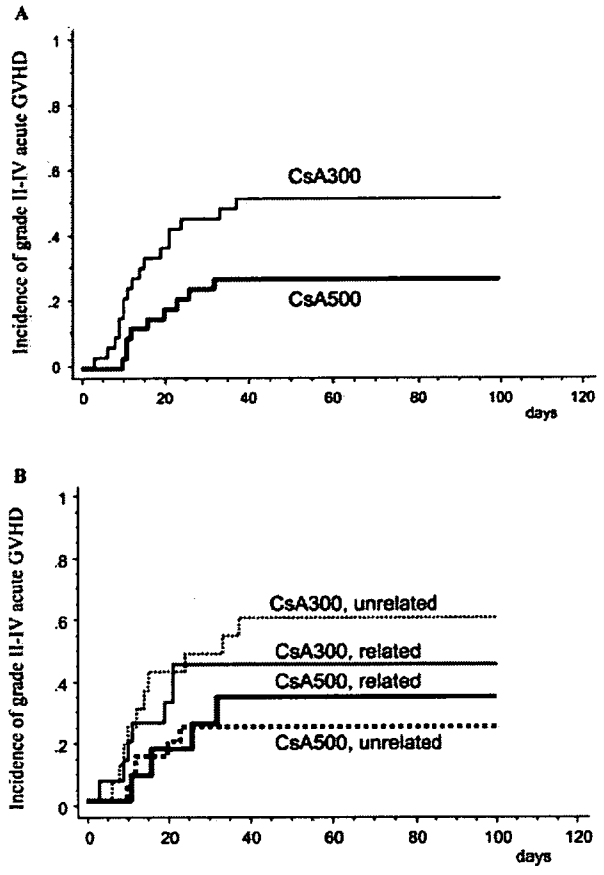

Figure 2. Incidence of Grades II–IV acute GVHD grouped according to the target level of cyclosporine. (A) all patients, (B) stratified by the donor type.

suggested that the measurement of blood concentration at 2–4 hr after infusion may be more appropriate [10]. In continuous infusion, the intradaily variation of the blood concentration of CsA should be minimal and we can evaluate the blood concentration regardless of the timing (steady-state concentration; C_{SS}). However, the relationship between Css in continuous infusion and C_{TL} in twice-daily infusion has not been clarified. Recently, Nakamura et al. reported that the target Css in the continuous infusion of CsA should be 2.55 times the C_{TL} to provide an equal AUC during the twice-daily infusion with a target C_{TL} [11]. Therefore, for example, the target C_{SS} in the continuous infusion of CsA should be 383–638 ng/ml to obtain a similar AUC during the twice-daily infusion with a target C_{TL} at 150–250 ng/ml, that is generally used in daily practice. However, the target blood concentration between 250 and 350 ng/ml is widely used in the continuous infusion of CsA [4]. The expected AUC will be far lower than that during the twice-daily infusion of CsA at the generally used target level. The target C_{SS} in this study at 500 ng/ml (450–550 ng/ml) would be appropriate according to the calculation model. In fact, the actual dose of CsA was maintained at 2.7 and 3.0 mg/kg on average. We had a concern that the incidence of renal dysfunction would be increased, since the relationship between the blood CsA level and drug-induced nephrotoxicity has been shown [12]. The incidence of renal dysfunction, however, was not increased by the dose adjustment and appropriate hydration when CsA levels above the target range were observed.

TABLE IV. Factors Associated the Incidence of Chronic GVHD

A. Univariate analyses

Factor	Chronic GVHD	*P*-value
Sex		
Male	24 (67%)	0.63
Female	10 (56%)	
Age		
<40 years	16 (60%)	0.31
≥40 years	18 (70%)	
Underlying disease		
CML	10 (77%)	0.12
Non-CML	24 (60%)	
Donor		
Related	14 (63%)	0.75
Unrelated	20 (66%)	
HLA		
Match	28 (64%)	0.74
Mismatch	6 (58%)	
Stem cell source		
BM	25 (60%)	0.20
PBSC	9 (81%)	
Regimen		
Non-TBI	27 (64%)	0.75
TBI	7 (65%)	
MTX dose		
<31mg/m^2	15 (64%)	0.81
≥31mg/m^2	19 (68%)	
Target levels of CsA		
CsA500	11 (47%)	0.016
CsA300	23 (73%)	

B. Multivariate analyses

Factor	RR	*P*-value
Target levels of CsA		
CsA300	1.00	0.014
CsA500	0.44 (0.23–0.85)	

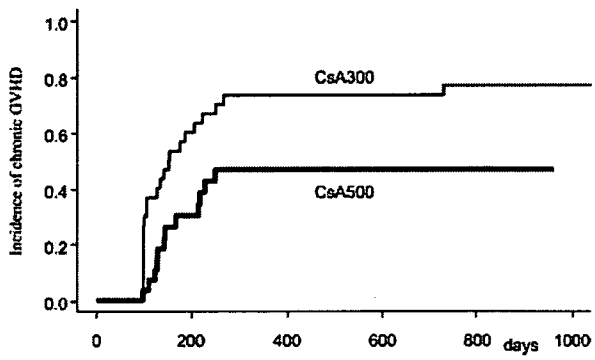

Figure 3. Incidence of chronic GVHD grouped according to the target level of cyclosporine.

Previous randomized control studies that compared continuous infusion of CsA and tacrolimus as GVHD prophylaxis had showed the superiority of tacrolimus to prevent acute GVHD [13–16]. However, these studies employed the lower target level of CsA between 150 and 400 ng/ml. Yanada et al. have also reported that tacrolimus-based regimen was better than cyclosporine-based regimen to prevent GVHD in unrelated bone marrow (BM) transplantation in Japan [17]. However, it was a retrospective analysis

TABLE V. Factors Associated the Incidence of Relapse and Disease-Free Survival

A. Univariate analyses

Factor	Relapse	*P*-value	Disease-free survival	*P*-value
Sex				
Male	5 (12%)	0.90	29 (58%)	0.054
Female	2 (15%)		18 (85%)	
Age				
<40 years	4 (16%)	0.72	25 (71%)	0.35
≥40 years	3 (10%)		22 (60%)	
Underlying disease				
CML	1 (7%)	0.51	11 (79%)	0.34
Non-CML	6 (15%)		36 (60%)	
Donor				
Related	1 (4%)	0.10	19 (60%)	0.53
Unrelated	6 (20%)		28 (67%)	
HLA				
Match	6 (13%)	0.74	37 (63%)	0.64
Mismatch	1 (10%)		10 (72%)	
Stem cell source				
BM	7 (16%)	0.15	35 (63%)	0.55
PBSC	0 (0%)		12 (76%)	
Regimen				
Non-TBI	0 (0%)	0.14	8 (51%)	0.41
TBI	7 (16%)		39 (69%)	
MTX dose				
<31 g/m^2	1 (4%)	0.071	21 (77%)	0.19
≥31 g/m^2	6 (21%)		26 (55%)	
Target levels of CsA				
CsA500	5 (20%)	0.069	26 (72%)	0.68
CsA300	2 (6%)		21 (63%)	

B. Multivariate analyses

Factor	RR of relapse	*P*-value	RR of disease-free survival	*P*-value
Target levels of CsA				
CsA300	1.00	0.065	1.00	0.68
CsA500	4.08 (0.92–18.1)		0.82 (0.32–2.12)	

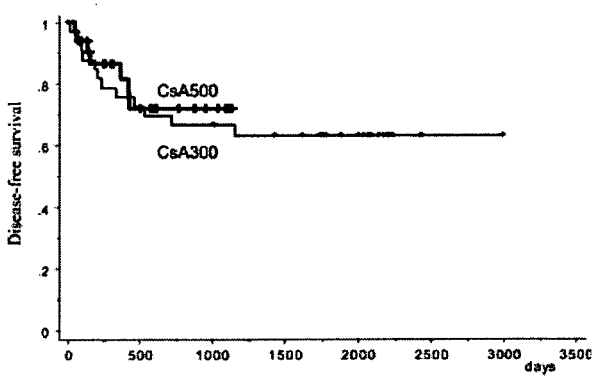

Figure 4. Disease-free survival grouped according to the target level of cyclosporine.

using the database of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and therefore the dose, target blood level, and infusion schedule of both cyclosporine and tacrolimus were various. Especially, the target level of CsA is generally low in the daily practice in Japan. Therefore, the results of these previous studies that compared CsA and tacrolimus as GVHD prophylaxis might have been affected by the target blood concentration [13–17].

The incidence of grades II–IV acute GVHD in the CsA500 group in this study was suppressed to 24% in unrelated HSCT including three HLA allele-mismatched transplants. This incidence was similar to that in the tacrolimus group of patients who underwent HSCT from an alternative donor (30 from an HLA-matched unrelated donor and 4 from the other alternative donor) in a Japanese randomized controlled trial (21%) [13]. Adverse drug reactions were more frequently observed in the tacrolimus group than in the CsA group in this Japanese randomized trial [13], whereas the toxicities in the CsA500 group were equivalent to those in the CsA300 group in the current study. Therefore, the continuous infusion of CsA with a target concentration at 500 ng/ml may provide similar efficacy of GVHD prophylaxis with less frequent toxicities compared to tacrolimus. Wingard et al. have reported that an important relationship between blood concentration of these agents and their efficacy and toxicity using data of a randomized controlled trial [16]. They showed that the efficacy of CsA to prevent GVHD could be improved by elevating the target blood concentration of CsA, whereas the toxicity of tacrolimus could be reduced by lowering the target blood concentration of tacrolimus. Therefore, a randomized controlled trial to compare CsA and tacrolimus with their appropriate target blood concentration is required to draw a definite conclusion.

Another concern about the elevation of the target concentration of CsA was the possible increase in the inci-

dence of relapse [18,19]. We previously showed that the incidence of relapse was significantly lower after the continuous infusion of CsA with the low target CsA concentration at 300 ng/ml compared to twice-daily infusions targeted to 150–300 ng/ml, because the actual dose of CsA was obviously decreased in the continuous infusion group [6]. In this study, the incidence of relapse tended to be higher in the CsA500 group (20 vs. 6%, $P = 0.065$), although there was no significant difference in disease-free survival. A possible explanation of the tendency toward higher relapse rate in the CsA500 group was the impaired graft-versus-leukemia effect due to the higher CsA concentration. Another explanation was the fact that the CsA300 group included significantly more patients with CML in the first chronic phase, the relapse rate of which is expected to be very low. Actually, the difference in the incidence of relapse became smaller when we excluded patients with CML. In addition, relapse in the CsA500 group mainly occurred in patients with relatively poor underlying diseases, including one with chronic neutrophilic leukemia in uncontrollable leukocytosis, one with acute myeloblastic leukemia with monosomy 7, and one with acute lymphoblastic leukemia with minimal residual disease detected by flow cytometry. Therefore, it might be important to make an appropriate definition of standard-risk disease. Currently, we are excluding acute leukemia in first remission with poor cytogenetic abnormalities, such as the presence of Philadelphia chromosome or monosomy 7, from standard-risk disease.

In conclusion, the continuous infusion of CsA with a target level of 450–550 ng/ml appeared to be safe and effective to prevent acute and chronic GVHD. A randomized controlled trial is being planned to confirm the appropriateness of this higher target level of CsA.

Patients and Methods

Patients

A continuous infusion of CsA with the target blood level between 450 and 550 ng/ml was started as GVHD prophylaxis for standard-risk patients at our institute in March 2003. We compared the safety and efficacy of this GVHD prophylaxis with those in the historical standard-risk patients in whom the blood CsA level was targeted to 250–350 ng/ml [6]. Standard-risk disease included acute leukemia in complete remission, CML in chronic phase, myelodysplastic syndrome without leukemic transformation, chemosensitive lymphoma, and nonmalignant disorders such as chronic active Epstein-Barr virus infection, while the others were considered high-risk diseases.

Transplantation procedure

Conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) with either busulfan (4 mg/kg/day for 4 days) or total body irradiation (TBI; 2 Gy twice daily for 3 days). BM was exclusively used as stem cell source in unrelated HSCT, whereas peripheral blood (PB) or BM was chosen in HSCT from a relative. GVHD prophylaxis consisted of CsA and short term methotrexate (MTX). The dose of MTX was 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6 in HLA-matched related HSCT. MTX at 7 mg/m^2 was added on day 11 in HLA-mismatched related HSCT and HLA-matched unrelated HSCT. In HLA allele-mismatched unrelated HSCT, the doses of MTX were increased to 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6, and 11.

CsA was administered as a 24-hr continuous infusion. The concentration of CsA was measured twice a week by fluorescence polarization immunoassay with a specific monoclonal antibody, using whole blood samples [20]. The dose of CsA was adjusted based on the ratio of the measured blood concentration and the target blood concentration of cyclosporine at 500 ng/ml to maintain the blood CsA concentration between 450 and 550 ng/ml. For example, when the measured blood concentration was 400 ng/ml using a daily cyclosporine dose of 200 mg, we multiplied the dose of cyclosporine by the ratio and determined the next cyclosporine dose at 200 mg × 500/400 = 250 mg. The route of CsA administration was converted to oral at a ratio of 1:2 when patients were able to tolerate oral intake after engraftment. Acute GVHD was graded as previously described [21]. Prophylaxis against bacterial, fungal, and *Pneumocystis carinii* infection consisted of fluconazole, tosufloxacin, and sulfamethoxazole/trimethoprim or inhalation of pentamidine. As prophylaxis against herpes simplex virus infection, acyclovir was given from days 7–35. Pre-emptive therapy with ganciclovir for cytomegalovirus infection was performed by monitoring cytomegalovirus antigenemia. The initial dose of ganciclovir was 5 mg/kg once daily and the dose was elevated to 5 mg/kg twice daily, when an increasing antigenemia was observed [22]. Other supportive procedures were not changed.

Statistical considerations

Toxicities were evaluated until the route of CsA was changed to oral. Renal dysfunction was defined as elevation of serum creatinine level above ×1.5 or ×2.0 the baseline value. Liver dysfunction was defined as elevation of the total bilirubin level above 2 mg/dl. Dichotomous variables of the patients' characteristics in the two groups were compared using Fisher's exact test. Overall survival, disease-free survival, and the cumulative incidence of acute GVHD were calculated using the Kaplan–Meier method, whereas the cumulative incidences of relapse and nonrelapse mortality were calculated using Gray's method considering each other event as a competing risk [23]. Potential confounding factors for the analyses included age, sex, donor types (related or unrelated), stem cell sources (BM or PB), conditioning regimens (TBI or non-TBI), HLA-mismatch, total doses of MTX, and the target levels of CsA. To evaluate the influence of the confounding factors on these events, the log-rank test and proportional hazards modeling were used for univariate and multivariate analyses, respectively. Factors that showed at least borderline significance ($P < 0.10$) in univariate analyses were included in the multivariate analyses and stepwisely deleted from the model, although the target level of CsA was persistently stayed in the model. All P-values were two-sided and P-values of 0.05 or less were considered statistically significant.

References

1. Ruutu T, Niederwieser D, Gratwohl A, Apperley JF. A survey of the prophylaxis and treatment of acute GVHD in Europe: A report of the European Group for Blood and Marrow, Transplantation (EBMT). Chronic Leukaemia Working Party of the EBMT. Bone Marrow Transplant 1997;19:759–764.
2. Yee GC, Self SG, McGuire TR, et al. Serum cyclosporine concentration and risk of acute graft-versus-host disease after allogeneic marrow transplantation. N Engl J Med 1988;319:65–70.
3. Przepiorka D, Shapiro S, Schwinghammer TL, et al. Cyclosporine and methylprednisolone after allogeneic marrow transplantation: Association between low cyclosporine concentration and risk of acute graft-versus-host disease. Bone Marrow Transplant 1991;7:461–465.
4. Kanda Y, Hyo R, Yamashita T, et al. Effect of blood cyclosporine concentration on the outcome of hematopoietic stem cell transplantation from an HLA-matched sibling donor. Am J Hematol 2006;81:838–844.
5. Martin P, Bleyzac N, Souillet G, et al. Relationship between CsA trough blood concentration and severity of acute graft-versus-host disease after paediatric stem cell transplantation from matched-sibling or unrelated donors. Bone Marrow Transplant 2003;32:777–784.
6. Ogawa N, Kanda Y, Matsubara M, et al. Increased incidence of acute graft-versus-host disease with the continuous infusion of cyclosporine A compared to twice-daily infusion. Bone Marrow Transplant 2004;33:549–552.
7. Miller KB, Schenkein DP, Comenzo R, et al. Adjusted-dose continuous-infusion cyclosporin A to prevent graft-versus-host disease following allogeneic bone marrow transplantation. Ann Hematol 1994;68:15–20.
8. Grevel J, Welsh MS, Kahan BD. Cyclosporine monitoring in renal transplantation: Area under the curve monitoring is superior to trough-level monitoring. Ther Drug Monit 1989;11:246–248.
9. Lindholm A, Kahan BD. Influence of cyclosporine pharmacokinetics, trough concentrations, and AUC monitoring on outcome after kidney transplantation. Clin Pharmacol Ther 1993;54:205–218.
10. Duncan N, Craddock C. Optimizing the use of cyclosporin in allogeneic stem cell transplantation. Bone Marrow Transplant 2006;38:169–174.
11. Nakamura Y, Takeuchi H, Okuyama K, et al. Evaluation of appropriate blood level in continuous intravenous infusion from trough concentrations after oral administration based on area under trough level in tacrolimus and cyclosporine therapy. Transplant Proc 2005;37:1725–1727.
12. Kagawa Y, Sawada J, Yamada S, et al. Relationship between development of nephrotoxicity and blood concentration of cyclosporine A in bone-marrow transplanted recipients who received the continuous intravenous infusion. Biol Pharm Bull 2003;26:1115–1119.
13. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. Bone Marrow Transplant 2001;28:181–185.
14. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood 2000;96:2062–2068.

15. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood 1998;92:2303–2314.
16. Wingard JR, Nash RA, Przepiorka D, et al. Relationship of tacrolimus (FK506) whole blood concentrations and efficacy and safety after HLA-identical sibling bone marrow transplantation. Biol Blood Marrow Transplant 1998; 4:157–163.
17. Yanada M, Emi N, Naoe T, et al. Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: a nationwide survey conducted in Japan. Bone Marrow Transplant 2004;34:331–337.
18. Bacigalupo A, Van Lint MT, Occhini D, et al. Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. Blood 1991;77:1423–1428.
19. Bacigalupo A, Vitale V, Corvo R, et al. The combined effect of total body irradiation (TBI) and cyclosporin A (CyA) on the risk of relapse in patients with acute myeloid leukaemia undergoing allogeneic bone marrow transplantation. Br J Haematol 2000;108:99–104.
20. Alvarez JS, Sacristan JA, Alsar MJ. Comparison of a monoclonal antibody fluorescent polarization immunoassay with monoclonal antibody radioimmunoassay for cyclosporin determination in whole blood. Ther Drug Monit 1992;14: 78–80.
21. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825–828.
22. Kanda Y, Mineishi S, Saito T, et al. Response-oriented preemptive therapy against cytomegalovirus disease with low-dose ganciclovir: A prospective evaluation. Transplantation 2002;73:568–572.
23. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: New representations of old estimators. Stat Med 1999;18:695–706.

Journal of Antimicrobial Chemotherapy (2008) **61**, 411–416
doi:10.1093/jac/dkm463
Advance Access publication 30 November 2007

JAC

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

Yuki Asano-Mori[1,2], Yoshinobu Kanda[1], Kumi Oshima[1], Shinichi Kako[1], Akihito Shinohara[1], Hideki Nakasone[1], Makoto Kaneko[1], Hiroyuki Sato[1], Takuro Watanabe[1], Noriko Hosoya[3], Koji Izutsu[1], Takashi Asai[1], Akira Hangaishi[1], Toru Motokura[1], Shigeru Chiba[3] and Mineo Kurokawa[1]*

[1]*Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan;* [2]*Department of Hematology, Japanese Red Cross Medical Center, Tokyo, Japan;* [3]*Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan*

Received 2 September 2007; returned 8 October 2007; revised 2 November 2007; accepted 4 November 2007

***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,

*Corresponding author. Tel: +81-3-5800-9092; Fax: +81-3-5840-8667; E-mail: kurokawa-tky@umin.ac.jp

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

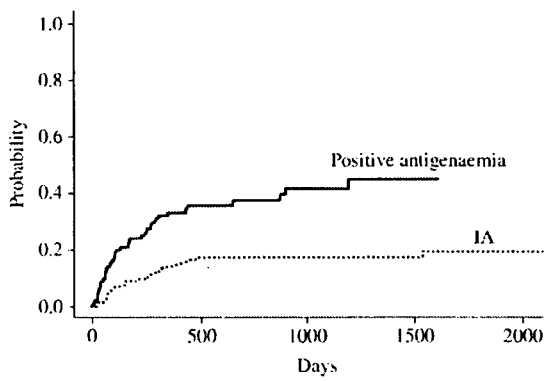

Figure 1. Cumulative incidences of IA and positive GM antigenaemia after HSCT.

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.

Acknowledgements

We thank all the clinicians who have assisted with the provision of data for this project.

Funding

This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare.

Transparency declarations

None to declare.

References

1. Herbrecht R, Denning DW, Patterson TF *et al.* Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–15.

2. Stynen D, Goris A, Sarfati J *et al.* A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. *J Clin Microbiol* 1995; **33**: 497–500.

3. Herbrecht R, Letscher-Bru V, Oprea C *et al. Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002; **20**: 1898–906.

4. Maertens J, Verhaegen J, Lagrou K *et al.* Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001; **97**: 1604–10.

5. Maertens J, Verhaegen J, Demuynck H *et al.* Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. *J Clin Microbiol* 1999; **37**: 3223–8.

6. Wheat LJ. Rapid diagnosis of invasive aspergillosis by antigen detection. *Transpl Infect Dis* 2003; **5**: 158–66.

7. Ascioglu S, Rex JH, de Pauw B *et al.* Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; **34**: 7–14.

8. Hamaki T, Kami M, Kanda Y *et al.* False-positive results of *Aspergillus* enzyme-linked immunosorbent assay in a patient with chronic graft-versus-host disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2001; **28**: 633–4.

9. Ansorg R, van den Boom R, Rath PM. Detection of *Aspergillus* galactomannan antigen in foods and antibiotics. *Mycoses* 1997; **40**: 353–7.

10. Murashige N, Kami M, Kishi Y *et al.* False-positive results of *Aspergillus* enzyme-linked immunosorbent assays for a patient with gastrointestinal graft-versus-host disease taking a nutrient containing soybean protein. *Clin Infect Dis* 2005; **40**: 333–4.

11. Swanink CM, Meis JF, Rijs AJ *et al.* Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. *J Clin Microbiol* 1997; **35**: 257–60.

12. Viscoli C, Machetti M, Cappellano P *et al.* False-positive galactomannan Platelia *Aspergillus* test results for patients receiving piperacillin–tazobactam. *Clin Infect Dis* 2004; **38**: 913–6.

13. Maertens J, Theunissen K, Verhoef G *et al.* False-positive *Aspergillus* galactomannan antigen test results. *Clin Infect Dis* 2004; **39**: 289–90.

14. Niiya H, Kanda Y, Saito T *et al.* Early full donor myeloid chimerism after reduced-intensity stem cell transplantation using a combination of fludarabine and busulfan. *Hematologica* 2001; **86**: 1071–4.

15. Kanda Y, Oshima K, Asano-Mori Y *et al. In vivo* alemtuzumab enables haploidentical HLA-mismatched hematopoietic stem cell transplantation without *ex vivo* graft manipulation. *Transplantation* 2002; **73**: 568–72.

16. Kawazu M, Kanda Y, Nannya Y *et al.* Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-β-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004; **42**: 2733–41.

17. Hughes WT, Armstrong D, Bodey GP *et al.* 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 2002; **34**: 730–51.

18. Gooley TA, Leisenring W, Crowley J *et al.* Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**: 695–706.

19. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006; **42**: 1417–27.

American Journal of Hematology 82:386–390 (2007)

Early Relapse of JAK2 V617F-Positive Chronic Neutrophilic Leukemia With Central Nervous System Infiltration After Unrelated Bone Marrow Transplantation

Shinichi Kako,[1] Yoshinobu Kanda,[1] Tomohiko Sato,[1] Susumu Goyama,[1] Naohiro Noda,[2] Eriko Shoda,[1] Kumi Oshima,[1] Morihiro Inoue,[1] Koji Izutsu,[1] Takuro Watanabe,[1] Toru Motokura,[1] Shigeru Chiba,[3] Masashi Fukayama,[2] and Mineo Kurokawa[1]*

[1] Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[2] Department of Pathology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan
[3] Department of Cell Therapy and Transplantation Medicine, University of Tokyo Hospital, Tokyo, Japan

Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative disorder characterized by a proliferation mainly of mature neutrophils. The prognosis is generally poor and an optimal therapeutic strategy remains to be determined. Allogeneic hematopoietic stem cell transplantation (HSCT) is expected to be the only curative therapy so far. We report a 46-year-old male with progressive CNL who underwent bone marrow transplantation from an HLA-matched unrelated donor. After engraftment was achieved on day 35, relapse of CNL was confirmed on day 50. The progression of CNL was very rapid afterward and infiltration to the central nervous system was observed. The Janus Kinase 2 (JAK2) V617F homozygous mutation was detected from the peripheral blood or bone marrow samples throughout the clinical course. From comparison with reports of successful HSCT for CNL in the literature, it was inferred that HSCT should be performed in a stable status before progression. Furthermore, JAK2 V617F-positive CNL may contain an aggressive disease entity in contrast to previous reports. Accumulation of experiences is required to establish a definite role of HSCT in the treatment of CNL and a prognostic significance of JAK2 mutation in CNL. Am. J. Hematol. 82:386–390, 2007.

Key words: chronic neutrophilic leukemia; allogeneic hematopoietic stem cell transplantation; intracranial invasion; JAK2 V617F mutation

INTRODUCTION

Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative disorder which predominantly affects older adults [1–3] and characterized by sustained mature neutrophilic leukocytosis in peripheral blood, neutrophilic granulocyte proliferation in the bone marrow, and the absence of the Philadelphia chromosome. Neutrophilia is usually progressive, and anemia and thrombocytopenia occur successively [1]. Most patients with CNL have a poor prognosis with a median survival of about 2 years [4]. Because of the rarity of this disease, an optimal therapeutic strategy remains to be determined. Allogeneic hematopoietic stem cell transplantation (HSCT) is expected to be the only curative therapy so far [1,4]. Several reports of successful HSCT for CNL have been reported in the literature, in which HSCT was performed during a controllable phase [5,6]. However, we experienced a 46-year-old male with JAK2 V617F-positive CNL who underwent bone marrow transplantation from an HLA-matched unrelated donor in the progressive phase and developed early systemic relapse associated with central nervous system infiltration.

*Correspondence to: Mineo Kurokawa, M.D., Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: kurokawa-tky@umin.ac.jp

Received for publication 20 May 2006; Accepted 19 August 2006

Published online 15 November 2006 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20805

TABLE I. Summary of Chromosome Analyses

Date	Total number of cells analyzed	Karyotype and number of cells with each karyotype	
2001/03/26	20	46, XY, inv. (9)	20
2001/10/22	20	46, XY, inv. (9)	20
2004/05/10	20	46, XY, inv. (9)	20
2004/10/05	20	46, XY, inv. (9)	20
2004/11/16	15	46, XY	15
2004/12/15	20	46, Y, r(X;6)(p11;p15),inv. (9)	2
		46, XY, r(1;11)(p34;q23), add(4)(p11), del(16)(p11), inv. (9)	2
		46, XY, add(1)(p11),del(1)(p36), add(3)(p21), inv. (9)	1
		46, XY, t(8;11)(q11;q23), inv. (9)	1
		46, XY, inv. (9)	14

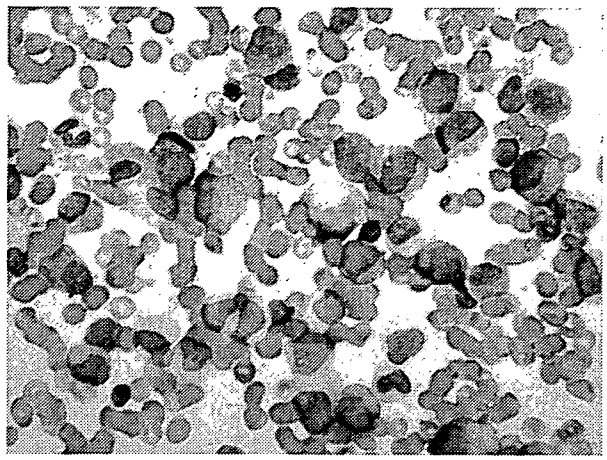

Fig. 1. Bone marrow aspiration on day 50 showed neutrophilic granulocyte proliferation without an increase in blast cells. (May–Giemsa stain, ×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CASE REPORT

A 46-year-old male was referred to our hospital in March 2001 due to leukocytosis and hepatosplenomegaly. White blood cell (WBC) count was 23.8 × 10^3/μL with 89% of neutrophils. Hemoglobin (Hb) level and platelet count were within normal limits (15.9 g/dL and 46.1 × 10^4/μL, respectively). Bone marrow aspiration showed hypercellular bone marrow, which was compatible with chronic myelogenous leukemia in chronic phase. However, the karyotype was normal except for inversion 9, which was considered to be a normal variant. The bcr/abl fusion mRNA was not detected by reverse-transcriptase polymerase chain reaction (RT-PCR). Morphological abnormality was not apparent. In the next month, WBC count was elevated to more than 25.0 × 10^3/μL and he fulfilled the diagnostic criterion of CNL according to the World Health Organization (WHO) classification. Thereafter, WBC count was stable and he was followed up without any treatment. In November 2001, WBC count increased to more than 40.0 × 10^3/μL. Therefore, we started hydroxyurea and WBC count was well controlled below 10.0 × 10^3/μL. In April 2004, however, WBC count increased again to more than 20.0 × 10^3/μL with 91% of neutrophils, associated with a progressive decline in Hb and platelet count, which was refractory to the increased dose of hydroxyurea. We added cytarabine ocfosfate, but it was also ineffective. He became dependent on frequent blood transfusions. The lower margin of his spleen was palpable beyond the navel level. Bone marrow aspiration showed marked neutrophilic granulocyte proliferation and there was no change in chromosome analysis and RT-PCR findings (Table I).

We performed bone marrow transplantation from an HLA-matched unrelated male donor on October 26th, 2004. The conditioning regimen consisted of cyclophosphamide (60 mg/kg for 2 days) and fractionated total body irradiation (2 Gy twice daily for 3 days). Prophylaxis against graft-versus-host disease (GVHD) consisted of cyclosporine (CsA) at 3 mg/kg and short-term methotrexate (10 mg/m^2 on day 1 and 7 mg/m^2 on days 3, 6, and 11). Bone marrow aspiration on day 19 showed hypocellular bone marrow, but karyotype was 46, XY without inversion 9. Therefore, hematopoiesis at that time was considered to be donor-origin. On day 35, neutrophil engraftment was achieved. Grade I acute GVHD of the skin was observed, but quickly disappeared with topical steroid. We performed bone marrow aspiration on day 50, which showed hypercellular bone marrow without an increase in blast cells (Fig. 1). Chromosome analysis showed complex abnormality including inversion 9 in all dividing cells analyzed (Table I). WBC count began to increase to more than 10.0 × 10^3/μL with a decline in platelet count. Therefore, he was diagnosed to have a relapse of CNL and hydroxyurea was started with a rapid tapering of CsA. The dose of hydroxyurea was increased to 3000 mg/day but WBC count increased up to 47.6 × 10^3/μL. Therefore, we started a continuous infusion of cytarabine at 100 mg/day from day 70. On the same day, however, he developed dysarthria and hyperesthesia of the right side of his body. We performed plain head CT scan, which revealed multiple high-density lesions (Fig. 2A). Cerebrospinal fluid obtained by lumber puncture on the next day did not reveal an increase in cell count, but we highly suspected of central nervous system infiltra-

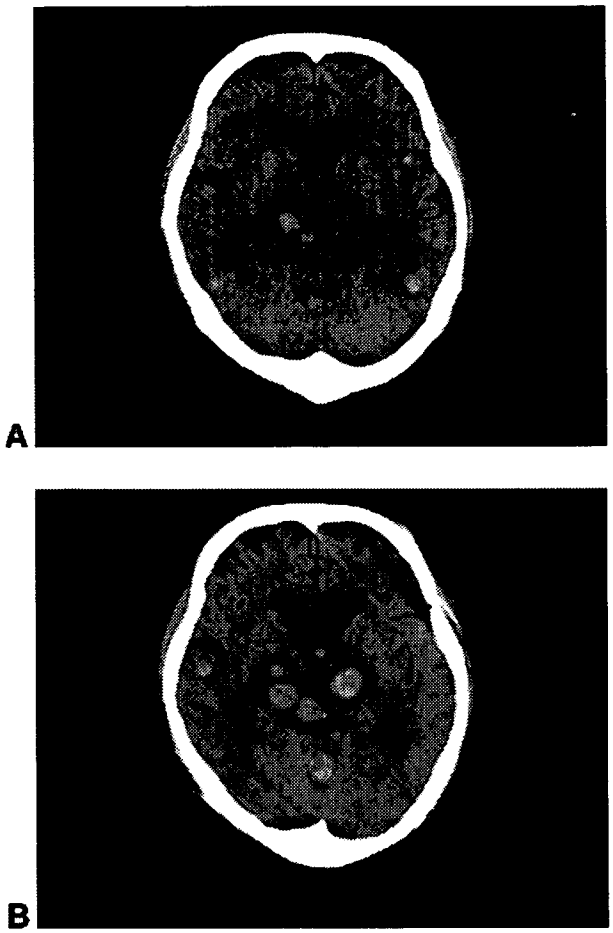

Fig. 2. Multiple high-density lesions, indicating hemorrhages, were observed in plain head CT scans on day 70 (A) and on day 74 (B).

tion of CNL and administered cytarabine at 1 g/m^2 by a 2 hr infusion. However, the neurological symptoms got worse and WBC count was persistently more than 40.0 $\times$ 10^3/μL with more than 60% of neutrophils. He became unable to communicate and anisocoria appeared on day 74. Plain head CT scan on that day showed an enlargement of the pre-existing lesions as well as an emergence of many new lesions (Fig. 2B). We started whole brain irradiation at 3 Gy/day but the heart rate and blood pressure were elevated suddenly on the night and his spontaneous ventilation stopped. Although we started mechanical ventilation, he died on day 79 probably due to intracranial hemorrhage. Autopsy revealed marked infiltration of mature and immature neutrophils in most organs, associated with multiple hemorrhagic lesions in the brain (Fig. 3).

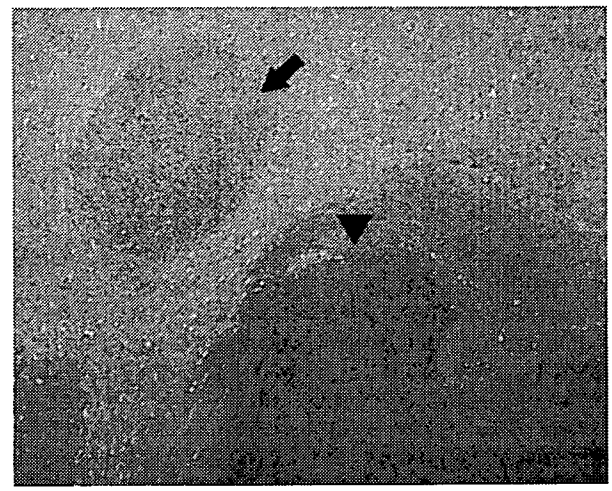

Fig. 3. Autopsy revealed invasion and mass formation of mature and immature neutrophils in the parenchyma of the brain (arrow). Hemorrhagic lesion was just close to the mass (triangle). (Hematoxylin–Eosin stain, ×100). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Janus Kinase 2 (JAK2) Mutation Analysis

We evaluated JAK2 V617F mutation as described previously [7]. In brief, genomic DNA was extracted from peripheral blood or bone marrow samples and amplified by polymerase chain reaction using sense and antisense primers (5′-TGCTGA- AAGTAGGA-GAAAGTGCAT-3′ and 5′-TCCTACAGTGTTTT-CAGTTTCAA-3′, respectively). Fluorescent dye chemistry sequencing was performed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystem, Foster City, CA) using the same primers. GenBank accession NM_004 972 (JAK2 mRNA) and the corresponding region from the NC_000 009 chromosome 9 contig were used for sequence analysis.

The JAK2 V617F homozygous mutation was detected in the peripheral blood sample obtained in December 2001, when the WBC count started to increase for the first time after the diagnosis (Fig. 4). The same mutation was detected in the bone marrow samples obtained in the progressive phase before HSCT (October, 2004) and at relapse of CNL on day 50 after HSCT (December, 2004). We could not evaluate the JAK2 V617F mutation on the CNS lesions because cerebrospinal fluid obtained by lumber puncture at CNS relapse did not contain leukemic cells and brain tissue obtained at autopsy was severely damaged and inappropriate for the JAK2 mutation analysis.

DISCUSSION

CNL is a new distinct entity which was defined in the WHO classification, and only 150 cases have been

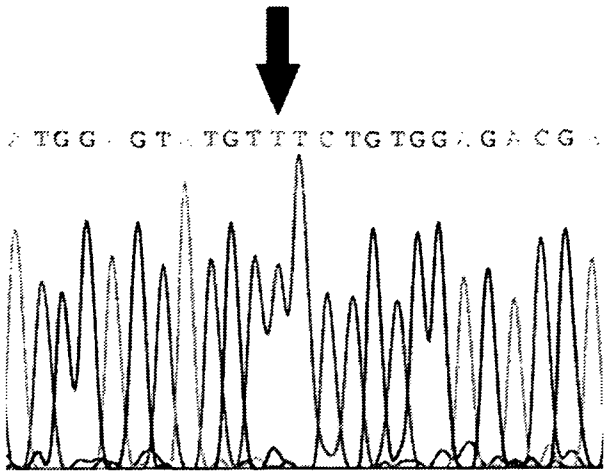

Fig. 4. Fluorescent dye chemistry sequencing detected JAK2 V617F homozygous mutation in the peripheral blood sample obtained in December 2001. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

reported [4]. Therefore, the clinical and pathological features of CNL, as well as an optimal therapeutic strategy, have not been well understood. Oral cytoreductive agents, such as hydroxyurea, can control neutrophilia only temporarily, while intensive chemotherapy has often resulted in death from infection or hemorrhage due to severe cytopenias [1,2,4]. Allogeneic HSCT has been expected to be the only curative therapy so far [1,4]. Several successful cases have been reported. Hasle et al. reported a long-term remission of CNL in two young patients who underwent allogeneic HSCT when the WBC count was stably controlled with hydroxyurea [5]. Piliotis et al. also reported a successful clinical course of 60-year-old male with CNL who underwent allogeneic HSCT in the stable phase [6]. In contrast, the current patient underwent allogeneic HSCT when the disease was progressive despite of administering hydroxyurea and had a relapse of CNL early after HSCT. The difference in the transplantation outcome might have resulted from the disease status at HSCT, which is similar to the difference in the outcome of HSCT for chronic myelogenous leukemia between patients who underwent HSCT in accelerated or blastic phase and those who underwent HSCT in chronic phase. Another explanation may be the "publication bias," that unsuccessful outcomes tend to be rejected by journal editors or are not submitted by clinicians.

The leading cause of death in patients with CNL is intracranial hemorrhage, the pathogenesis of which remains unclear [1,2,4]. Noguchi et al. reported a patient who died of CNL due to intracranial hemorrhage. The autopsy findings showed extensive infiltration of mature and immature neutrophils, which resulted in the destruction of vascular walls [8]. On the other hand, vascular destruction by mucormycosis was the cause of intracranial hemorrhage in a CNL patient reported by Yasui et al. [9]. Autopsy findings of the current patient demonstrated invasion and mass formation of mature and immature neutrophils in the parenchyma of the brain. Regrettably, we could not examine the infiltration of neutrophils to the vascular wall, because brain tissue was already severely damaged at autopsy.

The JAK2 V617F mutation was detected in most patients with polycythemia vera and one-third to one-half of patients with essential thrombocythemia or idiopathic myelofibrosis [10]. However, there have been only three reports as to the association between CNL which met the current WHO diagnostic criterion and the JAK2 V617F mutation. Steensma et al. evaluated the prevalence of JAK2 V617F homozygous mutation in six patients with CNL and the mutation was detected in one patient, whose clinical course was stable with hydroxyurea more than two years from the diagnosis of CNL [7]. In addition, Mc Lornan et al. reported a CNL patient with JAK2 V617F homozygous mutation who was surviving at 96 months after the diagnosis of CNL [11]. Recently, Lea et al. presented another CNL patient with JAK2 V617F homozygous mutation who was stably controlled with busulphan [12]. All these reports suggested a possible relationship between the presence of JAK2 mutation and a long survival of CNL. In contrast, the current patient showed progression of CNL 3 years after the initial diagnosis and the subsequent clinical course was uncontrollable. Therefore, an accumulation of data is required to clarify the relationship between the JAK2 mutation status and the clinical course of CNL.

In conclusion, CNL is associated with extremely poor prognosis and allogeneic HSCT has been considered to be the only curative strategy at the moment. Considering the difference in the transplant outcome between the current patient and those in the previous reports of successful HSCT for CNL, it was inferred that the status of the disease at HSCT might be important. A prospective study to perform allogeneic HSCT early after diagnosis of CNL is warranted to establish a role of allogeneic HSCT in the treatment of CNL. In addition, JAK2 would become a potential target for therapeutical intervention against a subset of CNL, especially in the early phase before the accumulation of additional genetic alterations.

REFERENCES

1. Elliott MA. Chronic neutrophilic leukemia: A contemporary review. Curr Hematol Rep 2004;3:210–217.
2. Bohm J, Schaefer HE. Chronic neutrophilic leukaemia: 14 new cases of an uncommon myeloproliferative disease. J Clin Pathol 2002;55:862–864.

3. Eliott MA, Dewald GW, Tefferi A, et al. Chronic neutrophilic leukemia (CNL): A clinical, pathologic and cytogenetic study. Leukemia 2001;15:35 40.
4. Eliott MA, Hanson CA, Dewald GW, et al. WHO-defined chronic neutrophilic leukemia: A long-term analysis of 12 cases and a critical review of the literature. Leukemia 2005;19:313 317.
5. Hasle H, Olesen G, Kerndrup G, et al. Chronic neutrophil leukaemia in adolescence and young adulthood. Br J Haematol 1996;94:628 630.
6. Piliotis E, Kutas G, Lipton JH. Allogeniec bone marrow transplantation in the management of chronic neutrophilic leukemia. Leuk Lymphoma 2002;43:2051 2054.
7. Steensma DP, Dewald GW, Lasho TL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both "atypical" myeloproliferative disorders and myelodysplastic syndromes. Blood 2005;106:1207–1209.
8. Noguchi T, Ikeda K, Yamamoto K, et al. Severe bleeding tendency caused by leukemic infiltration and destruction of vascular walls in chronic neutrophilic leukemia. Int J Hematol 2001;74: 437 441.
9. Yasui H, Adachi Y, Ishii Y, et al. Mucormycosis as an etiology of cerebral hemorrhage in patients with chronic neutrophilic leukemia. Am J Med 2003;115:674 676.
10. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779 1790.
11. Mc Lornan DP, Percy MJ, Jones AV, et al. Chronic neutrophilic leukemia with an associated V617F JAK2 tyrosine kinase mutation. Haematologica 2005;90:1696 1697.
12. Lea NC, Lim Z, Westwood NB, et al. Presence of JAK2 V617F tyrosine kinase mutation as a myeloid-lineage-specific mutation in chronic neutrophilic leukaemia. Leukemia 2006;20:1324–1326.