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ORIGINAL ARTICLE

Prediction of infectious events by high-sensitivity C-reactive protein level before undergoing chemotherapy for acute myeloid leukaemia

MIKI SATO, SHINICHI KAKO, KUMI OSHIMA, KEN SATO, KIRIKO TERASAKO, SHUN-ICHI KIMURA, HIDEKI NAKASONE, SHINYA OKUDA, RIE YAMAZAKI, TAKAKAZU HIGUCHI, JUNJI NISHIDA & YOSHINOBU KANDA

From the Division of Haematology, Saitama Medical Centre, Jichi Medical University, Saitama, Japan

Abstract

We retrospectively evaluated the serum high-sensitivity C-reactive protein (CRP) level before chemotherapy for the prediction of infectious events during neutropenia in patients with acute myeloid leukaemia. Thirty-eight patients who underwent first induction chemotherapy and 37 patients who underwent first consolidation chemotherapy were analyzed separately. A receiver-operating characteristic (ROC) curve revealed that the serum CRP level just before the first consolidation chemotherapy, but not just before the induction chemotherapy, had a significant predictive value for febrile neutropenia (FN) at a cut-off value of 0.19 mg/dl and documented infection (DI) at a cut-off value of 0.26 mg/dl. The high-sensitivity CRP measurement enabled the detection of slight increases in the serum CRP level, which might reflect a minute inflammation by occult infection, and discriminated high-risk patients for infectious events.

Introduction

Infection is the most common complication in neutropenic patients undergoing chemotherapy for haematological malignancies. Several measures, such as the use of high efficiency particulate air (HEPA) filters and the prophylactic administration of antibiotics, have been shown to be effective in preventing infectious events. However, considering the cost and the emergence of resistant bacteria, these interventions should not be applied to low-risk patients. Therefore, predictive factors before starting chemotherapy have been investigated to discriminate high-risk patients for infectious events during neutropenia.

C-reactive protein (CRP) is an acute phase reactant that is mainly produced in the liver. Serum CRP levels rapidly rise within 24 h in response to infection or tissue injuries [1]. Several reports have demonstrated that the prognoses of infectious events can be predicted by the serum CRP level measured at their onset in patients with neutropenia [2,3]. Other studies have demonstrated that changes in serum CRP levels reflect the response to antibiotic therapy [4,5]. However, it has been difficult to predict infectious events by the serial measurement of serum CRP levels [4]. High-sensitivity quantitation of serum CRP levels has recently become available; this can determine quantities of CRP of <0.3 mg/dl in sera, which is the detection limit in the conventional measurement of CRP, and enables the detection of slight inflammation. It has been reported that a slight increase in the serum CRP level in patients with atherosclerosis is associated with the risk of ischaemic heart disease, suggesting the clinical usefulness of the sensitive measurement of CRP [6]. Therefore, in this study, we examined the relationship between the high-sensitivity serum CRP level before chemotherapy and infectious events during neutropenia in patients with acute myeloid leukaemia (AML), to evaluate its predictive value for such events.

Patients and methods

High-sensitivity measurement of CRP became available in routine practice at our centre in October 2003. Therefore, we retrospectively analyzed consecutive patients

Correspondence: Y. Kanda, Division of Haematology, Saitama Medical Centre, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama City, Saitama 330-8503, Japan. Tel: +81 48 647 2111 ext. 5416. Fax: +81 48 648 5188. E-mail: ycanda-tky@umin.ac.jp

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with AML treated at our institution from October 2003 to January 2008. Patients who underwent first induction chemotherapy or first consolidation chemotherapy for AML and who had neutropenia (neutrophil count <500/μl) for 7 days or longer were included in the study. We excluded patients who had had fever or definite infection and those who had received intravenous antibiotics at the start of chemotherapy. All the included patients received oral antibacterial and antifungal agents prophylactically. Antibacterial prophylaxis was performed with levofloxacin or polymyxin B. Oral fluconazole, itraconazole, or amphotericin B was given for antifungal prophylaxis. Antibacterial agents were changed to intravenous antibiotics when the neutrophil count became <500/μl, at the discretion of the attending physician (in 8 patients in the induction group and 12 patients in the consolidation group).

The serum high-sensitivity CRP level was measured at least twice a week as routine practice by latex immunoagglutination assay (Nanopia CRP, Sekisui Medical, Tokyo, Japan; minimum detection level 0.01 mg/dl). We collected data on serum CRP levels just before the start of chemotherapy. Infectious episodes were categorized into 2 groups: febrile neutropenia (FN) defined as fever during neutropenia with axillary temperature ≥37.5°C, and documented infection (DI), which included microbiologically documented infection and presumed infection based on clinical and/or radiological findings [7].

Patients who received induction chemotherapy and those who received consolidation chemotherapy were analyzed separately. The predictive value of the serum CRP level was evaluated using a receiver-operating characteristic (ROC) curve. ROC curves were drawn by plotting the sensitivity (*y*-axis) against (1 − specificity) (*x*-axis). The points on the ROC curves closest to the left upper corner were considered to be the best cut-off values, with which the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. In addition, the predictive value of the following factors on the incidence of infectious events was also evaluated: sex, age, type of AML, performance status, history of recent infection, use of central venous catheter, prophylactic use of intravenous antibiotics after the start of chemotherapy, and duration of neutropenia. The incidence of FN was calculated using the Kaplan–Meier method. Univariate comparisons for dichotomous, continuous, and time-to-event variables between groups were performed with the Fisher's exact test, *t*-test, and the log-rank test, respectively. Factors associated with at least borderline significance ($p < 0.15$) in the univariate analysis were subjected to a multivariate analysis using proportional hazards modelling.

Results

Patient characteristics and infectious events during hneutropenia

Patient characteristics are summarized in Table I. Thirty-eight patients who underwent first induction chemotherapy and 37 patients who underwent first consolidation chemotherapy were analyzed separately. Seventeen patients were included in both analyses. In the induction group there were 26 males and 12 females with a median age of 54 y (range 16–78) and in the consolidation group there were 22 males and 15 females with a median age of 50 y (range 16–72). The median serum CRP level was 0.3 mg/dl (range 0.02–5.69) just before the first induction chemotherapy and 0.18 mg/dl (range 0.02–2.19) just before the first consolidation chemotherapy. The median durations of neutropenia <500/μl and <100/μl were 22 and 14 days, respectively, in the induction group and 18 and 10 days, respectively, in the consolidation group.

During the induction chemotherapy, 31 patients developed FN and 18 patients developed DI. During the consolidation chemotherapy, 26 patients developed FN and 11 patients developed DI. All patients who developed DI in both groups had experienced FN. Therefore, 18 patients in the induction group and 11 patients in the consolidation group had neither FN nor DI. The details of DI are summarized in Table II. In most of these patients, DI was documented by clinical or radiological evidence and the

Table I. Patient characteristics

	Induction (n = 38)	Consolidation (n = 37)
Age (range), y	54 (16–78)	50 (16–72)
Sex (M/F)	26/12	22/15
Performance status (0/1/2/3/4)	29/7/2/0/0	33/4/0/0/0
Antibiotic prophylaxis	38	37
HEPA filter	21	20
Central venous catheter	21	20
CRP level (range), mg/dl	0.3 (0.02–5.69)	0.18 (0.02–2.19)
Duration of neutropenia <500/μl (range), days	22 (7–69)	18 (9–51)
Duration of neutropenia <100/μl (range), days	14 (0–37)	10 (4–18)

HEPA filter, high efficiency particulate air filter; CRP, C-reactive protein.

Table II. Documented infections (DI)

	Induction chemotherapy	Consolidation chemotherapy
DI	18	11
Blood stream infection	5	4
Anorectal infection	4	2
Oral infection	3	0
Cutaneous infection	2	1
Upper respiratory tract infection	1	1
Lower respiratory tract infection	1	3
Peripheral venous catheter-related infection	1	2
Central venous catheter-related infection	0	1
Others	1	2

Some patients in the consolidation group developed 2 or more episodes of DI.

causative pathogen was not identified. However, in patients with blood stream infections, the most frequent causative pathogens were Staphylococcus epidermidis ($n = 4$), followed by Staphylococcus aureus, Staphylococcus capitis, Streptococcus mitis, and Enterococcus faecalis ($n = 1$ each).

Statistical analyses

The area-under-the ROC curve (AUC) plot, based on the serum CRP level just before induction chemotherapy was 0.48 for FN and 0.56 for DI, suggesting that the serum CRP level before induction therapy had poor predictive value for FN and DI. On the other hand, the serum CRP level just before consolidation chemotherapy had a better predictive value for FN and DI, with AUCs of 0.77 and 0.67, respectively (Figure 1). The best cut-off value of serum CRP level for FN was 0.19, which gave sensitivity, specificity, PPV, and NPV of 0.64, 0.82, 0.89, and 0.50, respectively. The best cut-off value of serum CRP level for DI was 0.26, which gave sensitivity, specificity, PPV, and NPV of 0.64, 0.80, 0.58, and 0.83, respectively. With these cut-off values, the likelihood ratio for a positive result of CRP for FN was 3.6 and for DI was 3.2. The likelihood ratio for a negative result of CRP for FN was 0.44 and for DI was 0.45.

In a univariate analysis, the prophylactic use of intravenous antibiotics after the start of chemotherapy and serum CRP level less than 0.2 mg/dl were associated with a lower incidence of FN during consolidation chemotherapy with at least borderline significance ($p = 0.12$ and $p = 0.07$, respectively; Table III). In a multivariate analysis, the adjusted p-value of the serum CRP level for the prediction of FN was 0.057. Cumulative incidences of FN calculated by the Kaplan–Meier method were 88% and 59% in patients with serum CRP levels of <0.2 mg/dl or in those with higher CRP levels ($p = 0.05$; Figure 2A). However, no difference in the incidence of FN was observed when the patients in the induction group were grouped according to the serum CRP level using the same cut-off value (Figure 2B).

Discussion

High-sensitivity measurement of the serum CRP level has enabled the detection of slight inflammatory change. We examined the relationship between the serum CRP level measured just before chemotherapy and the incidence of infectious events during neutropenia in patients undergoing first remission induction therapy or first consolidation therapy for AML. The serum CRP level before the first consolidation chemotherapy demonstrated a high predictive value for infectious events. High-risk patients for FN and DI could be discriminated by the serum CRP level, with cut-offs of 0.19 mg/dl and 0.26 mg/dl, respectively. This means that high-risk patients could not be discriminated by

Table III. Predictive factors for febrile neutropenia during consolidation chemotherapy

	Univariate analysis		Multivariate analysis	
	Incidence	p-Value	OR (95% CI)	p-Value
Sex (M/F)	68%/73%	>0.99		
Age (<50 y/≥50 y)	68%/72%	>0.99		
FAB (M3/other than M3)	63%/72%	0.67		
ECOG-PS (0/1)	68%/83%	0.65		
History of recent infection (+/−)	82%/60%	0.17		
Central venous catheter (+/−)	70%/71%	>0.99		
Prophylactic intravenous antibiotics (+/−)	50%/98%	0.12	0.26 (0.05–1.37)	0.11
Serum CRP level (<0.2 mg/dl/≥0.2 mg/dl)	57%/88%	0.07	5.70 (0.95–34.3)	0.057
Duration of neutropenia, <500/μl (<18 days/≥18 days)	63%/78%	0.48		
Duration of neutropenia, <100/μl (<11 days/≥11 days)	67%/75%	0.72		

OR, odds ratio; CI, confidence interval; FAB, French–American–British Cooperative Group criteria for the classification of acute myeloid leukaemia; ECOG-PS, performance status according to the Eastern Cooperative Oncology Group scale; CRP, C-reactive protein.

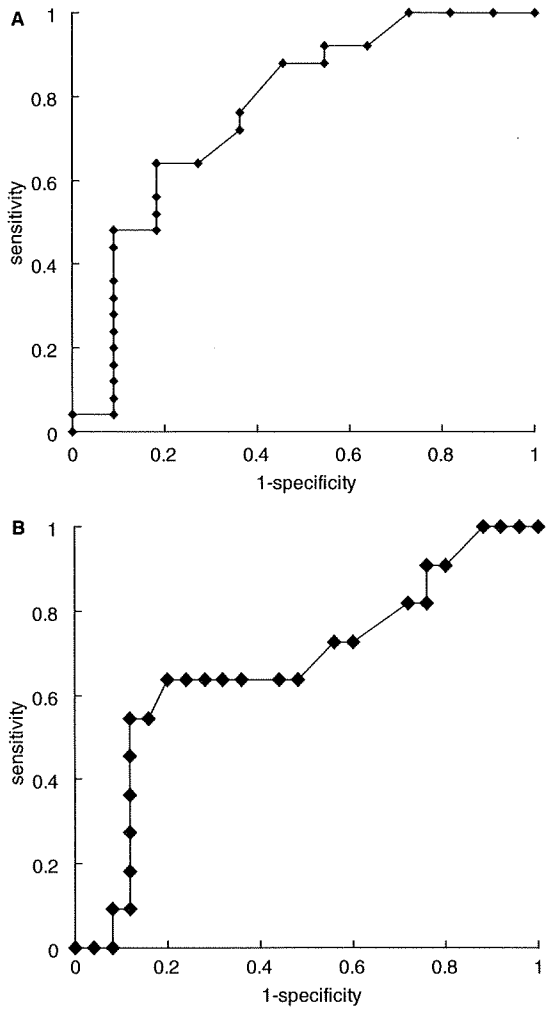

Figure 1. Receiver-operating characteristic (ROC) curve plot based on the serum C-reactive protein level before first consolidation therapy and the incidence of febrile neutropenia (A) and documented infection (B).

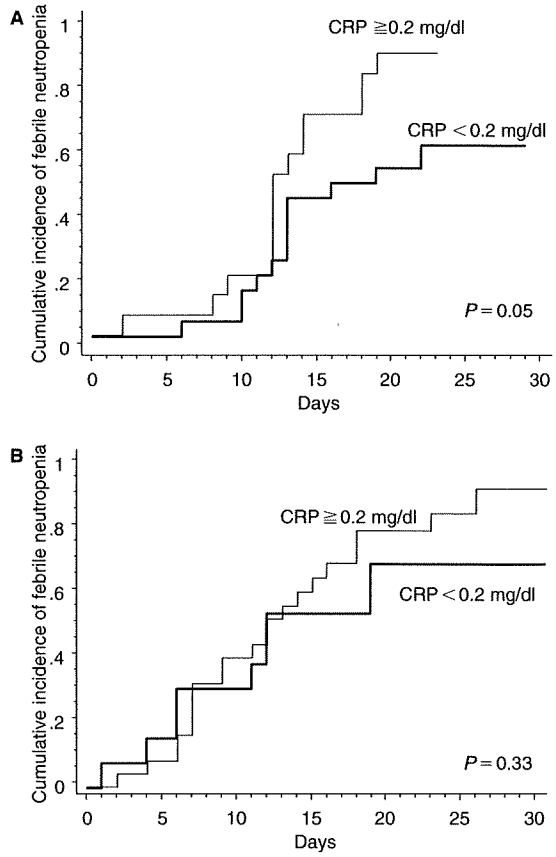

Figure 2. Cumulative incidence of febrile neutropenia grouped according to the serum C-reactive protein (CRP) level before chemotherapy in patients who underwent first consolidation therapy (A) and induction therapy (B).

the conventional measurement of the serum CRP level. The slight increase in the serum CRP level might reflect a minute inflammation by occult infection, leading to the overt infectious events during neutropenia.

On the other hand, serum CRP level before the first induction chemotherapy did not have a significant predictive value for infectious events. Serum CRP levels rise in response not only to inflammation, but also to tumour cells [8,9]. Patients before the first induction chemotherapy had a huge number of tumour cells in their bodies, which might have affected the serum CRP level and made it difficult to detect the slight inflammation. In fact, the median serum CRP level just before the first induction chemotherapy was significantly higher than that just before the first consolidation chemotherapy (median 0.3 mg/dl vs 0.18 mg/dl, $p = 0.031$). This might have reflected the difference in the number of leukaemic cells (average white cell count 22,202/μl vs 4488/μl, $p = 0.014$ and average blast cell count 15,587/μl vs 14/μl, $p = 0.0016$), although the difference in the CRP level was surprisingly small if we consider the huge difference in the number of leukaemic cells. Another possible explanation is the longer neutropenic duration after induction therapy than consolidation therapy, which increases the possibility of infectious events acquired after starting chemotherapy.

There have been many reports regarding other markers for infectious events. Interleukin-6 (IL-6) is a cytokine that is produced by T-cells or macrophages and regulates the humoral immune response. Procalcitonin (PCT) is a precursor of calcitonin, which is usually produced by thyroid c-cells, but is also produced markedly by monocytes or hepatocytes in response to proinflammatory cytokines. Both IL-6 and PCT have been reported to be useful markers of infection [10,11], especially as markers of bacterial infection [10].

Von Lilienfeld-Toal et al. reported that PCT and IL-6 were more reliable markers than CRP for bacteraemia in patients with FN [12], although Hambach et al. did not confirm the superiority of PCT in allogeneic hematopoietic stem cell transplantation recipients [13]. However, whether IL-6 or PCT has predictive value for infectious events has not been evaluated. If IL-6 and PCT are more specific to inflammation than CRP, the serum levels of IL-6 and PCT, or these factors in combination with CRP, would be more suitable to predict infectious events even during the first induction chemotherapy. This hypothesis should be tested in a future study.

Prophylactic antibiotics are widely administered in patients undergoing chemotherapy. A recent meta-analysis of randomized controlled trials revealed that antibiotic prophylaxis for neutropenic patients, especially with fluoroquinolones, reduced mortality [14]. However, such prophylaxis may lead to the emergence of antibiotic-resistant pathogens. Therefore, it is still important to limit the use of prophylactic antibiotics as much as possible. Identifying low-risk patients by serum inflammatory markers might prevent the excessive use of antibiotics.

In conclusion, serum high-sensitivity CRP levels measured just before consolidation chemotherapy for AML show significant correlation with the development of FN and DI during neutropenia. Therefore, it may become possible to discriminate high-risk patients for infectious events during neutropenia by the CRP level. Further studies are required to establish the appropriate prophylactic methods during neutropenia based on risk stratification according to baseline CRP level.

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ORIGINAL ARTICLE

A randomized controlled trial of plasma real-time PCR and antigenemia assay for monitoring CMV infection after unrelated BMT

Y Kanda[1], T Yamashita[2], T Mori[3], T Ito[4], K Tajika[5], S Mori[6], T Sakura[7], M Hara[8], K Mitani[9], M Kurokawa[10], K Akashi[11] and M Harada[11,12]

[1]*Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan;* [2]*Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan;* [3]*Division of Hematology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan;* [4]*Division of Hematology, Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan;* [5]*Division of Hematology, Department of Internal Medicine, Nippon Medical School, Tokyo, Japan;* [6]*Department of Hematology and Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan;* [7]*Division of Hematology, Saiseikai Maebashi Hospital, Maebashi, Japan;* [8]*Division of Hematology, Ehime Prefectural Central Hospital, Matsuyama, Japan;* [9]*Department of Hematology, Dokkyo Medical University School of Medicine, Tochigi, Japan;* [10]*Department of Hematology and Oncology, University of Tokyo, Tokyo, Japan;* [11]*Medicine and Biosystemic Science, Kyushu University School of Medical Science, Fukuoka, Japan and* [12]*NHO Ohmuta National Hospital, Fukuoka, Japan*

Preemptive therapy is the standard strategy for preventing CMV disease after allogeneic hematopoietic SCT. In this study, unrelated BMT recipients were randomly assigned to a plasma real-time PCR group or an antigenemia group to compare the value of these monitoring tools for CMV reactivation. Ganciclovir (GCV) was started at 5 mg/kg/day when PCR reached 300 copies per ml or when antigenemia reached three positive cells per two slides. A total of 88 patients were randomized into the antigenemia group ($n = 45$) or the PCR group ($n = 43$). A significantly higher number of patients reached the threshold in the antigenemia group than in the PCR group (73.3 vs 44.2%, $P = 0.0089$). However, only three patients (one in the antigenemia group and two in the PCR group) developed early CMV disease. These patients exclusively had colitis and were successfully treated with GCV or foscarnet. The median number of antigenemia-positive cells at the start of GCV was 47 in the PCR group. These findings suggest that antigenemia assay with the current cutoff was too sensitive and led to unnecessary use of GCV. However, the appropriateness of the threshold may be different by the methodology used, and therefore, it is difficult to generalize.

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Keywords: CMV; antigenemia; real-time PCR; preemptive therapy

Correspondence: Dr Y Kanda, Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma, Omiya-ku, Saitama-city, Saitama 330-8503, Japan.
E-mail: ycanda-tky@umin.ac.jp
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Introduction

Cytomegalovirus infection is a frequent complication after allogeneic hematopoietic SCT. Universal prophylaxis with ganciclovir (GCV) did not improve the transplantation outcome because of neutropenia caused by GCV.[1,2] Therefore, the initiation of GCV triggered by the detection of CMV reactivation is currently the standard strategy for preventing CMV disease.[3–5] A CMV antigenemia assay has been widely used to monitor CMV reactivation. However, the details of preemptive therapy still need to be clarified, including the threshold number of antigenemia-positive cells for deciding when to start GCV, the dose and duration of GCV and so on. We previously showed that a risk-adapted preemptive therapy, in which the cutoff number of antigenemia-positive cells for deciding when to start GCV was changed according to the risk for CMV disease, was appropriate in allogeneic SCT recipients, but the incidence of neutropenia was still high.[6] Therefore, in the next study, we evaluated the feasibility of preemptive therapy with low-dose GCV, and the findings showed that the initial dose of GCV could be safely decreased to 5 mg/kg.[7]

The PCR used to detect CMV DNA has also been investigated for its ability to monitor CMV reactivation.[8] PCR using whole blood samples might be too sensitive as a trigger for deciding when to start preemptive therapy compared with an antigenemia assay or PCR using plasma samples.[9,10] However, the recent development of real-time PCR has enabled the quantification of CMV DNA. Several studies have shown the feasibility of preemptive therapy guided by real-time PCR monitoring using either whole blood or plasma samples.[11–14] As for whole blood real-time PCR, Gerna *et al.* performed two randomized controlled trials of PCR and antigenemia, one in young patients (0–25 years old) and the other in older patients (20–67 years old).[12,13] They showed that a threshold value of 10 000

copies per ml for determining when to start GCV by whole blood PCR significantly reduced the use of GCV compared with a threshold in which GCV is started at any level of positive antigenemia. However, the study included heterogeneous patients in terms of donor type, stem cell source and GVHD prophylaxis. In particular, antithymocyte globulin was used in approximately half of the patients, and this may have strongly affected the incidence of CMV reactivation and disease.[15,16] In addition, preemptive therapy guided by antigenemia assay could be more appropriately performed by using a cutoff based on the number of positive cells.

Therefore, we performed a randomized controlled trial of plasma real-time PCR with a cutoff of 300 copies per ml and an antigenemia assay with a cutoff of three positive cells per two slides in a homogenous population of unrelated BMT recipients who received GVHD prophylaxis with a calcineurin inhibitor and MTX.

Patients and methods

Patients

Patients were eligible for the study if they were between 20 and 55 years old, would undergo BMT without *in vivo* or *ex vivo* T-cell depletion from an HLA-matched unrelated donor using a myeloablative conditioning regimen and had a good performance status without significant organ dysfunction, as defined in the protocol. Either the donor, the recipient or both must have been seropositive for CMV. Prophylaxis against GVHD was limited to a combination of CYA and MTX, but a combination of tacrolimus and MTX was allowed after June 2002. Patients were enrolled before starting a conditioning regimen, but randomization was performed between day 10 and day 12 after transplantation to exclude patients who developed significant organ dysfunction early after transplantation. This study was approved by the institutional review board of each participating center and a written informed consent was obtained from each patient (UMIN-CTR C000000347).

CMV monitoring methods

Cytomegalovirus antigenemia assay was performed as described previously.[17] In brief, 1.5×10^5 peripheral blood leukocytes were attached to a slide using a cytocentrifuge and fixed with formaldehyde. The cells were sequentially immunostained with MoAb C10/11 (Clonab CMV; Biotest, Dreieich, Germany) and reacted with goat alkaline phosphatase-labeled anti-mouse Ig (Mitsubishi Kagaku Iatron Inc, Tokyo, Japan). Under a light microscopy, CMV-positive cells were counted and the results are presented as the sum of the number of positive cells per two slides.

Real-time PCR was performed using primers and a TaqMan probe for immediate early genes using serum samples.[18] Briefly, DNA extracted from 100 μl of plasma was subjected to PCR using TaqMan Universal PCR Master Mix (PE Biosystems, Foster City, CA, USA) and the PCR product was detected as an increase in the fluorescent intensity using ABI Prism 7700 (PE Biosystems). Real-time fluorescent measurements were taken and a threshold cycle (CT) value for each sample was calculated by determining the point at which the fluorescence exceeded 10 times the baseline fluorescence. A standard curve was constructed using the CT values obtained from serially diluted DNA extracted from a plasmid that contains the respective region of CMV. The CT values from the clinical samples were plotted on the standard curve and the copy number was calculated automatically using Sequence Detection System version 1.6 (PE Biosystems).

Preemptive therapy against CMV disease

Patients were randomly assigned to the antigenemia group or the PCR group using a random block design. Assignment was stratified by the institute, age and the presence or absence of GVHD at the time of randomization. CMV reactivation was monitored weekly by both the antigenemia assay and PCR in all patients, but only the results of the assigned monitoring method were returned to the physicians. Preemptive therapy with GCV was started at an induction dose of 5 mg/kg/day when three or more CMV-positive cells per two slides were detected in the antigenemia group and 300 or more CMV DNA copies per ml were detected in the PCR group. The dose of GCV was increased to 10 mg/kg/day when a rising CMV load was observed. The dose of GCV was decreased to 5 mg/kg/day when a declining CMV load was observed in patients who were receiving GCV at 10 mg/kg/day. A rising and declining CMV load was defined as an increase and decrease in the CMV load by 50% or more of the previous value, respectively. However, changes in antigenemia-positive cells by less than five cells per two slides and changes in the DNA copy number by less than 500 copies per ml were regarded as a stable CMV load. When the CMV load fell below the threshold to start GCV, the dose of GCV was decreased to 5 mg/kg/day, if the patient was receiving GCV at 10 mg/kg/day, and GCV was discontinued if the patient was receiving GCV at 5 mg/kg/day. The dose of GCV was adjusted according to the renal function.[19] CMV monitoring was continued until all of the following three requirements were fulfilled: (i) More than 100 days had passed after transplantation; (ii) More than 2 weeks had passed after the last administration of GCV; and (iii) Absence of the use of (methyl-)prednisolone at 0.5 mg/kg/day or more.[20]

Definition of CMV disease

All patients with symptoms compatible with CMV disease such as interstitial pneumonia, colitis and gastritis underwent extensive pathological and microbiological examination of biopsy specimens. The diagnosis of CMV disease was made by histopathological examination and immunochemical staining of biopsy specimens. However, CMV retinitis was diagnosed when CMV DNA was detected by PCR using aqueous humor samples associated with characteristic retinal changes by ophthalmoscopy. Early and late CMV diseases were defined as those occurring before and after day 100, respectively.

Statistical considerations

The primary end point of the study was the incidence of early CMV disease. We defined success as the absence of CMV disease before day 100. Noninferiority was predefined as a difference in the success rates between the antigenemia group and the PCR group of no more than 10 percentage points. On the basis of the assumption of a success rate of 95% in the PCR group and 90% in the antigenemia group, 39 patients in each treatment group were required to show noninferiority with an alpha error of 5% and a power of 80%, which permitted a 10% difference in the success rate. On the basis of the assumption of a 20% loss of patients between the enrollment and randomization, a total of 96 patients needed to be enrolled in this study. Comparisons for dichotomous and continuous variables between groups were performed with Fisher's exact test and *t*-test, respectively. Pearson's correlation coefficient was calculated to compare the results of the two monitoring methods after logarithmic transformation.

Results

Incidence of CMV reactivation and the use of GCV

A total of 96 patients were enrolled in the study between January 2002 and March 2007. Among these patients, eight patients were excluded because of the use of tacrolimus as GVHD prophylaxis in one, negative CMV Ab in both the donor and recipient in one and organ dysfunction after the conditioning regimen in six. Therefore, a total of 88 patients were randomized into the antigenemia group ($n=45$) or the PCR group ($n=43$) (Figure 1). There were no differences in age, sex, background disease, CMV serostatus, conditioning regimen or GVHD prophylaxis between the two groups (Table 1). In addition, the incidence of grade II–IV acute GVHD was similar (42 vs 47%, $P=0.67$).

Cytomegalovirus reactivation, defined as a detection of CMV at any level, was more frequently observed in the antigenemia group (40 of 45 patients, 88.9%) than in the

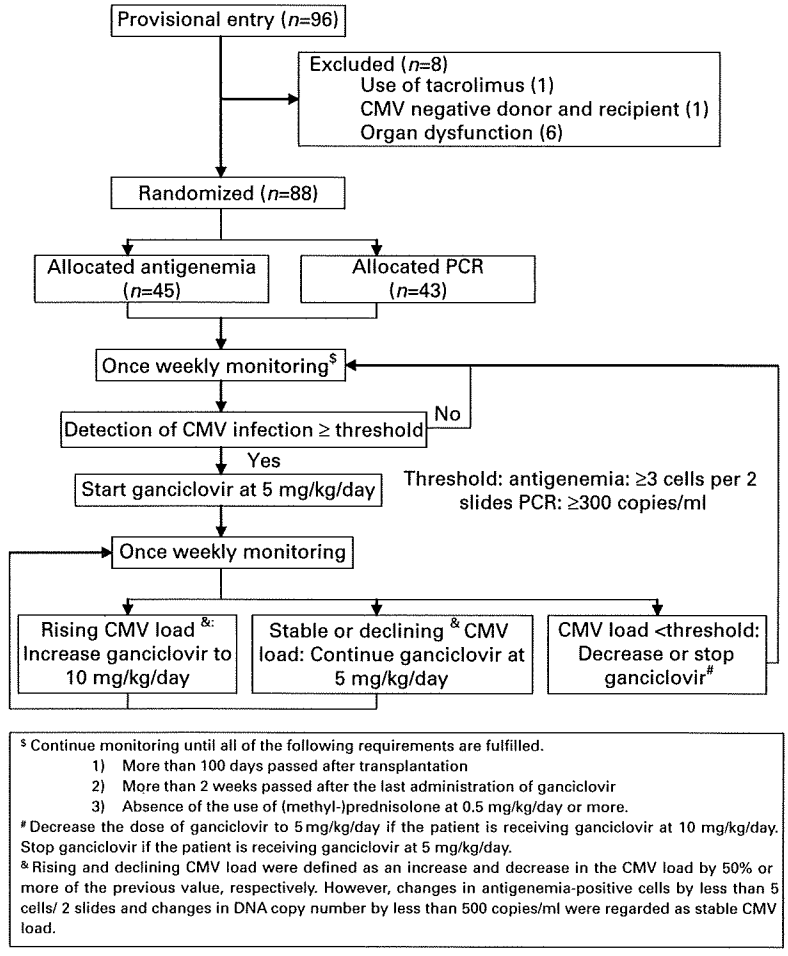

Figure 1 Design of the study.

PCR group (27 of 43 patients, 62.8%) ($P=0.0050$, Table 2). The probability of starting GCV was significantly higher in the antigenemia group than in the PCR group (73.3 vs 44.2%, $P=0.0089$, Figure 2). The results of PCR in the antigenemia group and those of the antigenemia assay in the PCR group were disclosed after the completion of the study. A good correlation was seen between the results of PCR and the antigenemia assay ($P<0.0001$, $r^2=0.38$, Figure 3). Of the 33 patients who received GCV in the antigenemia group, PCR and the antigenemia assay reached the threshold simultaneously in five patients and PCR reached the threshold before starting GCV in only four patients (Figures 4a and 5a). In the other 24 patients, the CMV DNA copy number was persistently below the threshold until GCV was started. On the other hand, in 11 of 19 patients who received GCV in the PCR group, the results of the antigenemia assay reached the threshold earlier in 11 patients and simultaneously in 7 patients (Figures 4b and 5b). The results of the antigenemia assay were persistently below the threshold until GCV was started in only one patient. The median number of antigenemia-positive cells at the start of GCV was 5 (range: 3–102) and 47 (range: 0–2921) in the antigenemia and PCR groups, respectively (Figure 6a, $P=0.0051$). The median CMV DNA copy number was negative (range: 0–4400) and 750 (range: 310–13000) in the antigenemia and PCR groups, respectively (Figure 6b, $P<0.0001$).

Among the 52 patients who received preemptive therapy with GCV at 5 mg/kg/day, only 13 and 7 patients in the antigenemia and PCR groups, respectively, experienced a rising CMV load and required dose-escalation to 10 mg/kg/day, suggesting that the initiation of GCV at 5 mg/kg was appropriate.

Table 1 Patient characteristics

	Antigenemia (n=45)	*PCR (n=43)*	P-*value*
Pre-transplantation factors			
Median age (range)	41 (20–55)	40 (20–53)	0.82
Sex (male/female)	25/20	24/19	>0.99
HLA mismatch	7 (16%)	9 (21%)	0.59
Background disease			
AML	17	18	
ALL	12	12	
CML	6	3	
MDS	5	7	
Others	5	3	0.57
Donor/recipient CMV status			
Pos./Pos.	28	26	
Pos./Neg.	5	4	
Neg./Pos.	8	6	0.74
Conditioning regimen			
TBI	39	36	
Non-TBI	6	7	0.77
GVHD prophylaxis			
CYA–MTX	25	25	
TAC–MTX	16	16	0.59

Abbreviations: MDS = myelodysplastic syndrome; Neg. = negative; Pos. = positive; TAC = tacrolimus.

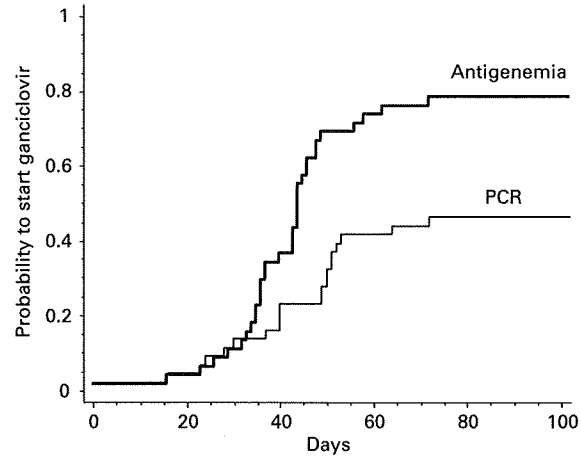

Figure 2 Days to start ganciclovir after transplantation.

Table 2 CMV-related events after engraftment

	Antigenemia (n=45)	*PCR (n=43)*	P-*value*
CMV reactivation[a]	40	27	0.0050
Start ganciclovir	33	19	0.0089
Duration of ganciclovir (days)	23.2 ± 19.4	20.8 ± 14.2	0.64
Total dose of ganciclovir (mg/kg)	140.8 ± 129.7	118.4 ± 91.2	0.51
Dose escalation to level II	13	7	>0.99
Neutropenia <500 per μl	5	3	>0.99
Stop ganciclovir because of neutropenia	1	0	>0.99
Increase in serum creatinine[b]	8	0	0.039
CMV disease			
Early (before day 100)	1	2	0.61
Late (after day 100)	0	1[c]	0.48

[a]Detection of antigenemia or DNA at any level.
[b]Increase in serum creatinine level by 0.5 mg per 100 ml or more from the baseline level.
[c]The patient developed early CMV disease, which was improved by ganciclovir. However, intestinal symptoms recurred after day 100 and CMV colitis was suspected because of positive antigenemia, although it was not confirmed by biopsy.

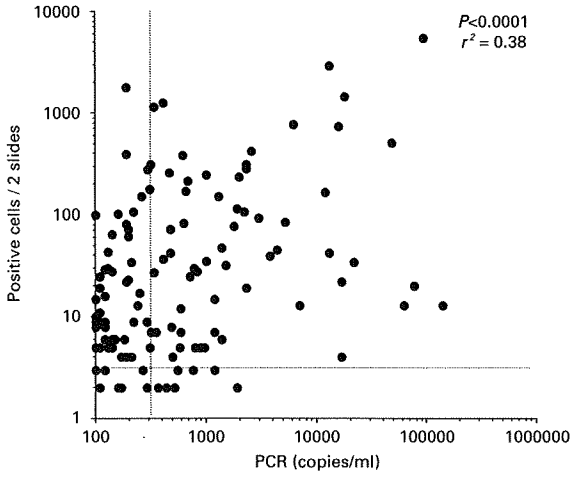

Figure 3 Correlation between the number of positive cells in the antigenemia assay and copy number by PCR.

CMV diseases

Early CMV disease was diagnosed in 1 of the 45 patients (2.2%) in the antigenemia group and 2 of the 43 patients (4.7%) in the PCR group ($P=0.61$). These patients exclusively developed CMV colitis. Another patient in the PCR group showed characteristic retinal changes and was presumptively treated with GCV, although CMV infection was not detected in either the aqueous humor or the peripheral blood. The 95% confidence interval for the difference in the success rate was −10.1 to 5.2%, and thus was just outside the predefined lower limit of −10%. However, as shown in Table 3, the development of CMV disease in the PCR group could not be avoided even if these patients were assigned to the antigenemia group, as either the antigenemia assay and PCR reached the threshold simultaneously (UPN32) or the antigenemia assay did not reach the threshold before the diagnosis of CMV disease (UPN35). All of these patients were successfully treated with GCV or foscarnet, although one patient (UPN35) showed the recurrence of colitis after day 100. None of the other patients developed late CMV disease.

Adverse events during preemptive therapy

The mean duration of preemptive therapy with GCV and the mean total dose of GCV was 23.2 ± 19.4 days and 140.8 ± 129.7 mg/kg in the antigenemia group and 20.8 ± 14.2 days and 118.4 ± 91.2 mg/kg in the PCR group ($P=0.64$ and $P=0.51$), respectively. Neutropenia with a neutrophil count of <500 per μl was observed in 5 of the 33 patients in the antigenemia group and 3 of the 19 patients in the PCR group ($P>0.99$). Only one patient in the antigenemia group required a discontinuation of GCV because of neutropenia. The total dose of GCV was higher in patients who developed neutropenia, but this difference was not statistically significant (163.8 ± 82.5 vs 126.9 ± 121.4, $P=0.42$).

An increase in the serum creatinine level by at least 0.5 mg per 100 ml was observed in 8 of the 33 patients in the antigenemia group and in none of the 19 patients in the PCR group ($P=0.039$). The total dose of GCV was significantly higher in patients who developed renal impairment (255.0 ± 198.0 vs 106.0 ± 45.5, $P=0.0004$).

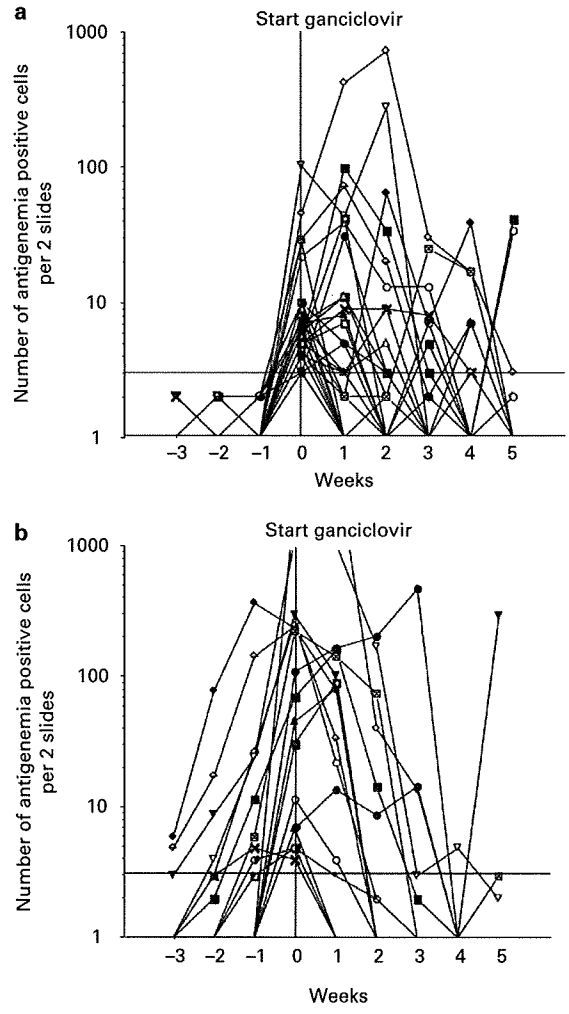

Figure 4 Serial changes in the number of antigenemia-positive cells in patients who received preemptive therapy in the antigenemia group (a) and in the PCR group (b). Week 0 represents the day ganciclovir was started.

Discussion

In this randomized controlled trial, we compared plasma real-time PCR with a cutoff at 300 copies per ml and an antigenemia assay with a cutoff at three positive cells per two slides as a trigger for deciding when to start preemptive therapy with GCV after unrelated BMT. GCV was used significantly less frequently in the PCR group. A comparison of the number of antigenemia-positive cells and the CMV DNA copy number at the start of GCV treatment clearly revealed that plasma PCR was significantly less sensitive than the antigenemia assay, at least with the current cutoff values. Although the 95% confidence

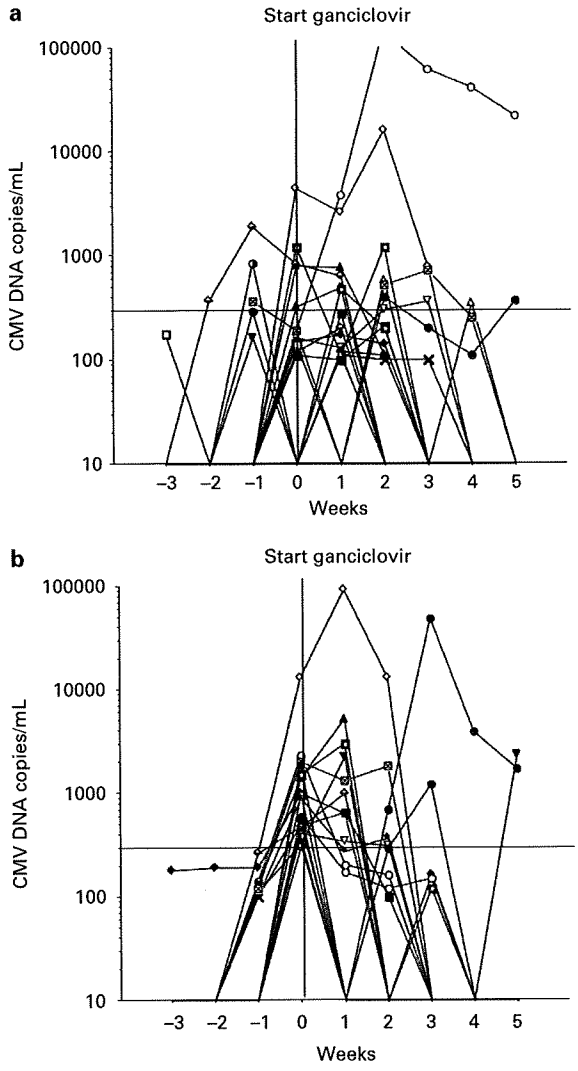

Figure 5 Serial changes in CMV DNA copy number in patients who received preemptive therapy in the antigenemia group (**a**) and in the PCR group (**b**). Week 0 represents the day ganciclovir was started.

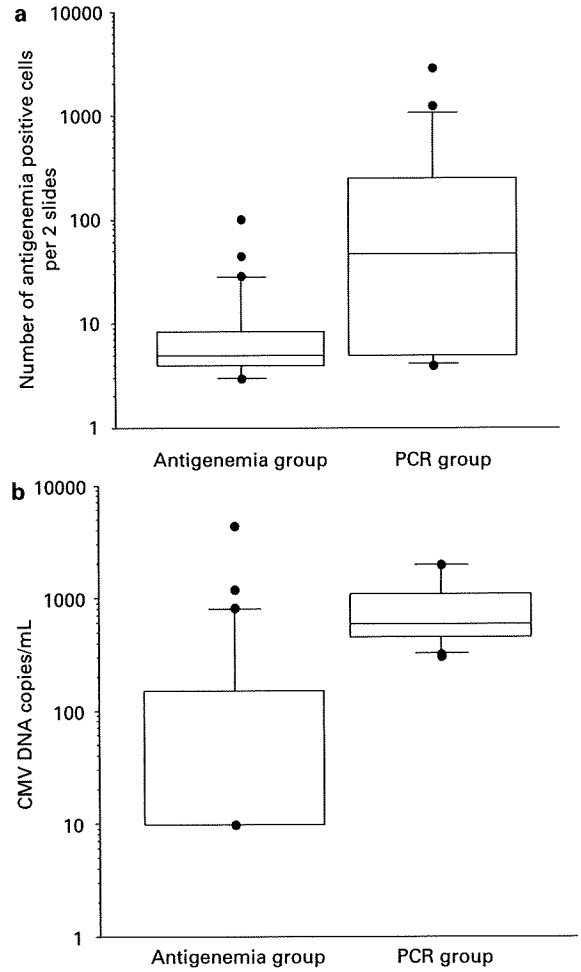

Figure 6 The number of antigenemia-positive cells (**a**) and the CMV DNA copy number at the start of preemptive therapy (**b**), grouped according to the randomization arm. The box-and-whisker plot shows 10, 25, 50, 75 and 90 percentile values. Outliers are indicated by dots.

interval for the difference in the successful prevention rate was just outside the predefined lower limit of −10%, and therefore, we could not show the noninferiority of the PCR group, the incidence of CMV disease was limited to two patients even in the PCR group. In addition, prevention of CMV pneumonia, the main aim of preemptive therapy, was completely achieved in both groups. These findings suggest that an antigenemia assay with a cutoff of three positive cells per two slides was too sensitive and resulted in the unnecessary use of GCV.

The unnecessary use of GCV may be reduced if the cutoff value for the antigenemia assay is increased. The antigenemia assay has already been shown to be not sensitive enough for detecting gastrointestinal involvement by CMV even with a low threshold.[21] In this study, the median number of antigenemia-positive cells at the start of GCV treatment was 47 in the 19 patients who received preemptive therapy in the PCR group. Figure 7 shows the serial changes in the number of antigenemia-positive cells in the patients of the PCR group who developed positive antigenemia that reached the threshold, but who did not receive GCV at that time. In about half of the patients, antigenemia spontaneously became negative without GCV treatment. On the other hand, seven patients developed high-grade antigenemia of over 100 positive cells per two slides. However, GCV was started when the number of positive cells was 260 (median, range: 73–1262 cells) and none of these patients developed CMV disease. Although patients who developed grade II–IV acute GVHD or who received steroid at 0.5 mg/kg or higher experienced high-grade antigenemia more frequently than those who did not

develop grade II–IV acute GVHD and did not receive steroid (Figures 7a and b), the use of GCV was comparable (54.5 vs 40%, $P=0.67$). Thus, although it is difficult to determine the appropriate cutoff value for the antigenemia assay, we thought that it may be worth trying to apply a cutoff value of 20 positive cells per two slides, which we are already safely using in allogeneic hematopoietic SCT from an HLA-matched sibling donor,[20] to transplantation from an unrelated donor.

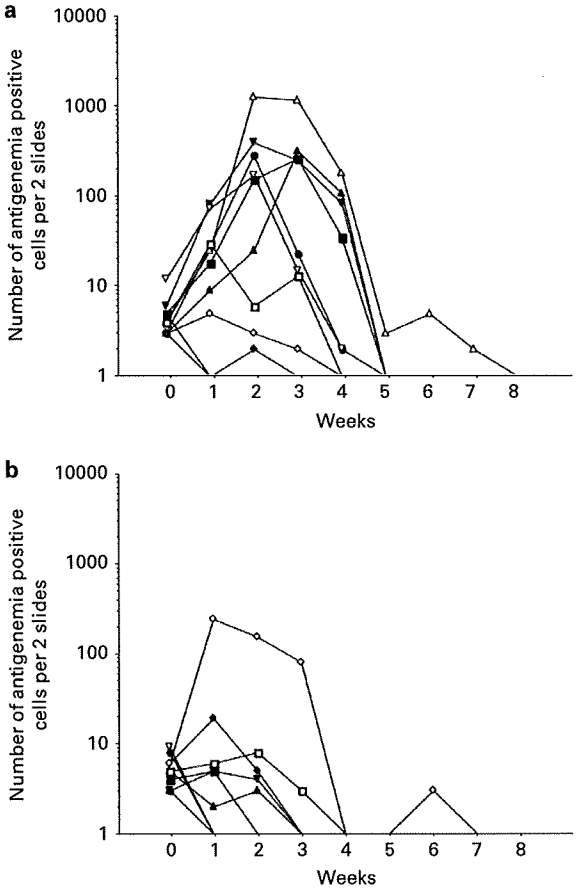

Figure 7 Serial changes in the number of antigenemia-positive cells in the PCR group patients who developed positive antigenemia that reached the threshold, but who did not receive ganciclovir. (a) Patients who developed grade II–IV acute GVHD or who received steroid at 0.5 mg/kg or more. (b) Patients who did not develop grade II–IV acute GVHD and did not receive steroid.

Although Boeckh *et al.*[3] reported a 14% incidence of early CMV disease using the same cutoff as in the current study, the incidences of positive antigenemia at any level and three or more positive cells per two slides were similar to those in this study (79 and 70% in Boeckh's study and 89 and 73% in the current study). Therefore, the higher incidence of early CMV disease probably resulted from the high incidence (35%) of grade III–IV acute GVHD in their study rather than from the difference in the method used for the antigenemia assay, as acute GVHD is one of the strongest risk factors for CMV disease.

Nevertheless, it is important to note that the sensitivity and specificity of these assays vary depending on the methodology used.[9,22–24] In fact, the unexpected differences in the sensitivities of the two assays in this study could be explained by the difference in the methodology used in the antigenemia assay. The cutoffs used for the antigenemia assay and real-time PCR were determined based on our previous study in which HRP-C7 Ab was used in the antigenemia assay.[18] In this study, however, we used C10/C11 Ab in the antigenemia assay, as this Ab has been used worldwide. Although we did not believe that there are clinical differences between these two antigenemia assays,[6,7,20] we should have tested the correlation between the results of plasma PCR and the antigenemia assay using C10/C11 Ab. Fortunately, the unexpected difference in the sensitivity in these assays contributed to the finding that the antigenemia assay with the current cutoff was too sensitive as a trigger for deciding when to start preemptive therapy. These data are valid only when the same methodology is used, and standardization of the methods is warranted.[25,26]

In conclusion, CMV colitis could not be completely prevented by the current preemptive strategy using the peripheral blood samples, but CMV pneumonia was completely prevented in both groups. The initiation of GCV at 5 mg/kg/day was confirmed to be safe, provided the CMV load continues to be monitored. Plasma PCR with a cutoff at 300 copies per ml seemed to be appropriate for monitoring CMV reactivation after transplantation. The cutoff number of positive cells should be raised above that used here when using an antigenemia assay. However, the appropriateness of the threshold of these assays may be different on the basis of the methodology and patient background, such as the risk of GVHD, and therefore, it is difficult to generalize.

Table 3 CMV load in patients who developed CMV disease

Age/sex	*Acute GVHD*	*Onset/affected organ of CMV disease*		*−3 weeks*	*−2 weeks*	*−1 week*	*Onset*
UPN32 38/M (PCR group)	Grade II	Day 56/colitis	PCR	(−)	260	13 000[a]	93 000
			Ag	(−)	(−)	2921	5467
UPN35 36/M (PCR group)	Grade II	Day 46/colitis	PCR	(−)	(−)	(−)	(−)
			Ag	0	0	2	12
UPN70 38/M (Antigenemia group)	Grade II	Day 50/colitis	PCR	(−)	(−)	110	100
			Ag	2	(−)	5[a]	99

[a]Preemptive therapy was started.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Ⅵ. 平成 21 年度研究成果作成資料

【資料 1】　末梢血幹細胞非凍結での移植の経験

厚生労働省科学研究事業
「同種末梢血幹細胞移植を安全且つ効率的に行う場合の基準、指針、社会的合意に関する研究」班
吉村 健一 班長

平成21年度　第2回　研究成果報告（2010/1/31）

末梢血幹細胞非凍結での移植の経験

都立駒込病院　造血細胞移植チーム
坂巻壽、真山英樹、比留間潤

背景・目的

同種造血幹細胞移植の幹細胞源として末梢血幹細胞（PBSC）の有用性が高まりつつあるが、十分なCD34陽性細胞の得られないドナーが少なからず存在することから、数日間にわたり採取、凍結保存して十分な細胞数を確保してから移植されることが多い。しかし、這等な移植細胞数に関しては必ずしも定まっているとは言えない。

当院では原則、採取末梢血は凍結保存せずに移植を行ってきた。採取末梢血を凍結保存せずに移植を行った症例を後方視的に解析したので報告する。

駒込病院における非凍結末梢血CD34+細胞を用いた同種移植

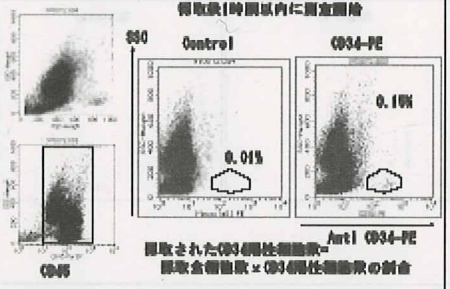
対象および方法

◆ 1998年8月～2009年12月、同種PBSCT（RISTを除く）72例。
患者年齢：中央値37才（17～60才）
患者性別：男/女=47/25
AML　31例　　MDS　5
ALL　15　　MM　4
NHL　8　　SAA　2
CML　7

◆ ドナーはHLA一致の血縁者で、G-CSF 10μg/kg/dayを皮下注し投与4～5日後にCobe Spectraで1～2日間PBSCを採取した。
処理量は150～200 ml/kg（ドナー体重）。
CD34+細胞数は2x10⁶/kgを目標、1x10⁶/kgを最少限界とすることを目安に採取した。

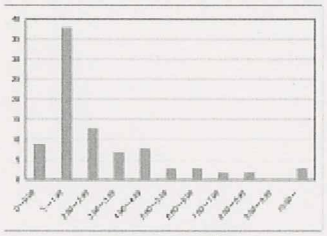
末梢血幹細胞中のCD34陽性細胞測定法

採取後1時間以内に測定開始



採取されたCD34陽性細胞数=
採取全細胞数×CD34陽性細胞数の割合

採取されたCD34陽性細胞数の度数分布

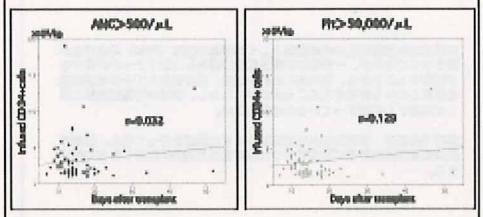


CD34+cells ×10⁶/Kg

初日採取細胞数の少なかったケースでの最終輸注細胞数

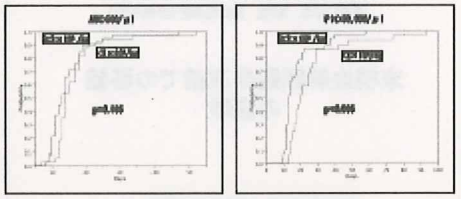
1日目採取CD34細胞数 (CD34⁺/kg)	2日目採取CD34細胞数 (CD34⁺/kg)	移植CD34陽性細胞数 (CD34⁺/kg)
0.28	0.81	1.09
0.29	1.41	1.8
0.58	0.84	1.42
0.65	1.03	1.68
0.67	0.32	0.99
0.75	0.38	1.13
0.93		0.93
0.95	1.21	2.16
0.98	0.85	1.83

輸注CD34+細胞と造血回復

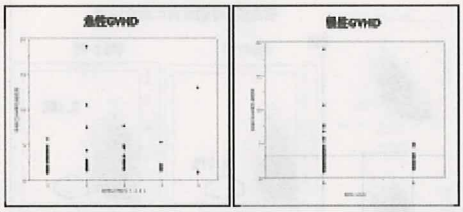


輸注CD34+細胞と造血回復

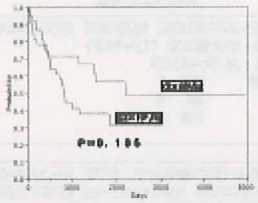
CD34+<2.0x10⁶とCD34+≧2.0x10⁶の比較



輸注CD34+細胞とGVHD



輸注CD34+細胞と生存率



まとめ

- 72回の移植の内、ドナーから2日にわたり採取したのは14例であった。
- 輸注されたCD34+細胞数は中央値2.16 X10⁶/Kg (0.63～18.92 X10⁶/Kg)
- 全例で生着を認めた。
- CD34+細胞>2.0 X10⁶/Kg輸注された症例では血小板の回復が有意に促進されたが、好中球数の回復には有意差を認めなかった。
- 急性・慢性GVHDとも輸注されたCD34+細胞数との相関は認めなかった

【資料 2】　非血縁者間末梢血幹細胞移植導入に向けての
施設調査アンケート用紙

非血縁者間末梢血幹細胞移植導入に向けての
施設調査アンケート用紙
（事前調査）

平成 21 年 3 月

厚生労働科学研究　免疫アレルギー疾患等予防・治療研究事業

『同種末梢血幹細胞移植を非血縁者間で行う場合等の
医学、医療、社会的基盤に関する研究』
宮 村 班

はじめに：

末梢血幹細胞移植は、ほとんどの諸外国ですでに行われており、ドナーと患者双方に恩恵をもたらす非血縁者間末梢血幹細胞移植を本邦において速やかに実施することを目的として、その医学、医療、社会的基盤を作ることを目的に本研究班が設置されました。この一年間、ドナー基準、コーディネートマニュアル、施設基準、採取マニュアル、臨床研究としての在り方について、議論を行い班としての原案を作って参りました。今回のアンケートは、非血縁者間末梢血幹細胞移植を導入するとなった場合にあたり、ボランティアドナーからの末梢血幹採取を実施していただける施設を把握するとともに、各種基準、マニュアルについて、方向性を決める上で重要なことについての、ご意見をいただきたいと思います。

導入によって期待される効果：

非血縁者間末梢血幹細胞移植が実用化されることにより、多くの患者に治癒する機会を与えることが期待されます。具体的には

1）末梢血幹細胞は骨髄移植と比較して造血幹細胞が多く移植できるため生着不全が少なく早期死亡率を減らすことができる。
2）骨髄非破壊的前処置において必要であり、高体重の患者においても移植を受ける機会を与える。
3）強い抗白血病効果が期待され、移植後の再発が減る。
4）ドナーの選択枝が増えることで、善意の気持ちを発揮しやすい環境を整え、登録数を増やし、途中の辞退を減らすことができる。
5）移植後の血球回復が早いため経済的である。
6）骨髄採取施設の不足による移植の遅れの問題が解決する。
7）手術室の確保、自己血保存がないため、速やかに移植が行える。我々の試算では最短 1 週間で採取が可能であり、放射線被爆事故等に際しての危機管理的側面からも、本邦において非血縁者間末梢血幹細胞移植法の確立は急務である。

などの利点・理由があります。
つきましては、少しでも多くの先生方にご理解賜り、ご協力を頂きたく存じます。

データ利用について：

今回のアンケート結果で集計したデータにつきましては、本研究事業以外で使用することはございません。

『同種末梢血幹細胞移植を非血縁者間で行う場合等の
医学、医療、社会的基盤に関する研究』
宮 村 班

○施設情報

施設名　：＿＿＿＿＿＿＿＿＿＿＿＿

診療科名　：＿＿＿＿＿＿＿＿＿＿科

記載医師名　：＿＿＿＿＿＿＿＿＿＿先生

(1)非血縁者間末梢血幹細胞採取について

これまで通り Give and Take の原則で、移植病院においては概ね施行した非血縁移植と同数の骨髄又は末梢血幹細胞の採取を行うことを原則とします。採取は可能であるが、自施設で CD34 を測定できない場合も a 可能か b 体制が整えば可能にチェックをしてください。

問1.　貴施設は DLI 採取施設ですか。

a. □ はい

b. □ いいえ

問2.　貴施設は日本輸血細胞治療学会認定施設ですか。

a. □ はい

b. □ いいえ

問3. 非血縁者間末梢血幹細胞採取の対応は可能でしょうか。

a.□ 可能　:(月　　件)

b.□ 体制が整えば可能　:(理由)＿＿＿＿＿＿＿＿

c.□ 将来にわたり不可能　:(理由)＿＿＿＿＿＿＿＿

d.□ その他　:(詳細)＿＿＿＿＿＿＿＿

問4. 貴施設で CD34 の測定はできますか。

a.□ 可能

b.□ 体制が整えば可能　:(理由)＿＿＿＿＿＿＿＿

c.□ 将来にわたり不可能　:(理由)＿＿＿＿＿＿＿＿

d.□ その他　:(詳細)＿＿＿＿＿＿＿＿

問5. 問4.で a.以外にお答えになった先生にお尋ねします。

貴施設で測定不可能な場合には、外注などで当日夕方までに結果を知ることが可能ですか。

a.□ 可能

b.□ 体制が整えば可能　:(理由)＿＿＿＿＿＿＿＿

c.□ 将来にわたり不可能　:(理由)＿＿＿＿＿＿＿＿

d.□ その他　:(詳細)＿＿＿＿＿＿＿＿

※問3.で a,b とお答えになられた先生は、次頁採取方法について、お答えください。

(2)採取方法について

【G-CSF 投与について】
G-CSF は day1-day3 を外来で行うことを原則とする予定です。

問6. 外来での G-CSF の投与を考えておりますが、対応は可能でしょうか。(day1-3 の投与時間は規定していません。問7で時間を尋ねます)

a. □ 可能
b. □ 不可能
c. □ その他 :(詳細)_____

問7. 可能な場合 G-CSF の投与は週末も可能でしょうか。a, b のどちらかをチェックしてください。

a. □ 週末も可能である。
b. □ 週末はできない。

また平日を含め可能な時間帯を複数でチェックしてください。(仕事を休まずに来れるかに関しての質問です。)

c. □ 午前 (時～ 時)
d. □ 午後 (時～ 時)
e. □ 夕方以降(17時より 時まで可能
f. □ その他 :(詳細)_____

問8. 問6で a とお答えになった先生にご質問いたします。夜間や休日などで G-CSF の投与による副作用が出現した場合の対応は可能でしょうか。

a. □ 外来時間内での対応なら可能
b. □ 週末・夜間共担当医師が対応可能
c. □ 週末・夜間共当直医師が対応可能
d. □ その他 :(詳細)_____

問9. 非血縁者間末梢血幹細胞採取の G-CSF 投与を入院させずに実施する方法について、先生のご意見はいかがでしょうか。

(3)末梢血幹細胞採取について

○末梢血幹細胞採取は、一日で終わる可能性の高い day5 での採取を考えていますが、日本で広く行われている day4 の方がよいという意見もあります。

問10.

a. □ Day 5 でよい。 :(理由)_____
b. □ Day4がよい。 :(理由)_____
c. □ Day4 または day5 どちらでもよいとする。
:(理由)_____
d. □ その他 :(詳細)_____

(4)入院について

採取前日または当日に入院とし、一日目採取後は入院いただき、翌日採取がなければ退院、あれば早朝に G-CSF を打ち昼過ぎには終了、問題なければ数時間後に退院とすることを計画しています。

問11.

a.□ これでよい :(理由)_____
b.□ その他 :(詳細)_____
c.□ これでは問題がある :具体的に