

Viral infections

Monitoring of cytomegalovirus reactivation after allogeneic stem cell transplantation: comparison of an antigenemia assay and quantitative real-time polymerase chain reaction

K Yakushiji¹, H Gondo², K Kamezaki², K Shigematsu³, S Hayashi³, M Kuroiwa⁴, S Taniguchi⁴, Y Ohno⁵, K Takase², A Numata², K Aoki², K Kato², K Nagafuji², K Shimoda², T Okamura^{1,2}, N Kinukawa⁶, N Kasuga⁷, M Sata¹ and M Harada²

¹Second Department of Internal Medicine, Kurume University School of Medicine, Kurume, Japan; ²Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; ³Department of Hematology, Harasanshin General Hospital, Fukuoka, Japan; ⁴Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; ⁵Department of Internal Medicine, Kitakyushu Municipal Hospital, Kitakyushu, Japan; ⁶Department of Medical Information Science, Kyushu University Hospital, Fukuoka, Japan; and ⁷Otsuka Assay Laboratories, Otsuka Pharmaceutical Co., Tokyo, Japan

Summary:

Cytomegalovirus (CMV) antigenemia and quantitative real-time polymerase chain reaction (PCR) were compared for monitoring of CMV reactivation after allogeneic stem cell transplantation. The number of CMV antigen-positive cells by the antigenemia assay and the level of CMV DNA by real-time PCR correlated well. The sensitivity and specificity of the antigenemia assay was 55.4% and 95.5%, respectively, using real-time PCR as the reference standard. The probability of positive antigenemia at day 100 was 76.5%, with a median of first detection at day 37 in 51 patients, compared with a positive PCR of 84.3% and day 33, respectively. When HLA-identical sibling donor transplant recipients and other donor transplant recipients were analyzed separately, there was no difference between the two tests. However, temporal patterns of first detection of CMV antigen-positive cells and CMV DNA differed between HLA-identical and alternative recipients; patients without CMV (29%) or with sporadic positive PCR results (14%) were more common in HLA-identical sibling transplants, whereas patients with simultaneous antigenemia and positive PCR occurred more in alternative transplants (48%). Two of 51 patients (4%) developed CMV colitis despite antigenemia-guided prophylaxis, but both were successfully treated with ganciclovir. Although PCR is more sensitive than antigenemia, both tests are useful in the early detection of CMV after allogeneic stem cell transplantation.

Bone Marrow Transplantation (2002) 29, 599–606. DOI: 10.1038/sj/bmt/1703513

Keywords: cytomegalovirus; stem cell transplantation; antigenemia; quantitative real time PCR

Viral infection due to immunodeficiency is a major concern in patients receiving allogeneic stem cell transplantation. Cytomegalovirus (CMV) disease is frequently observed after allogeneic stem cell transplantation.¹ CMV pneumonia, in particular, is a significant cause of morbidity and mortality after transplantation. Currently, prophylactic therapy with ganciclovir has been used to prevent active CMV disease following allogeneic stem cell transplantation.¹ Prophylactic ganciclovir is begun at engraftment and has resulted in effective prevention of CMV disease in the 100 days after transplantation, but has also been associated with invasive fungal infection and late CMV disease.² Pre-emptive therapy, on the other hand, calls for ganciclovir to be given only to those patients who are at high risk for CMV disease based on the detection of CMV. Therefore, a diagnostic test that accurately reflects active CMV infection is essential for the success of pre-emptive therapy. An antigenemia assay has been widely used for rapid diagnosis and monitoring of CMV after allogeneic stem cell transplantation.^{3–8} In antigenemia-guided treatment, however, a higher incidence of CMV disease has been demonstrated, compared to the prophylactic ganciclovir regimen.² Qualitative detection of CMV DNA by polymerase chain reaction (PCR) has been reported to be as sensitive as the antigenemia assay.^{9–13} Quantification of CMV DNA has recently been introduced and may be useful for monitoring CMV disease and for assessing the efficacy of antiviral therapy.^{14–18} In the present study, CMV antigenemia and quantitative real-time PCR were compared for monitoring CMV reactivation after allogeneic stem cell transplantation.

Materials and methods

Patients

Between May 1999 and May 2001, 51 consecutive patients who received myeloablative therapy and allogeneic stem cell transplantation for hematologic malignancy or aplastic anemia were enrolled. Characteristics of these patients are shown in Table 1. There were 29 men and 22 women with a median age of 44 years and a range of 17 to 59 years (Table 1). Seventeen patients had acute nonlymphoblastic leukemia (ANLL), four acute lymphoblastic leukemia (ALL), three acute mixed lineage leukemia, eight chronic myelogenous leukemia (CML), nine myelodysplastic syndrome (MDS), two adult T cell leukemia, seven non-Hodgkin's lymphoma, and one severe aplastic anemia. One patient with acute mixed lineage leukemia who received a second transplant from the same donor after relapse from the first transplant was included. The serostatus for CMV of donors and recipients before transplantation were as fol-

lows; donor positive/recipient positive results were observed in 42, donor negative/recipient positive in six, and donor positive/recipient negative in three. Twenty-eight patients received transplants from HLA-identical sibling donors and 23 patients from donors other than HLA-identical siblings. Donors other than HLA-identical siblings were defined as alternative donors, including 10 HLA-identical unrelated, four HLA-identical non-sibling relatives, three one-locus antigen mismatched relative, four two-loci antigen mismatched relative, and two haploidentical related donors. In six patients who received transplants from two-loci antigen mismatched or haploidentical related donors, CD34-positive cell selection from grafts was performed before transplantation. All patients achieved sustained engraftment.

Stem cell transplantation

Regimens for pretransplant conditioning and prophylaxis for graft-versus-host disease (GVHD) are shown in Table 1.

Table 1 Patient characteristics

	Stem cell donor		
	HLA-identical sibling donor	Alternative donor ^a	Total
No. of patients	28	23	51
Male/Female	15/13	14/9	29/22
Median age (range), years	47 (19–59)	29 (17–56)	44 (17–59)
Underlying disease			
Acute nonlymphoblastic leukemia	8	9	17
Acute lymphoblastic leukemia	2	2	4
Acute mixed lineage leukemia	3	0	3
Chronic myelogenous leukemia	4	4	8
Myelodysplastic syndrome	5	4	9
Adult T cell leukemia	2	0	2
Non-Hodgkin's lymphoma	3	4	7
Severe aplastic anemia	1	0	1
Pretransplant conditioning ^b			
Busulfan-based	15	7	22
Total body irradiation-based	13	16	29
Stem cell source			
Bone marrow	14	13	27
Peripheral blood	14	10	24
GVHD prophylaxis			
Cyclosporin/methotrexate	28	13	41
Tacrolimus/methotrexate	0	10	10
Grades of acute GVHD			
0	12	2	14
I	4	6	10
II	8	10	18
III, IV	4	5	9
CMV serostatus			
Donor(+) / Recipient(+)	23	19	42
Donor(-) / Recipient(+)	3	3	6
Donor(+) / Recipient(-)	2	1	3

^aAlternative donors include 10 HLA-identical unrelated, 4 HLA-identical related, 3 one-locus antigen mismatched related, 4 two-loci antigen mismatched related, and 2 haploidentical related donors.

^bPretransplant conditioning in HLA-identical sibling donor transplant recipients: Busulfan (BU)-based regimen; BU (16 mg/kg)/CY (120 mg/kg) 12, BU (16 mg/kg)/CY (120 mg/kg)/AraC (8 g/m²) 1, and BU (16 mg/kg)/VP (60 mg/kg) 2. TBI-based regimen; TBI (12–13.2 Gy)/CY (120 mg/kg) 4, TBI (12–13.2 Gy)/CY (120 mg/kg)/AraC (8 g/m²) 5, TBI (12–13.2 Gy)/CY (120 mg/kg)/VP16 (40 mg/kg) 2, and TBI (12–13.2 Gy)/CY (120 mg/kg)/thiotepa (800 mg/m²) 2. Pretransplant conditioning in alternative donor transplant recipients: BU-based regimen; BU (16 mg/kg)/CY (120 mg/kg) 4, BU (16 mg/kg)/thiotepa (800 mg/m²)/L-PAM (100 mg/m²) 1, BU (16 mg/kg)/AraC (700 mg/m²)/L-PAM (140 mg/m²) 1, and BU(16 mg/kg)/TBI (6 Gy)/L-PAM (140 mg/m²) 1. TBI-based regimen; TBI (12–13 Gy)/CY (120 mg/kg) 3, TBI (12–13.2 Gy)/CY (120 mg/kg)/AraC (8 g/m²) 7, TBI (12–13.2 Gy)/CY (120 mg/kg)/VP16 (40 mg/kg) 2, and TBI (12–13.2 Gy)/CY (120 mg/kg)/thiotepa (800 mg/m²) 4.

Total body irradiation (TBI)-based regimens and busulfan (BU)-based regimens were used in 29 and 22 patients, respectively. Details of the conditioning regimens are described in the footnote to Table 1. To prevent GVHD, cyclosporine (CSP) 3 mg/kg/day was given as a continuous intravenous infusion in 41 patients combined with methotrexate 10 mg/m² on day 1 and 7 mg/m² on days 3 and 6, vs tacrolimus 0.03 mg/kg/day by continuous intravenous infusion, combined with methotrexate as above in 10 patients. The diagnosis and grading of acute GVHD was based on the clinical criteria of Thomas *et al*¹⁹ with histologic confirmation as required.¹⁹ Each patient was isolated in a laminar air-flow room, and standard decontamination procedures were followed.²⁰ Prophylaxis for bacterial, fungal, and *Pneumocystis carinii* infection consisted of fluconazole, ciprofloxacin, and sulfamethoxazole/trimethoprim. All patients were given aciclovir 1000 mg/day orally from days -7 to 35 for the prevention of herpes simplex virus infection and intravenous immunoglobulin (10 g) at biweekly intervals for the first 3 months after transplantation. All blood products were irradiated and filtered.

CMV antigenemia assay

This assay was performed at least once a week after engraftment according to the method described previously.^{3,4,6,7} In brief, peripheral blood leukocytes were isolated by dextran sedimentation, and 1.5×10^5 leukocytes were cytocentrifuged on glass microscope slides. The slides were air-dried and fixed with acetone, then stained with peroxidase-conjugated monoclonal antibody, HRP-C7, which specifically binds the pp65 antigen of CMV. Under light microscopy, CMV antigen-positive cells were enumerated. The degree of antigenemia was expressed as the number of CMV antigen-positive cells per 5×10^4 leukocytes.

Extraction and amplification of viral DNA

This was performed according to the method described by Machida *et al*.¹⁸ Viral DNA was extracted from plasma using a QIAamp Blood mini-kit (Qiagen, Valencia, CA, USA) and then subjected to PCR. PCR primers and the probe were selected from the US17 region of CMV AD169. The TaqMan probe was labeled with 6-carboxyfluorescein at the 5' end as the reporter dye and 6-carboxytetramethylrhodamine at the 3' end as the quencher. The PCR product was detected as an increase in fluorescence using an ABI PRISM7700 (PE Biosystems, Foster City, CA, USA). The PCR was performed using TaqMan Universal PCR master mix (PE Biosystems).

Diagnosis of CMV reactivation and disease

CMV reactivation was defined as ≥ 1 antigen-positive cell or $\geq 2 \times 10^2$ CMV DNA copies/ml. For the diagnosis of CMV disease, such as pneumonia, colitis, or hepatitis, the above had to be accompanied by clinical symptoms, signs, and histologic confirmation.^{1,21}

Pre-emptive therapy with ganciclovir for prevention of CMV disease

The decision to use pre-emptive therapy was based entirely on a positive antigenemia test ($\geq 3/50\,000$ antigen-positive cells), independent of the quantity of CMV DNA detected. Intravenous infusion of ganciclovir at 10 mg/kg/day was started when more than $3/50\,000$ antigen-positive cells were detected. Ganciclovir was continued for as long as antigenemia persisted. Granulocyte colony-stimulating factor (G-CSF) was administered when the absolute neutrophil count was $< 500/\mu\text{l}$.

Statistical analysis

The number of antigen-positive cells and the level of CMV DNA were compared using Spearman's rank correlation test. Days to the first positive antigenemia and first positive PCR were calculated by the Kaplan-Meier method, and differences in the two groups were analyzed by the log-rank test. Temporal patterns of first detection of CMV antigen-positive cells and CMV DNA were compared by Fisher's exact test. A *P* value of < 0.05 was accepted as statistically significant.

Results

Relationship between the number of CMV antigen-positive cells and the level of CMV DNA

CMV antigen-positive cells and CMV DNA levels were assessed on the same samples obtained from engraftment to day 100 after transplantation. As shown in Figure 1, the number of antigen-positive cells and the level of CMV DNA correlated significantly ($r = 0.7195$, $P < 0.0001$ by Spearman's rank correlation test). One hundred and twelve of 202 samples (55.4%) with more than 2×10^2 CMV DNA copies/ml were also positive by the antigenemia assay, whereas 300 of 314 samples (95.5%) with less than 2×10^2 CMV DNA copies/ml were negative by the antigenemia assay (Table 2), yielding the sensitivity and specificity of the antigenemia, respectively, when the CMV DNA level of 2×10^2 copies/ml was used as the reference standard (Table 2). The positive predictive value of the

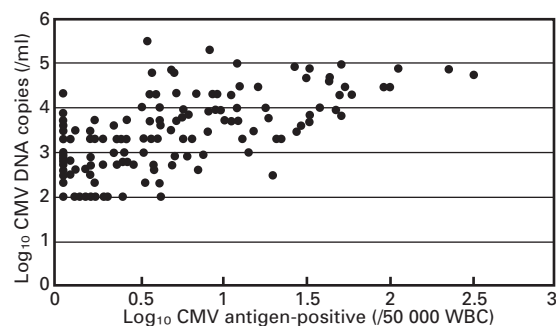


Figure 1 Relationship between the number of CMV antigen-positive cells and the level of CMV DNA. There was a statistically significant correlation between the two tests (Spearman's correlation coefficient 0.7195, $P < 0.0001$).

Table 2 Sensitivity and specificity of the antigenemia assay using quantitative real-time PCR as the reference

	No. of samples									
	$<2 \times 10^2 \geq 2 \times 10^2$		$<5 \times 10^2 \geq 5 \times 10^2$		$<1 \times 10^3 \geq 1 \times 10^3$		$<5 \times 10^3 \geq 5 \times 10^3$		$<1 \times 10^4 \geq 1 \times 10^4$	
Antigenemia										
Positive	14	112	26	100	39	87	72	54	94	32
Negative	300	90	339	51	357	33	374	16	388	2
Sensitivity (%)	55.4		66.2		72.5		77.1		94.1	
Specificity (%)	95.5		92.9		90.2		83.9		80.5	

PCR = polymerase chain reaction; CMV = cytomegalovirus.

antigenemia assay was 88.9% and the negative predictive value was 76.9%. The sensitivity of the antigenemia assay was increased from 55.4% to 94.1% and its specificity decreased from 95.5% to 80.5%, when the CMV DNA level was increased to 1×10^4 copies/ml as the reference (Table 2). The receiver operating characteristic (ROC) curve corresponding to Table 2 is demonstrated in Figure 2.

Probability of positive antigenemia and positive PCR after transplantation

Thirty-eight of 51 patients developed positive antigenemia at a median of day 37 (range 11 to 79 days), and 42 of 51 patients developed a positive PCR with a median at day 33 (range 11 to 72 days) after transplantation. Kaplan–Meier estimates of positive antigenemia and positive PCR by day 100 were 76.5% and 84.3%, respectively (Figure 3a). The difference between these two tests was not statistically significant. The probability of positive antigenemia and positive PCR was separately analyzed in patients with HLA-identical sibling transplants ($n = 28$, antigenemia 57.1% vs PCR 71.4%, not significant) (Figure 3b) and in those with alternative donor transplants ($n = 23$, antigenemia 100% vs PCR 100%, not significant) (Figure 3c).

Compared to HLA-identical sibling transplant patients,

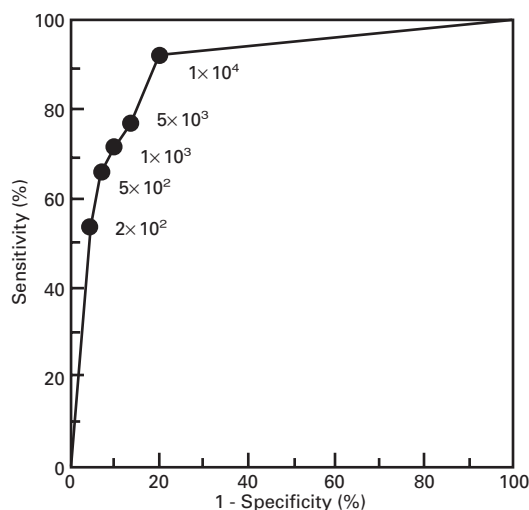


Figure 2 Receiver operating characteristic (ROC) curve corresponding to Table 2. Numbers in the figure indicate threshold levels of CMV DNA (copies/ml) as the reference.

alternative donor transplant patients developed reactivation of CMV infection more frequently. This was demonstrated by both the antigenemia assay (HLA-identical sibling patients 57.1% vs alternative patients 100%, $P < 0.01$) (Figure 3d) and the quantitative PCR (HLA-identical sibling patients 71.4% vs alternative patients 100%, $P < 0.05$) (Figure 3e).

When the incidence of CMV reactivation was analyzed according to the severity of acute GVHD, patients with grade II–IV acute GVHD developed more CMV reactivation than those with grade 0–I acute GVHD. The incidence of positive antigenemia was 88.9% in 27 patients with grade II–IV acute GVHD and 62.5% in 24 patients with grade 0–I acute GVHD ($P < 0.01$). Similarly, the incidence of positive PCR was 92.6% in 27 patients with grade II–IV acute GVHD and 75.0% in 24 patients with grade 0–I acute GVHD ($P < 0.01$). There were no differences between positive antigenemia and positive PCR when patients with grade 0–I and grade II–IV acute GVHD were analyzed separately (data not shown).

Longitudinal analysis for first detection of CMV antigen-positive cells and CMV DNA after transplantation

Temporal patterns of first CMV detection were longitudinally analyzed and classified into six groups (Figure 4); (1) neither antigenemia nor PCR was positive during monitoring, (2) antigenemia alone was positive, (3) PCR alone was positive, (4) positive antigenemia preceded positive PCR, (5) positive PCR preceded positive antigenemia, and (6) antigenemia and PCR became positive simultaneously. Among HLA-identical sibling donor transplants, neither CMV antigen-positive cells nor CMV DNA were detected in eight of 28 patients (29%) during monitoring. PCR alone became positive in four of 28 patients (14%). These 12 patients did not receive pre-emptive ganciclovir therapy. Antigenemia became positive prior to PCR in 2 of 28 patients (7%), whereas positive PCR preceded positive antigenemia by 7 days (range, 3 to 14 days) in 11 of 28 patients (39%). Antigenemia and PCR were positive at the same time in three of 28 patients (11%).

In alternative donor transplants, both tests became positive in all patients during monitoring; all were given pre-emptive ganciclovir therapy. Antigenemia became positive prior to PCR in three of 23 patients (13%). Positive PCR preceded positive antigenemia by 7 days (range, 5 to 21 days) in nine of 23 patients (39%), similar to HLA-identical

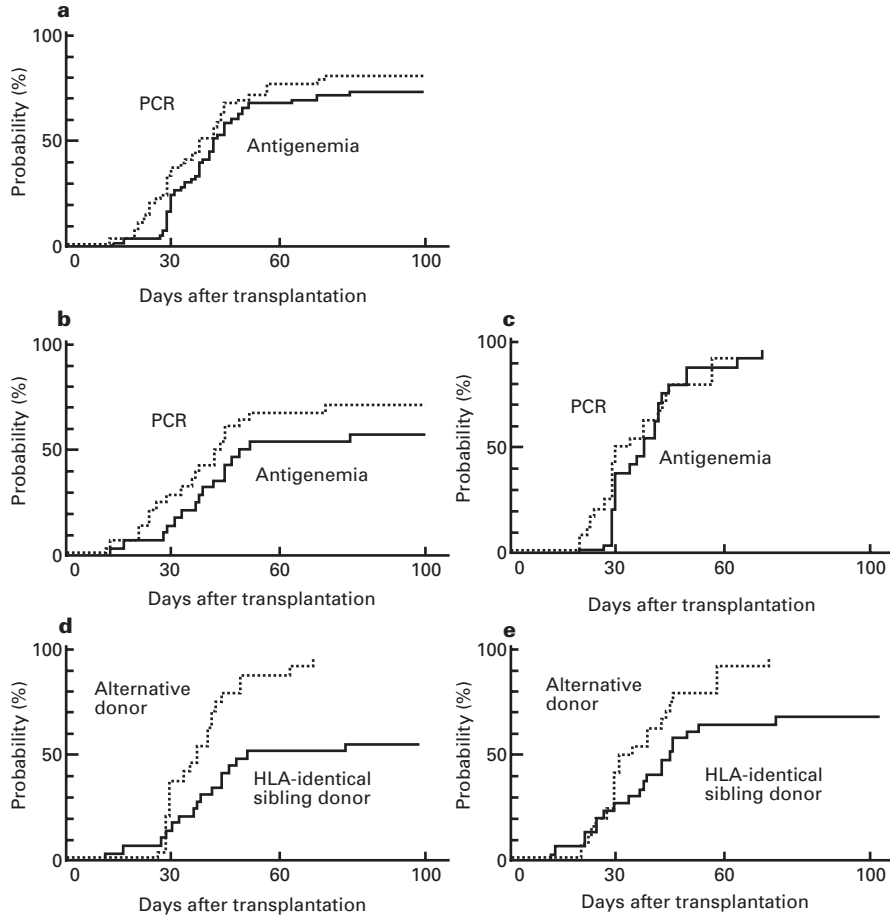


Figure 3 (a) Kaplan–Meier plot of the probability of developing positive antigenemia (—, $n = 51$) and positive PCR (---, $n = 51$) (not significant). (b) Kaplan–Meier plot of the probability of developing positive antigenemia (—, $n = 28$) and positive PCR (---, $n = 28$) in HLA-identical sibling donor transplant recipients (not significant). (c) Kaplan–Meier plot of the probability of developing positive antigenemia (—, $n = 23$) and positive PCR (---, $n = 23$) in alternative donor transplant recipients (not significant). (d) Kaplan–Meier plot of the probability of developing positive antigenemia in patients who received transplants from HLA-identical sibling donors (—, $n = 28$) vs alternative donors (---, $n = 23$) ($P < 0.01$). (e) Kaplan–Meier plot of the probability of developing positive PCR in patients who received transplants from HLA-identical sibling donors (—, $n = 28$) vs alternative donors (---, $n = 23$) ($P < 0.05$).

	Ag, PCR; negative	Ag alone; positive	PCR alone; positive	Ag → PCR	PCR → Ag	Ag and PCR simultaneously
Antigenemia						
Real-time PCR						
Total ($n = 51$)	8 (16%)	0 (0%)	4 (8%)	5 (10%)	20 (39%)	14 (27%)
Stem cell donor						
HLA-identical sibling ($n = 28$)	8 (29%)	0 (0%)	4 (14%)	2 (7%)	11 (39%)	3 (11%)
Alternative ($n = 23$)	0 (0%)	0 (0%)	0 (0%)	3 (13%)	9 (39%)	11 (48%)
Grade of acute GVHD						
0–I ($n = 24$)	6 (25%)	0 (0%)	3 (13%)	2 (8%)	6 (25%)	7 (29%)
II–IV ($n = 27$)	2 (7%)	0 (0%)	1 (4%)	3 (11%)	14 (52%)	7 (26%)

Figure 4 Longitudinal analysis for first detection of antigenemia and real-time PCR. Negative PCR is defined as CMV DNA $< 2 \times 10^2$ copies/ml. Ag, antigenemia; Ag → PCR, positive antigenemia preceded positive PCR; PCR → Ag, positive PCR preceded positive antigenemia; Ag and PCR simultaneously, antigenemia and PCR became positive at the same time. Temporal patterns of first detection of CMV antigen-positive cells and CMV DNA between HLA-identical sibling donor transplant patients and alternative donor transplant patients ($P < 0.01$) or between patients with grade 0–I acute GVHD and those with grade II–IV acute GVHD (not significant) were compared using Fisher’s exact test.

sibling donor transplants. However, CMV antigen-positive cells and CMV DNA were detected simultaneously in 11 of 23 alternative donor transplant patients (48%), indicating different temporal patterns between HLA-identical sibling and alternative donor transplants ($P < 0.01$).

When the pattern of first detection of CMV antigen-positive cells and CMV DNA was analyzed according to development of acute GVHD, neither antigen-positive cells nor CMV DNA was detected in six of 24 patients (25%) with grade 0–I acute GVHD, compared to two of 27 patients (7%) with grade II–IV acute GVHD (Figure 4). Sporadically positive PCR was observed in three of 24 patients (13%) with grade 0–I acute GVHD, and in one of 27 patients (4%) with grade II–IV acute GVHD. CMV antigen-positive cells and CMV DNA were detected simultaneously in seven of 24 patients (29%) with grade 0–I acute GVHD and in seven of 27 patients (26%) with grade II–IV acute GVHD (not significant) (Figure 4).

Incidence of CMV disease

Two of 51 patients (4%) developed CMV colitis despite pre-emptive therapy; one patient (diagnosed on day 33) was an HLA-identical sibling and the other patient (diagnosed on day 57) was an alternative donor transplant. Antigenemia and PCR became positive prior to development of CMV colitis. Both patients were successfully treated with ganciclovir.

Discussion

Quantitative detection of CMV DNA has been substituted for the antigenemia assay for the initiation of antiviral treatment in allogeneic stem cell transplant recipients.^{14–16,18,22} It has been suggested that quantitative PCR has several advantages over the antigenemia assay, including an increased sensitivity for detection of CMV reactivation, reliable detection of CMV reactivation during severe neutropenia in the early post-transplant period, shorter time required for the procedure, and convenient processing of large numbers of specimens.^{14–16,18,22} The number of antigen-positive cells and the level of plasma CMV DNA correlated well in the present study. When detection of ≥ 1 antigen-positive cell and $\geq 2 \times 10^2$ CMV DNA copies/ml were defined as positive results, this antigenemia assay showed 55.4%/95.5%/88.9%/76.9% for sensitivity/specificity/positive predictive value/negative predictive value, using the real-time PCR as the reference.

Gerna *et al*²³ compared qualitative results of immediate-early (IE) mRNA obtained by nucleic acid sequence-based amplification (NASBA) with those obtained by antigenemia assay and quantitative blood PCR in 51 marrow transplant patients. Qualitative determination of IEmRNA was the most sensitive for detection of CMV infection; the sensitivity and specificity of the antigenemia assay (sensitivity 59.0%, specificity 93.2%) and those of the blood PCR (sensitivity 67.7%, specificity 93.2%) were not different to NASBA as the reference standard.²³ Similarly, Boeckh *et al*¹² demonstrated that detection of CMV DNA in peripheral blood leukocytes by PCR (PBL PCR) was the most

sensitive test, followed by the antigenemia assay, detection of CMV DNA in plasma by PCR (plasma PCR), and viremia. Their antigenemia assay showed 55%/97%/90%/83% for sensitivity/specificity/positive predictive value/negative predictive value using the PBL PCR as the reference standard, and 62%/88%/45%/94%, respectively, using plasma PCR as the reference.¹² The sensitivity of our antigenemia assay was very similar to those obtained by Gerna *et al*²³ and Boeckh *et al*,¹² despite different reference standards. These findings suggest that the plasma quantitative real-time PCR may also be a sensitive test for detection of CMV reactivation.

Boeckh *et al*¹² demonstrated that the time to first detection of CMV reactivation by the antigenemia assay and plasma PCR was similar, whereas it occurred significantly earlier for PBL PCR. Gerna *et al*²³ similarly reported identical times to first CMV detection by antigenemia and quantitative blood PCR, 39.6 days and 40.9 days, respectively. In the present study, the median day to first CMV detection by the antigenemia assay and PCR was 37 and 33, respectively. There was a trend towards earlier detection by PCR in HLA-identical sibling donor transplant patients. However, any difference in sensitivity between the antigenemia assay and the real-time PCR does not appear to have a major clinical impact on early treatment strategies, especially in alternative donor transplant patients as shown in Figure 3.

Approximately one-third of HLA-identical sibling donor transplant patients did not develop reactivation of CMV infection, and four of 28 HLA-identical sibling donor transplant patients developed a sporadically positive PCR alone; they did not receive ganciclovir during monitoring. Pre-emptive strategies, rather than ganciclovir administration at engraftment, may be preferable in HLA-identical sibling donor transplant recipients to avoid ganciclovir-induced neutropenia. Of note, positive antigenemia preceded a positive PCR in five of 51 transplant patients (10%), furthermore, antigen-positive cells and CMV DNA were detected simultaneously in 40 to 50% of alternative donor transplant patients. These findings confirm the lack of a difference in first detection of CMV reactivation between these two tests. Differences in the temporal pattern of CMV reactivation between HLA-identical sibling transplants and transplants from alternative donors may be related to differential reconstitution of CMV-specific immunity. In recipients of transplants from unrelated donors, recovery of CMV-specific cytotoxic T lymphocytes (CTL) has been demonstrated to be delayed in comparison to that in recipients of transplants from siblings by direct visualization of CTL after allogeneic stem cell transplantation.²⁴

Pre-emptive therapy in the present study resulted in effective prevention of CMV disease after allogeneic stem cell transplantation. Antigenemia-guided early treatment at low levels of antigen-positive cells resulted in effective prevention of CMV disease without an increase in fungal infections, and the incidence of CMV disease in patients receiving antigenemia-guided treatment was comparable to that in patients receiving ganciclovir prophylaxis at engraftment.²⁵ Our incidence of CMV disease was 4%, comparable to recently reported incidences ranging from 0 to 16%.^{2,8,9,12,13,25–28} The antigenemia assay, as well as the

quantitative real-time PCR, may be useful in early detection of CMV reactivation and in prevention of CMV disease after allogeneic stem cell transplantation.

However, CMV colitis did develop in two patients during pre-emptive therapy with ganciclovir. The sensitivity and predictive value of the antigenemia assay in CMV colitis have been reported to be low,^{2,28} although it is sometimes difficult to differentiate CMV colitis from gastrointestinal GVHD presenting with nausea, diarrhea, or abdominal pain. Examination of the gastrointestinal tract may be delayed. PCR on stool samples may be helpful for ruling out intestinal CMV disease in patients at high risk.²⁹ Surveillance for CMV colitis is critical during antigenemia-guided early treatment.^{2,28}

Quantitative real-time PCR is more sensitive than the antigenemia assay in the detection of CMV reactivation. The number of patients evaluated in the present study was small, and patients did not receive different treatment strategies. However, the probabilities and time to first detection of positive antigenemia and positive PCR were not different, and the incidence of CMV disease was low. These results suggest that the difference in the sensitivity between these two tests is unlikely to have a major clinical impact on early treatment strategies for CMV disease. Both tests are useful in the early detection of CMV reactivation and in the prevention of CMV disease after allogeneic stem cell transplantation.

Acknowledgement

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

References

- 1 Zaia JA. Cytomegalovirus infection. In: Thomas ED, Blume KG, Forman SJ (eds). *Hematopoietic Cell Transplantation*. Blackwell Science: Malden, 1999, pp 560–583.
- 2 Boeckh M, Gooley TA, Myerson D *et al*. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. *Blood* 1996; **88**: 4063–4071.
- 3 van der Bij W, Torensma R, van Son WJ *et al*. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. *J Med Virol* 1988; **25**: 179–188.
- 4 The TH, van der Bij W, van der Berg AP *et al*. Cytomegalovirus antigenemia. *Rev Infect Dis* 1990; **12** (suppl. 7): S737–S744.
- 5 Boeckh M, Bowden RA, Goodrich JM *et al*. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. *Blood* 1992; **80**: 1358–1364.
- 6 Gondo H, Minematsu T, Harada M *et al*. Cytomegalovirus (CMV) antigenemia for rapid diagnosis and monitoring of CMV-associated disease after bone marrow transplantation. *Br J Haematol* 1994; **86**: 130–137.
- 7 Takenaka K, Gondo H, Tanimoto K *et al*. Increased incidence of cytomegalovirus (CMV) infection and CMV-associated disease after allogeneic bone marrow transplantation from unrelated donors. *Bone Marrow Transplant* 1997; **19**: 241–248.
- 8 Kanda Y, Mineishi S, Saito T *et al*. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. *Bone Marrow Transplant* 2001; **21**: 437–444.
- 9 Einsele H, Ehninger G, Hebart H *et al*. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. *Blood* 1995; **86**: 2815–2820.
- 10 Gozlan J, Laporte JP, Lesage S *et al*. Monitoring of cytomegalovirus infection and disease in bone marrow recipients by reverse transcription-PCR and comparison with PCR and blood and urine cultures. *J Clin Microbiol* 1996; **34**: 2085–2088.
- 11 Hebart H, Muller C, Loffler J *et al*. Monitoring of CMV infection: a comparison of PCR from whole blood, plasma-PCR, pp65-antigenemia and virus culture in patients after bone marrow transplantation. *Bone Marrow Transplant* 1996; **17**: 861–868.
- 12 Boeckh M, Gallez-Hawkins GM, Myerson D *et al*. Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation. *Transplantation* 1997; **64**: 108–113.
- 13 Ljungman P, Aschan J, Lewensohn-Fuchs I *et al*. Results of different strategies for reducing cytomegalovirus-associated mortality in allogeneic stem cell transplant recipients. *Transplantation* 1998; **66**: 1330–1334.
- 14 Ljungman P, Lore K, Aschan J *et al*. Use of a semi-quantitative PCR for cytomegalovirus DNA as a basis for pre-emptive antiviral therapy in allogeneic bone marrow transplant patients. *Bone Marrow Transplant* 1996; **17**: 583–587.
- 15 Kanda Y, Chiba S, Suzuki T *et al*. Time course analysis of semi-quantitative PCR and antigenemia assay for prevention of cytomegalovirus disease after bone marrow transplantation. *Br J Haematol* 1998; **100**: 222–225.
- 16 Caliendo AM, St. George K, Kao S *et al*. Comparison of quantitative cytomegalovirus (CMV) PCR in plasma and CMV antigenemia assay: clinical utility of the prototype Amplicor CMV Monitor test in transplant recipients. *J Clin Microbiol* 2000; **38**: 2122–2127.
- 17 Emery VC, Sabin CA, Cope AV *et al*. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. *Lancet* 2000; **355**: 2032–2036.
- 18 Machida U, Kami M, Fukui T *et al*. Real-time automated PCR for early diagnosis and monitoring of cytomegalovirus infection after bone marrow transplantation. *J Clin Microbiol* 2000; **38**: 2536–2542.
- 19 Thomas ED, Storb R, Clift RA *et al*. Bone marrow transplantation. *New Engl J Med* 1975; **292**: 895–902.
- 20 Gondo H, Harada M, Taniguchi S *et al*. Cyclosporine combined with methylprednisolone or methotrexate in prophylaxis of moderate to severe acute graft-versus-host disease. *Bone Marrow Transplant* 1993; **12**: 437–441.
- 21 Ljungman P, Griffiths P. Definitions of cytomegalovirus infection and disease. In: Michelson S, Plotkin SA (eds). *Multidisciplinary Approach to Understanding Cytomegalovirus Disease*. Elsevier Science Publishers: Amsterdam, 1993, pp 233–237.
- 22 Limaye AP, Huang ML, Leisenring W *et al*. Cytomegalovirus (CMV) DNA load in plasma for the diagnosis of CMV disease before engraftment in hematopoietic stem-cell transplant recipients. *J Infect Dis* 2001; **183**: 377–382.
- 23 Gerna G, Baldanti F, Lilleri D *et al*. Human cytomegalovirus immediate-early mRNA detection by nucleic acid sequence-based amplification as a new parameter for pre-emptive therapy in bone marrow transplant recipients. *J Clin Microbiol* 2000; **38**: 1845–1853.

- 24 Cwynarski K, Ainsworth J, Cobbold M *et al*. Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation. *Blood* 2001; **97**: 1232–1240.
- 25 Boeckh M, Bowden RA, Gooley T *et al*. Successful modification of a pp65 antigenemia-based early treatment strategy for prevention of cytomegalovirus disease in allogeneic marrow transplant recipients. *Blood* 1999; **93**: 1781–1782.
- 26 Verdonck LF, Dekker AW, Rozenberg-Arska M, van den Hoek MR. A risk-adapted approach with a short course of ganciclovir to prevent cytomegalovirus (CMV) pneumonia in CMV-seropositive recipients of allogeneic bone marrow transplants. *Clin Infect Dis* 1997; **24**: 901–907.
- 27 Manteiga R, Martino R, Sureda A *et al*. Cytomegalovirus pp65 antigenemia- guided pre-emptive treatment with ganciclovir after allogeneic stem cell transplantation: a single-center experience. *Bone Marrow Transplant* 1998; **22**: 899–904.
- 28 Mori T, Okamoto S, Matsuoka S *et al*. Risk-adapted pre-emptive therapy for cytomegalovirus disease in patients undergoing allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2000; **25**: 765–769.
- 29 Michel D, Marre E, Hampl W *et al*. Intestinal cytomegalovirus disease in immunocompromised patients may be ruled out by search for cytomegalovirus DNA in stool samples. *J Clin Microbiol* 1995; **33**: 3064–3067.