

*Full Length Research Paper*

# Incidence and clinical effects of Dynamic cytomegalovirus infectious disease in autologous stem cell organism transplant beneficiaries

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Cytomegalovirus (CMV) infection in patients after autologous stem cell transplant (ASCT) has become a major medical concern. However, few studies have reported active CMV infection before the transplantation. In this study, we retrospectively analyzed the incidence, clinical impact, risk factors, and outcome of CMV pp65 antigenemia in 44 consecutive patients who underwent ASCT between January 2005 and June 2011. CMV pp65 antigenemia assay was performed weekly, from 7 days before stem cell infusion until the patient was discharged. All patients were CMV seropositive before ASCT. Pre-transplantation antigenemia was detected in 19 patients (43.2%) and post-transplantation antigenemia in 17 patients (38.6%). Multivariate analysis could not identify any pre-transplantation risk factors for CMV antigenemia. When patients with and without pre-transplantation antigenemia were compared, we found that pre-transplantation active CMV infection had significant effects on post-transplantation platelet and neutrophil recovery, although the time of hospitalization and amount of blood transfusion were similar in both groups. CMV antigenemia was asymptomatic in all cases, and cleared spontaneously in 86.7% of patients who did not receive antiviral treatment. In conclusion, CMV reactivation before ASCT might occur frequently and even an asymptomatic infection could significantly delay engraftment. Therefore, ASCT candidates should be routinely evaluated for active CMV infection.

**Key words:** Cytomegalovirus, autologous stem cell transplant, CMV pp65 antigenemia, infection.

## INTRODUCTION

Human cytomegalovirus (CMV) is a major opportunistic infectious agent among allogeneic stem cell transplant (HSCT) recipients, and CMV infection is associated with high morbidity and mortality (Meyers et al., 1986). Although, the incidence of CMV infection is lower in autologous stem cell transplant (ASCT) recipients than in HSCT recipients, the fatality rates of patients with CMV disease are equally high in both groups (Focosi et al., 2009; Holmberg et al., 1999; Scaglione et al., 2005). Several studies investigated CMV infection in patients after ASCT (Boeckh et al., 1996b; Borchers et al., 2011; Jang et al., 2011; Konoplev et al., 2001; Reusser et al.,

1990; Wingard et al., 1988). However, active CMV infection before the transplantation has not been described. The presence of CMV pp65 antigenemia is an indicator of active CMV infection, and it is associated with an increased risk of CMV disease in hematopoietic stem cell transplant recipients (Boeckh et al., 1992; Boeckh et al., 1996a; Nichols et al., 2001).

Moreover, CMV pp65 antigenemia is predictive of the development of invasive diseases in transplant patients (Boeckh et al., 1996a; Lee et al., 2009; Nichols et al., 2001). The occurrence of CMV pp65 antigenemia and CMV disease following ASCT have been reported (Boeckh et al., 1996b; Luo et al., 2010; Rossini et al., 2005; Tormo et al., 2010). Unfortunately, these studies have not evaluated CMV reactivation before transplantation in patients who were CMV seropositive. In our center, we routinely perform the CMV pp65

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antigenemia assay to screen for CMV reactivation before and after HSCT.

The purpose of this study was to retrospectively analyze CMV pp65 antigenemia before and after ASCT, including the incidence, clinical impact, risk factors, and outcomes. The effect of pre-transplantation active CMV infection on the engraftment in ASCT recipients was also studied.

## MATERIALS AND METHODS

### Patients

Between January 2005 and June 2011, 44 consecutive ASCTs were performed in the bone marrow transplantation center of The First Affiliated Hospital of Zhejiang University, China. The median patient age was 45 years old (range: 12 to 63 years). Most of the patients had non-Hodgkin's lymphoma or multiple myeloma (MM), and others had Hodgkin's lymphoma, multiple sclerosis, or chronic myelogenous leukemia. With the exception of a 12-year-old boy, all patients were adults. The details on the diseases and conditioning regimens were listed in Table 1. Before ASCT, all patients were seropositive for CMV (IgG positive and IgM negative). All patients received peripheral blood as the source of stem cells and leukocyte-depleted blood products if required. However, blood products were not screened for the CMV antibody. This study was approved by the local institutional review board.

### CMV surveillance

A standard immunohistochemical method was used to detect the CMV pp65 antigen in peripheral blood leukocytes (PBLs), as previously described (Zhang et al., 2009). PBLs were separated from ethylenediaminetetraacetic acid-anticoagulated blood and spread on slides. Anti-CMV pp65 antigen monoclonal antibody (AAC10; DAKO, Denmark) and EnvisionTM+ System, peroxidase (DAB) kit (DAKO) were used. Cells stained brown or yellow were positive, whereas blue cells were negative.

The result was expressed as the number of positive cells detected in 50,000 PBLs. CMV antigenemia was defined as any degree of antigenemia ( $\geq 1$  positive cell/50,000 PBLs) (Ljungman et al., 2002). All patients were monitored by quantitation of pp65 antigenemia in PBLs, from the initiation of conditioning until the patients were discharged. Most of the patients were monitored on a weekly basis. Active CMV infection was defined as the presence of CMV pp65 antigenemia without clinical signs and symptoms. CMV disease was defined as a symptomatic CMV infection, including CMV pneumonia, CMV gastroenteritis, or CMV retinitis (Ljungman et al., 2002).

### Antiviral treatment

To prevent herpes simplex virus infection, most patients received intravenous low-dose acyclovir (250 mg bid), beginning at the initiation of conditioning. All but 2 patients received this treatment. Patients who tested positive for CMV pp65 antigenemia ( $\geq 5$  positive cells/50,000 PBLs) were treated with intravenous ganciclovir or foscarnet to prevent CMV disease. Ganciclovir (5 mg/kg bid, intravenously) or foscarnet (90 mg/kg bid, intravenously) was administered daily until the antigenemia was resolved.

### Engraftment

The recovery of neutrophil counts after transplantation was examined based on the number of days required to achieve sustained counts of  $0.5 \times 10^9/L$  or greater. The recovery of platelet counts was evaluated based on the number of days required to achieve self-sustaining counts of  $20 \times 10^9/L$  or greater, on at least 2 consecutive days without platelet transfusion support.

### Statistical analysis

Statistical analyses were performed using SPSS 13.0. The values were expressed as median values and mean  $\pm$  SD. A t-test was used to compare the quantitative data. Mann-Whitney U test was used for univariate analyses, and logistic regression was used for multivariate analyses. All P values were based on a 2-tailed test of significance ( $P < 0.05$ ).

## RESULTS

### CMV pp65 antigenemia

Among the 44 patients, 36 (81.8%) tested positive for antigenemia pre- or post-transplantation, and only 8 tested negative. At the conditioning time (pre-transplantation), 19 patients (43.2%) had antigenemia and the median antigenemia level was 5/50,000 PBLs (range, 1–20/50,000 PBLs). The other 17 (38.6%) patients had antigenemia after stem cell infusion.

The first positive antigenemia presented a median of 13 days after stem cell infusion (range, 3 to 22 days). The median antigenemia level at the first appearance was 3/50,000 PBLs (range, 1–9/50,000 PBLs). When the 2 groups (patients with pre- or post-transplantation antigenemia) were compared, the first group had a higher level of positive cells than the latter but the difference was not significant ( $P = 0.07$ ).

### Risk factors for CMV pp65 antigenemia

To reveal the risk factors for pre-transplantation CMV pp65 antigenemia, we analyzed 5 variables, including age, gender, underlying disease, time from diagnosis to transplantation, and time from the last chemotherapy to conditioning. These variables were assessed by multivariate regression analysis, but no significant differences in these parameters were observed (Table 2). Because of the small number of patients, we could not analyze the risk factors for post-transplantation antigenemia.

### CMV antigenemia and engraftment

To evaluate the effect of pre-transplantation active CMV infection on engraftment, we compared the groups with and without pre-transplantation antigenemia. We found that neutrophil count recovery ( $15.32 \pm 3.86$  days vs.

**Table 1.** The characteristics of the patients.

Characteristics	Cases (total 44)
<b>Age (years)</b>	
Median	45
Range	12–63
<b>Sex (male:female)</b>	
	23:21
<b>Disease</b>	
Hodgkin's disease	2
Non-Hodgkin's lymphoma	19
Multiple myeloma	21
Multiple sclerosis	1
Chronic myelogenous leukemia	1
<b>Type of conditioning (n)</b>	
BEAM	14
BEAM + Rituximab	8
Bortezomib + Melphalan	14
Melphalan	7
BUCY2	1

12.32 ± 1.60 days;  $P = 0.004$ ) and platelet recovery (16.17 ± 3.38 days vs. 13.0 ± 1.73 days,  $P = 0.001$ ) were significantly delayed in the group with pre-transplantation CMV antigenemia than in the group without. However, the length of hospital stay was similar (41.74 ± 25.12 days vs. 34.56 ± 4.62 days,  $P = 0.168$ ). Although engraftment was delayed, the amount of blood transfusion was similar (Mann–Whitney  $U = 232$ ;  $Z = -0.141$ ;  $P = 0.887$ ).

### Therapy

All of the patients who had CMV pp65 antigenemia were asymptomatic. Of the patients with pre-transplantation CMV pp65 antigenemia, 14 (73.7%) accepted drug treatment to prevent CMV disease (9 received ganciclovir and 5 received foscarnet). Antigenemia was resolved in all patients in a mean duration of 14.75 days (range, 6–30 days). Of the 5 patients who did not accept drug treatment, 3 experienced spontaneous resolution of antigenemia and 2 had higher post-transplantation antigenemia, but their condition could be successfully controlled using foscarnet.

In the group that had post-transplantation antigenemia, 3 patients were treated with ganciclovir and 4 with foscarnet, and the antigenemia in all patients was successfully resolved. The other 10 patients with post-transplantation antigenemia also experienced spontaneous resolution without drug treatment.

### Supportive care

All patients received empiric antibiotic therapy when fever

or a suspected infection developed. All patients received granulocyte-colony stimulating factor.

### DISCUSSION

CMV remains the most important viral infection after HSCT, including ASCT. This study aimed to investigate active CMV infection in patients before and after ASCT. We screened 44 ASCT patients for pre- and post-transplantation CMV pp65 antigenemia. Our results showed that 81.8% of the patients (36/44) presented with CMV pp65 antigenemia before or after transplantation; 43.2% of the patients (19/44) had pre-transplantation antigenemia and 38.6% (17/44) had post-transplantation antigenemia. Previous studies have not routinely screened for CMV reactivation before ASCT or examined pre-transplantation active CMV infection. Through pre-transplantation screening, we found that 43.2% (19/44) of the candidates for ASCT had active CMV infection. The rate of CMV pp65 antigenemia in the patients, preceding ASCT, has not been previously reported. There are some possible explanations for the high incidence of CMV pp65 antigenemia observed in our study.

First, all patients in our study were CMV seropositive before ASCT. A high seroprevalence of CMV has been found in the general population in China (Fang et al., 2009; Zhao et al., 2009), and we have not routinely selected blood products from CMV seronegative donors. Therefore, there is a risk of CMV transmission through frequent transfusions. Second, transplantation candidates always accept chemotherapy for their underlying malignancies such as MM and lymphoma, and these chemotherapy drugs such as rituximab and melphalan may increase the risk of developing CMV infection or disease in these patients (Fassas et al., 2001; Lee et al., 2008; Scaglione et al., 2005). Third, the pp65 antigenemia test is a sensitive method for screening active CMV infection (Boeckh et al., 1992; Tormo et al., 2010; Wingard et al., 1988).

Our analysis showed that the patients with pre-transplantation CMV reactivation had a significant delay in neutrophil count and platelet recovery, compared to those without pre-transplantation antigenemia. Several reports have documented CMV infection and prolonged thrombocytopenia after HSCT. Dominietto et al. (2001) found that thrombocytopenia was more frequent in patients with high-level CMV antigenemia (4 positive cells/200,000 PBLs). Nash et al. (1996) analyzed 523 autologous HSCT patients and found that delayed platelet recovery after transplantation was associated with a positive CMV serology and the presence of post-transplantation infection before engraftment. This delay may be attributed to several factors. CMV has been shown to have a direct or indirect inhibitory effect on the hematopoietic system (Bego and St Jeor, 2006; Maciejewski and St Jeor, 1999). Furthermore, treatment of CMV antigenemia with drugs such as ganciclovir or

**Table 2.** Risk factors for pre-transplantation CMV antigenemia.

Parameter	Positive	Negative	P value
All patients (media)	19	25	
Age	49	44	0.912
Gender			0.714
Male	11	12	
Female	8	13	
Underlying disease			0.319
Multiple myeloma	11	10	
Non-Hodgkin's lymphoma	7	12	
Hodgkin's lymphoma	1	1	
Other	0	2	
Time from diagnosis to transplantation (months)	11.21 ± 7.60	18.54 ± 18.36	0.188
Time from the last chemotherapy to conditioning (days)	54.68 ± 38.87	60.00 ± 34.42	0.618

foscarnet results in leukocytopenia and thrombocytopenia (Reusser et al., 2002). In the present study, 73.7% of the patients with pre-transplantation CMV pp65 antigenemia accepted drug treatment (9 with ganciclovir and 5 with foscarnet) to prevent CMV disease.

Our data show that CMV antigenemia was asymptomatic in all of the cases and all of the infections were successfully controlled. In patients that did not accept antiviral treatment (60.0% of pre-transplantation patients and all post-transplantation patients), infection was cleared spontaneously without antiviral treatment. Boeckh et al. (1996b) found that antigenemia after ASCT was self-limiting without antiviral treatment in about 75% of the patients who had low-level antigenemia (<5 positive cells/slide). In our study, post-transplantation antigenemia was more easily cleared than pre-transplantation antigenemia, possibly due to further immune suppression from pre-transplantation conditioning chemotherapy. Our findings raise questions regarding the suitable antigenemia cut-off values and time of therapy to avoid unnecessary antiviral treatment and its side effects.

We detected antigenemia in 43.2% of the patients before transplantation and in 38.6% patients after transplantation. Pre-transplantation active CMV infection significantly delayed the recovery of neutrophil counts and platelets. In 86.7% of patients, CMV reactivation was cleared spontaneously without antiviral treatment, and no patients died of CMV disease. Based on these findings, we speculate that CMV reactivation before ASCT might occur frequently and thus ASCT candidates should be routinely evaluated for CMV reactivation. In this aspect, the results of our study emphasize the importance of screening CMV reactivation before ASCT in order to achieve satisfactory effects of ASCT therapy. The small number of patients and short follow-up period are limitations of the present study. Patients in this study were routinely monitored for an average of 37 days post-transplantation for CMV infection. Further, carefully

designed prospective controlled studies are needed to assess CMV reactivation and disease, the risk factors for CMV infection and disease, the relationship between CMV infection and engraftment, and the efficacy and safety of prophylactic therapy before and after ASCT.

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