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## ORIGINAL ARTICLE

# Cytomegalovirus shedding in the oral cavity of allogeneic haematopoietic stem cell transplant patients

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**OBJECTIVE: This study was designed to investigate the effect of allogeneic haematopoietic stem cell transplantation (HSCT) on cytomegalovirus (CMV) shedding in the saliva by nested polymerase chain reaction (nested PCR) and its impact on patient survival.**

**PATIENTS AND METHODS: One hundred and twenty-four HSCT patients and 124 healthy volunteers were included in the study. Oral swabs were taken before, after 100 days and 1 year of HSCT at the buccal mucosa. Nested PCR was used to detect CMV in the saliva. Time of death after HSCT was displayed, by means of the Kaplan–Meier method, for the following parameters: age and gender of the patient, donor gender, primary disease, stem cell source, platelet number, chronic graft *vs* host disease (cGVHD) of salivary glands and oral mucosa, and oral CMV shedding. Cox proportional hazards model was used for multivariate survival analysis.**

**RESULTS: While none of the individuals in the control group showed positive swabs for CMV, the frequency of positive CMV oral swabs in patients at day + 100 after HSCT (45.2%) was statistically higher than before (7.2%) and 1 year after HSCT (17.5%). The presence of CMV was not associated with cGVHD and did not have any impact on post-transplant survival.**

**CONCLUSIONS: The present study shows that oral CMV shedding occurs after HSCT, especially at day +100 post-transplant. Identification of CMV in the saliva might be important for the early diagnosis of CMV infection in allo-HSTC.**

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**Keywords:** cytomegalovirus; haematopoietic stem cell transplantation; cytomegalovirus shedding

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## Introduction

Cytomegalovirus (CMV) belongs to the Herpesviridae family. As other viruses of this family, CMV is capable of establishing host latency after a period of infection (Sinclair and Sissons, 1996; Nichols and Boeckh, 2000; Yan and Fedorko, 2002). CMV is a pathogen that causes infection in paediatric and adult patients (Yan and Fedorko, 2002). The salivary glands, kidney and leucocytes are the major sites of CMV replication in primary infection (Kano and Shiohara, 2000). The exact sites that allow latent infection are unclear, but polymorphonuclear cells, dendritic cells, endothelial vascular tissue and salivary glands may contain the virus (Grefte *et al*, 1993). CMV may be found in oropharyngeal secretions (saliva), cervical and vaginal excretions, semen, breast milk, tears, urine, faeces and blood (Kano and Shiohara, 2000). Direct exposure to infected urine and saliva is considered to be the main mechanism responsible for transmitting CMV infection (Canto *et al*, 2000).

Cytomegalovirus infection is common in the human population and its seroprevalence has been reported to be as high as 80–90% (Kloover *et al*, 2002). CMV infections are ubiquitous in Brazil and about 95% of the adult individuals are CMV seropositives. After CMV infection in immunocompetent individuals, the disease occurs in a small number of cases and is usually manifested as an infectious mononucleosis-like syndrome (Cunha *et al*, 2002). In immununocompromised hosts CMV may result in severe opportunistic infectious with morbidity and mortality, especially in allogeneic haematopoietic stem cell transplantation (allo-HSCT) (Ammatuna *et al*, 2001; Boeckh *et al*, 2003).

Allo-HSCT is performed in patients for a range of underlying disorders, including haematological malignancy, severe aplastic anaemia and genetic diseases. Allo-HSCT recipients are immunosuppressed and the impaired cellular immunity seen in the first 3–7 months after transplantation can result in CMV reactivation of latent infection (Zekri *et al*, 2004). CMV reactivation or

disease may occur in up to 60% of cases of patients after allo-HSCT with particularly high incidence in the first 100 days after transplantation (Einsele *et al*, 1995; Osarogiagbon *et al*, 2000). The incidence of CMV infection increases with the intensity and duration of immunosuppression and is more frequent in allogeneic bone marrow recipients who are CMV seropositive and/or receive a transplant from a CMV-seropositive donor (Hebart *et al*, 2000). If untreated, CMV reactivation evolves to CMV disease. In allo-HSCT mortality caused by CMV disease is close to 30% (Hebart *et al*, 2000). CMV prophylaxis with ganciclovir or preemptive therapy can change the natural history of the disease, sometimes reducing CMV disease and mortality (Boeckh *et al*, 1996). However, this prophylaxis could also prolong the period of reactivation risk, probably by inhibiting the development of CMV-specific T-cell lymphocyte response, caused by general depression of T-cell immunity in patients receiving the drug (Li *et al*, 1994; Machado *et al*, 2001). The clinical manifestations could vary from completely asymptomatic infection, defined as active CMV replication in blood in the absence of clinical manifestations or organ failure abnormalities, to CMV disease, characterized by CMV infection with clinical symptoms or organ function abnormalities (Ammatuna *et al*, 2001; Castagnola *et al*, 2004). In transplant recipients CMV may cause allograft injury (Ammatuna *et al*, 2001; Dominietto *et al*, 2001) or it may influence the development of graft-*vs*-host disease (GVHD) (Söderberg *et al*, 1996; Takemoto *et al*, 2000).

The oral cavity is a frequent site of infectious, immunologic and toxic reactions associated with HSCT (Heimdahl *et al*, 1989). Oral manifestation of the CMV in immununocompromised individuals includes painful ulcers and erosions on the lips, tongue and buccal mucosa. Considering that the salivary glands harbour latent CMV infection and that evidences point that immunosuppression is related to its reactivation, the purpose of the present study was to investigate the presence and frequency of CMV DNA in the saliva of HSCT subjects and its impact on survival.

## Patients and methods

The study protocol was approved by the Ethics Committee of Universidade Federal de Minas Gerais, and informed consent was obtained from all the patients and from parents if the patient was less than 18 years.

*Patients and specimens*

Three groups of patients were included in the study. The first group included subjects who were being prepared to allo-HSCT (group 1; $n = 42$), while the second group comprised individuals 100 days after allo-HSCT (at day + 100) (group 2; $n = 42$). The third group included subjects at 1 year after HSCT (group 3; $n = 40$). All patients were attended to at the Dental Clinics in the School of Dentistry. Serological tests for detecting anti-CMV antibodies were performed in patients and donors before transplantation in order to access the serology status of CMV. Positive IgG to CMV was observed in 97% (group 1), 88% (group 2), and 94% (group 3) of the patients.

Patients in groups 2 and 3 were conditioned for transplantation according to the following protocol. Patients received cyclophosphamide (50 mg $kg^{-1}$ $day^{-1}$ for 4 days for patients with aplastic anaemia or 60 mg $kg^{-1}$ $day^{-1}$ for 2 days for patients with leukaemia or lymphoma) and busulfan (4 mg $kg^{-1}$ $day^{-1}$ for 1 day for patients with aplastic anaemia or 4 mg $kg^{-1}$ for 4 days for patients with leukaemia or lymphoma). Methotrexate and cyclosporin were used for GVHD prophylaxis and prednisone for GVHD treatment after allo-HSCT.

The medical records of the patients were reviewed and included the following information: gender and age of the patient, donor gender, primary disease, stem cell source (bone marrow or blood stem cell), leucocyte, neutrophil and platelet counts, and donor/recipient serologic CMV status before transplant. Biopsies of the lower lip were carried out in group 2 patients for chronic GVHD staging in the oral mucosa and salivary glands as described elsewhere (Gomez *et al*, 2001).

The control group was composed of 124 non-transplanted healthy volunteers attending the Restorative Dentistry Clinic. The sex and age of both groups were matched with the control group.

*Sample collection and DNA extraction*

Oral mucosa swabs were taken from the labial mucosa, buccal mucosa and tongue of HSCT subjects. The same procedure was performed in the oral mucosa of the control group. The swabs were taken with sterile plastic tips, placed immediately in Eppendorf microtubes containing 500 $\mu$l of Krebs buffer (NaCl 20%, KCl 2%, $CaCl_2 2H_2O$ 2%, $MgSO_4 KH_2PO_4 C_6H_{12}O_6$), and the pellet obtained after 10 min of centrifugation at 10 ***g*** was stored at −20°C until processing. DNA extraction was carried out as described by Boom *et al* (1990).

*Nested polymerase chain reaction (nested PCR)*

Three microlitres of DNA solution was subjected to PCR with two sets of primers pairs from a gene of glycoprotein B of the human CMV (Victória *et al*, 2005). The primers were 5′-ACA TGG AAT CCA GGA TCT GGT GCC-3′ and 5′-CCC TAT GAT ATG CCA CGA AAA CCG-3′. A 25 $\mu$l reaction mixture containing buffer, *Taq* DNA polymerase, primers and deoxyribonucleoside triphosphates was subjected to 30 cycles at 95°C for 30 s, 58°C for 45 s, and 72°C for 30 s performed in an Eppendorf-Master Cycler (Eppendorf, Hamburg, Germany). After the first round of PCR, 1 $\mu$l of the final product was used as a template for the second PCR with the inner primer pairs 5′-CAA CAC GTA ACG TCT TCT GAA GCC-3′ and 5′-TAG ACC ACC ATG ATG CCC TCA TCC-3′. The same procedure as described earlier was used except that 57°C was used as the annealing temperature. The human $\beta$-globin gene was amplified in order to assess the adequacy of each specimen (Gall-Troselj *et al*, 2001).

*Agarose gel electrophoresis*

Ten microlitres of each reaction product was added to 2 $\mu$l of gel loading dye (0.25% bromophenol blue, 30% glycerol, 10 mM EDTA) to visualize the specific product (224 bp) in a 1.5% agarose gel. Electrophoresis was carried out using 1x TAE buffer. DNA fragments were visualized after staining with ethidium bromide (0.5 $\mu$g ml$^{-1}$) and using the photo documentation system Vilber Lourmat (Vilber, Lourmat, Torcy, France). The molecular weight of the DNA was estimated using 100 bp ladder markers.

*Statistical analysis*

Chi-squared test and Fisher's exact test were used to test the association between variables for categorical data. The Mann–Whitney *U*-test was used for continuous variables. Time to death after allo-HSCT was displayed by means of the Kaplan–Meier method for the following variables: gender and age of the patient (≤30 and > 30 years), donor gender, primary disease (malignant *vs* non-malignant), stem cell source (bone marrow *vs* blood stem cell), platelet count (< 100 000 mm$^{-3}$ vs > 100 000 mm$^{-3}$, cGVHD at the salivary glands and oral mucosa (no *vs* mild *vs* moderate/severe), and presence of CMV in the saliva. The results of Kaplan–Meier plots were initially compared by the log-rank test. The factors that showed $\rho \leq 0.25$ along with CMV DNA (irrespective of the *P*-value) were included in the Cox proportional hazards model (Hosmer and Lemeshow, 2000; Kalbfleisch and Prentice, 2002). Statistical significance was set at $\rho < 0.05$. The records of each patient were reviewed 12 months later after the oral swab of all patients in the three groups of the study.

**Table 1** Distribution of HSCT patients and control subjects with oral mucosa swab positive for CMV

| | *CMV positive* | *CMV negative* | *ρ-value* ($\chi^2$ *test*) |
|---|---|---|---|
| Group 1 ($n = 42$) | 3 (7.2) | 39 (92.8) | < 0.0001 |
| Control ($n = 42$) | 0 (0) | 42 (100) | |
| Group 2 ($n = 42$) | 19 (45.2) | 23 (54.8) | < 0.0001 |
| Control ($n = 42$) | 0 (0) | 42 (100) | |
| Group 3 ($n = 40$) | 7 (17.5) | 33 (82.5) | < 0.0001 |
| Control ($n = 40$) | 0 (0) | 40 (100) | |

Values are expressed as *n* (%). CMV, cytomegalovirus. Group 1: before allo-HSCT; group 2: at day +100 after allo-HSCT; group 3: 1 year after allo-HSCT.

## Results

Group 1 included 20 women and 22 men with a mean age of 33.1 years (range 3–57). Group 2 ( + 100 after HSCT) included 18 women and 24 men with a mean age of 26.3 years (range 13–54). Group 3 consisted of 19 women and 21 men with a mean age of 25.5 years (range 3–64).

The frequency of CMV-positive swabs in each group is shown in Table 1. The frequency of CMV DNA detection in all groups, including before allo-HSCT, 100 days and 1 year after allo-HSCT was statistically higher than in the control group. None of the individuals in the control group showed positive oral swabs for CMV. The frequency of positive samples for CMV in group 2 (45.2%) (100 days after HSCT) was significantly higher than groups 1 (7.2%) and 3 (17.5%) ($P = 0.0001$). Despite the presence of CMV DNA on oral swabs, none of the patients presented oral lesions suggestive of CMV infection. The presence of CMV in the saliva of patients using or not immunosuppressive drugs at the time of sample collection was also evaluated but did not show significant association (data not shown).

Four of five recipients of HSCT IgG negative to CMV in group 2 received a transplant from a CMV-seropositive donor. Two of these patients showed positive oral swabs to CMV. None of the subjects of groups 1 and 3 who were IgG negative to CMV showed positive swabs for the virus on the PCR reaction.

The haematological counts of individuals in each group were compared according to the presence of CMV on the oral swabs (Table 2). The median number of platelet counts in group 3 (1 year after HSCT) subjects with positive CMV swabs (139 000 mm$^{-3}$) was significantly lower than from CMV-negative individuals (226 000 mm$^{-3}$) ($\rho = 0.02$). Forty-two patients were scored for chronic GVHD of salivary gland/oral mucosa and classified according to the presence of CMV in the oral swab (Table 3). The frequency of positive oral swabs for CMV was not related to the degree of chronic GVHD.

Baseline characteristics of living and dead patients are listed in Table 4. Survival according to the different variables analysed are shown in Table 5. In univariate analysis only chronic GVHD at salivary gland showed a $P = 0.05$. The multivariate analysis demonstrated significance only for chronic GVHD in the salivary gland. The relative survival risk, confidence interval and the *P*-value of the Cox proportional hazard model demonstrated an increased risk of death for patients

**Table 2** Median leucocyte, neutrophil and platelet counts in the subjects included in the study according to oral mucosa swab positive or negative for cytomegalovirus

| | *Group 1* | | | *Group 2* | | | *Group 3* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *CMV+* | *CMV−* | *P*-value | *CMV+* | *CMV−* | *P*-value | *CMV+* | *CMV−* | *P*-value |
| Leucocytes (mm$^{-3}$) | 2300 | 5700 | 0.08 | 5100 | 4830 | 0.61 | 8230 | 5800 | 0.20 |
| Neutrophils (mm$^{-3}$) | 1000 | 2952 | 0.08 | 2340 | 2200 | 0.54 | 2915 | 3008 | 0.47 |
| Platelets (mm$^{-3}$) | 180 000 | 169 000 | 0.31 | 139 000 | 185 000 | 0.16 | 139 000 | 226 000 | 0.02* |

Group 1: before allo-HSCT; group 2: at day +100 after allo-HSCT; group 3: 1 year after allo-HSCT.
*Statistically significant.

**Table 3** Distribution of patients in group 2 (100 days after allo-HSCT) according to cGVHD grading in the oral mucosa and salivary glands and presence of positive oral swabs to CMV

| | *Oral mucosa cGVHD* | | | *Salivary gland cGVHD* | | |
|---|---|---|---|---|---|---|
| | *CMV negative* | *CMV positive* | *P*-value | *CMV negative* | *CMV positive* | *P*-value |
| Absence | 7 (33) | 6 (31) | 0.70 | 3 (14) | 7 (37) | 0.23 |
| Mild | 10 (48) | 11(58) | | 12 (57) | 9 (47) | |
| Moderate /severe | 4 (19) | 2 (11) | | 6 (29) | 3 (16) | |

Values are expressed as *n* (%). cGVHD, chronic graft *vs* host disease; CMV, cytomegalovirus.

**Table 4** Baseline characteristics of living and dead patients

| | *Group 1 (n = 42)* | | *Group 2 (n = 42)* | | *Group 3 (n = 40)* | |
|---|---|---|---|---|---|---|
| *Parameters* | *Dead (25)* | *Living (17)* | *Dead (10)* | *Living (32)* | *Dead (4)* | *Living (36)* |
| Recipient gender | | | | | | |
| Male | 10 | 12 | 07 | 17 | 01 | 20 |
| Female | 15 | 05 | 03 | 15 | 03 | 16 |
| Donor gender | | | | | | |
| Male | 13 | 11 | 04 | 19 | 03 | 17 |
| Female | 12 | 06 | 06 | 13 | 01 | 19 |
| Age of patient (years) | | | | | | |
| ≤30 | 08 | 09 | 05 | 21 | 02 | 23 |
| >30 | 17 | 08 | 05 | 11 | 02 | 13 |
| Primary disease | | | | | | |
| Malignant | 21 | 13 | 08 | 25 | 03 | 25 |
| Non-malignant | 04 | 04 | 02 | 07 | 01 | 11 |
| Stem cell source | | | | | | |
| Blood stem cell | 18 | 10 | 08 | 18 | 02 | 20 |
| Bone marrow | 07 | 07 | 02 | 14 | 01 | 14 |
| Platelet number | | | | | | |
| ≤100 000 $mm^{-3}$ | 07 | 04 | 04 | 05 | 01 | 03 |
| > 100 000 $mm^{-3}$ | 18 | 13 | 06 | 27 | 02 | 33 |
| Oral mucosa cGVHD[a] | | | | | | |
| No | | | 04 | 09 | | |
| Mild | | | 04 | 17 | | |
| Moderate/severe | | | 01 | 05 | | |
| Salivary gland cGVHD[a] | | | | | | |
| No | | | 05 | 05 | | |
| Mild | | | 04 | 17 | | |
| Moderate/severe | | | 0 | 09 | | |
| CMV DNA | | | | | | |
| Negative | 23 | 16 | 05 | 18 | 04 | 29 |
| Positive | 02 | 01 | 05 | 14 | 0 | 07 |

cGVHD, chronic graft *vs* host disease; CMV, cytomegalovirus. Group 1: before allo-HSCT; group 2: at day + 100 after allo-HSCT; group 3: 1 year after allo-HSCT.
[a]GVHD staging in oral mucosa and salivary glands was performed only in group 2.

with a less GVHD degree (HR: 0.28; 95% CI 0.09–0.89; $P = 0.03$).

## Discussion

In the present study we took oral swabs to investigate the presence of CMV DNA from the saliva. As CMV is commonly detected in the salivary glands, endothelial cells, leucocytes, but not on oral mucosa epithelial cells (Kano and Shiohara, 2000), the oral swabs probably represent the presence of the virus in the saliva rather than in the oral mucosa.

Despite the high seroprevalence for CMV, including a Brazilian population (Cunha *et al*, 2002), the presence of this virus in the saliva of allo-HSCT patients has not been investigated. No individual in the control group presented CMV DNA in the oral mucosa swabs. These data demonstrate that asymptomatic reactivation of CMV in the oral mucosa represents a very uncommon event. On the other hand, the current study shows a high frequency of CMV in the saliva of allo-HSCT recipients. This reactivation was probably caused by HSCT therapy.

The salivary glands are the preferred sites for persistence of CMV. The production of infectious virus by the salivary glands, and subsequent shedding of infectious virus in the saliva, provides a crucial vehicle for horizontal transmission of CMV through the population (Canto *et al*, 2000; Kloover *et al*, 2002). By using rat models of CMV infection, it was demonstrated that CMV shedding from the salivary glands may result in reinfection of internal tissues and organs, possibly via small lacerations of the oral/pharyngeal and/or oesophageal mucosa, providing a continuous challenge for the host's immune system (Kloover *et al*, 2002). Infection by CMV in different organs may not only result in CMV disease, such as fatal pneumonia, gastrointestinal disease, hepatitis, encephalitis, retinitis, unexplained fever and endothelial damage, but have also been associated with graft failure (Castagnola *et al*, 2004), increased susceptibility to other infections, due to its immunomodulatory effects, and reactivation of previous controlled viral infections (Kano and Shiohara, 2000; Nichols and Boeckh, 2000). New studies are necessary to understand the importance of the asymptomatic reactivation of CMV in the saliva of allo-HSCT patients.

The present study demonstrates increased frequency of CMV DNA in the oral swabs of allo-HSCT patients 100 days after transplant (group 2) compared with before (group 1) and 1 year after transplant (group 3). As our study is cross-sectional in nature and the groups are not similar, results should be interpreted with caution. Previous studies have demonstrated high rates of CMV infection between 30 and 100 days after transplant (Zaia *et al*, 1984; Einsele *et al*, 1995; Boeckh *et al*, 2003). In the present study the samples were collected at an outpatient department, and no samples were collected until 100 days of allo-HSCT. Thus, we are unable to verify the presence of CMV in the oral mucosa in this time interval.

The diagnosis of CMV reactivation on HSCT is made by antigenaemia and/or PCR of plasma or blood (Zaia *et al*, 1997). Antigenaemia test detects between 1 and 1500 positive cells for the virus per 50 000 leucocytes $\mu l^{-1}$ (Piiparinen *et al*, 2001). As PCR of plasma or blood requires 247–1650 copies of CMV DNA per ml and 500 DNA copies per 100 ng of total

**Table 5** Survival according to different variables analysed – univariate analysis:

| | *Group 1* | | *Group 2* | | *Group 3* | |
|---|---|---|---|---|---|---|
| | *n = 42* | *P*-value *(log-rank test)* | *n = 42* | *P*-value *(log-rank test)* | *n = 40* | *P*-value *(log-rank test)* |
| Median follow-up (years; min-max) | 2.3 (1.5–3.5) | | 3.0 (1.3–3.7) | | 2.5 (1.4–3.6) | |
| Overall | 45 | | 78 | | 92 | |
| Recipient gender | | | | | | |
| Male | 54 | 0.08 | 75 | 0.34 | 100 | 0.23 |
| Female | 35 | | 83 | | 84 | |
| Donor gender | | | | | | |
| Male | 45 | 0. 70 | 87 | 0.28 | 89 | 0.39 |
| Female | 44 | | 68 | | 95 | |
| Age of patient (years) | | | | | | |
| ≤30 | 65 | 0.11 | 84 | 0.33 | 95 | 0.65 |
| >30 | 31 | | 69 | | 86 | |
| Primary disease | | | | | | |
| Malignant | 41 | 0.37 | 89 | 0.85 | 89 | 0.84 |
| Non-malignant | 60 | | 75 | | 100 | |
| Stem cell source | | | | | | |
| Blood stem cell | 39 | 0.30 | 69 | 0.17 | 90 | 0.76 |
| Bone marrow | 57 | | 94 | | 100 | |
| Platelet number | | | | | | |
| ≤100 000 $mm^{-3}$ | 36 | 0.46 | 56 | 0.09 | 75 | 0.20 |
| >100 000 $mm^{-3}$ | 48 | | 84 | | 97 | |
| Oral mucosa cGVHD[a] | | | | | | |
| No | | | 77 | | | |
| Mild | | | 81 | 0.71 | | |
| Moderate/severe | | | 83 | | | |
| Salivary gland cGVHD[a] | | | | | | |
| No | | | 58 | | | |
| Mild | | | 81 | 0.05 | | |
| Moderate/severe | | | 100 | | | |
| CMV DNA | | | | | | |
| Negative | 46 | 0.68 | 78 | 0.86 | 90 | 0.34 |
| Positive | 33 | | 77 | | 100 | |

cGVHD, chronic graft *vs* host disease; CMV, cytomegalovirus. Group 1: before allo-HSCT; group 2: at the day + 100 after allo-HSCT; group 3: 1 year after allo-HSCT.
[a]GVHD staging in the oral mucosa and in the salivary glands was performed only in group 2.

leucocytes DNA is equivalent to $10^4$ cells (Boriskin *et al*, 2002), identification of CMV before engraftment (usually before day +30) limits early CMV diagnosis. Thus, the identification of CMV in the saliva could be advantageous to the diagnosis of reactivation of this virus on period before engraftment. As no sample collection was performed between these intervals, further studies are needed to elucidate the same. Prompt and accurate diagnosis of CMV infection might help prevent and monitor treatment of these infections (Yan and Fedorko, 2002).

Oral CMV shedding may result from primary infection, reinfection or reactivation (Kano and Shiohara, 2000). As almost all allo-HSCT patients were serologically positive test for CMV before transplantation, the presence of the virus in oral swabs may be the result of virus reactivation, reinfection, asymptomatic shedding or subclinical shedding, but rules out the hypothesis of primary infection. No attempt was made to differentiate each of these conditions.

In group 3 (at 1 year after HSCT), the median platelet count for patients positive for CMV (139 000 $mm^{-3}$) was lower than for patients negative for CMV (226 000 $mm^{-3}$). This might suggest that CMV reactivation could also influence long-term engraftment. Decreased or low peripheral blood counts are associated with CMV infection/disease (Dominietto *et al*, 2001). CMV has been shown to have an inhibitory activity on the haematopoietic system and this inhibitory activity could be direct, via infection of myeloid cells, or indirect, via infection of stromal cells (Torok-Storb *et al*, 1997). Delay in immunological reconstitution probably represents the major cause of late-onset CMV reactivation or disease (Castagnola *et al*, 2004).

Graft-*vs*-host disease and infectious complications represent major obstacles to the success and use of allo-HSCT for the treatment of haematological diseases and other disorders (Jones *et al*, 1996). Interactions between CMV and donor and/or host elements may result in the potentiation of responses that promote the development and/or expression of GVHD (Cray and Levy, 1993; Söderberg *et al*, 1996). There is a suggestion that development of GVHD after transplant might be a risk factor for CMV reactivation or disease (Machado *et al*, 2001). However, in this study, no association between chronic GVHD severity of salivary gland/oral mucosa

and CMV shedding in the oral mucosa of patients at the +100 day was found.

Survival analysis demonstrated significance only for salivary gland GVHD. This is in accordance with the view that patients without GVHD do not show graft-*vs*-leukaemia effect, being more susceptible to relapse of the primary disease (Basara *et al*, 2001). Multivariate analysis also showed that CMV shedding in the oral mucosa has no effect on survival after allo-HSCT. This result is similar to another study that demonstrated that high-grade CMV antigenaemia did not affect survival after HSCT (Asano-Mori *et al*, 2005).

In conclusion, despite the preliminary nature of the data, the present study shows that immunosuppression related to allo-HSCT affects CMV reactivation in the oral mucosa with an especially high incidence in patients at +100 days after transplantation. Further studies are necessary to delineate the systemic consequences of CMV shedding in the oral mucosa of HSCT patients and the importance of its identification in the saliva for early diagnosis of CMV reactivation (before engraftment) after allo-HSCT.

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