



## Monitoring of cytomegalovirus infection after allogeneic stem cell transplantation

### Praćenje infekcije citomegalovirusom posle alogene transplantacije matičnih ćelija hematopoeze

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#### Abstract

**Background/Aim.** More than 90% of worldwide population is infected with human cytomegalovirus (CMV), one of the most common agents which complicate immunocompromised patients. Viral infections, in particular CMV ones are still a major cause of mortality and morbidity after stem cell transplantation (SCT). Monitoring is performed by detecting CMV-Ag or virus DNA in peripheral blood. Risk factors are donor/recipient CMV status, type of transplant and acute graft versus host disease. The aim of the study was to determine the extent of validity of CMV infection monitoring after transplantation as a reliable parameter of further CMV replication course in patients with hematopoietic stem cell transplantation. **Methods.** A total of 49 patients with stem cell transplantation were studied prospectively during a 2-year period after transplantation for the presence of CMV DNA. Polymerase chain reaction (PCR) CMV DNA was performed on 222 full blood samples using Cobas Amplicor assay. **Results.** Activation of CMV was detected in 10/49 (20.48%) of the patients. The median posttransplantation time for the first positive PCR result was 6 weeks for the stem cell transplant patients. Viremia became negative in all the cases after the antiviral therapy with ganciclovir. **Conclusion.** Our data show that the level of CMV-DNA load at the time of initial CMV detection after transplantation could be a possible predictor for further course of CMV replication in patients receiving hematopoietic stem cell.

**Key words:** cytomegalovirus; transplantation, homologous; polymerase chain reaction.

#### Apstrakt

**Uvod/Cilj.** Citomegalovirus (CMV) spada u najčešće uzročnike infekcija kod imunokompromitovanih osoba, dok je više od 90% svetske populacije inficirano ovim virusom. Virusne infekcije, posebno infekcije sa CMV čest su uzrok morbiditeta i mortaliteta posle transplantacije matičnih ćelija (*stem cell transplantation* – SCT). Praćenje ovih infekcija sprovodi se detekcijom virusnog antigena ili virusne DNK u perifernoj krvi. U faktore rizika spadaju CMV status donora i recipijenta, tip transplantata i akutno odbacivanje grafta. Cilj ove studije bio je da se utvrdi u kojoj meri je praćenje CMV viremije posle transplantacije validan parametar daljeg toka CMV replikacije kod bolesnika posle transplantacije matičnih ćelija hematopoeze. **Metode.** U ovom 2-godišnjem ispitivanju praćeno je 49 bolesnika posle transplantacije matičnih ćelija hematopoeze na prisustvo CMV DNA. Ukupno 222 uzorka krvi ispitivano je testom PCR Cobas Amplicor. **Rezultati.** Aktivacija CMV virusa dokazana je kod 10/49 bolesnika (20,48%). Srednje vreme pozitivnog testa bilo je šest nedelja posle transplantacije matičnih ćelija. Viremija je bila negativna posle primenjene terapije ganciklovikom. **Zaključak.** Dobijeni rezultati pokazuju da je praćenje CMV viremije posle transplantacije dobar pokazatelj daljeg toka CMV replikacije kod bolesnika posle transplantacije matičnih ćelija hematopoeze.

**Ključne reči:** infekcija, cytomegalovirus; transplantacija, homologna; polimeraza, reakcija lanaca.

#### Introduction

Human CMV is a common infection with 40% to 100% of adults seropositive in different geographic and socioeconomic populations. In common with other herpesvirus infec-

tions, primary infection which is often asymptomatic in the healthy host is followed by latency. CMV latency is maintained in several tissues including mononuclear cells and salivary glands. Persistent viral excretion and recurrences often result from the reactivation of a latent virus, although

reinfection with another strain of CMV can sometimes occur, particularly in the immunocompromised individuals. In the immunocompromised host, a primary CMV infection, reactivation and reinfection can thus occur and are all associated with a significant morbidity and mortality. Although the immunocompromised populations at greatest risk of CMV infection and disease are those with deficient cellular immunity, this often accompanies neutropenia during the period of maximal bone marrow suppression in patients receiving chemotherapy for malignant disease and before bone marrow engraftment in a stem cell transplantation (SCT) recipient<sup>1</sup>.

In solid organ transplant recipients (whether they are seropositive or seronegative for CMV) the most common source of virus is a transplanted organ, even in those such as liver transplant recipients who receive large volumes of (potentially infected) blood by transfusions. In contrast, the maximal risk of CMV disease in SCT recipients is associated with reactivation of their own latent virus (i.e. in seropositive recipients).

Until recently CMV was the single most important pathogen in SCT (and, indeed, other transplant) recipients, occurring in about 50% of patients. The incidence in the early post-transplant phase is now decreasing markedly as a result of the widespread use of chemoprophylaxis<sup>2,3</sup>. The aim of the study was to determine the extent of validity of CMV infection monitoring of the transplantation as a reliable parameter of further CMV replication course in patients with hematopoietic stem cell transplantation.

## Methods

A total of 49 patients who had undergone SCT at the Department of Hematology, Military Medical Academy between 2006 and 2007 were included in the study. Out of that number 29 patients were male and 20 female. All recipients were CMV immunoglobulin G (IgG) positive before transplantation (R+). CMV positive donors were chosen for CMV positive recipients, while one patient was R+ but received an organ from a CMV IgG negative donor (D-).

Condition therapy in most patients was according to the disease and consisted either of busulfan (four doses of 1mg/kg of body mass on days -7 to -4) and cyclophosphamide (two doses of 60 mg/kg of body mass on days -3 and -2). Oral acyclovir was administered in all cases at dosage of 400 mg four times a day for prophylaxis of herpes simplex virus infection until day 100 after SCT. The CMV-seropositive patients received a transplant from a seropositive donor, and one patient received a transplant from a seronegative donor.

The recipients of an allogeneic bone marrow or peripheral blood stem cell transplant who were at least 9 years of age were screened for the presence of CMV in peripheral blood specimens. Screening was performed at least one weekly posttransplant for up to 12 months. In our institution first peripheral blood leukocytes were screened for pp65 antigenemia. Since 2005 CMV DNA screening by PCR (Roche Diagnostics) has been undertaken, in addition to pp65 antigenemia. Here are the data with PCR test.

Totally 222 whole blood samples were collected from 49 SCT patients. Collection of serial blood samples started within the first month posttransplantation. For this screening, we used PCR for CMV DNA in peripheral blood leukocytes. The patients were eligible for the study if CMV was detected by PCR assay within the first 100 days after SCT.

Two millilitres of the same whole blood samples were processed for the extraction of plasma and stored at -70°C for the PCR analysis. The PCR assay was performed by using a Cobas Amplicor CMV Monitor test platform. In this procedure, plasma DNA was extracted and the amplified product detected by hybridization to a labeled oligonucleotide probe which comprises a sequence of 365 nucleotides located in the amino terminus of the CMV DNA polymerase gene. A CMV quantitation standard amplicon was added to each specimen, and the final PCR product was measured by a colorimetric reaction with a lower detection limit of 400 copies/ml of plasma<sup>4,5</sup>.

A preemptive antiviral approach was based on the results of the PCR test. Ganciclovir therapy was initiated and terminated as seemed necessary by the physicians in charge.

The positive patients received intravenous ganciclovir at a dose of 5 mg/kg every 12 hours for 2-3 weeks.

If CMV was undetectable in peripheral blood by the end of induction treatment, the study was discontinued. The course was continued if screening remained positive or if a patient redeveloped antigenemia.

## Results

We retrospectively investigated the course of CMV-DNA load in plasma after SCT with regularly available follow-up samples transplanted between 2006 and 2007 in Military Medical Academy.

Indications for SCT were in accordance with the criteria shown in table 1 and most patients had stem cells from peripheral blood.

**Table 1**  
**Diagnostic indications for stem cell transplantation (SCT)**

Indications for SCT	No of patients
Acute myelogenous leukemia (AML)	15
Acute lymphoblastic leukemia (ALL)	9
Hodgkin disease (HD)	6
Non-Hodgkin lymphoma (NHL)	5
Multiple myeloma	4
Chronic myeloid leukemia (CML)	3
Aplastic anemia (AA)	3
Syndroma myelodysplasticum (MDS)	2
Solid tumors	2

A total of 222 blood samples from 49 patients who had undergone an allogeneic SCT (mean 5 samples per patient; range 2 to 9) were analyzed. CMV viremia was diagnosed in 10 (20.41%) patients by the PCR (10 of 222 samples). CMV infection was diagnosed by the PCR in a median of 42.8 days (range 27 to 62 days).

Viral load was between  $5.37 \times 10^2$  to  $1.34 \times 10^5$  copies/mL.

A total of 10 patients received ganciclovir therapy based on the results of the PCR test, while 9 patients had CMV disease with non specific symptoms and one D+R+ patient developed reactivation with gastrointestinal disease and relapsed.

Viremia became negative in all the cases after the anti-viral therapy with ganciclovir.

Higher levels of CMV DNA seemed to be associated with CMV-related symptoms and a significant decrease in viral load was observed following the anti-viral therapy.

Not all patients with CMV positive DNA and reactivation develop clinical disease. It is the degree and the rate of CMV replication that predict impending CMV disease and, thus, the need for specific treatment.

However, it is generally accepted that higher CMV DNA copy levels or an increasing trend in viral loads predict clinical progression to a disease or clinical relapse.

## Discussion

While management of CMV infection in the peritransplant period has improved in recent years, this herpes virus remains a significant cause of morbidity and mortality in both children and adults. Current strategies aimed at reducing CMV disease in transplanted patients are focused on a) identifying the most appropriate donor, b) universal antiviral prophylaxis, c) improved surveillance accompanied by preemptive therapy and d) therapy which encompasses both pharmacological and immune modulated therapy.

Allograft recipients are at particularly high risk for severe CMV disease during the phase of profound combined immunodeficiency, which usually lasts 3 to 4 months after SCT. The introduction of prophylactic or preemptive antiviral drug treatment during this early posttransplantation period resulted in a marked reduction of the incidence of CMV pneumonia<sup>6-8</sup>.

Although CMV disease after SCT may present merely as a non-specific febrile illness, viremic spread to involve the viscera is the commonest manifestation. In SCT and heart-lung recipients, CMV most commonly manifests as pneumonia, with a mortality of 30-60% in both groups<sup>9</sup>.

CMV causes a direct lytic infection of cells and tissue damage but there is a considerable evidence that immunologically mediated damage may be the major pathogenic mechanism of CMV pneumonitis.

Given the mortality attached to CMV disease, every attempt should be made to obtain the diagnosis early in the clinical course of disease. This not only allows therapy to be started promptly but also prevents unnecessary (and potentially toxic) therapy in those without CMV disease.

The quantity of CMV in blood correlates with the likelihood of developing CMV disease in SCT recipients but high viral loads, although temporally associated with disease, provide no prognostic information<sup>10</sup>.

Early detection of viremia can be made by an antigen assay or by the PCR. Polymerase chain reaction can be used for the detection of CMV in a variety of specimens, including blood, plasma and extracts of mononuclear cells.

In our study, the PCR-CMV-test-guided strategy for the initiating of preemptive therapy (any positive result) resulted in an incidence of CMV disease before day +100 of 20.48% rate.

This assay detect free virions in plasma with high level of sensitivity.

Because a low CMV viral load is highly significant in the context of SCT, the primary objective should be on improving sensitivity. Therefore, in our study detection >400 CMV DNA copies/mL (lower detection limit of Cobas Amplicor CMV Monitor test) were defined as positive results. In our patients viral load was between  $5.37 \times 10^2$  to  $1.34 \times 10^5$  cop/mL.

Our data from a 2-year clinical study are in agreement with those already reported by other authors<sup>11</sup>.

There are some results that have been reported from a major US transplant center where the incidence of CMV disease in allogeneic stem cell transplantation recipients during the first 100 days posttransplant fell from 38% to 5% over an approximate 19-year interval. In some European countries rates of CMV disease were 13% to 2.2%<sup>12</sup>.

The most common clinical manifestations of CMV disease in allogeneic bone marrow transplant (BMT) recipients are pneumonitis and gastrointestinal disease. We had one patient with gastrointestinal symptoms.

Principal clinical variables associated with high risk for CMV disease include a donor and recipients CMV status, donor-recipient histocompatibility, a conditioning regimen used for a recipient, and the type of immunosuppression used after transplant. The risk of early CMV infection (first 100 days after transplant) is relatively high in D+/R- BMT; it is also elevated in R+ transplants<sup>13,14</sup>.

The length of preemptive therapy of CMV infection selected for this trial was a maximum of 3 weeks in order to reduce the risk for adverse events, late CMV disease, and antiviral drug resistance. Previous data indicated that preemptive treatment based on sensitive CMV assays permitted initiation of therapy at an early stage of CMV infection, and treatment longer than 4 weeks was thus rarely necessary<sup>10</sup>. Furthermore, the risk for late CMV disease increases with the duration of prior antiviral drug therapy. Ganciclovir is a potent inhibitor of CMV replication, and the intended use of this agent is thought to impair the reconstitution of posttransplantation CMV-specific cellular immunity, which plays an important role in mediating protection from CMV infection and CMV disease<sup>15,16</sup>.

The best management strategy for CMV infection and disease in immunocompromised individuals depends upon several factors: the diagnostic tests available, a risk for disease and a likely outcome of disease. For BMT recipients risks for disease are high and the outcome is poor.

Our data show that the level of CMV-DNA load at the time of initial CMV detection after transplantation could be a possible predictor for the further course of CMV replication in a patient receiving hematopoietic stem cell.

## Conclusion

The success of CMV prevention during an early post-transplant period and the improvement of CMV disease management are partly attributed to the advancement in diagnostic

virology. The development and the widespread implementation of a sensitive, specific, and reliable diagnostic assay for CMV detection is essential in achieving these goals. Moreover, the introduction of viral load quantification has greatly improved the clinical utility of diagnostic virology.

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