# Significance of qualitative PCR detection method for preemptive therapy of cytomegalovirus infection in patiens after allogeneic hematopoietic stem cell transplantation – single-centre experience

D. ŠKAPOVÁ, Z. RÁČIL, D. DVOŘÁKOVÁ, D. MINAŘÍKOVÁ, J. MAYER

*Department of Internal Medicine – Hematooncology, e-mail: dskapova@ibp.cz, University Hospital Brno, Czech Republic*

**Received July 28, 2004**

Both early cytomegalovirus (CMV) monitoring and prophylactic antiviral therapy can decrease clinical complications or can prevent them in patients after allogeneic hematopoietic stem cell transplantation (HSCT). Presented paper summarizes experiences with using regular monitoring of reactivation of CMV after allogeneic HSCT by qualitative polymerase chain reaction (PCR) method to prevent the development of symptomatic CMV disease. Samples of peripheral blood leukocytes (PBL) in 71 patients were monitored. Because of retransplantations in two patients, 73 transplantations, each followed by the monitoring, were performed. Patients were monitored weekly after the transplantation for CMV DNAemia in PBL. An episode of CMV infection representing an indication for pre-emptive ganciclovir (GCV) or foscarnet (FOS) therapy was defined as two consecutive positive PCR results in 4–7 days. Median time of monitoring was 313 days. The CMV infection was found in 28/73 monitorings (38.4 %) and always was followed by pre-emptive therapy. One recurrence of CMV infection was observed in 4/28 (14.3 %) monitorings and two recurrences in 1/28 (3.6 %) monitorings. Presented approach resulted in complete prevention of overt CMV disease and this study enable to show that qualitative PCR method for determination of incipient CMV infection followed by pre-emptive therapy is suitable for preventing patients after allogeneic transplantation from CMV disease.

*Key words: cytomegalovirus, polymerase chain reaction, allogeneic hematopoietic stem cell transplantation*

Cytomegalovirus (CMV) represents a serious infectious complication in patients after allogeneic hematopoietic stem cell transplantation. The outbreak of active CMV infection can lead to life-threatening diseases, including intersticial pneumonia.

Many precautions have been taken in order to decrease the rate of CMV infection and CMV disease, including the administration of blood products of CMV-seronegative donors to CMV-seronegative recipients and prophylactic administration of acyclovir (ACV) or ganciclovir (GCV) [24]. GCV is the most often used antiviral agent because of its effective suppression of CMV replication. Administration of GCV to patients is accompanied frequently by serious side effects (nefrotoxicity and myelosuppression). However, only a subset of patients is at substantial risk for CMV infection and CMV disease. In addition, long-termed use of GCV is followed by more frequent occurrence of late CMV disease (>100 days after the allogeneic transplantation) [8, 10, 22, 23]. Therefore, the prophylactic administration of this agent is often replaced by pre-emptive therapy in all patients, during which antiviral agents are used only in high-risk patients. This pre-emptive therapy should prevent CMV disease progression in the majority of patients.

The application of pre-emptive therapy demands the use of a sufficiently sensitive, rapid and specific diagnostic method. In 1988 an assay for detection of CMV antigens in PBL was developed, consisting in monitoring of antigenemia [38, 39, 40], which enables regular monitoring of patients and prompt obtaining of results. Monitoring of antigenemia proved to be fast, sensitive and specific [2, 3, 27]. Nevertheless, the serious problem with antigenemia detection is that in some patients CMV disease occurs without prior antigenemia positivity, or the positivity preceeds the disease for only a brief period of time [3].

The use of PCR for the diagnostics of incipient CMV infection represents a possible solution of these problems because PCR method detects the CMV positivity earlier than antigenemia [3, 4]. In presented paper, experiences with the

use of regular monitoring of CMV reactivation after allogeneic HSCT by a qualitative one-round PCR method for preventing the development of symptomatic CMV disease are summarized. The PCR method was introduced into clinical practice, e.g., by EHRNST et al [11], LJUNGMAN et al [20, 21] and GRUNDY et al [16], who introduced the qualitative nested PCR technique for detection of CMV DNAemia in PBL. The method used in our study represents a modification of their approach. Its advantages consist in a shorter time interval for obtaining results and in better prevention of contamination connected with false positivity, because of only one-round PCR. Regular monitoring of reactivation of CMV after allogeneic transplantation by qualitative PCR method is a useful tool for the prevention of development of symptomatic CMV disease.

## Patiens and methods

*Patiens.* From January 1, 1999 to December 1, 2002, 71 consecutive patients after 69 allogeneic peripheral blood stem cell transplantations (PBSCT) and 4 bone marrow transplantations (BMT) (median age 44 years, age range 19–65) were included in the study. In two patients retransplantations were performed, and therefore, in total 73 monitorings were realized. Patient characteristics are shown in Table 1. As a prophylaxis against CMV infection and disease we used filtered leukocyte-depleted blood products [7] and administered acyclovir (ACV) (3x750 mg/day intravenously (i.v.) until day +30, then 4x800 mg/day per os (p.o.) until day +100) [32]. An episode of CMV infection was defined as two consecutive positive PCR results in 4–7 days. The diagnosed CMV infection represented indication for pre-emptive GCV or foscarnet (FOS) therapy. FOS was indicated if myelosuppression was present. GCV was given i.v. at a dose of 5 mg/kg of body weight twice a day, until two consecutive PCR negative results were obtained. Patients still PCR-positive after 3–4 weeks of therapy with GCV received FOS (60–90 mg/kg i.v. three times a day).

*Samples.* All 71 patients were monitored by PCR weekly for signs of CMV reactivation using PBL DNA. Blood samples for examination by PCR were taken at least until day 100 after transplantation. A total of 1619 PCR testings were carried out. Examinations were performed promptly and the results were known on the second day after the blood was taken. Five ml of peripheral blood (PB) in EDTA (10 ml in patients with leukopenia, i.e. less than $4x10^9$ white blood cells/l) were used for isolation of DNA. PBL were isolated by osmotic lysis of erythrocytes followed by washing with saline. $2x10^6$ leukocytes were used for isolation of DNA using commercial kit (DNA Blood Mini Kit, Qiagen, Hilden, Germany). DNA from $2x10^6$ leukocytes was eluted into 100 µl sterile milliQ water (if patients had lower counts of leukocytes, i.e. $<2x10^6$, DNA was eluted into 50 µl sterile milliQ water) and was stored at 4 °C.

*PCR detection.* The method of qualitative one-round PCR for detecting CMV-DNA in PBL represents a modification of the PCR technique used in the studies of EHRNST et al [11], LJUNGMAN et al [20, 21], and GRUNDY et al [16]. The primers used were from the conserved region of the exon 3 of the CMV major immediate-early (IE) gene (IEP/3A: 5’-GACCAAGGCCACGACGTT-3’, IEP/3B: 5’-TCTGCCAGGACATCTTTCTC-3’) [29]. A master mix solution consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl (GeneAmp PCR Buffer II, Applied Biosystems, Foster City, CA, USA), 2.0 mM $MgCl_2$ (Applied Biosystems), 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems), 200 µM deoxynucleotidetriphosphates (Roche Diagnostics GmbH, Mannheim, Germany) and 0.2 µM each specific primers (IEP/3A, IEP/3B). Five µl of DNA were used as the template for the PCR reaction. The PCR mixture was placed in a thermocycler (MJ Reasearch, PTC-200, Waltham, MA, USA). The parameters were as follows: the first denaturation for 10 minutes at 95 °C, 35 cycles of denaturation for 50 seconds at 95 °C, annealing for 50 seconds at 55 °C, and extending for 50 seconds at 72 °C, closed by 7 minutes at 72 °C. After finishing PCR, 10 µl of the PCR reaction mixture was loaded onto a 8 % polyacrylamide gel. The gels were stained with ethidium bromide and specific PCR amplification products (167 bp) were detected using an UV-transilluminator. Each PCR analysis included positive control (CMV strain AD 169 obtained from National Institute of Public Health, National Reference Laboratory for Herpes Virus, Prague, Czech Republic or samples with evidence of CMV positivity) and negative control (sterile milliQ water).

The sensitivity of the method was ascertained using a cloned product prepared from positive control. The detection limit was set up at 10 copies of CMV plasmid DNA/ total DNA from $10^5$ leukocytes.

## Results

Because of 2 patients with HSCT retransplantations out of 71 patients included in the study (see Table 1), a total of 73 monitorings of CMV DNAemia were performed. Median time of monitoring was 313 days. The CMV infection with pre-emptive therapy developed in 28/73 monitorings (38.4 %). We observed one recurrence of CMV infection in 4/28 (14.3 %) monitorings and two recurrences in 1/28 (3.6 %) monitorings. In each monitoring in which CMV positivity was found, pre-emptive therapy with GCV (30 cases) or FOS (5 cases, four as primary treatments and one as secondary treatment) was introduced. In all patients the pre-emptive therapy was successful: by week 4 from starting the therapy, all patients with CMV detected originally were again free of CMV positivity. Twenty patients died (20/71, 28.1 %), five patients from the progress of the basic disease, eight patients from infectious complications other than CMV, two patients from GVHD, three patients for other causes and two patients due to unknown etiology. No patient developed overt CMV disease.

**Table 1. Characteristics of patients at risk of developing CMV disease**

| | All patients at risk of developing CMV disease (n = 71) |
|---|---|
| Total no. of patients | 71 |
| No. of patients/ transplantations | 71/73 |
| Median age (range) | 44 (19–65) |
| Gender | |
| Female | 31 |
| Male | 40 |
| CMV serostatus before transplantation | |
| R+/D+ | 42 |
| R+/D– | 14 |
| R–/D+ | 7 |
| R–/D– | 10 |
| Diagnosis | |
| Acute myelogenous leukemia | 24 |
| Acute lymphoblastic leukemia | 5 |
| Chronic myelogenous leukemia | 22 |
| Chronic lymphoblastic leukemia | 3 |
| Aplastic anemia | 2 |
| Myelodysplastic syndrome | 2 |
| Non-Hodgkin´s lymphoma | 9 |
| M. Hodgkin´s lymphoma | 2 |
| Multiple myeloma | 2 |
| Other diagnosis | 2 |
| Conditioning | |
| BUCY2 | 29 |
| TBI+CY | 6 |
| Non-myeloablative | 33 |
| Other | 5 |
| Type of transplantation | |
| BMT | 4 |
| PBSCT | 69 |
| Type of donor | |
| HLA-identical sibling | 1 |
| Mini allogeneic | 35 |
| HLA-matched family | 29 |
| Unrelated donor | 8 |
| GVHD | |
| Acute GVHD | 27 |
| Chronical GVHD | 28 |

D – donor; GVHD – graft-versus-host disease; R – recipient; TBI – total body irradiation

## Discussion

Literature data show that effective prevention of CMV disease after allogeneic HSCT is obtained if GCV or other antiviral drug is given prophylactically or as pre-emptive therapy. Currently the most common strategies are universal prophylaxis with ganciclovir and hybrid strategies utilizing both prophylaxis and pre-emptive therapy in different patient groups [1, 35].

Long-termed prophylaxis with GCV in all patients after allogeneic transplantation has good effects in preventing CMV reactivation. However, administration of GCV is frequently associated with neutropenia, late onset of CMV disease, opportunistic infections and risk of the development of GCV-resistant CMV strains. Low-potency anti-CMV prophylaxis (acyclovir or valacyclovir) is useful for permitting active CMV replication at a low level and may possibly favour immunologic priming decreasing thus the risk of late CMV disease [31].

Various types of pre-emptive strategies to decrease the number of treated patients have been developed. Pre-emptive therapy based on sensitive and early-detection markers of CMV reactivation can determine patients with higher risk of infection and enable the use of the rather toxic GCV treatment only in a lower number of patients. In addition, short courses of pre-emptive GCV therapy do not lead to the development of CMV UL97 mutations [14]. The pre-emptive administration of antiviral drugs for a short time interval has a good response [36]. In our study, 73.3 % of PCR-positive results disappeared already after seven days of GCV therapy (results not shown).

Different strategies for CMV prevention in allogeneic HSCT programmes utilize a variety of diagnostic tests for CMV. Detection of CMV antigenemia and/or CMV DNA or RNA has become a very effective means for detection of CMV reactivation [1, 30, 45].

Earlier the detection of CMV antigenemia was tested in our center as a way out for contingent pre-emptive therapy, but later it was changed for PCR detection [25]. The particular disadvantages of antigenemia are the need of quick preparation of samples, lower sensitivity, worse correlation with the course of CMV infection, laborious procedure and the effect of human subjectivity [13, 15, 18, 25, 34]. When neutrophil function is impaired or the number of neutrophils is decreased, the antigenemia assay may not be sufficient to detect active CMV infection.

In our laboratory very good results with PCR-guarded pre-emptive therapy were obtained. We have been attempting to reach the maximum standardization of the method for the detection of CMV reactivation. At first samples of whole blood were used (data not published). Because of differences in leukocyte counts in patients after transplantation, in the presented study counting of leukocytes was introduced and standardized count ($2x10^6$) was used for isolation of DNA. Commercial kits for isolation of DNA were employed to reduce the risk of PCR contamination.

Table 2 summarizes all PCR data in patients who showed at least one positive finding of CMV DNAemia. At least one positive result was detected during 41/73 (56.1 %) monitorings. As stated in Patients and methods, two consecutive positive findings of CMV DNAemia represented an indication for starting the pre-emptive treatment. Under these conditions, pre-emptive therapy was introduced during 28 monitorings. Four patients underwent two cycles and one patient three cycles of pre-emptive treatment. In 13 patients, positive PCR results appeared only sporadically and were not succeeded by the therapy (Tab. 2). The initiation of antiviral therapy after detection of CMV DNA in two consecutive test-

**Table 2. Schema of all positive PCR results**

| Patient No. | < Day +100 | > Day +100 |
|---|---|---|
| 1. | ●○○○○ ○○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○ † |
| 2. | ○○○○○ ○○●○○ | ○○○○○ ○○○○○ ○○○ |
| 3. | ○○○○○ ○○○○○ ○○○○○ | ○○○○○ ○○○○● ○○○○○ ○○○○ |
| 4. | ○○○○○ ○○●●○ ○●●○ | ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○ |
| 5. | ○○○○○ ○○○○○ ●○○○● | ○○○○○ ●●○○○ ○○○○○ ○○○○○ ○○○○○ ○○○ |
| 6. | ○○○○○ ●●●●○ ○○○○ | ○○○○● ○○○●○ ○○○○○ ○○○○○ ○●●○● ○○○○○ ○○○ |
| 7. | ○●○○○ ○○○○● ●●●○○ ○○ | ●○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○ |
| 8. | ○○○○○ ○○●●● ○○○○ | ○○○○○ ●○○○○ ○○○○○ ○○○○○ ○○○○○ ○ |
| 9. | ○○○○○ ○○○●● ○○○○○ ○ | ○○○○○ ○○○○○ |
| 10. | ○●○○○ ●○○○○ ○○○ † | |
| 11. | ●○○○○ ●●○○○ ○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○ |
| 12. | ○○○○○ ○○○●● ○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○ † |
| 13. | ○○●○○ ○○○○○ ○○○○ | ○○○○○ † |
| 14. | ○○○○○ ○○○●● ○○○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○ |
| 15. | ○○●○○ ○○○○○ ○○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○● ○○○○○ ○○○○○ |
| 16. | ○○○○○ ●○●○ ●○○○○ | ○○○○† |
| 17. | ○○○○○ ○○○○○ ○● | ●○○○ ●○○○○ ○○○○○ ○○○○○ ○○○○○ ○○ |
| 18. | ○○○○○ ●●○○○ ○○○ | ○○○○○ ○○○○○ ○○○○○ ○○●○○ ○ |
| 19. | ○○○○○ ○●●●● ●○○○● | ●○○○ ○○○○○ ○○○○○ ○○ |
| 20. | ○○○●○ ○○○○○ ○○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○○ |
| 21. | ○○○○○ ○○●○○ ○○○●● | ○○○○○ ○○○○○ ○○○○○ ○○○○ |
| 22. | ○○○○○ ○○○●● ●●○○○ | ○○○○○ ○●○●○ ○○○○○ ○○○○○ ○○○○○ ○○ |
| 23. | ○○○○○ ●●○○○ ○○○○○ ○ | ○○○○○ ○●○○○ ○○○○○ ○○ |
| 24. | ○○○○○ ○○○○○ ○○○○ | ○○○○○ ○○○○○ ○●○○○ ○○○○○ |
| 25. | ○○○○○ ●○●○● ●○○●○ ○ | ○○○○○ ○○○○○ ○○○●● ○○○○○ ○○○○ |
| 26. | ○○○○○ ○○○●○ ○○○ | ○○○○○ ○○ |
| 27. | ○○○○○ ○○○●● ○○○ | ○○○○○ ○○○○○ |
| 28. | ○○○○○ ○○○○● ○○○○○ | ○○○○○ ○○○○○ ○○○○○ ○○○○○ ○○○ |
| 29. | ○○○○○ ○○●●● ○○○ | ○○○○○ ○○○○○ ○○○ |
| 30. | ○○○○○ ●● auto.Tx ○○○○○ ○ | |
| 31. | ○○○○○ ○○○○○ ●○○ | ○○○○○ ○○○○○ |
| 32. | ○○○○○ ○○●○ ○○○○ | ○○ |
| 33. | ○○○○○ ○○●●● ●●○○ | ○●●●● ○●●○○ ○○ † |
| 34. | ○○○○○ ○○○○● ●○○○ | ○○○○○ ○ |
| 35. | ○○○○○ ○○●●● ○○ | ○○ |
| 36. | ○○○○○ ●●●○○ ○●○ | ○○○○ |
| 37. | ○○○○○ ●●○○ ○●○ | |
| 38. | ○○○○○ ○●●○○† | |
| 39. | ○○○○● ○●○○● | |
| 40. | ●●○○○ ○○○ | |
| 41. | ○○○○● ●● | |

○ – negative result, ● – positive result, auto. Tx – autologous transplantation, † – death

ings within a week interval seems to be a better indication for pre-emptive therapy than only one positive result, because some percentage of patients included in the pre-emptive treatment may be overtreated and their immune system would withstand CMV infection without antiviral therapy [12, 20, 28, 34]. The CMV infection developed during 28/73 monitorings (38.4 %) and pre-emptive therapy was introduced after the second positive result in 34 cases. Our approach to introduction of pre-emptive therapy correlates well with other published data on patients after allogeneic stem cell transplantation [17, 28, 33, 34].

The ideal material for PCR (whole blood, leukocytes or plasma) is still questionable. Many studies tested plasma (or serum) as a material for PCR detection of CMV reactivation. It is documented that the use of plasma may lead to a loss of sensitivity and to later CMV detection in comparison with the use of leukocytes [6, 41,43]. In addition, CMV is known to be predominatly cell-associated, and circulating antibodies may restrict the presence of the virus to the cellular compartment [41]. Therefore we decided to use leukocytes as the most suitable sample material for our needs.

Recently several studies have been published using quantitative real-time PCR methods for CMV detection [5, 9, 15, 19, 26, 37, 44]. Real-time PCR assay enables to determine exactly the number of copies of viral DNA in a sample. However, it is rather expensive and several other problems, e.g. those of the determination of the best material for CMV detection or setting the thresholds of the risk of CMV disease, remain to be solved before the method is introduced widely into clinical practice.

To summarize our study, we show that the qualitative PCR-guided pre-emptive therapy is suitable for prevention of patients after allogeneic HSCT from CMV disease, because of its sufficient sensitivity, quick obtaining of results, low overtreatment of patients and financial accessibility. This statement is supported also by the fact that used approach resulted in complete prevention of CMV disease in our HSCT patients. No one patient developed overt CMV disease. Recently published results on real-time PCR [5, 9, 15, 19, 26, 37, 44] represent further improvement of PCR detection of CMV reactivation. Therefore, we consider to supplement the qualitative PCR detection of CMV by introducting the real-time PCR detection which will serve for evaluation of the effects of therapy with antiviral agents by determination of changes in virus load.

The authors would like to thank the laboratory personnel for providing the samples and medical, nursing and clinical staff for significant contribution to this study through their dedicated care of the patients.

## References

[1] AVERY RK, ADAL KA, LONGWORTH DL, BOLWELL BJ. A survey of allogeneic bone marrow transplant programs in the United States regarding cytomegalovirus prophylaxis and pre-emptive therapy. Bone Marrow Transplant 2000; 26: 763–767.

[2] BOECKH M, BOWDEN RA, GOODRICH JM, PETTINGER M, MEYERS JD. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogenic marrow transplantation. Blood 1992; 80: 1358–1364.

[3] BOECKH M, GOOLEY TA, MYERSON D, CUNNINGHAM T, SCHOCH G et al. Cytomegalovirus pp65 antigenemia - guided early treatment with ganciclovir versus ganciclovir at engraftment after allogenic marrow transplantation: a randomized double-blind study. Blood 1996; 88: 4063–4071.

[4] BOECKH M, GALLEZ-HAWKINS GM, MYERSON D, ZAIA JA, BOWDEN RA. Plasma polymerase chain reaction for cytomegalovirus DNA after allogenic marrow transplantation: Comparison with polymerase chain reaction using peripheral blood leukocytes, pp65 antigenemia, and viral culture. Transplantation 1997; 64: 108–113.

[5] BOECKH M, HUANG M, FERRENBERG J, STEVENS-AYERS T, STENSLAND L et al. Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. J Clin Microbiol 2004; 42: 1142–1148.

[6] BOIVIN G, BELANGER R, DELAGE R, BELIVEAU C, DEMERS C et al. Quantitative analysis of cytomegalovirus (CMV) viremia using the pp65 antigenemia assay and the COBAS AMPLICOR CMV MONITOR PCR test after blood and marrow allogeneic transplantation. J Clin Microbiol 2000; 38: 4356–4360.

[7] BOWDEN RA, SLICHTER SJ, SAYERS M, WEISDORF D, CAYS M et al. A comparison of filtered leukocyte-reduced and cytomegalovirus (CMV) seronegative blood products for the prevention of transfusion-associated CMV infection after marrow transplantation. Blood 1995; 85: 3598–3603.

[8] BROERS AE, VAN DER HOLT R, VAN ESSER JW, GRATAMA JW, HENZEN-LOGMANS S et al. Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. Blood 2000; 95: 2240–2245.

[9] CORTEZ KJ, FISCHER SH, FAHLE GA, CALHOUN LB, CHILDS RW et al. Clinical trial of quantitative real-time polymerase chain reaction for detection of cytomegalovirus in peripheral blood of allogeneic hematopoietic stem-cell transplant recipients. J Infect Dis 2003; 188: 967–972.

[10] DE MEDEIROS CR, MOREIRA VA, PASQUINI R. Cytomegalovirus as a cause of very late interstitial pneumonia after bone marrow transplantation. Bone Marrow Transplant 2000; 26: 443–444.

[11] EHRNST A, BARKHOLT L, LEWENSOHN-FUCHS I, LJUNGMAN P, TEODOSIU O et al. CMV PCR monitoring in leucocytes of transplant patients. Clin Diagn Virol 1995; 3: 139–153.

[12] EINSELE H, EHNINGER G, HEBART H, WITTKOWSKI KM, SCHULER U 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.

[13] FLEXMAN J, KAY I, FONTE R, HERRMANN R, GABBAY E et al. Differences between the quantitative antigenemia assay and the cobas amplicor monitor quantitative PCR assay for detecting CMV viraemia in bone marrow and solid organ transplant patients. J Med Virol 2001; 64: 275–282.

[14] GILBERT C, ROY J, BELANGER R, DELAGE R, BELIVEAU C et al. Lack of emergence of cytomegalovirus UL97 mutations conferring ganciclovir (GCV) resistance following preemptive GCV therapy in allogeneic stem cell transplant recipients. Antimicrob Agents Chemother 2001; 45: 3669–3671.

[15] GRISCELLI F, BARROIS M, CHAUVIN S, LASTERE S, BELLET D et al. Quantification of human cytomegalovirus DNA in bone marrow transplant recipients by real-time PCR. J Clin Microbiol 2001; 39: 4362–4369.

[16] GRUNDY JE, EHRNST A, EINSELE H, EMERY VC, HEBART H et al. A three-center European external quality control study of PCR for detection of cytomegalovirus DNA in blood. J Clin Microbiol 1996; 34: 1166–1170.

[17] KAISER L, PERRIN L, CHAPUIS B, HADAYA K, KOLAROVA L et al. Improved monitoring of cytomegalovirus infection after allogeneic hematopoietic stem cell transplantation by an ultrasensitive plasma DNA PCR assay. J Clin Microbiol 2002; 40: 4251–4255.

[18] KANDA Y, MINEISHI S, SAITO T, SEO S, SAITO A 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; 27: 437–444.

[19] LI H, DUMMER JS, ESTES WR, MENG S, WRIGHT PF et al. Measurement of human cytomegalovirus loads by quantitative real-time PCR for monitoring clinical intervention in transplant recipients. J Clin Microbiol 2003; 41: 187–191.

[20] LJUNGMAN P, LORE K, ASCHAN J, KLAESSON S, LEWENSOHN-FUCHS I 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.

[21] LJUNGMAN P, OBERG G, ASCHAN J, EHRNST A, LONNQVIST B et al. Foscarnet for pre-emptive therapy of CMV infection detected by a leukocyte-based nested PCR in allogeneic bone marrow transplant patients. Bone Marrow Transplant 1996; 18: 565–568.

[22] MACHADO CM, DULLEY FL, BOAS LS, CASTELLI JB, MACEDO MC et al. CMV pneumonia in allogeneic BMT recipients undergoing early treatment of pre-emptive ganciclovir therapy. Bone Marrow Transplant 2000; 26: 413–417.

[23] MACHADO CM, MENEZES RX, MACEDO MC, MENDES AV, BOAS LS et al. Extended antigenemia surveillance and late cytomegalovirus infection after allogeneic BMT. Bone Marrow Transplant 2001; 28: 1053–1059.

[24] MAYER J, KRAHULOVA M, VORLICEK J. Prophylaxis of the early comlications associated with allogenic transplantation of peripheral blood or bone marrow progenitors cells. Klin Onkol 1997; 4: 110–115 (in Czech).

[25] MAYER J, DVORAKOVA D, KRAHULOVA M, VORLICEK J, ADLER J. Diagnosis of cytomegalovirus infection with the

polymerase chain reaction and antigenemia in patients after allogenic peripheral stem cell transplantation–comparison of 318 paired samples. Cas Lek Cesk 1999; 138: 436–440 (in Czech).

[26] MORI T, OKAMOTO S, WATANABE R, YAJIMA T, IWAO Y et al. Dose-adjusted preemptive therapy for cytomegalovirus disease based on real-time polymerase chain reaction after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2002; 29: 777–782.

[27] NICHOLSON VA, WHIMBEY E, CHAMPLIN R, ABI-SAID D, PRZEPIORKA D et al. Comparison of cytomegalovirus antigenemia and shell vial culture in allogenic marrow transplantation recipients receiving ganciclovir prophylaxis. Bone Marrow Transplant 1997; 19: 37–41.

[28] PEGGS KS, PREISER W, KOTTARIDIS PD, McKEAG N, BRINK NS et al. Extended routine polymerase chain reaction surveillance and pre-emptive antiviral therapy for cytomegalovirus after allogeneic transplantation. Br J Haematol 2000; 111: 782–790.

[29] PORTER-JORDAN K, ROSENBERG EI, KEISER JF, GROSS JD, ROSS AM et al. Nested polymerase chain reaction assay for the detection of cytomegalovirus overcomes false positives caused by contamination with fragmented DNA. J Med Virol 1990; 30: 85–91.

[30] PREISER W, BRAUNINGER S, SCHWERDTFEGER R, AYLIFFE U, GARSON JA et al. Evaluation of diagnostic methods for the detection of cytomegalovirus in recipients of allogeneic stem cell transplants. J Clin Virol 2001; 20: 59–70.

[31] PREISER W, BRINK NS, AYLIFFE U, PEGGS KS, MACKINNON S et al. Development and clinical application of a fully controlled quantitative PCR assay for cell-free cytomegalovirus in human plasma. J Clin Virol 2003; 26: 49–59.

[32] PRENTICE HG, GLUCKMAN E, POWLES RL, LJUNGMAN P, MILPIED N et al. Impact of long-term acyclovir on cytomegalovirus infection and survival after allogenic bone marrow transplantation. Lancet 1994; 343: 749–753.

[33] QAMRUDDIN AO, OPPENHEIM BA, GUIVER M, MUTTON KJ, CHOPRA R. Screening for cytomegalovirus (CMV) infection in allogeneic bone marrow transplantation using a quantitative whole blood polymerase chain reaction (PCR) method: analysis of potential risk factors for CMV infection. Bone Marrow Transplant 2001; 27: 301–306.

[34] SCHULENBURG A, WATKINS-RIEDEL T, GREINIX HT, RABITSCH W, LOIDOLT H et al. CMV monitoring after peripheral blood stem cell and bone marrow transplantation by pp65 antigen and quantitative PCR. Bone Marrow Transplant 2001; 28: 765–768.

[35] SINGH N. Preemptive therapy versus universal prophylaxis with ganciclovir for cytomegalovirus in solid organ transplant recipients. Clin Infect Dis 2001; 32: 742–751.

[36] SINGHAL S, MEHTA J, POWLES R, TRELEAVEN J, HORTON C et al. Three weeks of ganciclovir for cytomegaloviraemia after allogeneic bone marrow transplantation. Bone Marrow Transplant 1995; 15: 777–781.

[37] TANAKA N, KIMURA H, IIDA K, SAITO Y, TSUGE I et al. Quantitative analysis of cytomegalovirus load using a real-time PCR assay. J Med Virol 2000; 60: 455–462.

[38] VAN DER BIJ W, SCHIRM J, TORENSMA R, WILLEM J, VAN SON WJ et al. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. J Clin Microbiol 1988; 26: 2531–2535.

[39] VAN DER BIJ W, TORENSMA R, VAN SON WJ, ANEMA J, SCHIRM J et al. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. J Med Virol 1988; 25: 179–188.

[40] VAN DER BIJ W, VAN DIJK RB, VAN SON WJ, TORENSMA R, PRENGER KB et al. Antigen test for early diagnosis of active cytomegalovirus infection in heart transplant recipients. J Heart Transplant 1988; 7: 106–109.

[41] VON MULLER L, HAMPL W, HINZ J, MEISEL H, REIP A et al. High variability between results of different in-house tests for cytomegalovirus (CMV) monitoring and a standardized quantitative plasma CMV PCR assay. J Clin Microbiol 2002; 40: 2285–2287.

[43] WEINBERG A, SCHISSEL D, GILLER R. Molecular methods for cytomegalovirus surveillance in bone marrow transplant recipients. J Clin Microbiol 2002; 40: 4203–4206.

[44] YUN Z, LEWENSOHN-FUCHS I, LJUNGMAN P, VAHLNE A. Real-time monitoring of cytomegalovirus infections after stem cell transplantation using the TaqMan polymerase chain reaction assays. Transplantation 2000; 69: 1733–1736.

[45] ZAIA JA. Prevention of cytomegalovirus disease in hematopoietic stem cell transplantation. Clin Infect Dis 2002; 35: 999–1004.