

PCR analysis of CMV in hematology patients

Can Özlü¹, Ülkü Ergene²

¹Department of Hematology, Kütahya Health Science University, Evliya Çelebi Training and Research Hospital, Kütahya, Turkey

²Department of Hematology, Health Science University, Bozyaka Training and Research Hospital, Izmir, Turkey

ABSTRACT

Objectives: In this study we aimed to analyze cytomegalovirus (CMV) deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) results in non-transplant patients.

Patients and methods: Although we do not currently perform bone marrow transplant (BMT), we conducted a retrospective analysis of CMV DNA PCR monitoring (twice-weekly) of 91 hospitalized patients (with 306 peripheral blood) (47 males, 44 females; mean age 62±2.3 year; range, 25 to 85 year). For this purpose, we reviewed CMV DNA PCR records of patients in our clinic.

Results: The results of CMV DNA PCR in 91 non-transplant patients are analyzed retrospectively in this study. CMV DNA PCR test results were positive in 10 patients (a total of 17 peripheral blood) while negative in 81 patients (a total of 289 peripheral blood). We did not begin antiviral therapy in patients with positive CMV DNA PCR results. Because it is incompatible with the patients' clinical CMV DNA PCR positive CMV infection. None of the 91 patients enrolled in the study are transplant recipients, and they did not receive alemtuzumab treatment. Three of 10 CMV DNA PCR-positive patients had multiple myeloma (MM) with renal failure. One of the MM patients was diabetic. Three patients were diagnosed with acute myeloblastic leukemia and received chemotherapy; two patients were diagnosed with immune thrombocytopenic purpura; one patient was diagnosed with chronic lymphocytic leukemia, and one patient received therapy for the diagnosis of aplastic anemia. They were given treatment protocols based on their diagnosis. None of our patients has specific clinical CMV infection clinical findings.

Conclusion: We conclude that close CMV DNA PCR monitoring in non-transplant hematology patients is not cost-effective. Even though it is not recommended for other hematology patients, close monitoring of CMV DNA PCR is still performed in many clinics in Turkey, and we suspect it is still performed in clinics abroad.

Keywords: Cytomegalovirus, hematology, polymerase chain reaction.

Cytomegalovirus (CMV) is a herpesvirus family member that is a common community infection, with approximately 80% of adults showing seropositivity to the virus. In allogeneic stem cell transplantation patient CMV prophylaxis, early diagnosis, and treatment are critical. Its relevance in other patient populations is less well understood. Historically, the incidence of CMV disease in non-transplant cancer patients was very low, as demonstrated by a large autopsy study conducted at the MD Anderson Cancer Center, which reported

a 0.4% frequency of CMV pneumonitis.^[1] However, a recent publication from the same center found that CMV disease was diagnosed in 2.9% of 2,136 patients.^[2] This review will briefly discuss the clinical results and main issues concerning CMV, and the current role of CMV deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) detection in hematology clinic patients.

Cytomegalovirus

Human CMV is a beta herpesvirus related to human herpesvirus 6 and human herpesvirus 7. Cytomegalovirus is a large virus with around 200 proteins. Cytomegalovirus has been found in a wide range of human cells, including endothelial cells, epithelial cells, blood cells including neutrophils, and smooth muscle cells.^[3,4] The presence of CMV in these cells may be due to active replication within the cell, phagocytosis of CMV proteins, or abortive

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Correspondence: Can Özlü, MD.

e-mail: cozlu20@gmail.com

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(incomplete) replication, and it most likely contributes to dissemination and transmission. Cytomegalovirus, like the other herpesviruses, remains in the human body for the rest of one's life after primary infection. There is little known about the site or mechanisms of CMV latency and persistence. Cellular immunity mediated by T cells is the most important factor in controlling CMV replication. The role of humoral immunity in the control of CMV replication is unclear. The innate immune system seems to be involved in the control of CMV replication as well.^[4]

Definitions

Cytomegalovirus disease: Cytomegalovirus is detected in an organ in a biopsy or samples from other invasive procedures (bronchoalveolar lavage [BAL], cerebrospinal fluid [CSF]) together with symptoms and/or signs from the affected organ by a test with appropriate sensitivity and specificity. For CMV retinitis, typical ophthalmologic findings are sufficient.^[5] Cytomegalovirus infection can appear as a primary infection, reinfection, or reactivation. Cytomegalovirus infection is becoming more common as the number of immunocompromised patients rises, especially in transplant recipients. In allogeneic bone marrow transplant (BMT) cases, CMV infection is a major concern, with 30 to 50% of cases displaying clinically significant infection.^[6]

Cytomegalovirus prophylaxis: Antiviral agents are administered to patients to prevent either primary CMV or recurrent infection.^[5]

Preemptive therapy: An asymptomatic CMV infection detected by a screening assay is treated with antiviral agents.^[5]

Methods for detection of cytomegalovirus

The serologic determination of CMV-specific antibodies (immunoglobulin [Ig]G and IgM) is useful in determining a patient's risk for CMV infection after transplantation, but it cannot be used to diagnose CMV infection or disease. This technique is no longer useful for diagnosing CMV in hematopoietic stem cell transplant (HSCT) recipients since CMV grows in tissue culture for several weeks. The shell vial (rapid culture/DEAFF) technique, in which monoclonal antibodies are used to detect CMV immediate-early proteins in

cultured cells, is not sensitive enough to use for routine blood monitoring but is highly useful in the diagnosis of CMV pneumonia on BAL fluid.^[7,8] The detection of the CMV pp65 in peripheral blood leukocytes (antigenemia) is a rapid and semiquantitative method of diagnosing CMV infection. A positive CMV pp65 assay is predictive for the development of invasive disease in transplant patients. The most sensitive method for detecting CMV is polymerase chain reaction (PCR). Quantitative PCR (qPCR) relies on CMV deoxyribonucleic acid (DNA) amplification and quantification while maintaining high specificity. High levels of DNA in the blood (whole blood or plasma) is a good predictor of CMV disease in HSCT recipients.^[9-12] Although PCR has been used on BAL fluid, viral-load cut-offs have not been defined, and although the sensitivity and negative predictive values are extremely high, the specificity and positive predictive values are unknown.^[13-19]

Risk factors and prophylaxis

Serologic tests are also helpful in determining the risk of acquisition of CMV. Cytomegalovirus-seropositive recipients are at the highest risk of developing CMV disease after transplant.^[20] Other risk factors for CMV infection after allogeneic HSCT include the use of high-dose corticosteroids, T-cell depletion, acute and chronic graft-versus-host disease (GVHD), and the use of mismatched unrelated donors.^[21-26] Kim et al.^[2] strongly recommend that more than 100 days of CMV monitoring be conducted following stem cell transplant (SCT), particularly in patients receiving donor lymphocyte infusions (DLIs) or with a history of CMV infection. Patients with these risk factors are more likely to develop late CMV infection, so more comprehensive and continuous monitoring should be considered a necessity.^[2] Ozdemir et al.^[27] analyzed the clinical factors associated with late CMV reactivation in a group of 269 consecutive allogeneic stem cell transplant recipients for hematological malignancies. They found that important risk factors included lymphoid diagnosis, the occurrence of GVHD, a greater number of episodes of early reactivation, persistent day 100 lymphopenia, and the use of a CMV seronegative donor graft.^[27] Several factors predict the development of late CMV disease,

and extended monitoring and antiviral therapy are recommended in patients with risk factors to reduce the risk. After autologous HSCT, approximately 40% of seropositive patients develop CMV infection.

Although CMV infection is rare following autologous HSCT, the outcome of CMV pneumonia is similar to that after allogeneic HSCT. CD34 selection, high-dose corticosteroids, and total-body irradiation or fludarabine as part of the conditioning regimen are all risk factors for CMV disease after autologous HSCT.^[4] Alemtuzumab is an anti-CD52 monoclonal antibody that causes lymphopenia in CD41 and CD81 cells that can last up to nine months after administration. When compared to matched controls who did not receive alemtuzumab, patients who received it had a higher rate of CMV infection.^[28,29]

Prophylaxis

Without prophylaxis, approximately 80% of CMV-seropositive patients develop CMV infection after allogeneic HSCT. Cytomegalovirus incidence has been reduced as a result of current preventive strategies.^[5] The use of sirolimus for GVHD prophylaxis seems to have a protective effect against CMV infection, possibly due to the inhibition of cellular signaling pathways co-opted by CMV during infection for viral protein synthesis.^[22,26] Nonmyeloablative conditioning regimens generally have been shown to have a lower rate of CMV infection and disease early after HSCT when compared to standard myeloablative regimens. However, by one year after HSCT, the risks of CMV infection and disease are comparable. Umbilical cord blood transplantation (CBT) is becoming a more popular HSCT technology.^[24,30] Because most infants are born without CMV infection, the transplanted allograft is almost always CMV-negative. The rate of CMV infection after CBT among CMV-seropositive recipients who do not receive antiviral prophylaxis ranges from 40 to 80%, with one study reporting a 100% infection rate.^[31-35] When patients receive prophylaxis with high dose valacyclovir after CBT, it does not seem that CBT entails a significantly greater risk of CMV infection and disease than does peripheral blood stem cell or bone marrow transplantation.^[26]

Preemptive treatment of cytomegalovirus

Today, with the use of preemptive ganciclovir therapy, CMV disease has become a more serious problem after day 100 following allogeneic HSCT.^[36,37] The risk varies considerably between centers, presumably due to factors related to patient and donor selection, as well as the choices of transplantation modalities used at each center (stem cell source, GVHD prophylaxis, and treatment, conditioning regimens).^[4] Late CMV infection is strongly associated with non-relapse mortality.^[27] Quantitative PCR assays for CMV DNA are increasingly being used to avoid unnecessary treatment of patients who are at low risk of disease progression due to their performance characteristics that allow the development of institution-specific viral load thresholds for the initiation of preemptive treatment. The initial viral load and the viral load kinetics have been reported to be important risk factors for CMV disease.^[16] Currently, several variations are used, making it difficult to establish validated universal viral load thresholds due to differences in assay performance and testing material (whole blood *vs.* plasma).^[38] If the preemptive therapy strategy is used, all patients who have undergone allogeneic HSCT should be monitored for CMV infection on a weekly basis until day 100 posttransplant. Although CMV infection is rare in D-/R- (donor negative/recipient negative) patients, routine monitoring was effective in identifying CMV infection and preventing disease in a large cohort.^[39] The ideal duration and frequency of CMV monitoring after HSCT is unidentified.^[29,30,32,37,40-42] Various durations of preemptive antiviral treatment have been explored. Most centers now continue antiviral treatment until the designated viral marker is negative and the patient has been on it for at least two weeks. If an assay less sensitive than PCR, such as the pp65 antigenemia assay is used, then preemptive therapy should be continued until two negative results are obtained.^[42] If a patient's PCR or pp65 antigenemia assay results are still positive after two weeks of therapy, treatment should be extended until the clearance is achieved. A low rate of viral load decrease has been shown to be a risk factor for later-occurring CMV disease.^[18]

Table 1. Patients with CMV DNA PCR positive results, diagnosis, and treatment, and also CMV DNA PCR results

Age/Sex	Diagnosis	Treatment	CMV DNA PCR (IU/mL)
65/E	Myeloma-chronic renal insufficiency	Bortezomib + dexamethasone	6
45/E	Aplastic anemia-diabetes mellitus	Dexamethasone	11, 12
49/E	Chronic lymphocytic leukemia	Fludarabine + rituximab + cyclophosphamide	12
25/K	ITP	Prednisolone	11
47/E	Multiple myeloma-diabetes mellitus-chronic renal insufficiency	Lenalidomide + dexamethasone	14, 5, 35, 6, 30
63/K	ITP	Prednisolone	1,167
72/E	Multiple myeloma-chronic renal insufficiency	Bortezomib + dexamethasone	171
34/E	Acute myeloid leukemia	7+3 Induction therapy	12, 9
34/K	Acute myeloid leukemia	7+3 Induction therapy	10
65/K	Acute myeloid leukemia	7+3 and HDAC	15, 8

CMV: Cytomegalovirus; DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; ITP: Immune thrombocytopenic purpura; HDAC: Histone deacetylase.

PATIENTS AND METHODS

Although we do not currently perform BMT, we conducted a retrospective analysis of CMV DNA PCR monitoring (twice-weekly) of 91 hospitalized patients (with 306 peripheral blood) (47 males, 44 females; mean age 62+2.3 year; range, 25 to 85 year). For this purpose, we reviewed CMV DNA PCR records of patients in our clinic. The study was conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

We reviewed the clinical charts of all patients (Table 1). Cytomegalovirus PCR test results were positive in 10 patients (a total of 17 peripheral blood), but negative in 81 of our patients (a total of 289 peripheral blood). In patients with positive CMV DNA PCR results, we did not begin antiviral therapy. Because it is incompatible with the patients' clinical CMV DNA PCR positive CMV infection. None of our 91 patients enrolled in the study were transplant patients, and they did not receive alemtuzumab treatment. Three out of 10 CMV DNA PCR positive patients were diagnosed with multiple myeloma (MM) with renal failure. One MM patient was diabetic. Three patients were diagnosed with acute myeloblastic leukemia and received chemotherapy; two patients were

diagnosed with immune thrombocytopenic purpura; one patient was diagnosed with chronic lymphocytic leukemia, and one patient received therapy for the diagnosis of aplastic anemia. They were given treatment protocols based on their diagnosis. None of our patients has specific clinical CMV infection clinical findings.

DISCUSSION

Cytomegalovirus infection causes severe disease, especially in immunocompromised patients, and can even result in death. Chemotherapeutic agents, such as TNF alpha-blockers and steroids, can cause immunosuppression.^[43] The results of CMV DNA PCR in 91 non-transplant hematology patients were examined retrospectively in this study. Patients who are CMV-seropositive occurring before planned allogeneic HSCT have a very high risk of mortality.^[44] After transplantation, a patient with documented pretransplant CMV disease should be closely monitored for CMV (i.e. twice-weekly) or given prophylaxis with ganciclovir or foscarnet.^[2] All allogeneic SCT patients, whether or not they receive CMV prophylaxis, should have their peripheral blood tested for CMV at least once a week using the CMV antigenaemia, quantitative PCR, or a technique for detecting CMV DNA. Monitoring and antiviral treatment of patients with a positive CMV test and symptoms consistent with CMV

infection is one management option for patients receiving alemtuzumab. Patients with high-risk autologous SCT might potentially benefit from preemptive therapy and monitoring.

In other hematology patients, routine monitoring and preemptive therapy are not necessary.^[45] Finally, while CMV DNA PCR monitoring and close follow-up are recommended in patients who have previously received alemtuzumab and/or undergoing stem cell transplantation, it is not recommended in other patients.

In conclusion, we revealed that our findings are consistent with the literature. We conclude that close CMV DNA PCR monitoring in non-transplant hematology patients is not cost-effective. Even though it is not recommended for other hematology patients, close monitoring of CMV DNA PCR is still performed for all hematology patients in many clinics in Turkey, and we suspect it is still performed in clinics abroad.

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