

# Peripheral blood leukocytes and serum nested polymerase chain reaction are complementary methods for monitoring active cytomegalovirus infection in transplant patients

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**BACKGROUND:** Human cytomegalovirus is an important cause of morbidity and mortality in immunocompromised patients. Qualitative polymerase chain reaction (PCR) has proven to be a sensitive and effective technique in defining active cytomegalovirus infection, in addition to having low cost and being a useful test for situations in which there is no need for quantification. Real-time PCR has the advantage of quantification; however, the high cost of this methodology makes it impractical for routine use.

**OBJECTIVE:** To apply a nested PCR assay to serum (sPCR) and to evaluate its efficiency to diagnose active cytomegalovirus infection compared with PCR of peripheral blood leukocytes (L-PCR).

**METHODS:** Samples of 37 patients were prospectively evaluated. An internal control was created and applied to sPCR to exclude false-negative results.

**RESULTS:** In total, 21 patients (57%) developed active cytomegalovirus infection. After analyzing the two methods for the diagnosis of active infection, higher sensitivity and negative predictive value of the L-PCR versus sPCR (100% versus 62%), and higher specificity and positive predictive value of sPCR versus L-PCR (81% versus 50% and 72%, respectively) were observed. Discordant results were observed in 11 patients who were L-PCR-positive but sPCR-negative for active cytomegalovirus infection, five of whom developed clinical symptoms of cytomegalovirus. Clinical symptoms were observed in 14 patients, 12 of whom were diagnosed with active infection by nested L-PCR ( $P=0.007$ ) and seven by nested sPCR ( $P=0.02$ ). Higher specificity and a positive predictive value for sPCR were observed.

**CONCLUSION:** Nested L-PCR and sPCR were considered to be complementary methods for the diagnosis and management of symptomatic cytomegalovirus infection.

**Key Words:** CMV; Nested PCR; Serum nested PCR; Transplant

Human cytomegalovirus (CMV) is an important cause of morbidity and mortality in immunocompromised patients, such as transplant recipients (1-3). Early diagnosis of disease in these patients is essential to initiate antiviral therapy, thus enabling prompt and appropriate treatment. Therefore, the availability of a sensitive test, capable of detecting active CMV infection, is crucial.

The limited resources available for public health care lead to the need for important considerations regarding simplicity and cost of diagnostic assays (4). Qualitative polymerase chain reaction (PCR)

**La réaction en chaîne de la polymérase sur leucocytes du sang périphérique et sur sérum sont des méthodes complémentaires pour surveiller une infection active à cytomégalovirus chez des patients greffés**

**HISTORIQUE :** Le cytomégalovirus humain est une cause importante de morbidité et de mortalité chez les patients immunodéprimés. Il est démontré que la réaction en chaîne de la polymérase (PCR) qualitative est une technique sensible et efficace pour définir l'infection active à cytomégalovirus, sans compter qu'elle est peu coûteuse et qu'elle est pratique dans les situations où la quantification est inutile. La PCR en temps réel a l'avantage d'inclure la quantification, mais en raison de son coût élevé, il est impossible d'y recourir systématiquement.

**OBJECTIF :** Appliquer la PCR sur sérum (PCRs) et en évaluer l'efficacité pour diagnostiquer une infection active à cytomégalovirus par rapport à la PCR sur leucocytes du sang périphérique (PCR-L).

**MÉTHODOLOGIE :** Les chercheurs ont effectué une évaluation prospective de 37 patients. Ils ont créé un contrôle interne et l'ont appliqué au PCRs en vue d'exclure les résultats faux négatifs.

**RÉSULTATS :** Au total, 21 patients (57 %) ont développé une infection active à cytomégalovirus. Après l'analyse des deux méthodes diagnostiques de l'infection active, les chercheurs ont remarqué la plus forte sensibilité et la valeur prédictive négative de la PCR-L par rapport à la PCRs (100 % par rapport à 62 %) et la plus forte spécificité et la valeur prédictive positive de la PCRs par rapport à la PCR-L (81 % par rapport à 50 % et 72 %, respectivement). Ils ont constaté des résultats discordants chez 11 patients dont la PCR-L était positive, mais la PCRs était négative à l'infection active à cytomégalovirus, dont cinq ont manifesté des symptômes cliniques. Les chercheurs ont remarqué des symptômes cliniques chez 14 patients; dans 12 cas, l'infection active a été diagnostiquée par PCR-L ( $P=0,007$ ) et dans sept, par PCRs ( $P=0,02$ ). Ils ont également observé que la PCRs avait une plus forte spécificité et une valeur prédictive positive.

**CONCLUSION :** La PCR-L et la PCRs étaient considérées comme des méthodes complémentaires pour diagnostiquer et prendre en charge l'infection à cytomégalovirus symptomatique.

has proven to be a sensitive and effective technique in defining active CMV infection, and is especially useful in situations in which there is no need for quantification in addition to having a low cost (5). Real-time PCR has the advantage of quantification; however, the high cost of this methodology makes it impractical for routine use (6).

Peripheral blood leukocytes (PBL) PCR and serum/plasma PCR (L-PCR and sPCR, respectively) are assays largely used in the diagnosis of active CMV infection. Some reports suggest that leukocyte-based tests are superior for detecting CMV DNA (7,8); however, due

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to its high sensitivity, detection of CMV DNA in peripheral leukocytes using PCR does not always correlate with the development of CMV disease. Other studies have shown that plasma/serum positivity is more correlated with active infection, in addition to presenting easier processing (9,10).

Serological analysis methodologies are considered to be useful for the verification of a history of exposure of the patient to human CMV (11). The determination of active human CMV infection in transplant patients through serological methods has limitations due to the intense immunosuppression to which patients are subjected (12). Studies confirm disability and absence of antibodies in immunosuppressed patients with symptomatic and disseminated disease (13). Therefore, serological analyses were not applied in the present study for the diagnosis of active human CMV infection in the post-transplant period.

The aim of the present study was to develop and apply a nested PCR assay for serum (sPCR) and to evaluate its efficiency to diagnose active CMV infection, compared with L-PCR – the PCR-based available method. Thus, statistical parameters of the test, such as sensitivity, specificity and positive and negative predictive values, were calculated, as well as the synthesis of an internal control, which was used in sPCR, enabling major test reliability for eliminating the presence of possible inhibitors and the exclusion of false-negative results.

## METHODS

### Patients and samples

A total of 37 patients were monitored in the present study – 20 underwent kidney transplant and 17 hematopoietic stem cell transplant. Peripheral blood and serum specimens were drawn weekly during the first month after renal transplant and, after this period, every two weeks until completing four months; only serum was collected from those who were submitted to hematopoietic stem cell transplant until bone marrow engraftment (leukocyte count  $>0.5 \times 10^9/L$  for two or more consecutive days and platelet count  $>20 \times 10^9/L$  for five consecutive days). After observing these criteria, the peripheral blood and serum collection continued every two weeks until the fifth month after transplant, resulting in a total of 400 peripheral blood samples – an average of 10 specimens collected per patient.

Approximately 4.0 mL of EDTA-anticoagulated whole blood was collected from each patient and used for extraction of nucleic acid from PBLs, as described below. Sera were collected in appropriate tube(s) and, after 30 min, were centrifuged and stored at  $-20^\circ\text{C}$  until processed. Thus, the presence of viral particles in serum was avoided due to cell lysis because of delayed sample processing.

Clinical information was collected from medical records and prospectively analyzed. Active CMV infection, CMV recurrence and CMV disease were defined according to the recommendations published by Ljungman et al (14). Active CMV infection was defined by observing two consecutive L-PCR and/or sPCR results within an interval  $\leq 30$  days (15,16). CMV-related diseases were defined as signs or symptoms of these disorders in association with a confirmation of CMV infection by laboratory tests. CMV disease was defined by immunohistochemical analysis of biopsy specimens as well as clinical signs and symptoms, such as unexplained fever ( $>38^\circ\text{C}$ ), leukopenia (white blood cell count  $<3.5 \times 10^9/L$ ) and/or thrombocytopenia (platelet count  $<100 \times 10^9/L$ ), gastrointestinal symptoms, arthralgia, hepatitis, enteritis, retinitis, pneumonitis, colitis, esophagitis and encephalitis. Probable CMV disease was defined as exhibiting clinical signs and symptoms without biopsy. Symptoms were not attributed to CMV if another cause was suspected or there was concomitant rejection.

The research was approved by the Research Ethics Committee of the Faculty of Medical Sciences, University of Campinas (São Paulo, Brazil).

### PBL nucleic acid extraction

Extraction of nucleic acid from PBLs was accomplished using the method by Schmidt et al (17) with adaptations for CMV-DNA extraction. One millilitre of 6% dextran solution was added to every 4 mL of

PBLs to separate them by gravity sedimentation. Contaminating erythrocytes were lysed using a solution containing  $\text{NH}_4\text{Cl}$  (8 g/L) and  $\text{NH}_4\text{HCO}_3$  (0.8 g/L) at  $4^\circ\text{C}$ . After alkaline lysis with NaOH and heat denaturing for 30 min at  $100^\circ\text{C}$ , samples were adjusted to pH 8.4 by adding 1 M Tris-HCl.

### Serum nucleic acid extraction

Serum nucleic acid extraction was accomplished using the method described by Di Pentima et al (18), with some modifications.

Aliquots of 100  $\mu\text{L}$  of serum were incubated with 200  $\mu\text{L}$  of  $2\times$  buffer at  $100^\circ\text{C}$ , for 5 min (composition: 0.1 M KCl; 0.02 M Tris-HCl, pH 8.3; 0.005 M  $\text{MgCl}_2$ ; 0.2 mg/mL gelatin; 0.9% Nonidet P-40; 0.9% Tween 20). After 10 min centrifugation at 14,000 rpm, the supernatant was transferred to another tube and stored at  $-20^\circ\text{C}$  until PCR amplification.

### CMV nested PCR

**Avoiding contamination:** Procedures to avoid contamination were strictly followed: DNA extraction was conducted in a positive pressure laminar flow hood; reaction mix preparation and the DNA samples extracted were aliquoted in different cabins; and the product electrophoresis was conducted in a separate room equipped with separate pipettes, aerosol resistant tips and reagents. In addition, negative (water) and positive (strain AD169 aliquot) controls were included in each PCR experiment.

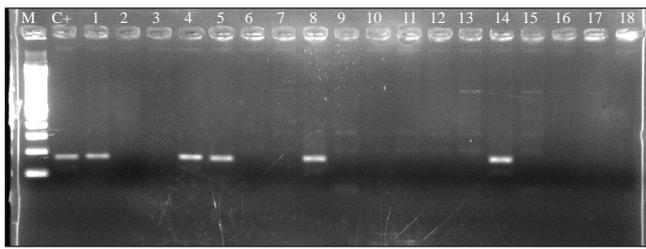
### Sensitivity

To determine the sensitivity of the nested PCR, a DNA fragment of 620 base pairs (bp) containing the immediate-early gene region 1, the same fragment amplified by the primers MIE-4 and MIE-5 and IE-1 and IE-2, was created from the AD169 strain and the primer set MIE' (MIE-1) 5'-GGTGCTCAGCACATTGATC-3' and MIE-5 5'-CAGCACCATCCTCCTCTTCTCTGG-3'. After cloning into a pGEM T easy vector (Promega, USA), the fragment was propagated in chemically competent *Escherichia coli* DH5 $\alpha$  cells. Plasmid DNA was purified from transformed cells using the Concert Rapid Plasmid Purification System (Life Technologies, USA) and quantified by spectrophotometric analysis at 260 nm, on the basis of plasmid size and the corresponding DNA mass. Plasmid dilutions, representing  $10^4$  to  $10^0$  copies of DNA, were used as a template. Similarly, the sensitivity of nested sPCR was determined using serum samples from CMV-seronegative patients.

### Nested L-PCR

To verify the integrity and quality of the DNA extracted, all samples were initially amplified using the primer set PCO3+ 5'-CCTCTGACACAAGTGTGTTCACTAGC-3' and PCO4+ 5'-TCACCACCAACTTCATCCACGTTACC-3' (19,20), according to the same reaction conditions of reagent concentrations in the first PCR reaction for detection of CMV-DNA by nested PCR, as described below. The samples were amplified for 30 to 35 cycles of DNA denaturation at  $94^\circ\text{C}$  for 45 s, followed by primer annealing at  $55^\circ\text{C}$  for 45 s and DNA extension at  $72^\circ\text{C}$  for 60 s. The initial cycle of PCR denaturation and final extension were conducted at  $94^\circ\text{C}$  for 5 min and at  $72^\circ\text{C}$  for 7 min, respectively. The amplification product consisted of a 110 bp sequence.

The outer primer set MIE-4 5'-CCAAGCGCCTCTGATAACCAAGCC-3' and MIE-5 5'-CAGCACCATCCTCCTCTTCTCTGG-3' (21,22), amplifying a 435 bp sequence; and the inner primer set IE-1 5'-CCACCCGTGGTGCCAGCTCC-3' and IE-2 5'-CCCGTCTCCTGAGCACCC-3' (22-24), amplifying a 159 bp sequence, were used for CMV nested PCR. All four primer sequences are complementary to the immediate-early gene region 1. The reaction mixture consisted of: 0.8  $\mu\text{L}$  of DNA, 2  $\mu\text{L}$  of buffer  $10\times$  (composition: 500 mM KCl, 200 mM Tris-HCl, pH=8.4), 1.5 mM  $\text{MgCl}_2$ , 2  $\mu\text{L}$  of each deoxynucleotide triphosphate (dATP, dCTP, dTTP and dGTP), 1.5 mM, 2  $\mu\text{M}$  of each primer and 0.5 U *Thermus aquaticus* (Taq) DNA polymerase recombinant (Life Technologies,



**Figure 1)** Amplicon seen in reaction amplification through peripheral blood polymerase chain reaction ('L-PCR'); M: 100 bp DNA ladder; C+: Positive control, lanes 1, 4, 5, 8 and 14: positive samples; 2, 3, 6, 7, 9–13, 15–17: negative samples; 18: water (blank)

USA) to a final volume of 20  $\mu$ L. The tubes were covered with mineral oil to prevent evaporation, and PCR was conducted in an automated thermal cycler (Robocycler<sup>TM</sup> 40, Stratagene, USA). The amplification reactions were run for 30 to 35 cycles of DNA denaturation at 94°C for 60 s, followed by primer annealing at 55°C for 60 s and DNA extension at 72°C for 90 s. The initial cycle of PCR denaturation and final extension were performed at 94°C for 5 min and at 72°C for 7 min, respectively.

In the nested PCR step (second reaction), 0.6  $\mu$ L of the product from the first amplification was added to a new reaction mixture with 2  $\mu$ M of each inner primer (IE-1 and IE-2) and amplified with 30 to 35 cycles, changing to 94°C for 40 s, followed by primer annealing at 57°C for 40 s and DNA extension at 72°C for 90 s.

The nested PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide, and the results were photographed using a Kodak Gel Logic 100 Imaging System over ultraviolet light, after staining with ethidium bromide (0.1  $\mu$ g/mL).

#### Nested sPCR

To verify the presence of inhibitors of *Taq* DNA polymerase by nested sPCR, a recombinant molecule of 377 bp (internal control) was constructed, in accordance with Zipeto et al (25), with the primer set 1 5'-CAAGCGCCTCTGATAACCAAGCCGGTTAT TGTCTCATGAGCGG-3' and 2 5'-CAGCACCATCCTCCTCTT CCTCTGGTGCTCTGATGCCGCATAGTT-3', containing the complementary regions of the MIE-4 and MIE-5 primers and 0.025  $\mu$ g of the plasmid PGEM-4Z (Promega, USA).

The samples containing DNA (5  $\mu$ L of alkaline lysates) were then initially amplified in the presence of an aliquot (1  $\mu$ L) of the molecule synthesized using the same reagent concentrations under amplification conditions of the first L-PCR reaction but in a final volume of 50  $\mu$ L.

Only the positive internal control serum samples were submitted to nested PCR for CMV DNA detection, following the same parameters of the reaction mixture and L-PCR amplification, with a final volume of 50  $\mu$ L and 5  $\mu$ L of alkaline lysates.

#### Diagnostic criteria

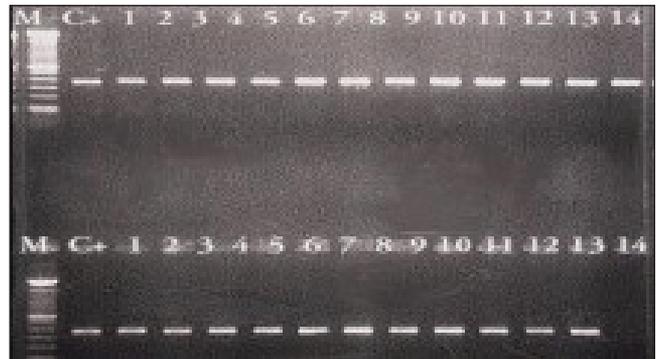
Active CMV infection was defined based on the following criteria: two or more consecutive positive L-PCR and sPCR results. For the diagnosis of CMV disease, the active infection had to be accompanied by clinical symptoms and histopathological identification of CMV. Recurrence of CMV infection was defined as active CMV infection occurring after negative L-PCR and sPCR following treatment of the initial episode of infection. Late active CMV infections and diseases were defined as those occurring more than 100 days after transplant.

#### Statistical analysis

The laboratory results were compared with the presence/absence of CMV-related disease by calculating the following four statistical parameters: sensitivity; specificity; positive predictive value; and negative predictive value (26). The Fisher's exact test was used for comparison between groups. The degree of concordance between the methods was



**Figure 2)** Amplicon seen in reaction amplification by serum polymerase chain reaction ('s-PCR'). M: 100 bp DNA ladder; C+: Positive control (strain AD169); Lanes 1–14, 16–19: negative samples; 15: positive samples; 20: water (blank)



**Figure 3)** Internal control (377 base pair amplicon) to verify the presence of inhibitors in serum samples. M: 100 base pair DNA ladder; C+: Positive control, lanes 1–14 (above) and 1–13 (below): positive internal control serum samples, absence of inhibitors; 14: water (blank)

determined using the kappa coefficient (results were interpreted according to the criteria established by Landis and Koch [27]).

## RESULTS

The target CMV DNA was detected at the level of  $10^2$  by single PCR and  $10^1$ , equivalent to 10 copies of target DNA, by nested PCR. Although 21 of 37 patients (57%) developed active CMV infection, all of these were positive according to L-PCR ( $P=0.0003$ ) (Figure 1). Only 10 PCR-positive serum patients (Figure 2) had active CMV infection (10 of 37 [27%];  $P=0.01$ ). Serum samples that showed no amplification of the internal control (377 bp fragment), due to possible presence of PCR inhibitors, were extracted again, and then evaluated for the presence of CMV DNA (Figure 3). Discordant results were observed in 11 patients who were L-PCR-positive but sPCR-negative for active CMV infection, five of whom developed clinical symptoms of CMV. The observed kappa coefficient of agreement for both assays was 0.44 (moderate agreement). Sixteen of 37 patients (43%) did not develop active CMV infection; however, two of these developed clinical symptoms of probable CMV disease and one presented later confirmatory biopsy for CMV disease.

Clinical symptoms were observed in 14 patients, 12 of whom were diagnosed with active infection by L-PCR ( $P=0.007$ ) and seven by sPCR ( $P=0.02$ ) (Table 1). Absence of clinical symptoms was observed in nine patients diagnosed with active CMV infection by L-PCR, and three patients diagnosed by sPCR (kappa coefficient 0.57, moderate agreement). Of the 14 symptomatic patients, two CMV-seronegative patients who received seropositive kidneys developed primary infection. Analyzing the two methods for the diagnosis of active infection, a higher sensitivity and a higher negative predictive value for was observed L-PCR (L-PCR 100% versus sPCR 62%), and a higher specificity and positive predictive value was observed for sPCR (sPCR 81% versus L-PCR 50% and 72%). The values of L-PCR- or sPCR-positive tests to predict CMV disease were 57% and 70%, respectively, and the value of negative tests to predict that CMV disease would not develop

**TABLE 1**  
**Chronology of cytomegalovirus infection detected in peripheral blood leukocytes (PBL) – nested polymerase chain reaction (PCR) and serum PCR and clinical manifestations**

Patient	Clinical manifestations	Day post-transplant	
		PBL – nested PCR (+)	Serum PCR (+)
1	Prolonged fever, liver enzyme alterations	8, 20, 27	8, 13, 20, 27, 34, 48
2	Fever, leukopenia	12, 21, 27, 33	5, 21, 27, 33, 47
3	Thrombocytopenia, liver enzyme alterations	7, 12, 19, 26	–
4	Renal function alteration, thrombocytopenia	18, 25, 38, 52, 59, 66, 73, 80	11, 18, 25, 38, 73, 126
5	Fever, myalgia, liver enzyme alterations, leukopenia	42, 52, 58, 70, 105	52, 58, 105
7	Fever, liver enzyme alterations, leukopenia	36, 50	50, 57
8	–	41, 48	48, 55
9	–	42, 49, 65	29, 42, 49, 65, 77
10	Liver dysfunction, renal function alteration	4, 17, 26	26
12	Fever, leukopenia	39, 46, 53	–
14	Pancytopenia	12, 19, 26	19
16	–	68, 80, 87, 94, 110	0, 68, 80, 87, 110
17	Leukopenia	48, 56, 62, 77, 84	48, 56, 62, 77, 84
21	–	32, 46, 61, 75, 119	–
22	Arthralgia	29, 47, 62, 77, 89, 105, 110, 117	62, 77
24	–	42, 63, 69	–
25	Chronic mucositis in esophagus, stomach and duodenum (gastric biopsy positive for cytomegalic disease: gastroduodenitis and acute esophagitis by cytomegalovirus)	–	–
27	–	29, 42	8, 42
28	–	0, 21, 35	–
30	–	48, 69, 84	–
33	Hepatosplenomegaly, liver insufficiency, hepatic encephalopathy	32, 40, 45, 47	–
35	–	18, 41	–
37	Oral herpetic mucositis, febrile neutropenia, acute esophagitis (gastric biopsy: acute esophagitis with alterations suggestive of herpesvirus)	–	–

*Patients 6, 11, 13, 15, 18, 19, 20, 23, 26, 29, 31, 32, 34 and 36 had no active infection or clinical symptoms of cytomegalovirus*

was 88% for L-PCR and 74% for sPCR. The comparative analysis between the first positive results for the presence of active CMV infection and the onset of symptoms showed that the L-PCR test preceded the onset of clinical symptoms by a mean of 19 days in eight patients. In two patients, the sPCR test preceded the L-PCR test by a mean of seven days for detection of symptomatic CMV infection.

## DISCUSSION

The search for establishing a better correlation between the detection of CMV viral genome and disease development has contributed to the improvement of amplification techniques (28). Questions related to the type of sample that better reflects active infection have also motivated scientific research (29).

Methodologies based on quantification of viral load, such as real-time PCR, have advantages including shorter time to results and lower possibility of contamination (5). However, these methods have elevated costs (4) – both the devices and the reagents necessary for their application (6). Regarding cost analysis, the nested PCR presents advantages when compared with real-time PCR; however, the high sensitivity associated with the method used to detect CMV DNA from leukocytes limits the clinical application of the technique because viral DNA may be detected in seropositive patients in the absence of disease (30).

The detection of CMV DNA from plasma and serum of immunocompromised patients suggests the existence of an alternative mechanism for virus dissemination within the host; plasma and serum, therefore, may be considered sources of material for detection of infection disseminated by CMV (31). Although there is no a consensus in the literature concerning the presence of CMV DNA in these clinical samples, they have been widely used in viral diagnosis (32,33).

Analyzing the two methods in relation to the detection of active infection and probable CMV disease, we verified that the sensitivity and specificity values, as well as positive predictive and negative predictive values corroborate the literature data. High sensitivity in the detection of CMV DNA and low specificity for disease diagnosis are associated with PCR in leukocytes (34,35), justifying the 100% sensitivity and negative predictive value obtained in the diagnosis of active infection, but specificity of 50%, because positive results were observed in eight of 16 patients without active infection. sPCR was less sensitive compared with L-PCR for the diagnosis of active infection, with a 62% sensitivity and negative predictive value; however, it demonstrated greater specificity, with specificity and positive predictive value of 81%. The higher sensitivity of PCR from leukocytes, compared with the use of serum in the present study, agrees with results reported in the literature (7,8,36).

Isolated positive results observed in the present study were detected by L-PCR and sPCR, which did not characterize active infection; however, they were not considered false positives because the necessary conditions to avoid contamination of samples during amplification reactions were stringently observed. The detection of CMV DNA by PCR in the plasma of patients who have undergone bone marrow transplant (7) confirms the possibility of virus replication in other sites than polymorphonuclear cells and, perhaps, endothelial cells, justifying some isolated positive results observed in serum samples (8,37).

Sixteen patients did not show active infection. Of these, 14 remained asymptomatic during the entire follow-up period; however, two of these 16 patients developed symptoms suggestive of infection by CMV. One patient (patient 25) was gastric biopsy positive for cytomegalic disease, while the other (patient 37) had a gastric biopsy suggestive of herpesvirus. However, it was not confirmed whether CMV was the pathogenic cause of infection. The two patients were treated with gancyclovir. These results are in accordance with the literature. Zipeto et al (31) observed negativity in DNA samples extracted from leukocytes of a patient who developed CMV disease, inferring that infections located in highly vascularized sites contribute to the absence of infection in PBLs.

For the diagnosis of probable disease, a higher sensitivity of L-PCR (86% versus 50% sPCR) and a higher negative predictive value (88% versus 74% sPCR) were also observed; however, a lower specificity (61% versus 87% sPCR) and positive predictive value (57% versus 70% sPCR) were also observed. Abecassis et al (38,39) found similar results and affirmed that qualitative PCR has a low positive predictive value for the development of disease. However, presenting results similar to ours, the detection of CMV DNA in plasma was considered by

Spector et al (9) as the most convincing risk sign of development of CMV disease. L-PCR detected active infection by CMV in nine of 16 patients who did not present clinical manifestations of probable CMV disease. These results corroborate those in the literature, because viral replication with high levels of viral charge is observed in patients who are asymptomatic for CMV (37 patients), which justifies the positive results observed that characterize active infection in nine asymptomatic patients. Five patients were diagnosed with active infection only by L-PCR (patients 3, 10, 12, 17 and 36). Therefore, sPCR presented a sensitivity of only 50% when detecting active infection in only seven of 14 patients who presented clinical manifestations.

According to the literature, PCR application in plasma is useful in situations of absolute neutropenia (7,34). We observed that, of 31 samples in which L-PCR application was not possible (not only due to neutropenia), four of 31 (13%) presented detectable CMV DNA in the serum, confirming the usefulness of this technique situations in which PCR of leukocyte DNA cannot be performed (40). The use of a recombinant DNA molecule as an internal control enabled the detection of the inhibitor effect in the sPCR in 13 of 101 samples of serum tested, which is equivalent to 13%.

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## CONCLUSION

L-PCR and sPCR were considered as complementary methods for the diagnosis and management of symptomatic CMV infection. The internal control used in sPCR allowed a greater reliability for testing and excluding false-negative results.

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