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Comparison of quantitative competitive polymerase chain reaction–enzyme-linked immunosorbent assay with LightCycler-based polymerase chain reaction for measuring cytomegalovirus DNA in patients after hematopoietic stem cell transplantation

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Abstract

Development of highly sensitive quantitative assays for cytomegalovirus (CMV) DNA detection is crucial for identification of immunodeficient patients at high risk of CMV disease. We designed 2 internally controlled competitive quantitative assays, enzyme-linked immunosorbent assay (ELISA)-based and real-time polymerase chain reaction (PCR) tests, using amplification of the same segment of the CMV genome. The aim of this study was to compare sensitivity, specificity, and laboratory performance characteristics of these assays. In both assays, a 159-bp segment of UL83 gene was amplified. External and internal controls were constructed by cloning the amplification product and heterogenous DNA segment flanked by target sequences for CMV-derived primers into bacterial plasmids, respectively. Real-time PCR was performed on LightCycler (Roche Diagnostics, Mannheim, Germany), and amplicons were detected using fluorescence resonance energy transfer probes. Alternatively, PCR products were labeled by digoxigenin, hybridized to immobilized probes, and detected by ELISA. The assays were tested on genomic DNA isolated from laboratory strains of CMV, QCMD control panel, and CMV DNA-positive peripheral blood DNA samples from hematopoietic stem cell transplant recipients, previously characterized by pp65 antigenemia and qualitative nested PCR. Real-time and ELISA-based PCR assays showed a linear course of $1–10^8$ and $10–10^5$ copies of CMV DNA per reaction, respectively. When compared with ELISA-based PCR, real-time PCR showed superiority in inter- and intra-assay reproducibility. Both assays were highly specific in detecting CMV DNA. No difference in amplification efficiency of internal or external standards and wild-type CMV DNA was found. The assays exhibited 83% concordance in CMV DNA detection from clinical samples, all discrepant samples having low CMV DNA copy numbers. There was a good correlation between viral DNA loads measured by the 2 assays. Statistically significant correlation was observed between the numbers of CMV DNA copies and pp65-positive leukocytes in the samples tested. Both variants of competitive PCR are adequately sensitive to be used for CMV DNA quantitation in clinical samples. LightCycler PCR, having superior performance characteristics and being less time-consuming, seems to be more suitable for routine diagnosis.
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Keywords: CMV; Quantitative competitive PCR; Real-time PCR; Hematopoietic stem cell transplant recipients

1. Introduction

Cytomegalovirus (CMV) infection is still a major cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients. Although antiviral prophylaxis has contributed to a reduction of CMV disease in these patients, the toxicity of currently available antiviral agents (i.e., ganciclovir, foscarnet, and cidofovir) remains a significant problem. Therefore, scientists focused effort on developing a highly sensitive and quantitative detection methods to identify all patients at risk for the disease before its onset, thereby focusing antiviral treatment on these patients ("preemptive" therapy).

These quantitative methods allow determining the viral load that is indicative not just of the CMV presence, but also of the degree of active viral replication. It appears to be a prognostic marker for assessing disease progression, and it

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is also valuable for monitoring the efficacy of antiviral therapy and for predicting treatment failure, often due to the emergence of drug-resistant CMV strains in patients on long-term antiviral treatment.

Various settings of real-time polymerase chain reaction (PCR) assay using LightCycler instrument have been published, but a major drawback for most of these assays is the lack of internally controlled amplification, leading to false-negative results. Many inhibitors to the PCR (such as hemoglobin, lactoferrin, leukocyte DNA, added anticoagulants, immunoglobulin G [IgG] in plasma, etc.) may be present in clinical samples and consequently affect the amplification reaction (Al-Soud and Radström, 2001). To overcome this drawback, we have designed a real-time quantitative competitive PCR assay, using competitive coamplification of internal standards (ISs). Data from LightCycler-based PCR assay and quantitative competitive PCR–enzyme-linked immunosorbent assay (ELISA) developed previously in our laboratory were compared.

The aims of this study were to compare these 2 quantitative competitive PCR assays and to evaluate their diagnostic significance in measuring CMV DNA levels in peripheral blood of HSCT patients. CMV DNA was retrospectively quantitated in consecutive blood samples from 14 HSCT recipients, which experienced CMV reactivation in the early posttransplant period.

2. Materials and methods

2.1. Patients' specimens and controls

Adult patients who underwent allogeneic HSC transplantation in the Transplantation Unit of Institute of Haematology and Blood Transfusion, Prague, Czech Republic, in 2000–2002 were followed up at weekly intervals during their hospital stay and afterward. Virologic examination included pp65-antigenemia test and the detection of CMV DNA in peripheral blood using qualitative nested PCR. Samples of DNA isolates from blood were archived at −70 °C. Fourteen patients who became positive for laboratory markers of CMV reactivation in at least 1 of the tests during the convalescent period were enrolled in the study. Control DNAs of herpes simplex virus (HSV) 1, HSV2, and varicella zoster virus (VZV) were isolated from laboratory strains of the viruses (strains 67/20, 610, and M, respectively; donated by Dr Kutinova, Prague, Czech Republic), which had been grown on primary human fibroblasts. Epstein–Barr virus (EBV) DNA was prepared from lymphoblastoid cell line Raji, latently infected with the virus. Human herpesvirus (HHV) 6 and HHV8 DNAs were purified from the cultures of J-Jhan cells infected with HHV6 and BCP1 cells infected with HHV8. The infected cell lines were kind gifts from Dr Cinatl, Hamburg, Germany, and Dr Weiss, London, UK, respectively. The amount of DNAs used in PCRs ranged from 10^4 to 10^5 copies per reaction.

2.2. Methods

2.2.1. pp65-Antigenemia test

Cytospin preparations of patient peripheral blood mononuclear cells (PBMCs) were fixed in 5% formalin with 2% sucrose in phosphate-buffered saline (PBS) and permeabilized in 1% Igepal (Sigma; Aldrich, Prague, Czech Republic) and 10% sucrose in PBS. CMV lower-matrix protein pp65 was stained in indirect immunofluorescence test using a monoclonal antibody against pp65 (Clonab, Biotest, Dreieich, Germany) and swine antimouse IgG–fluorescein isothiocyanate conjugate (Sevac, Prague, Czech Republic). The test was considered positive when more than 5 pp65-positive cells per 2×10^5 PBMCs displayed brilliant green fluorescence in the nuclei (Gerna et al., 1992).

2.2.2. DNA isolation

DNA was isolated from buffy coats (BCs) using the QIAamp DNA blood minikit (Qiagen, Hilden, Germany) and eluted with 50 μL of elution buffer.

2.2.3. Qualitative nested PCR

The specific CMV primers amplifying the region of UL55 gene encoding envelope glycoprotein gB were used (outer primers: 5′-TCCAACACCCACAGTACCCGT-3′, 5′-CGGAAACGATGGTGTAGTTCG-3′; inner primers: 5′-GTCAAGGATCAGTGGCACAGC-3′, 5′-GTAGCTGGCATTGCGATTGGT-3′) (Schäfer et al., 1993). Amplification reactions were carried out in a total volume of 50 μL, and reaction mixtures contained 10 mmol/L Tris–HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 250 μmol/L dNTPs, 0.5 μmol/L primers, and 1.25 U Taq DNA polymerase (Invitrogen, Groningen, Netherlands). Five microliters of the sample were added to 45 μL of the amplification mixture. Amplification conditions were as follows: first run—95 °C at 3 min, 35 cycles (94 °C at 30 s, 45 °C at 30 s, 72 °C at 45 s), 72 °C at 5 min; second run—95 °C at 3 min, 20 cycles (94 °C at 30 s, 45 °C at 30 s, 72 °C at 45 s), 72 °C at 5 min. Amplification product was detected by agarose electrophoresis after EtBr staining.

2.2.4. Construction of recombinant DNA molecules

For quantitation of viral DNA, 2 recombinant DNA molecules were constructed: one, called pK CMVpp65, was obtained by cloning the amplified UL83 gene region (159 bp) into plasmid pCR 2.1 by using Original TA Cloning Kit (Invitrogen). The other one, called internal standard (IS CMVpp65; 199 bp), was obtained by cloning a 149-bp fragment of Rous sarcoma virus DNA (bp 7755–7904; GenBank accession no. NC_001407; RSV-specific primers sequences 5′-GGCGGCTTCTACATCACCTC-3′ and 5′-GCGTCCTTGGCGAGTCCCTG flanked by the target sequences recognized by CMV pp65-specific primers). pK and IS amplification products were identified by electrophoretic mobility assays and hybridization with specific probes. In coamplification assays, pK and IS

showed the same amplification kinetics. Thus, the efficiency of amplification of the 2 molecules was comparable.

2.2.5. Quantitative competitive PCR-ELISA

The specific CMV primers amplifying the region of UL83 gene encoding pp65 were used (CMV 028: 5′-AAAGAGCCCGACGTCTACTACACGT-3′, CMV 029: 5′-CCAGGTACACCTTGACGTACTGGT C-3′; GenBank accession no. NC_001347) (Zaia et al., 1990). Amplification reactions were carried out in a total volume of 50 μL, and reaction mixtures contained 10 mmol/L Tris–HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L $MgCL_2$, 200 μmol/L dNTPs, 10 μmol/L digoxigenin-11-dUTP (Roche Diagnostics, Mannheim, Germany), 0.75 μmol/L primers, 2 U Taq DNA polymerase (Invitrogen), and 500 copies of pIS. Amplification conditions were as follows: 94 °C at 4 min followed by 45 cycles (94 °C at 30 s, 55 °C at 30 s, 72 °C at 30 s), 72 °C at 5 min.

Amplification products were identified by hybridization with specific probes and detected by colorimetric reaction on microtiter plates. Probe sequences were as follows (biotin-labeled at the 5′ end): pK probe, 5′-GTTCTCCATG-GAGCAAACCAG CTC; IS probe, 5′-CAGCAGCTG-GTGGCCTACTACTC. For each sample, 2 wells were required, 1 for detection of amplified wild-type CMV and 1 for detection of amplified IS. Ten microliters of PCR products (10-fold diluted in Tris–EDTA buffer) were denaturated with 20 μL of denaturation solution (Roche) in a sterile well for 10 min at room temperature. Two hundred microliters of hybridization buffer (Roche) with a 1:100 dilution of a biotin-labeled specific probe (6 μg/mL) were added to the wells coated with streptavidin (Roche). Samples were mixed and incubated at 37 °C for 1 h on a shaker. Microtiter plates were washed 5 times with washing buffer (Roche), then 200 μL of a 1:1000 dilution of horseradish peroxidase-labeled antidigoxigenin antibody (200 U/mL; Roche) was pipetted into each well. After another incubation of 1 h at 37 °C with shaking, the wells were washed 5 times and filled with 200 μL of ABTS substrate solution (Roche). Color reaction was allowed to develop at ambient temperature for 15 min. The optical density was immediately read at a wavelength of 414 nm (reference filter, 492 nm). Standard curve was constructed for each run by coamplification of pK containing 50–10^4 copies of target DNA with 500 copies of IS.

The amount of amplified products (pK/IS ratio) was plotted against the number of target pK copies in the log scale. The number of CMV genomes in the sample tested was obtained by extrapolating the amount of amplified products (sample/IS ratio) from the standard curve.

2.2.6. Quantitative competitive LightCycler PCR

For the LightCycler PCR, the same specific CMV primers were used as were for PCR-ELISA. The CMV-specific PCR products were detected with fluorescence resonance energy transfer (FRET) hybridization probes 5′-TGGTTTGCTCCATGGAGAACACGC-3′-FL and LC Red640-5′-CGCAACCAAGATGCAGGTGATAGGT-p-3′. The IS-specific PCR products were detected with FRET hybridization probes 5′-AGCTGCTGTAGGCTGCCG AACTGT-3′-FL and LC Red705-5′-TGCGTGAGGT-GATGTAGAAGCCGC-p-3′. The hybridization probes were designed and prepared by TIB MOLBIOL, Berlin, Germany.

The PCR mixtures were prepared by using LightCycler Fast Start DNA Master Hybridization Probes (Roche) supplemented with 4 mmol/L $MgCl_2$, 0.66 μmol/L primers, 0.2 μmol/L hybridization probes, and 100 copies of IS. The capillaries were loaded with 15 μL of reaction mixture and with 5 μL of a sample by a short centrifugation step (10 s at 700 × *g*). PCR was performed on the LightCycler instrument with the following cycling program: 95 °C at 10 min followed by 58 cycles (95 °C at 5 s, 55 °C at 10 s, 72 °C at 10 s). The products were analyzed by melting curve analysis by applying 95 °C at 0.1 s and 50 °C at 30 s, followed by an increase in temperature from 50 to 85 °C (0.1 °C/s) and continuous fluorescence recording. CMV- and IS-specific hybridizations were recorded on channels F2 and F3, respectively. Absence of the signal in both F2 and F3 channels indicated a presence of PCR inhibitors in the sample. The CMV- and IS-specific melting temperatures were 68 and 71 °C, respectively.

2.2.6.1. Data analysis. The fluorescence curve analyses were carried out in the "fit points" mode with 2 points of the

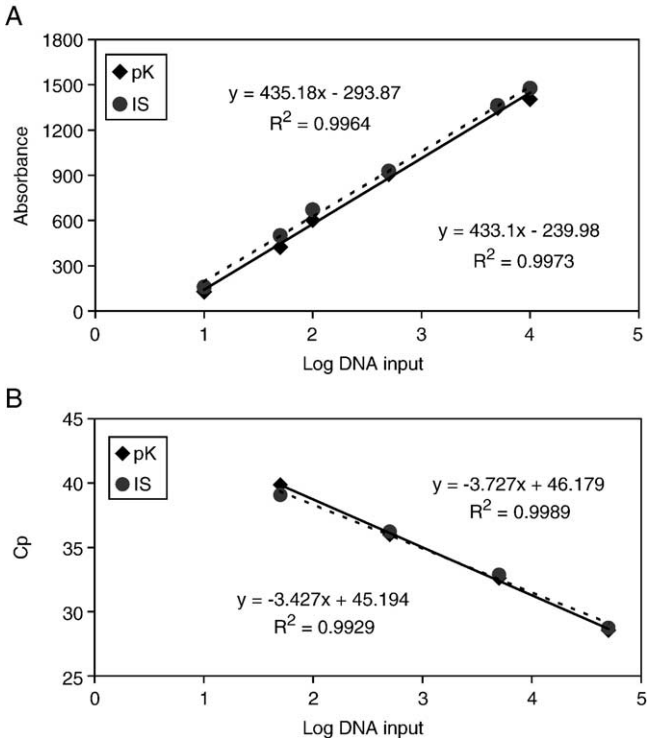

Fig. 1. (A) Standard curves for plasmids pK and IS PCR-ELISA. (B) Standard curves for plasmids pK and IS LightCycler PCR.

LightCycler software. The noise band for the crossing-point determination was adjusted at a mean F2/F1 fluorescence of 3 blanks plus 2 SDs at cycle 58. Samples with a fluorescence signal higher than the background signal were considered reactive.

Quantitation of CMV DNA was performed with four 10-fold serial dilutions of a plasmid standard pK, ranging from 50 to 50 000 copies per reaction. To generate a standard curve, the crossing point (C_p) of these standard dilutions was plotted against the number of plasmid copies used as input.

3. Results

3.1. Validation of PCR-ELISA and LightCycler PCR assays (specificity, sensitivity, and reproducibility)

Our goal was to design a PCR assay for quantitation of CMV in human samples. We developed 2 quantitative competitive PCR assays (PCR-ELISA and LightCycler PCR), based on the amplification of a 159-bp region of a sequence in the UL83 gene coding for the lower-matrix protein detected in the pp65-antigenemia test.

To test the 2 assays for specificity, a panel of other herpesvirus DNAs (i.e., HSV1, HSV2, VZV, EBV, HHV6, and HHV8) was used (results not shown). These control DNAs were isolated from laboratory strains of the viruses, which were grown on tissue cultures. The templates used in the reaction contained 10^4–10^5 copies of viral DNA. All herpesvirus DNA samples were nonreactive in both assays. No PCR inhibition was observed in samples with negative test results.

Standard curves obtained with the amplification of serial dilutions of pK and IS using PCR-ELISA and LightCycler PCR are shown in Fig. 1. In PCR-ELISA, a linearity of the product quantitation was observed between 10 and 10^5 copies of plasmid standard pK per reaction in the presence of 500 copies of IS. In LightCycler PCR, the standard curve was linear in the range of 1–10^8 copies of pK in the presence of 100 copies of IS.

Table 1
Intra-assay reproducibility of CMV DNA quantitation

Input (no. of copies) pK/reaction	PCR-ELISA	(n = 4)[a]	LightCycler	PCR (n = 7)[b]
	No. of copies measured (mean/SD)	Coefficient of variation (%)	No. of copies measured (mean/SD)	Coefficient of variation (%)
5000	4742 (418)	9	6732 (681)	10
500	477 (82)	17	674 (58)	9
100	85 (19)	23	ND	ND
50	ND	ND	58 (8)	13
10	ND	ND	10 (3)	25

ND = not determined.

[a] Amplifying different numbers of pK copies in the presence of 500 copies of IS.

[b] Amplifying different numbers of pK copies in the presence of 100 copies of IS.

Table 2
Interassay reproducibility of CMV DNA quantitation

Input (no. of copies pK/reaction)	PCR-ELISA	(n = 8)[a]	LightCycler	PCR (n = 7)[b]
	No. of copies measured (mean/SD)	Coefficient of variation (%)	No. of copies measured (mean/SD)	Coefficient of variation (%)
10000	9776 (1817)	19	ND	ND
5000	4613 (702)	15	4959 (140)	3
500	627 (112)	18	450 (26)	6
100	113 (35)	31	ND	ND
50	41 (14)	33	50 (3)	10

[a] Amplifying different numbers of pK copies in the presence of 500 copies of IS.

[b] Amplifying different numbers of pK copies in the presence of 100 copies of IS.

As shown in Fig. 1A, the linear correlations between the value of absorbance and the logarithm of the DNA copy number were identical for both pK and IS in PCR-ELISA (the slopes were 435 and 433 for pK and IS, respectively, and the correlation coefficients were 0.9964 and 0.9973, respectively). Similar results were obtained in LightCycler PCR (Fig. 1B), when C_p values were plotted against the logarithm of the DNA copy number (the slopes were −3.73 and −3.43 for pK and IS, respectively, and the correlation coefficients were 0.9989 and 0.9929, respectively).

To assess intra-assay variability of the tests, defined amounts of pK together with fixed amounts of IS were amplified at several parallel reactions in the same test run. The interassay variability was determined by amplifying defined concentrations of pK in several independent test runs. The results are summarized in Tables 1 and 2, respectively. As shown in Table 1, there was no difference between these 2 methods in coefficients of variation for

Table 3
Comparison of peak CMV DNAemia level detected by different PCR assays in BCs with antigenemia level in individual patients

Patient no.	Clinical symptoms	Peak CMV DNAemia level (PCR-ELISA)[a]	Peak CMV DNAemia level (LightCycler)[a]	Peak CMV antigenemia level[b]
1	Fever, LP	5.40	5.54	103
2	None	4.29	4.20	13
3	None	2.72	0	3
4	Organ disease	0	0	ND
5	Fever	1.87	0	0
6	Organ disease	3.50	0	0
7	Fever, LP	4.78	2.00	1
8	Organ disease	4.79	4.32	2
9	None	4.48	4.49	74
10	None	3.40	2.95	8
11	None	4.42	3.72	57
12	Organ disease	5.74	6.03	200
13	None	3.87	3.57	30
14	Fever, LP	4.30	4.38	1

LP = leukopenia.

[a] Results of PCRs are logarithm of number of copies per milliliter of BC.

[b] Results are number of positive cells per 200 000 leukocytes.

higher pK concentrations. For lower pK concentrations, the LightCycler PCR intra-assay reproducibility was higher. As for interassay reproducibility (Table 2), the LightCycler PCR has also shown superiority in quantitation accuracy.

When wild-type CMV DNA (AD 169) was quantified by LightCycler in different test runs, the mean copy number was 284, with a SD of 17 (5.84%). For C_p value, it was 30.99 ± 0.24 (0.79%). These results demonstrate that there is no difference in DNA quantitation accuracy when comparing wild-type CMV DNA and plasmid standard pK.

3.2. Comparison of PCR-ELISA with LightCycler PCR for CMV DNA detection from BC samples of HSCT patients (retrospective analysis)

CMV DNA was retrospectively detected in BC samples from HSCT patients. Sixty-eight samples from 14 patients were tested by both assay methods—PCR-ELISA and LightCycler PCR. Of 68 nested PCR-positive samples, there were 53 (78%) with detectable CMV DNA (positive samples) by either or both the assays (Table 3). Among the positive samples, CMV DNA was detected by the LightCycler PCR in 44 samples (83%) compared with 53 (100%) by PCR-ELISA. Discrepant samples typically contain low levels of CMV DNA (mean CMV DNA load was 750 copies per milliliter of BC using PCR-ELISA). None of the specimens negative in PCR-ELISA were positive in LightCycler PCR. Maximum CMV DNAemia levels detected by different PCR assays in BC and antigenemia levels of individual patients are summarized in Table 3. The viral loads detected by PCR-ELISA ranged from 65 to 5.5×10^5 copies per milliliter of sample, and the loads detected by the LightCycler ranged from 56 to 1.1×10^6 copies per milliliter of sample. The median CMV load for all samples tested was 4.16×10^4 copies per milliliter of sample by PCR-ELISA and 3.56×10^4 copies per milliliter of sample by LightCycler PCR. We found a good correlation between the quantitations of the viral load measured by PCR-ELISA and LightCycler PCR ($n = 68$; $r = 0.649$; $P < 0.0001$ by the Spearman rank test), as shown in Fig. 2.

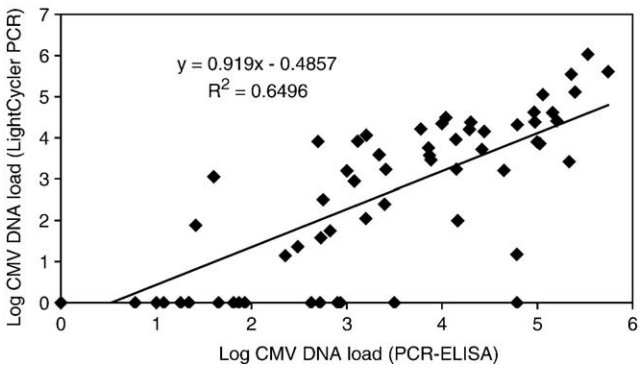

Fig. 2. Correlation of CMV DNA load (copies per milliliter of BC) measured by PCR-ELISA and LightCycler PCR ($n = 68$ pairs).

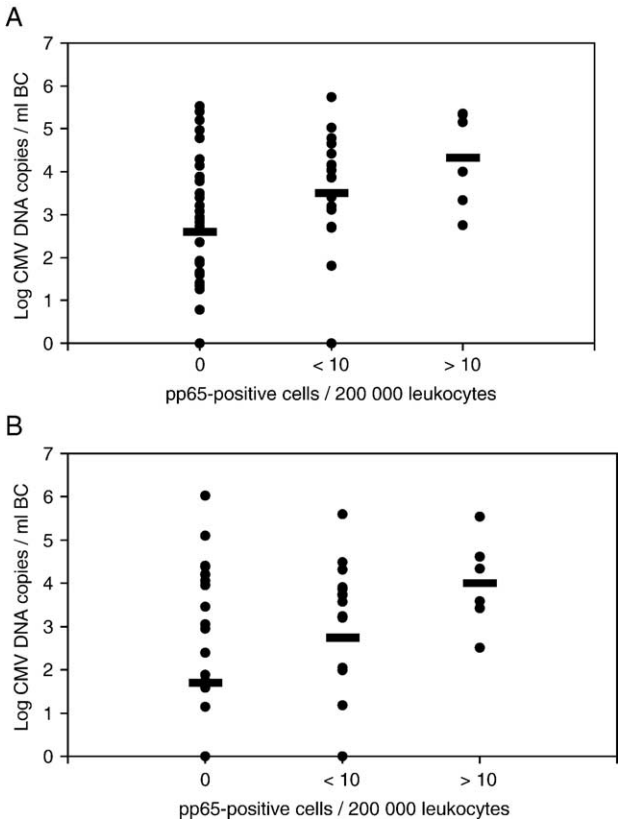

Fig. 3. Comparison of CMV DNA loads (detected by (A) PCR-ELISA and (B) LightCycler PCR) by the pp65-antigenemia assay in PBL samples. PCR-positive samples ($n = 57$) were classified into 3 groups according to the results of the pp65-antigenemia assay. Samples in group 1 ($n = 32$) were negative for pp65 antigenemia, samples in group 2 ($n = 19$) had <10 pp65-positive cells, and samples in group 3 ($n = 6$) had ≥10 pp65-positive cells. Bars show the mean CMV DNA copy numbers.

3.3. Comparison of PCR quantitation with pp65-antigenemia assay

Of the 68 samples tested by both the PCR quantitation assays, 57 were also screened by the pp65-antigenemia assay to be subsequently classified into 3 groups: group 1 ($n = 32$) negative for pp65-antigenemia; group 2 ($n = 19$) with <10 pp65-positive cells; and group 3 ($n = 10$) with ≥10 pp65-positive cells. As expected, the samples of group 3 had higher CMV DNA copy numbers detected by both of the PCR quantitation assays compared with group 1 ($P = 0.01$ by the Student t test for both PCR assays) (see Fig. 3). Furthermore, a statistically significant correlation was observed between the CMV DNA copy number and the number of pp65-positive cells ($n = 57$; $r = 0.337$, $P < 0.01$ and $r = 0.493$, $P < 0.001$ by the Spearman rank test for PCR-ELISA and LightCycler PCR, respectively).

4. Discussion

In the present study, diagnostic efficiency of 2 quantitative competitive PCR methods for monitoring CMV DNA load was evaluated. Both the methods use the same primers

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for amplification of an identical segment of the CMV genome and the same IS. In PCR-ELISA, the final reaction products were detected using hybridization to immobilized specific probes and colorimetric detection of digoxigenin-labeled amplicons. In LightCycler PCR, real-time detection of reaction products was performed using FRET probes. Both the tests were highly specific in detection of CMV DNA, with the range of CMV template quantitation being adequate for diagnostic purpose. When tested with pK, the analytical sensitivity of LightCycler PCR compared with PCR-ELISA was higher, reaching the cutoff value of 50 pK copies per milliliter. However, when tested on clinical samples, the diagnostic sensitivity of LightCycler PCR was only 83% when compared with PCR-ELISA. As PCR-ELISA testing preceded LightCycler PCR, a possible explanation of these discrepancies may be partial degradation of the template during the storage, precipitation due to freezing and thawing the samples, or DNA denaturation due to absorption on the walls of storage tubes (Gaillard and Strauss, 2001). In discrepant samples, PCR-ELISA only revealed low CMV DNA load. In view of lower accuracy and higher variability of this method observed for low-positive samples, we cannot exclude that CMV DNA copy numbers in these samples could be overestimated. As we had expected, LightCycler PCR compared with PCR-ELISA showed significantly lower intra- and interassay variation. The real-time PCR detection strategy based on the monitoring of reaction kinetics allows performing more accurate measurements than PCR-ELISA with the monitoring of end-point state of the reaction. LightCycler PCR showed a wider range of standard curve linearity, allowing the evaluation of samples with CMV DNA copy numbers higher than 10^8 copies per milliliter without their dilution.

The correlation between the amount of pp65 antigenemia-positive cells and CMV DNA load does not mean that the amount of pp65-positive cells and CMV DNA copy number was identical. It only says that the relation between both the parameters was linear: the samples with high DNA copy numbers had high numbers of pp65-positive cells and vice versa. Both CMV DNA and antigenemia positivity may precede the development of CMV disease. Our patients were preemptively cured with ganciclovir or foscavir once the first laboratory markers of CMV activation had occurred. This may be reason why some CMV DNA- and antigenemia-positive patients stayed asymptomatic.

In the last few years, many works, which compare various PCR methods for the quantitation of CMV DNA in clinical samples, were published (Diaz-Mitoma et al., 2003; Kearns et al., 2001; Pang et al., 2003; Piiparinen et al., 2004; Razonable et al., 2001; Schalasta et al., 2000; Weinberg et al., 2002). In most of them, a commercial ELISA-based quantitative competitive PCR test (CMV Amplicor Monitor) was compared with various home-brew real-time PCR assays. Generally, the authors reported results similar to ours, indicative of higher accuracy and a wider detection of a range of real-time PCR systems. Unlike most authors, we used identical primers amplifying the same segment of the CMV genome and identical amplification conditions (annealing temperature, etc.) in both real-time and ELISA-based PCR assays. We have shown that the 2 variants of quantitative competitive PCR, differing in the way of amplified product detection, are usable for CMV infection monitoring. LightCycler PCR, having superior performance characteristics and being less time-consuming, seems to be more suitable for routine diagnosis.

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