ASSESSMENT OF CYTOMEGALOVIRUS (CMV) LOAD IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS BY CMV ANTIGENEMIA AND TWO DIFFERENT REAL-TIME PCR ASSAYS

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INTRODUCTION

Cytomegalovirus (CMV) infection occurs frequently after stem cell or solid transplantation. Preemptive therapy is a strategy for preventing CMV disease in transplant recipients and requires good markers for CMV replication and also highly predictive tests to identify patients at greatest risk for CMV disease. CMV pp65 antigenemia assay has been associated with high sensitivity and specificity for the early detection of CMV disease and adopted by many transplant centers for guiding preemptive therapy in transplant recipients. However, this assay has some limitations; requires a sufficient number of leukocytes and rapid sample processing and experienced personnel for interpretation of slides. The measurement of CMV load by quantitative polymerase chain reaction (PCR) appears to be important for the diagnosis and prediction of CMV disease. A number of laboratory developed real-time PCR (RT-PCR) assays have been evaluated for monitoring CMV infection in stem cell transplant recipients. These assays, however, are not well standardized and use different target sequences, primer sets, and extraction and detection methods, which result in different analytical performance. This makes it difficult to compare studies conducted at different laboratories. In many stem cell transplantation center, preemptive therapy is initiated upon a positive antigenemia result (≥12x10⁵ leukocytes). In our transplantation center this cutoff value is defined to be ≥10 positive cells/2x10⁵ leukocytes. In RT-PCR assays, cutoff levels which predict CMV disease, have still to be defined. It is necessary, particularly when laboratories consider replacement of the antigen assay.

The aims of the study were to compare the results of two commercial CMV RT-PCR assays with pp65 antigenemia results and define cutoff levels for RT-PCR assays according to antigenemia results as a gold standard for preemptive therapy.

MATERIALS AND METHODS

Samples: A total of 170 samples derived from allogeneic stem cell transplant recipients were analyzed by pp65 antigenemia test and by two commercial RT-PCR assays. Simultaneously collected plasma samples and peripheral blood leukocytes were used for RT-PCR assays and antigenemia test, respectively.

Antigenemia test: The CMV antigenemia assay was performed with the CINA kit (Argene, Varilhes, France), according to the manufacturer’s instructions. Briefly, 2x10⁵ leukocytes were applied to a glass slide by cytospin, fixed, and permeabilized to allow subsequent detection of CMV pp65 antigen. The presence of pp65 antigen was detected by Anti-HCMV ppUL83 (pp65) antibody and visualized by means of a specific secondary fluorescein isothiocyanate-labeled antibody. The number of CMV antigen-positive cells per duplicate stain was counted.

RT-PCR assays: Two different commercial RT-PCR assays: CMV R-gene (Argene SA, Varilhes, France) and LightCycler CMV Quant Kit (Roche Diagnostics GmbH, Mannheim, Germany) were performed on plasma samples. For CMV R-gene test viral DNA extraction was performed manually with DNA extraction kit (Argene SA, Varilhes, France) from 200µl plasma samples, resulting with 50 µL of eluat. Ten microliter of the eluat was added to the master mix resulting with 25 µL of final volume. Rotor – Gene 6000 Instrument (Corbett Research, Australia) was used with CMV R-gene quantification kit according to the manufacturer’s instructions for real-time amplification. For LightCycler CMV Quant Kit test DNA extraction was performed with High Pure Viral Nucleic Acid kit (Roche Diagnostics GmbH, Mannheim, Germany) from 200 µL of plasma samples resulting with 35 µL of eluat. Ten microliter of the eluat was added to the master mix resulting with 25 µL of final volume. LightCycler 2.0 instrument (Roche Diagnostics GmbH, Mannheim, Germany) was used with LightCycler CMV Quant Kit according to the manufacturer’s instructions for real-time amplification.

Statistical analysis: Receiver-operating characteristic (ROC) plot analysis of PCR was performed to determine the cutoff values of the CMV DNA load in plasma by CMV R-gene and by LightCycler CMV Quant Kit. Current clinical practice is based on the pp65 antigenemia assay, and therefore this assay was chosen to determine the optimal cutoff value for RT-PCR assays. ROC curves for optimal cutoff values were calculated for two specific antigenemia values (>1 positive cells and >10 positive cells) using SPSS version 13.0.

RESULTS

A total of 170 samples derived from transplant recipients were analyzed by pp65 antigenemia test and by two RT-PCR assays. Via ROC curve analyses, the tentative cutoff value for 1 or more positive cells per 2x10⁵ leukocytes with pp65 antigenemia test was determined to be approximately 1434.5 copies/ml (sensitivity 90.2%, specificity 80.3%) for CMV R-gene assay and 423 copies/ml (sensitivity 73.7%, specificity 79.5%) for LightCycler CMV Quan Kit (Figure 1, 2).

Using a higher cutoff value, >10 positive cells per 2x10⁵ leukocytes, the corresponding cutoff values were found 5803 copies/ml (sensitivity 82.4%, specificity 88.7%) for CMV R-gene and 7645 copies/ml (sensitivity 78.9%, specificity 90.1%) for LightCycler CMV Quant Kit (Figure 1, 2). The Pearson correlation coefficient between two RT-PCR assays was r=0.993 (p<0.001). Comparison of the qualitative results of two CMV RT-PCR assays and antigenemia test was shown in Table 1 (r=0.471 for CMV R-gene assay and r=0.435 for LightCycler CMV Quant Kit).

CONCLUSION

• Commercially available RT-PCR assays are easy-to-handle, standardised and reproducible methods and allow testing of large number of samples. They also have the ability to detect possible PCR inhibitors with an internal control DNA which is included in the master mix.

• We determined tentative thresholds for preemptive therapy for two commercially available RT-PCR assays. However, further studies are needed for clinical validation.

• In developing countries, because of the high CMV seropositivity rate, optimal cutoff values for preemptive therapy in transplant recipients might be different than observed in developed countries so the determination of their own cutoff value has a great importance.

REFERENCES: