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Short communication

Whole blood real-time quantitative PCR for cytomegalovirus infection follow-up in transplant recipients☆

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Abstract

Background: Cytomegalovirus (CMV) remains a major opportunistic agent among transplant recipients. While detection of CMV pp65-lower matrix protein (pp65Ag) is still widely used for monitoring CMV infection, real-time PCR assays have been recently developed for routine quantitation of CMV DNA. However, correlations are lacking between results of pp65Ag and quantitative PCR assays and there is no consensus yet as to the more appropriate blood compartment (whole blood (WB), leukocytes, plasma) to be tested with PCR assays.
Objectives: The aims of the study were to determine, in a population of transplant recipients: (i) the correlation between pp65Ag and CMV quantitative real-time PCR in our setting and (ii) the utility of plasma CMV DNA quantitation in comparison to WB quantitation.
Methods: In 170 blood samples (from 61 solid organ or bone marrow transplant recipients) with pp65Ag results, CMV quantitation was performed in WB and plasma using an in-house real-time quantitative PCR.
Results: Real-time PCR and pp65Ag results in WB were correlated: thresholds of 10 and 50(+) cells/200,000 cells were equivalent to 3.3 $\log_{10}$ copies/mL (2000 copies/mL) and 3.8 $\log_{10}$ copies/mL (6300 copies/mL), respectively. When WB viral load was $\geq$3.6 $\log_{10}$ copies/mL, the risk to have a negative plasma CMV DNA result was $\leq$2.5%.
Conclusions: For the routine exploration of a single compartment, whole blood would represent a suitable compromise: easy processing for a sensitive assay. The 3.6 $\log_{10}$ copies/mL threshold, which could help in identifying active CMV infection, deserves further evaluation.

Keywords: Cytomegalovirus; Transplantation; pp65 Antigenemia; Quantitative real-time PCR; Whole blood; Plasma

Abbreviations: BMT, bone marrow transplant recipients; CMV, cytomegalovirus; PBL, peripheral blood leukocytes; pp65Ag, CMV phosphoprotein 65 antigenemia; SOT, solid organ transplant recipients; WB, whole blood

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1. Introduction

Detection of CMV phosphoprotein 65 (pp65Ag) is still widely used for monitoring CMV infection in immunocompromised patients such as bone marrow (BMT) and solid organ transplant (SOT) recipients. Molecular assays development and recently real-time methodology have reduced the turnaround time with automated, simplified and high dynamic ranged assays.

There is no consensus as to the optimal blood compartment (whole blood (WB), peripheral blood leukocytes (PBL), plasma). Even though plasma positivity is indicative of an active infection, plasma load monitoring is of modest clinical utility to predict CMV disease and results in delayed detection of CMV DNA (Boeckh and Boivin, 1998; Humar et al., 2004; Pellegrin et al., 2000). For a sensitive viral detection, WB and PBL appear more appropriate. WB specimens offer the advantage of easier processing. In addition, the suitability of WB in BMT recipients who are often aplasic or leukopenic has been shown (Mengelle et al., 2003).

To implement CMV PCR as pp65Ag in clinical practice, correlations have been investigated. The threshold of 50 pp65Ag(+) cells/200,000 PBLs has been correlated to 4 $\log_{10}$ genome copies/mL WB (Gouarin et al., 2004; Li et al., 2003; Mengelle et al., 2003). However, firstly, many physicians treat their patients before they present with 50 pp65Ag(+) cells while only few authors have correlated lower thresholds of pp65Ag (Li et al., 2003); secondly, there is no available international standard for CMV PCR and studies evaluating these tests are highly heterogeneous. Each clinical laboratory therefore needs to determine its own assays characteristics for an appropriate patients' monitoring.

In the present work, in comparison to pp65Ag, we have studied a real-time PCR assay to quantitate CMV DNA in WB and plasma, using a target sequence located in the UL83 gene which codes for the lower matrix protein detected in the pp65Ag test. The aims of our study were: (i) to determine in transplant patients and with our setting, the correlation between WB and plasma CMV loads, and thresholds of 50 or 10 pp65Ag(+) cells/200,000 cells; (ii) to identify the utility of plasma CMV DNA quantitation in comparison to WB quantitation.

2. Materials and methods

As part as the routine follow-up after transplantation, EDTA blood samples were collected from SOT and BMT recipients. pp65Ag was carried out within 4 h of collection. WB and plasma samples were frozen at −80 °C for retrospective quantitative real-time PCR.

2.1. CMV assays

CMV pp65Ag was performed following the manufacturer's recommendations (CINAkit, Argène Biosoft). The test was considered to be positive when ≥1 fluorescent cell(s)/200,000 PBL was observed.

2.1.1. DNA extraction

DNA was extracted from 200 μl WB or plasma using the MagNA™ Pure instrument (Roche Molecular Biochemicals) with the MagNA™ Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics). After elution in 100 μl buffer, 10 μl were further used for PCR.

2.1.2. Standards

For the CMV external quantitave standard curve for PCR, a plasmid (pGEM-T Easy Vector, Promega) containing one copy of the UL83 target sequence (263 bp length) was constructed from the CMV AD169 strain. The pGEM-UL83 was diluted from 5×10^2 (2.69 $\log_{10}$) to 5.10^6 (6.69 $\log_{10}$) copies/mL.

2.1.3. Quantitative CMV PCR in WB and plasma

WB and plasma samples were assayed for CMV DNA quantitation as described elsewhere (Gault et al., 2001; Mengelle et al., 2003) with real-time PCR using TaqMan technology on the LightCycler™ instrument (version 1.0, Roche Diagnostics) with the Fast Start DNA™ master hybridization probes (Roche Molecular Biochemicals). A positive control was included from extraction to quantitation in each run as well as a distilled water sample to check the absence of contamination. Quantitation linearity was obtained down to 2.69 $\log_{10}$ copies/mL (500 copies/mL). Below 2.69 $\log_{10}$ copies/mL, samples could have a positive result with unreliable quantitation. The results obtained in 2004 and 2005 for the Quality Control for Molecular Diagnostics (QCMD, Glasgow, Scotland) in our laboratory were in agreement with the expected results.

2.2. Statistical analysis

Concordance among the assays was statistically assessed with the Kappa (κ) coefficient. The "r" Pearson's coefficient provided the different correlations between quantitative variables.

3. Results

One hundred and seventy blood samples were collected from 61 patients (35 men, 26 women, median (IQR) age of 51 (41:59) years). There were 39 renal, 11 BMT, four lung, three liver, two heart, one heart–lung and one liver–lung recipients.

3.1. Comparison of pp65Ag to WB CMV loads (Table 1)

Concordance was low ($\kappa = 0.40$), because of the higher sensitivity of PCR assay. Altogether, 27.3% (45/165) samples were Ag pp65(−)/WB CMV(+) with low viral load (median < 2.69 $\log_{10}$ copies/mL), except for two samples; 4.2% (7/165) samples were Ag pp65(+)/WB CMV(−) with