# Presence of human cytomegalovirus DNA in blood of patients with community-acquired pneumonia

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## Abstract

After a primary infection, human cytomegalovirus (HCMV) remains latent in certain human cells. Different stimuli, including immune deficiency and severe infection, can trigger the reactivation of latent HCMV infection. In the last decade, the role of the reactivation in immunocompetent patients with serious illness has been intensely studied; however, the knowledge of the potential role of moderately severe infections on HCMV dynamics is limited. In the prospective study, 80 HCMV-seropositive, immunocompetent adult patients with community-acquired pneumonia (CAP), treated outside the intensive care unit (ICU), were monitored with real-time polymerase chain reaction (PCR) for the presence of HCMV DNA. Detection of HCMV DNA in whole blood and/or plasma was interpreted as reactivation of latent HCMV infection. HCMV DNA was detected in 6 of 80 (7.5%) patients. All HCMV DNA–positive patients were classified according to the pneumonia severity index (PSI) as high-risk classes IV or V; thus, HCMV DNAaemia rate within these two PSI classes was 16.7%. All of the patients had positive whole blood samples, whereas plasma samples were positive in a single patient. We did not detect any significant differences comparing six patients with proven HCMV DNAaemia and 74 patients in whom HCMV DNAaemia was not demonstrated regarding the levels of inflammatory parameters on admission, length of treatment with supplemental oxygen, and length of hospital stay. In conclusion, the finding of HCMV DNAaemia in patients with CAP treated outside the ICU is a rare event and occurs only in patients with PSI classes designating more severe pneumonia.



**Keywords:** Blood, community-acquired pneumonia, HCMV DNAaemia, human cytomegalovirus, plasma, pneumonia severity index, reactivation

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## Introduction

Human cytomegalovirus (HCMV) is the member of a Betaherpesvirinae subfamily [1]. Infection with the virus in humans is ubiquitous, with seroprevalence rates in adults between 60% and 100% [2]. After a primary infection, HCMV establishes a latent infection in certain human cells. Different stimuli, including immunosuppression and inflammation, can trigger the reactivation of latent HCMV infection [3]. For a long time HCMV has been recognized as one of the most important pathogens in immunocompromised patients, such as solid organ transplant recipients, patients with haematological malignancies, and HIV patients. In the last decade the role of serious infections in activation of latent HCMV infection in immunocompetent patients also is being scrutinised. The studies comprised critically ill patients treated at intensive care units (ICU) [4-10].

The aim of our study was to determine the rate of HCMV DNAaemia in immunocompetent patients with community-acquired pneumonia (CAP) treated outside the ICU, to



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identify factors associated with the presence of HCMV DNAaemia, and to assess its effect on the clinical course of CAP. To the best of our knowledge, this is the first study on HCMV dynamics in patients with CAP treated outside the ICU.

## Materials and methods

### Patients

This prospective study was conducted at the Department of Infectious Diseases of the University Medical Centre Maribor, Slovenia, between July 2011 and December 2013. The inclusion criteria were the presence of CAP in an adult patient without a known immunodeficiency, who provided an informed consent. The diagnosis of CAP was made according to the criteria of the British Thoracic Society [11]. Exclusion criteria were: age younger than 18 years, pregnancy, HCMV seronegativity, known tuberculosis, hospitalisation in the last 10 days before admission, treatment with certain antiviral drugs within the last week (cidofovir, foscarnet, ganciclovir, valganciclovir, acyclovir, valacyclovir), known congenital or acquired immune defects (HIV infection, immunosuppressive therapy within the last month, known haematologic malignancies or metastatic carcinoma), absolute neutrophil count below 500/μL on admission or during follow-up, and blood transfusion within the last month and during study performance.

### Study protocol

The study was approved by Slovenian National Medical Ethics Committee (No. 110/01/11). Informed consent was provided by all patients included in the study. Patients who fulfilled inclusion criteria and did not have any exclusion criteria were tested for the presence of anti-HCMV antibodies within 24 hours. The presence of IgG anti-HCMV was determined with the LIAISON CMV IgG II assay (DiaSorin S.P.A., Saluggia, Italy), following the manufacturer's instructions. Patients with positive anti-HCMV IgG were screened for the presence of HCMV DNA on days 1, 5, 10 and 20 using commercial real-time polymerase chain reaction (PCR)–based assay. According to the manufacturer's data, the limit of detection 95% is 22 copies/PCR reaction or 555 copies/mL, and limit of detection 5% 1 copy/PCR reaction or 30 copies/mL. HCMV DNA load was measured in each patient in both plasma and whole blood. Total DNA was extracted from 200 μL of whole blood and 200 μL of plasma samples using MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics, Pleasanton, CA, USA) on MagNA Pure Compact Instrument (Roche Diagnostics), and HCMV viral load was subsequently determined using CMV R-gene kit (Argene, Verniolle, France), following manufacturer's instructions. The presence of measurable HCMV DNA in either whole blood or plasma sample was interpreted to represent the reactivation of latent HCMV infection.

At admission chest radiograph and the arterial blood gas analysis were performed, and the severity of pneumonia was assessed according to the pneumonia severity index (PSI). The patient's inflammatory parameters (white blood cell count [WBC] and differential, C-reactive protein [CRP] and procalcitonin concentrations, erythrocyte sedimentation rate), basic kidney and liver function tests, and concentration of electrolytes were determined on days 1, 3, 5, 7, 10 and 20. The regression of lung infiltrates was followed up using chest radiograph during the hospital stay and according to the patient's clinical condition also during follow-up after discharge from the hospital. The first chest radiograph was performed on admission and the second on day 7 in the majority of patients.

On admission, two pairs of blood cultures were obtained from each patient. Analysis of blood cultures was performed at a microbiological laboratory with the automatic system BacT ALERT 3D. Pretreatment Gram stain and culture of sputum were attained according to standard procedures. Sputum samples were cultured only if they fulfilled quality criteria: 25 or more neutrophils and 10 or fewer squamous epithelial cells per low-power field. In the vast majority of patients, tests for the detection of urinary antigens of *Streptococcus pneumoniae* and *Legionella pneumophila* were performed. Urinary antigen of *S. pneumoniae* was determined with rapid immunochromatographic assay BinaxNOW (Alere Scarborough Inc., Scarborough, ME, USA). For the detection of urinary antigen of *L. pneumophila,* Legionella urine antigen Enzyme-Immunoassay (Bio-Rad, Marnes-la-Coquette, France) was used.

Patients included in the study were clinically followed up on a regular basis. During the hospital stay, they were checked and had their vital signs (temperature, blood pressure, heart rate, respiratory rate, and pulse oximetry) measured at least twice daily. The decision for the empirical antimicrobial treatment on admission was made by the attending physician or physician on duty. In patients with an established aetiology of CAP, antimicrobial treatment was directed by the results of microbiological studies.

### Statistics

Data were analysed using the statistical package SPSS (version 20). Numerical data were analysed with the Mann-Whitney *U* test. Fisher's exact test was performed to compare categorical variables. A value of $p < 0.05$ was considered statistically significant.

## Results

### Study population

Of 192 patients with CAP admitted to our department during the study period (July 2011 to December 2013), 117 met the

 