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VIROLOGICAL AND MOLECULAR-BIOLOGICAL METHODS OF DIAGNOSING CYTOMEGALOVIRUS INFECTION

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Abstract
The gold standard is the determination of CMV in cell culture. As a rule, human foreskin fibroblast cells are used for this purpose. Blood, urine, saliva, cervical-vaginal secretion, cerebrospinal fluid, BAL and tissue from biopsy specimens are the materials for the study. Reproduction of CMV in cell culture is accompanied by a characteristic CMV-associated cytopathic effect [7,8,9].

Keywords: Diagnosis, identification, verification, cytomegalovirus infection, immunofluorescence analysis.

Introduction

However, the virus multiplies much more slowly than is useful for diagnosis, so the method is usually combined with either PCR or immunofluorescence analysis aimed at finding early antigens. In the latter case, highly specific monoclonal antibodies are used [1,2,3].

Usually a positive result in the study of infected cell cultures is proof of active CMVI. However, the isolation of virus from urine, saliva in cell culture does not always indicate acute infection. Caution should be exercised when interpreting the result of diagnostic studies of CMV in young children. The diagnosis of CMV infection requires identification of virus in a cell culture sample before 3 weeks of age, but perinatally acquired infection may also begin to manifest within this time frame [4,5,6]. Among immunocompromised patients, reactivation of latent CMV in the absence of overt CMV infection is quite common [7,8].

In this case, the detection of CMV by culture in urine or saliva may reflect only chronic excretion of the virus and is difficult to interpret in cases of severe organ involvement, such as pneumonia or hepatitis. The most commonly used methods for confirming CMV infection are detection of CMV antigen (PP65) and quantification of CMV DNA (viral load) by PCR. Usually, CMV

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antigenemia in neutrophils correlates with viral load. This test is relatively easy to perform and does not require expensive equipment, although there are limitations to standardisation, including interpretation of the result and the need for an adequate neutrophil count (more than 1000 cells/ml). Determination of PP65 antigen is also very important during the first week of CMVI therapy. CMV DNA can be detected in both plasma and whole blood, with results varying depending on the type of sample material (plasma versus whole blood) [9,10,11,12].

Determination of viral load in blood is important for monitoring CMV concentrations in transplantology. Serial weekly blood testing is usually performed in most children before and after transplantation. Detection of CMV DNA in the liquor by PCR is highly sensitive for confirming CNS involvement [13,14,15].

DNA quantification by PCR is applicable as a marker of disease risk and monitoring of response to therapy. For this purpose, the National Institute of Standards and Technology and the WHO Expert Committee on Standardisation of Biological Products have developed standards for PCR diagnosis of CMV DNA. Detection of CMV DNA in the amniotic fluid of a pregnant woman allows assessment of the possibility of antenatal transmission of CMV to the foetus [16,17,18].

The test is performed one week after the onset of the disease (in the presence of symptoms) or after 21 weeks of gestation to reduce false negatives. A viral load of 105 or higher copies/ml in amniotic fluid increases the likelihood of vertical transmission of CMV. Detection of CMV antigenemia or CMV DNA by PCR is a predictor of CMV infection, except in recipients after alloHSCT with CMV pneumonia. According to literature data, patients with high viral load and/or persistent viraemia on the background of pronounced clinical symptoms have a high risk of lethal outcome in CMV infection [19,20,21].

The combined use of PCR and CMV antigenemia testing is used to monitor the status of CMV replication and establish the diagnosis of CMV infection in immunodeficient patients. The magnitude of the CMV viral load in the blood of such patients is an important factor [22,23,24].

Serological methods show that the absence of IgM in newborn infants with CMV infection is not a reason to dismiss the clinical diagnosis. This fact is probably due to the fact that the IgM response may be masked by a high concentration of IgG antibodies or there is immunological tolerance due to physiological features of the immune system in infants. Determination of the avidity of anti-cytomegalovirus IgG is used to detect primary CMV infection in adults, especially during pregnancy. Low-avidity anti-cytomegalovirus IgG indicates primary infection. High-avidity anti-cytomegalovirus IgGs indicate a mature immune response and a history of infection [25].

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