

## CYTOMEGALOVIRUS INFECTION IN INFANTS WITH HEPATOSPLENOMEGALY

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**Summary.** Cytomegalovirus (CMV) infection is the most common congenital infection with the incidence varying from 1% to 3%. At birth, approximately 10% of intrauterine infected infants demonstrate clinical symptoms and signs of infection which carries a risk of morbidity and mortality. Perinatal infection occurs in the contact of an infant with secretions and excretions of the mother with active CMV infection either by passing the birth canal or by excreting the virus into breast milk. The aim of this paper was to determine the involvement of CMV infection in the occurrence of hepatosplenomegaly in 1-5 month-old-infants and a possible involvement of recurrent maternal CMV infection in symptomatic congenital and perinatal infection. The investigated group included nine 1-5 month-old-infants with hepatosplenomegaly, hyperbilirubinemia, and increased transaminases. None of the investigated infants had evident malformations at birth. All hepatotropic viruses and HIV infection were excluded in all infants.

ELISA test for detection of CMV IgM and IgG antibodies was performed on blood samples taken from mothers and their infants and antigenemia assay for CMV pp65 antigen detection in the blood. By CMV pp65 antigen detection in the blood, active CMV infection was established in 5 of the total of 9 infants with the syndrome of hepatosplenomegaly referred to the virology laboratory. One out of 5 infants was positive for anti CMV IgM antibodies. In 2 of 5 mothers, primary CMV infection in pregnancy was excluded. The presence of hepatosplenomegaly in 1-5 month old infants is indicative of congenital or perinatal CMV infection. Recurrent maternal infection can result in symptomatic, congenital or perinatal infection.

**Key words:** Cytomegalovirus, hepatosplenomegaly, congenital, perinatal, antigenemia

### Introduction

Primary Cytomegalovirus (CMV) infection is a common disease, and in a high percentage, it occurs at the early stage of life. Usually, it is asymptomatic or presents as a mild mononucleosis syndrome. However, a spectrum of diseases and damages caused in immuno-compromised hosts and, above all, congenital and perinatal infections, classify CMV into a group of significant human pathogens. CMV infection is classified according to the time of occurrence: infection occurring before birth (congenital), at birth or immediately after birth (perinatal), and later in life (postnatal). As in other herpes viruses, a primary CMV infection results in establishing persistent infection. Virus reactivation (recurrent infection) can occur in response to different stimuli. Re-infection caused by different CMV strains is also possible.

CMV infection is the most common congenital infection with the incidence varying from 1%-3%. At birth, approximately 10% of intrauterine infected infants demonstrate clinical symptoms and signs of infection which carry the risk of morbidity and mortality. Primary infection in pregnant women brings the highest risk for transplacental virus transmission (40-50%). In a recurrent infection that risk is much lower (1%). The infec-

tion of fetus can occur over the whole period of pregnancy. The clinical manifestations and the severity of damage depend on the gestational age of the fetus and the mother's immune response. The most frequent manifestations at birth are hepatosplenomegaly with jaundice and petechiae. In addition, microcephalia and other damages to visceral organs can be present. A high percentage of infants (15%) with asymptomatic infection develop different sequels later in childhood.

Perinatal infection occurs in the contact of a newborn/an infant with secretions and excretions of the mother with active CMV infection either by passing the birth canal or by excreting the virus into breast milk. The most frequent symptoms are pneumonitis and sepsis-like syndrome.

### Aim

The aim of the study was to determine the involvement of CMV infection in the occurrence of hepatosplenomegaly in 1-5-month-old infants and a possible involvement of recurrent CMV of the mother in a symptomatic congenital and perinatal infection.

## Patients and Methods

### 1. The investigated group

The investigated group included 9 1-5-month-old infants with hepatosplenomegaly, bilirubinemia (dir. bilirubin up to 30 mg/dl) and increased transaminase (up to 170 IU). Only in one of 9 (1/9) infants hepatosplenomegaly with jaundice was established in the second week of life. In 8 healthy-born infants hepatosplenomegaly was established 1-5 months after their birth. The investigated infants were classified into three categories:

- Term infants with normal birth weight (2/9);
- Term infants with birth weight less than 2,600g (4/9); and
- Pre-term infants with gestational age at birth of 28 and 32 weeks (3/9).

None of the investigated infants had malformations established at birth. The presence of hepatotropic viruses and HIV infections was excluded in all infants.

### 2. Serologic diagnostics

A commercial ELISA test (Launch Biokit) was performed on the blood samples taken from mothers and their infants for the detection of CMV IgM and IgG antibodies.

### 3. CMV antigenemia assay

The test is based on detecting the structural matrix protein pp65 of the virus in the infected PMN leukocytes by using monoclonal antibodies. Leukocytes were separated from full blood within 6 hours of taking the sample for a detectable amount of antigen is reduced by storage of blood. CMV pp65 monoclonal antibody (clone 12D10) was used to detect and identify matrix protein pp65 in isolated peripheral blood leukocyte. Anti-mouse IgG (FITC) conjugate was used as a secondary antibody (Chemicon).

## Results

Active CMV infection was established in 5 of 9 infants with hepatosplenomegaly syndrome referred to the virology laboratory. The results obtained in the investigation are demonstrated in Table 1.

Table 1. CMV infection markers in infants with hepatosplenomegaly

	Total	IgG	IgM	pp65
Term infants with normal birth weight	2	2	0	1
Term infants with low birth weight	4	4	1	2
Pre-term infants (born at 28-32 weeks' gestational age)	3	3	0	2

In 1 of 2 term infants (1/2) with normal birth weight, CMV pp65 antigen was detected in peripheral blood leukocytes.

In the category of term infants with low birth weight, 2 infants (2/4) had CMV infection. One of them, with hepatosplenomegaly established in the second week of life and a virology diagnosis done at the same time, had a positive finding for CMV IgM antibodies and pp65 antigen in blood. In the second infant in this category, CMV infection was confirmed by detecting pp65 antigen in blood.

In 2 of 3 (2/3) pre-term infants, CMV infection was confirmed by detection of pp65 in blood. Anti CMV IgG antibodies were present in all investigated infants.

## Discussion

According to available data, more than 40,000 infants are born with congenital CVM infection in USA annually, 100 times more than with congenital toxoplasmosis (1,2). In most cases, it is transplacental virus transmission after primary infection of the mother. Intrauterine infection can occur during the course of recurrent infection. Previous investigations pointed out that congenital infection which occurred as the consequence of recurrent infection always resulted in the asymptomatic infection at birth (3,4). Results of the latest investigations have provided the evidence on symptomatic congenital infection which occurs equally frequently both as the consequence of recurrent infection of the mother and after primary infection (2,5,6,7,8). A great number of authors also point to the significance of recurrent maternal infection and to the danger it presents for the occurrence of congenital perinatal infection (6,7). The possibility of re-infection by some of the CMV homologue strains and the possibility of virus transmission to the fetus during this infection are not negligible.

The incidence of perinatal infection in a population of infants varies from 0,2% to 2,2% (9). Perinatal infection occurs 1-6 months after birth, 10 times more frequently than congenital infection. The results of the investigation proved the presence of CMV in the mother's milk as the consequence of virus reactivation in 22% of sera-positive mothers who breastfed their infants, because of which the authors point out to a serious risk of the occurrence of perinatal infection. Particularly endangered are pre-term infants and infants with low birth weight (10,11).

It is rather difficult to determine the type of CMV infection (primary, recurrent, re-infection) in pregnant women with unknown preconception serological status (IgG). The presence of CMV IgG antibodies prior to conception excludes the occurrence of primary infection in pregnant women.

Primary infection is characterized by CMV IgM antibodies. In recurrent infection and re-infection, IgM antibodies are undetectable or, more rarely, low levels of these antibodies are present. CMV IgM antibodies have low specificity for and sensitivity to diagnosing congenital infection (12,13). The detection of CMV IgM in the newborn blood (blood taken up to the 2nd week of life) is a safe indicator of congenital infection.

Nevertheless, the presence of CMV IgM does not exclude congenital infection. In these infants, IgG antibodies (conventionally passively-acquired antibodies) do not exhibit a decreasing tendency. After 6 months, they maintain stable levels. Also, in infants with perinatal infection, CMV IgG are detected after this period. All this makes serologic diagnostics less valid for establishing CMV infection and, above all, for the categorization of this infection. For this reason, it is necessary to apply diagnostic procedures detecting the presence of virus in the investigated sample (virus isolation, antigenemia assay, molecular biology - PCR techniques and hybridization). Infants with congenital infection excrete the virus in secretions and excretions long after birth. In infants with perinatal infection, it is possible to detect CMV in blood and urine very early, in the first months of life (not before 2-3 weeks), which supports the method of direct virus detection.

CMV antigenemia assay is a highly sensitive and specific method which enables a quick and early diagnosis of active CMV infection. It allows the diagnosis of viremia up to two weeks prior to the occurrence of the disease symptoms. It is always connected with circulating leukocytes (PMN and monocytes). The demonstration of CMV antigens in blood is particularly significant for CMV viremia is a safe marker of active infection, and it is significant for CMV disease (14,15). Results of the test are in a significant correlation with the detection of CMV DNA by means of sensitive PCR technique. Many investigations point out that CMV antigenemia assay is a much faster and sensitive method than cell culture (16,17,18). Above all, it has demonstrated a high sensitivity in diagnosing symptomatic CMV infection (19). According to the results obtained in our investigation, all patients proved positive by PCR technique had asymptomatic CMV infection. In symptomatic CMV infection, a good correlation between these two tests was found (20). Relationships exist between levels and duration of CMV antigenemia and the host immunity and CMV-related syndromes.

The test can be used as a semi-quantitative if there is a minimum of 50,000 leucocytes enabling the application of this test in determining anti-viral therapy, as well as in therapy monitoring (20). The test is particularly adequate for laboratories with fewer possibilities, i.e. those with no PCR technique available. Our investigation included 9 infants with hepatosplenomegaly, transaminases > 170 iu and direct bilirubin > 30 mg/dl. In 1/9 of infants from the category of newborns with lower birth weight, hepatosplenomegaly with jaundice was established in the second week of life. In 8/9 infants, this syndrome was diagnosed 1-5 months after birth. There were no evident malformations in any infant. Hepatotropic viruses and HIV infection were excluded in all investigated infants.

Active CMV infection was confirmed in 5 (55.55%) of the total of 9 investigated infants. Hepatosplenomegaly, along with purpura, is the most frequent syndrome diagnosed in infants with congenital infection at birth. It can

persist 3-6 months after birth and sometimes up to one year of age.

In the category of term infants with normal birth weight (2 infants), CMV infection was confirmed by the detection of pp65 antigens in the blood in one infant. In this infant, hepatosplenomegaly was diagnosed in the 4<sup>th</sup> month of age. The mother of this infant had a history of previous miscarriage (5 months before a new pregnancy). Maternal serological status was determined before pregnancy (IgG+) which excludes maternal primary infection and speaks in favor of recurrent infection. Virology diagnostics in the newborn was not performed immediately after birth, which makes it impossible to define infection as congenital or perinatal.

In the category of term infants with lower birth weights (> 2600 g) in 2 of 4 infants (2/4) CMV infection was confirmed. In one of two infants (1/2), hepatosplenomegaly with jaundice was diagnosed in the second week of life when the virology laboratory diagnostics was done. In this case, all CMV infection markers were positive. The presence of CMV IgM antibodies in the newborn's blood confirms intrauterine virus transmission, that is, congenital infection. Maternal serological status was unknown before pregnancy. CMV IgM antibodies were not confirmed in the mother after delivery. IgM antibodies in the mother after delivery can be present or can be independent of the period of pregnancy in which the infection occurred and the immune response. CMV IgG were positive. In this case, congenital infection was confirmed; however, in the absence of data on maternal serological status, it was impossible to define the type of infection (primary, recurrent). CMV infection in the second infant in this category was established by detection of pp65 antigens in the blood sample taken 5 months after birth. The maternal serological status was not known in this case either.

In the category of pre-term infants born at 28-32 weeks' gestational age, CMV infection was confirmed in 2 out of 3 infants (2/3) 2-4 months after birth. One infant (1/2) was hospitalized in the second month of age for diagnosed nonspecific neonatal sepsis when the presence of hepatosplenomegaly was established as well, and the infant underwent a diagnostic procedure. A sepsis-like syndrome occurs very often in perinatal CMV infection. In the case of this infant, infection was confirmed by detection of pp65 antibodies in the blood. The infant was breastfed. The preconception serological status in the mother was known before pregnancy (IgG+), which suggests the possibility of recurrent maternal infection. In the second infant in this category, CMV infection was confirmed at the age of 4 months by detection of pp65 antibodies in the blood. The mother was IgG positive. The maternal serological status was unknown before pregnancy.

Pre-term infants and infants with low birth weights belong to the group of infants at higher risk of perinatal infection transmitted from maternal secretions and excretions (breast milk) due to a high percentage of virus reactivation during breastfeeding (10,11).

A total of 7 pre-term infants or infants with low birth weight were included in our investigation. CMV infection was confirmed in 4 (57,14%) infants. On the basis of our investigation, it is not possible to determine if there is a correlation between the risk of the occurrence of symptomatic CMV infection in infants and the type of maternal infection (primary, recurrent) because of the unknown preconception serological status in all mothers (7/9) and the absence of a continuous follow-up of pregnant women at higher risk of primary (IgG neg. pregnant women) and recurrent CMV infection (IgG+ pregnant women). The investigation was carried out on a small sample, which reduces the possibility of drawing conclusions. In two cases, recurrent maternal infection and symptomatic CMV infection in the infant, occurring as a consequence of that infection, were con-

firmed thus supporting the investigations of those authors who have reported data on symptomatic CMV infection in infants after maternal recurrent infection.

## Conclusion

CMV infection in 1-5-month-old infants with hepatosplenomegaly is confirmed as an etiological agent in a high percentage (55.55%). The presence of hepatosplenomegaly in infants is indicative of congenital or perinatal CMV infection. Recurrent maternal infection can result in symptomatic congenital or perinatal infection.

In order to define the type of infection, it is necessary to know the preconception serological status of the mother, as well as prenatal and early postnatal diagnostics.

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## **CITOMEGALOVIRUSNA INFEKCIJA KOD DECE SA HEPATOSPLENOMEGALIJOM**

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*Kratak sadržaj: Citomegalovirusna (CMV) infekcija je najčešća kongenitalna infekcija sa incidencom 1-3%. Na rođenju oko 10% intrauterino inficirane dece ispoljava kliničke simptome i znake infekcije sa rizikom po morbiditet i mortalitet. Perinatalna infekcija dešava se u kontaktu novorođenčeta sa sekretima i ekskretima majke koja ima aktivnu CMV infekciju, bilo prolaskom kroz porodajni kanal ili majčinim mlekom. Cilj rada bio je da se utvrdi učešće CMV infekcije u nastanku hepatosplenomegalije kod dece starosti 1-5 meseci i eventualno učešće rekurentne CMV infekcije majke u simptomatskoj kongenitalnoj i perinatalnoj infekciji. Ispitivana grupa obuhvatala je 9 dece starosti do 5 meseci koja su imala hepatosplenomegaliju, hiperbilirubinemiju i povećane transaminaze. Nijedno od ispitivane dece nije imalo evidentirane malformacije na rođenju. Kod sve dece bili su isključeni svi hepatotropni virusi i HIV infekcija. Iz uzorka krvi, uzetog od dece i njihovih majki, radjen je ELISA test za detekciju CMV IgM i IgG antitela i test za detekciju CMV pp65 u krvi. Od ukupno 9 dece koja su bila upućena u virusološku laboratoriju sa sindromom hepatosplenomegalija kod 5 dece utvrđena je aktivna CMV infekcija detekcijom CMV pp65 antigena. Jedno od petoro dece imalo je anti CMV IgM. Kod dve od pet majki isključena je primarna CMV infekcija u trudnoći. Prisustvo hepatosplenomegalije kod dece do 5 meseci života indikativno je za kongenitalnu ili perinatalnu CMV infekciju. Rekurentna infekcija majke može da rezultira simptomatskom kongenitalnom ili perinatalnom infekcijom.*

*Ključne reči: Citomegalovirus, hepatosplenomegalija, kongenitalna, perinatalna, antigenemija*