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RELEVANCE OF CMV DNA DETECTION IN SYMPTOMATIC CHILDREN UP TO 3 MONTHS OF AGE

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Abstract

Cytomegalovirus (CMV) is the most frequent cause of congenital infection worldwide. The burden of disease related to congenital CMV (cCMV) is substantial, as it is the leading cause of sensorineural hearing loss and an important cause of neurodevelopmental disabilities in children. Despite its clinical significance, cCMV infection often goes undetected because the majority of infected infants are asymptomatic at birth and screening programmes have not been implemented in any country.

The aim of this study is to define the role of CMV in congenital and early postnatal morbidity in clinically relevant symptomatic children up to 3 months of age in Varna region, comparatively in ELISA (anti CMV IgM/IgG) and PCR.

We found CMV DNA in 12 out of 50 tested children (24%, 95% CI: 12.16–35.84%). The viral load ranged in 65–1 628 879 IU/ml, average viral load 144 707.2 IU/ml.

It is important to note that only half of the PCR positive children 6/12 (50%) had an anti CMV IgM positive result.

Our data demonstrate that serological tests defining active CMV infection with CMV IgM detection are not sufficiently indicative in all newborns, and it is therefore mandatory to conduct modern PCR for all relevant infants after birth.

Key words: CMV, PCR, neonates, cCMV infection, CMV screening

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Introduction. Cytomegalovirus (CMV) is an ubiquitous DNA virus, belonging to the *Herpesviridae* family. The majority of CMV infections are asymptomatic or self-limited in healthy children, though in infected fetuses CMV produces a high burden of morbidity with different organs involvement. Congenital CMV infection is the most common congenital infection worldwide, and it is the leading non-genetic cause of sensorineural hearing loss (SNHL) in children and an important cause of neurodevelopmental delay [1,2]. The clinical spectrum of congenital CMV infection varies widely, from the complete absence of signs of infection to potentially life-threatening end-organ disease. At birth, 85–90% of infected infants are asymptomatic, and 10–15% present with clinical apparent infection [3]. The most common findings in this last group are petechiae, jaundice, hepatomegaly, splenomegaly, microcephaly, and other neurologic signs. Laboratory and imaging findings include thrombocytopenia, elevated transaminases, direct hyperbilirubinemia, chorioretinitis, abnormalities indicative of central nervous system involvement, and sensory-neural hearing loss [4]. According to the literature, most of the full-term congenitally infected newborns are asymptomatic at birth because they receive a full range of maternal transplacental anti CMV IgG from their seropositive mothers. These antibodies significantly modify the congenital infection and make the symptoms relatively mild from a clinical point of view. About 15–20% of infected premature infants, who do not receive the full set of maternal antibodies, can develop clinical CMV disease [5]. Another study reported that 22.1% of preterm infants developed symptomatic infection [6]. This places prematurity as one of the main signs in which congenital or intranatal CMV infection should be actively sought. It is considered that CMV horizontal infection of healthy full-term infants does not usually have serious clinical consequences.

The aim of this study is to define the role of CMV in congenital and early postnatal morbidity in clinically relevant children up to 3 months of age, comparatively in ELISA and PCR.

Materials and methods. ***Patients and clinical samples.*** We investigated 50 children in a single plasma sample. The children were divided into two age groups: A ($n = 25$) and B ($n = 25$), with the limit being 21 days after birth, in order to be more precise about the type of infection – congenital or postnatal. The blood samples were collected in EDTA vacutainers and the resulted plasma was stored at $-20\,^{\circ}\mathrm{C}$ before ELISA test, DNA extraction and QRT-PCR analysis. The demographic and clinical-morphological characteristics of the enrolled infants are summarized in Table 1.

Indirect ELISA. Anti CMV IgM/IgG (Euroimmun, Germany) according to the standard instructions of the manufacturer were used. When calculating the IgM results, the semiquantitative method was applied: Ratio = Extinction of the sample/Extinction of calibrator. Positive samples had a ratio > 1.1; negative samples had a ratio of < 0.8; and ratios between 0.8 and 1.1 were considered borderline. For IgG, we used the quantitative method for defining positive

Table 1

Characteristics of the tested children, $n = 50$

Variable	N (%)	CMV DNA positive
Age (median/range)	27.18 days (1–90 days)	A total of 12 positive
Gender (Male/Female)	30/20 (60%/40%)	7/5
Age < 21 days (group A)	25 (50%)	1
Age ≥ 21 days (group B)	25 (50%)	11
Leading clinical/laboratory manifestation		
Prematurity and low gestational age	21* (42%)	3
Hepatitis or icterus neonatorum prolongatus	6 (12%)	—
Sepsis		
Congenital abnormalities	5 (10%)	2
Vomiting and not gaining weight	3 (6%)	—
Rash	3 (6%)	2
Hydrocephaly	3 (6%)	1
Hemolytic anemia	2 (4%)	—
Thrombocytopenia	2 (4%)	1
Unspecified seizures	2 (4%)	1
Gastrointestinal bleeding	1 (2%)	1
Pneumonia	1 (2%)	1

*The babies were born prematurely between 25 and 37 g.w., with an average of 31.66 g.w. (SD ± 4.19), with a low gestational weight of 700 to 2600 g, an average weight of 1552 g (SD ± 665). In addition to prematurity, some of the infants also had other manifestations suspicious for congenital CMV infection (hydrocephaly, cerebral abnormalities, microcephaly, pneumonia, thrombocytopenia)

and negative samples by constructing a calibration curve (Cal 1 = 200 RU/ml, Cal 2 = 20 RU/ml, Cal 3 = 2 RU/ml, where RU/ml is relative units/ml). Samples with results > 22 RU/ml were considered positive; with results < 16 RU/ml were considered negative; and the borderline results are between 16 RU/ml and 22 RU/ml.

PCR method. DNA was extracted from 150 µl plasma using Kit Ribo Virus (Sacace Biotechnologies S.r.l., Italy). All PCR reactions were performed with Taqman Real time Quantitative PCR for CMV-DNA detection (Sacace Biotechnologies S.r.l., Italy) in the presence of Internal Control to identify possible inhibition of the reaction and endogenous internal control (b-globine gen) to evaluate the adequacy of the material and its storage. The results were converted to IU/ml using the 1st WHO Standard (5 000 000 IU/ml) to calibrate the test. The amplification was performed with a PCR instrument Quant Studio Dx in a final volume of 25 µl reactions.

Statistical methods. The results obtained were processed with McCallum Layton calculators [`www.mccallum-layton.co.uk`] to calculate the median age of the children, the relative proportions and the confidence intervals.

Results. According to the serological analysis, 92% (95% CI: 84.48–99.52%, $n = 46$) of the children were anti CMV IgG positive and the titers of their specific IgG antibodies ranged from 26 RU/ml to 244 RU/ml (a mean of 100.87 RU/ml).

Anti CMV IgM positive results were found in 16% (95% CI: 5.84–26.16%, $n = 8$), anti CMV IgM borderline were 6% (95% CI: 0.58–12.58%, $n = 3$), and anti-CMV IgM negative were 78% (95% CI: 66.52–89.48%, $n = 39$) of the children. Semi-quantitative measurement in samples with positive anti CMV IgM results showed a range of 1.3 R to 6.8 R (average 3.88 R).

According to the PCR analysis, we found CMV DNA in 12 out of 50 tested children (24%, 95% CI: 12.16–35.84%). The viral load ranged 65 (1.8 log) – 1 628 879 IU/ml (6.2 log), average viral load 144 707.2 IU/ml (5.1 log).

It is important to note that only half of the PCR positive children 6/12 (50%) had an anti CMV IgM positive result. The average viral load in IgM positive infants was log 4.06, and the average viral load of the IgM negative infants was log 2.92 (Fig. 1).

Positive viral load was detected in one out of 25 children in group A (4%), which confirms congenital infection, and in 11 out of 25 children in group B (44%) symptomatic children up to 3 months old, probably infected intranataly or early postnataly.

In addition, children were tested in ELISA for multiple other pathogens (TORCH) – anti-HIV ½ Ag/Ab, Syphilis Ab, HBsAg, anti-HCV, anti Rubella-virus IgM, anti HSV1 IgM and anti HSV2 IgM, and showed negative results.

Discussion. According to literature, CMV infection is a huge health problem worldwide because it is the most common congenital infection and the most common non-hereditary cause of deafness and neurodevelopmental delay in newborn

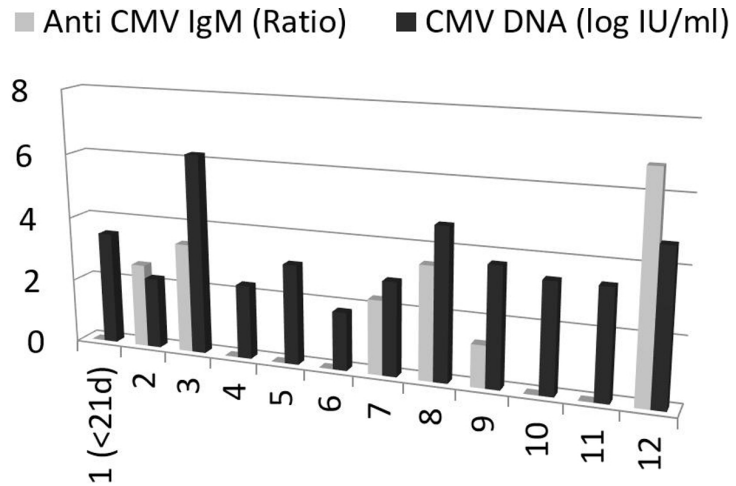

Fig. 1. Correlation of anti CMV IgM (R) with CMV DNA (log IU/ml) in PCR positive children, $n = 12$

children [7]. Intrauterine CMV transmission occurs because of primary maternal infection during pregnancy, or in women with preexisting antibodies to CMV either by reactivation of a previous infection or by acquisition of a different CMV strain (non-primary infection) [8]. Primary CMV infections are associated with the greatest risk of in-utero transmission at 30–35%, while for non-primary infections the transmission rate is significantly lower – at 1.1–1.7% [1]. Congenital CMV infection resulting from non-primary maternal infections constitute nearly two-thirds of infected infants because of the high CMV seroprevalence in society [9]. According to regional data, the average CMV seroprevalence is high – 78.4% [10]. Such seroprevalence implies a high proportion of CMV involvement in congenital and early postnatal morbidity. A previous study, based on the serological data (anti CMV IgM positivity) in infants, found CMV infection in 23.26% (10/43) related to low birth weight and 16.87% (14/83) in children with congenital abnormalities up to 3 months of age [11]. Using modern PCR analysis we demonstrated that the relative deal of CMV in neonatal and early postnatal morbidity in the region is 24% (12/50 children). In comparative terms, the results mean, that CMV continues to play a significant role in the morbidity of newborns in the region and is somewhat neglected.

In the present study, we divided the children into two age groups, with the limit being 21 days, in order to be more precise as to the type of infection – congenital or intranatal/early postnatal. Detection of viral load in children up to 21 days (group A) would confirm congenital CMV infection acquired vertically, whereas the detection of CMV-DNA at $>$ 21 days of age (group B) does not exclude intranatal and early postnatal (horizontal) mode of transmission of the CMV infection. In this case, the guideline for differentiation may be the severity of the clinical manifestation [12].

In our study, we found only one congenitally infected symptomatic infant in group A (4%). The infant was born of a 21-year-old mother, after first normal pregnancy. The infant was male, born at 38 gw, with low for gestational age weight (2000 g) with difficult adaptation with respiratory problems consistent with pneumonia. Serological results in a 2-day-old baby were: anti CMV IgM – negative; anti CMV IgG 116 RU/ml – positive; PCR for CMV-DNA – 3360 IU/ml. The serological profile of the mother showed anti CMV IgM 1.5 R $>$ 1.1 R – positive; anti CMV IgG 227 RU/ml – positive and RAI = 82%, high avidity of IgG antibodies. The newborn was anti CMV IgM negative, but had a significantly lower titer of anti CMV IgG antibodies than his mother. This speaks in favour of the phenomenon of depletion of specific antibodies in case of active infection. It is more likely, a reactivated maternal infection, or a primary infection at a very early stage of pregnancy, to have led to the fetus infection. We hypothesize that the congenital infection in this baby is modified by the passively transferred anti-CMV IgG antibodies, which is why the clinical symptoms were relatively mild. The child responded well to the treatment with Ganciclovir i.v. 5 mg/kg

for 10 days. He started to gain weight, and was dehospitalized in a good general condition.

In our study, we detected CMV-DNA in 11 infants of group B (44%). Due to the age of the children in group B (21 days – 3 months), we cannot be specific about the type of CMV infection (vertical or horizontal), but the severity of the clinical symptoms would allow us to make a reasonable assumption. Horizontal infection in healthy term infants does not usually lead to severe clinical consequences. A similar statement was expressed in a study by Dworsky et al. [13] among randomly selected women and their newborn infants, which found that 69% of infants became infected with CMV with breast milk and radiated the virus for a long time afterwards, without serious health consequences. Only two of the children who were born prematurely developed pneumonia, which resolved without chronic consequences [13]. In our study we found 11 positive infants by PCR (44%). Their viral load ranged from 65 (log 1.81) to 1 628 879 IU/ml (log 6.21). All children (6 males and 5 females, two of the children were twins) with a positive viral load were anti CMV IgG positive also. The most common clinical manifestations in children with a positive viral load were as follows: prematurity and low gestational age (3/110, vomiting and not gaining weight (3/11), sepsis (2/11), pneumonia (2/11), thrombocytopenia (2/11), hemolytic anemia (1/11) and rash (1/11). Symptoms are greater than the number of PCR positive children because some of them have presented a complex of clinical manifestations. In summary, only half of PCR positive children (6/12) had anti CMV IgM (Fig. 1). These data lead us to conclude that a negative serological anti CMV IgM result solely does not exclude active CMV infection and PCR test is mandatory in all symptomatic children.

Conclusion. The relative deal of CMV involvement in neonatal and early postnatal morbidity in symptomatic infants in Varna region determined by PCR is 24%. Compared to previous serologically based studies, this relative proportion shows stability and does not tend to decrease. Our data demonstrate that serological tests defining active CMV infection (IgM) are not sufficiently indicative in all newborns, and it is therefore mandatory to conduct modern PCR investigation in all relevant infants after birth.

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