Cytomegalovirus quantity and genotypes in infants below 1 year of age – a single centre experience

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Key words: cytomegalovirus, genotype

The study was performed in the Teaching Hospital Motol, Prague, Czech Republic

Running title: CMV in sucklings

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Abstract:
Cytomegalovirus (CMV) remains an important cause of congenital and early postnatal infections both symptomatic and asymptomatic leading occasionally to severe long term sequels such as sensoneural hearing loss, or visual impairment. We tested 592 samples from 95 neonates and infants below 1 yr of age at the time of the first sampling. Median quantity of CMV positive whole blood samples from 12 neonates was 6.42 CMV normalised viral copies (NVCs) and 6.50 NVCs in the rest of the positive infants (equals the median quantity of 7.5x10^3/ml in 9 neonates and median quantity 8.5x10^5/ml in 30 sucklings respectively). In the positive samples, CMV genotyping using gB and gH types was performed. At least one glycoprotein was detected in 77 patients (in 9 one glycoprotein only, 9 mixed strain infections and 59 patients with only one CMV strain detected).
Introduction:

Importance of human cytomegalovirus (CMV) in general population increases due to wider impact of CMV described both in immunocompetent and immunocompromissed host. One of the major highlights of the CMV presently is the impact of the infection on developing fetus during the pregnancy, same as the unknown impact on the sucklings during the early postnatal infection. Recently, congenital CMV (cCMV) infection is described in about 0.5-1.0% of the living neonates[7, 8, 10]. Clinical symptoms described in cCMV are intrauterine growth retardation, prematurity, hepatopathy, splenomegaly, microcephaly, CNS calcifications, petechiae and pulmonary symptoms. The lost term sequels of this infection are mainly sensoneural hearing loss, visual impairment, mental retardation, seizures and motoric dysfunction and rarely death. Unfortunately such clinical symptoms can be observed in about 10-20% of cases only; 80-90% of the infants with congenital CMV are born in term and asymptomatic[7, 8, 10]. Despite the frequency of long term sequels, especially progressive sensoneural hearing loss which can develop during first years of age, are observed in symptopatic children much more frequently (about 40-60%) comparing to about 10% in asymptomatic neonates, total numbers of children suffering of the sequels of cCMV is higher among the asymptomatic neonates[7, 8, 10]. Recent focus on cCMV is connected also with details of the CMV infection, such as different CMV genotypes, might be important for development of the sequels[3, 4, 9].

Therefore, we aimed on quantification and genotyping of CMV in the sucklings positive during testing in Virology laboratory of Motol University Hospital within the years 2002-2014.
Patients and methods:

We tested 69 whole blood and 30 urine samples from 17 neonates at the time of the first sample (median 4 days of age at the first sampling) and 387 whole blood and 64 urine samples from 78 sucklings below 1 year of age at the first sampling and 42 additional biological samples (most frequently CFS) from 17 children (4 neonates). All children (61 boys and 33 girls) were sampled due to clinical suspicion and possible symptoms of the CMV infection, including 4 children that where after allogeneic Haematopoietic stem cell transplantation. Urine samples were extracted using UltraClean Blood Spin DNA Isolation Kit (MoBio, Carlsbad, CA, USA) and whole blood samples were extracted using QiaAmp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacture’s instructions. Quantitative real-time PCR detection of CMV was performed as published previously[6] at the time of sampling. Results were normalised to 10,000 human genome equivalents and expressed as normalized viral copies (NVCs) as published previously and as copies/ml of urine. Samples were subsequently stored at -20°C. After introduction of the genotyping assays discriminating the CMV genotypes by glycoprotein B and H (gB and gH) into 4 types of gB and 2 of gH, we tested the stored samples according the previously published assays[2, 5].

Results:

CMV was detected in 317 samples. Median quantity of CMV in 18 positive whole blood samples from 12 positive neonates was 6.42 CMV NVCs (range 2.24-376.65 NVCs) and 6.50 NVCs (range 0.19-13,095 NVCs) in the rest of the positive whole blood samples and children respectively. Calculating the CMV results to 1 ml of the whole blood sample shows the median quantity 7.5x10^3/ml (range 5x10^2-8.1x10^5) in our patients.

CMV in urine was detected in 9 neonates with median quantity 8.14x10^6/ml (range 1x10^4-5.6x10^9) and in 30 sucklings with median quantity 8.5x10^5/ml (range 3.1x10^3-9.2x10^8). In 33
patients, we tested both whole blood and urine sample and in all patients both samples were positive. CMV was detected also in 3 samples of CSF from 2 neonates and 1 suckling with a quantity of \(2 \times 10^3\) CMV/ml, \(5 \times 10^3\) CMV/ml and \(1.6 \times 10^6\) CMV/ml respectively. In one of these neonates, huge porencephalic cysts were detected 3 days after the delivery by magnetic resonance imaging (MRI) (Figure 1). Longest continual detection in case of cCMV was 355 days from the first positive sample in peripheral blood samples and 421 days in urine in the same patient; median of observed continuous CMV positivity among the patients with postnatal infection was 625 days (range 13-989 days).

CMV genotypes were tested in 231 samples from presented patients and determined in 187 samples 11 neonates and 66 sucklings. In 9 patients, genotype of gB or gH was detected only and in 9 infants (1 neonate), infection with more than one CMV strain was detected. Most frequently detected genotypes were gB1gH2 in 14 children (1 neonate) and gB1gH1 in 13 children (1 neonate). Frequency of genotypes is shown in Table 1.
Discussion:

CMV infection among the neonates and young infants remains at the focus of the research[7, 8, 10]. Long term sequels are of major importance due to severe impairment of the hearing and visual ability, and likely also the other more distinct impacts of the cCMV on the brain. Detected quantity of CMV is in agreement with the lower CMV quantities detected in peripheral blood and urine samples in neonates same as in transplant recipients described [1, 3, 6]. Long lasting shedding in the urine samples is also well described and our results confirm such observation in our cohort[1].

According to the data published by deVries et al., our result confirm the observation of higher frequency of gH2 detected among our patients, but different proportion of the gB types because we observed most frequently gB2 among the neonates and gB1 among the rest of the patients, we also did not observed higher proportion of the gB3[3].

Interestingly, even early detection of the CMV in the CSF and morphological abnormities are not certain of severe clinical sequels. Girl presented in Figure 1 (having mixed CMV strain infection detecting the gB2,3 gH1 in the urine; only gB3 gH1 was detected in the CSF) was treated with ganciclovir 10 mg/kg/day in two doses for 3 weeks and in one year of age, there was no CMV attributable impairment observed and the psychomotorical development was almost as supposed to be too.

Our more or less epidemiological data further documents the complexity of CMV infection during the early life. It is certain, that more detailed studies aimed on impact and pathogenicity of different CMV strains and the biological characteristics are needed.

Support by grant of the Ministry of Health of the Czech Republic NT-13691.
References:
Figure 1.

Porencephalic cysts shown in frontal brain indicated by the arrow on MRI at 3 days of age.
Table 1.

Frequency of different CMV genotypes in tested infants.

<table>
<thead>
<tr>
<th>CMV genotype</th>
<th>No. of neonates (%)</th>
<th>No. of sucklings (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gB1 gH1</td>
<td>1 (9.1%)</td>
<td>12 (15.2%)</td>
<td>13</td>
</tr>
<tr>
<td>gB1 gH2</td>
<td>1 (9.1%)</td>
<td>13 (19.7%)</td>
<td>14</td>
</tr>
<tr>
<td>gB2 gH1</td>
<td>2 (18.2%)</td>
<td>2 (3.0%)</td>
<td>4</td>
</tr>
<tr>
<td>gB2 gH2</td>
<td>3 (27.3%)</td>
<td>6 (6.0%)</td>
<td>9</td>
</tr>
<tr>
<td>gB3 gH1</td>
<td>1 (9.1%)</td>
<td>5 (7.6%)</td>
<td>6</td>
</tr>
<tr>
<td>gB3 gH2</td>
<td>0</td>
<td>3 (4.5%)</td>
<td>3</td>
</tr>
<tr>
<td>gB4 gH1</td>
<td>0</td>
<td>7 (10.6%)</td>
<td>7</td>
</tr>
<tr>
<td>gB4 gH2</td>
<td>2 (18.2%)</td>
<td>1 (1.5%)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Infection with more genotypes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gB1 gH1,2</td>
<td>0</td>
<td>2 (2.0%)</td>
<td>2</td>
</tr>
<tr>
<td>gB1,2,4 gH2</td>
<td>0</td>
<td>1 (1.5%)</td>
<td>1</td>
</tr>
<tr>
<td>gB1,4 gH1,2</td>
<td>0</td>
<td>1 (1.5%)</td>
<td>1</td>
</tr>
<tr>
<td>gB2,4 gH1,2</td>
<td>0</td>
<td>1 (1.5%)</td>
<td>1</td>
</tr>
<tr>
<td>gB2,3 gH1</td>
<td>1 (9.1%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>gB2,3 gH2</td>
<td>0</td>
<td>2 (3.0%)</td>
<td>2</td>
</tr>
<tr>
<td>gB3 gH1,2</td>
<td>0</td>
<td>1 (1.5%)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Detection of gB or gH only</strong></td>
<td>0</td>
<td>9 (13.6%)</td>
<td>9</td>
</tr>
</tbody>
</table>
CMV genotypes in patients after allogeneic haematopoietic stem cell transplantation.

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Introduction:
Human cytomegalovirus is one of the most important viral pathogens in patients after haematopoietic stem cell transplantation (HSCT). Better characterisation of CMV infections might give us the informations about impact of different CMV genotypes on the prognosis off the patient, including survival, presence of severe complications or risk of development of resistance and so help for better patient’s tailored therapy in the future. Genotypisation was performed using UL55 gene (coding glycoprotein B) and UL74 gene (coding glycoprotein H).

Methods:
We genotyped 1697 CMV positive samples from 135 children and 332 adult patients after allogeneic HSCT at Department of Paediatric Haematology and Oncology of Motol University Hospital and Institute of Haematology and Blood Transfusion between January 2002 and January 2013. DNA was extracted using Qiagen QiaAmp DNA Blood Mini and DNA Mini Kits from biological samples (from whole blood and in minor cases from other biological tissues). Samples were primary used for prospective CMV DNA testing and were subsequently stored at -20°C. Genotypisation was performed using real-time PCR technology on Applied Biosystems 7500 and Bio-Rad CFX96 machines using specific primers and MGB-probes aimed at specific sequence of gB1-4 and gH1 and gH2 genotypes.

Results:
CMV genotype was detected 1213 samples from 116 children and 297 adult patients. A single CMV strain was detected in 1,021 (84.17 %) samples from 89 (76.72%) children and 200 (67.34%) adult patients. Mixed infection caused by two or more CMV strain was detected in another 192 samples (15.83 %) from 27 children and 97 adult patients. Most frequently detected genotypes in “single strain” infection were gB1gH2 (detected in 350 samples from 25 children and 69 adults) and gB1gH1 (detected in 250 samples from 23 children and 44 adults). Most frequently detected strains in “mixed strains” infections were gB1gH1gH2 (in 41 samples from 21 patients) and gB1gB3gH1gH2 (in 43 samples from 19 patients). Likely due to long lasting storage and slow DNA degradation or due to enormously low CMV quantity in the samples, we were not able to detect any CMV genotype in samples from 54 patients.

Conclusions:
Mixes CMV strain infections are quite frequent among patients after allogeneic HSCT patients with higher frequency among adult patients. Detailed analysis of clinical consequences of viral infections including CMV are necessary for better understanding of both direct and indirect impact of the CMV on the outcome of HSCT recipients.

Supported by grant of Internal Grant Agency of Ministry of Health of Czech Republic NT/13691-4.