Transmission of human cytomegalovirus via breastmilk and potential risks to very preterm infants

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Breastfeeding has clear short-term benefits for the baby1. Additionally, based on a prospective long-term cohort study from Brazil, breastfeeding is associated with improved IQ scores and increased educational attainment 30 years later2. During lactation, mother-to-infant transmission of viral infections like HIV, hepatitis B (HBV), and human cytomegalovirus (HCMV), may occur. The article presented here will focus on the dynamics of HCMV shedding into breastmilk, describe the short- and long-term risks of HCMV infection of small preterm infants, and options for prevention.

HCMV reactivation during lactation

HCMV, a β-herpesvirus, persists following primary infection for lifetime in hematopoetic CD34+ precursor cells and may be reactivated by stress, transient loss of CD4+ and CD8+ T-cell immunity, IL-6 signalling, cell cycle arrest, and DNA damage3. Interestingly, HCMV is also reactivated in healthy immunocompetent seropositive women during lactation3. The ratio of HCMV reactivation at any stage of breastfeeding during the first three months after birth is very high (>95%) and nearly equals the maternal seroprevalence5,6. The mechanisms leading to viral shedding exclusively into breastmilk are not understood. HCMV seroprevalences in Western Europe, USA, Canada, and Australia range from 40–60%, and are above 90% in South Africa, Brazil, India, Japan, and Turkey7.

Maternal HCMV reactivation of seropositive mothers during lactation with shedding of viral DNA and virolactia can be detected already in colostrum and normally ends after about three months after birth. According to our experience with individual kinetics of HCMV reactivation in breastmilk of more than 500 healthy HIV-negative breastfeeding mothers of preterm infants, the onset of viral shedding begins with low viral load (<1000 copies/mL) and low infectivity (without detectable infected fibroblast nuclei in short-term microculture) at the end of the first week post partum (p.p.). The onset, the dynamics, and the end of virus shedding into milk is inter-individually different and describes mostly unimodal kinetics (Fig. 1). The viral reactivation during lactation is a strictly self-limited process8. Using overnight microculture from cell- and fat-free milk whey, virolactia peaks coincide with viral DNA peak values, varying from 103–106 copies HCMV DNA/mL of milk whey9.

The initiation of viral shedding into colostrum shows divergent results. While in Gambia HCMV excretion in colostrum and genital tract were observed in 100% of congenitally infected, and 48% of early infected infants10, a study from Japan showed, that in seven cases of very low birthweight (VLBW) infants the initial viral load in breastmilk in the first week p.p. ranges between 10 and <1000 copies/mL HCMV DNA11. In contrast, an Italian group detected viral DNA in 31 out of 57 (54%) colostrum samples12.

The HCMV reactivation of HIV-negative mothers during lactation is a local process without detection of a disseminated or compartmentalised infection in plasma, throat or cervical swabs13–15. Therefore, HCMV DNA, viral late pp65 transcripts and virions can only be detected in breastmilk cells and cell-free milk whey9,16,17. Viral cells involved in HCMV reactivation include CD14+ macrophages9. However, HCMV-infected milk cells are not essential for virus transmission13,15. Several reports found an association between high HCMV viral load in breastmilk and risk of transmission18,19. However, other observations seem to be important in the context of prevention14. An inverse correlation between milk HCMV-specific IgG avidity and HCMV load was also found20.
In HIV/HCMV coinfected breastfeeding mothers many of the findings in the HIV-negative population are altered. Shedding of HCMV and potentially Epstein–Barr virus (EBV) in breastmilk is associated with HIV-1 transmission by breastfeeding. About 5% of HIV-1-positive breastfeeding mothers had detectable HCMV DNA perinatally in plasma. There was a strong correlation between cervical HCMV DNA detection during pregnancy and later breast milk HCMV levels. Maternal HCMV DNA breastmilk levels and CD4 < 450 cells/mm³ were determinants of HCMV transmission. All HIV-1-infected inocula like genital secretions, breastmilk and blood contain cell-free virus and infected cells.

Postnatal HCMV infection of very preterm infants: neonatal entity and prevention

Sepsis-like symptoms (SLS) have been introduced as a term to describe symptomatic postnatal HCMV disease in VLBW preterm infants, comprising apnea and bradycardia, hepatosplenomegaly, grey pallor and distending bowels. Following maternal HCMV reactivation and shedding into breastmilk, the incidence of HCMV transmission to the infant feeding raw, untreated breastmilk was 38% at the age of 3 months corrected age, 18% of the infants had one or more SLS. Virological data could be almost exactly confirmed by a recent report and clinical findings of postnatal CMV infection by many other studies worldwide. The main risks for symptomatic neonatal disease are extremely low birthweight, early transmission, low GA and low infantile IgG titers. In a controlled study VLBW preterm infants had a significantly higher incidence of thrombocytopenia, neutropenia and slightly increased C-reactive protein (10–20 mg/L) than matched controls. For the first time in this study clinical parameters could be confined to the entity of postnatal CMV infection in preterm infants (but additional prospective studies with standardized protocols are warranted). All additional parameters included were self-limiting and there was no impact on neonatal outcome parameters like intracranial hemorrhage, periventricular leukomalacia, retinopathy of prematurity and necrotizing enterocolitis (NEC). However, a large number of cases and case series – including a case from our department which was observed later on – describe severe illness in VLBW infants including pneumonia requiring artificial ventilation, hepatitis and gastrointestinal involvement, with some infants needing antiviral treatment with (val)ganciclovir.

Options for prevention of virus transmission

Effective prevention of HCMV transmission is only possible through heat treatment of BM. Both long-term (30 min, 63°C) and short-term (5 s, 62°C) pasteurisation methods are effective, but short-term pasteurisation conserves nutritional and immunological relevant...
components in milk like HCMV-specific antibodies and enzymes\(^{36,39}\), while Holder pasteurisation does not. Using this method the benefits of BM feeding can be preserved without the disadvantages of CMV transmission.

Freeze–thawing at \(-20^\circ C\) for time intervals ranging from 18 h to 10 days is not efficient in viral elimination\(^{36,40,41}\). Extended duration of cryopreservation of native breast milk at \(-20^\circ C\) from 18 h to 60 days is not sufficient for complete virus killing (see Table 1) during decrease of viral DNAlactia and virolactia from peak level to baseline viral shedding, as shown in Fig. 1.

### Long-term outcome

An earlier study revealed a significant association of postnatal HCMV infection in infants with birthweight <2000 g and neurologic sequelae and handicaps at 3 years of age\(^{42}\). In a more recent study, there was no difference in the neurodevelopmental outcome between VLBW infants with postnatal HCMV infection acquired during their stay in the neonatal intensive care unit and their matched controls at the age of 2–4 years\(^{43}\). However, this changed at the age of 4–10 years and 11–16 years: the cognitive outcome of the HCMV infected infants was significantly lower than their controls using K-ABC and HAWIK, the latter with a difference of 93 \(v\) vs. \(103 (P < 0.05)\)^{34,46}.

### Summary

In conclusion, there is a relevant entity of postnatally acquired symptomatic CMV infection and disease of very preterm infants through raw breastmilk. Actual data are supporting negative influence on long-term cognitive development. Concerning prevention, only heat-inactivation is effective and short-term heat-inactivation preserves the nutritional and immunological capacity of breast milk\(^{39}\).

### References


### Table 1. Why cryostorage for 10 days is not able to destroy efficiently human cytomegalovirus (HCMV) infectivity in human milk.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control N(\text{IFN/mL}) Copies/mL</th>
<th>18 h (-20^\circ C) N(\text{IFN/mL}) Copies/mL</th>
<th>4 days (-20^\circ C) N(\text{IFN/mL}) Copies/mL</th>
<th>10 days (-20^\circ C) N(\text{IFN/mL}) Copies/mL</th>
<th>30 days (-20^\circ C) N(\text{IFN/mL}) Copies/mL</th>
<th>60 days (-20^\circ C) N(\text{IFN/mL}) Copies/mL</th>
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<tbody>
<tr>
<td>Day 19</td>
<td>10 729 124 000</td>
<td>2053 (19.1%) 136 000</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>Day 20</td>
<td>10 926 150 000</td>
<td>1966 (18.0%) 123 000</td>
<td>767 (7.0%) 122 000</td>
<td>300 (2.8%) 122 000</td>
<td>13 (0.1%) 117 000</td>
<td></td>
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<tr>
<td>Day 23</td>
<td>22 360 136 000</td>
<td>1001 (4.5%) 241 000</td>
<td>421 (1.9%) 178 000</td>
<td>n.d.</td>
<td>51 (0.2%) 129 000</td>
<td>6 (0.02%) 113 000</td>
</tr>
<tr>
<td>Day 28</td>
<td>3568 103 000</td>
<td>1544 (43.3%) 111 000</td>
<td>119 (3.3%) 94 500</td>
<td>96 (2.7%) 92 000</td>
<td>n.d.</td>
<td>0 (0%) 96 000</td>
</tr>
<tr>
<td>Day 47</td>
<td>7 10 000</td>
<td>1 (14.3%) 10 600</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Day 49</td>
<td>6 2230</td>
<td>3 (50%) 3620</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
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Course of lactation of the HIV-negative mother of preterm twins from Fig. 1. Duration of cryopreservation ranged from 18 h to 2 months at \(-20^\circ C\). Freeze–thawing was performed with aliquots of the raw milk. The results from Fig. 1 correspond to the untreated control. Milk whey was prepared after the given cryostorage time. Using quantitative PCR (Cobas Roche Amplicor) no significant difference related to controls during cryopreservation can be detected. However, short-term 18 h microculture revealed the effect of reduction in infectivity. Residual viral infectivity after cryostorage is given in % of the untreated freshly expressed control time. Using quantitative PCR (Cobas Roche Amplicor) no significant difference related to controls during cryopreservation can be detected. However, short-term 18 h microculture revealed the effect of reduction in infectivity. Residual viral infectivity after cryostorage is given in % of the untreated freshly expressed control milk. Efficiency strongly depends from stage of lactation (see Fig. 1).


Biographies

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