Article

Postnatal cytomegalovirus infection in extremely preterm infants receiving raw mother's own milk: clinical course and neurodevelopment at two years

Hélène Dumonceau, Anne-Britt Johansson, Aline Vuckovic

aline.vuckovic@huderf.be

Keywords

Human cytomegalovirus, Breast milk, Pasteurization, Extremely low birth weight infants, Neurological outcomes

Abstract

Objective. To investigate neonatal manifestations and neurological outcomes after postnatal cytomegalovirus (CMV) infection in extremely preterm infants receiving raw mother's own milk.

Methods. This was a retrospective analysis of 45 lactating mothers and their 53 infants born ≤ 28 weeks gestational age. Maternal serologies and screening for CMV in infants were obtained. CMV-positive and CMV-negative groups were compared regarding clinical course, raw mother's own milk intake, as well as growth, neurological, and hearing outcomes until 24 months corrected age.

Results. Maternal seroprevalence was 90%. CMV viruria occurred at a median age of 33.4 weeks in 10 infants, all born to CMV-seropositive mothers. CMV-positive infants were more exposed to daily raw mother's own milk (70% vs. 28%; P = .02). Five infants with a history of prolonged ventilation and systemic corticosteroids increased their ventilator needs concurrently with CMV infection. The rates of bronchopulmonary dysplasia, necrotizing enterocolitis and retinopathy were similar in both groups. There was no fatal outcome among CMV-positive infants. However, hospital stay was prolonged by 26 days on average (P = .02). At 24 months, 7 CMV-positive infants and 22 CMV-negative infants scored similarly on Bayley-III scales. Sensorineural hearing loss was not detected after CMV infection.

Interpretation. Postnatal CMV infection in extremely preterm infants had no impact on hearing and neurodevelopment within the first 2 years of life. However, postnatal CMV infection might worsen respiratory status and increase length of hospital stay.

Introduction

Mother's own milk (MOM) is encouraged in preterm infants since its immunomodulating and nutritional properties combine to decrease inflammation, thereby attenuating the risk of bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis (1). Breast milk is also beneficial in long-term health and psychomotor development (1).

Various pathogens can be transmitted through breast milk, including cytomegalovirus (CMV) (1,2). In industrialized countries, CMV seroprevalence varies between 50 and 90% in pregnant women (2). During lactation, most seropositive mothers experience viral reactivation in the mammary glands, which begins around day 3 postpartum and peaks for 4–8 weeks (2,3). Once infected, full-term newborns usually remain asymptomatic, likely due to the complete transplacental transfer of maternal anti-CMV antibodies. By contrast, CMV transmission can reach 20–35% in preterm infants receiving raw MOM (4,5). Approximately 20% of very low birth weight (VLBW; < 1500 g) infants will develop self-limited illness ranging from abnormal laboratory findings to clinical deterioration mimicking sepsis (4,5). Despite high transmission and infection rates, deaths have rarely been reported (4). These reassuring outcomes were recently challenged by observations in extremely preterm (≤ 28 weeks gestational age) and/or extremely low birth weight (ELBW; < 1000 g) infants (4,5). In this category, postnatal CMV infection was more frequent and more often associated with severe acute disease (6). Increased risks of bronchopulmonary dysplasia and retinopathy of prematurity were also reported (7-9). By contrast with the well-known neurodevelopmental sequelae of congenital CMV infection, conflicting long-term outcomes are reported after postnatal CMV infection in VLBW infants (10-12). While neurological outcomes and sensorineural hearing seem preserved in toddlers and preschool children (11-13), older children can possibly display subtle alterations of cognitive performances (10,14,15).

Strategies of CMV inactivation in MOM, such as freezing and pasteurization, are used to limit transmission at the price of detrimental effects on the nutritional and immunological quality of milk (16). In 2012, considering contemporary evidence, the American Academy of Pediatrics recommended promotion of raw MOM in all preterm infants (1). However, the possibility of severe illness and emerging uncertainties regarding long-term outcomes have led some scientific committees to argue against the administration of raw MOM to the most immuno-incompetent infants (2,16,17). In 2016, the Belgian Superior Health Council recommended systematic pasteurization of MOM expressed from CMV-seropositive mothers until 32 weeks postmenstrual age for infants born ≤ 28 weeks gestational age and/or with an ELBW (18). Whether this recommendation might be excessive remains questionable when balancing the risks of postnatal CMV infection with the benefits of raw MOM.

Therefore, this retrospective study conducted in a Belgian neonatal intensive care unit (NICU) before the implementation of the national guidelines recapitulates the evolution of a previous cohort of extremely preterm infants, regularly receiving raw MOM from their CMV-seropositive mothers, and screened for CMV infection. The first aim was to describe the characteristics of postnatal CMV infection in this vulnerable cohort. Because follow-up data are scarce in ELBW infants, the second aim was to compare neonatal comorbidities and neurodevelopmental outcomes until 24 months corrected age in infected and non-infected infants.

Materials and methods

Study population

This was a retrospective study including all infants born ≤ 28 weeks gestational age and admitted to the NICU of Queen Fabiola Children's University Hospital (Brussels, Belgium) between June 1, 2014 and December 31, 2018.

Exclusion criteria were death before screening for CMV, congenital CMV (defined as a positive culture in urine within the first 3 weeks of life), incomplete data due to transfer to another hospital, major congenital anomaly, exclusive formula milk, and unknown maternal CMV status. Medical records were reviewed by 2 investigators (H.D. and A.V.). The Institutional Review Board approved the study protocol (No. 19/19). The need for parental informed consent was waived due to retrospectively collected data. Anonymity was preserved.

Local particularities regarding mother's own milk handling

Due to local organization and adaptation of infrastructures in our milk bank, the Belgian 2016 recommendations were implemented in January 2019. Before this date and during the study period, raw MOM expressed in the NICU was directly administered within the first hour of expression or transported to the milk bank where bacteriological cultures were performed. In the case of a negative culture, raw MOM was stored at 4°C for a maximum of 4 days before being frozen at –18°C. Holder pasteurization was applied (i.e., 62.5°C for 30 minutes then back under 10°C within less than 2 hours) if MOM cultures showed > 10^5 CFU/mL for saprophytic strains including coagulase-negative staphylococci, and/or < 10^4 CFU/mL for pathogenic bacteria including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and the *Enterobacteriaceae* family. MOM was discarded in any case of *Bacillus sp* and/or if > 10^4 CFU/mL for pathogenic bacteria. MOM expressed outside the NICU was transported to the milk bank for pasteurization.

After birth, raw colostrum was administered for 3 days. In addition to parental nutrition, enteral feeds increased by 20 mL/kg/day according to tolerance. When reaching 60 mL/kg/day of feeding volume, MOM was enriched with multicomponent fortifier (PreNAN human milk fortifier, Nestlé, Vevey, Switzerland). Fortification was not individualized given the unavailability of a bedside infrared analyzer. Parental nutrition was stopped when enteral feeding volumes reached 120 mL/kg/day. Volumes of fortified MOM were then increased to 160–180 mL/kg/day. In the case of insufficient MOM, pasteurized donor milk was offered as the best alternative. Otherwise, preterm formula milk (PreNAN, Nestlé) was administered.

Data collection

Demographics and perinatal characteristics were collected, such as gestational age and anthropometric data at birth, cause of prematurity, antenatal steroids, type of delivery, 5-minute Apgar score, Clinical Risk Index for Babies, and maternal sociodemographic data. CMV surveillance included maternal serology obtained during the last trimester of pregnancy or immediately after delivery, screening in urine scheduled on a weekly basis, and age at positive screening. Postnatal CMV infection was defined as detection of viruria after 21 days of life with a previous negative culture. The onset of viruria was calculated as the date at mid-point between the last negative and the first positive viral culture. Clinical and laboratory findings were retrieved in a 7-day period before and after identified CMV viruria.

Regarding feeding practice, the type of milk at discharge was recorded. The number of feeds containing raw MOM was counted for each day between day 3 of life and 32 weeks postmenstrual age. These data were used to calculate the proportion of days where infants were fed raw MOM at least once a day and the proportion of days where infants were exclusively fed raw MOM.

Characteristics of postnatal management were obtained throughout the NICU stay, including duration of invasive ventilation, systemic corticosteroid use, and administration of leukoreduced blood components. Comorbidities were retrieved, such as death before 36 weeks postmenstrual age, bronchopulmonary dysplasia defined as ventilatory and/or oxygen needs at 36 weeks postmenstrual age, retinopathy of prematurity ³ stage 3, necrotizing enterocolitis ³ stage 2 according to Bell criteria, intraventricular hemorrhage ³ grade 3 according to the Papile classification, periventricular leukomalacia, and proven late-onset sepsis. Variables were obtained at discharge, including length of stay, anthropometric data, brain imaging, auditory evoked potentials, fundus examination, and electroencephalogram.

At 12 months and 24 months corrected age, infants were classified as having normal or abnormal neurological findings. The level of disability in children with cerebral palsy was based on the Gross Motor Function Classification System. Beside anthropometric data, cognitive, language and motor functions were evaluated with the Bayley Scales of Infant and Toddler Development–Third edition by the same pediatrician responsible for the follow-up of preterm infants. Infants were screened for auditory and visual impairment at least once during the first 24 months of life. Hearing was investigated by wideband tympanometry and auditory evoked potentials. Sensorineural hearing loss was defined as a threshold > 20 dB (19).

Statistical analyses

Data were expressed as numbers and percentage or median and interquartile range (IQR). Two-group comparisons were performed between CMV-positive and CMV-negative groups, and between asymptomatic and symptomatic infants in the CMV-positive group. Because most data were not normally distributed, the Mann-Whitney U test was used to compare continuous variables between the two groups. Fisher's exact test was applied to categorical variables. Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). Significance of two-tailed tests was considered at $P < .05$.

Results

Study population

Seventy-five infants born to 63 mothers were initially considered (Figure 1). Twenty-two infants were excluded because of death or transfer before 21 days of life with unknown CMV status ($n = 13$), incomplete data ($n = 5$), major congenital anomaly ($n = 1$), or no breastfeeding ($n = 3$). Among the 53 included infants born to 45 mothers, 10 (19%) were diagnosed with CMV viruria (CMV-positive group), while 43 infants remained negative (CMV-negative group). Both groups displayed comparable perinatal and demographic characteristics (Table 1). All CMV-positive infants were singletons. After discharge, 7 CMV-positive infants and 22 CMV-negative infants completed their follow-up until 24 months corrected age (Figure 1). One infant with postnatal CMV infection was only evaluated at 24 months corrected age.

Screening for CMV and characteristics of postnatal infection

Maternal seroprevalence was 91% ($n = 41/45$). The screening for CMV viruria started at a median of 7 days (IQR 3.7–7.2) in the CMV-positive group and was performed 6 times (IQR 4–8) during the NICU stay. In the CMV-negative group, the first analysis was obtained at 8 days (IQR 4–13; $P = .17$) and a median number of 5 analyses (IQR 4–7) were done ($P = .35$). No infant was diagnosed with congenital CMV infection. The 5 infants born to 4 CMV-seronegative mothers did not acquire CMV infection.

In the CMV-positive group, viruria was detected at 8 weeks (IQR 6.4–8.4) after birth, which corresponded to 34.5 weeks postmenstrual age (IQR 32–35.3). Based on the date of the last negative culture, the onset of CMV viruria was estimated at 33.4 weeks postmenstrual age (IQR 32.3–35). There was no positive screening before 31 weeks postmenstrual age. Five infants developed mild signs of illness such as fever and/or increased ventilator or oxygen needs, corresponding to an incidence of 12% of symptomatic ELBW infants born to CMV-seropositive mothers. Endotracheal intubation was not required. There was no severe acute illness or death. Thrombocytopenia (minimum 80,000/mm^3) and neutropenia (minimum 400/mm^3) occurred in one and 3 infants respectively. Liver enzymes remained within the normal range, with a median value for GOT of 25 IU/L (IQR 21–31) and a median value for GPT of 11 IU/L (IQR 9–13). The level of C-reactive protein was reported in 3 febrile infants and was < 20 mg/L. No antiviral therapy was administered because CMV-positive infants were either mildly affected or not symptomatic.

Breastfeeding practice

Between day 3 and day 15 of life all CMV-positive infants received raw MOM every day, whereas 56% of CMV-negative infants were daily fed raw MOM (Table 2). The proportion of days where infants received raw MOM at least once a day was also higher in the CMV-positive group (Table 2). Likewise, the proportion of days where raw MOM was the only type of feeding given tended to be twofold higher in CMV-positive infants (69% vs. 37%; $P = .054$). The rate of exclusive breastfeeding decreased over time, resulting in 30% of infants from both groups exclusively fed breast milk at discharge (Table 2).

Neonatal comorbidities

Figure 1 : *Study flow diagram. GA, gestational age; CA, corrected age.*

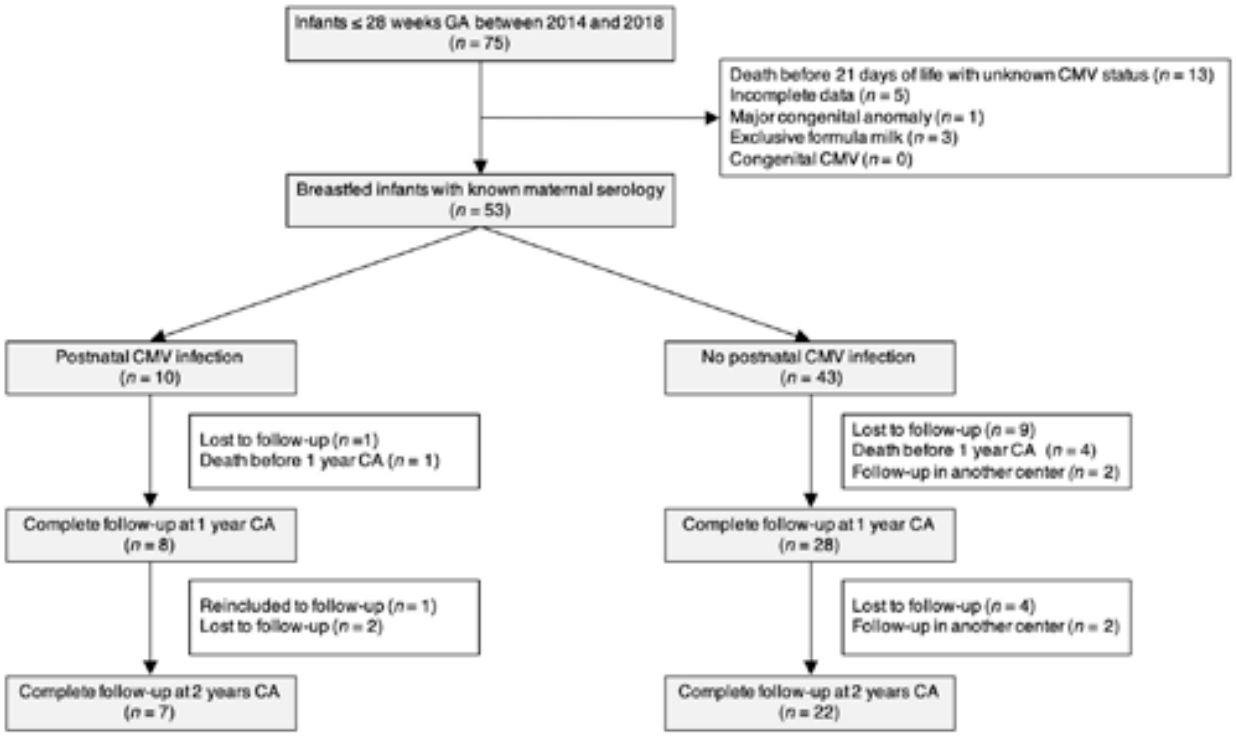

Table 1. : *Demographic and perinatal characteristics of extremely preterm infants according to the diagnosis of postnatal CMV infection*

	CMV-positive (*n* = 10)	*CMV-negative* (*n* = 43)	*P* value
Maternal variables			
CMV seropositivity, *n* (%)	10 (100)	38 (88)	.57
Age, years, median (IQR)	28.1 (24–33.3)	29.5 (24.3–32.3)	.82
Education < high school, *n* (%)	2 (20)	8 (19)	.99
Singleton, *n* (%)	10 (100)	24 (56)	.009
Chorioamnionitis, *n* (%)	1 (10)	5 (12)	.99
Pre-eclampsia, *n* (%)	3 (30)	4 (9)	.11
Placenta abruption/previa, *n* (%)	0	4 (9)	.99
Antenatal steroids, *n* (%)	9 (90)	41 (95)	.47
Cesarean section, *n* (%)	6 (60)	31 (72)	.47
Outborn delivery, *n* (%)	0	3 (7)	.99
Infant variables			
Male gender, *n* (%)	5 (50)	24 (56)	.99
Gestational age (weeks), median (IQR)	26.7 (25.8–27.2)	27 (26.1–27.7)	.18
Birth weight (g), median (IQR)	765 (695–1008)	885 (770–1075)	.25
Small for gestational age, *n* (%)	0	3 (7)	.99
Microcephaly, *n* (%)	0	2 (5)	.99
5-min Apgar score, median (IQR)	9 (6.8–9)	8 (7–9)	.29
CRIB, median (IQR)	4.5 (1.8–7.3)	3 (1–6.3)	.53

CRIB, Clinical Risk Index for Babies; IQR, interquartile range. Chorioamnionitis was diagnosed based on histology and/or microbiology. Small for gestational age and microcephaly were defined respectively as birth weight and head circumference Z-scores < -1.29 based on the 2013 Fenton growth charts.

Table 2. : *Feeding practices in extremely preterm infants according to the diagnosis of postnatal CMV infection*

	CMV-positive	*CMV-negative*	*P*
	(*n* = 10)	(*n* = 43)	value
Parenteral nutrition (full or partial), days, median (IQR)	10.5 (9–15.3)	11 (10–18)	.74
Infants receiving daily raw MOM			
From day 3 to 15, *n* (%)	10 (100)	24 (56)	.009
From day 16 to 32 weeks postmenstrual age, *n* (%)	7 (70)	12 (28)	.024
% of days with raw MOM intake			
At least for one feed per day, median (IQR)	100 (91–100)	74 (59–100)	.014
For all feeds per day, median (IQR)	69 (49–80)	37 (21–73)	.054
Feeding at discharge*			.17
Exclusive breastfeeding, *n* (%)	3 (30)	12 (28)	
Mixed feeding, *n* (%)	4 (40)	6 (14)	
Formula milk, *n* (%)	3 (30)	22 (51)	

*IQR, interquartile range; MOM, mother's own milk. *Including 40 infants alive at discharge in the CMV-negative group.*

Table3. : *Neonatal outcomes of extremely preterm infants according to the diagnosis of postnatal CMV infection*

	CMV-positive (*n* = 10)	*CMV-negative* (*n* = 43)	*P* value
Outcomes before discharge			
Invasive ventilation, *n* (%)	9 (90)	34 (79)	.66
Invasive ventilation, days, median (IQR)	10 (1–23.5)	4 (1–16.5)	.49
Systemic corticosteroids, *n* (%)	6 (60)	12 (28)	.07
Leukoreduced blood transfusions, *n* (%)			.74
1 transfusion	4 (40)	11 (25.6)	
2 transfusions	2 (20)	11 (25.6)	
≥ 3 transfusions	2 (20)	11 (25.6)	
Day of life at the first blood transfusion, median (IQR)	19 (15–45)	19 (10.3–24.5)	.38
Late-onset proven sepsis, *n* (%)	2 (20)	9 (21)	.99
Necrotizing enterocolitis ≥ grade 2, *n* (%)	0	3 (7)	.99
Moderate to severe BPD, *n* (%)*	6 (60)	20 (50)	.72
Intraventricular hemorrhage ≥ grade 3, *n* (%)	0	2 (4.7)	.99
Periventricular leukomalacia, *n* (%)	0	3 (7)	.99
Any retinopathy, *n* (%)	3 (30)	6 (14)	.34
Retinopathy ≥ grade 3, *n* (%)	0	2 (5)	.99
Death before 36 weeks postmenstrual age, *n* (%)	0	3 (7)	.99
Outcomes at discharge*			
Length of stay, days, median (IQR)	108 (83–146)	82 (67–100)	.02
Postmenstrual age, median (IQR)	41.4 (38.5–46.6)	37.7 (37–40.9)	.02
Weight, g, median (IQR)	2815 (2520–4560)	2720 (2383–3000)	.31
Head circumference, cm, median (IQR)	34.4 (33.2–37.6)	33.5 (32–35)	.08
Abnormal brain imaging, *n* (%)	2 (20)	17 (42)	.28
Abnormal EEG, *n* (%)	2 (20)	3 (7)	.26
Abnormal auditory evoked potentials			.31
Abnormal transmission, *n* (%)	1 (10)	3 (7)	
Cochlear impairment, *n* (%)	0	1 (2)	
Abnormal central conduction, *n* (%)	2 (20)	8 (20)	

*EEG, electroencephalogram; IQR, interquartile range. Abnormal brain imaging included persistent periventricular echogenicity, subependymal hemorrhage, ventriculomegaly, and focal parenchymal lesions. *Including 40 infants alive at discharge in the CMV-negative group.*

CMV-positive and CMV-negative infants were similarly treated in terms of mechanical ventilation and patent ductus arteriosus management (Table 3). The rate of leukoreduced blood cell transfusions and the timing of the first transfusion were superimposable in both groups (Table 3). Systemic corticosteroids were used twice as often in CMV-positive infants, but this trend failed to reach statistical significance (Table 3). Three CMV-negative infants died before 36 weeks postmenstrual age of bacterial sepsis ($n = 2$) and complications of intraventricular hemorrhage ($n = 1$). At discharge, there were no differences in the rate of retinopathy of prematurity, bronchopulmonary dysplasia, and necrotizing enterocolitis (Table 3). Especially, none of the CMV-infected infant was diagnosed with severe retinopathy requiring treatment or proven necrotizing enterocolitis. Besides, CMV-positive and CMV-negative infants had comparable growth, brain imaging, electroencephalogram, and auditory evoked potentials (Table 3). However, the length of NICU stay was higher in CMV-positive infants, resulting in an increased postmenstrual age at discharge (Table 3).

Subgroup analyses within the CMV-positive group were performed to understand this finding better (Table 4). As compared to asymptomatic infants who remained clinically stable, those who experienced clinical deterioration during CMV infection had a history of prolonged invasive ventilation requiring systemic corticosteroids. All of them were diagnosed with moderate or severe bronchopulmonary dysplasia and stayed longer in the NICU. Symptomatic and asymptomatic infants exhibited similar gestational age, birth weight, timing of viruria, and growth characteristics at discharge (Table 4).

Long-term neurodevelopment

At 12 months and 24 months corrected age, growth pattern, neurological examination, and Bayley-III scores were similar in CMV-positive and CMV-negative groups (Table 5). In the CMV-negative group, hearing was not evaluated in 6 infants. In the remaining infants, one was diagnosed with sensorineural hearing loss, and 2 were diagnosed with conductive hearing loss. Eight CMV-positive infants were evaluated at least once until 24 months corrected age. No sensorineural hearing loss was detected, and transmission was impaired in 3 infants. Overall, hearing tests did not differ between the groups ($P = .39$; Fisher's exact test). Severe bilateral visual deficit was diagnosed in one CMV-negative infant, while CMV-positive infants did not show any deficit ($P = .99$; Fisher's exact test). Finally, albeit the very small sample size precludes from any robust conclusion, growth and neurodevelopmental outcomes at 12 months and 24 months corrected age appeared similar in infants with symptomatic and asymptomatic postnatal CMV infection (Table 6).

Discussion

In this 4-year retrospective study focusing on extremely preterm infants mostly fed raw MOM during the first weeks of life and regularly screened for CMV, postnatal CMV viruria occurred in one-fifth of cases after 32 weeks postmenstrual age. Although infected and non-infected infants shared analogous outcomes, postnatal CMV infection was associated with a prolonged NICU stay without altering growth, hearing, or neurodevelopment until 24 months corrected age. These observations are consistent with the idea that most preterm infants born to CMV-seropositive mothers could benefit from raw MOM without devastating health consequences. However, these data should be cautiously interpreted given the limited size of the cohort.

Our findings are relevant for populations with high maternal CMV seroprevalence, which typically present CMV reactivation during lactation (3). In the case of extreme prematurity, recent guidelines recommend systematic pasteurization until 32 weeks postmenstrual age for MOM expressed from CMV-seropositive mothers (16,18). This would imply the pasteurization of MOM expressed from the majority of mothers, with subsequent higher workload, organizational constraints, and costs (20). In a recent French audit, only one-fourth of neonatal units complied with the French recommendations of systematic pasteurization of MOM in VLBW infants born to CMV-seropositive mothers, which indirectly suggests difficult implementation in clinical practice (17). In Belgium, there are no official reports regarding adherence to such recommendations. One could speculate that implementation would be difficult as most Belgian NICUs do not have access to a milk bank or pasteurization (21).

In Belgium, screening for CMV antibodies throughout pregnancy is no longer recommended, considering that congenital CMV can occur after reactivation in previously CMV-seropositive women (22). This was one of the rationales for incorporating CMV screening in all preterm infants and their mothers as part of the routine monitoring in our NICU. The median onset of CMV viruria was estimated at 8 weeks of life, which was consistent with previous reports in VLBW and ELBW infants (3,5,6). Viral load in MOM, a critical factor for CMV transmission, could not be evaluated in this study and, therefore, the correlation between virolactia and infant virologic data could not be established (3). Yet, MOM as the main source of transmission was reasonably supported by the higher proportion of raw MOM feeds received in infected infants during their first weeks of life. Besides, the systematic use of leukoreduced blood transfusions likely decreased the transmission of CMV in the present cohort, as previously reported (23). High rates of leukofiltrated blood cell transfusions were similarly found in CMV-positive and CMV-negative groups, making transfusions play a minimal role in the acquisition of CMV infection. Finally, the contribution of maternal cervical secretions to postnatal CMV infection was limited by a high rate of cesarean section in this study.

Despite high reactivation rates in MOM, not all extremely preterm infants acquire CMV infection. First, the presence of lactoferrin and anti-CMV IgG might provide raw MOM with some antiviral properties, at least experimentally (2). Second, MOM handling and storage can affect CMV activity and influence the rate of postnatal transmission (2). Herein, raw MOM was predominantly administered to CMV-positive infants during the first weeks postpartum, possibly explaining the higher rate of infection than in ELBW infants mainly receiving frozen MOM (24). Third, the risk of symptomatic infection is related to the infant's immaturity, as denoted by the association with lower birth weight and earlier age at viral detection (6). In the present cohort, postnatal CMV infection was diagnosed in about 20% of extremely preterm infants. This was in line with studies including ELBW infants, but still lower than in infants born at the limit of viability (4,6).

Half of postnatal CMV infections presented as self-limited illness, while hematologic disturbances remained asymptomatic. Antiviral therapies by ganciclovir or valganciclovir have been suggested for symptomatic congenital CMV infection to improve long-term neurodevelopmental outcomes. However, such treatments were not considered in the present study given the absence of severe symptoms in our cohort, potential immunological and liver toxicity of (val) ganciclovir, as well as the absence of prospective clinical trials supporting a use for symptomatic postnatal CMV infection acquired via MOM (2). As CMV copy count in blood and evolution of laboratory tests were not monitored, our data remained inconclusive regarding the timing of recovery and possibility of long-lasting disease (6). In accordance with previous reports, postnatal CMV infection did not affect the overall rate of death, bronchopulmonary dysplasia, necrotizing enterocolitis or retinopathy of prematurity (9,11,12). The increased length of stay could suggest that CMV infection, even if mildly symptomatic, augments the need for additional support and prolongs the length of stay (8,24). Of note, among CMV-positive infants, those who exhibited respiratory deterioration concurrent with CMV infection were more often diagnosed with bronchopulmonary dysplasia than asymptomatic ones. To date, studies exploring the association between postnatal CMV infection and bronchopulmonary dysplasia have yielded controversial results (7,11,25). A toxic effect on immature lungs was hypothesized, but the causality between CMV and bronchopulmonary dysplasia was not demonstrated (26). Consistent with the timing of CMV infection in this cohort, infants eventually diagnosed with bronchopulmonary dysplasia carried a pre-existing respiratory vulnerability, which was further destabilized by the viral episode. Based on this rationale, postnatal CMV infection could be an aggravating factor of ongoing bronchopulmonary dysplasia, retinopathy of prematurity or intestinal inflammation, depending on individual frailty. Compatible with the protective role of MOM, necrotizing enterocolitis and retinopathy of prematurity were rare outcomes in the present cohort of breastfed infants. The sample size of this study was thus too small to highlight differences after CMV infection.

The debate on whether postnatal CMV infection has an adverse effect on neurodevelopment is ongoing. Several studies do not support a role for postnatal CMV infection in neurodevelopmental delay during the first 2 to 4 years of life (5,11,27). In line with the most recent and large prospective study, our

study corroborated these findings, whether or not the patients were symptomatic, and at least until 24 months corrected age (13). Likewise, a systematic review compiling hearing outcomes in more than 1500 individuals failed to show any association between postnatal CMV infection and sensorineural hearing loss (28). This contrasted with a large retrospective study showing higher rates of failed hearing screen in CMV-positive infants (8). Yet, a failed hearing screen is frequent during the neonatal period and is not necessarily indicative of sensorineural etiology (19). Moreover, cases of congenital CMV could not be formally excluded in this study (8). Finally, discrete changes in complex cognitive functions after acquired CMV infection, still scoring within normal ranges, have been identified in 6 year-old children and adolescents (10,14,15). This must draw attention to the pursuit of long-term vigilance.

Because of the aforementioned controversies in extremely preterm infants fed raw MOM, recommendations regarding MOM handling to prevent CMV transmission vary between countries, depending on whether health authorities choose to favor the precautionary or the proportionality principle (17,18,20). Holder pasteurization suppresses the risk of CMV transmission but affects nutritional and immunological properties of MOM, including decreased concentration of bile salt-stimulated lipase activity, lactoferrin, immunoglobulins, growth factors, interleukins, and cellular components (16). It is still unclear whether pasteurization might be deleterious for postnatal growth or increase the risk of late-onset sepsis or necrotizing enterocolitis (29,30). By contrast, short-term freezing preserves the main properties of breast milk, but does not suppress the risk of CMV transmission (2). Furthermore, high-temperature short-term pasteurization has been alternatively developed to decrease the impact of MOM handling on its quality (2,20). A recent prospective cohort study showed a 10-fold reduction in the proportion of postnatal CMV infection (31). Although effective, this technique requiring time, training and specific medical device is not yet used in most NICUs (20). Therefore, instead of a generalized and strict recommendations difficult to implement in most NICUs, encouraging raw MOM in all extremely preterm infants and promoting early sucking during skin-to-skin holding at any age could be an acceptable option after informed consent regarding milk treatment.

Apart from the limitations stated above, we acknowledge that the retrospective design, small sample size and high dropout rates in our study were major shortcomings. Retrospective analysis may have an impact on the quality of retrieved data. However, the follow-up of extremely preterm infants during and after the NICU stay was prospectively collected in our institution, which likely limited such bias. The small sample size was inherent to the co-existence of 19 official NICUs in Belgium sharing the management of a limited number of ELBW infants annually. We can speculate that missed appointments in the SARS-CoV-2 pandemic context could explain unavailable data at 24 months corrected age for infants born in 2018. Finally, as this retrospective study focused on a recent cohort, follow-up data are limited to the first 24 months of life. Given uncertainties regarding long-term outcomes, cognitive performances should be monitored in older children with a previous CMV infection. Nevertheless, the strengths of our study include the homogeneity of the cohort and the screening for CMV available throughout hospital stay. This led to better characterization of phenotypes and related outcomes after postnatal CMV infection in the most fragile infants. This distinguishes our study from larger cohorts that focused on symptomatic infants and mixed those with negative CMV testing and those without any testing (and possibly asymptomatic) in the same control group (7,8,11). Interpretation of such studies can be difficult as misclassification of infants could not be ruled out.

Conclusion

In the absence of registries reporting postnatal CMV screening and long-term outcomes of CMV infection as part of the prospective surveillance of VLBW infants, recommendations for the use of raw MOM are heterogeneous in preterm infants born to CMV-seropositive mothers. Even in the case of persistent divisions about long-term risks, the evolution of extremely preterm infants after postnatal CMV infection seems globally reassuring. Therefore, reconsideration of the current Belgian guidelines and uniformization between countries regarding MOM handling could be envisaged to allow most preterm infants to benefit from raw MOM. Nevertheless, our study suggested an association between postnatal CMV infection, prolonged NICU stay, and mild worsening of the respiratory status in ELBW infants eventually diagnosed with bronchopulmonary dysplasia. These findings raised the unresolved question of restricting MOM pasteurization in the few ELBW infants with pre-existing susceptibilities prone to deteriorate after CMV infection. Due to the timing of CMV viruria in ELBW infants, the question whether the critical period during which pasteurization should be extended in these particularly vulnerable infants remains open.

Acknowledgements

The authors would like to thank Camille Brans and Marjorie Debande from the Department of Dietetics at Queen Fabiola Children's University Hospital for their support in providing protocols for milk handling.

Conflict of interest

The authors have no conflict of interest to disclose.

REFERENCES:

1. Johnston M, Landers S, Noble L, Szucs KV, L. Breastfeeding and the use of human milk. Pediatrics. 2012;129(3):e827-e841.
2. Osterholm EA, Schleiss MR. Impact of breast milk-acquired cytomegalovirus infection in premature infants: Pathogenesis, prevention, and clinical consequences. Rev Med Virol. 2020;30(6):1-11.
3. Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet. 2001;357(9255):513-518.
4. Lanzieri TM, Dollard SC, Josephson CD, Schmid DS, Bialek SR. Breast milk-acquired cytomegalovirus infection and disease in VLBW and premature infants. Pediatrics. 2013;131(6):e1937-e1945.
5. Capretti MG, Lanari M, Lazzarotto T, Gabrielli L, Pignatelli S, Corvaglia L et al. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study. J Pediatr. 2009;154(6):842-848.
6. Mehler K, Oberthuer A, Lang-Roth R, Kribs A. High rate of symptomatic cytomegalovirus infection in extremely low gestational age preterm infants of 22-24 weeks' gestation after transmission via breast milk. Neonatology. 2014;105(1):27-32.
7. Kelly MS, Benjamin DK, Puopolo KM, Laughon MM, Clark RH, Mukhopadhyay S et al. Postnatal Cytomegalovirus Infection and the Risk for Bronchopulmonary Dysplasia. JAMA Pediatr. 2015;169(12):e153785.
8. Weimer KED, Kelly MS, Permar SR, Clark RH, Greenberg RG. Association of Adverse Hearing, Growth, and Discharge Age Outcomes With Postnatal Cytomegalovirus Infection in Infants With Very Low Birth Weight. JAMA Pediatr. 2020;174(2):133-140.
9. Martins-Celini FP, Yamamoto AY, Passos DM, do Nascimento SD, Lima EV, Di Giovanni CM et al. Incidence, Risk Factors, and Morbidity of Acquired Postnatal Cytomegalovirus Infection Among Preterm Infants Fed Maternal Milk in a Highly Seropositive Population. Clin Infect Dis. 2016;63(7):929-936.
10. Goelz R, Meisner C, Bevot A, Hamprecht K, Kraegeloh-Mann I, Poets CF. Long-term cognitive and neurological outcome of preterm infants with postnatally acquired CMV infection through breast milk. Arch Dis Child Fetal Neonatal Ed. 2013;98(5):F430-3.
11. Turner KM, Lee HC, Boppana SB, Carlo WA, Randolph DA. Incidence and impact of CMV infection in very low birth weight infants. Pediatrics. 2014;133(3):e609-e615.
12. Jim WT, Chiu NC, Ho CS, Shu CH, Chang JH, Hung HY et al. Outcome of Preterm Infants With Postnatal Cytomegalovirus Infection via Breast Milk: A Two-Year Prospective Follow-Up Study. Medicine (Baltimore). 2015;94(43):e1835.
13. Gunkel J, de Vries LS, Jongmans M, Koopman-Esseboom C, van Haastert IC, Eijsermans MCJ et al. Outcome of Preterm Infants With Postnatal Cytomegalovirus Infection. Pediatrics. 2018;141(2):e20170635.
14. Bevot A, Hamprecht K, Krägeloh-Mann I, Brosch S, Goelz R, Vollmer B. Long-term outcome in preterm children with human cytomegalovirus infection transmitted via breast milk. Acta Paediatr. 2012;101(4):e167-e172.

Table 4. : *Neonatal outcomes of extremely preterm infants diagnosed with postnatal CMV infection depending on their symptomatology*

	Asymptomatic	*Symptomatic*	*P*
	(*n* = 5)	(*n* = 5)	value
Birth variables			
Gestational age (weeks), median (IQR)	26.7 (25.6–27.4)	26.3 (25.6–27.1)	.65
Birth weight (g), median (IQR)	950 (690–1080)	750 (685–885)	.55
Male gender, *n* (%)	1 (20)	4 (80)	.21
Postmenstrual age at CMV detection, median (IQR)	34.7 (33.5–37.9)	32.1 (31.5–33.5)	.24
Respiratory outcomes			
Bronchopulmonary dysplasia, *n* (%)	1 (20)	5 (100)	.047
Invasive ventilation, *n* (%)	4 (80)	5 (100)	.99
Invasive ventilation, days, median (IQR)	3 (1–19)	21 (5–25)	.31
Invasive ventilation > 7 days, *n* (%)	1 (20)	4 (80)	.21
Systemic corticosteroids, *n* (%)	1 (20)	5 (100)	.047
Length of stay, days, median (IQR)	83 (73–108)	139 (109–203)	.032
Growth variables at discharge			
Weight (z-score), median (IQR)	-0.97 (-2.27,-0.41)	-2.23 (-2.84,-0.4)	.69
Length (z-score), median (IQR)	-1.04 (-1.77,0. 23)	-2.63 (-3.2,0.19)	.42
Head circumference (z-score), median (IQR)	-0.18 (-0.92,0.44)	-1.03 (-2.1,-0.25)	.22

IQR, interquartile range. As gestational age at discharge varied between groups, growth variables were expressed as z-scores following the 2013 Fenton growth charts.

Table 5. : *Developmental outcomes at 12 months and 24 months corrected age in extremely preterm infants according to the diagnosis of postnatal CMV infection*

	12 months corrected age			24 months corrected age		
	CMV-positive (*n* = 8)	*CMV-negative* (*n* = 28)	*P* value	*CMV-positive* (*n* = 7)	*CMV-negative* (*n* = 22)	*P* value
Growth variables						
Weight, kg, median (IQR)	8.1	8.7	.69	11	11.7	.69
	(7.4–10.1)	(8–9.8)		(9.5–14)	(10.4–12.5)	
Head circumference, cm, median (IQR)	45.3	45.5	.8	47.5	48	.69
	(43.1–48)	(44–46.9)		(45–49.2)	(46–49.5)	
Neurological examination			.61			.53
Normal, *n* (%)	7 (88)	23 (82)		6 (86)	19 (86)	
Motor deficit, *n* (%)	1 (13)	3 (11)		1 (14)	1 (5)	
Cerebral palsy, *n* (%)	0	2 (7)		0	2 (9)	
BSID-III*						
Cognitive score, *n* (%)	8 (100)	26 (93)	.99	7 (100)	18 (82)	.98
≥ 85, *n* (%)	7 (87)	24 (92)		6 (86)	14 (78)	
< 85, *n* (%)	1 (13)	2 (8)		1 (14)	4 (22)	
Motor score, *n* (%)	7 (87)	24 (85)	.33	7 (100)	17 (77)	.99
≥ 85, *n* (%)	4 (57)	19 (79)		5 (71)	13 (77)	
< 85, *n* (%)	3 (43)	5 (21)		2 (29)	4 (23)	
Language score, *n* (%)	6 (75)	25 (89)	.99	6 (86)	18 (82)	.99
≥ 85, *n* (%)	6 (100)	22 (88)		4 (67)	11 (61)	
< 85, *n* (%)	0	3 (12)		2 (33)	7 (39)	

*BSID-III, Bayley Scales of Infant and Toddler Development-Third edition; IQR, interquartile range. The BSID-III motor score includes fine and gross motor skills. A standardized BSID-III score < 85 indicates mild impairment. *Excluding the 2 infants with cerebral palsy in the CMV-negative group, who could not perform the tests.*

15. Brecht KF, Goelz R, Bevot A, Krägeloh-Mann I, Wilke M, Lidzba K. Postnatal human cytomegalovirus infection in preterm infants has long-term neuropsychological sequelae. J Pediatr. 2015;166(4):834-839.e1.

16. Picaud JC, Buffin R, Gremmo-Feger G, Rigo J, Putet G, Casper C et al. Review concludes that specific recommendations are needed to harmonise the provision of fresh mother's milk to their preterm infants. Acta Paediatr. 2018;107(7):1145-1155.

17. Lopes AA, Champion V, Mitanchez D. Nutrition of Preterm Infants and Raw Breast Milk-Acquired Cytomegalovirus Infection: French National Audit of Clinical Practices and Diagnostic Approach. Nutrients. 2018;10(8):1119.

18. Brasseur D, Rigo J, Melin P, Cossey V, Daube G, Goossens L et al. Report 8734: Recommendations on the use of raw own mother's milk for preterm infants (≤ 28 weeks and/or < 1000 g) in Neonatal Intensive Care Units [Internet]. Brussels: Superior Health Council; 2016 [Cited 2021 May 14]. Available from: https://www.health.belgium.be/en/superior-health-council .

19. Paludetti G, Conti G, Di Nardo W, De Corso E, Rolesi R, Picciotti PM et al. Infant hearing loss: from diagnosis to therapy Official Report of XXI Conference of Italian Society of Pediatric Otorhinolaryngology. Acta Otorhinolaryngol Ital. 2012;32(6):347-370.

20. Klotz D, Jansen S, Gebauer C, Fuchs H. Handling of Breast Milk by Neonatal Units: Large Differences in Current Practices and Beliefs. Front Pediatr. 2018;6:235.

21. Cossey V, Johansson AB, de Halleux V, Vanhole C. The use of human milk in the neonatal intensive care unit: practices in Belgium and Luxembourg. Breastfeed Med. 2012;7:302-306.

22. Van Laethem Y, Dubois J, Braeckman L, Delforge M, Donner C, de Mol P et al. Report 9262: Specific recommendations regarding the prevention of cytomegalovirus infections in mothers and newborns [Internet]. Brussels: Superior Health Council; 2015 [Cited 2021 May 14]. Available from: https://www.health.belgium.be/en/superior-health-council .

23. Josephson CD, Caliendo AM, Easley KA, Knezevic A, Shenvi N, Hinkes MT et al. Blood transfusion and breast milk transmission of cytomegalovirus in very low-birth-weight infants: a prospective cohort study. JAMA Pediatr. 2014;168(11):1054-1062.

24. Doctor S, Friedman S, Dunn MS, Asztalos EV, Wylie L, Mazzulli T et al. Cytomegalovirus transmission to extremely low-birthweight infants through breast milk. Acta Paediatr. 2005;94(1):53-58.

25. Mukhopadhyay S, Meyer SA, Permar SR, Puopolo KM. Symptomatic Postnatal Cytomegalovirus Testing among Very Low-Birth-Weight Infants: Indications and Outcomes. Am J Perinatol. 2016;33(9):894-902.

26. Prösch S, Lienicke U, Priemer C, Flunker G, Seidel WF, Krüger DH et al. Human adenovirus and human cytomegalovirus infections in preterm newborns: no association with bronchopulmonary dysplasia. Pediatr Res. 2002;52(2):219-224.

27. Vollmer B, Seibold-Weiger K, Schmitz-Salue C, Hamprecht K, Goelz R, Krageloh-Mann I et al. Postnatally acquired cytomegalovirus infection via breast milk: effects on hearing and development in preterm infants. Pediatr Infect Dis J. 2004;23(4):322-327.

28. Martinez-Gomez E, Perez-Carpena P, Flook M, Lopez-Escamez JA. A Systematic Review on the Association of Acquired Human Cytomegalovirus Infection with Hearing Loss. J Clin Med. 2020;9(12):4011.

29. Cossey V, Vanhole C, Eerdekens A, Rayyan M, Fieuws S, Schuermans A. Pasteurization of mother's own milk for preterm infants does not reduce the incidence of late-onset sepsis. Neonatology. 2013;103(3):170-176.

30. Stock K, Griesmaier E, Brunner B, Neubauer V, Kiechl-Kohlendorfer U, Trawöger R. Pasteurization of breastmilk decreases the rate of postnatally acquired cytomegalovirus infections, but shows a nonsignificant trend to an increased rate of necrotizing enterocolitis in very preterm infants--a preliminary study. Breastfeed Med. 2015;10(2):113-117.

31. Bapistella S, Hamprecht K, Thomas W, Speer CP, Dietz K, Maschmann J et al. Short-term Pasteurization of Breast Milk to Prevent Postnatal Cytomegalovirus Transmission in Very Preterm Infants. Clin Infect Dis. 2019;69(3):438-344.

Table 6 . : *Developmental outcomes at 12 months and 24 months corrected age in extremely preterm infants with postnatal CMV infection depending on their symptomatology*

	12 months corrected age			24 months corrected age		
Growth	*Symptomatic* (*n* = 4)	*Asymptomatic* (*n* = 4)	*P* value	*Symptomatic* (*n* = 3)	*Asymptomatic* (*n* = 4)	*P* value
Weight, kg, median (IQR)	7.6	9.3	.77	9.7	11.5	.38
	(7.4–10.2)	(7.1–10.8)		(9.5–9.9)	(9.8–14.7)	
Head circumference, cm, median (IQR)	44.8	45.3	.84	46	48	.38
	(43–48)	(43.6–48)		(44.5–47.5)	(45.8–50.1)	
BSID-III*						
Cognitive score, *n* (%)	4 (100)	4 (100)	.99	3 (100)	4 (100)	.43
≥ 85, n (%)	3 (75)	4 (100)		2 (67)	4 (100)	
< 85, *n* (%)	1 (25)	0		1 (33)	0	
Motor score, *n* (%)	4 (100)	3 (75)	.99	3 (100)	4 (100)	.99
≥ 85, *n* (%)	2 (50)	2 (67)		2 (67)	2 (67)	
< 85, *n* (%)	2 (50)	1 (33)		1 (33)	1 (33)	
Language score, *n* (%)	2 (50)	4 (100)	.99	2 (67)	4 (100)	.99
≥ 85, *n* (%)	2 (100)	4 (100)		2 (100)	3 (75)	
< 85, *n* (%)	0	0		0	1 (25)	

BSID-III, Bayley Scales of Infant and Toddler Development-Third edition; IQR, interquartile range. The BSID-III motor score includes fine and gross motor skills. A standardized BSID-III score < 85 indicates mild impairment.