

Introduction

Neonates can acquire cytomegalovirus (CMV) antenatally *in utero* (congenital CMV infection, cCMV) or *postnatally* through mucosal exposure (postnatal CMV infection, pCMV). Over 90% of CMV-seropositive women excrete the virus in their breastmilk (BM) during the first weeks of lactation.[1] The proportion of women shedding CMV into BM is therefore closely linked to CMV-seroprevalence, the latter is globally approximately 80%.[2] CMV-positive fresh human BM is the main mode of infant acquisition of pCMV, defined as detection of the virus after 21 days from birth after exclusion of cCMV.[3] Healthy term neonates generally remain asymptomatic after infection, likely due to maternal antibody.[4,5] However, preterm infants <32 weeks of gestational age (GA) are at risk of pCMV.[1,6] There are conflicting reports about the incidence (ranging from 5.7 to 58.6% [4]) and clinical effects of pCMV infection, largely due to heterologous study design and populations.[7] Common clinical manifestations that have been associated with CMV include sepsis-like syndrome, respiratory illness (e.g. pneumonitis and bronchopulmonary dysplasia (BPD)) and gastrointestinal symptoms (e.g. necrotising enterocolitis (NEC)).[4,8–13] However, these diseases are common in preterm infants and a causal relationship with pCMV is difficult to ascertain. Infants with pCMV can develop long-term neurodevelopmental sequelae but data are contradictory.[14–16] Some infants exposed to CMV-positive BM never develop pCMV.[8]

Postnatal CMV infection is increasingly detected in Neonatal Intensive Care Units (NICU) due to the improving survival of extremely preterm infants and increasing awareness amongst clinicians.[17] However, the evidence base on which to manage pCMV is limited due to a lack of clinical and epidemiological data. Our study assessed the incidence and clinical

burden of pCMV in a prospective cohort of preterm and very low birth weight (VLBW) infants.

Methods

Study setting

This prospective cohort study was undertaken at The Royal Women's Hospital (RWH), a tertiary NICU in Melbourne, Australia. The study was approved by the Royal Women's Hospital Research and Ethics Committee.

Participants

Mothers of infants <32 weeks of GA and <1250 g birth weight (BW), born between 15th Aug 2001 and 16th Dec 2003, were invited to join the study during the first seven days of life.

Maternal CMV status established thereafter with an extra blood test or adding on the test to existing blood samples. The infants of mothers who tested CMV IgG positive were included.

Weekly clinical and laboratory data were collected for a period of 14 weeks.

Infants were included in the analyses if their CMV status could be established using urine CMV PCR and expressed BM (EBM) culture results. Mothers were considered potential CMV BM-transmitters if they had at least one CMV-positive EBM culture. Mothers were considered a CMV-non-transmitter if all their EBM cultures were CMV-negative, and they had at least three EBM-samples tested in the first six weeks after giving birth.

Infants were considered to have pCMV if they had at least one positive urine CMV PCR in the first 14 weeks of life and congenital CMV was excluded. Infants were considered CMV-uninfected if: (i) they had urine CMV PCR results at least until week six of life available with all samples testing negative (this interval being the mean incubation time for postnatal CMV infection [1]); (ii) all urine samples tested after the first 6 weeks remained CMV-negative; and

(iii) if the child remained urine PCR CMV-negative, they had been exposed to EBM which tested positive at least once during the first six weeks of life. Perinatal CMV transmission was excluded by a negative ear swab CMV PCR at birth and congenital CMV infection by negative urine CMV PCR results in the first three weeks of life.[18] None of the infants included in this study received treatment of pCMV infection with valganciclovir or ganciclovir during the study period.

Data and specimen collection

Demographic and perinatal data were collected upon inclusion in the study (table 1 and supplemental table 1), and clinical and laboratory data were collected daily or as indicated until discharge or death of the infant (table 2). Where possible, data and specimen collections were continued after inter-hospital transfer. Breast milk from the mother together with urine from the infant were tested weekly for CMV by culture and PCR, respectively, when available until discharge or death.

Procedures

Breast milk was given either fresh, refrigerated at 4°C for <24 hours, or frozen at -20°C for up to three months. Fresh BM was used as a preference when available; formula milk was occasionally used as a supplement. None of the infants received donated BM. All blood product transfusions were CMV-negative and leucocyte depleted as standard of care in the unit. Maternal CMV serology and CMV EBM deposit (cells) culture, as well as infant CMV PCR in urine and ear swabs, were done as previously described.[19] A positive result was considered as EBM- or urine CMV-positive.

Data analysis

Mothers of twins were included as one entity for EBM analysis. For urine analysis, twins were treated as individual participants. Only clinical data after the first two weeks of life were included in the analysis to exclude confounding factors in the immediate perinatal period.

Urine CMV-positive infants were categorised into three symptom severity groups including five symptom categories (infection, bone marrow suppression, gastrointestinal or respiratory symptoms and general appearance): asymptomatic, mild (symptoms in one or two of five categories \pm abdominal distension without any major clinical deterioration) and severe (defined as sepsis-like symptoms or symptoms in at least three of five categories). As urine analysis was done weekly, symptoms in the week of the first CMV-positive urine as well as those one week before and one week after were considered to be associated with pCMV.

Thrombocytopenia was defined as a platelet count $\leq 150 \times 10^9/L$, neutropenia as a neutrophil count $\leq 1.5 \times 10^9/L$. Urine CMV-negative infants exposed to CMV-positive BM were used as the control group for urine CMV-positive infants.

Statistical analysis

Statistical analysis was done using SPSS v 25. Results were analysed using Chi-Square and Fisher-Exact tests for categorical variables and ANOVA-test for continuous variables. Graphs were prepared using Prism 8 (GraphPad Software, LLC).

Results

Study Population

Of 370 potentially eligible infants born, the mothers of 247 infants were approached for the study and informed consent was obtained from the mothers of 202 infants. Of those, 47% (95/202) were born to CMV-seropositive mothers and included in the study. A total of 65

infants of 56 mothers (nine sets of twins) were included into the final analysis (figure 1). Of these 65 infants, 89% (58/65) were exposed to CMV-positive BM and 47% (27/58) of those exposed infants became urine CMV-positive (figure 2).

CMV excretion into BM

There were no statistically significant differences in the characteristics between BM CMV-negative and CMV-positive women (supplemental table 1). Nine women were mothers of twins with concordant CMV detection in their EBM deposit. Of the 56 mothers, 88% (49/56) had at least one CMV-positive EBM sample during the 14 weeks observation period. Of these mothers, 91% (45/49) shed the virus in their milk within the first four weeks, and 98% (48/49) had their first positive sample by week six (figure 3). CMV was first detected in BM at a median of 3.0 weeks (IQR 1) after birth (table 1).

CMV infection of infants

58 infants of 49 mothers were exposed to CMV-positive BM. The infants of seven mothers who never excreted CMV into their BM did not acquire pCMV and remained urine PCR CMV-negative. Of the 58 infants exposed to CMV-positive BM, 47% (27/58) became urine PCR CMV-positive at one point during the observation period (figure 4). The proportion of infants who were <29 weeks gestation was similar in the urine CMV-positive and the CMV-negative groups (85% (23/27) vs. 74% (23/31)).

Most infants excreted CMV into urine for the first time between week six and nine (15/27), with a median detection time of 7.0 weeks after birth (IQR 2, range 3-13). Of the 27 infants, 23 remained urine CMV-positive up to the last available sample, although two infants had at least one intermittent negative sample. In eight of nine twin pairs, CMV urine PCR was

positive in at least one sample with concordant results. In one twin pair, results were discordant. One twin tested negative, although follow-up urine samples were not available.

Clinical impact of CMV infection

There were no statistically significant differences in the demographic or perinatal characteristics between the 27 CMV-positive (symptomatic or asymptomatic) and 31 urine CMV-negative infants exposed to CMV-positive BM (table 1). All 58 infants of BM CMV-positive mothers were exposed to fresh BM at some point during their hospital stay. The mean intake of fresh BM for infants was 45% of all feeds. Virus was first detected in EBM at a median of 3 weeks in non-infected infants (IQR 1, range 1-10) and 2 weeks in infected infants (IQR 1, range 1-6).

A higher proportion of CMV-positive infants had a new episode of neutropenia (18 vs. 8 infants, $p<0.01$), and trend towards thrombocytopenia (5 vs. 1 infant, $p=0.06$) after week 2 of life. CMV-positive infants had a trend towards a longer duration of CPAP and endotracheal tube ventilation (19.9 vs. 14.5 days, $p=0.27$ and 11.9 vs 9.8 days, $p=0.64$, respectively) as well as an oxygen requirement at 36 weeks of GA (9 vs. 5, $p=0.13$). The rate of NEC was similar in both groups, although only 6 of the 58 infants exposed to CMV-positive BM developed clinical NEC during the observation period. Urine CMV-positive infants had a significantly longer length of hospital stay (LOS) (93.6 vs. 79.2 days, $p=0.03$) (table 2).

Symptomatic vs. asymptomatic infection

In total, 30% (8/27) of the CMV-positive infants were asymptomatic, 48% (13/27) mildly symptomatic and 22% (6/27) severely symptomatic at the time of the first CMV-positive urine sample (table 3). Neutropenia was one of the most common presentations (44%, 12/27),

followed by respiratory deterioration (33%, 9/27; apnoea (3), new CPAP requirement (1), new intubation (2), increasing oxygen requirement (7)). Nine out of 27 infants had a partial or full septic work-up.

Discussion

In our study, approximately half of VLBW infants <32 weeks, exposed to CMV-positive BM acquired pCMV, and approximately half developed mild clinical symptoms and one-fifth severe symptoms. Infected infants had a significantly increased length of hospital stay (LOS).

This transmission rate of 47% is amongst the highest described.[1,4] This may relate to the preferential use of fresh BM in the NICU during the study period accounting for approximately 50% of all feeds. In contrast, in a Canadian NICU that reported a pCMV incidence of only 6.2%, the proportion of fresh BM intake was only 15.3% in CMV-positive infants and 3.2% in CMV-negative infants.[20] A rate of 65% of symptomatic pCMV infections in CMV-positive BM-exposed preterm infants of 22 to 24 weeks GA to was observed in a German NICU after a change of feeding policy from routine Holder pasteurised BM to fresh BM.[21]

Our finding of a longer LOS and duration of respiratory support is consistent with other studies that reported an association between pCMV and longer duration of respiratory support, slower weight gain and an increased corrected GA and decreased weight-for-length ratio at discharge.[10,20,22]. In our study, it would be less likely that neonates born 20 years ago would be discharged on home oxygen and the time taken to stop low flow oxygen could have influenced increased LOS in pCMV infected cases.

We found no significant increase in BPD or NEC in CMV-positive infants. However, other studies have reported varying rates, likely attributable to differences in study design, small cohorts and inconsistent clinical definitions.[9,10,12,23,24]

Postnatal CMV infection was associated with a septic work-up in one third of CMV-positive infants underlining the importance of the recognition of pCMV as a possible cause of clinical deterioration in preterm infants.[22] Under-recognition of pCMV as a cause of culture-negative sepsis may contribute to the varying incidence in the literature: in a recent retrospective study only 1.3% of VLBW infants were diagnosed with symptomatic pCMV.[22] The clinical significance of asymptomatic and mildly symptomatic pCMV remains uncertain. Prolonged neutropenia was one of the main and occasionally the only presenting symptom of pCMV in our study. Distinguishing pCMV as a primary or contributing (by causing neutropenia and subsequent bacterial infection [25–27]) factor to clinical deterioration needs further investigation in prospective studies with a control group.

Early identification of mother-infant pairs at risk of pCMV transmission is important for any pCMV prevention strategy. Of the CMV-seropositive mothers in our study, almost all had their first BM CMV culture-positive sample in the first six weeks after birth. CMV reactivation and excretion into BM usually occurs between three days and three months postpartum.[1,28] National guidelines in Germany, France, Austria and Sweden recommend identifying CMV-seropositive mothers of preterm and VLBW infants at birth and treating their BM.[29] The AAP also now suggests a similar approach. [30,31] Holder pasteurisation is the gold-standard treatment, but a newer technique of short-term pasteurisation with lower temperatures might maintain BM properties better [32,33] However, in addition to potentially reducing the nutritional benefits of BM, pasteurising BM is time consuming and

expensive.[34,35] Half of the infants in our study exposed to CMV-positive BM remained CMV-negative; therefore, understanding the pathophysiology of pCMV and identifying those infants at risk might help guide the risk-benefit assessment of prevention strategies.

The effects of pCMV on neurodevelopment in later life are unproven and it remains uncertain which infants are most affected, and whether asymptomatic and mild pCMV in the neonatal period has the same long-term consequences.[36] The Tübingen group followed 41 of 44 children in their original cohort into early adolescence.[14] They found no difference in neurodevelopment in pCMV-infected infants in early childhood but lower (within normal range) values in cognitive and motor function assessment tools at school age. Infected infants also scored significantly lower in overall cognitive abilities in adolescence.[14,16,37] A Dutch study also found that infants with pCMV showed normal but lower cognitive scores at the age of six years compared to matched non-infected peers.[24] Microstructural brain changes on MRI have been postulated as an early correlate of pCMV, though at 16 months of age neurodevelopment was normal[38]. Although these studies suggest an association with pCMV, their small size precludes definitive conclusions.

The main strength of our study is the rigorous sample collection of urine and BM allowing early detection of asymptomatic and mildly symptomatic pCMV, which would likely have been missed in other observational studies. Limitations include the relatively small sample size from a single centre, incomplete data collection (i.e. pregnancy history, time to establish feeds, weight at discharge, cranial imaging data, maternal seropositivity at birth/seroconversion), sample collection necessitating exclusion of some infants and incomplete follow-up due to discharge or death before the end of the observation period. The inability to match CMV-positive infants with CMV-negative infants precluded a comparison

of symptom severity between the groups meaning it is difficult to attribute clinical manifestation to CMV infection or complications of prematurity. In addition, preterm infants of CMV-negative mothers were not included for comparison. Finally, pCMV acquisition after six weeks may have been missed because of discontinuation of urine screening, hospital transfer or discharge.

In conclusion, the last two decades have seen a significant improvement of survival rates of extremely premature infants.[39] The increased preference for BM feeding has led to an increased awareness of the potential risks of CMV transmission through BM.[17] Our study highlights that pCMV may have clinical implications for preterm and VLBW infants. The rate of asymptomatic and mildly symptomatic infections is high and long-term consequences remain uncertain.[30] Guidelines to manage pCMV are therefore predominately pragmatic using limited epidemiological data.[3,40] Future research should include sufficient infants to allow matching of CMV-positive and CMV-negative infants to better enable the distinction between symptoms attributable to CMV infection and those resulting from prematurity. Universal screening studies, in extremely preterm cohorts, should be prioritised to understand if there are any prognostic markers that may help inform when antiviral treatment should be started and which neonates would benefit most. In addition, the benefit of screening for pCMV in preterm and VLBW infants to identify those who might benefit most from treatment and to better understand long-term outcomes needs further evaluation.

References

- 1 Hamprecht K, Maschmann J, Vochem M, *et al.* Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. *Lancet* 2001;**357**:513–8. doi:10.1016/S0140-6736(00)04043-5
- 2 Zuhair M, Smit GSA, Wallis G, *et al.* Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. *Rev Med Virol* 2019;**29**:e2034. doi:10.1002/rmv.2034
- 3 Kadambari S, Whittaker E, Lyall H. Postnatally acquired cytomegalovirus infection in extremely premature infants: How best to manage? *Arch Dis Child Fetal Neonatal Ed* 2020;**105**:F334–9. doi:10.1136/archdischild-2019-317650
- 4 Kurath S, Halwachs-Baumann G, Müller W, *et al.* Transmission of cytomegalovirus via breast milk to the prematurely born infant: A systematic review. *Clin Microbiol Infect* 2010;**16**:1172–8. doi:10.1111/j.1469-0691.2010.03140.x
- 5 Coclite E, Di Natale C, Nigro G. Congenital and perinatal cytomegalovirus lung infection. *J Matern Neonatal Med* 2013;**26**:1671–5. doi:10.3109/14767058.2013.794207
- 6 Martins-Celini FP, Yamamoto AY, Passos DM, *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**:929–36. doi:10.1093/cid/ciw394
- 7 Josephson CD, Caliendo AM, Easley KA, *et al.* Blood transfusion and breast milk transmission of cytomegalovirus in very low-birth-weight infants: A prospective cohort study. *JAMA Pediatr* 2014;**168**:1054–62. doi:10.1001/jamapediatrics.2014.1360
- 8 Tengsupakul S, Birge ND, Bendel CM, *et al.* Asymptomatic DNAemia heralds CMV-associated NEC: Case report, review, and rationale for preemption. *Pediatrics*

- 2013;**132**:e1428-34. doi:10.1542/peds.2013-0087
- 9 Kelly MS, Benjamin DK, Puopolo KM, *et al.* Postnatal cytomegalovirus infection and the risk for bronchopulmonary dysplasia. *JAMA Pediatr* 2015;**169**:e153785. doi:10.1001/jamapediatrics.2015.3785
 - 10 Weimer KED, Kelly MS, Permar SR, *et al.* Association of Adverse Hearing, Growth, and Discharge Age Outcomes with Postnatal Cytomegalovirus Infection in Infants with Very Low Birth Weight. *JAMA Pediatr* 2020;**174**:133–40. doi:10.1001/jamapediatrics.2019.4532
 - 11 Fischer C, Meylan P, Bickle Graz M, *et al.* Severe postnatally acquired cytomegalovirus infection presenting with colitis, pneumonitis and sepsis-like syndrome in an extremely low birthweight infant. *Neonatology* 2010;**97**:339–45. doi:10.1159/000260137
 - 12 Patel RM, Shenvi N, Knezevic A, *et al.* Observational study of cytomegalovirus from breast milk and necrotising enterocolitis. *Arch Dis Child Fetal Neonatal Ed* 2020;**105**:F259–65. doi:10.1136/archdischild-2018-316613
 - 13 Omarsdottir S, Agnarsdottir M, Casper C, *et al.* High prevalence of cytomegalovirus infection in surgical intestinal specimens from infants with necrotizing enterocolitis and spontaneous intestinal perforation: A retrospective observational study. *J Clin Virol* 2017;**93**:57–64. doi:10.1016/j.jcv.2017.05.022
 - 14 Bevot A, Hamprecht K, Krägeloh-Mann I, *et al.* Long-term outcome in preterm children with human cytomegalovirus infection transmitted via breast milk. *Acta Paediatr Int J Paediatr* 2012;**101**:e167-72. doi:10.1111/j.1651-2227.2011.02538.x
 - 15 Nijman J, van Loon AM, de Vries LS, *et al.* Urine viral load and correlation with disease severity in infants with congenital or postnatal cytomegalovirus infection. *J Clin Virol* 2012;**54**:121–4. doi:10.1016/j.jcv.2012.02.017

- 16 Brecht KF, Goelz R, Bevot A, *et al.* Postnatal human cytomegalovirus infection in preterm infants has long-term neuropsychological sequelae. *J Pediatr* 2015;**166**:834-839.e1. doi:10.1016/j.jpeds.2014.11.002
- 17 Wright CJ, Permar SR. Preventing postnatal cytomegalovirus infection in the preterm infant: Should it be done, can it be done, and at what cost? *J Pediatr* 2015;**166**:795–8. doi:10.1016/j.jpeds.2014.12.062
- 18 Haslam R. Management of Perinatal Infections. In: Palasanthiran P, Starr M, Jones C, *et al.*, eds. *Journal of Paediatrics and Child Health*. Sydney: : Australasian Society of Infectious Diseases (ASID) Inc. 2003. 482–3. doi:10.1046/j.1440-1754.2003.t01-3-00198.x
- 19 Curtis N, Chau L, Garland S, *et al.* Cytomegalovirus remains viable in naturally infected breast milk despite being frozen for 10 days. *Arch Dis Child Fetal Neonatal Ed* 2005;**90**:F529-30. doi:10.1136/adc.2004.067769
- 20 Doctor S, Friedman S, Dunn MS, *et al.* Cytomegalovirus transmission to extremely low-birthweight infants through breast milk. *Acta Paediatr Int J Paediatr* 2005;**94**:53–8. doi:10.1080/08035250410022332
- 21 Mehler K, Oberthuer A, Lang-Roth R, *et al.* High rate of symptomatic cytomegalovirus infection in extremely low gestational age preterm infants of 22-24 weeks' gestation after transmission via breast milk. *Neonatology* 2013;**105**:27–32. doi:10.1159/000355306
- 22 Mukhopadhyay S, Meyer SA, Permar SR, *et al.* Symptomatic Postnatal Cytomegalovirus Testing among Very Low-Birth-Weight Infants: Indications and Outcomes. *Am J Perinatol* 2016;**33**:894–902. doi:10.1055/s-0036-1581080
- 23 Neuberger P, Hamprecht K, Vochem M, *et al.* Case-control study of symptoms and neonatal outcome of human milk-Transmitted cytomegalovirus infection in premature

- infants. *J Pediatr* 2006;**148**:326–31. doi:10.1016/j.jpeds.2005.09.030
- 24 Gunkel J, De Vries LS, Jongmans M, *et al.* Outcome of preterm infants with postnatal cytomegalovirus infection. *Pediatrics* 2018;**141**:e20170635. doi:10.1542/peds.2017-0635
 - 25 Capretti MG, Lanari M, Lazzarotto T, *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**:842–8. doi:10.1016/j.jpeds.2008.12.046
 - 26 Lombardi G, Garofoli F, Manzoni P, *et al.* Breast milk-acquired cytomegalovirus infection in very low birth weight infants. *J Matern Neonatal Med* 2012;**25**:57–62. doi:10.3109/14767058.2012.712345
 - 27 Tran L, Ferris M, Norori J, *et al.* Necrotizing enterocolitis and cytomegalovirus infection in a premature infant. *Pediatrics* 2013;**131**:e318-22. doi:10.1542/peds.2011-1971
 - 28 Hamprecht K, Goelz R. Postnatal Cytomegalovirus Infection Through Human Milk in Preterm Infants: Transmission, Clinical Presentation, and Prevention. *Clin Perinatol* 2017;**44**:121–30. doi:10.1016/j.clp.2016.11.012
 - 29 Haiden N, Greber-Platzer S, Haiden N, *et al.* Prevention of CMV infections in preterm babies (<28 + 0 weeks gestation or birth weight <1000g) from maternal milk—Update 2018: Consensus paper of the committee on nutrition jointly with the working group neonatology and pediatric intensive care medicine. *Monatsschr Kinderheilkd* 2019;**167**:323–8. doi:10.1007/s00112-018-0626-8
 - 30 Schleiss MR. Breast Milk-Acquired Cytomegalovirus in Premature Infants: Uncertain Consequences and Unsolved Biological Questions. *JAMA Pediatr* 2020;**174**:121–3. doi:10.1001/jamapediatrics.2019.4538
 - 31 Kimberlin DW, Brady MT, Jackson MA, *et al.*, editors. American Academy of

- Pediatrics. Human milk. In: *Red Book: 2018 Report of the Committee on Infectious Diseases*. Itasca, IL: : American Academy of Pediatrics 2018. 113–21.
- 32 Bapistella S, Hamprecht K, Thomas W, *et al*. Short-term Pasteurization of Breast Milk to Prevent Postnatal Cytomegalovirus Transmission in Very Preterm Infants. *Clin Infect Dis* 2019;**69**:438–44. doi:10.1093/cid/ciy945
- 33 Maschmann J, Müller D, Lazar K, *et al*. New short-term heat inactivation method of cytomegalovirus (CMV) in breast milk: Impact on CMV inactivation, CMV antibodies and enzyme activities. *Arch Dis Child Fetal Neonatal Ed* 2019;**104**:F604–8. doi:10.1136/archdischild-2018-316117
- 34 Peila C, Moro GE, Bertino E, *et al*. The effect of holder pasteurization on nutrients and biologically-active components in donor human milk: A review. *Nutrients* 2016;**8**:477. doi:10.3390/nu8080477
- 35 Bryant P, Morley C, Garland S, *et al*. Cytomegalovirus transmission from breast milk in premature babies: Does it matter? *Arch Dis Child Fetal Neonatal Ed* 2002;**87**:75–7. doi:10.1136/fn.87.2.f75
- 36 Osterholm EA, Schleiss MR. Impact of breast milk-acquired cytomegalovirus infection in premature infants: Pathogenesis, prevention, and clinical consequences? *Rev Med Virol* 2020;**30**:1–11. doi:10.1002/rmv.2117
- 37 Vollmer B, Seibold-Weiger K, Schmitz-Salue C, *et al*. Postnatally acquired cytomegalovirus infection via breast milk: Effects on hearing and development in preterm infants. *Pediatr Infect Dis J* 2004;**23**:322–7. doi:10.1097/00006454-200404000-00009
- 38 Nijman J, Gunkel J, De Vries LS, *et al*. Reduced occipital fractional anisotropy on cerebral diffusion tensor imaging in preterm infants with postnatally acquired cytomegalovirus infection. *Neonatology* 2013;**104**:143–50. doi:10.1159/000351017

- 39 Moore T, Hennessy EM, Myles J, *et al.* Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: The EPICure studies. *BMJ* 2012;**345**:1–13. doi:10.1136/bmj.e7961
- 40 Hamele M, Flanagan R, Loomis CA, *et al.* Severe morbidity and mortality with breast milk associated cytomegalovirus infection. *Pediatr Infect Dis J* 2010;**29**:84–6. doi:10.1097/INF.0b013e3181b6dbb5

Figure Legends

Figure 1. Enrolment of study participants

BW – birth weight, CMV – cytomegalovirus, EBM – expressed breast milk, GA – gestational age, RWH – Royal Women’s Hospital

Figure 2. Demographic data with expressed breast milk and urine results for all 65 infants

Study numbers with asterisks represent twin pairs. Urine CMV-positive infants are further marked as being asymptomatic (A), mildly symptomatic (M) and severely symptomatic (S). A – asymptomatic, BM – breast milk, BW – birth weight (in grams), EBM – expressed breast milk, GA – gestational age (in weeks), neg – negative ear swab CMV PCR result, M – mildly symptomatic, NS – no swab, S – severely symptomatic, SN – study number, S_y – symptoms.

Figure 3. CMV excretion in expressed breast milk deposit

Proportion of EBM CMV-positive samples for 56 mothers by week post-delivery. The grey shaded bars represent the proportion of EBM CMV-positive samples by week. The dark grey bars show the proportion of first-time positive samples. The continuous line shows the cumulative proportion of EBM CMV-positive samples. Absolute sample numbers are shown in the table below with total numbers of all samples including negative results by week. POS – CMV-positive EBM samples.

Figure 4. CMV excretion in infants’ urine

Proportion of urine CMV-positive samples of infants exposed to CMV-positive BM. Samples from 58 infants were examined. The dark grey bars represent the proportion of first-time

positive samples. The continuous line shows the cumulative proportion of urine CMV-positive samples. Absolute sample numbers are shown in the table below with total numbers of all samples including negative results by week. POS – CMV-positive urine samples.

Table 1. Demographic and perinatal data of infants exposed to CMV-positive EBM

C/S – caesarean section, ROM – rupture of membranes

Table 2. Clinical data of infants exposed to CMV-positive breast milk

ABX – antibiotic therapy, CPAP – continuous positive airway pressure therapy, CRP – C-reactive protein, ETT – endotracheal tube, GA – gestational age, NEC – necrotising enterocolitis, NICU – neonatal intensive care unit

Table 3. Clinical data of the 27 urine PCR CMV-positive infants

Data from the week of the first CMV-positive urine as well as one week before and after.

Study numbers with asterisks represent twin pairs.

ABX – antibiotics, BCx – blood culture, BW – birth weight, FiO₂max – maximum additional oxygen requirement, GA – gestational age, LP – lumbar puncture, neg – negative, pos – positive, SN – study number, SPA – suprapubic aspirate, 1st pos urine – week of first CMV-positive urine PCR.