Asymptomatic DNAemia Heralds CMV-Associated NEC: Case Report, Review, and Rationale for Preemption

abstract

Human cytomegalovirus (CMV) infection may be acquired in very low birth weight and extremely low birth weight (ELBW) infants from breast milk. The clinical relevance of such infections is uncertain. There is no consensus on whether screening breast milk for CMV, freezing/pasteurizing milk before feeding, or performing virological monitoring on at-risk infants is warranted. We describe an ELBW infant who acquired CMV postnatally from breast milk and developed CMV sepsis syndrome and clinical evidence of necrotizing enterocolitis (NEC) at ~5 weeks of age. The availability of serial dried blood spots from day of life (DOL) 4 to 21, coincidentally obtained for a metabolic study, provided the novel opportunity to retrospectively test for and quantify the magnitude of CMV DNAemia. DNAemia was present for several weeks before the onset of severe CMV disease, first being noted on DOL 18 and increasing in magnitude daily to 4.8 $\log_{10}$ genomes/mL on DOL 21, approximately 8 days before the onset of abdominal distension and 15 days before the onset of CMV sepsis syndrome and NEC. After surgical resection, supportive care, and ganciclovir therapy, the infant recovered. This case underscores the importance of including CMV infection in the differential diagnosis of sepsis and NEC in premature infants. This case also suggests the value of prospective virological monitoring in at-risk low birth weight and ELBW infants. Future studies should examine the potential utility of preemptive monitoring for, and possibly treatment of, CMV DNAemia in premature infants, which may herald the onset of serious disease. *Pediatrics* 2013;132:e1428–e1434

AUTHORS: Supatida Tengsupakul, MD,[a] Nicole D. Birge, MD,[a] Catherine M. Bendel, MD,[a] Robyn C. Reed, MD, PhD,[a] Beth-Ann Bloom, MS, CGC,[b] Nelmary Hernandez, MS,[a] and Mark R. Schleiss, MD[a]

[a]Department of Pediatrics, Amplatz Children's Hospital and University of Minnesota Medical School, Minneapolis, Minnesota; and [b]Minnesota Department of Health, Newborn Screening Program, 601 North Robert Street, St Paul, Minnesota

KEY WORDS
cytomegalovirus, premature infant, necrotizing enterocolitis (NEC), DNAemia (viral load), breast milk

ABBREVIATIONS
AAP—American Academy of Pediatrics
CMV—cytomegalovirus
DBS—dried blood spots
DOL—day of life
ELBW—extremely low birth weight
GCV—ganciclovir
HSCT—hematopoietic stem cell transplant
NEC—necrotizing enterocolitis
PCR—polymerase chain reaction
SOT—solid organ transplant
VLBW—very low birth weight

INTRODUCTION

Very low birth weight (VLBW) and extremely low birth weight (ELBW) infants are at risk for symptomatic postnatal cytomegalovirus (CMV) infections, most commonly acquired from breast milk. Most CMV-seropositive women reactivate virus postpartum in the mammary gland upon lactation, and shed CMV in breast milk.[1] Breast milk-acquired CMV infections are typically asymptomatic in term infants, but can cause serious disease in VLBW and ELBW infants. Such infections may present with sepsis-like syndrome, thrombocytopenia, neutropenia, pneumonitis, and cholestatic hepatitis.[1,2] Enteritis may also be a manifestation of CMV and can mimic necrotizing enterocolitis (NEC), occasionally requiring surgical intervention.[2–15]

There is no consensus to guide surveillance, prevention, and therapy of CMV infections acquired via breast milk in the premature infant. Some experts stress the generally innocuous course of these infections, and argue that special precautions to prevent transmission are unnecessary. A recently published policy statement by the American Academy of Pediatrics (AAP) Section on Breastfeeding stressed the benefits of breast milk in reducing the risk of complications of prematurity, and stated that "the value of routinely feeding human milk from seropositive mothers to preterm infants outweighs the risks of clinical disease" attributable to CMV.[16] The AAP also noted that although freezing breast milk before feeding can reduce the viability of CMV in milk, it does not eliminate the risk of transmission. Pasteurization, although capable of completely inactivating CMV, unfortunately inactivates some of the salutary components of milk.[17–20] Therefore, the AAP recommends fresh maternal breast milk for routine feeding in premature infants.

We report here a case of sepsis syndrome and NEC in a premature infant infected with CMV acquired via maternal breast milk. The enrollment of the infant in a clinical study requiring daily heel-stick blood samples in the weeks before the onset of CMV disease presented us with the unique opportunity to retrospectively identify CMV DNAemia, by polymerase chain reaction (PCR) of DNA extracted from dried blood spots (DBS), before symptom onset. We show that high-grade CMV DNAemia was present several weeks before the onset of severe CMV end-organ disease. PCR of control blood spots was negative for CMV DNA, confirming the specificity of our PCR assay. We speculate that surveillance PCR for CMV DNA could serve as an important prognostic and predictive marker for preterm infants at high risk of developing end-organ disease, and suggest that this is testable hypotheses that could be evaluated in future studies of preemptive monitoring and possibly antiviral intervention.

PATIENT PRESENTATION

A preterm male infant born at 24 and 5/7 weeks' gestation and weighing 800 g was admitted to a tertiary care NICU with respiratory distress syndrome. Physical examination excluded microcephaly, hepatosplenomegaly, and rash. Empirical antibiotics were discontinued at 48 hours of age after documentation of negative blood and urine cultures. Congenital CMV infection was excluded by negative urine CMV PCR obtained at day of life (DOL) 12.[21] Feeding with breast milk initiated on DOL 14. The infant was fed frozen maternal breast milk, stored at −20°C, via gavage. On DOL 26 his abdomen became distended. Abdominal radiograph revealed dilated bowel loops. Antibiotics were administered because of concern for NEC. Bacterial cultures of blood and urine were negative. Seven days later (on DOL 33)

he had tachycardia and temperature instability. His abdomen remained distended. White blood cell count was 12×10^9/L. Platelet count was 24×10^9/L. Exploratory laparotomy revealed perforations in the jejunum, and histology demonstrated variable ischemic necrosis and CMV inclusions. Immunohistochemical stain of bowel using a monoclonal antibody to CMV (DDG9/CCH2) was positive (Fig 1). The diagnosis of systemic CMV infection was confirmed by PCR of urine and blood, which demonstrated 4.4 $\log_{10}$ copies/mL and 6.2 $\log_{10}$ copies/mL, respectively. Ganciclovir (GCV) was commenced intravenously on DOL 35, at 6 mg/kg/dose every 12 hours for 3 weeks. Response to therapy was confirmed by clinical improvement and resolution of DNAemia (Fig 2).

Of interest, daily DBS had been collected from this infant on DOL 4 to 21 for an ongoing clinical study of immunoreactive trypsinogen and its potential association with NEC and intestinal perforation in ELBW infants (http://clinicaltrials.gov/ct2/show/NCT01530828). After Institutional Review Board approval and informed consent, these DBS were examined for CMV DNA by PCR. Five 3-mm punches were collected from each DBS, and DNA extraction and PCR amplification were performed as previously described.[22] This analysis demonstrated that CMV DNAemia commenced on DOL 18, 8 days before onset of symptoms and 4 days after initiation of breast milk feeds (Fig 2). As a control for the PCR, 10 DBS obtained in a comparison of CMV infections in infants who pass or fail newborn hearing screening were tested in parallel with this infant's samples. These DBS (from CMV-uninfected controls with normal hearing) were all negative for CMV DNA.[22] PCR analyses of 4 samples of frozen breast milk, independently obtained at different dates, all demonstrated high levels of
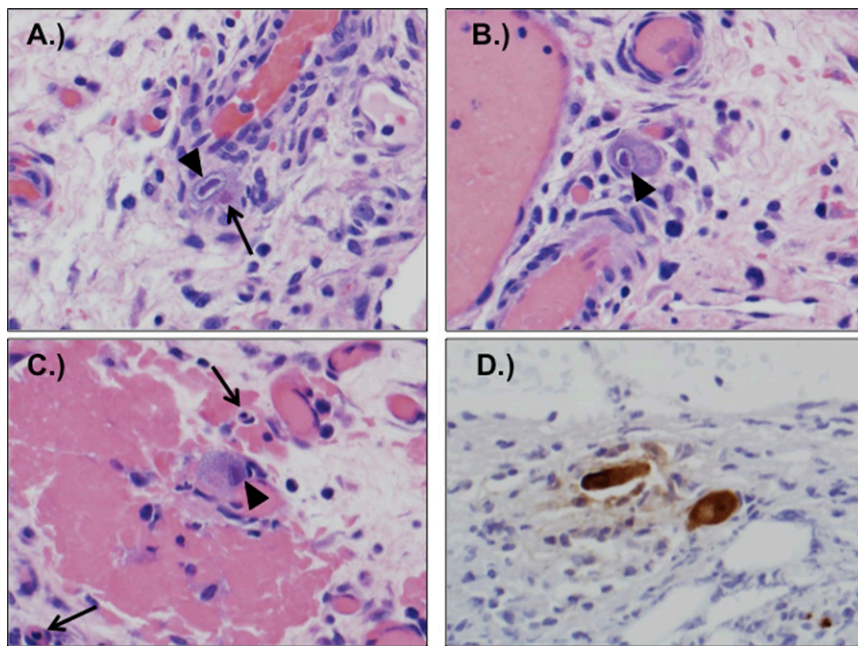
FIGURE 1

A–C, Sections of surgically resected bowel showed enlarged endothelial cells with viral cytopathic changes, including eosinophilic cytoplasmic inclusions and basophilic nuclear inclusions. Arrows point to cytoplasmic (open arrows) and nuclear (arrowheads) inclusions. D, Immunoperoxidase staining with anti-CMV antibody was positive in affected cells.

CMV DNA, with a range in viral load from 5.7 to 6.6 $\log_{10}$ copies/mL (data not shown). The breast milk and blood CMV isolates were identical (gB genotype group 1 strains; data not shown).[23]

DISCUSSION

Postnatal CMV infections are transmitted via breast milk in 6% to 59% of breastfed premature infants, and up to 34% of infected infants become symptomatic.[24–26] Early appearance of viral DNA in milk, the presence of viral DNA in milk whey, and the overall magnitude of the breast milk viral load are risk factors for transmission.[24] In this case report, we demonstrate by retrospective analyses of DBS samples that CMV DNAemia may be present well in advance of the onset of symptoms in the VLBW infant. DNAemia occurred quickly after initiation of feeds, with low-grade
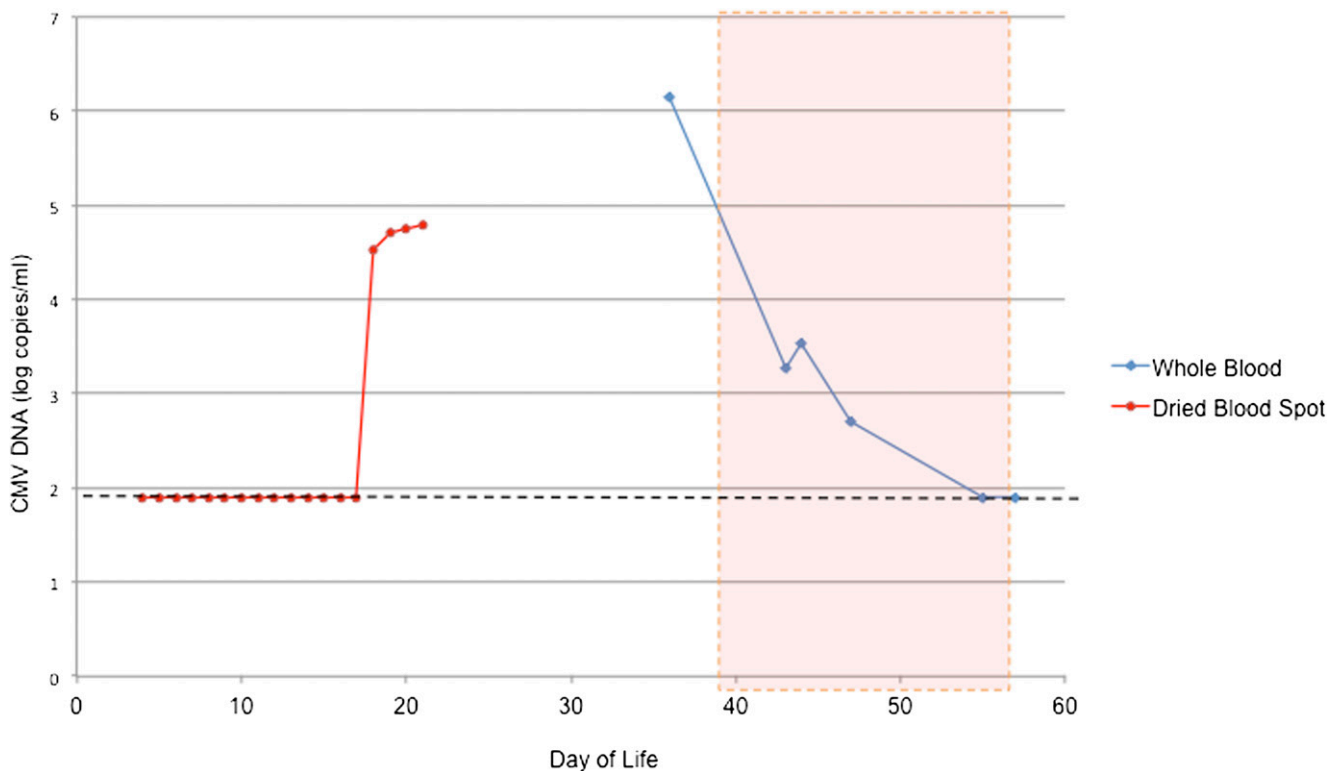

FIGURE 2

CMV viral load by real-time PCR from dried blood spot (analyzed retrospectively) and whole blood. The dotted line shows the detection limit of the assay. Asymptomatic CMV DNAemia was noted as early as DOL 18, preceding the development of clinical evidence of CMV end-organ disease by 8 days. The dotted red box shows duration of ganciclovir treatment.

levels of viral DNA noted in blood within 4 days of initiation of breast milk (DOL 18; Fig 2). CMV PCR of a urine sample obtained on DOL 12 almost certainly excluded the possibility of congenital infection, given the known sensitivity and specificity of urine PCR for the diagnosis of congenital CMV.[21,27] Despite an initial absence of symptoms, this infant's systemic viral load continued to rise to 4.8 $\log_{10}$ genomes/mL on DOL 21 (the last day that DBS samples were available through the immunoreactive trypsinogen study protocol). Although no samples were available to test for CMV DNA between DOL 21 and DOL 33, the viral load on DOL 33 when the patient demonstrated a CMV sepsis syndrome and required surgical intervention for NEC was ~6 $\log_{10}$ genomes/mL. Whether earlier recognition and possibly treatment of asymptomatic (but high-grade) DNAemia could have prevented the development of severe end-organ disease in this patient is speculative, but this question may warrant further research in future prospective studies.

It was of interest that this patient had an NEC-like syndrome caused by CMV infection. We identified 14 previously reported cases of perinatal CMV infections associated with NEC or NEC-like syndrome (Table 1).[3] This infant's intraoperative specimens demonstrated histopathological findings of ulceration, inflammation, granulation tissue, necrosis, and stricture.[8] The pathogenesis of NEC-like syndrome triggered by CMV infection is unclear. CMV enteritis often occurs in immunocompromised adults, including solid organ transplant (SOT) or hematopoietic stem cell transplant (HSCT) patients, but it is uncommon in the neonate and only rarely has been associated with severe enough pathology to require surgical resection. In a recent report of CMV-associated NEC, it was proposed that CMV infection may increase

TABLE 1 Summary of Reported Cases of CMV Associated With NEC

Ref	Gestational Age (wk)	Weight (g)	Clinical Presentation	Breast Milk Given	Operative Findings	Histopathology	Other Clinical Features	GCV
3	29	1490	Vomiting, distended abdomen, lethargy	Yes	Stricture in ascending and transverse colon	Ulcerating and granulating inflammation of colonic mucosa	None	No
4	Term	3500	Fever, diarrhea, distended abdomen, hepatosplenomegaly	No	Ileal perforation	Ileal perforation, transmural inflammation and granulation tissue formation	None	No
5	34	2695	Distended abdomen	NA	Colonic strictures	Severe colitis with mucosal ulceration and transmural acute and chronic inflammation	None	No
6	36	NA	Distended abdomen	NA	Ileal ulceration	Ileal ulcer with inflammatory changes	Hearing impairment	No
7	33	2200	Vomiting, distended abdomen	NA	Stricture of descending colon	Ischemic colonic stricture	Hearing impairment	No
8	37	2490	Distended abdomen, biliary vomiting mimicking Hirschsprung disease	Yes	Stricture at colon-sigmoid junction	Inflammatory colonic stricture with vasculitis, endothelialitis, mucosal ulceration covered by fibrinopurulent exudate	Chorioretinitis	Yes
9	30	NA	Distended abdomen, vomiting	NA	Inflamed ileocecal area, perforated tip of appendix	Ileocecal necrosis with acute and chronic inflammation	None	No
10	27	490	Distended abdomen, pale and mottled skin, temperature instability, tachycardia	Yes	Stricture of ascending and transverse colon	Inflamed, ulcerated cecum and colon with area of full thickness necrosis	None	No
11	28	790	Distended abdomen, hepatosplenomegaly	NA	Stricture of colon	NA	NA	No
	30	990	Distended abdomen, hepatosplenomegaly, sepsis	NA	Colonic ulceration	NA	None	No
12	23$^{5/7}$	580	Vomiting, diarrhea, distended abdomen	Yes	Ileal ulceration	Ileal ulceration and mixed inflammation	None	No
13	37	2490	Biliary vomiting, distended abdomen	Yes	Colonic stricture with multiple ulcers	Colonic stricture with multiple ulcers	Chorioretinitis	Yes
14	27$^{4/7}$	1130	Blood stool	Yes	Distal ileum	NA	None	No
15	25$^{1/7}$	653	Pneumatosis intestinalis	Yes	Fulminant	NA	None	No
16	24$^{5/7}$	800	Distended abdomen	Yes	Jejunum	CMV inclusions; positive viral antigen staining	None	Yes

CMV, cytomegalovirus; GCV, ganciclovir; NEC, necrotizing enterocolitis.

vulnerability to secondary bacterial invasion and may also drive proinflammatory immune responses,[15] further exacerbating the pathology of NEC. Some of the other potential mechanisms for CMV-associated NEC proposed in this report[15] included increased intestinal mucosal permeability,[28] enhanced proinflammatory cytokine production,[29–31] and alterations in the normal interactions between host cells and commensal microorganisms.[32,33] In addition to inducing proinflammatory cytokines, CMV also encodes gene products that modulate the function of host leukocytes, including T cells, neutrophils, and natural killer cells.[34,35] CMV-induced modulation of the host immune response could in turn lead to disruptions in mucosal immune homeostasis, potentiating the development of NEC.

As reviewed previously, the availability of a collection of DBS obtained sequentially between DOL 4 and 21 allowed for retrospective examination of DNAemia that evolved during the course of illness, well before the onset of any signs or symptoms of disease. We propose a possible analogy between premature infants and immunocompromised SOT or HSCT patients with respect to CMV DNAemia analysis. Monitoring for DNAemia in HSCT and SOT recipients is of critical importance in identifying patients who are at high risk for CMV disease, and initiation of preemptive antiviral therapy dramatically reduces CMV morbidity and mortality in these settings.[36–38] Similarly, it is tempting to speculate that this same preemptive therapy approach could be applied if future studies confirm that DNAemia heralds a substantial risk for subsequent end-organ disease in premature infants. Interestingly, in our patient the doubling time of CMV DNAemia was 1.2 days, similar to that reported in transplant patients.[39,40] Weekly screening for CMV DNAemia as a part of routine laboratory studies in premature infants might identify those at high risk for development of symptomatic CMV disease and guide preemptive antiviral therapy. Studies in HSCT patients indicate that a viral load of 1000 copies/mL ($3 \log_{10}$) is an appropriate cut-off for the initiation of preemptive therapy.[41–43] Whether an approach aimed at preemptively treating asymptomatic high-grade DNAemia in the ICU setting could prevent the development of CMV end-organ disease or yield additional benefits is unproven, but may merit future research.[44]

We chose to treat the infant described in this report with GCV, based on recommendations by the infectious diseases consultant involved in this case. In infants with congenital CMV infection involving the central nervous system, 6 weeks of GCV therapy has been associated with improved audiologic and neurodevelopmental outcomes.[45,46] There are no clinical trials to date suggesting a benefit of GCV therapy for postnatally acquired CMV infections, outside of the setting of severe immune compromise. However, given the severity of this infant's CMV sepsis syndrome and end-organ disease, the clinicians caring for this child believed that GCV therapy was warranted. A 3-week course of therapy was recommended, rather than 6 weeks, because this was sufficient both to resolve DNAemia and to produce other clinical signs of improvement. It is unclear whether GCV therapy has any impact on the neurodevelopmental prognosis of premature infants who acquire CMV infection postnatally from breast milk. A recent study suggested less favorable cognitive and motor function outcomes in preterm infants with postnatally acquired CMV infection, as compared with those without CMV infection,[47] but other studies have not demonstrated an increased risk.[48] Future clinical studies are needed to determine whether the appearance of DNAemia in asymptomatic preterm infants is predictive of the development of end-organ disease, and to assess whether there are any short- or long-term benefits to be realized from the use of preemptive antiviral interventions, such as GCV and/or CMV immune globulin, in the absence of clinical evidence of CMV disease.

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