Detection of Cytomegalovirus in Intestinal Tissue of Infants with Necrotizing Enterocolitis or Spontaneous Intestinal Perforation

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Objective To determine the frequency of detection of cytomegalovirus (CMV) in surgical or autopsy intestinal tissue from infants with necrotizing enterocolitis (NEC) or spontaneous intestinal perforation (SIP) of the small bowel.

Study design This was a retrospective cohort study of infants in the neonatal intensive care unit at Nationwide Children's Hospital, Columbus, Ohio, with NEC (Bell stage ≥2B) or SIP from 2000 to 2016. Paraffin-embedded surgical or autopsy intestinal tissues were examined for CMV by polymerase chain reaction (PCR) and immunohistochemistry (IHC), and clinical characteristics of CMV-positive vs CMV-negative cases were compared.

Results CMV was detected by PCR or IHC in 7 (4%) of 178 infants with surgical or autopsy- confirmed NEC (n = 6) or SIP (n = 1). Among 143 NEC cases (123 surgical, 20 autopsy), CMV was detected in 6 (4%): 4 (2 surgical, 2 autopsy) by both PCR and IHC, and 2 (surgical) by PCR only. Among 35 SIP cases (32 surgical, 3 autopsy), 1 (3%) surgical case was positive, by PCR only. CMV-associated NEC cases had lower median gestational age (24 vs 28 weeks; P = .02), birth weight (649 vs 1121 g; P = .04), and platelet count (16 000/mm^3 vs 50 000/mm^3; P = .018) compared with CMV-negative cases, respectively. No association was found with receipt of maternal milk, age at NEC diagnosis, male sex, cholestasis, or mortality.

Conclusions CMV was detected in intestinal tissue from 4% of NEC or SIP cases (NEC, 4%; SIP, 3%). Lower gestational age, lower birth weight, and thrombocytopenia were significantly associated with detection of CMV in NEC or SIP cases. *(J Pediatr 2019;214:34-40)*.

Necrotizing enterocolitis (NEC) and focal spontaneous intestinal perforation (SIP) of the small bowel remain major causes of morbidity and mortality among infants in the neonatal intensive care unit (NICU), affecting up to 10% and 1%, respectively, of infants born preterm.[1,2] Both of these conditions are associated with worse neurodevelopmental outcomes and/or death in infants born preterm.[3] The etiology of NEC and SIP remains unknown,[4] although intestinal dysbiosis with resultant intestinal inflammation may be a contributing mechanism for NEC and possibly SIP.[5]

Congenital cytomegalovirus (CMV) infection affects about 0.5%-1% of all live births and 0.4% of infants born preterm with very low birth weight (birth weight <1500 g).[6] Postnatal acquisition of CMV in the NICU is an increasingly recognized occurrence, and although the infection may be clinically silent,[6] it has been associated with severe clinical illness and even death.[7-9] Postnatal CMV transmission usually occurs via ingestion of unpasteurized human milk in up to 32% of breastfed infants born premature with very low birth weight, and up to 12% of infected infants develop clinical symptomatology.[7,10,11] Case reports have identified an association between CMV infection and neonatal gastrointestinal pathology including enterocolitis, enteric ulceration, ileal and colonic perforation (eg, SIP), colonic strictures, abdominal compartment syndrome, and NEC.[9,12-23] However, the frequency of CMV infection among infants with surgical NEC and/or SIP is not known.

Our hypothesis was that CMV is an under-recognized pathogen in infants with surgical NEC and/or SIP. Therefore, the objectives of this study were to determine the frequency of CMV detection in intestinal tissue obtained at surgery or autopsy from infants with NEC or SIP and to identify clinical characteristics associated with CMV-associated NEC or SIP that could serve as clinical indicators of CMV infection in these patients.

CMV	Cytomegalovirus
IHC	Immunohistochemistry
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
SIP	Spontaneous intestinal perforation

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Supported in part by Nationwide Children's Hospital intramural grant (#20066515 [to D.M. and P.S.]). The sponsors had no role in study design, data collection, data analysis, data interpretation, decision to publish, or preparation of the manuscript. The authors declare no conflicts of interest.

Portions of this study were presented at the Pediatric Academic Societies annual meeting, May 6-9, 2017, San Francisco, California.

https://doi.org/10.1016/j.jpeds.2019.07.038

Methods

This was a retrospective cohort study of infants with NEC (Bell stage ≥2B)[24] or SIP who were treated in the NICU at Nationwide Children's Hospital, Columbus, Ohio, from 2000 to 2016. Cases were identified by review of the surgical pathology database of the Nationwide Children's Hospital Pathology Department, using the diagnoses of "necrotizing enterocolitis" or "small bowel perforation." NEC or SIP was confirmed after histopathologic review by a pediatric pathologist. Inclusion criteria were (1) histopathologically confirmed diagnosis of NEC or SIP; and (2) sufficient paraffin-embedded tissue available in the pathology archive for CMV testing. Exclusion criteria were (1) histopathologic diagnosis of non-NEC or SIP-associated gastrointestinal disease (eg, atresia, volvulus, omphalocele, gastroschisis); (2) presence of congenital heart disease; or (3) no retrievable specimens in the pathology archives.

The medical records of patients were reviewed for pertinent demographic, clinical, laboratory, and radiographic data, with particular attention to results of neuroimaging studies as well as ophthalmologic and hearing evaluations. Real-time polymerase chain reaction (PCR) testing for the presence of CMV DNA and staining by immunohistochemistry (IHC) for CMV inclusions were performed on paraffin-embedded tissue specimens. The study was approved by the institutional review board of Nationwide Children's Hospital.

DNA Extraction and PCR Testing

Paraffin-embedded small intestinal tissues were cut into eight 10 μM scrolls to procure approximately 150 mm^2 of tissue surface. De-paraffinization was performed with a 1-hour incubation using heptane and methanol with paraffin lysis buffer and proteinase K. DNA was extracted using the All-Prep DNA/RNA formalin-fixed, paraffin-embedded kit (QIAGEN Inc, Germantown, Maryland). Real-time quantitative PCR for CMV was performed using published primers and probe for human CMV immediate early 2 protein and quantitated using a standard curve based on amplification of a plasmid containing the immediate early 2 sequence (gift of M. Prichard, University of Alabama at Birmingham).[25] To confirm DNA integrity and to exclude PCR inhibitors, 28 samples were analyzed by real-time PCR using primers and probe specific for human zinc finger gene (forward primer, 5′-TCT GTC TTC ATC CAG CAT CG; reverse primer, 5′-CTT TGC ACT CGT AGG GCT TT; probe, VIC-GAA GAC CTT CAC CTA CCG CTC TGT TTT-MGBNFQ; gift of H. Wang, Nationwide Children's Hospital, Columbus, Ohio). Each sample was run in triplicate using the StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, California) under published cycling conditions.[26] Each PCR plate included a no-target control, a negative control sample (DNA extracted from paraffin-embedded histologically normal, CMV negative intestinal tissue), and a positive control sample (DNA extracted from paraffin embedded lung tissue from a patient with CMV pneumonitis).

Immunohistochemical Staining

The hematoxylin and eosin–stained slides for each case were reviewed by a clinical pathologist to confirm the diagnosis of NEC or SIP. A representative paraffin block from the affected small intestine was selected and a 4-μm thick section was cut and used for CMV IHC. Primary antibodies against CMV (clones DDG9 and CCH2; Dako, Santa Clara, California) were used at a dilution of 1:20, followed by an indirect biotin streptavidin system (iVIEW DAB Detection Kit; Ventana Medical Systems, Tucson, Arizona). Intestinal tissue was classified as positive or negative for CMV by a pathologist blinded to the PCR results and clinical data. A positive result was defined as visualization of definitive strong staining for viral inclusions. Positive and negative controls were included with each case: CMV-positive placental tissue was used as a positive control, and CMV-negative intestinal tissue was used as a negative control.

Statistical Analyses

Descriptive analyses were used to summarize patients' demographic characteristics using means with SD or medians (IQRs) and frequency distributions as appropriate. Categorical variables were analyzed using χ^2 or Fisher exact tests, and continuous variables using *t*-test or Mann–Whitney *U* tests according to data distribution. Correlations were performed using Spearman rank correlation coefficient because most of the data did not follow a normal distribution. All analyses were performed using Prism 7.03 (GraphPad Software, La Jolla, California). Two-sided *P* values <.05 were considered statistically significant.

Results

From 2000 to 2016, 310 infants were identified with a diagnosis of "necrotizing enterocolitis" or "small bowel perforation" in the pathology database. Of these, 178 cases (57%; 143, NEC; 35, SIP) were included in the study (**Figure 1**) based on intraoperative surgical reports, histopathologic confirmation of the diagnosis by pathologist review, and availability of formalin-fixed, paraffin-embedded tissue. Overall, CMV was detected in small intestinal tissue from 4% (7/178) of infants with either NEC or SIP. Of the 143 infants with NEC, intestinal tissue samples had been obtained at the time of surgery for NEC (86%, n = 123) or at autopsy (14%, n = 20). Among the 35 infants with SIP, 32 (91%) intestinal tissue samples had been obtained at surgery and 3 (9%) were from autopsy (**Figure 1**).

CMV Detection by PCR

Of the 178 small intestinal tissue samples, 7 (3.9%; 6, NEC; 1, SIP) were positive for CMV DNA (**Table I**), representing 4.2% (6/143) of NEC and 2.9% (1/35) of SIP cases. Of the 6 infants with NEC, 5 (83%) were of extremely low

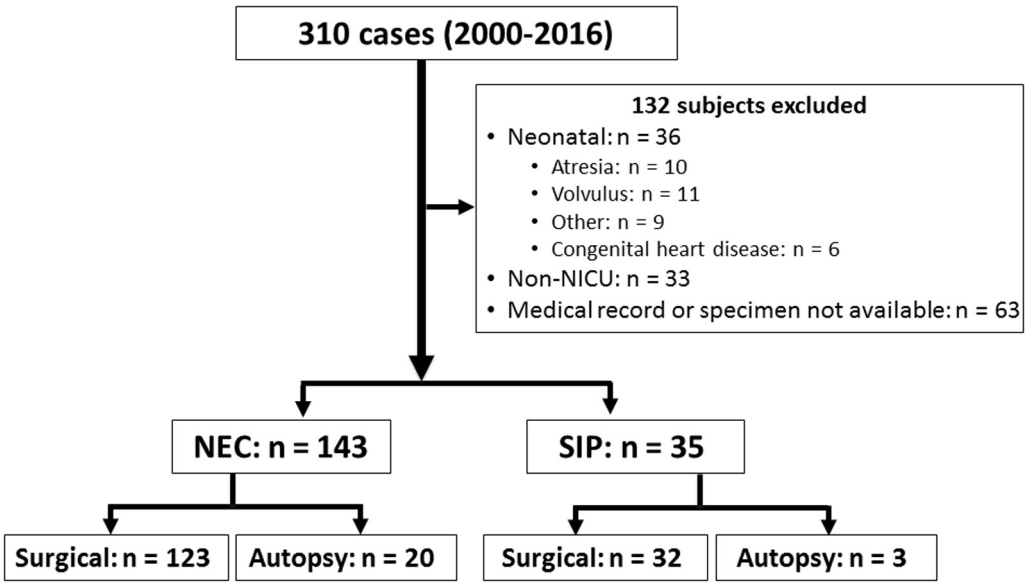

Figure 1. Study population. Of 310 cases of "necrotizing enterocolitis" or "small bowel perforation" in the pathology department's database, 178 (57%) infants with histopathologically confirmed NEC (n = 143) or SIP (n = 35) had available paraffin-embedded small intestinal tissue. Cases of NEC or SIP were classified by outcome (surgical vs autopsy). "Other" reasons for exclusion were ileal strictures, omphalocele, and abdominal hernia.

gestational age (<28 weeks) and had clinically ordered urine PCR testing for CMV in the medical records, of which 4 were positive. Only 1 of these 5 infants (Table I; case 2) had a urine CMV PCR test performed before 21 days of age; the result was negative. At 41 days of age, this infant had surgical NEC and the intestinal tissue was positive for CMV by both PCR and IHC, consistent with previously unrecognized, postnatally acquired CMV infection. The remaining 4 infants were tested for CMV after 3 weeks of age and thus the timing of infection could not be determined. Two infants (Table I; case 6 [NEC], and case 7 [SIP]) with positive intestinal tissue CMV PCR test before 3 weeks of age were never tested for CMV during the NICU hospitalization.

One infant (Table I; case 6) with a positive intestinal tissue CMV PCR presented at 7 days of age with NEC, direct hyperbilirubinemia (total, 8.1 mg/dL; direct, 8.1 mg/dL), and thrombocytopenia (14 000/mm^3). Cranial ultrasound scan and other supportive tests for congenital CMV infection were not performed. The 1 infant with SIP and a positive intestinal tissue CMV PCR (Table I; case 7) presented at 10 days of age with direct hyperbilirubinemia (total, 10.9 mg/dL; direct, 8.9 mg/dL), thrombocytopenia (71 000/mm^3), grade 1 intraventricular hemorrhage, and ventriculomegaly. No additional testing for congenital CMV infection was performed. On follow-up, this infant developed cerebral palsy and hydrocephalus with placement of a ventriculoperitoneal shunt. Of the 171 infants whose intestinal tissue was CMV DNA PCR-negative, only 1 had a positive urine PCR test performed clinically at 108 days of age.

Table I. Detection of CMV in small intestinal tissue of infants with surgical- or autopsy-confirmed NEC or SIP

Infants	Gestational age, wk	Birth weight, g	Age (d) at surgery or autopsy	Intestinal tissue			
				CMV PCR	CMV IHC	Age (d) at urine CMV PCR*	Urine CMV PCR
NEC							
#1	23	649	40†	Positive	Positive	33	Positive
#2	23	510	41‡	Positive	Positive	13	Negative
#3	24	740	74‡	Positive	Positive	68	Positive
#4	25	601	58†	Positive	Negative	38	Positive
#5	27	896	41†	Positive	Positive	45	Positive
#6	33	2271	7†	Positive	Negative	Not done	–
SIP							
#7	24	605	10†	Positive	Negative	Not done	–

*Age of infant when CMV DNA PCR testing was performed.
†Surgical specimen.
‡Autopsy specimen.

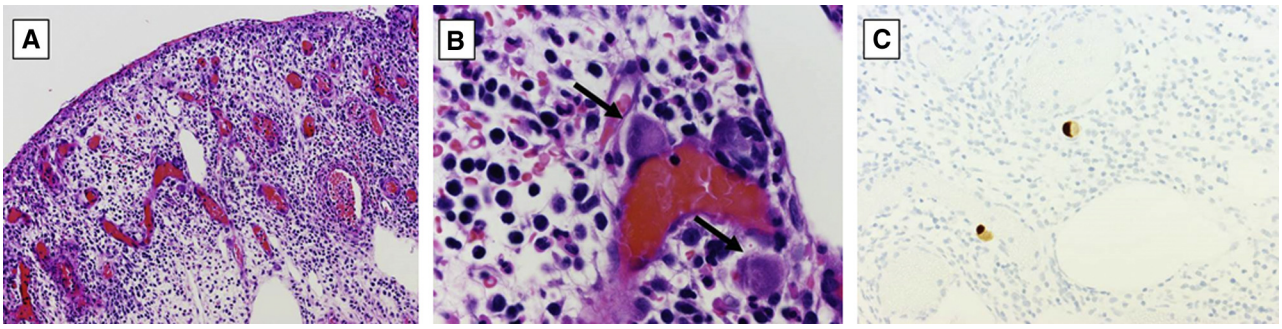

Figure 2. CMV immunohistochemistry findings in small intestinal tissue of infants with NEC. **A,** NEC of the small bowel shows mucosal ulceration, granulation tissue, and mixed inflammatory infiltrate of neutrophils and lymphocytes (H&E, 20× objective magnification). **B,** Cells with nuclear and cytoplasmic viral inclusions (*black arrows*) (H&E, 100× objective magnification). **C,** Viral inclusions identified by CMV immunohistochemistry (40× objective magnification). *H&E*, hematoxylin and eosin.

CMV Detection by IHC

Of the 178 small intestinal tissue samples, 4 (2.2%; 4, NEC; 0, SIP) were positive for CMV inclusions by IHC (Table I and Figure 2), representing 2.8% (4/143) of NEC cases and no SIP case. All of the IHC-positive intestinal tissues also were positive for CMV by tissue PCR (Table I). However, 3 intestinal tissues (2, NEC; 1, SIP; Table I) with a positive CMV PCR test were negative for CMV by IHC.

Two infants (Table I, cases 2 and 3) with CMV IHC-positive intestinal tissue died with NEC totalis, and autopsy showed evidence of disseminated CMV infection by viral cytopathic effects noted in gastrointestinal and respiratory tract, liver, pancreas, spleen, and brain (case 2) and CMV inclusions detected in lungs, esophagus, pancreas, liver, and spleen (case 3). The latter infant (case 3) was diagnosed with CMV infection secondary to worsening thrombocytopenia, direct hyperbilirubinemia, and elevated serum hepatic enzymes 6 days before development of NEC. Five days later and 1 day before the diagnosis of NEC, blood CMV DNAemia was >300 000 IU/mL (CMV log10 DNA >5.5 IU/mL), and he received 2 doses of ganciclovir (6 mg/kg/dose) intravenously. One other infant (Table I; case 1) was diagnosed with CMV infection at 33 days of age secondary to hyperbilirubinemia (blood CMV DNAemia 9111 IU/mL; log10 DNA 4.0 IU/mL) and developed NEC and *Pseudomonas aeruginosa* septicemia 6 days later. Ten days after the NEC diagnosis, blood CMV DNAemia was >300 000 IU/mL (CMV log10 DNA >5.5 IU/mL), CMV CSF PCR was positive, and infant received ganciclovir for 14 days with diminution of CMV DNAemia. The infant developed BPD and, at 4 years of age, has cerebral palsy and global developmental impairment.

Clinical and Laboratory Characteristics

Of the 178 infants, 171 had intestinal tissue (NEC/SIP) negative for CMV and 7 had CMV-positive intestinal tissue by either PCR or IHC (Table II). Infants with CMV-positive NEC/SIP had a lower median gestational age (IQR, 24 vs 28 weeks; P = .017) and birth weight (649 vs 1121 g; P = .04) compared with infants with CMV-negative NEC/SIP. They did not differ in postnatal age at NEC/SIP diagnosis, male sex, small for gestational age, receipt of human milk, physical examination findings at the time of NEC/SIP diagnosis (abdominal distention, bloody stools, or jaundice), or mortality.

Comparison of laboratory tests (worst values during the NEC/SIP illness) between infants whose intestinal tissue was CMV-positive vs negative revealed no differences in total white blood cell count and differential (Table II). Although the platelet count was low in both groups, infants with intestinal tissue positive for CMV had a significantly lower platelet count than infants negative for CMV (16 000/mm^3 vs 50 000/mm^3, respectively; P = .018). Infants with CMV-positive intestinal tissue had greater serum direct bilirubin (6.8 vs 1.8 mg/dL; P = .08) and similar serum transaminase concentrations (Table II).

The 2 groups of infants did not differ with respect to hearing, bronchopulmonary dysplasia, and neuroimaging findings (Table II). Of the infants who had neuroimaging studies performed, none had periventricular calcifications. Significantly more infants whose intestinal tissue was positive for CMV had retinopathy of prematurity (Table II; 83% vs 30%, respectively; P = .016), but none had chorioretinitis.

Discussion

There is increasing concern for the occurrence of CMV-associated disease among infants in the NICU, and in particular, the possible association with NEC and possibly SIP.[27,28] In this large, retrospective cohort study of 178 infants with NEC or SIP, CMV was detected in intestinal tissue of 4% (6/143) of surgical or autopsy specimens from stage ≥2B NEC cases and 3% (1/35) of SIP cases. Of the 7 CMV-positive small intestinal tissues from 7 infants (6 NEC and 1 SIP), CMV was detected by PCR in all 7 and IHC identified CMV in only 4 of the 7 cases (4, NEC; Table I). This finding is consistent with previous studies that showed greater

Table II. Clinical and laboratory characteristics of infants with CMV-positive vs CMV-negative NEC or SIP during hospitalization in the NICU

Characteristic	CMV-positive NEC/SIP (n = 7)	CMV-negative NEC/SIP (n = 171)	*P* Value
Demographic characteristics			
Age at NEC diagnosis, d	6 (5-39)	9 (5-23)	.996*
Male sex, n (%)	5 (71)	97 (57)	.700†
Race/ethnicity, n (%)			.012‡
White	2 (29)	94 (55)	
Black	1 (14)	50 (29)	
Hispanic	2 (29)	11 (6)	
Biracial	1 (14)	7 (4)	
Asian	1 (14)	2 (1)	
Other	0 (0)	7 (4)	
Gestational age, wk	24 (23-27)	28 (25-33)	.020*
Birth weight, g	649 (601-896)	1121 (713-1818)	.040*
Human milk ingestion, n (%)	5 (71)	97 (57)	.700†
Mortality, n (%)	3 (43)	61 (36)	.703†
Clinical signs at admission			
SGA	0 (0)	34 (20)	.349†
Jaundice	1 (14)	52 (30)	.676†
Lethargy	5 (71)	120 (70)	.999†
Hypotonia	2 (29)	54 (32)	.999†
Seizures	1 (14)	12 (7)	.418†
Clinical signs at time of NEC/SIP			
Abdominal distention	6 (86)	161 (94)	.365†
Gastric residuals	1 (14)	59 (35)	.426†
Bloody stools	4 (57)	71 (42)	.457†
Respiratory support, n (%)			.776†
Room air	2 (29)	39 (23)	
Mechanical ventilation	7 (71)	122 (71)	
CPAP	0 (0)	10 (6)	
Absent bowel sounds	4 (57)	125 (73)	.396†
Abdominal tenderness	6 (86)	157 (92)	.466†
Complete blood count (worst value)			
White blood cells, #/mm^3	31 100 (10 100-33 400)	18 400 (6400-31 200)	.358*
Neutrophils (%)	49 (33.8-51.3)	36 (16-55)	.374*
Bands (%)	13.5 (8.5-17.8)	12 (5-20)	.895*
Lymphocytes (%)	17.5 (6-25.8)	22 (13-42)	.303*
Metamyelocytes (%)	3 (1.5-9.8)	1 (0-3)	.113*
Platelets (# $\times$1000/mm^3)	16 (13-65)	50 (30-91)	.018*
Liver function (worst value)			
ALT, U/L	51 (32-175)	38 (21-85.5)	.182*
AST, U/L	53 (27-383)	57 (32.5-124)	.755*
Total bilirubin, mg/dL	8 (2.8-9.2)	5.5 (2.9-9.6)	.894*
Direct bilirubin, mg/dL	6.8 (1.5-8.1)	1.8 (0.2-4.2)	.083*
Complete blood count (at NEC diagnosis)			
White blood cells/mm^3	17 750 (6200-24 200)	8100 (4100-15 600)	.146*
Neutrophils (%)	16.5 (4.3-41.5)	27 (9-42)	.515*
Bands (%)	25 (16-32.5)	13 (6-26)	.094*
Lymphocytes (%)	30 (13.8-37)	33 (18-45)	.515*
Metamyelocytes (%)	8.5 (2-15)	2 (0-5)	.010*
Platelets (# $\times$1000/mm^3)	71 (42-122)	159 (91-257)	.057*
Retinopathy of prematurity§	5/6 (83)	26/86 (30)	.016†
Failed hearing screening§	0/5 (0)	15/116 (13)	.996†
Abnormal cranial ultrasound§	4/6 (67)	71/144 (49)	.681†
Abnormal brain CT§	1/1 (100)	7/11 (64)	.999†
Abnormal brain MRI§	1/2 (50)	14/23 (61)	.995†
BPD	2 (29)	23 (13)	.255†

ALT, alanine aminotransferase; *AST*, aspartate aminotransferase; *BPD*, bronchopulmonary dysplasia; *CPAP*, continuous positive airway pressure; *CT*, computed tomography; *MRI*, magnetic resonance imaging; *SGA*, small gestational age.
*Mann–Whitney *U* rank sum test.
†Fisher exact test.
‡χ^2 test.
§Number abnormal/number done.

sensitivity of PCR than histopathology or IHC staining for detection of CMV in tissues.[29]

The contribution of CMV infection to NEC and SIP is not well defined. Several investigators have reported the presence of CMV inclusions in intestinal and colonic tissues from infants with NEC, intestinal and colonic strictures, intestinal perforation, and volvulus.[12,14,16,18,19,21,30] A recent retrospective histopathologic analysis of 70 intestinal tissues from 61

infants with NEC, SIP, or related surgical complications detected CMV in 81% (57/70) of intestinal tissues by IHC compared with 20% (2/10) of 10 control autopsy specimens from infants without bowel disease.[31] In contrast to this high rate of CMV detection in NEC and SIP cases, retrospective PCR testing for CMV DNA in blood among a cohort of 17 infants with NEC did not identify CMV DNAemia in any infant.[32] Furthermore, in a case series of 40 infants born preterm who acquired CMV from maternal milk, Neuberger et al found no association of postnatal acquisition of CMV with NEC.[33] The current study represents the largest cohort of NEC and SIP cases to be assessed systematically for the presence of CMV in intestinal tissue and suggests that, in some cases, CMV may be associated with these conditions. Differences in detection of CMV among published reports and our study may reflect differences in the patient population, maternal CMV seroprevalence, treatment of human milk (freezing or pasteurization),[34,35] and methodology (PCR and IHC conditions).

Acquisition of CMV by infants born preterm has been attributed mostly to receipt of fresh or frozen human milk, although transmission also can occur secondary to transfusion of CMV-positive blood products.[36] In a recent meta-analysis, Lanzieri et al reported that among 299 infants fed untreated breast milk, 19% (11%-32%) acquired CMV infection and 4% (2%-7%) developed CMV-related sepsis-like syndrome.[7] Among 212 infants fed frozen breast milk, 13% (7%-24%) acquired CMV infection and 5% (2%-12%) developed CMV-related sepsis-like syndrome. In this study, CMV detection was not associated with receipt of human milk, although the characteristics of the human milk (eg, fresh/frozen or pasteurized, donor or maternal milk) could not be ascertained from the medical records. CMV infection of infants born preterm also has been associated with bronchopulmonary dysplasia and severe retinopathy of prematurity,[10,37] although this study found only an association of positive CMV intestinal tissue with retinopathy of prematurity (**Table II**).

Infants whose intestinal tissue contained CMV were of lower gestational age and birth weight than the CMV-negative group. It may be that the infants born most premature are the ones at greatest risk for compromise of the intestinal mucosal barrier by an infectious agent such as CMV that results in NEC or SIP.[38] Moreover, autopsy findings consistent with clinically unsuspected disseminated CMV infection in 2 NEC cases suggest that viral sepsis during acquired CMV infection may be a factor in fulminant and fatal NEC. Similar autopsy findings of disseminated CMV infection have been seen among infants with sudden unexplained death.[39]

Platelet counts were significantly lower among infants whose intestinal tissue contained CMV, and thrombocytopenia could serve as an indication for CMV testing in patients with NEC or SIP. However, although both infants with NEC with unsuspected disseminated infection were thrombocytopenic, only 1 had a positive urine CMV PCR, and the other was negative. In this study, testing for CMV was infrequent. Only 1 infant with NEC and CMV-positive intestinal tissue had a negative urine CMV PCR test before 21 days of age, suggesting that infection was acquired postnatally. Conversely, 2 of the CMV-positive intestinal tissues were from infants aged <14 days, suggesting previously undiagnosed congenital infection (**Table I**, cases 6 and 7). Neither NEC nor SIP previously has been associated with congenital CMV infection. Universal screening of newborns who are admitted to the NICU could clarify the mode of transmission of CMV, so that future studies could elucidate a previously unknown association of NEC or SIP with congenital CMV infection, or alternatively, determine whether postnatal CMV acquisition is temporally associated with NEC or SIP onset.[40]

Limitations of this study include its retrospective design, with an inability to ascertain specific timing of the CMV infection and its relationship to the NEC or SIP occurrence. Because the majority of infants in this study were not tested for CMV, the prevalence of congenital or acquired CMV infection in the CMV intestinal tissue-negative group is unknown. Intestinal tissue tested for CMV was paraffin-embedded, and this may have lowered the sensitivity of detection compared with testing on a fresh sample. In addition, of the 132 cases excluded from the final study population, the medical record or tissue specimen was not available in 63 of the infants, which could have resulted in selection bias. The lack of CMV testing of intestinal tissues from a control population of infants with intestinal pathologies unrelated to NEC or SIP does not allow knowledge of whether the 4% rate of CMV detection in this study was greater or lower than would be seen in infants born preterm. Moreover, maternal CMV seroprevalence and rates of CMV shedding in breast milk were not characterized. The contribution of CMV to the gastrointestinal manifestations was not analyzed mechanistically in any of the infants. Given these limitations, causation of NEC or SIP cannot be attributed to CMV infection. Universal screening for CMV in infants admitted to the NICU may assist in determining a causal relationship between CMV infection and NEC or SIP that ultimately could lead to therapeutic or preventive strategies.[34] ■

We thank Asuncion Mejias, MD, Department of Pediatrics, Division of Pediatric Infectious Diseases, Nationwide Children's Hospital and the Center for Vaccines and Immunity, Abigail Wexner Research Institute at Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, Ohio, for invaluable statistical and formatting assistance.

Submitted for publication Mar 7, 2019; last revision received May 28, 2019; accepted Jul 12, 2019.

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