

## EDITORIAL

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## Is there a link between viruses and necrotising enterocolitis?

Despite advances in the management of preterm infants, necrotising enterocolitis (NEC) continues to cause significant morbidity and mortality. Recent studies have confirmed that mortality rates from NEC have not decreased substantially over time (1). Factors associated with NEC include preterm gestational age, low birth weight, pharmacologic interventions – such as intrapartum antibiotics, indomethacin, H-2 receptor antagonists and prolonged empiric antibiotic use – feeding regimens such as formula feeding, bacterial infections and aberrations of the intestinal microbiome.

Bacteria associated with NEC include the Enterobacteriaceae such as *Escherichia coli* (*E. coli*) and *Klebsiella* species, *Pseudomonas* species, *Clostridia* and *Staphylococcus* species (2). Outbreaks of NEC due to *Cronobacter sakazakii*, formerly *Enterobacter sakazakii*, have occurred after infants were fed with contaminated powdered formula (2). More recently, key differences in the intestinal microbiome of preterm and term infants have been identified. The stools of term infants contain greater quantities of *Bifidobacterium* species, as measured by colony-forming units per gram of stool, and quantitatively lower numbers of *E. coli*, *Clostridium difficile*, *Bacteroides fragilis* and *Lactobacillus* species (3). Lower diversity is found in the preterm intestinal microbiome, mostly consisting of *Staphylococcus*, *Enterococcus*, *Enterobacter*, *Clostridia* and *Lactobacillus* species, but with lower quantities of *Bifidobacterium* and other anaerobes than are found in the intestines of term infants (3,4). In one study that investigated the microbiomes of preterm infants, those with NEC had lower diversity, decreased Firmicutes and increased Gammaproteobacteria than those without NEC (4). These studies suggest that changes in the predominant bacterial species inhabiting the intestinal microbiome may predispose some preterm infants to NEC.

The role of viral agents in the pathogenesis of NEC is largely uncharacterised. Viruses that cause gastroenteritis in healthy children and adults have also been associated with NEC in preterm infants. For example, the rotavirus has been reported in association with neonatal gastroenteritis and in several NEC outbreaks in neonatal intensive care units (NICUs), as have norovirus, echovirus and coronavirus (2). In addition, the human astrovirus and adenovirus have been identified by stool enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) in sporadic NEC cases in NICUs, when clinical tests for viral infections were ordered for infants with symptoms compatible with NEC (2,5). In a single-centre retrospective review for infectious aetiologies of NEC, the torovirus was identified by stool electron microscopy in

48% of NEC cases, but in only 17% of the stools from patients without NEC (6). NEC-like gastrointestinal syndromes caused by the cytomegalovirus (CMV) have been described in case reports, largely attributed to postnatally acquired infection (2). In one case, NEC occurred in a preterm twin shortly after initiating breast milk feedings. The CMV was detected in the maternal breast milk by PCR, as well as in the resected intestine by immunohistochemical staining (7). However, no prospective studies have systematically investigated the possible viral aetiologies of NEC.

In this issue, Skeath et al. (8) sought to address the unanswered question of whether viral agents could contribute to the occurrence of sporadic NEC. The authors used the novel United Kingdom Supporting Enhanced Research in Vulnerable InfantS (SERVIS) biobank, which was initiated in 2011. The SERVIS study prospectively enrolled preterm infants who were under 32 weeks of gestational age or weighed less than 1500 g at birth at a single tertiary care NICU and salvaged remnant clinical blood samples and stools from diapers. Samples were frozen and archived in a biobank linked to the infants' clinical data (9). This innovative approach of remnant salvage and prospective biobanking permitted the collection and storage of many more specimens than could be procured from preterm infants for any individual research study, with subsequent linkage to the clinical course of each subject. For this study, Skeath et al. (8) identified 22 NEC cases in the SERVIS database, of which 17 (77%) had appropriate biological samples available for study. Stool and blood samples obtained in temporal proximity to the onset of NEC were recovered from the biobank and tested by multiplexed PCR in a clinical microbiology laboratory. The blood samples were tested for CMV, Epstein–Barr virus (EBV) and adenovirus, and the stool samples were tested for the norovirus, sapovirus, astrovirus, adenovirus and rotavirus. None of the samples tested positive. Controls were selected among gestationally matched infants who had postnatally age-matched samples, but these samples were not tested due to the negative results found in the cases. The authors concluded that none of the investigated viral pathogens contributed to the onset of NEC in this case series.

One powerful aspect of this study was the use of the SERVIS biobank to address an important research question. Although the study was retrospective in nature, the authors utilised specimens that were prospectively collected from enrolled patients. This study highlights the value of using a biobank to answer difficult research questions. A second novel aspect of this study was the use of multiplexed PCR to test for several viruses in a single sample. Most published work investigating viral aetiologies of NEC has

relied upon single PCR assays, ELISA-based tests or electron microscopy to detect specific viruses in samples. In this report, the multiplexed PCR served the dual purpose of testing for multiple viruses in a single assay and efficiently processing limited sample volumes from the biobank. This innovative approach by Skeath et al. introduces the possibility of whether next-generation sequencing (NGS) technologies could in future yield even more information regarding the aetiology of NEC. NGS is currently limited by technical and methodological challenges, such as the identification of viral nucleic acid sequences among bacteria-laden stool specimens and the number of available viral genome reference sequences for classification. However, this technology could eventually permit broader analysis of viral enteric pathogens and perhaps provide insight into the intestinal virome, similar to the recent work that has expanded our understanding of the intestinal bacterial microbiome in preterm infants.

Although the authors are to be commended for their study, there are limitations that need to be mentioned when interpreting their results. The small sample size of 17 infants of this negative study raises the question of adequate statistical power and whether testing a larger series of patients would have yielded different results. Another limitation of this study was the nature of the tested samples. Several viruses were interrogated either in blood samples or in stool samples. Although viruses causing intestinal disease may be detected in blood, PCR analysis of both blood and stool samples could have strengthened the conclusion that viruses were not detected in the subjects at the time of NEC. For example, as noted by the authors, CMV intestinal disease may not be accompanied by viraemia in immunocompromised hosts, so negative blood testing alone may have missed purely enteric diseases (8,10). Finally, the performance characteristics – sensitivity and specificity – of the PCR assays would have been valuable in the interpretation of this negative study, particularly with regard to the limit of detection of each PCR assay for the given pathogen from a stool sample. Thus, caution should be exercised in the interpretation of this study.

Despite the negative results, this work by Skeath et al. paves the way for future studies. This research area might benefit from larger multicentre studies that have not been performed to date. The multiplexed PCR approach and future advances in NGS technologies could enable the detection of a broad array of pathogens in a single limited sample, as was illustrated in the SERVIS study. As discussed by the authors,

advanced molecular techniques could also elucidate interactions between viruses, bacteria and perhaps so far undetected organisms of low pathogenicity, such as bacteriophages and commensal viruses, in the intestinal microbiome (8). This innovative study by Skeath et al. highlights the extraordinary value of biobanks and data repositories, such as the SERVIS study, and the role of new molecular techniques that will enable many important questions to be addressed in neonatology and other populations.

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