Clinical characteristics of viral intestinal infection in preterm and term neonates

S. Bagci, A. M. Eis-Hübinger, A. F. Yassin, A. Simon, P. Bartmann, A. R. Franz, A. Mueller

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Abstract: Objectives: The clinical presentation of the viral enteric pathogens in newborn infants has not been adequately examined. The aim of this study was to evaluate the clinical characteristics of viral intestinal infections in newborn infants.
Methods: Clinical data of all term and preterm infants admitted to our tertiary neonatal intensive care unit from 1998 to 2007 with clinical signs of gastroenteritis (GE) or necrotizing enterocolitis (NEC) were retrospectively reviewed and compared between infants with different viral enteric pathogens in stool specimens.
Results: In thirty-four infants with signs of GE or NEC, enteropathogenic viruses were found in stool specimens. Rotavirus was detected in 12 cases, of which 2 infants had NEC. Compared with infants with rotavirus or norovirus, infants with astrovirus more frequently suffered from NEC ($p<0.05$). In addition, an acute systemic inflammatory response was significantly more common in patients with astrovirus infection (astrovirus vs. rotavirus and astrovirus vs. norovirus $p<0.01$ and $p<0.05$, respectively). Of 8 children infected with norovirus, one infant had a systemic acute inflammatory response and NEC.
Conclusions: This study demonstrates that in newborn infants, intestinal rotavirus, norovirus, and astrovirus infections may be associated with severe illness such as hemorrhagic enteritis resulting in bloody diarrhea or even NEC.

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We thank you for reviewing the manuscript.

Yours sincerely,
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Running title: Viral intestinal infection in neonates

ABSTRACT

Objectives: The clinical presentation of the viral enteric pathogens in newborn infants has not been adequately examined. The aim of this study was to evaluate the clinical characteristics of viral intestinal infections in newborn infants.

Methods: Clinical data of all term and preterm infants admitted to our tertiary neonatal intensive care unit from 1998 to 2007 with clinical signs of gastroenteritis (GE) or necrotizing enterocolitis (NEC) were retrospectively reviewed and compared between infants with different viral enteric pathogens in stool specimens.

Results: In thirty-four infants with signs of GE or NEC, enteropathogenic viruses were found in stool specimens. Rotavirus was detected in 12 cases, of which 2 infants had NEC. Compared with infants with rotavirus or norovirus, infants with astrovirus more frequently suffered from NEC ($p<0.05$). In addition, an acute systemic inflammatory response was significantly more common in patients with astrovirus infection (astrovirus vs. rotavirus and astrovirus vs. norovirus $p<0.01$ and $p<0.05$, respectively). Of 8 children infected with norovirus, one infant had a systemic acute inflammatory response and NEC.

Conclusions: This study demonstrates that in newborn infants, intestinal rotavirus, norovirus, and astrovirus infections may be associated with severe illness such as hemorrhagic enteritis resulting in bloody diarrhea or even NEC.

INTRODUCTION

Viral pathogens are the most frequent cause of endemic and outbreak-related GE and are responsible for significant morbidity and mortality in infants and children world-wide, both outbreak-related and endemic. Most episodes are due to infections with rotavirus (RV), human astrovirus (HAstV), norovirus (NV), and adenovirus (AdV) [1-2].

Etiologic studies of acute GE showed that RV is the major cause of clinically severe, epidemic and sporadic viral GE in young children, immunocompromized patients, and in the elderly [1]. NV and HAstV are usually associated with cases of mild and self-limiting GE in children [3-6].

In contrast to the usually self-limiting course of GE in healthy children, NEC, a major and life-threatening gastrointestinal emergency, has occasionally been reported associated with RV infection or colonization in preterm infants [7-9]. Moreover, we have previously shown that colonization or infection with HAstV may also be associated with severe confirmed NEC in preterm infants [10], and recently, Armbrust et al. [11] reported a NV outbreak in 11 premature infants in a tertiary Neonatal Intensive Care Unit (NICU), of whom 2 developed NEC.

With more sensitive virus identification techniques, viral GE has been increasingly reported in infants, especially in pediatric hospitals and child-care centers. The aim of this retrospective investigation was to evaluate the clinical presentation of viral intestinal infections especially with NV in newborns admitted to a NICU.

METHODS

Medical records of 3402 newborn infants admitted between January 1998 and December 2007 to a tertiary NICU of the Department of Neonatology, University Hospital of Bonn were retrospectively reviewed for the diagnoses of GE or NEC. As several studies showed the relationship between pathogenic microorganisms and NEC [12] , it has been routine care at our institution during this 10 year period to examine fecal specimens of all infants with one or more acute gastrointestinal symptoms such as abdominal distension, feeding difficulties, increased gastric residuals (> 5 ml/kg per feeding), vomiting, bloody stools, loose stools, or diarrhea by culture for enteric bacterial pathogens, by cell culture test (human foreskin fibroblasts) for Clostridium difficile cytotoxin, and by ELISA or PCR for a series of viral pathogens including RV, AdV, NV, and HAstV. When a new case with viral pathogen was identified, all patients who shared a room with the infected infant were immediately tested. All infants who were diagnosed to have NEC or GE were eligible for analysis. Infants without NEC or GE were excluded from the study if no viral or bacterial pathogens were detected in their stool specimens.

Case Definitions and Laboratory Methods: NEC was staged according to modified Bell's criteria [13], a definite case of NEC (stage ≥ IIA) is an infant with one of the following intestinal or systemic signs: visible blood from rectum, apnoea, shock, hypotension or X-rays with positive signs of pneumatosis intestinalis; a suspected case of NEC (stage I) is a infant with at least two of the following clinical signs: abdominal distension, gastric residual (> 5 ml/kg per feeding), occult blood in stool or a radiological finding of persistent intestinal distension [14].

Although there was no clear definition to differentiate between NEC stage I and GE, we defined viral GE in this study by the presence of diarrhea defined as loose, watery

stools that occur more than three times per day for at least three consecutive days and a positive ELISA or RT-PCR for a viral enteric pathogen but negative culture results for bacterial enteric pathogens and *Clostridium difficile* cytotoxin [14].

From all infant records, that contained the diagnoses GE or NEC with positive ELISA or RT-PCR for an enteric virus, the following data were extracted: Baseline characteristics; clinical features of the acute episode, standard laboratory tests, including complete blood cell count, basic metabolic profile, acute phase reactant including C-reactive protein and interleukin 6, reports of stool cultures, virus detection, therapeutic interventions, and complications. An acute systemic inflammatory response was defined as either C-reactive Protein >10 mg/l [15] or Interleukin 6 >100 pg/ml [16] and lactic acidosis was defined as lactate >1.8 mmol/l.

Examinations of viral pathogens were done by antigen-specific enzyme-linked immunabsorbent assay (ELISA) for RV (Premier Rotaclone, Meridian Bioscience, Inc. Cinncinati, USA), AdV (Premier Adenoclone, Meridian Bioscience, Inc. Cinncinati, USA) and HAstV (Amplified IDEIA Astrovirus, DakoCytomation, Carpinteria, CA, USA). All ELISAs were performed according to the manufacturer's instructions. NV was detected by RT-PCR as previously described [2, 17-19].

Statistical Analyses: Statistical analysis was performed by SPSS 17.0 software package for windows. Infants with evidence of different viral pathogens were compared using Fisher's Exact Test for dichotomous variables, Mann Whitney U test for continuous variables which were not normally distributed and t-test for continuous variables with normal distribution. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 906 stool specimens from 567 infants were examined for gastrointestinal viruses and enteropathogenic bacteria. Of 220 infants, 108 (3.2%) had NEC and 112 (3.3%) had GE. In the other 347 infants, no viral or bacterial pathogens were detected and these infants were excluded from analysis.

Gastrointestinal viral agents were detected in 34 infants out of 220 (15.5%) infants with GE and NEC; of these, 26 (77 %) were preterm infants at the onset of clinical signs. All patients with virus detection showed signs of GE (n=22) or NEC (n=12), of whom 6 had Bell's stage ≥ IIA NEC. There was no statistically significant difference in the gestational age between infants with and without viral enteric pathogens both in the GE group (median [range]: 34 [26-40] vs. 31 [23-40] weeks; p=0.064), and in the NEC group (29 [25-35] vs. 28 [23-41] weeks; p=0.207), respectively.

RV was detected in stool specimens of 12 infants, HAstV in 14, and NV in 8 infants. No adenovirus was identified. RV could be identified only during an outbreak of gastrointestinal rotavirus infections between January 1998 and June 1998. Moreover, RV was detected in the second stool specimen of an infant with HAstV who was admitted to the group of HAstV. The majority of cases with RV infection have been mild and self-limiting GE, but 2 preterm infants had signs of NEC.

Infants with detection of HAstV more frequently suffered from NEC ≥ IIA if compared with infants with detection of RV or NV (RV n=2/12. NV n= 1/8, HAstV n=9/14; HAstV vs. RV and HAstV vs. NV $p < 0.05$, respectively). In addition, analysis concerning the infants with GE (without NEC), HAstV GE showed more frequently an acute systemic inflammatory response (RV n=0/10, NV n=1/7, HAstV n=4/5; HAstV vs. RV and HAstV vs. NV, $p < 0.01$ and $p < 0.05$, respectively). The clinical features of all neonates with clinical GE or NEC in whom viral pathogens were detected are summarized in Table 1.

NV was detected in 8 newborns with a median gestational age of 29 weeks (range 26-36 weeks). Of these 8 children, 5 (63%) were still preterm at the onset of illness. Predominant clinical signs in infants in whom NV was detected were abdominal distension, diarrhea, and apnea. Four neonates (n=4/8, 50%) with detection of NV showed bloody stools and one of them displayed an acute inflammatory response with elevated values for C-reactive protein (maximum value 42.1 mg/l), lactate (4.5 mmol/l) and clinical findings of NEC stage IB. In this infant oral feedings were withheld and antibiotics were administered for seven days. In one infant, repetitive stool specimens revealed a positive result for NV for 21 days after the onset of illness. The clinical features of infants in whom NV was detected are shown in Table 2.

DISCUSSION

Although viral pathogens have been identified as cause of epidemics of hospital-acquired GE for many years, the association of viral agents with NEC in preterm infants has only recently been recognized [8-10, 20-21]. Our study demonstrates that intestinal RV, NV and HAstV infection in preterm and term neonates may be associated with severe illness such as hemorrhagic enteritis resulting in bloody diarrhea or even NEC and provides further evidence for the role of gastrointestinal viral infections in this most common gastrointestinal emergency in premature infants. Nevertheless, the etiologic role of gastrointestinal viral agents in the pathogenesis of hemorrhagic enteritis or NEC remains largely unknown [1, 22-23].

Rotavirus is the main viral pathogen that causes severe diarrheal diseases in infants and young children up to two years. In addition, several reports have described the association of NEC with RV infection or colonization [7-9]. In our cohort we found two patients with RV infection and NEC. Hypotheses of the mechanism of diarrhea include reduced absorptive surface, functionally impaired absorption, cellular damage impairing absorption, enterotoxic effects of rotavirus protein NSP4, and stimulation of the enteric nervous system [1, 22]. However, the role of RV in the pathogenesis of NEC has not been established. The authors suggested that a possible synergism between RV and other microorganism can worsen the infection in the intestine and cause necrotizing enterocolitis [1, 7-8]. Although all patients in our NICU received a regular screening for viral agents and enteric bacterial pathogens during an outbreak of RV infections, no other gastrointestinal pathogen microorganisms were detected. Furthermore, we found no asymptomatic RV carriers in our NICU at that time.

Although HAstV is usually associated with cases of mild and self-limiting GE in older children, we previously reported that colonization or infection with HAstV may be

associated with the development of severe NEC in preterm infants [10]. Previously, Sirinavin et al. [24] described a HAstV outbreak in healthy neonates in a nursery of a maternity ward. However, so far neonatal cases are rare and outbreaks have not been reported from NICUs. Enteropathogenic viruses generally infect epithelial cells and result in cytolysis and cell destruction [25]. In symptomatic HAstV infection, viral particles have been detected in the low villous, and on the surface of the intestinal epithelium as well as in macrophages of the lamina propria. Investigations in an animal astrovirus model revealed vacuolization followed by degeneration and cell death leading to villous atrophy [26]. However, it is unclear which pathogenetic mechanisms are involved in the occurrence of acute systemic inflammatory response and intestinal ischaemia, i.e. in NEC, in infants with HAstV infection / colonization. Of our 14 infants infected with HAstV, 5 (36%) had Bell's stage ≥ IIA NEC and 4 (80%) of 5 infants with clinical GE showed an acute systemic inflammatory response. In contrast to older children, the intestinal epithelium in premature infants may not have the same regenerative capacity. Exaggerated inflammation caused by immature intestinal innate immunity may lead to the generation of toxic metabolites, enhance the epithelial damage and result in intestinal lesions recognized as necrosis. On the other hand, one could speculate that NEC or the systemic inflammatory response may result from bacterial translocation after mucosal damage as a result of a possible synergism between HAstV and bacteria or from the systemic spread of virus particles. However, we are unable to identify another bacterial enteropathogenic agent in stool and blood cultures in these infants.

Generally, NV infections are mild and self-limiting and diarrheal stool during NV infection is non-bloody, lacks mucus, and may be loose or watery [4, 6, 27]. In contrast, four of our 8 newborn infants with NV infection (50%) had bloody stools and one of them

had NEC [6]. Human noroviruses are thought to have an enteric tropism limited to the upper intestinal tract, because intestinal biopsies from volunteers revealed villous broadening and atrophy, crypt cell hyperplasia and cytoplasmic vacuolization in the jejunum and duodenum and an infiltration of polymorphonuclear and mononuclear cells into the lamina propria [28-31]. Similar pathology is seen in biopsies from pediatric transplant patients with diarrhea associated with human NV infection [32]. Histopathologic examination in the intestine of gnotobiotic pig's also showed mild lesions in the upper intestinal tract, but no cytopathic lesions [33]. Cheetham et al. [33] speculated that extensive histopathologic lesions may not be apparent in the intestine because the NV-infected enterocytes may be dying by apoptosis rather than necrosis. Recently, Turcios-Ruiz et al. [21] and Armbrust at al. [11] reported cases of NEC associated with NV in newborn infants similar to the case we describe in this report. Unfortunately none of these studies provided information on the histopathologic examination of colons to establish the involvement of the colon with NV infection / colonization in premature newborn infants.

In summary, just like other authors [34-36], we can only speculate that viral infections in preterm infants may increase epithelial cell damage leading to weakening of the mucosal barrier and occasionally resulting in NEC. The limitations of our study are the retrospective design, the lack of data on the prevalence of gastrointestinal viruses in stool specimen of infants without gastrointestinal symptoms and the lack of data concerning the time course of the development of GE or NEC with regard to the onset of colonization / infection with viruses. In order to understand the prevalence of enteric viruses in neonatal intensive care units and their role in the pathophysiology of NEC, gastrointestinal viruses should be included in the microbiological examination of stool specimens in patients with gastrointestinal symptoms and further studies are necessary.

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Table 1

Table1. Neonatal Characteristics and Clinical Findings of Newborns with Enteric Viral Infection

Characteristics	Total (n=34)	Rotavirus (n=12)	Astrovirus (n = 14)	Norovirus (n = 8)	*P* Value*
Birth weight (g)[#]	1620 (618-3790)	1700 (940-3080)	1690 (618-3340)	1335 (670-3790)	n.s.
Gestational age (weeks)[#]	32 (25-40)	33 (27-40)	33 (25-40)	29 (26-36)	n.s.
Male gender (%)	20 (59)	7 (58)	11 (79)	2 (25)	p <0.05 ‡
Surfactant treatment (%)	8 (24)	3 (25)	2 (14)	3 (38)	n.s.
Postnatal age at onset of illness (d)[#]	15 (2-92)	26 (6-45)	13 (2-45)	25 (8-92)	p <0.05 § ‡
Gastroenteritis (%)	22 (65)	10 (83)	5 (36)	7 (88)	p <0.05 § ‡
NEC (%)	12 (35)	2 (17)	9 (64)	1 (12)	p <0.05 § ‡
NEC Bell's stage ≥ IIA (%)	6 (18)	1 (8)	5 (36)	-	n.s.
Surgery (%)	5 (15)	1 (8)	4 (29)	-	n.s.
Duration of illness (d)[#]	3 (2-21)	3 (2-21)	2 (2-3)	5 (2-11)	p <0.05 ‡
Mortality (%)	-	-	-	-	
Symptoms of newborns with gastroenteritis[##]	**22**	**10**	**5**	**7**	
Diarrhea (% of those with gastroenteritis)	22 (100)	10 (100)	5 (100)	7 (100)	n.s.
Gastric residue ((% of those with gastroenteritis))	7 (32)	2 (20)	3 (60)	2 (29)	n.s.
Vomiting (% of those with gastroenteritis)	9 (41)	5 (50)	1 (20)	3 (43)	n.s.
Abdominal distension (% of those with gastroenteritis)	11 (50)	3 (30)	2 (40)	7 (100)	p <0.05 ‡ p <0.01 †
Blood in stool (% of those with gastroenteritis)	4 (18)	1 (10)	-	3 (43)	n.s.
Apnea (% of those with gastroenteritis)	9 (41)	3 (30)	3 (60)	4 (57)	n.s.
Acute systemic inflammatory response (% of those with gastroenteritis)	5 (23)	-	4 (80)	1 (14)	p <0.05 ‡ p <0.01 §

[#] Data are expressed as median (range), [##] n= Gastroenteritis

n.s. not significant (p>0,05) (Rotavirus vs. Astrovirus, Rotavirus vs. Norovirus, Astrovirus vs. Norovirus)

§ Rotavirus vs. Astrovirus; † Rotavirus vs. Norovirus; ‡ Astrovirus vs. Norovirus

Table 2

Table 2. Neonatal Characteristics and Clinical Findings in Patients with Gastroenteritis or NEC and Detection of Norovirus

Characteristics	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Birth weight (g)	1830	885	670	2800	1620	1050	3790	910
Gestational age (weeks)	33+3	26+5	27+1	34+0	29+5	28+4	36+1	26+1
Gender	F	F	F	F	F	F	M	M
Age at onset of illness (d) (W)	11 (34+6)	92 (39+5)	59 (35+4)	36 (39+1)	17 (32+1)	23 (31+6)	28 (40+1)	7 (27+1)
Month/ year at onset of illness	March 04	August 04	August 04	August 04	August 04	September 04	January 05	October 07
Increased gastric residue (>5 ml/kg)	-	-	-	-	+	-	+	-
Vomiting	+	-	-	+	-	-	+	-
Abdominal distension	+	+	+	+	+	+	+	+
Diarrhea	+	+	+	+	+	+	+	-
Duration of occult blood in stool	3	11	-	-	-	-	8	1
Apnoea	-	+	+	-	+	+	-	+
Acute systemic inflammatory response*	-	-	-	-	-	-	-	+
Lactic acidosis[#]	-	-	+	-	-	-	-	+
Diagnosis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	gastroenteritis	NEC

***** CRP >10mg/l and/or IL-6 >100pg/ml, [#] Lactate >1.8mmol/l, NEC: Necrotizing enterocolitis