Case study

*Clostridium difficile* infections in young infants: Case presentations and literature review

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**A R T I C L E  I N F O**

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**A B S T R A C T**

It has been assumed that symptomatic *Clostridium difficile* infections do not occur in young infants, as this specific group would lack specific *C. difficile* toxin receptors. As a consequence, it is often current practice not to test for *C. difficile* in neonates and young infants up to 2 years of age presenting with (bloody) diarrhea. The evidence to support this is, however, weak and largely based on small, poorly designed animal studies. We present two young infants with recurrent bloody diarrhea following antimicrobial therapy, positive testing for toxigenic *C. difficile* and successfully treated with metronidazole and vancomycin, and provide an overview of the literature on *C. difficile* infections in children under two years of age. Both our case histories and the literature search provide evidence for *C. difficile* infection as a potential cause of bloody diarrhea in neonates and young infants, in particular after previous treatment with antimicrobials.

**Introduction**

Bloody stools in neonates and small infants may be the presenting symptom of anal fissures, cow’s milk allergy, juvenile polyps, congenital venous malformation and especially bacterial infection of the gastrointestinal tract [1]. *Clostridium difficile* infection is a relatively frequent cause of (bloody) diarrhea and pseudomembranous colitis in older children and adults, especially when its occurrence was preceded by antibiotic treatment, but is considered extremely rare in neonates and young infants. Percentage of bloody diarrhea (cases) in which *C. difficile* was the cause vary from 8%, 13% and 37.5% in patients below 1, 0–18 and 3–16 (median 2.1) years of age, respectively [2–4]. In adult patients, the number varies from 8.3%, 13.7% and 33% in patients younger than 65 years, older than 65 years and patients with a median age of 64 years (IQR 52–74), respectively [5,6]. *C. difficile* is an anaerobic, rod-shaped, spore-forming Gram-positive bacterium belonging to the phylum *Firmicutes*. *C. difficile*-associated diarrhea is caused by toxigenic strains, characterized by the production of toxins, including enterotoxin (*C. difficile* toxin A) and cytotoxin (*C. difficile* toxin B). In neonates, only a minority of *C. difficile* strains are considered pathogenic. Toxigenic *C. difficile* provokes mucosal inflammation and destruction of colonic epithelial cells, resulting in watery or bloody diarrhea, the latter case being a sign of pseudomembranous colitis [7]. Dysbiosis following the use of antibiotics is considered the most important underlying cause. This disturbance of the precarious balance of the commensal gut microbiota may induce overgrowth of toxigenic *C. difficile*, eventually leading to symptomatic infection [7–9].

Between 25 and 70% of neonates are colonized with *C. difficile* [7,8,10,11]. Within this specific age group, *C. difficile* may therefore be considered a component of the commensal microbiota. After two years colonization rates have decreased to 3–10%, remaining at that level throughout childhood and into adulthood [12]. In most, but not all [13], studies, nontoxigenic strains are reported to be more prevalent than toxigenic strains [11]. This was substantiated by the review of Jangi and Lamont, demonstrating that in pooled data of nine studies with a total of 928 healthy infants, 13% were carrier of toxigenic *C. difficile* strains and 17% of nontoxigenic strains [12]. The subgroup of toxigenic *C. difficile* strains consisted of different ribotypes with highly variable virulence phenotypes, including the hypervirulent 027 ribotype [14].

Although in adults and older children, toxigenic *C. difficile* is widely accepted as cause of (bloody) diarrhea, less consensus exists on the potential of *C. difficile* as causative pathogen of (bloody) diarrhea in neonates and young infants up to 2 years of age. We present two young infants with recurrent symptomatic infection with toxigenic *C. difficile*, who recovered completely following targeted antibiotic treatment.
Case presentation

Case. A term, male neonate was born following uncomplicated pregnancy and delivery, birth weight 4860 g (> 2 SD). He was found to have hydrocephalus, spina bifida aperta and bilateral clubfeet. Postnatal diagnostic work-up, including MRI of the head and spine, revealed Arnold-Chiari malformation type 2, hydrocephalus and a myelomeningocele. Surgical closure of the spina bifida was performed at the age of four days. Up to twelve days of age the patient was treated with intravenous amoxicillin and subsequently discharged in good condition. As he needed intermittent catheterization because of bladder retention as a result of spina bifida, trimethoprim-sulfamethoxazole was prescribed as antibiotic prophylaxis. Nonetheless he experienced recurrent urinary tract infections, for which antibiotic courses were given with ceftriaxone, flucloxacillin and amoxicillin/clavulanic acid, respectively. At nine months of age the patient presented with frequent bloody diarrhea. Physical examination revealed no cause for the blood loss; length and weight were adequate. C. difficile toxin testing on feces by Enzym Immunoassay (EIA) was positive, and C. difficile was cultured from feces and from a biopsy of the sigmoid colon. Fecal culture for Salmonella, Shigella, Yersinia and Campylobacter (SSYC) species was negative. Tests for parasites, adenovirus and rotavirus were also negative. Metronidazole prescription led to complete resolution of symptoms within a few days. EIA toxin testing of feces was negative at four weeks follow-up. Six weeks later, the bloody diarrhea recurred, accompanied by a body temperature of 39.5 °C. Fecal culture again was positive for toxigenic C. difficile. A second course of metronidazole was successful again, but two weeks later, symptoms and positive culture and EIA on feces had recurred. This time, oral vancomycin was prescribed, resulting in complete and definite resolution of the bloody stools and in negative results for C. difficile culture and EIA.

Case. An eighteen-month old boy presented with one week of bloody, foul-smelling diarrhea. Two months before she had been admitted because of acute tubular necrosis-induced renal insufficiency, treated with hemodialysis for two weeks as well as several antibiotics including third-generation cephalosporins and flucloxacillin. Fecal culture for SSYC was repeatedly negative, although from these cultures an Aeromonas species was isolated on one occasion. Real-time PCR for rota-, adenovirus, and norovirus, and a triple feces test for parasites were negative. C. difficile testing on fecal samples was positive: C. difficile toxin gene PCR testing was positive, C. difficile could be cultured from feces, and toxins could be demonstrated in the feces by EIA. Abdominal ultrasound showed diffuse thickening of the colonic walls, suggesting colitis. After five days of unsuccessful treatment with oral metronidazole, vancomycin was prescribed. Bloody diarrhea resolved completely within a few days. At follow-up, C. difficile PCR, culture and toxin testing were negative.

Discussion

For considerable time it has been assumed that symptomatic infections do not occur in neonates and young infants, although colonization rate with C. difficile is very high in this specific age-group [10,15,16]. It has been suggested that the neonatal gut lacks C. difficile toxin receptor sites and has poorly developed cellular signaling pathways [17]. Consequently, testing for (toxigenic) C. difficile in neonates and infants below the age of two years has frequently been discouraged by clinicians and clinical microbiologists [18]. Policy statement of the American Academy of Pediatrics include the recommendation to limit testing for C. difficile in infants younger than 12 months of age presenting with diarrhea to those cases with severe motility disorders, Hirschsprung’s disease or in outbreak situations. It was advised to look for alternative etiologies even in those infants with a positive test result for C. difficile [19].

However, reports discussing pathophysiological mechanisms on how young infants may be protected from developing symptomatic C. difficile infections have not been substantiated by robust scientific data and are merely hypothetical. A study that is often referred to was performed by Eglov et al., who found a decreased concentration of toxin A receptors in the ileum of newborn rabbits compared to adult rabbits, they predicted a comparable outcome in humans [20]. However, in C. difficile associated diarrhea it is commonly the colonic mucosa and not the small intestines which is affected. Furthermore, generalization of findings in animals towards humans is complex and may be questionable, in particular since observations in these studies were contradictory. For example, in contrast to the findings in newborn rabbits, Keel et al. described a high concentration of toxin receptors in the small and large intestines of neonatal pigs [21]. Claims on impossibility of development of symptomatic C. difficile infections in young infants due to the absence of intestinal toxin receptors in this specific population seem to be based on cross-references [22], ultimately referring to one small-scaled experimental study by Chang et al. [23]. This study described uptake of Clostridium toxin in a suspension containing adult colonic cells (n = 1 adult colonic sample), but no toxin uptake in a suspension containing fetal intestinal mucosal cells (n = 2 fetal intestines), obtained from two aborted fetuses in the second trimester [23]. It could be questioned whether the applied study design is sufficient to allow extrapolation of findings to the general (neonatal) population.

Alternative theories regarding the observation that young infants colonized with C. difficile do not suffer from symptoms direct to the potential positive effect of maternal antibodies transferred to the child by breastmilk, and the composition of the neonatal gut environment [11,12,24].

A review of the literature yielded at least eight studies including children below one year of age that strengthen the hypothesis that symptomatic C. difficile infections do occur in neonates and infants, with (bloody) diarrhea as the most frequently reported presenting symptom (Table 1) [2,4,13,25-28]. Majority of described cases had, similar to our presented cases, used antibiotics prior to onset of clinical symptoms. Despite these observations, the possible causality between toxigenic C. difficile and clinical symptoms remains subject of debate. The Bradford Hill criteria for causation states that causality is likely if there is a very specific population at a specific site and disease with no other likely explanation [29]. Hypothetically, the presence of toxigenic C. difficile could merely have been a biomarker of intestinal dysbiosis, while other non-cultivable pathogens, which also may react on antibiotic treatment, may have provoked the clinical symptoms. To establish a causative relationship between toxigenic C. difficile and presence of clinical symptoms, detailed insight in the pre- and post-treatment composition of the intestinal microbiota, preferably by means of molecular microbiota detection techniques, could shed light on this dilemma.

Alternatively, demonstration of the presence of intestinal C. difficile toxin receptors in neonates and young infants could scientifically support the hypothesis that symptomatic C. difficile infections do occur in this specific population, and should therefore be the focus of future studies.

Clostridium testing

Currently, several tests are available for the detection of toxigenic C. difficile in feces (Table 2), based on culture, enzyme immunoassay or polymerase chain reaction [30]. To distinguish between C. difficile infection and colonization, it is of importance to assess whether toxins or toxigenic C. difficile strains are present. This can be done by directly demonstrating the toxin or toxin genes in feces, or by demonstrating the toxigenic nature of cultured C. difficile isolates (toxigenic culture). To achieve an optimal diagnostic yield, a combination of testing modalities could be useful. When a diagnosis of C. difficile infection in a young
| Patient group | Study design | Test | Test results | Treatment/response | Comment |
|--------------|-------------|------|--------------|-------------------|---------|
| Vesikari et al. 1984 [25] | Hospitalized children 0–2 years – 52 with diarrhea – 52 without diarrhea | Prospective case study | Toxin EIA | – 11/52 (21%) with diarrhea positive for Clostridium difficile, 1/11 toxin positive – 17/52 (33%) without diarrhea positive for Clostridium difficile, 5/17 toxin positive | NA | 23/28 children tested positive for Clostridium difficile had previous antibiotic treatment |
| Enad et al. 1997 [13] | 87 NICU patients admitted during 4-month period and tested 2-weekly for Clostridium difficile toxin A | Prospective cohort study | Toxin (A) EIA | 45/87 (52%) toxin A positive Blinding broken in 3 patients, all toxin A positive, treated with vancomycin: 1. Frequent foul stools 2. Frequent loose green stools, abdominal distention, dehydration 3. Abdominal distention, bloody stools (diagnosed with NEC) | Blinding broken in 3 patients, all toxin A positive, treated with vancomycin: 1. Frequent foul stools 2. Frequent loose green stools, abdominal distention, dehydration 3. Abdominal distention, bloody stools (diagnosed with NEC) | No difference in previous antibiotics between toxin positive and toxin negative group |
| Ferreira et al. 2003 [26] | Hospitalized children 0–5 years – 90 with acute diarrhea – 91 controls | Retrospective case study | CCNA | Positive tests in – 2/44 patients 0–5 months – 2/22 patients 6–11 months – 1/24 patients 1–5 years 0/91 controls | NA | Some patients had previous antibiotic therapy |
| Morinvill et al. 2005 [2] | 64 patients < 1 year with Clostridium difficile-associated diarrhea (positive assay and GI symptoms) – 45 watery stools/diarrhea; – 5 bloody stools; – 14 no diarrhea | Retrospective case study | CCNA | – 64/64 positive tests – 7/64 (11%) rotavirus positive – 1 inflammatory bowel disease – 2 Hirschsprung disease | 31/64 metronidazole 5/31 retreated for recurrent or persistent symptoms | 46/64 were hospitalized within prior month; 54/64 (84%) had antibiotics within prior two months |
| Pai et al. 2012 [27] | 75C. difficile toxin-positive children 2 days-14 years (median 2 years) Based on severity criteria: – 9 mild infection – 8 moderate infection – 5 severe infection – 1 life threatening | Retrospective descriptive study | EIA followed by cytotoxin assay | – 75/75 positive tests – 3 adenovirus positive – 3 norovirus positive – 2 rotavirus positive – 1 enterovirus positive | 30/75 metronidazole 3 treatment failures with solely metronidazole, escalation to vancomycin | 55/75 (73%) had used antibiotics one month prior to infection |
| Khanna et al. 2013 [28] | 92 children 1 month-17 years (median 2.3 years; 16 children < 1 year) with toxigenic C. difficile diarrhea | Population based cohort study | Toxin in EIA or PCR | 92/92 positive tests | 92 metronidazole (13 failures); 7 vancomycin, 2 rifaximin (no failures) Overall recurrence rate 20% | 72/92 (78%) had antibiotics prior to infection |
| Duleba et al. 2014 [4] | 64 children 3 months-16 years (median 2.1 years; 9 children < 1 year) with C. difficile diarrhea | Retrospective case study | PCR | – 64/64 positive test – 8 rotavirus positive – 2 Salmonella positive – 2 Yersinia positive – 1 adenovirus positive – 1 norovirus positive – 1 Campylobacter positive | 36/64 metronidazole, 8/64 vancomycin and metronidazole, 6/64 vancomycin, 1/64 rifaximin (Salmonella coinfection), 13/64 no treatment Good response in 49/51 Recurrence in 3 patients | 61/64 (95%) had previous antibiotics |
| Samady et al. 2014 [3] | 134 patients 0–18 years (28 children < 1 year) with C. difficile diarrhea – 274 controls (58 < 1 year) with C. difficile-negative diarrhea | Retrospective case-control study | Toxin EIA | NA | 88/134 (66%) C. difficile patients and 55/274 (20%) controls had previous antibiotics |
infant is established, initial treatment with metronidazole orally (30 mg/kg/day for 10 days) is recommended [19,31]. In case of therapy failure or relapse, vancomycin orally (40 mg/kg/day for 2 weeks or tapering) can be prescribed. A prolonged treatment course with tapering may be chosen in selected cases [19,31]. Although fecal transplantation in recurrent C. difficile infections in adults has been proven superior as therapy compared to prolonged antibiotic treatment, studies on efficacy of this relatively novel therapeutic approach in children with recurrent C. difficile infections are currently lacking [32].

Conclusion

Based on the assumption that symptomatic C. difficile infections do not occur in children below two years of age, current policy of several microbial laboratories is not to test for these infections in this specific population. The evidence to support this regime is weak and largely based on small scaled, poorly designed animal studies. Based on a literature search and illustrated by two case reports, we recommend to include symptomatic C. difficile infections in the differential diagnosis of (bloody) diarrhea in neonates and infants, especially following (prolonged) use of antibiotics. Demonstration of toxin receptors in this population should be the focus of future studies to scientifically support this recommendation.

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