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VIROQAS

Fatal case of acute gastroenteritis with multiple viral coinfections☆

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ABSTRACT

We report a fatal case of acute gastroenteritis in a child with autism spectrum disorder. Multiple viral coinfections were detected by PCR in the patient's stool and digestive biopsy specimens. As viral detection is not necessarily associated with symptomatic disease, a semi-quantitative approach using cycle treshold values was proposed for the clinical interpretation of PCR. We discuss whether concomitant viral infections could be a risk factor for severe outcome in gastroenteritis cases. Individual risk factors are also addressed.

1. Case presentation

The patient was a 3-year-old girl born at term. Autism spectrum disorder (ASD) had recently been diagnosed. The child was in a day-care centre where no case of gastroenteritis had been reported in the previous days or in her family. She had not been vaccinated against measles, mumps and rubella. No food allergies had been suspected in the child during her first years. The family had not recently travelled in developing countries. At 4 pm, the child vomited and nursery nurses described her as weak. At home, her parents noted diarrhoea, but no bloody stools, and found their daughter warm. No rash was observed. At 6 pm, her condition had not improved but oral rehydration solution was given successfully. At 8 pm, the parents noted mottling on her face and thighs. Her condition deteriorated with persistence of abundant and watery stools, a decreased heart rate and then loss of consciousness. At 10 pm, the parents contacted emergency services but, despite resuscitation attempts, the child died of cardiac arrest a few minutes after their arrival. Postmortem microbiological investigations and autopsy were conducted with the parents' consent. The autopsy revealed significant abnormalities only in the intestine with inflammation and adenopathies visible in the colon. The histopathology showed inflammation characterized by significant infiltration of lymphocytes in the intestinal mucosa. These observations were compatible with the diagnosis of acute gastroenteritis (AGE). No signs of myocarditis were observed. None of the microbiological cultures yielded any pathogenic bacteria in the patient's cerebrospinal fluid (CSF), blood and lung specimens. Cultures for *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Campylobacter coli*, *Clostridium difficile* and *Yersinia* were negative in stool and intestine biopsy specimens. Rapid tests for rotavirus, adenovirus 40–41 and norovirus were negative in the stool specimen. Serological findings showed a recent cytomegalovirus (CMV) primary infection with presence of CMV IgM (index 6, CMV-IgM-ELA test PKS, medac) and IgG-specific antibodies (OD 0.584, Enzygnost® assay) combined with a low IgG avidity test (avidity 30%, VIDAS® assay). Serology was negative for Epstein-Barr virus, HIV, measles, parvovirus B19, *Mycoplasma pneumoniae* and herpes simplex virus, but a high level of enterovirus IgG antibodies was found (index 207, SERION ELISA Classic assay). PCR for herpes simplex virus, varicella zona virus and enterovirus were negative in CSF. Respiratory multiplex PCR detected *M. pneumoniae* and coronavirus 229E in the

☆ Virology Question and Answer Scheme (VIROQAS)

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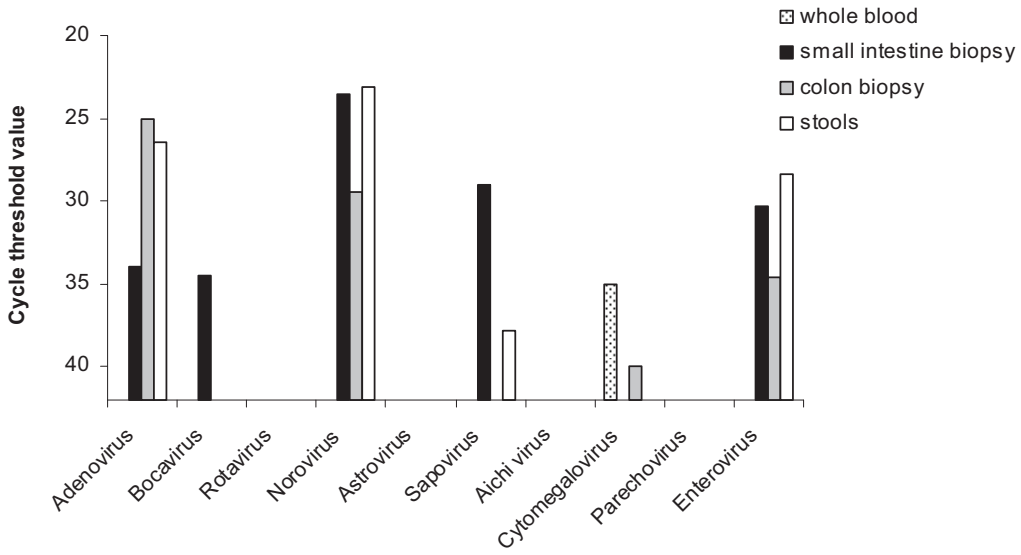

Fig. 1. Graph showing enteric viruses and viruses with digestive tropism detected in the patient's specimens by real-time PCR. All specimens were tested for the ten viruses sought. PCR results were all negative in the serum specimen. PCR for adenovirus was designed to detect all types (Adenovirus R-gene ARGENE).

tracheal aspirate and lung biopsy. In this AGE context, the patient's frozen specimens were sent to the French National Reference Centre for enteric viruses. Mixed infections were detected by PCR analysis. Semi-quantitative results estimated by the cycle threshold (Ct) values showed presence of adenovirus (genotyped as A-31), bocavirus, norovirus (genotyped as GII.e/II.2), sapovirus, CMV and enterovirus (genotyped as enterovirus A-5) in the patient's stool and/or digestive biopsy specimens. Only CMV DNA was detected in whole blood (Fig. 1).

2. Evidence-based opinion

2.1. What is your interpretation of laboratory results? Which virus(es) could be the causative agent(s) of this fatal case of AGE?

Viruses such as rotavirus, norovirus, enteric adenovirus, human astrovirus and sapovirus are the major etiological agents of AGE in children under 5 years of age. Our investigations also included other viruses with digestive tropism such as aichivirus, human parechovirus, human bocavirus, CMV and enterovirus, but their association with AGE is less documented [1,2]. This case was unusual because an extraordinary number of pathogens were found in the patient's specimens by PCR analysis (Fig. 1). New molecular PCR methods can detect diarrheal pathogens with better specificity and sensitivity than rapid antigen tests [3]. However, the presence of a pathogen in a fecal sample is not necessarily associated with a gastrointestinal disease, since high detection rates have also been reported for asymptomatic individuals [1,4–7]. Many studies have also reported mixed infections in children with AGE or in asymptomatic cases making the interpretation of PCR results very difficult [1,4,8,9]. Indeed, it is not possible to determine which pathogen is related to the disease at the time the sample is analyzed. Previous studies have assessed the usefulness of a semi-quantitative approach using the PCR Ct values to establish a causality link between a pathogen and the clinical manifestation, even if Ct cutoff values for a specific pathogen differ between laboratories [5,7,8,10–12]. For example, Phillips et al. proposed a Ct value cutoff of 31 in fecal specimens for attributing AGE to norovirus [10]. In our patient, the more abundant viruses were norovirus and adenovirus detected in biopsy and stool specimens with a Ct value less than 30. Sapovirus was detected with a low Ct value only in the small intestine biopsy (Fig. 1). To our knowledge, few studies have reported analysis of Ct values in specimens other than stools.

Other pathogens were detected in the patient's specimens. A recent infection with enterovirus was suspected by serology findings and positive PCR results (Fig. 1). Note that PCR for enterovirus was negative in CSF and serum samples. Since asymptomatic infections are frequent, it is difficult to ascertain a pathogenic role of enterovirus in our patient. Colitis caused by CMV is unusual in immunocompetent children, but the virus was detected in blood and colon biopsy specimens with a low viral load, making a direct role of CMV in the pathogenesis of the gastrointestinal disease unlikely. *M. pneumoniae* and coronavirus 229E were found in the patient's respiratory specimens, but this finding should be interpreted with caution considering the clinical context and the possibility of also detecting these pathogens in asymptomatic cases [13–15].

2.2. Which viral and host factors could be associated with severe outcome in gastroenteritis cases?

AGE occurring at extreme ages (>85 and <2 years of age) and in immunocompromised hosts are associated with more severe outcomes [16]. Our patient was affected by ASD and many studies support the possibility that this disease can be associated with immune dysregulation [17,18]. However, little is known about the increased risk of these patients for severe gastrointestinal diseases. Viral factors could contribute to the severity of AGE. Severe outcomes are associated with norovirus genotype II.4 outbreaks occurring in healthcare facilities [19]. Mixed infections raise the question as to whether a single pathogen is responsible for the illness or whether several pathogens act in synergy and may predict a more severe outcome in children [20]. Gonzales et al. showed no differences between severity or clinical presentation when comparing single to multiple infections [9], but further studies are needed to better assess this controversial point. Even if Ct values can be useful to discriminate AGE and asymptomatic cases, Gonzales et al. showed no correlation between viral load and severity of disease [9]. Given that viremia with enteric virus has been described and may be associated with more severe disease [21],

PCR for enteric virus was made in the patient's blood specimen, but only CMV was detected, which was compatible with a recent infection (Fig. 1). During primary infection, CMV leads to transitory immunosuppression, which could inhibit the immune response against other pathogens such as enteric viruses [22].

3. Conclusion

In developed countries, AGE-associated death is uncommon in immunocompetent children [23,24]. This report describes a fatal case of AGE in a 3-year-old girl with a medical history of ASD. We cannot rule out that this patient had an underlying immune disorder, but we could not explore this possibility. Extensive investigations showed multiple viral infections in the patient's specimens. Using PCR cycle threshold values, viral load for each enteric virus was estimated. Until now, few studies have focused on the clinical interpretation of PCR results, but high viral load seems to correlate with symptomatic cases. In the case presented herein, a strict comparison of our results with Ct values from other studies should be interpreted with caution since the patient's underlying medical condition may interfere with the quantification of enteric pathogens. Recent studies suggest that enteric viruses are not only pathogens, but could also be symbiotic modulators of host physiology [25]. Considering the emerging view of enteric virome, it is possible that our clinical interpretation of enteric infections will change in the future.

Competing interests

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Ethics approval

Not required.

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