Prevalence and Clinical Impact of Norovirus Fecal Shedding in Children with Inherited Immune Deficiencies

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We report the first prospective study describing the prevalence and clinical consequences of norovirus infection in hospitalized children with primary immunodeficiencies. Fecal samples from 62 children were systematically screened for virus. Norovirus was the most frequent pathogen (11 of 24 positive samples) found in both combined and humoral immunocompromised children. Norovirus shedding was associated with gastrointestinal symptoms and concomitant viremia in 54.5% and 25% of cases, respectively. Norovirus excretion was prolonged: 57.1% of fecal samples were still positive after a median of 9.5-months follow-up. Further large longitudinal studies are needed to evaluate the clinical consequences of norovirus shedding in patients with primary immunodeficiencies.

Noroviruses have been identified as a cause of significant morbidity related to chronic gastroenteritis in solid organ [2, 3] and hematopoietic stem cell transplantation (HSCT) recipients [4, 5]. No prospective data have been reported about norovirus shedding and/or infection in children with inherited immune deficiencies; these genetic diseases potentially compromise the norovirus-specific immune response. The aim of this prospective study was therefore to describe the prevalence and consequences of norovirus in such patients.

PATIENTS AND METHODS

From February through November 2011, all children aged <18 years who were admitted for a primary immunodeficiency (PID) to the Pediatric Immunology Unit of Necker Hospital (Paris, France) were included. Hospitalized children were included within 2 days after admission to avoid detection of nosocomial viral transmission. Children who previously received an HSCT were excluded to avoid issues relating to the potential consequences of the procedure on viral persistence. For each child, the following characteristics were recorded: age, sex, type of immunodeficiency, any known chronic enteropathy secondary to immune dysregulation, presence of recent clinical symptoms (nausea/vomiting, diarrhea, abdominal pain, or fever), and intestinal endoscopic findings, if performed.

Stool samples collected at inclusion were systematically screened for norovirus, adenovirus, enterovirus, and rotavirus with use of the norovirus real-time reverse-transcription polymerase chain reaction (RT-PCR) Kit (TaqMan, AnDiaTec Argene) for semi-quantitative results, real-time PCR for adenovirus [6], real-time RT-PCR for enterovirus [7], and an immunochromatographic test for rotavirus (RIDAQuick Rotavirus/Adenovirus Combi, r-biopharm). For patients presenting with gastrointestinal symptoms (diarrhea, nausea/vomiting, and/or abdominal pain), we also tested for the following enteric pathogens: (1) cytomegalovirus in the blood by PCR, (2) Salmonella, Shigella, Campylobacter, Yersinia, and Clostridium difficile by stool culture (with tests for toxin if C. difficile was isolated), and (3) Microsporidium and Cryptosporidium by fluorescence staining of stool samples.

For patients positive for norovirus stool shedding, the norovirus RT-PCR assay was performed on additional stool samples, if the patient was reassessed in our unit during the study period. Any blood or cerebrospinal fluid (CSF) samples available were also tested for norovirus.
RESULTS

From February through November 2011, 62 children were enrolled; most were boys (n = 38; 61.3%), and the median age was 3.5 years. Patients had combined PID (n = 34; 54.8%), antibody deficiencies (n = 10; 16.1%), defects of phagocyte number and/or function (n = 2; 19.4%), or other types of PID (n = 6; 9.7%), as detailed in Table 1. All of the children followed up for combined or isolated antibody deficiency were treated with substitutive intravenous immunoglobulin therapy. None of the children were vaccinated against rotavirus. Eight patients (12.9%) presented with a histologically proven chronic enteropathy due to immune dysregulation.

At the time of enrollment, 18 children (29.0%) presented with diarrhea, which had already lasted >2 months in 13 cases (72.2%). A history of vomiting, fever, and abdominal pain in the previous 2 weeks was found in 8.1% (n = 5), 8.1% (n = 5), and 3.2% (n = 2) of the patients, respectively. Most of the children (n = 43; 69.4%) had no gastrointestinal symptoms (Table 2).

Detection of fecal samples for viruses gave positive results in 24 patients (38.7%), 13 of whom were asymptomatic. Tables 1 and 2 summarize the results of the microbiological screening according to the type of immunodeficiency and the clinical symptoms in the children, respectively. Norovirus was the most frequently detected pathogen (in 45.8% of the 24 positive cases). Dual viral infection was documented for 2 children: adenovirus and norovirus (n = 1) and adenovirus and enterovirus (n = 1). A larger proportion of infected (45.8%) than uninfected (21.1%) children had gastrointestinal symptoms (the difference was not statistically significant). Norovirus tended to be more frequently isolated from symptomatic than from asymptomatic patients (31.6% vs 11.6%) and was more frequently associated with clinical symptoms than was adenovirus (54.5% vs 22.2%). Norovirus was the most prevalent virus among the 19 symptomatic children (31.6% vs 15.8% for enterovirus, 10.5% for adenovirus, and 5.3% for rotavirus). Clinical and microbiological follow-up of the 11 norovirus-infected children is depicted in Supplementary Table 1. Norovirus viremia was detected in 2 of the 8 patients excreting norovirus in their feces and for whom blood or plasma samples were available. CSF samples tested positive by norovirus RT-PCR for a child with concomitant detectable norovirus RNA in stool and plasma samples (child 7). This patient presented with hemiplegia, which was attributed to an intracerebral Epstein-Barr virus–induced lymphoproliferative disease.

Endoscopy of the upper gastrointestinal tract was performed in 3 patients with norovirus shedding and without known enteroepathy secondary to immune dysregulation (children 2, 3, and 8) within the first month after enrollment. The findings were normal in one case (child 2). Moderate esophagitis with an antral gastritis was observed in one case (child 3) and duodenal villous atrophy in 2 cases (children 3 and 8). Duodenal biopsies showed moderately higher than normal intraepithelial CD3 + CD8+ lymphocyte counts in 2 cases (children 3 and 8), slightly higher than normal numbers of apoptotic epithelial cells with mild polymuclear neutrophil infiltration in 1 case (child 3), and villous blunting in 1 case (child 8). The child #2 also underwent sigmoidoscopy, and the appearance of the intestinal mucosa was found to be normal.

Norovirus fecal shedding was prolonged: among the 7 patients for whom subsequent fecal samples were available, substantial viral excretion was detected in 4 after a median 9.5-months follow-up. None of these 7 children received a HSCT during the study period. In 3 patients with norovirus shedding and severe gastrointestinal symptoms, high-dose intravenous immunoglobulin treatment (≥1 g/kg/week) was started (children 1, 2, and 8). In one case, treatment with oral immunoglobulins was added (child 8). There was no improvement of clinical symptoms, and fecal shedding continued in the 3 cases.

Finally, results of virological screening of fecal samples were negative for the 38 remaining children, although 8 of them presented with gastrointestinal symptoms. In 2 of these 8 patients, other microbiological pathogens were detected: cytomegalovirus (n = 1) and Campylobacter (n = 1).

DISCUSSION

We report here the detection of fecal shedding of norovirus in 11 of 62 children hospitalized for inherited immunodeficiency. This is, to our knowledge, the first such report in a population of this type, potentially more susceptible than healthy children to norovirus infection.

Norovirus was the most frequently detected enteric pathogen. This finding is consistent with previous studies, suggesting that norovirus is not only the most common cause of gastroenteritis outbreaks in both immunocompetent children and adults [1], but also a major pathogen in immunocompromised patients [2–5]. We found rotavirus in few of our study population, although rotavirus is the virus most frequently involved in pediatric gastroenteritis. We cannot exclude the possibility that the real-time RT-PCR test that we used has a better sensitivity for norovirus detection than does the immunochromatographic test for rotavirus. However, our results are consistent with previous studies, indicating that chronic rotavirus infection is rare, even in severely immunocompromised children [8].

Norovirus shedding in stool persisted for long periods. We cannot estimate the true duration of viral shedding, because fecal screening for norovirus was not routinely performed before 2011. Thus, in norovirus-infected children, excretion of the virus could have begun many months or years before the beginning of this study. Consequently, the duration of norovirus shedding in our population is probably underestimated. Many previous studies reported shorter durations of norovirus...
Table 1. Results of the Microbiological Screening of 62 Children According to the Type of their Inherited Immunodeficiency

<table>
<thead>
<tr>
<th>Type of immunodeficiency</th>
<th>Norovirus</th>
<th>Adenovirus</th>
<th>Enterovirus</th>
<th>Rotavirus</th>
<th>Dual Viral Infection</th>
<th>CMV Viremia(^a)</th>
<th>Bacterial Pathogens in Stools</th>
<th>Parasites in Stools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined immune deficiency (n = 34)</td>
<td>7 (20.6%)</td>
<td>5 (14.7%)</td>
<td>5 (14.7%)</td>
<td>1 (2.9%)</td>
<td>2 (5.9%)</td>
<td>11 (32.4%)</td>
<td>16 (47.1%)</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>Severe CID (n = 6)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MHC II expression deficiency (n = 5)</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wiskott Aldrich syndrome (n = 3)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ICF syndrome (n = 3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD40L deficiency (n = 3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other CID(^b) (n = 14)</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1 (Cryptosporidia)</td>
<td>0</td>
</tr>
<tr>
<td>Antibody deficiency (n = 10)</td>
<td>2 (20%)</td>
<td>3 (30%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Agammaglobulinemia (n = 5)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1 (Campylobacter)</td>
<td>1 (Giardia intestinalis)</td>
</tr>
<tr>
<td>Hypogammaglobulinemia (n = 5)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Defect of phagocyte number and/or function(^c) (n = 12)</td>
<td>1 (8.3%)</td>
<td>1 (8.3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (8.3%)</td>
<td>1 (8.3%)</td>
<td>Microsporidia</td>
</tr>
<tr>
<td>Other immune deficiency (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophagocytic lymphohistiocytosis (n = 5)</td>
<td>1 (16.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (16.7%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyskeratosis, congenital (n = 1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: MHC II, major histocompatibility complex class II; ICF syndrome, Immunodeficiency, Centromere Instability and Facial anomalies syndrome; CMV, Cytomegalovirus; CID, combined immune deficiency.

\(^a\) Data not available for 2 cases.

\(^b\) Including ORAI1 deficiency (n = 2), hyper-IgE syndrome with STAT3 mutation (n = 2), CD25 deficiency (n = 1), Cartilage Hair Hypoplasia syndrome (n = 1) and CID of unknown origin (n = 8).

\(^c\) Including chronic granulomatous disease (n = 6), aplastic anemia (n = 2), congenital neutropenia (n = 1), leukocyte adhesion deficiency (n = 1), interferon gamma receptor 1 mutation (n = 1) and unknown deficiency of innate immunity (n = 1).
Table 2. Clinical Characteristics of the 62 Children with Inherited Immunodeficiency According to the Results of Systematic Fecal Screening for Virus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 62)</th>
<th>Patient with Norovirus Shedding (n = 11)</th>
<th>Patient with Adenovirus Shedding (n = 9)</th>
<th>Patient with Enterovirus Shedding (n = 5)</th>
<th>Patient with Rotavirus Shedding (n = 1)</th>
<th>Patient Without Documented Viral Shedding (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of male patients</td>
<td>38 (61.3%)</td>
<td>7 (63.6%)</td>
<td>6 (66.7%)</td>
<td>2 (40%)</td>
<td></td>
<td>25 (65.8%)</td>
</tr>
<tr>
<td>Median age (y) (range)</td>
<td>3.5 (0.1–15.5)</td>
<td>1.5 (0.5–14.5)</td>
<td>4.5 (1–13)</td>
<td>1.0 (0.5–2.5)</td>
<td></td>
<td>4.0 (0.1–15.5)</td>
</tr>
<tr>
<td>Chronic enenteropathy due to immune dysregulation a</td>
<td>8 (12.9%)</td>
<td>1 (9.1%)</td>
<td>1 (11.1%)</td>
<td>1 (20%)</td>
<td></td>
<td>5 (13.2%)</td>
</tr>
<tr>
<td>Clinical symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>18 (29.0%)</td>
<td>6 (54.5%)</td>
<td>2 (22.2%)</td>
<td>3 (60%)</td>
<td></td>
<td>7 (18.4%)</td>
</tr>
<tr>
<td>- including recent diarrhea (for less than 2 mo)</td>
<td>5/18 (27.8%)</td>
<td>1/6 (16.7%)</td>
<td>1 (9.1%)</td>
<td>0</td>
<td></td>
<td>6/37 (16.2%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (8.1%)</td>
<td>1 (9.1%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>3 (7.9%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2 (3.2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>2 (5.3%)</td>
</tr>
<tr>
<td>Absence of intestinal symptoms</td>
<td>43 (69.4%)</td>
<td>5 (45.5%)</td>
<td>7 (77.8%)</td>
<td>2 (40%)</td>
<td></td>
<td>30 (78.9%)</td>
</tr>
<tr>
<td>Fever</td>
<td>5 (8.1%)</td>
<td>1 (9.1%)</td>
<td>0</td>
<td>0</td>
<td></td>
<td>4 (10.5%)</td>
</tr>
<tr>
<td>Other microbiological documentation of enteric pathogens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus viremia b</td>
<td>13/60 (21.7%)</td>
<td>6/11 (54.5%)</td>
<td>3/8 (37.5%)</td>
<td>1/5 (20%)</td>
<td></td>
<td>3/37 (8.1%)</td>
</tr>
<tr>
<td>Isolation of bacteriological pathogens from stools</td>
<td>3</td>
<td>0</td>
<td>1 (Clostridium difficile)</td>
<td>1 (Clostridium difficile)</td>
<td>2 (Clostridium difficile, Campylobacter)</td>
<td></td>
</tr>
<tr>
<td>Isolation of parasites from stools</td>
<td>3</td>
<td>1 (Giardia intestinalis)</td>
<td>1 (Microsporidia)</td>
<td>1 (Cryptosporidia)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

a In patients suffering from chronic granulomatous disease (n = 2), CD25 deficiency (n = 1), cartilage-hair hypoplasia (n = 1), dyskeratosis congenita (n = 1) and combined immune deficiency of unknown origin (n = 3).
b Data not available for 2 cases.
c With additional positive test for toxin in the stool.
shedding by immunocompromised pediatric and adult populations [4, 9, 10]. However, these reports involved patients receiving chemotherapy and/or immunosuppressive therapy, leading to substantial variation of the immunodeficiency over time, and this may have had consequences for viral clearance. The situation is different in our population, whose level of immunodeficiency was stable and could not be modified to limit the duration of viral shedding and/or infection. The delay between the collection of consecutive stool samples was in some cases long. Consecutive positive samples may therefore have been attributable to prolonged infection or, alternatively, to reinfec-
tion. Nevertheless, chronic fecal shedding is the most probable explanation, consistent with previous reports of prolonged viral excretion in stools by immunocompromised patients.

The frequency of norovirus shedding was similar in children hospitalized for combined PID and exclusively humoral PID. However, the viral susceptibility of the different sub-

groups of PID cannot be compared because of the overrepre-
sentation of the patients with combined PID in our population and the substantial diversity of the genetic diseases included. Viruses were isolated from the feces from all of the 5 children with major histocompatibility complex class II expression deficiency (including norovirus in 3 cases), consistent with the known susceptibility of patients with this genetic disease to chronic fecal infectious shedding [11]. Norovirus was also isolated from 40% of children with agammaglobulinemia, in line with previous reports of chronic enterovirus shedding by such patients [12].

The clinical consequences of norovirus shedding in immu-

nocompromised hosts remain unclear because of the high frequency of both norovirus infection and asymptomatic shedding in the general population. The consequences are diffi-
cult to assess from our study for 2 reasons. First, the isolation of >1 potential enteric pathogen from several patients makes it difficult to establish any independent causal link between pathogen and symptoms. Nevertheless, in 5 of 6 patients with norovirus excretion and gastrointestinal symptoms, no other pathogens were found in stool samples; this strongly implicates the norovirus in the clinical symptoms. Second, almost 13% of the children had chronic enteropathy secondary to immune dysregulation, which could mimic symptoms of viral enteritis. However, because exacerbations of inflammatory bowel disease associated with norovirus have been described in children [13], we cannot exclude the possibility that norovi-

rus infection worsened pre-existing immune-mediated intesti-
nal damage in these patients. Of note, to our knowledge, ours is the first study describing histological esophageal and antral abnormalities in norovirus-infected patients [1]. Further pro-
spective studies are needed to evaluate the evolution of the clinical symptoms and/or the histological findings at and from the time of norovirus acquisition in children with chronic immune-mediated enteropathy.

Norovirus viremia was detected in 25% patients with noro-

virus fecal excretion, as previously described [14]. We also de-
teCTed norovirus in the CSF from one patient who had an intracerebral EBV-induced lymphoproliferative disorder. It was not possible to conclude whether the norovirus in the CSF was attributable to intracerebral viral replication or to passive viral diffusion through a lesion in the blood-brain barrier. However, the isolation of norovirus from extra-
digestive sites and the recent description of neurological symp-
toms attributed to norovirus [15] make a case for careful screening for this pathogen in immunocompromised patients with atypical extra-digestive symptoms and chronic fecal shedding of norovirus.

To summarize, norovirus was the virus most frequently de-
tected in fecal samples from children with PID. It can be

involved in chronic shedding and associated with severe gastro-

intestinal symptoms and may possibly have a role in the exacerba-
tion of pre-existing immune-mediated enteropathy. Our results suggest that systematic fecal screening for norovi-

rus in children with PID may be useful in 2 situations: (1) in cases of chronic gastrointestinal symptoms, especially when potential dysimmune and/or infectious etiologies are being considered, and (2) before hematopoietic stem cell transplan-
tation (a possible approach for the treatment of several inher-

ited immunodeficiencies). In addition, in cases of known intestinal shedding and unexplained extra-digestive symptoms, blood and/or plasma and local samples should be systematically screened for norovirus, because viral spread to outside the intestinal tract appears to be possible.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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