

Noroviruses as a Potential Cause of Protracted and Lethal Disease in Immunocompromised Patients

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(See the article by Roddie et al, on pages 1061–8.)

Recognition of the significance of noroviruses as enteric pathogens continues to unfold as more sensitive molecular diagnostic techniques become more widely available. Noroviruses are already recognized as the most common cause of foodborne disease in the United States [1], attributing to more than two-thirds of foodborne gastroenteritis cases [2] and leading to an estimated 23 million cases of norovirus gastroenteritis each year [3].

Gastrointestinal infections can result in serious illness in immunocompromised hosts. Defining the cause of vomiting and diarrhea in immunocompromised hosts can be challenging, with myriad potential, interrelated causes, including medications, infections, chronic gastrointestinal disorders, medical procedures, and immune phenomena, such as allograft rejection [4] and graft-versus-host disease (GVHD) [5]. The role of viral enteric pathogens in this population has not been well established, with only adenoviruses and rotaviruses detected by electron microscopy (EM) and enzyme-linked immunosorbent assay on a routine basis [6]. Our current understanding of the epidemiology of norovirus

infections in immunocompromised hosts is limited and based primarily on case reports.

In this issue of *Clinical Infectious Diseases*, Roddie et al [7] describe the first case series demonstrating noroviruses as a cause of persistent gastroenteritis in adult allogeneic hematopoietic stem cell transplant (HSCT) recipients. Previous reports have identified prolonged norovirus gastroenteritis and shedding in immunocompromised pediatric populations [4, 8]. Four previous norovirus cases in pediatric HSCT recipients [9–11] have been associated with prolonged gastroenteritis and viral shedding, with 1 patient dying “as a result of poor health complicated by gastroenteritis” [11]. One adult HSCT recipient with norovirus has also been described [12].

Although the number of cases studied by Roddie et al [7] is relatively small (12 cases), their investigation provides some important insights into a potentially profound impact that noroviruses may have in immunocompromised hosts. Ten of 12 patients had chronic diarrhea that persisted for a median of 3 months. Two of the HSCT recipients died, 1 secondary to complications of malnutrition and chronic norovirus gastroenteritis. Six patients required supplemental enteral or parenteral nutrition because of their persistent diarrheal illness. The norovirus-infected patients with gastroenteritis were hospitalized for a median of 73 days.

However, despite the significant morbidity and mortality attributed to noroviruses in this study [7], it is difficult to determine the exact role of noroviruses in the development of the severe gastrointestinal manifestations experienced by HSCT recipients. Information from this case series was collected retrospectively. In addition, one problem encountered by physicians attempting to detect norovirus infections in a nonoutbreak situation (7 of 12 cases) is distinguishing asymptomatic infection from clinical disease. It has been shown that up to 32% of immunocompetent hosts challenged with noroviruses develop asymptomatic infection [13]. The relative occurrence of symptomatic to asymptomatic infection in immunocompromised hosts is unknown. Roddie et al [7] evaluated fecal viral loads in a semiquantitative manner. Norovirus RNA detected in the first round of nested polymerase chain reaction (PCR) for conventional PCR or a cycle threshold of <30 PCR cycles of amplification for real-time PCR was used as a marker of increased viral load and to distinguish asymptomatic infection from clinical disease. Most researchers studying norovirus infections prefer to establish numerical quantitative viral loads as an objective measurement of infection. Potential problems with the assignment of high versus low norovirus viral loads based on cycle threshold include difficulty in interpreting and extrapolating these data to other laboratories, where cy-

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cle thresholds and amplification efficiencies for real-time PCR assays may differ.

Atmar et al [14] demonstrated that in healthy adult volunteers challenged with Norwalk viruses, individuals who did not meet the criteria for gastroenteritis (>200 g of unformed stool or 1 vomiting episode per day associated with an additional symptom of enteric infection such as abdominal pain) could still shed high fecal viral loads (up to 10^{12} norovirus copies per gram of feces). Even if norovirus infections caused the initial gastroenteritis symptoms in the HSCT recipients, it is difficult to demonstrate that the prolonged high norovirus fecal viral loads were responsible for the protracted gastrointestinal symptoms. Challenged volunteers experiencing norovirus gastroenteritis were capable of prolonged fecal viral shedding even after clinical resolution (up to nearly 8 weeks with high viral loads) [14]. Asymptomatic shedding in immunocompromised hosts after clinical resolution may be even longer. Distinguishing between clinical disease and asymptomatic norovirus shedding in immunocompromised hosts, who have deficient cellular immunity and are unable to clear viruses and who are at risk for gastrointestinal symptoms from other causes, represents a diagnostic challenge. Evaluation of tissue can be helpful but may not definitively differentiate non-infectious processes, such as allograft rejection or GVHD from norovirus gastroenteritis. Histopathologic findings, such as crypt apoptosis and inflammation, are associated with both noninfectious processes and norovirus infection [4].

Roddie et al [7] highlight the important therapeutic implications of establishing the correct diagnosis in these HSCT recipients. Immunosuppression was reduced or stopped in 8 patients in an attempt to clear the protracted norovirus gastroenteritis, as opposed to increasing immunosuppression to treat presumed gut GVHD in 3 patients, later found to have norovirus gastroenteritis.

The median time to diagnosis of no-

rovirus infection in the HSCT patients was 1 month after the onset of gastroenteritis. The insensitivity of EM (only 2 of 9 patients tested positive for noroviruses by EM) originally performed at the hospital contributed to a delay in diagnosis in at least 1 patient. There was a delay of up to 6 months in establishing the diagnosis in HSCT patients with diarrhea, when reverse-transcription PCR was later used to detect norovirus infection among the 12 patients. A lack of clinical suspicion for norovirus infections most likely contributed to a delay in testing for noroviruses. This study underscores 2 important diagnostic points: (1) reverse-transcription PCR is currently considered the gold standard for the diagnosis of norovirus infection [15] and should be performed early in the disease to accurately identify this enteric pathogen; and (2) norovirus gastroenteritis should be considered in susceptible populations, such as children, immunocompromised hosts, and travelers [16]. Norovirus gastroenteritis should especially be suspected when common enteric pathogens have been excluded or in patients with presumed GVHD refractory to prolonged steroid therapy, as was seen in this study [7].

Human mechanisms for protective immunity and clearance of noroviruses are not well defined. Past studies have shown that humoral immunity with antibody production does not provide long-lasting protection, even if the host is challenged with the same norovirus strain [17]. More recent studies indicate that the antigenic diversity of this group of viruses allows them to evade the host immune response, with antibodies cross-reactive to only noroviruses of the same genotype group [18]. In the current study [7], 2 patients were infected with a second norovirus variant genotype after their initial norovirus diagnosis. It is hypothesized that cellular immunity plays an important role in clearance of norovirus infections. Studies have shown a predominant T_H1 immune response in humans with elevated levels of interferon- γ and interleukin-2 [19]. All of

the patients in this study [7] were allogeneic HSCT recipients. One of the main complications of allogeneic HSCT is GVHD. T cell depletion strategies are often used to reduce the risk of GVHD but have been associated with increased rates of graft rejection, cytomegalovirus infection, invasive fungal infections, and Epstein-Barr virus-associated post-transplantation lymphoproliferative disease [20]. In vitro stimulation of peripheral blood mononuclear cells with norovirus-like particles in $CD4^+$ T cell depletion has been associated with significant decreases in interferon- γ production [19]. Most HSCT recipients (9 of 12) in this study received T cell-depleting conditioning regimens. It is likely that T cell depletion and the immunosuppressant treatment, administered to 11 of the 12 patients, suppressed both cell-mediated and humoral immunity in the HSCT patients, rendering them particularly susceptible to noroviruses and impairing their ability to clear the norovirus infections. This immunodeficiency likely led to persistent norovirus shedding and gastrointestinal symptoms. Future studies are needed to determine which immunosuppressive agents are more likely to be associated with prolonged symptomatic norovirus infection. Host genetic susceptibility based on histo-blood group antigen status is also an important determinant of infection for some norovirus strains [21] but was not examined in this study.

Eleven of 12 HSCT recipients acquired their norovirus infections from the community. Noroviruses have a low infectious dose (<100 viral particles for healthy adults [22]) and are environmentally stable and resistant to many cleaning agents [23]. As a result, noroviruses are infectious, with a high secondary attack rate. Appropriate measures should be considered to decrease the risk of acquiring norovirus infections among the immunocompromised. Immunocompromised individuals should avoid contact with persons acutely ill with gastroenteritis. Current recommendations for HSCT pa-

tients to prevent infections with enteric pathogens such as *Salmonella*, *Campylobacter*, *Cryptosporidium*, and *Strongyloides* species are to avoid contact with feces-contaminated surfaces; to avoid eating uncooked meats, eggs, or egg products; to thoroughly wash produce before consumption; and to consider boiling tap water [24]. More stringent guidelines for HSCT patients may need to be established during periods of increased immunosuppression if future studies confirm noroviruses as a significant cause of morbidity and mortality in immunocompromised hosts. Guidelines for selection of safe foods for this immunosuppressed population should follow principles developed for eating safely in developing regions of the world: “Boil it, cook it, peel it, or forget it” [25].

Another important aspect of patient care addressed by this article is the implementation of infection control measures for immunocompromised hosts who persistently shed noroviruses. These immunocompromised patients may serve as reservoirs and require contact isolation to prevent nosocomial norovirus occurrences. Although it is prudent to isolate patients who continue to shed noroviruses, the virulence of persistently shed noroviruses in such immunocompromised patients should be questioned in view of the lack of secondary cases [7].

Prospective cohort studies with larger sample sizes are needed to confirm that noroviruses are an important cause of morbidity and mortality among immunocompromised hosts such as allogeneic HSCT recipients. Roddie et al [7] provide us with important preliminary evidence that noroviruses may cause a protracted diarrheal illness, leading to significant complications, including extended hospitalizations, severe weight loss, malnutrition, and death. Additional epidemiologic studies are needed to investigate the role of norovirus infection in other clinical settings as well. We recently demonstrated that noroviruses are the second most common pathogen group causing travelers’ di-

arrhea, after diarrheogenic *Escherichia coli* [16], and are an uncommon cause of non-*Clostridium difficile* antibiotic-associated diarrhea in a hospital in Houston, Texas [26]. As our understanding of the epidemiology and pathogenesis of norovirus infections continues to broaden with the use of more sensitive molecular assays in susceptible populations, such as immunocompromised hosts, the substantial impact of these viral enteric pathogens is becoming more evident.

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