Chronic norovirus infection and common variable immunodeficiency

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Summary

Chronic infection with norovirus is emerging as a significant risk for patients with immunodeficiency – either primary or secondary to therapeutic immunosuppression. Patients with primary immunodeficiency present a range of pathological responses to norovirus infection. Asymptomatic infections occur and differentiating viral carriage or prolonged viral shedding after self-limiting infection from infection causing protracted diarrhoea can be challenging, due to relatively mild pathological changes that may mimic other causes of diarrhoea in such patients (for instance pathogenic bacteria or parasites or graft-versus-host disease). However, a subset of patients with common variable immunodeficiency (CVID) experience a severe norovirus-associated enteropathy leading to intestinal villous atrophy and malabsorption. Symptomatic infection of up to 8 years has been demonstrated with clinical and histological recovery on viral clearance. Although oral immunoglobulins and nitazoxanide have been used to treat noroviral infections associated with immunosuppression, ribavirin is the only agent to date that has been linked to viral clearance in the Noroviral enteropathy associated with CVID.

Keywords: enteropathy, immunodeficiency, norovirus, ribavirin

Norovirus

Norovirus is an unenveloped, positive stranded RNA virus, a member of the Caliciviridae [1]. It was first identified in stool isolates from an outbreak of ‘winter vomiting disease’ in Norwalk, Ohio in 1968 [2], where the majority of patients experienced nausea and vomiting and approximately a third also had profuse diarrhoea [3]. Norovirus is thought to be responsible for approximately 21 million cases of acute gastroenteritis in the United States every year, constituting approximately 60% of all cases with a known cause [4]. High viral loads in stool and vomit combined with a low infective dose [5] and short incubation period result in a high attack rate, averaging 50% (9–78%) in close environments such as health-care facilities or cruise ships [6]. Food (particularly fruit, oysters and other food eaten raw) and water-borne outbreaks are reported [7,8].

The viral genome encodes two capsid proteins and six non-structural proteins organized in three open reading frames (ORF 1–3) [9]. There is enormous diversity within and between strains, with six broad genotypes identified (G I–VI) based on the amino acid sequence of the VP1 capsid protein (which differs as much as 38% between GI and GII isolates) [10,11]. Of these, G II (predominantly G II.4) is responsible for the majority of human infections, followed by G I and G IV [12]. Specific strains affect animals (cows, pigs, dogs, rodents) and antibodies to these strains can be detected in asymptomatic humans, raising the possibility of norovirus being at least partially zoonotic [13].

Our understanding of norovirus biology is limited by the lack of a suitable ex-vivo culture for the human virus, and is dependent upon animal models [14]. The attachment receptor for virus entry into the cell is possibly a carbohydrate moiety in view of the protection afforded by a common null mutation of FUT2 (encoding fucosyl transferase) [15,16], although this is strain-specific, with some strains capable of infecting the resultant ‘non-secretor’ phenotype [17,18]. While transcytosis may occur through enterocytes, epitheliolymphorrhagism is difficult to demonstrate, and current evidence favours intestinal immune cells of the lamina propria as the cellular target in which viruses replicate – including dendritic cells, macrophages and Peyers patch B cells [19–22]. In murine infection, norovirus can be detected along the length of the gastrointestinal tract.
from stomach to colon [14] and dissemination to other organs such as spleen and liver may occur, possibly as a result of viraemia or via dendritic cells [23–25].

**Norovirus infection in the immunocompetent**

In most individuals norovirus infection results in a short-lived intense gastroenteritis, with two-thirds of patients experiencing nausea and vomiting, and approximately three-quarters have profuse diarrhoea [26]. Abdominal pain, fever and leucocytosis are reported, but unusual [27]. Following an average 48-h incubation period [28], symptoms last for 2–3 days but can be more prolonged and serious in elderly or very young people. One study reported a 30-day mortality rate of 7% in patients of a median age of 77 years [29]. Central nervous system involvement has been reported, and infants appear to be particularly at risk of (afebrile) seizures associated with norovirus gastroenteritis [30]. Norovirus continues to be shed for prolonged periods following resolution of symptoms – a recent study revealed 47% of individuals excreting virus for 21 days or more [31].

Similarly, asymptomatic infection is common, with high proportions of stool samples from unaffected individuals revealing the presence of norovirus [32].

Despite the severity of the symptoms, pathological changes are sparse. One human study has demonstrated epithelial tight junction dysfunction and increased permeability along with stimulated anion flux. There were minimal changes in villous architecture but a doubling of intraepithelial lymphocytes, related to an increase of cytotoxic intraepithelial lymphocytes [33].

Postinfective functional gastrointestinal symptoms have been described [34].

**Immune responses to norovirus**

Experimental rechallenge of volunteers has demonstrated that short-lived strain-specific immunity occurs (waning between 6 months and 2 years) [35] and mathematical modelling based on infection rates has suggested postinfective immunity to last for 4.1–8.7 years [36]. Humoral immunity is key as viral protection coincides with the detection of immunoglobulin (Ig)A antibodies in saliva, and passive immunity is transferred to infants by maternal antibody in breast milk [37]. Furthermore, humoral immunity drives selection with emerging strains developing mutations induced at codons that lead to amino acid changes at known antibody binding epitopes in capsid proteins [38].

The extent to which murine experimental studies can be extrapolated to humans (and indeed even between different human-infecting genotypes) is unclear [14]. Mice deficient in type 1 interferon receptors or downstream signalling pathways [signal transducer and activator of transcription 1 (STAT-1)] succumb to a multi-system infection with severe diarrhoea, gastric bloating and extra-intestinal pathology [39]. Recombination activating gene (RAG) knock-out mice are unable to clear the virus, but the infective load is reduced substantially following transfer of immune serum, B cells, CD4+ T cells or CD8+ T cells [40], which suggests that both antibody and cytotoxic T cell responses play a part in viral clearance.

**Norovirus in immunosuppressed individuals**

Immunosuppression changes the course of norovirus infection significantly, leading to prolonged symptoms and viral shedding [41,42]. An initial report of two renal transplant recipients in 2009 demonstrated persistent norovirus excretion [43]. One case was asymptomatic, but shed virus for more than 7 months before spontaneous clearance, and the other showed features of severe enteritis with diarrhoea, fever and weight loss and required a reduction of immunosuppression to clear the infection. A subsequent report from Paris found norovirus (or sapovirus, a related calicivirus) infection to be responsible for 80% of cases of otherwise unexplained diarrhoea in renal transplant recipients, with significant consequences of graft failure (in 81%) or rejection following reduction of immunosuppression (in five patients). Norovirus excretion was detected in stool for up to 581 days [44].

A series of nine renal transplant recipients reported from Zurich with chronic symptomatic noroviral infection reported chronic intermittent diarrhoea [45]. Molecular analysis of viral isolates showed the majority to be separate strains of GII.4, indicating sporadic infection rather than nosocomial transmission from a common source. Significant intrahost evolution occurred, with one virus mutating at least 25 capsid protein amino acid residues during an 898-day period of viral shedding.

Chronic noroviral infection has also been reported in recipients of other solid organs, including heart [46], lung [47], pancreas [48] and intestine [49], and also after bone marrow transplantation [50–53], where it presents a particular challenge as it may be misdiagnosed as intestinal graft-versus-host disease (GVHD) and lead to intensification of immunosuppression rather than reduction. Histological features of the two conditions overlap and there is no certainty that ongoing viral excretion represents symptomatic infection; furthermore, noroviral infection may accompany GVHD as a result of treatment with increased immunosuppression.

A report from London included 12 patients with chronic noroviral infection following allogeneic haemopoietic stem cell transplantation (HSCT) in whom diarrhoea persisted for a median of 3 months, and six patients required artificial nutrition support [50]. Two patients died, one of whom was related directly to malnutrition associated with the infection. Similarly, two of 10 patients with noroviral
enteritis died following HSCT in a Japanese centre, with a duration of symptoms up to 135 days (median 41). Infection was found to be more common after a second graft [51].

In Hong Kong, eight of 55 children undergoing HSCT developed chronic noroviral infection with time to clear the virus of up to 263 days (median 145 days) [52]. Six children required parenteral nutrition support and three died. All six patients who engrafted successfully with allogeneic cells developed acute GVHD, although not statistically significant, but there was an association with chronic GVHD in these patients. The intensity of the conditioning regimen (with fludarabine or almetuzumab in particular) and the use of peripheral or cord blood stem cells (that require heavier immunosuppression than bone marrow-derived cells) were risk factors for development of noroviral infection. The recovery of donor T cells in peripheral blood was strikingly associated with clearance of infection in 13 children from Manchester, even when delayed to 13 months after transplant [53]. Artificial nutritional support was required in all patients, with two patients requiring parenteral nutrition for almost a year, stopped on symptomatic recovery only after viral clearance.

Reports of severe complications associated with chronic noroviral infection after transplant, including agranulocytosis [54] and haemophagocytic lymphohistiocytosis [55], are difficult to link directly to the infection itself in view of the clinical context, but may hint at the development of extra-intestinal pathology in heavily immunosuppressed individuals.

Despite the relatively small number of case reports and series, the overall extent of the problem posed by chronic norovirus infection is demonstrated by a report from Texas of 22% of all solid organ and bone marrow transplant recipients developing the infection, more than 50% of whom required hospitalization, and in this institution it was the most common enteropathogen [56]. It is now apparent that chronic noroviral infection poses a substantial risk to transplant recipients, either from sporadic or nosocomial infection.

**Common variable immunodeficiency (CVID) enteropathy**

CVID is the most common identified symptomatic primary antibody deficiency in Europeans, affecting approximately 1 : 25 000. The immunopathology is variable, and probably relates to defects in a variety of different pathways that lead to terminal B cell differentiation [57,58]. A description of the different attempts to classify CVID subtypes is beyond the scope of this paper; however, a subtype associated with T cell defects that occurs in approximately 5% of cases appears to present with granulomatous disease, splenomegaly, gastrointestinal involvement and lymphoma and carries a worse prognosis [59]. A number of single gene defects have been identified in CVID but, together, only amount to approximately 2–10% of cases [60]. A subtype of CVID is associated strongly with autoimmune conditions either through a shared genetic predisposition that includes human leucocyte antigen (HLA) or mechanistically due to associated immune dysregulation [61].

Gastrointestinal disturbances are common in CVID, with diarrhoea being the most common symptom reported [62–68]. Common causes include enteropathogens such as *Giardia, Salmonella, Campylobacter* and *Cryptosporidium*, with a variety of case reports of other common or unusual organisms [63,66,67,69–71]. However, a range of inflammatory gastrointestinal pathologies are described [63,72]. In rare cases, histological appearances and clinical progression can mimic ulcerative colitis or Crohn’s disease and respond similarly to immunosuppressive medication. However, a clearly defined entity of malabsorption and villous atrophy of unknown cause (including non-responsiveness to gluten withdrawal) known as ‘CVID enteropathy’ appears to be peculiar to this condition [62].

CVID enteropathy occurs in approximately 5% of patients with CVID, and appears to be associated with the subtype of patients found to have defective T cell function [65]. The clinical presentation resembles that of coeliac disease, with diarrhoea, malabsorption and villous atrophy (Fig. 1) on mucosal biopsy of the proximal duodenum [65,66]. Symptoms may be profound, with fat malabsorption sufficient to cause clinical steatorrhoea, protein-losing enteropathy and malnutrition sufficient to require parenteral nutrition support. Nausea and vomiting are associated
frequently and the nausea can be persistent and troublesome. Histological features are described as resembling the appearance of acute intestinal GVHD, with mild to severe villous atrophy. Enterocytes become flattened and vacuolated and there is increased epithelial apoptosis. Chronic inflammatory infiltrates in the lamina propria lack plasma cells and may include neutrophils that may also infiltrate the epithelium (Fig. 2a). The only features that distinguish CVID enteropathy clearly from coeliac disease are the presence of lymphoid follicular hyperplasia and the lack of plasma cells; however, these are underlying features of the CVID mucosa itself, and they do not permit discrimination of CVID enteropathy from coeliac disease co-existent with CVID [65]. A significant degree of intestinal mucosal enhancement and wall thickening may be seen on cross-sectional imaging with computerized tomography (CT) or magnetic resonance imaging (MRI) demonstrating a pan-enteritis (Fig. 3). Diagnosis of CVID enteropathy requires an exhaustive search for known pathogens and exclusion of coeliac disease. This can be challenging (as serological tests are unhelpful), and depends upon demonstration of symptomatic and histological response to gluten withdrawal in patients with the susceptibility HLA haplotypes (HLA-DQ2 or 8). Histological recovery to gluten withdrawal in coeliac disease may take a year or more [73], and there is a learning curve for patients to be able to exclude effectively all gluten from the diet, therefore characterization of the response to gluten withdrawal may be prolonged. While GVHD and coeliac disease remain the most difficult to differentiate histologically from CVID enteropathy, other pathogens may cause a milder form of enteropathy in CVID. Giardia may lead to villous blunting but without severe inflammation, although excess eosinophils may be present in the lamina propria, and the parasites are clearly detectable on routine histopathological specimens and in stool [74]. Bacterial infections such as Campylobacter are more likely to lead to an ulcerative distal enterocolitis and the clinicopathological features of co-existent inflammatory bowel disease are generally sufficiently distinct to distinguish from CVID enteropathy. A recent review of viruses associated with primary immunodeficiencies identified a number that are shed over prolonged time-periods and may have been associated with exacerbation of underlying inflammatory enteropathy [75], and stool samples should be examined to exclude agents such as enteroviruses, rotavirus, astrovirus and adenovirus.

The cause of CVID enteropathy is currently unknown, but in the setting of CVID, infective or autoimmune pathologies have been considered. The diagnosis requires exclusion of known enteric pathogens and treatment to date has largely comprised immunosuppressive therapies, including steroids, thiopurines, cyclosporin A and anti-tumour

**Fig. 2.** (a) Close-up of duodenal biopsy in patient with common variable immunodeficiency and chronic norovirus infection [haematoxylin and eosin (H&E) stain, \( \times 40 \)]. Norovirus RNA was detected by polymerase chain reaction (PCR) in stool and also from the duodenal biopsy specimen. Marked villous atrophy is apparent with a chronic inflammatory infiltrate in the lamina propria (lacking plasma cells in view of the immunodeficiency). The enterocytes are reduced in height and vacuolated. (b) Close-up of duodenal biopsy in the same patient following norovirus clearance with a prolonged course of ribavirin (H&E stain, \( \times 40 \)). Note that the inflammatory infiltrate in the lamina propria has resolved, there is restitution of villous architecture and the enterocytes are columnar.

**Fig. 3.** Abdominal cross-sectional contrast-enhanced computerized tomography (CT image) from a patient with longstanding chronic norovirus infection with common variable immunodeficiency (CVID). Small bowel loops appear thickened with hyperenhancement of the mucosa (arrowheads), representing a pan-enteritis of the small intestine in contrast to the normal appearance of the colon (arrow).
necrosis factor (TNF)-α monoclonal antibodies [65,76]. Partial symptom responses (with improvement in nausea more than diarrhoea) have been demonstrated by such an approach but without resolution of intestinal inflammation or villous atrophy, and patients usually remain dependent upon nutritional support.

**Norovirus infection and CVID enteropathy**

As many of the pathological features of CVID enteropathy are in keeping with an infective origin, and following the coincidental finding of prolonged norovirus excretion in one of our patients, we investigated the possibility of chronic norovirus as a cause of CVID enteropathy in our patients [77]. A previous review of chronic and persistent viral infection in immunodeficiency noted the possibility that enteropathy might be triggered by a viral infection, such as is known to be the case with childhood rotavirus in infection and coeliac disease, but were unable to identify any reports of a viral association with CVID enteropathy [75].

All eight of our patients with CVID enteropathy demonstrated persistent norovirus excretion for periods of up to 1200 days, and none of the asymptomatic controls that were tested had norovirus present in stool. No other known enteric pathogens or enteroviruses were present in the patients with enteropathy. Sequencing of the isolates demonstrated separate strains of GII.4 in all cases. Three patients had a compatible haplotype for coeliac disease, but two made no symptomatic response and the other had only a short-lived benefit from gluten withdrawal. Five patients required intravenous nutritional support. Reverse transcription–polymerase chain reaction (RT–PCR) of archived paraffin-embedded intestinal biopsies demonstrated the presence of norovirus RNA sequences in the duodenum in seven of eight cases. Norovirus was detected in all intestinal biopsies in two patients during the entire 5- or 8-year course, respectively, of their symptomatic presentation with CVID enteropathy. Furthermore, sequence analysis demonstrated that in one case the same virus had been present throughout the course of the infection, rather than the occurrence of intermittent infection with different strains. Clearance of norovirus (in two cases following prolonged treatment with oral ribavirin and in one case spontaneously) was accompanied by rapid resolution of symptoms, resolution of intestinal inflammation and restoration of duodenal villous morphology (Fig. 2a,b). In addition, serum immunoglobulin levels were maintained with normal therapeutic dosage, whereas previously high-dose replacement was required, due presumably to enteric loss. Both patients receiving parenteral nutritional support were able to recommence full oral nutrition.

In our series, the evidence is strongly suggestive of chronic noroviral infection being the predominant cause of CVID enteropathy, and since publication of our report we have become aware of a large number of cases of CVID enteropathy associated with noroviral infection from different centres worldwide. However, other enteroviruses, such as parechovirus, have been detected in this setting [78] and it remains to be seen whether norovirus is just one of several viruses that can result in this entity. The clinical introduction of new technologies such as chip-based rapid PCR for multiple organisms may shed new light upon the associated viral infections with CVID enteropathy in the near future.

**The spectrum of norovirus infection in immunodeficiency**

A survey from Paris [79] of 62 children (median age 3.5 years) suffering from a range of inherited primary immunodeficiency conditions found norovirus excretion in stools in 24 patients (38.7%). Thirteen of these were asymptomatic; however, symptomatic presentation was twice as common in those with norovirus infection as those without. Norovirus excretion was three times as common in symptomatic patients as those without symptoms. Viraemia was identified in two children. Of 10 patients with undefined ‘enteropathy’ in this study, five had no virus identified and three had norovirus, although the histological features described in two patients were mild. CVID is not diagnosed in children below the age of 4 years, and the findings of this study cannot be extrapolated to adults, where CVID is the most common form of primary immunodeficiency.

There is clearly a wide spectrum of responses to norovirus that include acute self-limiting infection, asymptomatic carriage, prolonged or persistent shedding following acute infection, a severe sprue-like enteropathy and even viraemia, leading to the possibility of extra-intestinal organ involvement. Given that the majority of infections are caused by the same genotype – II.4 – it is unlikely that the range of responses relates to separate viral strains of differing virulence, and therefore most probably reflects altered host immune responses. Immunoglobulin replacement in patients with antibody deficiency appears insufficient to clear norovirus, which suggests that secretory IgA is required. To date, prolonged viral shedding has not been described in isolated IgA deficiency, which may relate to residual secretory IgA or compensation with IgM at mucosal surfaces. Prolonged asymptomatic viral shedding in immunodeficient patients may act potentially as a reservoir of strains for longer than specific herd immunity persists and has implications for recurrent community outbreaks [14].

The enteropathy associated with CVID – and with norovirus infection in all patients in our cohort – appears not to occur in other forms of primary immunodeficiency, where enteritis is rare and phenotypically distinct [80]. Notably, the severity of the symptomatic and
histopathological features of norovirus enteropathy in CVID (resulting in subtotal villous atrophy and malabsorption) is far greater than is seen in patients with acute infection or chronic infection associated with immunosuppression. This could be due simply to a greater burden of viral infection; however, patients with even more profound combined immunodeficiency do not demonstrate such features and it has been difficult to demonstrate viral cytopathic effects in enterocytes. It is more likely that the unique noroviral enteropathy associated with CVID results from a dysregulated host response, which may account for the similarity of the lesion to that seen in coeliac disease. This possibility is supported by the host response to Helicobacter pylori in patients with CVID, where infection appears to lead to more severe gastritis, atrophy and a higher risk of gastric adenocarcinoma than in other forms of immunodeficiency or in immunocompetent individuals [81]. Recent evidence from the CVID transcriptome would also suggest the possibility of higher baseline inflammatory responses [82].

**Treatment of norovirus infection in immunodeficiency**

It is only with the recent identification of chronic noroviral infection and asymptomatic carriage that efforts have been directed towards treatment. There are no anti-virals effective against norovirus that are approved currently for human use, and experience is limited to case reports.

In view of the apparent requirement for luminal antibody for viral clearance, oral immunoglobulin administration has been employed in cases of persistent norovirus excretion in immunodeficient patients post-transplant. Two days of administration appeared to clear the virus in 11 of 12 patients with norovirus gastroenteritis following lung transplantation [83], and was successful in a subsequent case report [84], but failed to clear the virus in one patient treated with alemtuzumab for chronic lymphocytic leukaemia [85]. There are no studies or reports of the successful use of oral immunoglobulin in norovirus infection associated with CVID.

Nitazoxanide was commenced in one patient with a 14-day history of symptomatic norovirus infection following haemopoietic stem cell transplantation, with symptomatic improvement within 24 h and complete resolution within 4 days [86].

On the basis of *in-vitro* studies [87], we evaluated ribavirin in our patients with CVID enteropathy and chronic norovirus infection [77]. Viral clearance occurred in two patients, with a treatment duration of up to 6 months. Notably, therapeutic drug level monitoring was required in view of reduced mucosal absorption. Three further patients failed to achieve viral clearance, despite the use of additional pegylated interferon-α in two. There were no clear differences that could discriminate responders from non-responders, and we hypothesize that a slow attainment of adequate plasma levels may lead to anti-viral resistance. Therefore, the efficacy of ribavirin with or without interferon in this setting requires further investigation; however, the hope is that newer, less toxic, approaches will be forthcoming as a result of new advances in targeted molecular therapeutics and a greater knowledge of norovirus biology from murine infections [88]. Until such time as anti-noroviral therapies are developed successfully, understanding of the risk posed by norovirus to patients with immunodeficiency (particularly in the setting of a subset of CVID) should lead to heightened awareness and precautions to prevent infection and secondary transmission.

**Disclosure**

The authors report no disclosures.

**References**
