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Chronic norovirus infection in immunodeficiency: a UK national case series.

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Clinical implications

Chronic norovirus infection can lead to significant diarrhoea, malabsorption, and weight loss in immunodeficiency. The clinical and histological picture in these patients is remarkably similar, suggesting that the virus is a main driver for the enteropathy.

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Keywords: chronic norovirus infection; primary immunodeficiency; secondary immunodeficiency; diarrhoea; malabsorption; weight loss; enteropathy; favipiravir; nitazoxanide; ribavirin; IgA; IgM; IgG; B cells; CD4+ T cells; CD8+ T cells; parenteral nutrition; villous atrophy; intraepithelial lymphocytosis

resolves in a few days, but certain populations are at higher risk of chronicity, including those with immunodeficiency. Chronic norovirus infection (CNI) can be debilitating in these patients, causing significant diarrhoea, malabsorption, and weight loss. Infection control measures are usually required, resulting in significant psychosocial impact. To gain more information on the clinical and laboratory picture of CNI in patients with immunodeficiency we collated relevant cases from Immunology centres across the UK.

A request was circulated via a mailing list, which includes 41 UK Clinical Immunology centres. Immunodeficiency patients with ≥2 positive stool samples for norovirus, detected by Polymerase Chain Reaction (PCR) >4 weeks apart, were included in the study. A data capture form was circulated. Successful clearance was defined as ≥2 stool samples negative, >2 weeks apart.

Forty-eight cases of CNI were reported from 11 UK centres (Table 1). Patients were mainly male (65%) adults (96%). Common Variable Immunodeficiency (CVID) was the most common immunological diagnosis (n=27), followed by secondary immunodeficiency (n=11). Twenty-seven patients underwent genetic testing, and a pathogenic mutation was detected in five: in BTK, XIAP, NFKB1, PTPN2 and TACI (heterozygous) genes. The total follow up time for norovirus infection ranged from 3 to 190 months, with a mean of 63. Ten patients also had another pathogen detected in their stool, in 2 of whom this was chronic (*Campylobacter* and enterovirus). All but one patients were on immunoglobulin replacement (intravenous in 35 and subcutaneous in 12) when CNI was diagnosed and in 78% (35/45) serum IgG trough was ≥8g/L at the time. Patients that required higher doses (>0.4g/kg/week, n=9) had a lower mean level of serum albumin (23.9 vs 31.6g/L), but not worse diarrhoea.

Laboratory investigations revealed very low pre-treatment serum immunoglobulin levels in 87% (41/47) for IgA (<0.22g/L), 62% (36/47) for IgM (<0.21g/L), 48% (15/31) for IgG (<1.34g/L) where data was available, and 84% were panhypogammaglobulinemic (26/31, Fig. 1). B cells were reduced ($<0.1*10^9$ cells/L) in 78% (36/46) and in those with normal levels, class-switched memory B cells were very low (<2%) in half. In 46% (22/48) T cells were also reduced ($<0.67*10^9$ cells/L), with CD4+ and CD8+ T cell subsets being equally affected (54% and 56% respectively). Low NK cells ($<0.1*10^9$ cells/L) were seen in 67% (31/46, Fig. 1).

exhibited significant weight loss (>20kg) and another suffered from malabsorption. In most (79%) weight loss was present, severe in 60%. Serum albumin was low in 67% and parenteral nutrition (PN) was required in 29%. In 94% (44/47) evidence of malabsorption was seen (≥1 serum nutrient reduced). In 45% (21/47) ≥4 nutrients were low and zinc was most often found to be low in those patients (96%). Elevated faecal calprotectin was seen in 83% (24/29). Stool alpha 1 antitrypsin was measured in 8 patients and was raised in half.

Villous atrophy and intraepithelial lymphocytosis were the two most common small bowel histological findings, in 82% and 62% respectively. In 35% an inflammatory cell infiltrate was also seen and in 24% plasma cells were absent or very low. Twenty patients underwent a colon biopsy and various forms of inflammation were reported in half (intraepithelial lymphocytosis, absent B cells, granulomatous inflammation etc.)

Treatment was given in 73%, either alone, in combination, or sequentially; 54% of patients being given more than one treatment. Treatment led to viral clearance in only 6 (17%): who respectively received favipiravir monotherapy, nitazoxanide monotherapy, ribavirin monotherapy, nitazoxanide with ribavirin, nitazoxanide with favipiravir, or hematopoietic stem cell transplantation (HSCT). No late recurrences were seen, with the follow up time for testing ranging from 4 months to 9 years. The primary diagnosis in those patients was CVID (n=4), XLA (n=1) and combined immunodeficiency(n=1). One patient cleared the infection spontaneously, infected with genotype G1. In all cases where treatment was successful, this was associated with significant improvement in the clinical picture (diarrhoea/ weight loss) and/or normalisation of absorption and gut mucosa. An improvement in the lymphocyte count was noted in two cases and it was possible to halve the dose of immunoglobulin replacement in another two. Twelve patients died, 6 of whom had previously required parenteral nutrition. In two cases the cause of death was deemed to be directly linked to CNI.

To our knowledge this is the largest reported series of patients with CNI and immunodeficiency. In the 7 previously published case series[1–7], CVID was the most common diagnosis (47/99), followed by combined immunodeficiency (20/99). CVID was the most common diagnosis in our cohort too, followed by secondary immunodeficiency, likely due to different inclusion criteria. Case series in secondary immunodeficiency have also been published, including patients with cancer[8] and after transplantation[9]. There were no obvious

despite previous reports implicating norovirus in 'CVID enteropathy'.

In our cohort very low baseline serum immunoglobulins and lymphocyte subsets were noted in the majority. E.g. B cells were reduced in 73% of CVID patients, while this is only expected to affect 10-20% of these patients. T cells were also frequently low and HSCT studies have shown that these cells play an important role in the clearance of CNI. Other factors likely also contribute, e.g. the only patient who spontaneously cleared CNI in our cohort was infected with genogroup G1.

Diarrhoea and malabsorption were the most common findings in our cohort, with serum zinc and albumin very often being low. Weight loss was also common, with PN required in a substantial proportion. Several patients required high dose immunoglobulin replacement to maintain adequate IgG trough levels, with lower serum albumin levels also seen in those, suggestive of a protein losing enteropathy state. The average immunoglobulin dose used, at around 1.3g/kg per month, was much higher than standard in antibody deficiency, emphasising the high healthcare cost of this condition. Notably, the absence of diarrhoea did not preclude the development of malabsorption, weight loss or the need for high dose immunoglobulin therapy. As previously shown[5,6], villous atrophy and intraepithelial lymphocytosis are common histological findings in the small bowel of infected patients, likely underlying malabsorption. The fact that the clinical and histological picture is remarkably similar regardless of the immune deficiency suggests that the virus is a main driver for the enteropathy, rather than the underlying immune disorder.

Favipiravir, nitazoxanide, and ribavirin, either used alone, or in combination, are potential treatment options for these patients. Cure is however often unachievable and management has to focus on supportive measures. HSCT should be considered in primary immunodeficiency.

This study is limited by its retrospective nature, and potential for reporting bias. Nevertheless, our survey identifies the burden of CNI and highlights clinical features that should prompt testing in immunodeficient patients including, diarrhoea, weight loss, low serum nutrients/ albumin, and the requirement for higher dose immunoglobulin replacement. Further studies are required to elucidate the pathogenesis and guide therapy.

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Figure 1: Pre-treatment immunoglobulin levels (top panel) and lymphocyte subset absolute counts (bottom panel) in patients with chronic norovirus infection.

Age at diagnosis (years)- median (range)	45 (6-70)	**Nutrient deficiency**- ratio (%)	
male -number (%)	31 (65%)	Calcium	29/48 (60%)
Immunodeficiency type- number (%)		Copper	4/4 (100%)
CVID	27 (58%)	Iron	16/18 (89%)
Secondary*	11 (23%)	Ferritin	16/25 (64%)
Combined	7 (15%)	Folate	12/31 (39%)
Other primary**	3 (6%)	Magnesium	5/19 (26%)
Norovirus strain -number (%)		Phosphate	4/26 (15%)
unknown	10 (21%)	Selenium	7/11 (64%)
1	1 (2%)	Zinc	24/25 (96%)
2	37 (77%)	Vitamin A	10/24 (42%)

Gastrointestinal coinfection (previous or current)- median (range)***	10 (21%)	Vitamin D	14/24 (58%)
Laboratory Parameters (pre-treatment)- median (LQ-UQ)		Vitamin E	15/27 (56%)
B cells (x10^9 cells/L)	0.02 (0.004-0.088)	Vitamin K	3/4 (75%)
T cells (x10^9 cells/L)	0.75 (0.38-1.2)	Vitamin B12	8/33 (24%)
CD4 T cells (x10^9 cells/L)	0.32 (0.2-0.49)	**Small bowel histology** -ratio (%)	
CD8 T cells (x10^9 cells/L)	0.27 (0.12-0.52)	Villous atrophy	24/34 (82%)
NK Cells (x10^9 cells/L)	0.07 (0.04-0.12)	Intraepithelial lymphocytes	21/34 (62%)
IgG (g/L)	1.6 (0.62-3.2)	Inflammatory cell infiltrate	12/34 (35%)
IgM (g/L)	0.1 (0.06-0.2)	Plasma cell depletion/absence	8/34 (24%)
IgA (g/L)	0.1 (0.03-0.11)	**Treatment** -ratio (%)	35 (73%)
Immunoglobulin replacement dose per kg of weight per week-median (range)	0.31 (0.09-0.71)	Nitazoxanide	24 (50%)

Trough IgG (g/L)- median (LQ-UQ)	9 (8-10.1)	Ribavirin	23 (48%)
Diarrhoea- ratio (%)	44/47 (94%)	Corticosteroids	6 (13%)
Mild (stool frequency <5 per day)	9/28 (32%)	Enteral immunoglobulin	2 (4%)
Moderate (stool frequency 5-10 per day)	14/28 (50%)	Interferon	5 (10%)
Severe (stool frequency >10 per day)	5/28 (18%)	Pentaglobin IV	3 (6%)
Weight loss- ratio (%)	35/44 (79%)	Favipiravir	13 (27%)
Mild to moderate (<20kg)	14 (40%)	Other	2 (4%)
Severe (>20kg)	21 (60%)	**Outcome** -ratio (%)	
Parenteral nutrition -ratio (%)	14/48 (29%)	Cleared	7 (15%)
Hypoalbuminemia -ratio (%)	32/48 (67%)	Ongoing	24 (51%)
Mild/moderate (>25g/L)	19 (59%)	Died	12 (26%)
Severe (<25g/L)	13 (41%)		

Table 1. Baseline patient characteristics, immunological profile, malabsorption parameters, histological findings and treatment of chronic norovirus infection patients. *Due to haematological malignancies (lymphoma n=3, leukaemia n=2, monoclonal gammopathy of uncertain significance n=1), immunosuppressive treatment (n=2), and Good's syndrome (n=2). **X-linked agammaglobulinemia (n=1), X-linked lymphoproliferative disease (n=1), other primary immunodeficiency (n=1). ***_Campylobacter_ n=6, _Giardia_ n=2, _Salmonella_ n=1, _C. difficile_ n=1, echovirus E11 n=1, untypable enterovirus n=1, adenovirus n=1, sapovirus n=1.