

## Faecal calprotectin indicates intestinal inflammation in COVID-19

GI symptoms such as diarrhoea, nausea and vomiting are frequent coronavirus disease (COVID-19) symptoms and affect up to 28% of patients.<sup>1–5</sup> The pathophysiology of COVID-19-associated GI symptoms is currently unclear. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-RNA was detected in the faeces in ~50% of patients with COVID-19<sup>3,5,6</sup>; SARS-CoV-2 viral particles were observed by electron microscopy in stool samples from two patients without diarrhoea<sup>2</sup>; and one study reported SARS-CoV-2 infection of the oesophagus, stomach, duodenum and rectum.<sup>5</sup>

Faecal calprotectin (FC) has evolved as a reliable faecal biomarker allowing detection of intestinal inflammation in IBD and infectious colitis.<sup>7</sup> In this pilot study, we explored a relation between GI symptoms, intestinal inflammation (determined

by FC) and faecal SARS-CoV-2-RNA in hospitalised patients with COVID-19 who did not require intensive care measures.

We analysed 40 patients with COVID-19 hospitalised at the University Hospital of Innsbruck, Austria. Confirmation of SARS-CoV-2 infection was performed by nasopharyngeal swab, and faecal SARS-CoV-2-RNA detection was performed using previously described real-time PCR<sup>6</sup> as recommended by the Centers for Disease Control and Prevention (DeKalb, Georgia). Diarrhoea was defined as loose stools >3 times/day. We excluded other causes of acute GI infection by stool analysis for common viral, bacterial, parasitic and protozoan pathogens in all patients with diarrhoea, and no other chronic intestinal disease was documented for any patient. FC concentration was determined by the Calprest ELISA (Eurospital, Trieste, Italy) according to the manufacturer's specification. The lower detection limit of the assay is 16 µg/g. Faecal RNA isolation (for SARS-CoV-2 PCR) was performed using RNeasy PowerMicrobiome Kit (Qiagen, Venlo, The Netherlands) according to the

manufacturer's specifications. Data were expressed as mean ± SEM. Statistical significance, determined by one-way analysis of variance or Spearman's rank correlation in GraphPad Prism, was assumed at  $p < 0.05$ .

The characteristics, frequency of GI symptoms and biochemical parameters of 40 hospitalised patients with COVID-19 are listed in table 1. In this cohort, 18 (45%) patients did not report diarrhoea (group A), while 22 patients (55%) reported diarrhoea. We separated these patients into two groups because in 13 patients diarrhoea had ceased (>48 hours) before we collected the faecal samples (group B), while 9 patients still reported diarrhoea (symptom onset <48 hours) (group C). COVID-19 patients with ceased diarrhoea (group B) and to a larger extent patients with ongoing diarrhoea (group C) displayed elevated FC concentrations when compared with COVID-19 patients without diarrhoea (group A) (figure 1A,  $p < 0.001$ ). FC concentration significantly correlated with serum interleukin-6 (IL-6) concentration (figure 1B,  $p < 0.001$ ), but not C reactive protein (CRP) or ferritin (data not shown). SARS-CoV-2-RNA was detected in stools from 12 of 40 (30%) patients with COVID-19 (figure 1C). Notably, SARS-CoV-2-RNA was not detected in stools from patients with ongoing diarrhoea (group C), but in eight patients with ceased diarrhoea (group B) and in four patients without diarrhoea (group A). In line with this, no relation between faecal SARS-CoV-2-RNA and FC, IL-6, CRP or ferritin was noted (figure 1D; data not shown).

Previous studies indicated that SARS-CoV-2 binds to cells in the GI tract (eg, small and large intestinal epithelial cells), likely via specific receptors such as ACE2 and the transmembrane serine protease 2.<sup>8,9</sup> It is conceived that this virus infects epithelial cells causing cytokine and chemokine release, instigating acute intestinal inflammation characterised by infiltration of neutrophils, macrophages and T cells. We report evidence that SARS-CoV-2 infection in patients with COVID-19 indeed instigates an inflammatory response in the gut, as evidenced by diarrhoea, elevated FC (largely expressed by neutrophil granulocytes<sup>7</sup>) and a systemic IL-6 response. Faecal SARS-CoV-2 RNA was not detected during acute diarrhoea but could be detected in asymptomatic patients with or without previous diarrhoeal symptoms. It is currently unknown if SARS-CoV-2 infection affects the course of patients with IBD and whether

**Table 1** Patient characteristics and biochemical parameters

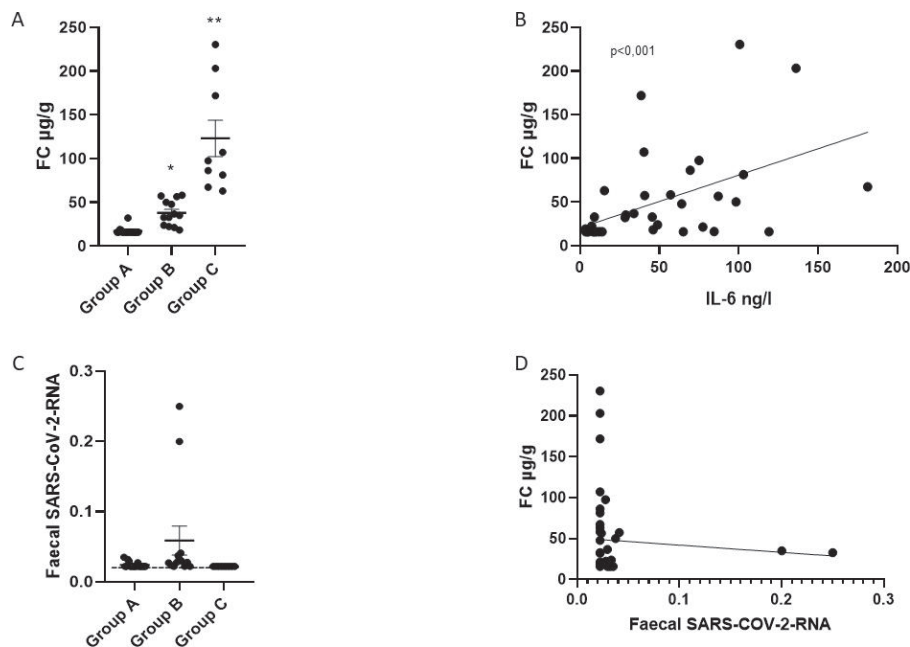
	Group A: COVID-19 patients without diarrhoea (n=18)	Group B: COVID-19 patients with ceased diarrhoea (>48 hours)* (n=13)	Group C: COVID-19 patients with acute diarrhoea (<48 hours) (n=9)
Onset of symptoms before admission to hospital (days)	4 (±1.7)	8.6 (±4.8)	4.3 (±2.8)
Faecal sample after hospital admission (days)	1.7 (±1.4)	4.8 (±3.1)	1.3 (±1.2)
Underlying condition†	50% (n=9)	54% (n=7)	78% (n=7)
Age	58.4 (±17.1)	66.3 (±13.1)	78.3 (±13.8)
Sex (male)	50% (n=9)	69% (n=9)	67% (n=6)
Nausea	11% (n=2)	8% (n=1)	89% (n=8)
Vomiting	6% (n=1)	15% (n=2)	22% (n=2)
Diarrhoea	0% (n=0)	100% (n=13)	100% (n=9)
Fever (≥37.3°C)	89% (n=16)	84% (n=11)	78% (n=7)
COVID-19-associated respiratory syndrome‡ and cough	100% (n=18)	100% (n=13)	100% (n=9)
X-ray (showing infiltrate)	28% (n=5)	31% (n=4)	55% (n=5)
Muscle ache and fatigue	88% (n=16)	54% (n=7)	89% (n=8)
Antibiotic therapy	28% (n=5)	15% (n=2)	33% (n=3)
Antiviral therapy	6% (n=1)	0% (n=0)	0% (n=0)
ACE1 inhibitor therapy	11% (n=2)	8% (n=1)	0% (n=0)
ACE2 inhibitor therapy	6% (n=1)	15% (n=2)	20% (n=2)
Faecal SARS-CoV-2-RNA	22% (n=4)	61.5% (n=8)	0% (n=0)
Calprotectin (µg/g)	17.3 (±4.8)	37.2 (±14.4)	123.2 (±58.8)
Interleukin 6 (ng/L)	25.7 (±21.2)	45.0 (±29.3)	84.3 (±49.1)
LDH (U/L)	266.1 (±129.3)	270.0 (±71.4)	262.8 (±61.7)
CRP (mg/dL)	6.0 (±5.0)	5.4 (±3.7)	5.9 (±5.0)
Leucocytes (10 <sup>9</sup> /L)	5.5 (±1.8)	5.5 (±1.3)	5.1 (±1.8)
Haemoglobin (g/L)	123.3 (±18.4)	121.5 (±18.6)	121.5 (±20.7)
Platelet count (10 <sup>9</sup> /L)	254.5 (±96.3)	257.85 (±81.9)	282.0 (±96.9)
Quick %	97.3 (±9.2)	98.2 (±8.1)	97.2 (±9.5)
Ferritin (µg/L)	736.9 (±568.2)	646.3 (±517.9)	895.4 (±604.6)

\*Patients who reported diarrhoea during COVID-19 but without diarrhoea 48 hours before FC testing.

†Including smoking, allergies, arterial hypertension, type 2 diabetes, malignant diseases, chronic heart disease, chronic liver disease, chronic obstructive respiratory disease and immunosuppressive therapy.

‡Including cough, sputum, haemoptysis, sore throat, nasal obstruction and shortness of breath.

COVID-19, coronavirus disease 2019; CRP, C reactive protein; FC, faecal calprotectin; LDH, lactate dehydrogenase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



**Figure 1** Increased FC and serum IL-6 feature acute diarrhoea in COVID-19. (A) FC concentration determined by ELISA. Group A (n=18): COVID-19 patients without diarrhoea. Group B (n=13): COVID-19 patients who reported diarrhoea which ceased 48 hours before stool collection. Group C (n=9): COVID-19 patients with acute diarrhoea (onset <48 hours) (\*p<0.001, group B versus group A; \*\*p<0.001, group C versus groups B and A). (B) Correlation of serum IL-6 concentration with FC concentration reported in A. (C) Detection of faecal SARS-CoV-2-RNA by PCR, reported as 1/cycle threshold value. Dashed line indicates threshold of RNA detection. (D) Correlation of faecal SARS-CoV-2-RNA (as in C) with FC. COVID-19, coronavirus disease 2019; FC, faecal calprotectin; IL-6, interleukin-6; SARS-CoV-2, severe acute respiratory syndrome corona virus 2.

immunosuppressive treatment affects their susceptibility to (or the course of) COVID-19.<sup>10</sup> Our data support the notion that SARS-CoV-2 infection exerts gut tropism characterised by an acute inflammatory response that potentially deteriorates the course of human IBD.

Maria Effenberger,<sup>1</sup> Felix Grabherr,<sup>1</sup> Lisa Mayr,<sup>1</sup> Julian Schwaerzler,<sup>1</sup> Manfred Nairz,<sup>2</sup> Markus Seifert,<sup>2</sup> Richard Hilbe,<sup>2</sup> Stefanie Seiwald,<sup>2</sup> Sabine Scholl-Buergi,<sup>3</sup> Gernot Fritsche,<sup>2</sup> Rosa Bellmann-Weiler,<sup>2</sup> Günter Weiss,<sup>2</sup> Thomas Müller,<sup>3</sup> Timon Erik Adolph,<sup>1</sup> Herbert Tilg<sup>1</sup>

<sup>1</sup>Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology and Metabolism, Medical University of Innsbruck, Innsbruck, Tirol, Austria

<sup>2</sup>Department of Internal Medicine II, Infectious Diseases, Pneumology, Rheumatology, Medical University of Innsbruck, Innsbruck, Tirol, Austria

<sup>3</sup>Department of Pediatrics, Medical University of Innsbruck, Innsbruck, Tirol, Austria

**Correspondence to** Professor Herbert Tilg, Department of Internal Medicine I, Gastroenterology, Hepatology, Endocrinology & Metabolism, Medical University Innsbruck, Innsbruck 6020, Austria; herbert.tilg@i-med.ac.at

**Contributors** ME, FG, TA and HT wrote the letter. LM, JS, SS, MN, SS-B, RH, MS and TM performed the laboratory analyses. GF, RB-W and GW were involved in patient care and contributed to the preparation of the manuscript.

**Funding** This study is supported by the excellence initiative VAScage (Centre for Promoting Vascular Health in the Ageing Community), an R&D K-Centre (COMET programme - Competence Centers for Excellent Technologies) funded by the Austrian Ministry for Transport, Innovation and Technology, the Austrian Ministry for Digital and Economic Affairs, and the federal states Tyrol, Salzburg and Vienna. TA was supported by the Austrian Science Fund (FWF): FP3070-B.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not required.

**Ethics approval** The study protocol was approved by the institutional ethics commission with an amendment to AN2017-0016 369/4.21, and written informed consent was obtained from all patients.

**Provenance and peer review** Not commissioned; internally peer reviewed.



## OPEN ACCESS

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made

indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

© Author(s) (or their employer(s)) 2020. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.



**To cite** Effenberger M, Grabherr F, Mayr L, et al. *Gut* 2020;**69**:1543–1544.

Received 9 April 2020

Accepted 13 April 2020

Published Online First 20 April 2020

*Gut* 2020;**69**:1543–1544. doi:10.1136/gutjnl-2020-321388

## ORCID iD

Herbert Tilg <http://orcid.org/0000-0002-4235-2579>

## REFERENCES

- Liang W, Feng Z, Rao S, et al. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. *Gut* 2020;**69**:1141–3.
- Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020. doi:10.1001/jama.2020.1585. [Epub ahead of print: 07 Feb 2020].
- Xiao F, Tang M, Zheng X, et al. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* 2020.
- Jin X, Lian J-S, Hu J-H, et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020;**69**:1002–9.
- Lin L, Jiang X, Zhang Z, et al. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020;**69**:997–1001.
- Pan Y, Zhang D, Yang P, et al. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis* 2020;**20**:411–2.
- Magro F, Lopes J, Borralho P, et al. Comparison of different histological indexes in the assessment of UC activity and their accuracy regarding endoscopic outcomes and faecal calprotectin levels. *Gut* 2019;**68**:594–603.
- Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020. doi:10.1016/j.cell.2020.02.052. [Epub ahead of print: 04 Mar 2020].
- Zhang H, Kang Z, Gong H, et al. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut* 2020;**69**:1010–8.
- Mazza S, Sorce A, Peyvandi F, et al. A fatal case of COVID-19 pneumonia occurring in a patient with severe acute ulcerative colitis. *Gut* 2020;**69**:1148–9.