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on behalf of the All Ireland Infectious Diseases (AIID) Study Group

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Research Letter: Faecal calprotectin as a potential biomarker of disease severity in SARS-CoV-2 infection.

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PM and GD contributed to the conception and design of the study. NOM, MT and AGL contributed to the acquisition, analysis, and interpretation of data. NOM drafted the article. JD and RS contributed to critically revising the article. PM and GD contributed to revising the article and gave final approval of the version to be submitted.

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This study has been approved by the Research Ethical Committee of St. Vincent's University Hospital

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Dear Editor,

We read with interest the article by Ijaz et al. regarding the mapping of SARS-CoV-2 IgM and IgG in gingival crevicular fluid (GCF), and its linkage to disease severity in hospitalised COVID-19 patients. The authors report some evidence that higher levels of antibody in GCF in the first 14 days post symptom onset were associated with severe disease and poor outcome (1). The utility of less invasive, non-venous analytes has been increasingly recognised during the global pandemic. These may be used for both disease detection and prediction of severity. The identification of biomarkers of disease severity in COVID-19 would be of considerable clinical value, particularly with the current limited availability of novel antiviral therapy, which are known to exert a maximal efficacy when given early in the disease course.

While predominantly recognised as an infection of the respiratory tract, gastrointestinal symptoms are frequently reported, with the incidence of diarrhoea and abdominal pain ranging from 2 to 49% (2). Both the SARS-CoV-2 receptor, angiotensin-converting enzyme 2 (ACE-2), as well as the SARS-CoV-2 nucleocapsid protein have been detected in gastrointestinal epithelial cells (enterocytes), suggesting a role for direct viral infection resulting in cytokine release and neutrophil activation (3). Furthermore, SARS-CoV-2 RNA has been detected in stool samples and colonic biopsies, implicating the gut as a site of viral replication (4).

The hypothesised effects of SARS-CoV-2 infection on enterocytes include a direct viral insult resulting in gastrointestinal inflammation and disruption to intestinal barrier function, with subsequent release and up regulation of inflammatory cytokines causing an exaggerated inflammatory response (5). Indeed, alterations in intestinal barrier function, supporting this hypothesis, have been demonstrated in patients with COVID-19 (6). Whether inflammation and disruption of the intestinal barrier by COVID-19 results in upregulation of the host immune response and a resultant increased severity of disease remains unknown.

Faecal calprotectin (FCP), a measure of neutrophil activation, is a recognised correlate of intestinal injury which rises in response to both acute and chronic inflammatory conditions. This non-invasive measurement of intestinal inflammation is frequently employed in the assessment of inflammatory bowel conditions. Elevated levels of FCP have been demonstrated in patients with symptomatic COVID-19, with the presence of both acute and resolved diarrhoea (7), and in the absence of gastrointestinal symptoms (8), regardless of whether SARS-CoV-2 RNA is detected in stool samples. Elevated levels of serum calprotectin (SCP) have previously been demonstrated in patients with severe complications of SARS-CoV-2 infection (9), however in a large ambulatory cohort of individuals with COVID-19, SCP was not predictive of developing severe disease (10).

We performed an analysis within a prospective observational study to assess the presence and significance of intestinal inflammation in a cohort of individuals with mild-moderate, PCR-confirmed SARS-CoV-2 infection (classified as per the World Health Organisation classification of severity), with and without gastrointestinal symptoms. We compared these findings with an age and sex-matched control group with a respiratory infection but who were confirmed SARS-CoV-2 PCR negative. We sought to determine whether intestinal inflammation was more prevalent in those with COVID-19, and what association, if any, this had with disease severity and clinical outcome.

Patients admitted to hospital due to complications of COVID-19 during the first wave of the SARS-CoV-2 pandemic (March–November 2020) were recruited to a prospective, multicentre cohort study (the All-Ireland Infectious Disease Cohort (AIID) Study). Patients with mild to moderate disease severity as per WHO severity index (WHO 1-2) were included and asked to provide a stool sample for analysis. Demographic and biochemical data were collected, as well as details of clinical course and use of steroid and antibiotic therapy. Systemic serum markers of inflammation (C-reactive protein, ferritin, platelets) were recorded. Requirement for non-invasive ventilation, escalation to intensive care and death were recorded and combined as a composite outcome of severity. Length of stay was also recorded as a surrogate marker of severity. Stool samples were tested for the presence of faecal

calprotectin using a qualitative immunochromatographic assay (output as elevated or negative) and a quantitative enzyme-linked immunosorbent assay (ELISA) (reported in ug/g). Use of proton-pump inhibitors (PPI), known to cause an elevation in FCP, was recorded as a potential confounder.

During the study period, we collected stool samples from 37 subjects, of whom 22 (59%) had SARS-CoV-2 detected on PCR from nasopharyngeal swab, with 15 (41%) SARS-CoV-2 negative controls. The median ages (62 vs. 67, p=0.5), gender (female 50% vs. 53%, 0.5) and median BMI (25.1 vs. 26.3 kg/m^2) were similar between the two groups. While GI symptoms were more common in the COVID-19 group (32% vs 0%, p=<0.05), there was no significant difference in qualitative FCP (45% vs. 40%, p=0.5) with similar median quantitative FCP (73ug/g vs. 95ug/g, p=0.7). There was also no between-group difference in PPI use (23% vs. 25%, p=0.5), co-morbidities (92% vs. 89%), LOS (16.5 days vs. 6, p=0.4) or markers of systemic inflammation (data not shown).

Within the COVID-19 group, a positive FCP qualitative assay (n=13) corresponded to a median FCP of 119ug/g (IQR 84-532). Interestingly, an elevated FCP was associated with a statistically significantly higher ferritin (638ug/L vs. 240ug/L, p=0.05) and a longer hospital length of stay (54 vs. 9 days, p=0.02). Although an elevated FCP was not linked to reported GI symptoms (16% in those with elevated FCP vs. 33% in those with a negative FCP, p=0.4), need for supplemental oxygen (median Fi02 28%) or need for steroid therapy (dexamethasone 6mg), there was a trend towards a more severe clinical disease course for those with evidence of intestinal inflammation. In the group with elevated FCP, 2 were referred to intensive care, both of whom died. All patients with a negative FCP were discharged home and did not reach the composite outcome of severity.

Our study suggests that while GI symptoms are commonly reported in patients with SARS-CoV-2 infection, these correlate poorly with non-invasive markers of intestinal inflammation. Faecal calprotectin, in our study, was not a sensitive marker of SARS-CoV-2 related intestinal injury, with similar levels detected in both COVID-19 subjects and control groups. Nonetheless, elevated FCP levels in those with mild to moderate SARS-CoV-2 did demonstrate an association with elevated

systemic inflammation as measured by serum ferritin levels, a significantly longer length of hospital stay, and a more severe clinical course. This suggests its utility may lie as a biomarker of disease severity in those with confirmed SARS-CoV-2 infection. Further larger prospective cohort studies are required to confirm this association.

The All Ireland Infectious Diseases (AIID) Study Group members:

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COVID +ve (n=22)	FCP +ve (n=13)	FCP –ve (n=9)	P value
Age (median) [IQR]	70 [63 – 82]	54 [48 – 56]	0.17
Gender (female)	38% (n=5)	67% (n=6)	0.19
BMI (kg/m2)	24	28	0.18
Co-Morbidities - Hypertension - GI Disease	92% (n=12) 62% (n=8) 0%	89% (n=8) 33% (n=3) 44% (n=4)	
Respiratory Symptoms	62% (n=8)	66% (n=6)	0.58
Viral Symptoms	33% (n=5)	89% (n=8)	0.25
Nausea	0	0	
Abdominal Pain	13% (n=2)	0	
Diarrhoea	13% (n=2)	33% (n=3)	
Time to stool sample (days) [IQR]	11 [4 – 35]	7 [3 – 19]	
Faecal Calprotectin Quantitative (+ve) (median) [IQR]	119 [84 – 532]	26 [12 – 29]	<0.01
CRP (median)	15	6	0.12
Ferritin	638	240	0.053
Leukocytes	5.95	5.3	0.95
PPI use	20% (n=3)	22% (n=2)	0.68
Length of Stay (days) [IQR]	54 [13 – 71]	9 [3 – 11]	*0.017*

Table 1. Demographics, Patient Characteristics, Biochemical Data of patients with COVID-19

COVID +ve (n=22)	FCP +ve (n=13)	FCP –ve (n=9)	P value
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