Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Correspondence

COVID-19 and intestinal inflammation: Role of fecal calprotectin ☆

Dear Editor,

At the end of January 2020, the World Health Organization (WHO) declared the SARS-Cov-2 (or Coronavirus 19 [COVID-19]) infection a global health emergency [1]. The virus, belonging to the β-Coronaviruses, spreads mainly through the respiratory tract from person to person, using the transmembrane serine protease 2 (TMPRSS2) [2] for S spike-protein priming and the angiotensin-converting-enzyme 2 (ACE2) receptors, receptors sited not only in the pulmonary epithelium but also in others districts such as in the renal and gastrointestinal ones [3]. SARS-Cov-2 infection typically occurs with fever, cough and interstitial pneumonia to more severe pictures of respiratory failure and ARDS [4]. Increasingly, patients experience nausea and vomiting, abdominal pain, anorexia and diarrhea [5] in addition to respiratory symptoms or, in some cases, patients are asymptomatic [1].

Several studies suggest that the virus actively infects the cells of the gastrointestinal district [6], replicating itself in the epithelium of the small and large intestine [5,7] and producing an excessive immunological reaction in the host [8], with the consequent production of many cytokines [interleukin 6 (IL-6) 11, Tumor Necrosis Factor (TNF-alpha) and interferon (INF) -alpha] by activated leukocytes [8,9]. The latter, especially neutrophils, also produce calprotectin [10], a calcium and zinc binding protein of the S-100 family of proteins, widely studied in inflammatory bowel disease, which is a useful tool to identify the damage of the intestinal mucosa. Calprotectin is indeed involved in many cellular physiological functions including cellular differentiation, migration, adhesion, and phagocytosis of neutrophils and is considered a positive acute phase protein. Therefore, the presence of calprotectin in the stool is a consequence of the migration of neutrophils into the gastrointestinal tissue due to an infection or an inflammatory process [3,10].

We conducted an observational study in the Emergency Department (ED) of the A. Gemelli University Hospital Foundation. The primary endpoint of our study was to evaluate the degree of intestinal inflammation by measuring the fecal calprotectin in symptomatic patients with a positive swab for COVID-19 and a radiological imaging of interstitial pneumonia compared to asymptomatic patients with a positive swab for COVID-19 and without evidence of interstitial pneumonia. The secondary endpoint was to identify the potential link between the level of fecal calprotectin and the severity of pulmonary manifestations from COVID-19 (including respiratory failure).

Each patient performed a chest x-ray or a chest computed tomography (CT) scan and collected a stool sample analyzed for fecal calprotectin in the Clinical Chemistry laboratory of the hospital.

The stool samples were extracted using the DiaSorin LIAISON Q.S.E.T. stool extraction device; measurements were subsequently performed using Liaison Calprotectin assay on a Liaison XL analyzer. The DiaSorin LIAISON Q.S.E.T. calprotectin assay is a sandwich chemiluminescent immunoassay (CLIA) that uses two monoclonal antibodies for capture and detection of calprotectin. Calprotectin was first extracted from stool samples The assay incubates the extracted sample, with the assay buffer and the paramagnetic particles coated with a monoclonal antibody that specifically recognizes the calprotectin heterocomplex. Following incubation, a wash cycle is performed to remove any unbound material. An isoluminol conjugated monoclonal antibody that recognizes calprotectin is then added to the reaction and incubated. The unbound conjugate is removed with a second wash step. Starter reagents are then added, and a flash chemiluminescent reaction started. The light signal is measured by a photomultiplier as relative light units (RLU) and is proportional to the concentration of calprotectin present in the samples. The concentration was expressed in µg/g. The normal value was considered ≤ 50 µg/g. We excluded patients with age < 18 years; pregnant women; patients receiving hydroxychloroquine; terminal oncological diseases; patients with heart disease, severe nephropathy and active inflammatory bowel diseases; patients with a history of pulmonary fibrosis, advanced interstitial disease, severe COPD (GOLD 3–4; group C and D); patients on antibiotic therapy or who have taken it in the last month.

We compared study variables between patients with normal calprotectin values (≤ 50 µg/g) and patients with elevated calprotectin values. We expressed non parametric variables as absolute numbers (%) and compared by Chi^2 test (with Fisher's test as appropriate); continuous variables are expressed as median [interquartile range] and compared by Mann–Whitney U test while parameters with significant association to an elevated calprotectin values were entered into a logistic regression model in order to individuate independent predictors associated to elevated calprotectin. We considered a p value ≤ 0.05 as statistically significant. We analyzed data by SPSS v25® (IBM, IL, USA).

We enrolled a total of 65 consecutive patients (15 women and 50 men) with an age of 38 years old (34–55). They were admitted with a positive swab for COVID-19 to our ED from the 1st to 30th April 2020. The demographic, clinical features and lab tests of the patients are summarized in Table 1.

We found elevated fecal calprotectin values (> 50 µg/g) in 19 patients (29.2%). Among these 11/19 (57.9%) had a pathologic chest X-ray/CT scan, compared to 5/46 (10.9%) in the group with normal fecal calprotectin level (≤ 50 µg/g) $p < 0.001$. The median

☆ The members of the **GEMELLI AGAINST COVID-19 group** can be found in the Appendix.

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Table 1
Demographic and clinical features of the enrolled patients. Values are expressed as absolute number (%). Continuous variables are expressed as median [interquartile range]. Continuous variables are compared by Mann–Whitney U test; Absolute numbers are compared by Chi2 test (with Fisher's test as appropriate). Multivariate analysis is performed by logistic regression (model Log likelihood-2 = 40.01).

	All Patients n° 65	Normal Calprotectin level (≤ 50 µg/g) n° 46	Elevated Calprotectin level (> 50 µg/g) n° 19	Univariate analysis (p value)	Multivariate analysis (*p* value)
Age (Years)	38 [34–55]	36 [33–44]	56 [36–73]	0.024	0.520
Sex (Male)	50 (76.9%)	40 (87.0%)	10 (52.6%)	0.003	0.065
Pathologic chest X-Ray/CT scan	16 (24.6%)	5 (10.9%)	11(57.9%)	< 0.001	0.028
*Gastrointestinal symptoms	16 (24.6%)	7 (15.2%)	9 (47.4%)	0.006	0.241
White-cell count (× 10^9/L)	5.8 [4.7–9.1]	5.8 [5.2–8.9]	5.8 [3.2–10.8]	0.733	/
Lymphocyte count (× 10^9/L)	1.6 [0.6–2.0]	0.9 [0.6–2.1]	1.6 [0.6–2.3]	0.898	/
C-reactive protein (mg/L)	22.7 [7.0–62.8]	21.3 [5.9–31.2]	32.5 [5.5–154.9]	0.492	/
Procalcitonin	0.09 [0.05–0.75]	0.06 [0.05–0.75]	0.50 [0.05–2.3]	0.171	/
D-Dimer	413 [301–2829]	391 [248–606]	917 [301–2990]	0.073	/
Ferritin	320 [48.5–700]	314 [61–384]	500 [310–685]	0.116	/

* Include: abdominal pain, diarrhea, vomiting, bloating, jaundice.

calprotectin value was 71.3 µg/g [Interquartile range18.8–248.0] in patients with a chest X-ray/CT scan of interstitial pneumonia (16/65), compatible with an acute intestinal inflammation, compared to 11.9 µg/g [5.8–32.0], in patients with a normal chest X-ray/CT scan ($p < 0.001$)

Patients with normal calprotectin (≤ 50 µg/g) were younger than patients with high calprotectin level (36 years old [33.0–44.0] vs. 56.0 years old [36–73] p 0.024). Furthermore, most of them were male 40/46 (87%) vs. only 10/19 (52.6%) with an elevated calprotectin level (> 50 µg/g).

Finally, we found that in patients with elevated calprotectin levels, gastrointestinal symptoms were more frequent (9/19 (47.4%) vs. 7/46 (15.2%); $p = 0.006$).

No differences were observed as regards other laboratory tests (Table 1).

When these factors were entered into a multivariate logistic regression model, we found that an elevated calprotectin level was independently associated with a pathological chest X ray (OR 11.2 [CI95% 1.29–28.2], p 0.028.). Conversely, age, gender, and gastrointestinal symptoms were not independently associated to increase calprotectin (Age OR 1.01 [0.97–1.07], p 0.520; Sex OR 5.05 [0.90–28.2], p 0.065; symptoms OR 3.18 [0.46–22.0], $p = 0.241$, respectively).

This study presents very interesting data regarding the significant correlation between Covid-19 pneumonia and high level of fecal calprotectin (expression of gastrointestinal involvement) in patients with Covid-19 infection. The presence of pneumonia is expression of disease's severity. Patients with Covid-19 infection, may experience both lung involvement than systemic impairment, including gastrointestinal one (documented by an increase of fecal calprotectin) not necessarily associated with gastrointestinal symptoms. In fact, in the population we studied, the gastrointestinal involvement (with high level of fecal calprotectin) occurred also in asymptomatic patients. Moreover, we interestingly found that women had higher calprotectin level compared to men. This is a very important result because Covid-19 infection has usually a more severe presentation in males compared to females. So, this let us think that a male with high calprotectin level could have an even worse prognosis than estimated. Our work provides new insights in understanding the association between lung involvement and gastrointestinal one by SARS-Cov-2. In our idea, the digestive system could be a potential route for Covid-19 infections and the monitoring of intestinal markers of inflammation as calprotectin could help physician know the degree of SARS-Cov-2 infection, the potential progression of it, and the possibility of Covid-19 transmission also by asymptomatic patients. Moreover, new studies are needed to explore this field and to find an easy and simple to collect- marker useful for the follow-up of patients who have had Sars-Cov-2 infection.

Conflict of interest

All the authors declare no conflicts of interest and no grant support.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2020.09.015.

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