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GUT MICROBIOTA' EFFECTS IN COVID-19 PATIENTS ACCESSING THE EMERGENCY DEPARTMENT: A 2022-UPDATE OF GASTROINTESTINAL MANIFESTATIONS

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Abstract – *Objective:* What we learned from two years of pandemic is that COVID-19 disease can appear with a wide spectrum of clinical symptoms ranging from the "typical" respiratory symptoms, such as fever, cough, interstitial pneumonia, respiratory distress/ARDS, to "less-typical" ones including abdominal pain, diarrhea, nausea/vomiting, anorexia. This turns the spotlight on the role of microbiota gut and gut barrier in preventing or predisposing to Sars-Cov-2 infection, its complications and outcomes. Although many studies have been conducted, many questions remain still opened today.
Material and Methods: We performed literature research on PubMed®, Up-To-Date®, Web of Science®, and collected metanalysis, systematic reviews, clinical trials, case control studies, retrospective and prospective articles published in the last two years.
Results: Our review article suggests that changes in the gut microbiota composition with an altered gut permeability ("leaky gut") may lead to a systemic involvement with more severe COVID-19 manifestations, need of intensive care and may be associated with worse outcomes.
Conclusions: The use of strategies and treatments (as probiotics), to prevent and keep the gut microbiota balanced in the Emergency Department may improve both the symptoms of patients, multiorgan complications and the length of hospitalization of COVID-19 patients.

Keywords: COVID-19, Sars-Cov-2, Gut microbiota, Abdominal pain, Diarrhea, ACE2, Probiotics.

INTRODUCTION

The coronavirus Sars-Cov-2 is responsible of coronavirus disease 2019 (COVID-19). The virus is internalized in the host cell through the binding of S protein to ACE-2 receptors facilitated by TMPRSS2 (transmembrane protease, serine 2)[1-5]. Then, the RNA-genome of the virus is replicated and translated, generating two proteins PP1A and PP1AB with various active functions. Moreover, the viral genome encodes for spike proteins, nucleocapsid, membrane and envelope. After the production of all the Sars-Cov-2 components, coronavirus is assembled and released outside the host cell. ACE-2 receptors have been found initially in lung tissue, but further studies have underlined their ubiquitous presence, also in the gastrointestinal (GI) tract. About that, some authors emphasized the relationship between lung and gut (known as lung-gut-axis)[6]. In fact, gut microbiota has a fundamental role in regulating the innate

immunity[7-9] response against the viruses in the respiratory tract. It influences the activities of alveolar macrophages, epithelial lung cells, dendritic respiratory cells and modulating both cellular and humoral adaptive immunity. Moreover, gut microbiota exerts various effects directly on the lung mucosa, controlling the viral replication in the lung cells. So, starting from these considerations, it is easier to understand that COVID-19 patients may present GI symptoms due to a missed regulation by gut microbiota of the lung immune system against Sars-Cov-2 infection or a direct viral damage of intestinal cells through ACE2-receptors6. In fact, Sars-Cov-2 has been detected in the stool of some patients, also for a period longer than 4 weeks, suggesting that stool may be an additional diagnostic font. On the faecal sample, it is possible to perform PCR test to detect COVID-19 infection also in case of negative upper respiratory results of PCR. Literature studies have underlined that the inclusion of stool PCR, together with nose-pharyngeal swab, may improve clinical sensitivity. Despite this, it is important to remember that some COVID-19 patients have not Sars-Cov-2 RNA in stool. Therefore, until now, the research of virus in the stool as diagnostic means should be limited to selected cases, and utilized only after having results of upper respiratory Sars-Cov-2 PCR. In 202010, an interesting study on faecal samples of 65 COVID-19 patients (15 women and 50 men) was conducted. It was found that in the group of patients with elevated calprotectin levels, GI symptoms were more frequent [9/19 (47.4%) *vs.* 7/46 (15.2%); $p = 0.006$] and these patients 11/19 (57.9%) showed pneumonia to chest X-ray or CT scan compared to 5/46 (10.9%) patients with a normal fecal calprotectin level (≤ 50 μg/g) $p < 0.001$. This study has led to understand that digestive system is a potential route for COVID-19 infections. Moreover, the monitoring of intestinal markers of inflammation as calprotectin could help to know the degree of Sars-Cov-2 infection, and the possibility of COVID-19 transmission by asymptomatic patients.

MATERIALS AND METHODS

We performed literature research on PubMed®, Up-To-Date®, Web of Science®, and collected meta-analysis, systematic reviews, clinical trials, case control studies, retrospective and prospective articles published in the last two years. We searched "COVID-19" OR "Sars-Cov-2" AND "Gut Microbiota", AND/OR "Gut-Lung axis" AND/OR "GI symptoms"; moreover, we included "COVID-19" AND "Gastrointestinal injury" AND/OR "Sars-Cov-2 pneumonia", AND/OR "microbiota gut-dysbiosis" AND/OR "leaky gut". The main limit of our research is based on the heterogeneity of studied included, on the different population studied and on the various setting of management of COVID-19 patients (outpatients, Emergency Department, Intensive Care Unit *etc.*).

RESULTS

Studies Reported GI Symptoms in COVID-19 Patients

At the beginning of pandemic, some literature studies started reporting the presence of GI symptoms in COVID-19 patients (Table I) in addition or without respiratory symptoms. For example, Lin et al[11] investigated 95 Sars-Cov-2 infected patients reporting 58/95 cases (61.1%) with GI symptoms with diarrhea (24.2%), nausea (17.9%), vomiting (4.2%) and alteration in liver function with elevated transaminases (32.6%). Jin et al[12] examined 74 patients infected with Sars-Cov-2 with GI symptoms, such as diarrhea, nausea and vomiting. They showed that about 28% of them did not suffer from respiratory symptoms. Xiao et al[13] investigated 73 Sars-Cov-2 infected hospitalized patients: >20% of infected patients had positive virus in faeces (not clear correlation with GI symptoms). Ong et al[14-15] reported that 50% of their COVID-19 patients had detectable faecal virus but only approximately half of these patients suffered from symptoms, such as diarrhea. After these initial studies, GI manifestations, such as nausea, vomiting, diarrhea, and abdominal pain, have been added to the list of common COVID-19 symptoms (Figure 1). Others as Ramachandran et al[16] observed in nineteen COVID-19 patients anorexia, nausea, vomiting, diarrhea, and abdominal pain. Perisetti et al[17] summed up the typical and atypical GI symptoms, reporting loss of appetite, anorexia, nausea

TABLE 1. 2020-STUDIES REPORTING GASTROINTESTINAL SYMPTOMS IN COVID-19 PATIENTS.

Covid-19 2020-Studies	GI symptoms reported
Lin et al[11]	58 patients: diarrhea (24.2%), nausea (17.9%), vomiting (4.2%) and alteration in liver function with elevated transaminases (32.6%).
Jin et al[12]	74 patients: diarrhea, nausea and vomiting.
Xiao et al[13]	73 patients: >20% of infected patients had positive virus in faeces (no clear correlation with GI symptoms).
Ong et al[14]	50% of COVID-19 patients had detectable faecal virus and diarrhea.
Ramachandran et al[16]	19 patients: anorexia, nausea, vomiting, diarrhea, and abdominal pain.
Perisetti et al[17]	GI symptoms: loss of appetite (anorexia), nausea and vomiting, diarrhea, abdominal pain vs altered taste (dysgeusia), *Clostridium Difficile* infection, GI bleeding.
Mao et al[18]	6064 patients: overall prevalence of 15% of GI symptoms: nausea/vomiting, diarrhea, and anorexia.

and vomiting, diarrhea, abdominal pain as typical, and altered taste (dysgeusia), *Clostridium Difficile* infection, GI bleeding of COVID-19 patients as atypical. In a meta-analysis[18] of about 60 studies for a total of 4243 patients, most of them Chinese, the prevalence of all GI symptoms was 17.6%. Anorexia was most common (26.8%), then diarrhea (12.5%), vomiting/nausea (10.2%), and finally, abdominal pain (about 9.2%). Another meta-analysis[19] comprising 18,000 patients, from different countries around the world, found diarrhea in 11.5%, nausea and vomiting in 6.3%, abdominal pain in 2.3%. Diarrhea was found also in 1/3 of COVID-19 patients who were managed in intensive care unit and nausea and vomiting in 1/5 of them. Mao et al[20] reported in 29 studies (of about 6064 COVID-19 patients) GI symptoms. The overall prevalence was 15%, the most frequent included nausea, vomiting, diarrhea and anorexia. The authors reported that around 10% of patients presented GI symptoms without respiratory manifestations. Literature data reported not only GI symptom but also abnormalities

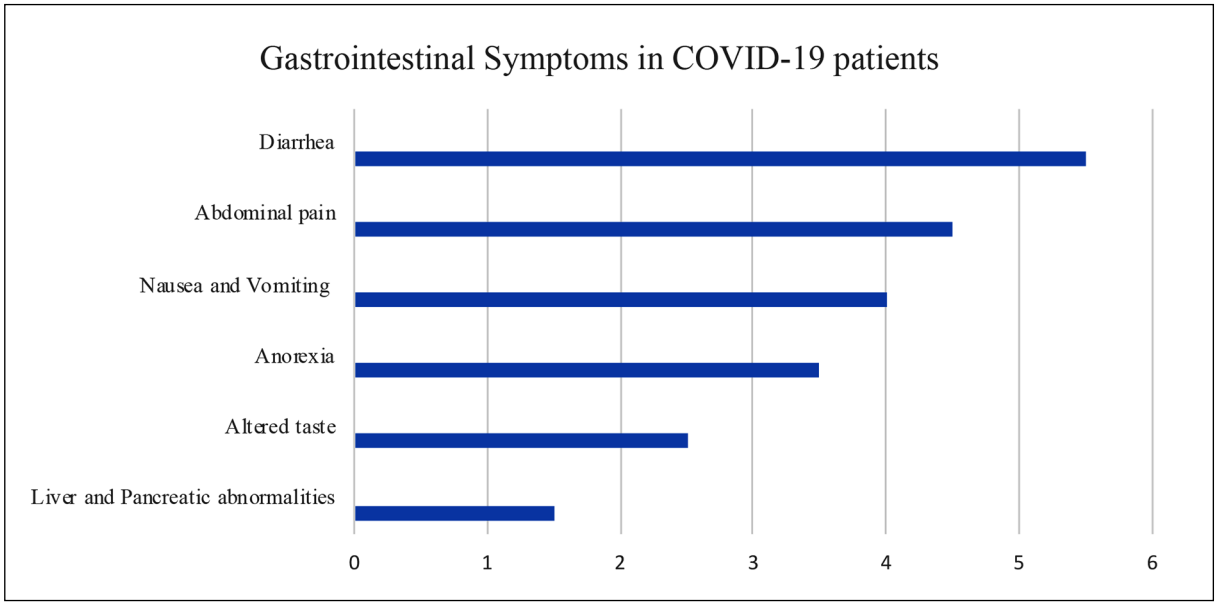

Figure 1. Main gastrointestinal symptoms in COVID-19 patients.

in liver biochemistries[3,21-22] in approximately 15-65% of Sars-Cov-2 infected individuals, even if the precise influence of COVID-19 on the liver remains unclear. Liver biochemistry abnormalities in COVID-19 patients are generally characterized by mild elevations of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (1-2 times the upper limit of normal) as reported in about 29-39% and 38-63% of patients, respectively. Hypoalbuminemia has also been documented in COVID-19 patients, representing a non-specific marker of illness severity but associated with worse outcomes. In addition, literature studies[22,23] reported elevation in serum bilirubin level and liver synthetic dysfunction. Interestingly, alteration in liver biochemistries is reported with the same frequency regardless of the presence of pre-existing liver disease[22,23]. As regards pancreas, the association between COVID-19 and pancreatic diseases as acute pancreatitis has not found literature evidence[24,25]. The available data are not easy to interpret and many questions remain opened. More studies are needed to better understand any relationship and mechanism between COVID-19 and pancreatitis, but for now their association has not been proven yet. Different pathogenic mechanisms have been described to explain GI injuries and GI symptoms in COVID-19 patients. First, the virus can directly mediate cytotoxic damages in the gut epithelium. Secondly, several researchers[21,26-28] reported a dysregulation of the renin-aldosterone-angiotensin system (RAAS) in the intestinal epithelium of COVID-19 patients for an imbalance in ACE/ACE2 receptors levels. In addition, the malabsorption of tryptophan in the gut has been recognized among the mechanisms of GI injuries[17,22,29-30] in case of Sars-Cov-2 infection; or the endothelial damages and thrombo-inflammation of vessels. Finally, other mechanisms identified have been the dysregulation of the immune system[8,31] (modulated by gut microbiota), the gut dysbiosis with intestinal inflammation and/or hypoxic events that are critical for intestinal homeostasis.

Probiotics for Gut Homeostasis in COVID-19 Patients

The treatment for Sars-Cov-2 infection has evolved in the last two years[32]. To date, recommendation included oxygen therapy, non-invasive (NIV) and invasive mechanical ventilation (IMV) in cases of severe respiratory distress and anti-viral drug as Remdesivir, an RNA-dependent RNA-polymerase inhibitor of Sars-Cov-2. However, with the increased mutation rate of Sars-Cov-2, the susceptibility to specific drugs frequently changes and sometimes there is a failure of this antiviral therapy. Despite prevention, that is the best measure to adopt, some literature data focused the attention on "immunity-boosting foods" for example multi-vitamins, fruits, prebiotics, antioxidants, and probiotics that are considered to have beneficial health properties. In particular, probiotics are generally considered safe and able to ameliorate the gut health and the immune system of the host. Some researchers have revealed that some probiotic strains and their metabolites (known as bacteriocins) have potential anti-viral agents and could speed up the healing in COVID-19 patients. So, probiotics[33,34] seem to be promising nutraceutical agents for prevention and possible treat of Sars-Cov-2 infection, but more studies are needed to explore this field.

Implications for Public Health

The presence of GI symptoms in COVID-19 patients has implications for public health[3,35-37].

The characteristics of GI symptoms are more insidious than the respiratory ones, and sometimes these are overlooked. Moreover, some COVID-19 patients might have only GI symptoms during the whole course of this disease, and some continue to eliminate the virus in stool, despite a respiratory negative swab. Further investigations are necessary to determine whether these patients represent a potentially means of transmission of the infection.

DISCUSSION

GI symptoms may be the initial presentation of Sars-Cov-2 infection in patients admitted to the Emergency Department with diarrhea, abdominal pain, nausea, vomiting, anorexia[4]. Re-

spiratory manifestations may not be present or can appear later[5]. Many studies have showed that the presence of GI symptoms can correlate with the disease' prognosis. These patients may often have a high risk of pneumonia, need of oxygen support, mechanical ventilation and/or ICU admission. As described in literature studies, ACE2 receptors, that allow the entry of the virus, are distributed on intestinal epithelial cells[1,13]. In the context of viral infection, the gut microbiota plays a crucial role in regulating the immune response of the host, in mediating the inflammatory response against the pathogen and maintain the "barrier" and "homeostasis" of the gut[28]. The increase of gut permeability secondary to viral infection and the alteration of the gut balanced-composition due to Sars-Cov-2 entry, are responsible of hyperinflammation, bacterial translocation, with increase of systemic dysfunction and worsening of lung infection (gut-lung communication)[7]. Literature data underline that the administration of probiotics strains as *Lactobacillus acidophilus, L. reuteri, L. rhamnosus. L. plantarum, or Streptococcus thermophilus, Bifidobacterium breve, B. lactis B. longum* etc…) can improve the composition and function of gut microbiota with reduction of gut inflammation[33,34]. Probiotics are able to strength gut barrier, restore a balanced intestinal environment, and also improve local and systemic immune system[34]. Trials about the effects of these probiotics' strains on lung inflammation, faecal-oral transmission and disease progression in patients with COVID-19 disease are ongoing, also in the context of ED.

CONCLUSIONS

Our review article suggests that changes in the gut microbiota composition with an altered gut permeability ("leaky gut") may lead to systemic involvement with severe COVID-19 manifestations, need of intensive care and worse outcomes. The use of strategies and treatments (as probiotics) to prevent and keep the gut microbiota balanced starting from the Emergency Department may improve the symptoms of patients, multiorgan complications and the length of hospitalization of COVID-19 patients.

Conflict of Interest

The author declares no conflict of interest.

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