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COVID-19 AND FECAL MICROBIOTA TRANSPLANTATION: LIMITATIONS AND POTENTIALITIES ARE TWO SIDES OF THE SAME COIN

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Abstract – ***Objectives:*** The global scientific community is struggling to understand the features of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and its related disease (coronavirus disease-2019, COVID-19). Even if the main symptoms of COVID-19 are respiratory, gastrointestinal manifestations have been described too. The disease shows a highly variable clinical course, from asymptomatic cases to lethal ones. Scientists are questioning the reasons that may explain these different disease courses. It is reasonable to speculate that the intestinal and pulmonary microbiota, and their mutual interaction in the "gut-lung axis", might influence the severity of COVID-19. In this context, the modification of the microbiota through fecal microbiota transplantation (FMT) might constitute a rescue therapy. Unfortunately, no randomized clinical trial has been conducted to prove the efficacy of FMT in COVID-19 patients and its role is only potential; to date, FMT is only recommended for recurrent infections of *Clostridium difficile*. Since the risk of transmission of SARS-CoV-2 with cells, tissues, and organs has not been demonstrated but cannot be excluded, many authorities have proposed stricter screening measures for donors. FMT is a potential means of SARS-CoV-2 transmission and the spread of the virus is creating concern among FMT experts worldwide. This review explores the "two sides of a coin", analyzing both the potential applications of FMT during COVID-19, also by revising the role of the microbiota during the infection and by discussing the limitations to FMT that have emerged during the pandemic and the proposal of the scientific community to overcome them.

Keywords: SARS-CoV-2, COVID-19, Gut-lung axis, Microbiota, Fecal microbiota transplantation.

INTRODUCTION

Soon after its outbreak in December 2019 in Wuhan (China), the novel coronavirus called "Severe Acute Respiratory Syndrome Coronavirus 2" (SARS-CoV-2) has spread all over the world, resulting in over 10 million cases and more than 500 thousand deaths[1].

SARS-CoV-2 Shows Intestinal Tropism

COVID-19 mainly displays respiratory symptoms like fever, dry cough, fatigue. In more severe cases it can also cause respiratory distress resembling an acute distress respiratory syndrome (ARDS)[2]. The main transmission route is the respiratory one, through the emission of droplets; in fact, the SARS-CoV-2 virus is usually detected in the respiratory secretions[3,4]. For this reason, the diagnosis relies on the RT-PCR (Real-Time Protein Chain Reaction) on nasopharyngeal swabs or bronchoalveolar lavage (BAL)[5]. 5-10% of patients present with enteric manifestations like nausea, vomiting, and diarrhea[6]. Some authors have demonstrated that SARS-CoV-19 RNA can be detected in the stool[3,7,8] or saliva[9] of some patients. SARS-CoV-2 pulmonary tropism is due to the binding to the angiotensin-converting enzyme-2 (ACE2) on the cellular membrane of pneumocytes[3,10]. Interestingly, this enzyme is expressed on the enterocytes cells too, where it plays an important role in the microbial ecology and regulation of the inflammation[11-14]. These observations suggest that the gastrointestinal tract may be an extra-pulmonary site of viral replication and activity[13,15].

Gut and Lung Microbiota in Health and Disease

The gut microbiota consists of almost 10^{14} resident microorganisms, including bacteria, archaea, viruses, and fungi[16]. Among bacteria, the main phyla are *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*[17]. These microorganisms play an important role in homeostasis because, while getting their habitat and nourishment from the host, they participate in dietary digestion and protect the host from pathogens in many ways[3]. First, they occupy a microbial niche, compete for nutrients, and produce antimicrobial peptides[18]. Also, gut microbiota-derived signals modulate the immune cell function in a pro- or anti-inflammatory fashion, promoting a balanced immune response and consequently affecting the susceptibility to several diseases and their clinical course[19]. A hyper-reactive or a hypo-reactive immune response can equally enhance the disease progression and predispose to complications[3].

While bacteria forming the gut microbiome have been widely studied, less is known about the viruses forming the "virome". The virome is composed of bacteriophages as well as eukaryotic viruses[20]. There is evidence that the virome significantly differs among healthy patients and it may play a role in some illnesses both by interacting with some risk genes (e.g., IL-10 in inflammatory bowel diseases) and by changing the bacterial microbiome through predator-prey relationships[21].

The growing interest in the study of the human microbiota has led to the evidence that many organs, that were once supposed to be sterile, also host their resident flora. Besides, microorganisms residing in different body districts are not compartmentalized but crosstalk thanks to the release of endotoxins and metabolites that can reach other microbial populations through the bloodstream[3].

New, culture-independent techniques have allowed scientists to find that, like the gut and many other organs that are in continuous communication with the external environment, also the airways and the lung – once considered sterile – host their resident flora, mainly consisting of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*[22].

Lung microbiota comes mainly from the oral cavity through inhalation and microaspiration. Indeed, the microbiota of healthy lungs overlaps with that of the mouth. The prominent genera in BAL samples of healthy patients are *Streptococcus* and *Veillonella* – belonging to the *Firmicutes* – and *Prevotella*, a member of *Bacteroidetes*[23].

Healthy lung microbiota promotes pulmonary immune system homeostasis. For example, after birth, the microbiota colonization of lungs enhances the polarization of naïve T cells from Th2 to Th1, thus protecting against neonatal asthma and allergy; *Bacteroidetes* seem to promote the proliferation of T-reg (regulatory T lymphocytes) that protects from allergies in adulthood; *S. Aureus* enhances the differentiation of M2 alveolar macrophages, protecting from potentially lethal pulmonary inflammation during caused by influenza infection[24].

Lung microbiota undergoes some modifications during respiratory illnesses like asthma[25], chronic obstructive pulmonary disease (COPD)[26], idiopathic pulmonary fibrosis (IPF)[27], and cystic fibrosis (CF)[24].

An important role of microbiota has been described also considering critical illnesses, including acute respiratory distress syndrome (ARDS). Normally, the alveoli are an unfavorable environment for bacteria because of the lack of nourishment and the presence of the bactericidal layer of surfactant. During an alveolar injury, like in pneumonia and ARDS, the alveoli are filled with protein-rich fluid. This fluid is an energy source for microbes, inactivates the surfactant, and impairs the mucociliary clearance[28].

The most important limitation in the study of microbiota during illnesses is the difficulty in understanding if the dysbiosis is the cause or the consequence of the disease itself. The second hypothesis is supported by some studies on animal models. In such models, the transfer of microbiota from ill animals to healthy animals without microbiota (i.e., "germ-free" animals), causes the same disease of the donor in the receiving animal[29].

Many studies have demonstrated that the impact of microbiota is not only local but can also affect distant organs, both by modulating the immune system and by the transfer of microbial products in the bloodstream. This relationship seems to be better understood for the intestinal and respiratory tracts, to the point that a "gut-lung axis" has been described (Figure 1). The existence of this "axis" can be explained by the fact that the gastrointestinal and respiratory tract share a common embryonic origin and so have structural similarities[30]. The interaction between the gut and lungs is bidirectional. Chronic respiratory pathologies – like COPD, asthma, CF – can induce intestinal manifestations. Chronic intestinal diseases – like irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) – show pulmonary involvement[24,30]. The gut-lung axis seems to be involved not only in chronic pathologies but also during acute diseases. During acute infections, for example, the axis is supposed to influence the modulation of the immune

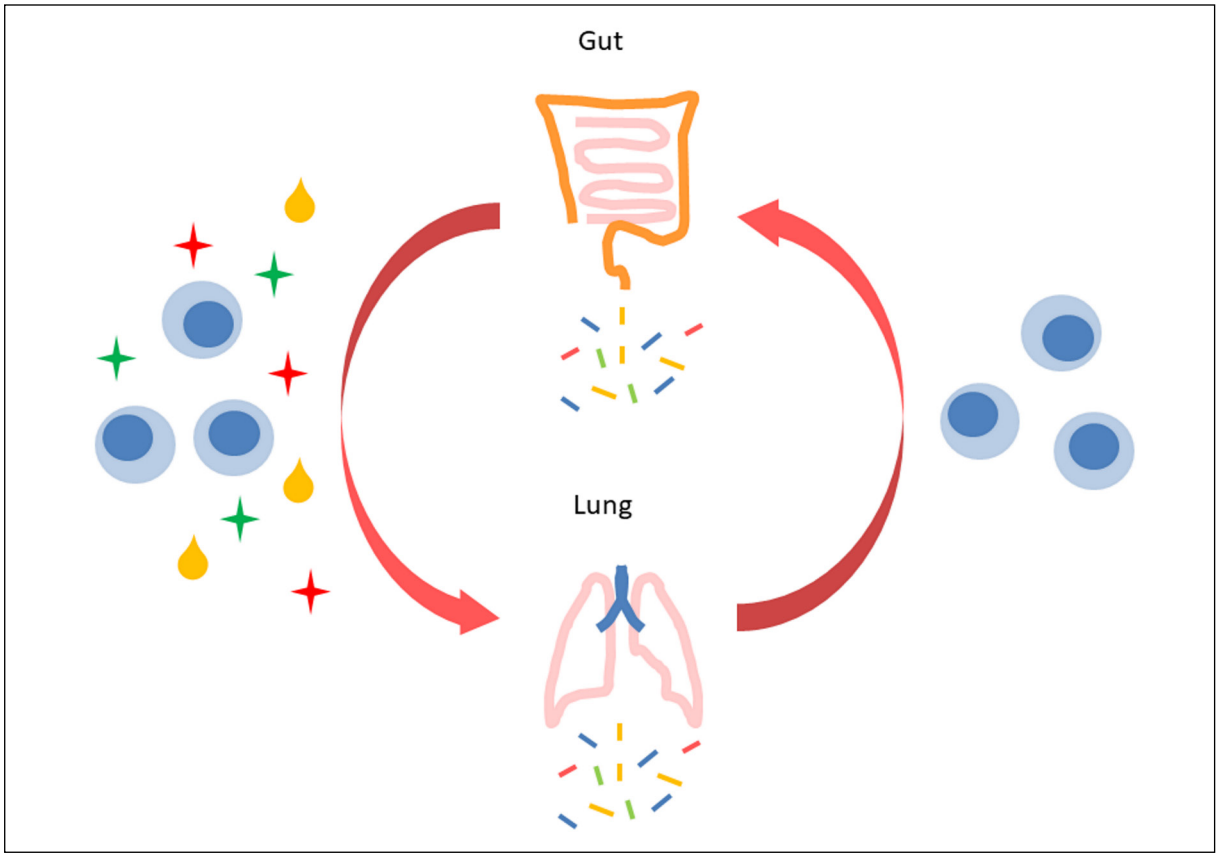

Figure 1. The «gut-lung axis». Gut and lung share similar microbiota composition (multicolor rods). Microorganisms participate in health and disease by modulating the immune system (gut- or lung-derived lymphocytes – blue circles) and by producing antimicrobial peptides (yellow drops) and anti-/pro-inflammatory molecules (green and red stars). Both lymphocytes and bacterial products may transfer in the bloodstream (red arrows) and reach distant organs where they can promote eu- or dysbiosis and consequently health and disease.

response. For example, Wang et al[31] have demonstrated that, during the influenza infection, no virus can be detected in the intestinal mucosa. Anyway, the intestinal damage can be explained by the recruitment of lung-derived specific T-lymphocytes that, once in the intestinal mucosa, cause gut microbiota disruption and subsequent inflammation. A protective role of gut microbiota against respiratory bacterial or viral infections has been reported too. Mice with depleted or absent intestinal microbiota show impaired lung immune responses with a higher incidence of infections and worse outcomes. Microbial products promote the proliferation of specific lymphocyte lines that then explicate their function not only in the intestine but in the lung too. Besides, some microbial products, like the SCFAs (short-chain fatty acids), have antimicrobial and anti-inflammatory effects and may reach the lung through the bloodstream[30].

AIM OF THE REVIEW

COVID-19 displays a range of manifestations that go from asymptomatic presentation to mild symptoms, to acute distress respiratory syndrome (ARDS). Besides respiratory symptoms, many patients present with gastrointestinal manifestations. Many scientists worldwide are questioning the reasons that may explain these different disease courses[32]. Recently, many authors have demonstrated the existence of the intestinal and pulmonary microbiota, their influence on the immune response, and their interaction in the "gut-lung axis". In the light of this, it seems reasonable to think that different compositions or disruption of the pulmonary and gastrointestinal microbiota may influence the severity of COVID-19 by interacting with the immune system and with the virus itself. Answering this question may lead to new interesting insights about the modulation of the microbiota, hopefully with the fecal microbiota transplantation (FMT), to prevent the infection and to promote faster recovery. On the other hand, the spread of SARS-CoV-19 across the globe is creating concern about the risks of transmission of the pathogen through the transplantation of cells and tissues, but also through the FMT, whose main indication is the treatment of recurrent *Clostridioides difficile infection* (CDI). The aim of this review is to revise the potential applications of FMT during COVID-19 and the role of the microbiota during the infection, discussing both the limitations to FMT that have emerged during the pandemic and the proposal of the scientific community to overcome them.

MATERIALS AND METHODS

The terms searched are "SARS-CoV-2", "covid", "covid-19", "2019-nCov", "coronavirus" AND "microbiota", "fecal transplantation", in all their possible combinations. These combinations have been searched in PubMed and Cochrane Library. The research produced some redundant results that have been eliminated; only the remainder 47 articles have been subjected to the abstract analysis. After abstract analysis 25 have been selected for full-text reading. From the reference analysis of those 25 articles, other 29 works have been added to the review process, for a total of 54 articles reviewed (Table 1).

DISCUSSION

COVID-19 and Lung Microbiota

The interaction between SARS-CoV-2 and lung microbiota is still unclear. Shen et al[33], reported that the composition of the BAL of 8 patients with SARS-CoV-2 pneumonia, is different from that of healthy individuals. Even if no specific microbiota alteration was observed in patients with COVID-19 pneumonia comparing to community-acquired pneumonia (CAP) or other suspected viral infections, this study suggests two important considerations about the role of lung microbiota in the development and progression of COVID-19 respiratory involvement. First, dysbiosis might promote viral infection through the liberation of a microbiological niche, the reduction of innate immune function, and the development of a pro-inflammatory habitat. Second, dysbiosis might promote a common complication of viral infections, which is a secondary bacterial infection that can contribute to a poor outcome[23].

TABLE 1. REVIEW PROCESS. THE RESEARCH HAS BEEN PERFORMED ON PUBMED AND THE COCHRANE LIBRARY. THE TERMS SEARCHED ARE LISTED ON THE HIGHER PART OF THE TABLE. THE MIDDLE PART OF THE TABLE REPORTS ALL THE POSSIBLE KEY WORDS COMBINATIONS: THE FIRST NUMBER IS THE TOTAL OF ARTICLES FOUND FOR EACH COMBINATION AND IS THE SUM OF PUBMED OUTPUT (THE FIRST NUMBER IN THE ROUND BLANKET) AND COCHRANE LIBRARY OUTPUT (THE SECOND NUMBER IN THE ROUND BLANKET). AT THE END OF THE ANALYSIS, A TOTAL OF 54 ARTICLES HAVE BEEN REVIEWED.

Research on PubMed and Cochrane Library		
• Covid • Covid-19 • SARS-CoV-2 • 2019-nCoV • Coronavirus	AND	• Microbiota • Fecal transplantation

AND	Microbiota	Fecal Transplantation
Covid	17 (16+1)	5 (4+1)
Covid-19	20 (19+1)	5 (4+1)
SARS-CoV-2	15 (15+0)	3 (3+0)
2019-nCoV	5 (5+0)	3 (2+1)
Coronavirus	35 (35+0)	4 (4+0)

Elimination of redundant results
47 articles

Title and abstract analysis
25 articles

Full-text reading and reference analysis
54 articles reviewed

COVID-19 and Gut Microbiota

The role of gut microbiota in the course of SARS-CoV-2 is complex and still far from being completely understood. On the one hand, a normal microbiota is known to be protective against viral infections[30]; on the other hand, viral infections may promote gut dysbiosis[31].

Studies on animal microbiota[34-39] have demonstrated that intestinal manifestations of coronavirus infection might depend not only on a direct effect of the virus on the enterocytes but also on the alteration of intestinal.

Concerning the gastrointestinal manifestations of SARS-CoV-2 infection, they might, at least in part, be explained by the modulation of ACE2 both by the virus and by the microbiota.

As previously exposed, SARS-CoV-2 enters the cell by binding the ACE2, a transmembrane glycoprotein with a short intracellular cytoplasmic tail and a long extracellular domain that exhibits carboxy-mono-peptidase activity. The main function of ACE2 is to cleave the C-terminal phenylalanine of angiotensin II to produce angiotensin 1-7, a vasodilator. On the membrane of enterocytes of the small intestine, ACE2 binds and stabilizes the protein B^0AT1, a transporter of neutral amino acids like tryptophan. ACE2 deficient mice cannot express B^0AT1 and therefore display a severe reduction of tryptophan with subsequent increase in the susceptibility to experimental chemical-induced colitis[14,40]. Interestingly, ACE2 is highly expressed in the small intestine but poorly present in the colonic mucosa, so that the major susceptibility to colitis can appear contradictory. However, there is evidence that a reduction of tryptophan levels causes a reduction of mTOR activity with subsequent impaired production of antimicrobial peptides by the Paneth cells of the small intestine. Once in the colon, these antimicrobial peptides cause a dysbiosis that promotes intestinal inflammation and explains the susceptibility to colitis[41].

This chain of events may also explain the intestinal manifestation of SARS-CoV-2 infection (Figure 2). Studies on SARS-CoV[41,42], the coronavirus responsible for MERS (Middle East Respiratory Syndrome), have demonstrated that the binding of the virus with ACE2 promotes the

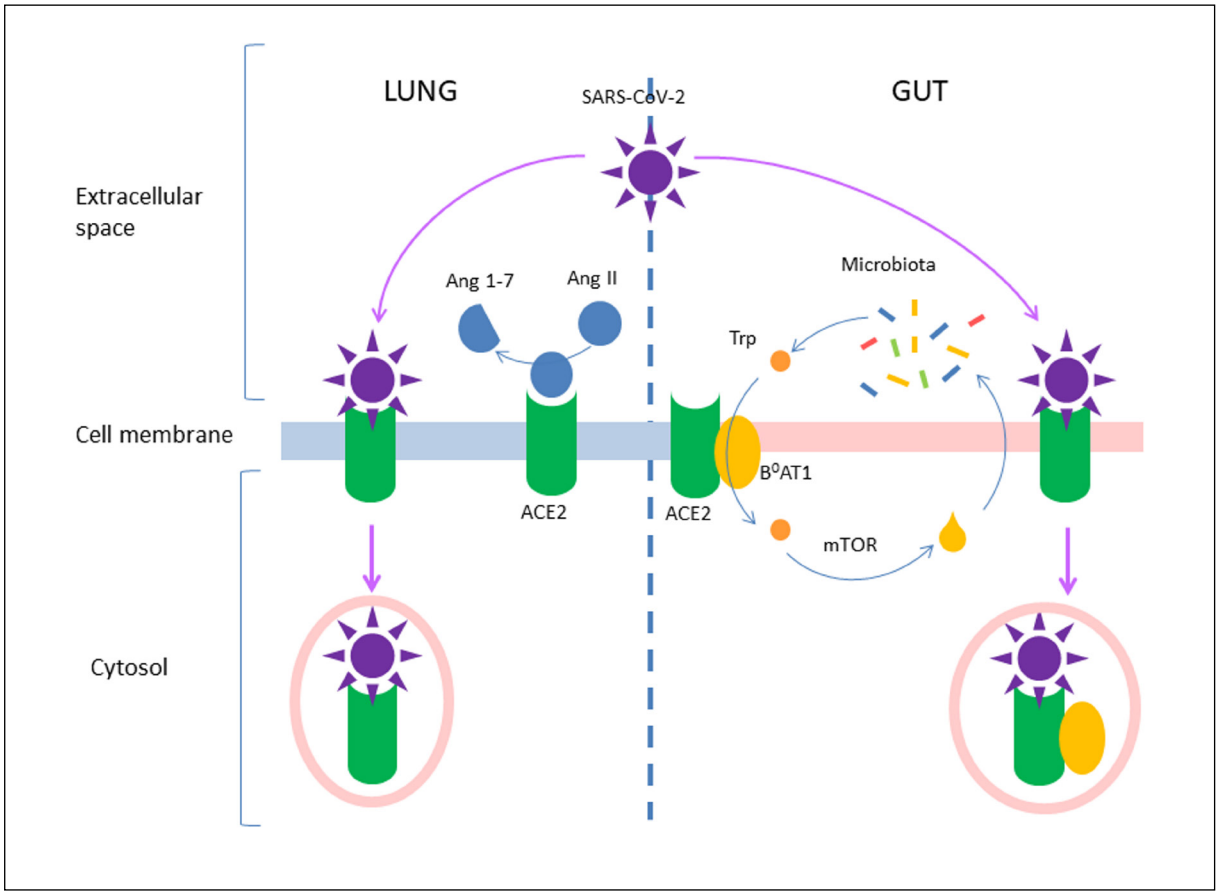

Figure 2. The role of ACE2 receptor in the lung and gut and its potential modulation during SARS-CoV-2. ACE2 is a transmembrane glycoprotein. In the lung, ACE2 transforms angiotensin II into angiotensin 1-7, a vasodilator. In the gut, ACE2 is necessary for the expression of the amino acid transporter B^0AT1. B^0AT1 transports tryptophan in the cytosol where it interacts with the mTOR pathway. This pathway regulates the production of antimicrobial peptides that, once in the intestinal lumen, modulate the microbiota composition. SARS-CoV-2 binds to the extracellular domain of ACE2 and this binding promotes endocytosis of the ACE2/virus complex and subsequent enzymes downregulation. In the lung, these events cause angiotensin II increase, which promotes ARDS. In the gut, the reduction of B^0AT1 causes a decrease of tryptophan and its action on the mTOR pathway. The subsequent alteration of antimicrobial signals causes intestinal dysbiosis and predisposition to colitis and diarrhea. Abbreviations: ACE2 – angiotensin-converting enzyme 2; Ang II – angiotensin II; Ang 1-7 – angiotensin 1-7; B^0AT1 – Broad neutral Amino acid Transporter; mTOR – mammalian target of rapamycin; SARS-CoV-2 ¬– severe acute respiratory syndrome coronavirus 2; Trp – tryptophan. Adapted from Perlot T, 2013.

endocytosis of the ACE2-virus complex, thus causing not only the virus entry in the host cell but also ACE2 downregulation.

This downregulation explains the local increase of angiotensin II that has been described as a cause of acute respiratory distress syndrome (ARDS) during SARS-CoV infection[41,43]. A similar binding process has been described for SARS-CoV-2[44]. Even if it has not been demonstrated, it is reasonable to speculate that the SARS-CoV-2 causes ACE2 downregulation that may impair the B^0AT1 expression on the enterocytes with subsequent tryptophan depletion, antimicrobial peptides increase, gut dysbiosis, and intestinal inflammation.

Moreover, not only SARS-CoV-2 might promote gut dysbiosis through downregulation of ACE2 but also ACE2 expression might be modulated by gut microbiota. Yang et al[40] have demonstrated that the gut microbiota might influence the expression of the ACE2 receptor in the

colonic mucosa of rats. In particular, the colonization of the germ-free gut by microbiota causes the reduction of ACE2 expression. The authors conclude that these findings may discourage the administration of empirical antibiotics that might provoke a gut microbiota depletion and, consequently, an increase of ACE2 expression, i.e., the site of virus binding and entry.

A prospective study[13] on 15 patients with various degrees of severity of COVID-19 has highlighted significant differences in gut microbiota composition of infected patients, compared with controls, both at the baseline and during the disease course. In particular, patients with COVID-19 displayed enrichment of opportunistic pathogens and depletion of beneficial commensals, at the time of hospitalization and at all time points during hospitalization. Moreover, the baseline abundance of *Coprobacilli* and *Clostridia* correlated with the disease severity, while the abundance of *Faecalibacterium prausnitzii*, an anti-inflammatory bacterium, was associated with a less severe course. During the hospitalization, the abundance of some *Bacteroides*, which downregulate the expression of ACE2 in the murine gut, correlated inversely with SARS-CoV-2 load in fecal samples.

COVID-19 and Modulation of Microbiota: The Role of Diet, Prebiotics, and Probiotics

A balanced diet is crucial to maintaining a healthy immune system and the equilibrium between a pro-inflammatory and an anti-inflammatory status. For example, insufficient protein intake causes a reduction in antibody production. Low levels of vitamins and cofactors are associated with a higher risk of infection and inflammation because of oxidative stress and the downregulation of anti-inflammatory pathways. Fibers are also important because they are fermented by the gut microbiota into short-chain fatty acids that show anti-inflammatory effects[45]. Moreover, the gut microbiota is malleable and is influenced by diet, therefore, its modulation through diet, probiotic and prebiotic has been proposed as a supplement to current routine therapies in COVID-19 patients[3], even if there is no clear evidence.

It is widely accepted that the administration of probiotics is useful in critically ill patients[46,47]. A systematic review and meta-analysis[46], which included a total of 30 randomized controlled trials (n = 2,972), reported that the administration of probiotics in such patients caused a significant reduction in infection and ventilator-associated-pneumonia[46].

In February, China's National Health Commission and National Administration of Traditional Chinese Medicine suggested using probiotics in patients with severe COVID-19[48]. Anyway, there is scarce evidence supporting this recommendation, coming from small case series and animal studies[49]. For example, in their case series, Xu et al[50] reported a microbial dysbiosis with decreased *Lactobacillus* and *Bifidobacterium* in some patients with COVID-19.

Lactobacillus species are the dominant microorganism of the vagina and, together with *Enterococcus faecalis*, produce reutericyclin which inhibits the growth of a variety of pathogens, including enveloped viruses like coronaviruses. An analog of reutericyclin, the glycerol monolaurate (GML), has shown potential therapeutic effects against enveloped viruses in animal models and *in vitro* studies[51]. Even if the role of reutericyclin and GML in the case of SARS-CoV-2 infection has not been much investigated so far, it is reasonable to hypothesize that the alteration of microbiota and, consequently, of its products, might influence the instauration and progression of the disease.

Other reasons to recommend probiotics in COVID-19 patients are the massive use of antibiotics that might promote antibiotic-related diarrhea (2-36% of patients)[49,52] and the gastrointestinal presentation of the disease (5-10% of patients)[32].

Some studies[53-55] also suggest the existence of gut-lung crosstalk that plays a role in respiratory tract infections and whose modulation might influence the clinical evolution of the infection.

The major morbidity and mortality of COVID-19 in the elderly can depend on the fact that gut microbiota diversity decreases in old age. Based on this observation, the use of prebiotics and probiotics may play a role both in the prophylaxis of COVID-19 by improving immunity, and in the treatment of the disease, along with current therapies[3].

As previously stated, Streptococcus is one of the most important genera of healthy lung microbiota. Among streptococci, *S. salivarius* seems to be protective against infections. A strain of *Streptococcus salivarius*, known as *K12*, creates a stable respiratory microbiota, thus reducing

infections caused by bacteria like *S. pyogenes*, *S. pneumoniae*, *H. influenzae,* and *M. catarrhalis* as well as viral infections like rhinitis, pharyngitis, laryngitis tracheitis, enteritis, and influenza. The anti-bacterial function of strain K12 derives from the release of bacteriocins that destabilizes the bacterial membrane; the antiviral action depends on the enhancement of the adaptive immune response with the increase of IFN-γ (interferon-gamma) in the saliva. Moreover, this strain plays an anti-inflammatory role in the bronchi by inhibiting the NF-κB pathways, which are usually modulated by viruses to enhance their replication. These observations suggest that the role of probiotics in the course of respiratory viral infections is not only in the gut microbiota modulation but also in the lung modulation. In particular, given its clinical potential and its safety, some authors[23] suggest the use of K12 to help control the viral infection, even if its efficacy has never been specifically evaluated for Sars-CoV-19 infection.

The Fecal Microbiota Transplantation in the COVID-19 Era

Since the potential transmission of COVID-19 through a transplant is unknown, there is concern about the screening of donors of human cells, tissues, or cellular or tissue-based products. Many institutions have suggested adopting stricter precautions and improving screening protocols. The US Food and Drug Administration has released an alert suggesting considering the donor's history of travel, cohabitation with infected individuals, or diagnosis of suspicion within the 28 days before the hospitalization[56]. The Global Alliance of Eye Bank Associations and the Joint United Kingdom Blood Transfusion Services Professional Advisory Committee have proposed similar measures[57,58]. The European Society for Blood and Marrow Transplantation suggests excluding potential donors who have been diagnosed with COVID-19, and waiting at least 21 days before donation in high-risk subjects (i.e., history of travel or contact)[59]. Stricter recommendations have been released by the Italian National Center for Transplants that states that all the potential donors with documented infection can donate only after 4 weeks from the virological remission confirmed by two negative nasopharyngeal swabs and only if a BAL, performed 24-48 hours before the donation is negative too. Also, potential donors with suspected infection (i.e., compatible symptoms) or a history of strict contacts with COVID-19 positive patients are subject to similar limitations[60].

The fecal microbiota transplantation (FMT) is an emergent treatment for the management of recurrent *Clostridioides difficile infection* (CDI). Given the fact that its use is spreading because of its advantage over antibiotics, there is a growing need for standardization, mostly to prevent the transmission of infectious diseases[6,61].

In 2019 a task of twenty-nine international experts in the field of FMT[62] has drafted guidelines regarding the standardization of FMT. The group analyzed different aspects of the topic, going from the need for new stool banks worldwide to the hierarchical organization of the stool banks, to the rules for donor's recruitment and screening. The task also provided a list of all the blood exams and stool exams needed, and the checklists for sample collection, storage, and preparation.

As with cells, tissues, and organs, the spread of COVID-19 has represented a problem for the FMT. The risk of transmitting SARS-CoV-2 by fecal microbiota might be higher than in other tissues. It is known that the virus can be found in feces, and stool samples can remain positive for the virus even when it is no longer detectable in the respiratory tract[63].

Because of this evidence, some centers like the University of Birmingham Microbiota Treatment Centre, have decided not to actively process new donors until a validated SARS-CoV-2 stool test is available[64].

Moreover, some stool banks have been required to separately store the stool collected after December 1, 2019, from those obtained and banked before this date, considering the latter reasonably not infected by the virus[65].

However, although preventing the transmission of SARS-CoV-2 represents a priority, CDI has not ceased to be a burden during the COVID-19 pandemic and many patients still need FMT. Anyway, since some centers have stopped processing samples and considering that stool viability is about 6 months[65], there may be a lack of stools for transplantation.

For these reasons, to ensure a safe FMT even during the COVID-19 pandemic, an international group of experts[6] has updated the recommendation for the screening of fecal transplan-

tation, proposing additions to the current recommendations. Considering the previous 30 days, the donors should be investigated epidemiological and clinical features. The epidemiological features are a history of travel in high-risk areas and contact with suspected or proven infected patients. The clinical features are typical COVID-19 symptoms like fever, fatigue, dry cough, myalgia, dyspnoea, and headache. If either of these items is positive, the potential donor should either be rejected or tested with an RT-PCR assay for SARS-CoV-2. Moreover, in endemic countries, the RT-PCR assay should be considered in all donors, independently of their epidemiologic or clinical features. Alternatively, stools should be stored and quarantined for 30 days before use and released only if the donor has not developed symptoms.

These recommendations collide with some limitations given by the characteristics of the pandemic: the lack of enough tests to diagnose symptomatic people, the fact that respiratory swab-based tests are not validated for asymptomatic people (i.e., the donors) and that validated stool assays for asymptomatic carriage are not available. Moreover, the absence of a respiratory coronavirus in an asymptomatic person does not rule out fecal carriage and transmission[65].

To overcome these limitations some authors have elaborated specific screening protocols.

For example, Khanna et al[65] have proposed a step-by-step algorithm. The algorithm includes epidemiologic and clinical evaluation, microbiological test (RT-PCR on nasopharyngeal swab), 14 days specimen embargo, and further rescreening and retesting for COVID-19. They suggest delaying the FMT in patients with recurrent CDI that may benefit from an antibiotic course and wait until the pandemic is better controlled. For patients with fulminant CDI with no response to maximal guideline-based combination therapy, they propose to use stools collected before the pandemic spread.

Ng et al[66] have proposed a protocol including epidemiological screening of donors and a SARS-CoV-2 RT quantitative PCR on donors' stool. Interestingly, they found that a single negative test, as in the current practice for screening other pathogens, does not exclude SARS-CoV-2 presence in the stool. In such samples, the level of viral RNA can fluctuate around the limit of laboratory detection. Therefore, they recommend testing donors multiple times during the donation period.

To overcome some of the difficulties and risks of FMT, some authors[67] have proposed an alternative method, the washed microbiota transplantation (WMT) in which the preparation is based on the automatic microfiltration machine. The study has been conducted *in vitro* and animal models and has shown a reduction in the adverse events versus FMT. Even if these results are encouraging, further studies on humans are needed to understand the implementation of this method.

The Potential Therapeutic Applications of FMT as Rescue Therapy in Patients Affected by SARS-CoV-2

There are no studies about the efficacy of FMT during COVID-19. Anyway, there is growing interest in this therapy in critically ill patients.

Zhang et al[68] registered a randomized clinical trial to investigate the potential of WMT in patients with COVID-19 but, unfortunately, the trial stopped early, before enrolling its first participant.

There is evidence that, in the critical care setting, many factors like the use of antibiotics, aberrant nutrition, bloodstream infections, bowel ischemia, and abnormal bowel motility, contribute to intestinal dysbiosis which is associated with worse clinical outcomes in the intensive care unit (ICU)[69]. Critically ill patients show dysbiosis in many organs, like the skin, gastrointestinal tract, and the lungs with loss of microbial diversity and overrepresentation of potentially pathogenic microorganisms that transform the health-inducing microbiome in disease-promoting "pathobiome"[69,70]. The intestinal microbiota is the reservoir for most multidrug-resistant bacterial bacteria (MDRB), such as extended-spectrum β-lactamase-producing Enterobacteriaceae, carbapenemase-producing Enterobacteriaceae, and vancomycin-resistant enterococci[71]. Moreover, ICU patients present a hyperpermeable gut barrier and dysregulation of the inflammatory response that favor pathogens translocation and promote sepsis[72].

The dysbiosis might be a consequence of the disease itself, but other factors can contribute to it. The use of antibiotics in critical illness, in particular, can cause antibiotic-associated diarrhea (AAD) that may worsen the clinical outcome. In such patients, rescue FMT – i.e., an "off-label" practice – may improve the clinical course, like it has been reported in a clinical trial on twenty critically ill patients with AAD[73]. Recent studies on animal models of sepsis have reported that FMT might reverse the course of otherwise lethal sepsis by enhancing pathogen clearance *via* the restoration of host immunity[74]. In the light of these observations, being COVID-19 a potentially fatal disease for which we do not have targeted therapies, the use of FMT as rescue therapy for severe cases in the ICU should be considered.

CONCLUSIONS

Microbiome modulation can be a good strategy to prevent SARS-CoV-2 infection, since it is becoming clearer and clearer that a healthy microbiome promotes a balanced immune response, by enhancing the equilibrium between a pro- and an anti-inflammatory status[3,45]. There are no studies about the modulation of the lung microbiome while more is known about the gut microbiome, whose composition can be influenced in many ways, from the diet to the administration of prebiotics and probiotics[3,23,46,50,73]. These strategies may play a role during the SARS-CoV-2 infection too, since we know that the infection is more common and severe in patients whose microbiota is depleted and disrupted, like the elderly, the immunocompromised, and the seriously ill (i.e., patients with diabetes, cancer, cardiovascular diseases)[3,47-49]. Moreover, to prevent secondary bacterial infections, patients with COVID-19 are frequently treated with massive doses of antibiotics, that may cause intestinal dysbiosis and antibiotic-related diarrhea. In such patients, the nutritional assessment is always recommended, and the administration of probiotics might restore the intestinal flora and rebalance the immune response[71]. Even if the FMT is now approved only in patients with CDI, this treatment might represent a promising strategy to rebalance the gut microbiota in critically ill patients like the ones with severe forms of COVID-19[6,67-69,72,73]. If on one side the worldwide spread of SARS-CoV-2 is highlighting the potential application of FMT in severe cases of COVID-19, on the other side it is rising concern about the risks connected to the transmission of the disease through the FMT itself in those patients who require this treatment for its sole current indication, i.e., the CDI. This concern has remarked the absence of a unique, unambiguous, protocol for FMT and the need to develop new and effective ways to screen stool donors, also for SARS-CoV-2[55-61,63-65]. The study of the viral way of transmission has highlighted that besides the respiratory way of transmission there is an oral-fecal one[8]. This evidence has stimulated the development of screening techniques able to detect the virus in materials different from the respiratory secretion, like the saliva or the stool[9,62,63]. As previously stated, there are no methods that directly modulate the lung microbiota, but the existence of a "gut-lung axis" allows us to speculate that the modulation of gut microbiota through the FMT might influence the lung flora and the pulmonary immune response, too. In conclusion, the diffusion of COVID-19 has constituted a new challenge in the understanding of the human microbiome physiopathology and has raised many questions about the potential applications of its modulation, mainly through the FMT. Unfortunately, we only have small case series and expert opinion; in the future, randomized clinical controlled trials may clarify the effectiveness of this treatment in the course of COVID-19[75].

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Conflict of Interest

The Authors declare to have no conflict of interests.

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