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REVIEW

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Gut microbiota in COVID-19: new insights from inside

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ABSTRACT

The epidemic of coronavirus disease-19 (COVID-19) has grown to be a global health threat. Gastrointestinal symptoms are thought to be common clinical manifestations apart from a series of originally found respiratory symptoms. The human gut harbors trillions of microorganisms that are indispensable for complex physiological processes and homeostasis. Growing evidence demonstrate that gut microbiota alteration is associated with COVID-19 progress and severity, and post-COVID-19 syndrome, characterized by decrease of anti-inflammatory bacteria like *Bifidobacterium* and *Faecalibacterium* and enrichment of inflammation-associated microbiota including *Streptococcus* and *Actinomyces*. Therapeutic strategies such as diet, probiotics/prebiotics, herb, and fecal microbiota transplantation have shown positive effects on relieving clinical symptoms. In this article, we provide and summarize the recent evidence about the gut microbiota and their metabolites alterations during and after COVID-19 infection and focus on potential therapeutic strategies targeting gut microbiota. Understanding the connections between intestinal microbiota and COVID-19 would provide new insights into COVID-19 management in the future.

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Introduction

Since late 2019, a novel coronavirus severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spreads out worldwide and results in a complicated multisystem disease named coronavirus disease-19 (COVID-19), imposing an emerging and huge challenge to human society. Although preventive vaccines have been developed to protect the populations, the virus continues to evolve rapidly and spread widely in the populations. Clinical data have shown that the mortality and severity were much higher in the elderly and patients with some morbidities like diabetes and cardiovascular disease. As of January 30, 2023, more than 670 million confirmed cases and 6.8 million deaths have been reported by Johns Hopkins University & Medicine[1]. Apart from originally found systemic and respiratory systems including fever, myalgias, fatigue, cough, and sore throat, gastrointestinal manifestations such as abdominal pain and diarrhea are gradually mentioned in infected patients.

Billions of microorganisms, mainly including bacteria and fungi, inhabit in human gut and participate in numerous physiological activities. Gut microbiota play a crucial role in maintaining health through modulating metabolism, immune function, and some other pathways. It has been found that gut microbiota alteration existed in the intestine of COVID-19-infected patients compared with healthy individuals, characterized by increased abundance of bacteremia-associated bacteria (e.g., *A. viscosus, C. hathewayi*, and *Streptococcus*) and decreased level of symbionts contribute to host immunity (e.g., *B. adolescentis*, *F. prausnitzii*, and *E. rectale*), and the disturbance persisted for a long time even if respiratory manifestations disappeared and throat swab turned negative[2,3]. What is more, disturbance of gut microbiota has been reported to relate to the disease severity and development of post-COVID-19 syndrome. Better clinical outcomes were associated with anti-inflammatory bacteria such as *Bifidobacterium* and *Ruminococcus*

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and beneficial microbial metabolites like short-chain fatty acids (SCFAs)[3].

With the in-depth study of COVID-19, the importance of gut-lung axis has attracted more and more attention. Although the gut and lung function differently, they share some common structural features because the tissues both develop from the same embryo tissue. The gut and lung are all covered with mucous membranes, which secrete mucin and form a common mucosal immune system to defend against pathogens. Furthermore, growing evidence shows that there is a crosstalk of microbiota between the respiratory tract and intestine. Gut microbiome is found to be altered in patients with certain respiratory diseases, such as chronic obstructive pulmonary disease and allergic asthma[4,5]. Gut microbiota and microbial metabolites participate in regulating immunity and pulmonary microbiota in response to respiratory tract infection. And microbiota-derived metabolites pathways can act distally and play an important role in anti-inflammation in the airway[6,7].

Some publications demonstrate that targeting gut microbiota for relieving COVID-19 could be a possible strategy. Supplementation of fermented vegetables was found to be associated with low death rate in some countries, probably due to *Lactobacillus*-induced increase in antioxidant capacity[8]. A randomized controlled research showed that probiotics supplementation alleviated both digestive and non-digestive manifestations in infected patients and reduced the signs of lung infiltration, accompanied with SARS-COV-2 load decrease in the nasopharynx[9]. Another well-known way for modulating gut microbiota, fecal microbiota transplantation (FMT), has also been reported to influence the clinical course in COVID-19 patients[10]. In addition, a multitude of herbal medicines can affect the structure and composition of gut microbiota and protect intestinal barrier function, providing some possibilities for COVID-19 treatment[11].

This article summarizes recent studies showing the alteration of gut microbiota including some special microbiota species and microbiota-derived metabolites in regulating the severity and disease progression in patients with COVID-19 infection and subsequent complications after recovery. Secondly, we discuss the complicated connections among gut microbiota, gut, and lung. Finally, we review clinical evidence targeting gut microbiota to alleviate clinical symptoms in patients and provide new insights into further COVID-19 management.

Gut microbial characteristics in COVID-19

Human gut microecosystem contains vast[12] microbial cells, which is 10 times more than the number of human cells. The enormous microecosystem mainly consists of a spectacular number of bacteria but also comprises commensal fungi, viruses, archaea, etc. In addition to playing an important role in maintaining intestinal homeostasis, the gut microbiota and microbiota-derived products interact with the host to regulate intestinal physiology and extra-intestinal function via regulation of several pathways, such as immunity and metabolism. It is reported that respiratory virus invasion affects not only respiratory immunity but also intestinal microbiota and microbiota-derived metabolites. Here we summarized the microbiota and its metabolites differentially presented in COVID-19-infected patients compared to non-COVID-19 individuals and shown in Tables 1 and 2, respectively.

Intestinal bacterial changes in COVID-19 progression

Based on existing sequencing methods and in-depth studies of COVID-19 cases, gut microbiota alteration is gradually observed in infected patients. As early as May 2020, Zuo T *et al*. first reported the gut microbiota alteration in infected patients after detecting fecal samples with shotgun metagenomics analysis. The researchers revealed that gut microbiota alteration was characterized by enrichment of human infection and bacteremia-related microbiota and reduction of symbionts beneficial to host immunity and decreased microbiota diversity, revealing a significant difference in fecal microbiota between healthy controls and hospitalized COVID-19 patients. It is interesting to note that a variety of bacteria such as *Coprobacillus*, *C. ramosum*, and *C. hathewayi* were shown to be positively associated with disease severity of patients, whereas a known butyrate-producing bacterium, *F. prausnitzii*, was negatively related to the

Table 1. Gut microbiota alterations in COVID-19 patients during hospitalization.

Country	Sample type	Enriched microbiota	Reduced microbiota	Drug application	Reference
China	5 COVID-19 patients, 6 patients with community-acquired pneumonia, and 15 healthy individuals	Patients without antibiotic treatment: *A. viscosus*, *C. hathewayi*, *B. nordii*	Patients without antibiotic treatment: *E. ventriosum* patients without antibiotic treatment: *D. formicigenerans*, *R. obeum*, *E. rectale*, *F. prausnitzii*	8 COVID-19 patients received empirical antibiotics; 13 COVID-19 patients received antiviral drug	[2]
China	57 COVID-19 patients with pneumonia	*Enterococcus*, *Enterobacteriaceae*	*F. prausnitzii*, *C. butyricum*, *C. leptum*, *E. rectale*, *Lactobacillus*, *Bifidobacterium*	A total of 50.9%, 5.3%, and 12.3% of patients received antibiotics, antifungal drugs, and probiotics	[13]
China	30 COVID-19 patients, 9 patients with pneumonia, and 30 healthy individuals	*C. albicans, C. auris, A. flavus*	NA	16 COVID-19 patients received antibiotics; 20 received antiviral therapy	[14]
China	13 COVID-19 patients and 5 healthy volunteers	*Eggerthella, Coprobacillus, Clostridium ramosum, E. lenta, R. gnavus*	*R. intestinalis, E. hallii, P. excrementihominis, A. indistinctus, C. fastidiosus, E. eligens, B. salyersiae, O. splanchnicus, A. shahii, R. bromii, B. massiliensis*	All the patients received antiviral therapy; eight were antibiotic-naive and the remaining five were treated with antibiotics	[15]
China	67 COVID-19 patients, 35 H1N1-infected patients, and 48 healthy controls	*C. albicans*	*P. citrinum, P. polonicum, C. parapsilosis, T. wortmannii, M. yamatoensis, R. mucilaginosa, M. aphidis, W. sebi, M. racemosus, Aspergillus, Trechispora*	All COVID-19 patients were treated with antiviral drugs; 49 COVID-19 patients received glucocorticoids	[16]
China	9 COVID-19 children aged between 7 and 139 months and 14 age-matched healthy control children	*Pseudomonas, Herbaspirillum, Burkholderia, Bacteroides, Parasutterella*	*Proteobacteria, Prevotella, Porphyromonas, Finegoldia, Anaerococcus*	NA	[17]
China	53 COVID-19 patients and 76 healthy individuals	*Streptococcus, Weissella, Enterococcus, Rothia, Lactobacillus, Actinomyces, Granulicatella, C. citroniae, B. longum, R. mucilaginosa*	*Blautia, Coprococcus, Collinsella, B. caccae, B. coprophilus, B. obeum, C. colinum*	More than 60% of COVID-19 patients were given antibiotics; more than 80% COVID-19 patients were given antiviral therapy	[18]
China	13 patients with COVID-19 and 21 healthy controls	*Ruminococcus, Fusobacteriota, B. coprophilus, B. graminisolvens, B. uniformis, B. stercoris*	*Lachnoclostridium, Ruminococcus, Butyrivibrio, Dorea, Eubacterium, B. hansenii, R. lactaris, T. nexilis*	NA	[19]
China	66 COVID-19 patients and 70 non-COVID-19 controls	*B. ovatus*, *B. dorei*, *B. thetaiotaomicron*	*B. adolescentis*, *R. bromii*, *F. prausnitzii*	All patients were antibiotic-naive; 35 patients received at least 1 antiviral drug	[20]
China	100 COVID-19 patients and 78 non-COVID-19 subjects	*R. gnavus, R. torques, B. dorei*	*B. adolescentis, F. prausnitzii, E. rectale*	34 patients received antibiotics; over 39 patients received antiviral drug	[21]
China	63 COVID-19 patients and 8 controls	*B. ovatus*, *A. bereziniae*, *C. innocuum*, *B. contaminans*, *B. nordii*, *B. longum*	*B. uniformis*, *F. prausnitzii*, *B. pseudocatenulatum*, *E. eligens*, *B. eggerthii*, *A. shahii*, *L. asaccharolyticus*, *B. cellulosilyticus.*	13 patients received moxifloxacin; 7 patients received other antibiotics. More than 50% of patients received antiviral drugs.	[22]
Germany	108 COVID-19 patients, 22 patients with post COVID-19, 20 symptomatic pneumonia controls, and 26 matched asymptomatic controls	*C. innocuum*, *R. lactatiformans*, *A. finegoldii*, *Parabacteroides*, *Lachnoclostridium*	*F. prausnitzii*, *B. luti*, *D. longicatena*, *G. formicilis*, *A. putredinis*, *Fusicatenibacter*, *Blautia*, *Faecalibacterium*, *Ruminococcus*	54 COVID-19 patients received antibiotics; 40 patients received immunosuppression; over 15 patients were given specific SARS-COV-2 treatment	[15]
Germany	30 SARS-CoV-2-positive patients and 23 SARS-CoV -2-negative patients	*Ascomycota*, *Agaricomycetes*, *Dothideales*, *Rhytismataceae*, *Nakaseomyces*, *Debaryomyces*, *Aureobasidium*, *Lophodermium*	NA	1 patient were treated with antibiotics; 1 received remdesivir; 3 received steroid; no patient received antifungal therapy;	[23]
China	66 COVID-19 patients and 18 healthy donors	*Enterococcus*	*Streptococcus*, *Bacteroides*	All the patients received antiviral treatment; 28 patients received antibiotic treatment; 53 patients received glucocorticoids	[24]

(*Continued*)

Table 1. (Continued).

Country	Sample type	Enriched microbiota	Reduced microbiota	Drug application	Reference
Japan	112 COVID-19 patients and 112 non-COVID-19 control individuals	*Streptococcus*, *Rothia*, *Actinomyces*	*Bifidobacterium*, *Dorea*, *Roseburia*, *Butyricicoccus*	NA	[12]
Switzerland and Ireland	172 COVID-19 patients and 29 controls	*Enterococcus*, *Coprobacter*, *Streptococcus*	*Anaerostipes*, *Ruminococcus_2*, *Faecalibacterium*, *Agathobacter*, *Ruminococcus_1*, *Bifidobacterium*, *Collinsella*	56 COVID-19 patients received antibiotics; 89 patients received immunosuppressives	[3]
America	13 COVID-19 infected infants and 582 controls aged 0–24 months	NA	*B. bifidum*, *A. muciniphila*, *B. hominis*, *E. clostridioformis*, *E. limosum*, *E. cloacae*, *V. dispar*	4 infants were recently given antibiotics	[25]

Abbreviation: COVID-19, coronavirus disease-19; NA, not available.

Table 2. Changes of intestinal microbiota-derived metabolites and metabolic pathways in COVID-19 patients during hospitalization.

Country	Sample type	Characteristics of fecal microbial metabolites and metabolic pathways	Reference
China	66 COVID-19 patients and 70 non -COVID-19 controls	Enriched capacity in COVID-19 patients: urea cycle Decreased capacity in COVID-19 patients: SCFAs and L-isoleucine production	[20]
China	9 COVID-19 children aged between 7 and 139 months and14 age-matched healthy control children	Enriched capacity in COVID-19 children: bacterial secretion system, lipoarabinomannan biosynthesis	[17]
China	13 patients with COVID-19 and 21 healthy controls	Enriched metabolites in COVID-19 patients: phenylacetyl glutamine, salsolinol, uric acid, ethyl glucuronide Decreased metabolites in COVID-19 patients:chenodeoxycholic acid, muricholic acid, glycochenodeoxycholic acid, glycocholic acid, ursodeoxycholic acid, hyodeoxycholic acid, phenylalanocholic acid	[19]
China	63 COVID-19 patients and 8 controls	Enriched capacity in COVID-19 patients: preQ0 biosynthesis glycolysis, fermentation, methionine biosynthesis, vitamin B12 biosynthesis, and teichoic acid biosynthesis	[22]
Japan	112 COVID-19 patients and 112 non-COVID-19 control individuals	Enriched metabolites in COVID-19 patients: glutamine, threonine, proline, glycine, tryptophan, phenylalanine, tyrosine, aspartic acid, leucine, valine Decreased metabolites in COVID-19 patients: :maltose, isomaltose, sucrose, glyoxylic acid, xylobiose, N-acetylmannosamine, glutaric acid, SCFAs, γ-aminobutyric acid, dopamine, serotonin, pyridoxal 5′ -phosphate, pyridoxine, nicotinic acid, 3-hydroxybutyric acid, lactulose, homoserine, glucosamine	[12]
America	71 patients with COVID-19 associated critical illness	Enriched metabolites in survival COVID-19 patients: deoxycholic acid, lithocholic acid, isodeoxycholic acid, desaminotyrosine	[26]

Abbreviation: COVID-19, coronavirus disease-19; SCFAs, short-chain fatty acids.

severity[2], which might be due to its potential anti-inflammatory ability. Subsequent studies using 16S rRNA sequencing and shotgun DNA sequencing found that a significant decrease of microbial diversity existed in the intestine of COVID-19 patients, and the intestinal microbiota composition differed markedly in patients with mild, moderate, severe, and critical diagnosis[21,27]. The alteration was consistent with the degree of disease and increase of multiple blood inflammation markers.

On the one hand, several commensal bacteria with potential immunomodulatory capacity were found to be decreased or even depleted. Yeoh YK *et al.* reported that patients with COVID-19 infection presented with depletion of *F. prausnitzii*, *E. rectale*, and several genera from *Bifidobacteriaceae* and *Lachnospiraceae* family such as *B. adolescent* and *E. hallii* [21]. The abundance of a variety of SCFAs-producing taxa from *Bifidobacterium*, *Dorea*, *Roseburia*, *Lachnoclostridium*, *Butyricicoccus*, *Blautia*, *Coprococcus*, *Faecalibacterium*, and *Eubacterium* and species with potential immunomodulatory effects comprising *B. hansenii*, *R.lactaris*, *C. colinum*, *B. obeum*, and *T. nexilis*, were reduced in COVID-19-infected patients[12,18,19,28]. Similar results were demonstrated in infants with COVID-19, manifested by decrease of *B. bifidum* and *A. muciniphila*, both of which were considered as anti-inflammatory bacteria[25]. Another study evaluated 10 kinds of major intestinal microbiota with the method of quantitative polymerase chain reaction. The results performed that the relative abundance of various

butyrate-producing bacteria, mainly *F. prausnitzii*, *C. butyricum*, *C. leptum*, and *E. rectale*, was reduced in most patients. These taxa presented a negative correlation with the levels of inflammatory factors and markers of tissue damage in peripheral blood including interleukin-6, tumor necrosis factor-α, C-X-C motif ligand 10, C-reactive protein, and aspartate aminotransferase, suggesting that the decrease of these species might aggravate inflammatory response and help distinguish between patients with normal symptoms and those with severe disease[13]. Further study by Zuo T *et al.* found that SCFAs producers such as *A. onderdonkii* were enriched and were top taxa to be negatively correlated with disease severity in fecal samples from COVID-19 patients with low to none infectivity compared to fecal samples with high SARS-COV-2 infective characterization[2,29]. The genus *Alistipes* mainly exists in the intestines of healthy people, and *A. onderdonkii* is one of the most detected species of *Alistipes* isolated from human feces[30]. Furthermore, another SCFAs-producing bacterium, *Fusicatenibacter*, was dramatically decreased in severe cases especially in those who were died. And the taxa, *F. prausnitzii*, was less abundant in patients with acute kidney injury, acute cardiovascular events, acute respiratory distress syndrome, and some other complications, and furthermore showed an inverse correlation with mortality[31]. Of great significance is that a higher level of *Bifidobacterium* was shown to benefit to maintaining cardiopulmonary function[13].

On the other hand, a high-risk intestinal microbiota composition was found to be correlated with a fatal inflammation and metabolic dysfunction in response to COVID-19 infection. The relative abundance of *Streptococcus*, *Rothia*, *Veillonella*, *Actinomyces*, *Neisseria*, and *Campylobacter* was elevated in patients, especially in severe subjects, and these bacteria were related to intestinal immune dysfunction, intestinal barrier disruption, and increased susceptibility to pneumonia and subsequent secondary bacterial infections[3,15,32]. In addition, a significant enrichment of *Pseudomonas* was reported in some cases, implying severe gut microbiota alteration[33]. The available data at present showed that alterations of gut microbiota composition were related to the complications and mortality in patients. Death-related factors such as tryptophan metabolism, coagulation-related fibrinopeptides, and bile acids (BAs) are associated with a variety of genera enriched with increased distance from healthy individuals like *Enterococcus* [3]. Fecal *Proteobacteria* expansion showed an obviously positive correlation with higher mortality among those who died of severe COVID-19[26]. In fecal samples with high viral infectivity, pro-inflammatory bacteria like *C. aerofaciens* and *M. morganii* were enriched[29]. Researchers also reported that the overgrowth of *Enterococcus* and *Enterobacteriaceae* in critically ill patients indicated poor outcomes[13,24]. Several species enriched in COVID-19 patients (e.g., *B. dorei*, *A. muciniphila*, *B. contaminans*, and *B. nordii*) were responsible for a multitude of immune biomarkers including levels of C-reactive protein, interleukin-6, interleukin-1β, and even affected T cell response[21,22]. Furthermore, some bacteria present in the oral cavity and upper respiratory tract were found in the feces of patients such as *S. infantis* [29]. Its presence demonstrated that airway bacteria could be transmitted into the digestive tract, revealing a complicated microbial crosstalk during COVID-19 infection. Recent data from France showed that a one-log increase in concentrations of *Enterococcus* spp. and *S. aureus* in the oropharynx or in the rectum was correlated with a 17% higher mortality in patients hospitalized in intensive care unit (ICU)[34].

Furthermore, gut microbiota alteration at baseline may have long-time effects on the clinical outcomes in infected patients. Patients with low microbial richness during hospitalization had poorer pulmonary function after recovery, manifested by decreased levels of forced vital capacity and forced expiratory volume in the first 1 s of expiration. And low microbial richness was correlated with higher inflammatory markers such as C-reactive protein during the acute phase[35]. The abundance of *Actinomyces sp S6 Spd3*, *A. johnsonii*, and *A. parvulum* at admission showed a positive correlation with prolonged clinical manifestations at 6 months after recovery[36]. And intestinal bacterial changes were shown to be partially improved but persisted in patients after recovery. Yeoh YK *et al.* collected fecal samples from 27 recovered patients to evaluate the gut microbiota composition and found that *B. dentium* and *L. ruminis* were enriched, whereas *E. rectale*, *R. bromii*, *F. prausnitzii* as

well as *B. longum* were decreased at an average of 6 days after nasopharyngeal swab turned negative[21]. Of note, the key window for SARS-COV-2 clearance from nasopharyngeal and fecal samples might be inconsistent, with differences in some patients lasting up to a week or more. After the fecal infective signatures turned negative from SARS-COV-2, an expansion of salutary species including *P. distasonis* and *B. uniformis* and a reduced abundance of intestinal inflammation-related bacteria *R. gnavus* were revealed. However, one patient performed a considerable increase of nosocomial infection-associated bacteria *K. pneumoniae* in feces[29].

It is worth noting that the drugs used to treat COVID-19 also showed effects on gut microbiota, especially the antibiotics. In fact, antibiotics are not the primary treatment option for SARS-COV-2 itself but are prepared for secondary bacterial infections. Administration of antibiotics modulates the composition and structure of gut microbiota, affects host immunity and antibody production, thereby inducing antibiotics-associated diarrhea and even delayed virus elimination[37]. For patients at admission, antibiotics treatment further depleted many bacterial species that contribute to host immunity, including *F. prausnitzii*, *L. bacterium 5_1_63FAA*, *E. rectale*, *R. obeum*, and *D. formicigenerans*. Administration of antibiotics contributed to a more heterogeneous microbiota composition in COVID-19 patients, and aggravated and prolonged gut microbiota changes, resulting in further separation from the gut microbiota composition of healthy controls[2,21]. Some findings demonstrated that antibiotics were unlikely to participate in improving clinical outcomes in patients without bacterial coinfections, but drove gut microbiota translocate from the intestine into blood and triggered secondary bloodstream infections in severe cases[21,28].

Intestinal fungal changes in the progression of COVID-19

Although the number of fungal cells is lower than that of bacterial microbiome, their effect on modulating disease and health is obvious. Fungal microbiome mainly consists of *Ascomycota* and *Basidiomycota*. Fungal dysregulation and homeostasis are dynamic processes that continuously shape immune response in the host. Obviously different fungal microbiome compositions could help distinguish SARS-COV-2-negative controls from SARS-COV-2-positive patients. Zuo T *et al*. conducted a pilot study on fungal changes during hospitalization using metagenomic sequencing analysis. Like the microbial alterations in patients with community-acquired pneumonia, greater individual variabilities in fecal microbiota were shown in the fecal samples from COVID-19 group compared to the control group, revealing a higher heterogeneity. *C. albicans*, *C. auris*, and *A. flavus* multiplied during disease course, and it is interesting to note that every hospitalized patient who had fecal *A. flavus* performed cough, implying the possible links in gut-lung connections[14]. Infection of *Candida* was found to promote host inflammatory response and aggravate sepsis either alone or in cooperation with *S. aureus* [38,39]. However, another study was inconsistent with the findings by Zuo T *et al*. The researchers found that *Aspergillus* species and *P. citrinum* and *P. polonicum* belonging to *Ascomycota*, and *M. yamatoensis* and *R. mucilaginosa* belonging to *Basidiomycota*, were obviously depleted in infected patients, accompanied with an increase of fungal load. There was a positive correlation between *A. niger* and diarrhea symptom as well as *Mucoromycota* and *Fusicatenibacter*, and a negative correlation between *P. citrinum* and C-reactive protein[16]. But it was not clear whether the higher fungal load was a predisposition factor for patients or a secondary change after infection.

In addition, the fungal gut microbiome of critically ill patients showed significantly reduced α diversity and specific microbial clustering compared with non-critically ill patients, with *Ascomycota* phylum predominated with the proportion as high as 96.5%. The genus *Bipolaris* was abundant in severe illness and positively correlated with COVID-19 severity, whereas the abundance of *Lophodermium* and *Aureobasidium* were higher in patients with non-severe COVID-19 illness[23]. The latter was reported to produce metabolites with antimicrobial effects and performed high capacity against *C. albicans* infection[40]. While

Bipolaris is a plant pathogen that secretes certain products, has been showed to have immunosuppressive effects and can induce cell death in mammals[41,42].

There is limited evidence that persistent changes in intestinal fungi exist despite the elimination of SARS-COV-2 in infected patients. Certain patients with remission of respiratory symptoms continued to show intestinal fungal alterations than healthy controls at 0–12 days after nasopharyngeal clearance of SARS-COV-2. Two fungal species, *A. flavus* and *A. niger*, which are known to relate to respiratory infections, were still detected in the stool samples from recovered patients[14]. Persistent fungal changes may do harm to human health in the long run.

Intestinal microbial metabolism changes in the progression of COVID-19

Microbial function analysis was performed based on metagenomics sequencing analysis, and fecal metabolites were also evaluated and associated with disease progression. It was found that beneficial metabolic function changed in COVID-19 patients, characterized by impaired biosynthesis pathways of SCFAs and L-isoleucine and enhanced urea production capacity, and the functional impairment was related to the severity of the disease. Correspondingly, the concentrations of SCFAs and L-isoleucine were decreased in feces. A variety of pathways involved in SCFAs production were significantly negatively correlated with N-terminal pro-B-type natriuretic peptide, a marker of heart failure related to adverse clinical outcomes. L-isoleucine biosynthesis pathway showed a negative correlation with C-X-C motif ligand 10 and C-reactive protein, two blood inflammatory markers[20]. Generated from dietary fiber in the food with the effect of intestinal microbiota, SCFAs protect intestinal barrier function and reduce inflammation by activating their receptors, thereby maintaining intestinal homeostasis[43]. L-isoleucine is a kind of branched amino acid that confers an important role in regulation and maintenance of innate and adaptive immune system in *vivo* as well as in *vitro* [44]. These pathways might protect patients against inflammatory cytokine storms and poor prognosis, demonstrating a key role of microbiota in modulating host immunity. Similar to the results of urea from this study, Liu Q *et al.* conducted research of 133 patients hospitalized for COVID-19 and demonstrated that urea cycle-associated pathway was enriched in severe patients, and this process was mainly driven by *Klebsiella* species. Higher blood urea levels suggested impaired renal function and were associated with a poor prognosis[45]. Bacterial secretion system and lipoarabinomannan biosynthesis related to virulence factor secretion and pathogen infection were also enriched in children with COVID-19[17].

In addition, the decreased levels of desaminotyrosine (DAT) and secondary BAs containing deoxycholic acid, lithocholic acid, and isodeoxycholic acid in feces were found to be associated with improved mortality from respiratory failure and clinical outcomes, probably due to their involvement in the regulation of interferon and T cells[26]. It is interesting to note that sulfonated BAs levels in serum altered with disease severity of COVID-19, demonstrating the pathway disruption of BAs in severely ill patients because sulfonation is an important detoxification mechanism in the host[3]. As for the other metabolites, Nagata N *et al.* showed that multiple fecal amino acids (e.g., phenylalanine, glutamine, and glycine) were elevated and positively correlated with enriched microbiota and increased inflammatory cytokines in COVID-19 patients. While carbohydrate metabolism-related metabolites (e.g., maltose SCFAs, sucrose, and glyoxylic acid) as well as neurotransmitters (e.g., serotonin, γ-aminobutyric acid, and dopamine), indicated a positive association with COVID-19 depleted microbiota[12]. Some other functional pathways including biosynthesis and metabolism of nucleotide remained controversial, while some possibilities could still be described.

In addition to the effects of COVID-19 on gut microbiota described above, other respiratory viruses can also disrupt the structure of intestinal microbiota and are widely discussed. Some studies demonstrated the decrease in diversity and alterations of microbial composition in patients with influenza infections, manifested by the overgrowth of *E. coli* and *E. faecium*, as well as the decrease in *Bifidobacterium*, *Roseburia* and some butyrate-

producing bacteria[32,46]. Respiratory syncytial virus (RSV) is the principal cause of bronchiolitis in early childhood. Animal experiments demonstrated that RSV infection could lead to the increase in *Bacteroidetes* and decrease in *Firmicutes* abundance of gut microbiota in murine[47]. Harding *et al*. examined the fecal microbiota of infants with RSV infection, and reported the reduction in the α diversity of the gut microbiota[48]. Meanwhile, the growing abundance of *Clostridiales*, *Lactobacillaceae*, *Actinomyces*, and *Odoribacteraceae* was observed in the RSV-infected infants. These findings may be related to the changes of intestinal environment after infection, such as intestinal inflammation and barrier damage[49].

The stable status of intestinal microbiota is beneficial to host health. Certain individuals containing older age populations and people with chronic diseases (e.g., diabetes, cardiovascular diseases, and inflammatory bowel disease) perform changes of intestinal microbiota diversity and composition[50]. The microbial diversity is decreased in the elderly accompanied with an enrichment of Proteobacteria[51]. And the microbial structure differs significantly in individuals with chronic diseases compared to healthy controls, mainly manifested by low microbial diversity and high levels of *S. aureus* and *C. difficile*[52]. These alterations of intestinal microbiota are associated with enhanced susceptibility to COVID-19, increased disease severity, poor prognosis, and higher mortality. Based on the evidence described above, it is important to emphasize that SARS-COV-2 exerts a huge threat to gut microbiome. In the research process of COVID-19, the gut microbiota has gained enormous attention, but there is not enough evidence to reveal the key gut microbiota and metabolites associated with COVID-19 susceptibility and disease severity. Meanwhile, there are still many limitations. First, a variety of interfering factors can significantly affect the gut microbiome, such as diet, underlying diseases, comorbidities, genetic characteristics, gender, ethnicity, geographic environment, etc. These confounding factors must be noticed and controlled in human studies. Second, analyses based on stool samples cannot accurately reflect the function and structure of the gut microbiome due to the longer passage of stool through the colon. Third, the large differences in the individual gut microbiota make it difficult to reach an effective conclusion of microbial alteration between patients and healthy controls[53]. Fourth, further research on the key microbiota or metabolites during COVID-19 in human and animal models are needed. Therefore, the changes of gut microbiota and its metabolites during COVID-19 infection still have many unknown areas, which need to be studied with larger sample size and more precise exploration.

Long-term gut microbiota alteration after recovery from COVID-19

Prolonged clinical symptoms and comorbidities always occurred in COVID-19 survivors, which are collectively referred to as post-COVID-19 syndrome (PCS). World Health Organization defines PCS as a series of symptoms that appear 3 months after COVID-19 infection, last for at least 2 months, and cannot be explained by other diagnoses[54]. The most common manifestations of PCS were shortness of breath, fatigue, dyspnea, myasthenia, anxiety, cognitive dysfunction, sleep disorders, and a variety of multisystem symptoms[55–57]. Owing to high expression of angiotensin converting enzyme 2 (ACE2) receptors in the digestive tract, gastrointestinal symptoms are also involved in PCS, including diarrhea, nausea, abdominal pain, heartburn, etc[58]. Up to 76% of patients still had at least one symptom 6 months after clearance of SARS-COV-2, and the symptoms persisted for more than 2 years[36,59]. There was a higher rate of PCS in hospitalized patients compared with non-hospitalized patients, especially in the ICU hospitalized patients, due to pneumonia and secondary pathological lesions[59,60]. It is worth noting that SARS-COV-2 might persist for a long time in the digestive tract after the respiratory tract cleared virus. A most recent study revealed that 12.7% of subjects were still positive for fecal viral RNA 4 months after infection, and 3.8% of subjects had fecal SARS-CoV-2 RNA positive for at least 7 months[61]. Another study found that antigen of SARS-COV-2 could still be detected in the intestine of most patients with inflammatory bowel disease up to 7 months after infection, suggesting that the virus had not been completely cleared[62].

Persistence of viral RNA or antigens in the gastrointestinal tract, prolonged abnormal intestinal immune status, intestinal microbiota imbalance, and maladaptive neuroimmune interactions may synergistically promote the development of PCS[58].

Existing studies reported that gut microbiota alteration could last up to 14 months after recovery from COVID-19[63]. The alterations of gut microbiota and microbial metabolites are shown in Table 3. At an average of 28 days after discharge, low abundance of the immunomodulatory bacteria (e.g., *B. adolescentis*, *R. bromii*, and *F. prausnitzii*) and the capacity of SCFAs and L-isoleucine production kept impaired[21]. Vestad B *et al.* found that a trend of lower relative abundance of *Erysipelotrichaceae UCG-003* and higher relative abundance of *Veillonella* and *Flavonifractor* was observed in COVID-19 survivors 3 months after recovery, and these taxa showed a strong connection to persistent respiratory impairment. Some butyrate-producing species from *Lachnospiraceae* and *Ruminococcaceae* families were still at a low level[64,65]. And *F. prausnitzii* showed a negative correlation with long-term chest tightness after activity[66]. Six-month follow-up studies revealed that gut microbiome was in correlation with prolonged clinical symptoms in COVID-19 patients with PCS. In comparison to non-SARS-COV-2 infected controls, it was observed that multiple genera including *S. anginosus*, *S. vestibularis*, *C. innocuum*, and *A. naeslundii* showed positive correlations with prolonged respiratory and neuropsychiatric symptoms, whereas butyrate-producing bacteria including *B. pseudocatenulatum* and *F. prausnitzii* showed a negative correlation with hair loss and had the strongest negative association with PCS at 6 months. In addition, urea cycle pathway and biosynthesis pathways of certain amino acids (L-citrulline and L-ornithine) were increased in these patients. It is interesting to note that the intestinal microbiome profile at admission of individuals might be related to subsequent PCS progression[36,45]. For patients with 1-year recovery period, butyric-producing bacteria (e.g., *Eubacterium*, *Faecalibacterium*, and *Bifidobacterium)* was gradually enriched, accompanied with increased fecal α diversity and lower abundance of lipopolysaccharide (LPS)-producing bacteria such as *Intestinibacter* and *Prevotellaceae*. Of great significance is that the gut microbiome recovered more slowly than oral microbiome[67].

The role of gut microbiota in the pathogenies of COVID-19

According to existing knowledge on COVID-19, SARS-COV-2 entry into the host lead to gut microbiota and microbial metabolites alteration and a variety of clinical manifestations. Nevertheless, the exact mechanism is not fully understood at present. Here we reviewed the possible mechanisms by which gut microbiota participate in the immunomodulatory response and disease progress during SARS-COV-2 infection (Figure 1).

Gut microbiota participates in regulating ACE2 expression

ACE2 is the main active peptide of systemic and local renin-angiotensin system, and is expressed in a multitude of organs such as heart, kidney, lung, and intestine. Previous data have confirmed that ACE2 is associated with cardiovascular and cerebrovascular diseases, diabetes, and other diseases by regulating blood pressure, hydro-electrolyte balance, inflammation, cell proliferation, hypertrophy, and fibrosis[68]. ACE2 has already been considered as the key target of COVID-19 infection due to its specific binding to the spike protein of the SARS-COV-2 virus. Originally, SARS-COV-2 was only thought to be transmitted through respiratory droplets and contaminants, since the virus was initially detected in upper respiratory tract samples. And there is abundant expression of ACE2 in Clara cells, type I and II alveolar epithelial cells, and ciliated bronchial cell in the lung.

In fact, the expression of ACE2 in the intestine was more than four times higher in comparison to that in other tissues, especially in brush border of the proximal and distal enterocytes in the small intestine[69], and thus the human gut is more susceptible to COVID-19. Results from in *vitro* experiments have shown that SARS-COV-2 can readily infect human enterocytes in small intestinal organoids[70]. And two mucosa-specific serine proteases transmembrane serine protease 2 and transmembrane serine protease 4

Table 3. Long-term gut microbiota and metabolites dysbiosis in patients after recovery from COVID-19.

Country	Sample type	Time	Alterations of gut microbiota	Characteristics of fecal metabolites	Reference
China	66 COVID-19 patients and 70 non – COVID-19 controls	an average of 28 days after discharge	Enriched microbiota in patients: *B. thetaiotaomicron*, *B. caccae* Decreased microbiota in patients: *B. adolescentis*, *R. bromii*, *F. prausnitzii*	Persistent depletion of SCFA and L-isoleucine biosynthesis	[20]
Norway	149 COVID-19 patients	3 months after hospital admission	Enriched microbiota in patients with respiratory dysfunction: *Veillonella, Flavonifractor* Decreased microbiota in patients with respiratory dysfunction: *Erysipelotrichaceae UCG-003* and several members of the *Lachnospiraceae* family and *Ruminococcaceae* family	NA	[64]
China	7 COVID-19 patients and 7 non-COVID -19 controls	3 months after discharge	Enriched microbiota in patients: *Flavonifractor, Eubacterium, Rothia, C. Microthrix, Erysipelatoclostridium* Decreased microbiota in patients: *Faecalibacterium*, *E. hallii* group, *Collinsella*, *Erysipelotrichaceae UCG-003*, *NK4A214* group	SCFAs levels of recovered patients were normal	[65]
China	15 COVID-19 patients and 14 non-COVID -19 controls	3 months after discharge	Enriched microbiota in patients: *Escherichia, Flavonifractor, Intestinibacter* Decreased microbiota in patients: *Faecalibacterium, Roseburia, Fusicatenibacter, Ruminococcus, Clostridium XVIII, Dorea, Butyricicoccus, Romboutsia, Intestinimonas, Bilophila*	NA	[66]
China	68 COVID-19 patients and 68 non-COVID -19 controls	6 months after hospitalization	Enriched microbiota in patients with PCs: *R. gnavus, B. vulgatus* Decreased microbiota in patients with PCs: *C. aerofaciens, F. prausnitzii, B. obeum*	Increased abundance of urea cycle pathway, L-citrulline biosynthesis pathway, L-ornithine biosynthesis II pathway	[36]
China	133 COVID-19 patients	6 months after viral clearance	Enriched microbiota in patients with PCs: *E. ramosum, C. bolteae, C. innocuum*	NA	[45]
China	35 COVID-19 patients and 166 healthy controls	1 year after recovery	Increased microbiota in the process of recovery: *Eubacterium, Fusicatenibacter, Agathobacter, unclassified Lachnospiraceae, Faecalibacterium* Decreased microbiota in the process of recovery: *Fusobacterium, Intestinibacter, Prevotellaceae*, *Muribaculaceae,Mitsuokella*	NA	[67]
China	155 patients with COVID-19 and 155 matched subjects without COVID-19	an average of 14 months after SARS-CoV-2 viral clearance	Enriched microbiota in patients with PCs: *R. gnavus, C. bolteae, F. plautii, E. ramosum* Decreased microbiota in patients with PCs: *G.formicilis, B. adolescentis*	NA	[63]

Abbreviations: COVID-19, coronavirus disease-19; PCS, post-COVOD-19 syndrome.

can promote the entry of SARS-COV-2 into intestinal epithelial cells to infect small intestinal organoids[71]. When ACE2 binds to SARS-COV-2, it exhausts and down-regulates ACE2 expression[72]. Under physiological conditions, ACE2 interacts with broad neutral amino acid transporter 1 (B0AT1) in the gut, and then participates in maintaining intestinal homeostasis through regulating the homeostasis of amino acids, the expression of antimicrobial peptides, and the dynamics of the gut microbiome[68]. ACE2 is required for the expression and function of B0AT1, and deficiency of B0AT1 can lead to the occurrence and development of Hartnup disease, which is mainly manifested by diarrhea[73]. Results of an animal experiment have revealed that intestinal amino acid malabsorption due to ACE2 depletion is linked to intestinal inflammation and diarrhea. Compared with wild-type mice, ACE2-defective mice did not show changes in intestinal villi structure, but aggravated dextran sodium sulfate as well as trinitrobenzene sulfonic acid-induced experimental colitis. Due to the absence of ACE2 receptor, B0AT1 cannot be normally expressed in the intestine of deficient mice, and various dietary amino acids such as tryptophan are mainly absorbed through the B0AT1/ACE2 transport pathway on the surface of the intestinal epithelium[74]. Tryptophan metabolism is involved in the immunomodulation in the gut, microbiota,

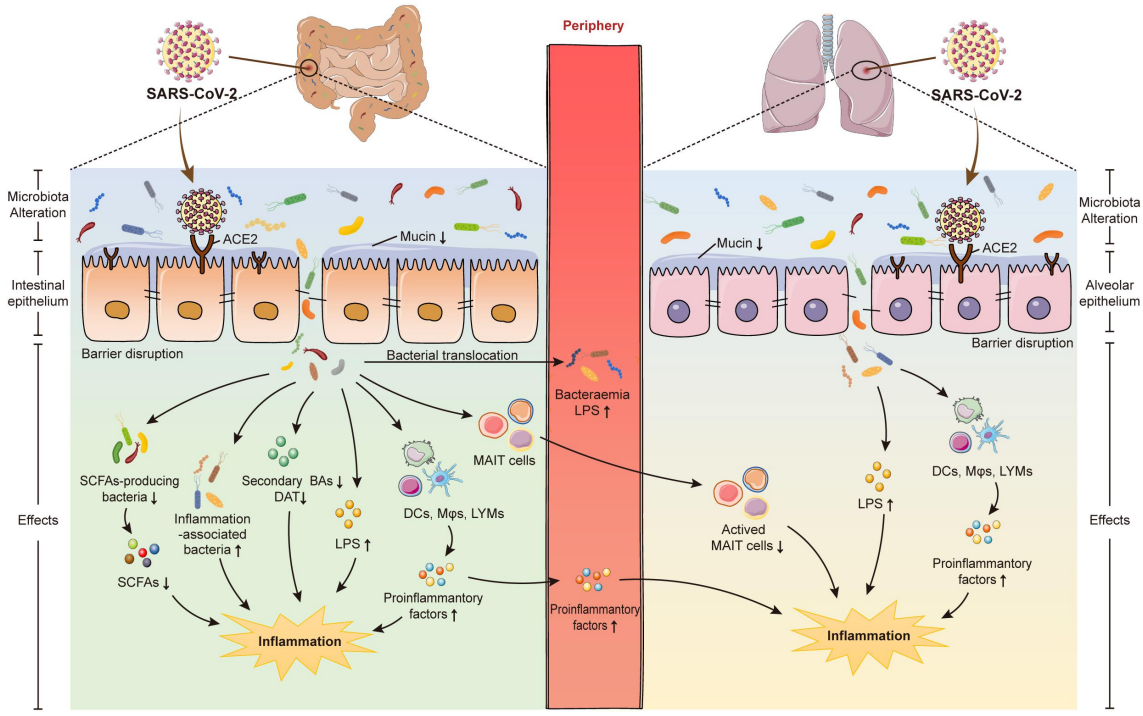

Figure 1. The role of gut microbiota in the pathogenies of COVID-19. Gut microbiota alteration contributes to immune dysfunction and severe disease in COVID-19 course. ACE2 in the gastrointestinal and respiratory tracts mediates SARS-COV-2 entry into the human body and subsequently triggers microbiota alteration and barrier function impairment in the gut and lung. Gut microbiota alteration is characterized by enrichment of inflammation-associated bacteria and decrease of SCFAs-producing bacteria. Gut microbiota alteration and barrier disruption provide opportunities for bacteria translocation, LPS and pro-inflammatory factors increase, SCFAs and secondary BAs and DAT decrease, taken together resulting in intestinal inflammation. Depleted commensal metabolites influence activation of MAIT cells in the host. Intestinal bacteria and inflammatory factors transfer into the blood and cause bacteremia and systemic inflammation. In the lung, microbiota alteration and barrier disruption increase LPS levels and trigger respiratory inflammation. COVID-19, coronavirus disease-19; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SCFAs, short-chain fatty acids; DAT, desaminotyrosine; BAs, bile acids; LPS, lipopolysaccharide; ACE2, angiotensin converting enzyme 2; DCs, dendritic cells; LYMs, lymphocytes; Mφs, macrophages; MAIT, mucosal associated invariant T.

immune cells, liver, and brain[75]. Impairment of tryptophan uptake results in imbalanced local tryptophan homeostasis in the gut, down-regulated expression of antimicrobial peptides, and then increases the susceptibility to intestinal inflammation[76].

What's more, there are bidirectional regulatory links between ACE2 receptor and gut microbiota. Transplantation of fecal bacteria from ACE2 defective mice into germ-free mice could transmit intestinal inflammation to germ-free mice, suggesting the regulatory effect of ACE2 on intestinal microbiota[74]. Edwinson A *et al.* first reported that gut microbiota could alter ACE2 expression in animals, and their research found that the expression of colonic ACE2 was significantly reduced in mice with humanized intestinal microbiota in comparison to germ-free mice[77]. And four *Bacteroides* spp. were shown to be related to downregulation of ACE2 receptor[2]. *Clostridia* species and microbiota-derived metabolites, SCFAs, could reduce the levels of ACE2 receptor both in the intestine and lung[78,79]. Taken together, down-regulation of ACE2 expression after SARS-CoV-2 infection may partially explain the gastrointestinal symptoms of COVID-19, and gut microbiota may serve as a key determinant of the susceptibility to COVID-19 infection and clinical outcomes in disease course by its effects on modulating ACE2 expression.

The intestinal barrier dysfunction in COVID-19

With the deepening of intestinal studies, intestinal barrier is composed of mucous layer, epithelial barrier, and immune barrier, which is involved in the regulation of microbiota-gut-lung axis, and its damage will trigger the occurrence and

development of numerous respiratory diseases. Plenty of human and animal research has confirmed that SARS-COV-2 interacts the gut and leads to loss of intestinal barrier integrity, known as leaky gut.

For in *vitro* test, monolayer cell culture assay results demonstrated the fact that SARS-CoV-2 affected tight junctions, adherens junctions, and gap junctions after infection of human colon cancer epithelial cell line-Caco-2 cells and *T*-84 cells[80,81]. Guo Y *et al.* established a three-dimensional co-culture model including human intestinal epithelial and mucosal and vascular endothelial barrier. The results showed that SARS-CoV-2-infected intestinal epithelium and damaged the integrity of the intestinal barrier, characterized by injury of intestinal villi, diffuse distribution of mucus-secreting cells, and decrease of cadherin expression. Abnormal cell morphology and tight junction disruption were performed in vascular endothelial cells[82]. For in *vivo* studies, results from animal models including rhesus monkeys and hamsters suggested that SARS-CoV-2 infection led to mucus barrier dysfunction by affecting goblet cells. The cell proliferation marker Ki67 and intestinal damage marker intestinal fatty-acid binding protein were also significantly inhibited in the digestive tract in response to SARS-CoV-2 infection[83,84]. In human, metabolomics analysis identified human-origin proteins from stool and blood samples of infected patients. Among them, the expression of hemoglobin-associated protein components was increased, with enrichment of cell immunoglobulin in stools of patients, indicating the possibility of gastrointestinal cell damage and bleeding. As for changes in blood, the plasma concentration of intestinal damage-related biomarker, LPS-binding protein, was elevated in COVID-19 patients, especially in severe cases. Moreover, LPS-binding protein was found to have a positive connection with inflammation, the percentage of immune cells, and markers of cardiac injury, which suggested a trend toward severe diseases, even multisystem inflammatory syndrome[22,85,86]. And gastrointestinal bleeding may be a clinical response to intestinal injury and barrier dysfunction during disease progress[87]. Intestinal proteome analysis showed immune disturbance, proteolytic and redox homeostasis, and the disturbance persisted possibly more than 2 months[19].

In the host, gut barrier disruption provides opportunities for pathogenic bacteria and endotoxins such as LPS to translocate from the intestinal tract to the systemic circulation and even other organs, increasing the risk of secondary bloodstream infections and other severe diseases like respiratory distress syndrome[28,88]. Therefore, it is necessary to demonstrate the potential mechanisms by which SARS-COV-2 defects gut. As previously described, ACE2 binds to B0AT1 to play a key role in intestinal homeostasis, including influencing the production and release of antimicrobial peptides, mediating intestinal nutrient uptake, and inhibiting inflammatory cytokines to regulate immune function. ACE2-mediated barrier dysfunction induced by SARS-COV-2 infection may result in the development of immune disorders, and then gastrointestinal symptoms may be shown. On the other hand, intestinal homeostasis depends on the benign interaction of intestinal microbiota, intestinal barrier, and immune system. Intestinal epithelial cells extract microbiota-derived signals to regulate intestinal barrier and innate and adaptive immune maturation, and thus maintain intestinal homeostasis[89]. Gut microbiota alteration may interfere with the normal function of the intestinal barrier, leading to increased intestinal permeability[90]. Generally speaking, protecting gut barrier function is expected to be a possible target for COVID-19 management.

Gut microbiota modulates host immune function during COVID-19 infection

During COVID-19 infection, the increased severity of clinical signs in patients was not only attributed to SARS-COV2 entry and replication in human, but also associated with subsequent inflammatory storm, taken together triggering systemic inflammatory response, increased vascular permeability, and multiple organ damages. The excessive immune response promoted the release of many inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α, and aggravated the manifestations in COVID-19[91]. There is a bidirectional

complicated connection between gut and lung, named as gut-lung axis, and the connection has implications for the progress of COVID-19[92]. The microbiome in the gastrointestinal and respiratory tract is essential for the development of immune function and physiological homeostasis, and influences the course of disease. Here we describe the possible links among SARS-COV-2 and gut microbiota alteration and severe disease.

On the one hand, SARS-COV-2 infection destroys gut microbiota homeostasis and increases levels of pro-inflammatory factors. The invasion of SARS-COV-2 into the host could be recognized by a multitude of immune cells including dendritic cells (DCs) and macrophages *via* activation of pattern-recognition receptors (PRRs), and promoted production and release of pro-inflammatory factors. These changes in turn resulted in gut barrier dysfunction and inflammation-associated bacteria enrichment in the intestine[93,94]. And The downregulation of ACE2 and its molecular chaperone expression induced by SARS-COV-2 infection promoted bacteria invasion and upregulates pro-inflammatory response[95].

On the other hand, it is known that alterations of the structure and composition of gut microbiota have recently been thought to be connected to dysregulated immune responses and COVID-19 disease development. Signals derived from the gut microbiota are known to modulate the pro-inflammatory and anti-inflammatory responses of immune cells, thereby influencing susceptibility and severity to various respiratory diseases. Ichinohe T *et al*. showed that antibiotic treatment-induced depletion of gut commensal bacteria, especially Gram-positive bacteria, impaired DCs activation in the respiratory tract and reduced DCs migration from the lung to draining lymph nodes and T-cell priming, which led to immunodeficiency. And the key process was mediated by inflammasome activation[96]. Gut microbiota is employed as the source of microbial associated molecular patterns and pathogen-associated molecular patterns. Both the molecular patterns are perceived by PPRs like toll-like receptors (TLR)[97]. Pathogen-associated molecular patterns released from pathogens can also be recognized by DCs and macrophages *via* PRRS, and induce the expression of pro-inflammatory factors[94]. LPS is a crucial virulence factor produced by Gram-negative bacteria, with strong ability of promoting pro-inflammatory response *via* microbial associated molecular patterns[98]. LPS produced by gut microbiota (mainly derived from Gram-negative bacteria) promotes nuclear transcription factor-κB activation by TLR4/myeloid differentiation factor 88/ nuclear transcription factor-κB signaling pathways and increases downstream pro-inflammatory factors in respiratory and digestive tract. What's more, LPS is reported to inhibit the expression of downregulating colonic mucosal resolution D1 levels in colonic cluster cells through the TLR4-myeloid differentiation factor 88 pathway, thereby increasing intestinal sensitivity to mechanical stimuli and low-grade inflammation[99]. Moreover, microbiota-derived signaling influences the effector function of mucosa-associated invariant T cells involved in defense against infectious pathogens. COVID-19-induced depletion of commensal symbionts can negatively affect the balance and recruitment of mucosa-associated invariant T cells, leading to the development of respiratory infections[100,101].

What is more serious is that impairment of intestinal barrier function promoted intestinal bacteria and their harmful metabolites such as LPS and pro-inflammatory factors to translocate to lung and many other organs through systemic circulation. The accumulation of these undesirable substances aggravates the inflammatory response in the lungs and exacerbates respiratory symptoms, and even induces acute respiratory distress syndrome[102]. It is interesting to note that multiple enterogenic bacteria was shown in the lung microbiota in COVID-19 patients with acute respiratory distress syndrome, which directly indicated the crosstalk between the intestine and lung[103].

The effects of microbial metabolites in COVID-19 course

Some effects of gut microbiota in the gut-lung connection may be mediated by numerous microbial metabolites in the intestine and have attracted much attention. SCFAs as well as BAs and some amino acids have been found to show some positive effects on the host.

As series of known metabolites, SCFAs provide energy for colonic cells and harbor beneficial anti-inflammatory effects. Although some evidence regarding the ability of SCFAs to resist SARS-COV-2 infection and replication is controversial, SCFAs promoted regulatory-T cell development through G protein coupled receptors and regulated the coagulation response to enhance adaptive immunity to COVID-19 infection[78,104]. SCFAs especially butyrate is the most crucial component for maintaining intestinal barrier function. They are mainly involved in orchestration of several kinds of tight junction proteins including claudins, occludin, and zonula occludens, and are able to promote production and secretion of mucin and antimicrobial peptides, thus contributing to decrease of translocation of bacteria and toxins and resistant of pathogens infection[105,106]. In animal studies, reduction of intestinal and blood SCFAs (especially acetate) levels after influenza A virus infection inhibited the bactericidal activity of alveolar macrophages and promoted subsequent double bacterial infection. While the negative effects could be inhibited by acetate supplementation by activating free fatty acid receptor 2[107]. What's more, SCFAs limited megakaryocyte proliferation and platelet production, leading to thrombocytopenia and coagulation limitation, which in turn prevented patients from evolving to severe infection[78].

Another kind of well-known signaling metabolite, BAs, participate in host immunity modulation, which is synthesized in the liver and then transferred into the gut and metabolized by gut microbiota. BAs own important signaling functions regulating nutrient absorption and lipid metabolism, shaping gut microbiota, and maintaining intestinal barrier with classical nuclear receptors such as farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5[108,109]. In a recent study, FXR was identified as an essential regulator of ACE2 transcription in the tissues invaded by SARS-COV-2, and ursodeoxycholic acid could decrease the levels of ACE2 and protect from COVID-19 infection *via* suppressing FXR signaling[110]. BAs such as tauroursodeoxycholic acid and glycoursodeoxycholic acid showed an antagonistic action on FXR, whereas FXR could be activated by cholic acid, chenodeoxycholic acid, lithocholic acid, and deoxycholic acid[111,112]. Furthermore, in the lung, ursodeoxycholic acid is beneficial to inhibit the migration of abnormal airway epithelial cells and enhance the repair capacity, thus promoting restoration of respiratory epithelium[113]. Moreover, it is showed that reduced secondary BAs levels were associated with poor prognosis in patients. Secondary BAs mainly comprising deoxycholate and lithocholate are crucial immune modulators in inflammatory response, wound healing, and the host's susceptibility to certain infections[114]. Specific new secondary BAs 3β-hydroxydeoxycholic acid can effectively induce regulatory-T cell differentiation in colon[115]. An interesting study from mice demonstrated that antibiotic administration promoted accumulation of primary BAs and induced bile salt hydrolase activity impairment, resulting in inhibition of the endoribonuclease Nsp15 and exerting antiviral effect[116].

Several other microbial metabolites have also shown therapeutic potential for COVID-19. Leupeptin is a family of secondary metabolites, which are produced by *actinomycetes* and consist of serine, cysteine, and threonine proteases. The results of enzyme assay and cell culture both demonstrated leupeptin could inhibit SARS-COV-2 replication through blunt of SARS-COV-2-induced proteases[117]. DAT is a kind of the product of amino acids and flavonoids with the effects of commensal bacteria such as *C. orbiscindens*. DAT prevented influenza and maintained mucosal immunity by enhancing type I interferon signaling, and protected mice from bacterial endotoxin-induced septic shock[118,119]. More detailed studies are needed to further investigate the effects of microbial metabolites during COVID-19 progress.

In addition, results from in *vitro* experiments showed that SARS-CoV-2 could replicate in bacterial growth medium isolated from COVID-19 patients' fecal samples and directly infected the bacteria in the intestine, demonstrating its possibility as a bacteriophage[120,121]. Subsequent research confirmed the presence of SARS-CoV-2 nucleocapsid protein both inside and outside of the bacteria. And the virus could replicate and act as a bacteriophage in at least two bacterial species including anti-inflammatory bacterium *F. prausnitzii* and *D. formicigenerans*, which

provided evidence for the negative correlation between depletion of anti-inflammatory symbionts and COVID-19 severity[122]. These data have important role for our understanding of SARS-COV-2, and further studies based on animal or human models will make it more convincing.

COVID-19 Management: Focus On Gut Microbiota

Dietary intervention

The nutritional status of COVID-19 patients was taken seriously because of its strong connection with clinical outcomes. The assessment of nutritional status in infected patients should not be ignored, as it has influence on susceptibility, course, severity of virus infection and response to treatment[123].A healthy, balanced, and anti-inflammatory diet, mainly characterized by sufficient intake of vegetables, fruits, and other healthy foods, is a crucial part of subsequent care. Complex carbohydrates and dietary fiber are fermented by gut microbiota to produce a large amount of SCFAs, which help reduce inflammatory response and enhance host immunity[124,125]. Bousquet J *et al.* found that cabbage and fermented vegetables were helpful to mitigate the severity of COVID-19 patients, and the clinical results might be associated with increased antioxidant effects[8]. And vice versa, a clinical study consisting nearly 600,000 subjects demonstrated a significant synergistic association between poor diet quality and lower socioeconomic status and COVID-19 risk[126]. Alcohol consumption inhibits the nature killer cell response and depletes other types of lymphoid cells which increased susceptibility to viral infections[127].

Focus on nutrition, there are also some studies on specific types of diets. Proinflammatory diets including high-fat and high-sugar and high-salt diets continuously reduce the abundance of *Bifidobacterium* and *Lactobacillus*, activate mechanistic target of rapamycin via PRRs, and trigger intestinal dysfunction[128]. Data from animal experiments showed that high-fat high-sugar diet increased disease severity and delayed recovery in COVID-19 infected Syrian hamster model[129]. However, the ketogenic diet, characterized by highly restrict in carbohydrates and high in fat, is an effective strategy for weight loss and visceral fat loss, suppression of respiratory and intestinal inflammation, and regulation of immune function, which may be beneficial to alleviation of severe disease and poor outcomes[128,130]. Low-fat diet is reported to promote the expansion of anti-inflammatory bacteria *F. prausnitzii* and enhance acetate levels in the stool of patients with ulcerative colitis[131]. And there was a protective effect of low-fat dairy products on COVID-19[132]. For high-protein diet, it down-regulates SCFAs contents, particularly butyrate, and enriches several metabolites that are averse to mucosal homeostasis[133]. And a clinical study found that a low- carbohydrates, high-protein diet was associated with a higher risk of moderate-to-severe COVID-19[134]. Besides, mediterranean diet, a recognized healthy diet model with high consumption of plant foods, is revealed to relate to reduction of inflammatory biomarkers and risk of respiratory infections. In regard to its role in COVID-19, the lowest absolute risks of COVID-19 were always present in the group with better adherence to the mediterranean diet[135,136]. Although studies have shown some results, stronger clinical studies and microbiota analysis are needed for dietary intervention.

Furthermore, certain kinds of non-nutritive bioactive substances (such as polyphenols) and vitamins as well as trace elements also play an important role in modulation of gut-lung axis. As is well known, ACE2 is the key receptor for invasion, and tea polyphenols could support the prevention and intervention of SARS-CoV-2 infections *via* inhibiting the expression of ACE2 and transmembrane serine protease 2. At the same time, tea polyphenols not only own a positive effect on protecting intestinal barrier, but also promote growth of beneficial bacteria and have an inhibitory action on numerous pathogenic bacteria[137,138]. Today, the role of vitamins for COVID-19 intervention remains controversial. Data from prospective research showed that there was a negative correlation between the risk of COVID-19 infection and dietary intake of vitamin C, vitamin D, vitamin B9 and vitamin K, and a positive association between the COVID-19 infection and calcium intake[139,140]. A study of 254 elderly patients with COVID-19 found that oral supplementation with a high dose of vitamin D3

within 3 days of diagnosis significantly reduced 14-day mortality[141]. While some other studies revealed that vitamin D supplementation had no obvious effect on preventing COVID-19 and a single high dose of vitamin D3 did not reduce the hospitalized time in patients with moderate to severe COVID-19[142,143]. More detailed analysis needs to be conducted to develop effective strategies for dietary intervention, and individual dietary programs can be considered.

Administration of probiotics/prebiotics and beneficial microbial metabolites

As already mentioned, microbiota alteration exists in a number of COVID-19 patients. Automatically, probiotics or prebiotics administration has been the following way to defend against the virus. Studies have suggested that probiotics/prebiotics play a role in improving the mucosal barrier and accommodating immunologic function, thence SARS-CoV-2 infection can be defended. The potential effects of probiotics/prebiotics therapy have been evaluated in a growing number of clinical and experimental studies in humans, which make effort to reduce the risk, duration, and severity of influenza, rhinovirus, and respiratory syncytial virus[144–146]. Though the safety and effect of probiotics among COVID-19 patients or in preventing infection are still questionable, a lot of people support probiotics therapy because they themselves benefit on intestinal homeostasis, inflammation, and immunologic function[9,147,148]. Now, new evidence of probiotics/prebiotics therapy on SARS-CoV-2 has come out gradually. Probiotics showed significant positive impacts on SARS-CoV-2 in patients, such as easing symptoms, decreasing the incidence of respiratory failure, and shortening the mortality and stay in hospital[149–151]. And on ClinicalTrials.gov, there are dozens of clinical trials registered and in progress to assess the probiotics for COVID-19. It is worth noting that the use of probiotics probably resulted in adverse outcomes like bacteremia in certain populations including ICU patients, the elderly, and immunocompromised individuals. It is necessary for rational administration of probiotics[152,153].

In addition, prebiotics, fructans, and galactans are recommended to provide nutrition for beneficial microbes. On account of hard-digested, prebiotics produce SCFAs that modulate gut microbiota and the immunologic function[154]. Accordingly, prebiotics are recommended for defending against virus infections. Indeed, the metabolites of microbiota can be beneficial mediators for viral infection. In COVID-19 course, SCFAs showed benefits on inhibiting SARS-COV-2 infection and upregulating the ability of adaptive immune response and alleviating clinical symptoms in animals and human[3,78]. Ursodeoxycholic acid was reported to suppress FXR signaling and decrease ACE2 levels and protect host from COVID-19 infection[110]. Leupeptin could inhibit SAR-COV-2 replication through blunt of SARS-COV-2-induced proteases in intestinal cells[117]. And DAT was demonstrated to fight against influenza and decrease lung immunopathology and was related to respiratory failure in severe COVID-19 patients[119].

Fecal microbiota transplantation

FMT is an emerging method for the treatment of intestinal and extra-intestinal diseases by transplanting functional intestinal microbiota extracted from healthy volunteers into the patients' intestine to rebuild their microecological environment. FMT has been widely recognized as a clinical strategy for recurrent *C. difficile* infection, and its application in cancer and its complications have been increasingly emphasized[155]. Since that there are remarkable changes in the gut and airway microbiota in patients with COVID-19, it has become a hot topic of discussion whether FMT can repair the intestinal microbiota alteration and restore microbiota-gut-brain axis and then treat COVID-19.

However, there are not many published studies of FMT in the treatment of COVID-19, which may be limited by the complexity of operation process and the strict requirements for safety. Back in 2021, FMT was first reported to be used on two Polish patients with *C. difficile* infection co-infected with COVID-19. It is surprising to note that both two patients showed rapid resolution from COVID-19, demonstrating that FMT seems to be safe and effective in

the treatment of COVID-19 in patients[10]. A Chinese study enrolled 11 COVID-19 patients, and four consecutive days of FMT were given one month after they were discharged from the hospital. Gastrointestinal symptoms were relieved in 5 patients, and fecal microbiota analysis showed that FMT increased the richness of gut microbiota in these patients, especially increased the abundance of *Bifidobacterium* and *Faecalibacterium*[156]. In addition, Zhang *et al.* performed washing microbiota transplantation in patients with COVID-19 complicated with refractory intestinal infection, and the disease severity of the patients was relieved after treatment[157]. The above results indicate that FMT holds promise as a potential treatment for COVID-19, but its safety and dose selection still require further research and discussion. Given the existence of possible fecal-oral transmission routes, establishing stricter screening standards for donors and employing FMT for treatment with caution at this unusual period are indispensable. To decrease the risk, the US Food and Drug Administration recommends that obtained microbiota from fecal bank donated before December 1, 2019, was able to be prepared for relatively safe use, while subsequent stool donations cannot be used for clinical treatment temporarily until additional screening and testing procedures were completed[158–160].

Herbal medicine

Herbal antiviral therapy has a long history. Previous studies have also confirmed the effectiveness of herbal medicine in the treatment of severe acute respiratory syndrome and influenza A infection[161]. Due to the safety (low toxicity) and availability of herbal medicine, screening and extraction of beneficial compounds targeting viral or host from herbal medicine may become a potential strategy for treating COVID-19. According to reports, almost 85% of COVID-19 patients in China received herbal medicine treatment and their symptoms were obviously relieved[162].

Huang *et al.* predicted by molecular docking that some herbal derived compounds, such as quercetin, glycyrrhizin, andrographolide and curcumin, could exert antiviral utility by targeting proteins such as spike protein, 3CLpro, PLpro, RdRp, and ACE2[163]. Lianhua Qingwen, a traditional and classic herbal medicine, has played an indelible role in the fight against the COVID-19. *In vitro*, Lianhua Qingwen significantly inhibited SARS-CoV-2 replication and resulted in abnormal virus morphology, accompanied by a downregulation of proinflammatory cytokine levels[164]. Further investigation suggested that Lianhua Qingwen could help relieve respiratory symptoms, effectively reduce the proportion of common type progressing to severe type, and shorten the recovery time in COVID-19 patients[165]. Extracts of some herbal medicine, such as lamiaceae, asteraceae and theaceae, have been found to reduce the cytopathogenic effect of SARS-CoV-2 on Vero E6 cells[166]. What's more, certain types of herbal medicine have been found to regulate the structure and metabolism of gut microbiota and restore intestinal barrier function, thus contributing to improving the pathological conditions in the host[11,167]. At present, several randomized controlled studies and cohort studies are under way, and the preliminary results show the advantages of herbal medicine in the treatment of COVID-19[168,169]. We believe that herbal medicine, which has both antiviral and gut microbiota-regulating effects, can lead to better treatment results when combined with approved western medicine.

Conclusion and perspective

COVID-19 pandemic exerts a dramatic impact worldwide. Many studies reveal that SARS-CoV-2 infection leads to pathophysiological changes in a variety of organs and systems, but the mechanisms are not well defined. Some patients infected with SARS-COV-2 perform gastrointestinal symptoms besides respiratory and systemic manifestations. Alterations of gut microbiota and microbial-derived metabolites and dysregulation of microbiota-gut-lung axis have been proposed

to play a crucial role in disease progression, disease severity, and subsequent PCS development during virus infection. The key gut microbiota and metabolites associated with COVID-19 susceptibility, disease severity, and PCS need to be determined. In future, larger sample sizes and more detailed studies are needed to exclude numerous confounding factors on gut microbiota including medication, underlying diseases, geographic environment, and individual differences.

The potential role of gut microbiota offers an unprecedented chance to explore new effective treatments for COVID-19. Since SARS-COV-2 will last for a long time and even recur periodically, targeting gut microbiota for relieving clinical symptoms may promise a road ahead to alleviate clinical manifestations and comorbidities in infected patients. In the present review, we propose dietary intervention, probiotics/prebiotics, microbial metabolites supplementation, FMT, and the application of herbs to combat COVID-19. Diet is known to participate in shaping the gut microbiota, and further research can focus on individual dietary intervention that regulate gut microbiota disruption and host immune function dysregulation. Until now, there is more evidence that reveals the benefits of probiotics as well as prebiotics in treating COVID-19, but the optimal strains and dose remain to be determined. Probiotics must be used with caution in immunocompromised patients. As for microbial metabolites supplementation and FMT, despite the limited evidence for relieving COVID-19, there are some possibilities in the future. While quite several results show the advantages of herbal intervention, more compelling evidence grades are needed and the specific active ingredients in herbal medicine remain to be elucidated.

Abbreviations

COVID-19	coronavirus disease-19
SARS-CoV-2	severe acute respiratory syndrome coronavirus-2
SCFAs	short-chain fatty acids
FMT	fecal microbiota transplantation
DAT	desaminotyrosine
BAs	bile acids
PCS	post-COVID-19 syndrome
LPS	lipopolysaccharide
ACE2	angiotensin converting enzyme 2
DCs	dendritic cells
PRRs	pattern-recognition receptors
TLR	toll-like receptors
FXR	farnesoid X receptor
RSV	respiratory syncytial virus
B0AT1	broad neutral amino acid transporter 1
MAIT	mucosa-associated invariant T.

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