

ROLE OF GUT MICROBIOTA IN ACUTE COVID-19 AND POST-ACUTE COVID-19 SYNDROME

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Abstract – Coronavirus disease-2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) mostly affects the respiratory system but emerging data showed that the human gut is also involved. Microbes in the gut play crucial roles in maintaining immune regulation and metabolic homeostasis. The imbalance of the gut microbiota composition, also known as dysbiosis, has been linked to COVID-19 severity and post-acute COVID-19 syndrome (PACS). In this review, we summarise data from studies published from 2020 to 2021 that have explored the interplay of gut microbiota and COVID-19 susceptibility and severity. We discuss the role of gut microbiota in regulating immunity against SARS-CoV-2 infection and highlight new associations between specific bacteria species and SARS-CoV-2 vaccine efficacy. Therapeutic approaches that target gut dysbiosis in alleviating acute COVID-19 symptoms and preventing PACS are rapidly being developed and tested in clinical trials.

Keywords: COVID-19, Gut microbiota, Microbiota, Long COVID, PACS.

INTRODUCTION

The novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]), responsible for coronavirus Disease 2019 (COVID-19), continues to cause a global toll. By May 2022, more than 520 million cases of SARS-CoV-2 infection have been reported worldwide¹. Apart from respiratory involvement, studies have shown that SARS-CoV-2 leads to multiorgan dysfunction, especially in high-risk patients.

The gastrointestinal (GI) tract has emerged as an important organ influencing the severity of, and susceptibility to COVID-19 infection. Several lines of evidence have suggested the involvement of the GI tract such as replication of SARS-CoV-2 in human enterocytes²⁻⁴, detection of viruses in faecal samples^{5,6} and altered gut microbiota composition including increased abundance of opportunistic pathogens and reduced abundance of beneficial symbionts in the gut of patients with COVID-19⁷⁻¹⁰. Furthermore, the gut microbiome has been linked to



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the regulation of local and systemic immune and inflammatory activity, and hence may be pivotal in influencing the immune response to COVID-19. Beyond regulation of the host immune system, the gut microbiota influences many other normal functions including regulation of host homeostasis, physiological processes, and the assembly of co-residing gut bacteria, which could potentially play an important role in the pathophysiological mechanisms determining COVID-19 outcomes. Prior data^{11,12} showed that downregulation of angiotensin-converting enzyme 2 (ACE2), caused by the entry of SARS-CoV-2, can consequently lead to an altered microbiota that confers susceptibility to inflammation of the gut. Collectively, SARS-CoV-2 invades gut enterocytes through ACE-2 and causes changes in gut microbiota and their metabolites, impaired epithelial barrier function, and bacterial translocation into the circulation, leading to aggravated systemic inflammation and multiple-organ involvement.

In this review, we discuss the latest data from papers published between April 2021 and March 2022 that have reported a link between gut microbiome and pathogenesis and disease progression of COVID-19 infection. The gut microbiome has emerged as a potential therapeutic target and could represent a resource for the restoration of SARS-CoV-2 intestinal mucosa damage through modulation of gut microbiota and related inflammation.

Alterations of Gut Microbiome in Acute COVID-19

Indigenous microbiota are essential components for maintaining human health and mediating diseases¹³, and its composition is influenced by both environmental and host factors¹⁴. Gut microbiota dysbiosis may trigger immune dysregulation and pro-inflammatory effects associated with various diseases. Dysbiosis is defined as a disruption of the normal microbiota ecology which involved the loss of beneficial microbes or metabolites and an expansion of pathobionts¹⁵. Studies⁸ have reported that faecal samples from COVID-19 patients showed marked alterations in faecal microbial communities (based on metagenomic sequencing) compared with those of healthy controls. The gut microbiome of COVID-19 patients showed marked dysbiosis correlating with dysfunctional immune responses and elevated inflammatory markers compared with controls. In studies from different populations and geography, microbial diversity in faecal samples of patients with COVID-19 was found to be decreased and characterised by depletion of short-chain fatty acid (SCFA; crucial for maintaining intestinal homeostasis)-producing bacteria from the *Lachnospiraceae*, *Ruminococcaceae*, and *Bifidobacteriaceae* families, and enrichment of opportunistic pathobionts. In addition, the gut microbiota composition of patients during the acute infection was correlated with the abnormal immune response to COVID-19. Depletion of several bacterial species such as *Faecalibacterium prausnitzii*, *Eubacterium rectale* and bifidobacteria was linked to elevated blood levels of TNF- α , CXCL10, CCL2 and IL-10 indicating that these depleted taxa may have a role in preventing overaggressive inflammation⁸. Opportunistic pathogens such as *Enterococcus faecalis* and *Saccharomyces cerevisiae* were enriched in COVID-19 patients with fever. *E. faecalis* was positively correlated with blood levels of LDH and D-dimer and negatively correlated with CD8+T cells and IL-4, while *S. cerevisiae* was positively correlated with symptoms of diarrhea¹⁶. Furthermore, several species with anti-inflammatory and protective effects, such as *Bacteroides fragilis* and *Eubacterium ramulus*, were enriched in patients who did not have fever¹⁶. Enrichment of *Ruminococcus gnavus*, *Ruminococcus torques*, *Bacteroides dorei* and *Bacteroides vulgatus* found in COVID-19 was also consistent with the inference of a microbial-mediated immune dysregulation. For example, *R. gnavus* and *R. torques* have been reported to be abundant in the gut of patients with inflammatory bowel disease^{17,18}, *Bacteroides dorei* and *Bacteroides vulgatus* have been implicated in several inflammatory gut diseases such as irritable bowel disease and ulcerative colitis¹⁹.

The loss of beneficial microbial is thought to trigger pro-inflammatory effects and immune dysregulation associated with various disease states. Overall gut microbiome in patients with COVID-19 was characterised by a significant reduction of gut commensals such as *F. prausnitzii*, *E. rectale*, *Blautia obeum*, *Bifidobacteria adolescentis*, and *Bacteroides caccae*^{8,20}, which has been linked to reduced host inflammatory response in other inflammatory-related diseases. For instance, *F. prausnitzii* has been shown to induce priming of human colonic regulatory T cells that secrete the anti-inflammatory cytokine IL-10²¹, and *B. adolescentis* can suppress activa-

tion of nuclear factor κ B that promotes expression of proinflammatory cytokines²². However, it remains unclear whether inflammatory-associated gut microorganisms enriched in COVID-19 play an active part in disease or simply flourish opportunistically due to a depletion of other gut microorganisms.

Children appeared to be less susceptible to COVID-19 and they also manifested lower morbidity and mortality after the infection, for which a multitude of mechanisms may be considered. Intestinal dysbiosis has been reported in children with COVID-19. Two small cohorts have described the gut microbiome in children with COVID-19. Nashed et al²³ reported decreased abundances of *Bifidobacterium bifidum* and *Akkermansia muciniphila* in SARS-CoV-2 positive faecal samples; these two bacteria were linked to protection against inflammation. *Bifidobacterium* is a pioneering coloniser of the gut microbiota and has immunomodulatory properties. *B. bifidum* was found to be inversely correlated with COVID-19 severity in adults. It has also been shown that gut microbiome changes are detectable in asymptomatic infants infected with SARS-CoV-2²³, accompanied by a decrease in anti-inflammatory bacteria taxa similar to that seen in symptomatic adults. The impact of microbial alterations and their impact on subsequent immune and inflammatory responses in children is unknown but deserves further exploration given the risk of development of autoimmune and autoinflammatory conditions in children with COVID-19. A recent study evaluated the longitudinal dynamics of the upper respiratory tract and the gut microbiome in children and found that the respiratory and the gut microbiomes presented a contemporaneous and persistent dysbiosis from acute infection to a long period after acute infection. Alterations of the microbiome were dominated by the genus *Pseudomonas*, and they lasted for up to at least 19-24 days after discharge from the hospital²⁴. They found dominant opportunistic pathogens had a negative correlation with some common commensals (including *Faecalibacterium*, *Roseburia*, *Prevotella* and unclassified *Clostridia*) in the acute and convalescent phase. Disturbed development of both the gut and the upper respiratory microbiome implies possible a slow improvement of the microbiome in these COVID-19 children and increased health risk of the post-COVID effects on these COVID-19 children²⁴.

Additionally, several studies determining the functional capacity of gut microbiota in patients with COVID-19 revealed that SARS-COV-2 infection was associated with impairment of amino acid metabolism and carbohydrate metabolism, especially production of short-chain fatty acids, arginine and L-isoleucine, tryptophan which are crucial mediators in the microbiota-host crosstalk and play important roles in regulating host innate and adaptive immunity²⁵⁻²⁷. In a gnotobiotic mice model, variable ACE2 expression levels in the GI tract and respiratory tracts were modulated by gut microbiota suggesting that the gut microbiome may be one crucial factor determining the risk of COVID-19 infection²⁸.

Dysbiosis in Post-Acute COVID-19 Syndrome (PACS)

Recent evidence has shown that over three-quarters of COVID-19 patients suffered debilitating symptoms after the acute infection²⁹, known as post-acute COVID-19 syndrome (PACS), characterized by persistent symptoms or long-term complications beyond 4 weeks from the onset of symptoms³⁰. This population is at high risk of persisting health impairments six months after discharge associated with reduced physical function and health-related quality of life²⁹. Studies²⁹⁻³¹ have shown that PACS can affect the whole spectrum of people with COVID-19, from those with very mild disease to the most severe forms. Like acute COVID-19, long covid can involve multiple organs and can affect many systems including, but not limited to, the respiratory, cardiovascular, neurological, gastrointestinal, and musculoskeletal systems. It has been hypothesized that perturbations of immune and inflammatory responses, the presence of residual viral particles, and cellular damage by acute viral infection or critical illness may contribute to the pathogenesis of PACS. As the gut microbiota plays a crucial role in the maturation of the immune system³¹, aberrant immune response to COVID-19 infection induced by resident microorganisms may also affect the recovery process. A recent study⁸ has shown that gut dysbiosis in patients with COVID-19 persisted for up to 30 days after clearance of SARS-CoV-2, suggesting it may increase susceptibility to reinfections or predispose subjects to long-term health issues. The influence of the microbiome configuration on susceptibility and complications is an area under intense

investigation, as studies have reported decreased abundances of *Bacteroides* species, which are thought to have a protective role in SARS-CoV-2 infection in patients with metabolic comorbidities as compared to healthy subjects^{9,20}. The gut microbiome of patients with COVID-19 also showed an impaired capacity for SCFA and L-isoleucine biosynthesis that persisted after disease resolution⁸.

Altered gut microbiota of COVID-19 patients, including decreased alpha diversity and under expression of certain bacterial species, was shown to be associated with impaired pulmonary function³². Vestad et al³² found that several members of the *Lachnospiraceae* and *Ruminococcaceae* families, such as *Coproccoccus* and *Ruminococcus*, which are known butyrate producers, were reduced in the gut of patients with COVID-19 who also suffered from pulmonary impairment at three months after admission. The decreased microbial diversity and compositional gut microbiota alterations in patients with respiratory dysfunction after 3 months together with persistently raised plasma levels of lipopolysaccharide-binding protein levels pinpoint a potential involvement of the gut-lung axis in patients with PACS. Gut microbiota richness of COVID-19 patients also failed to restore to normal levels 6 months after recovery. The relative abundance of members of *Ruminococcus* and *Bifidobacterium* remained significantly lower in patients with COVID-19 compared with non-COVID-19 controls in the longer term³³.

In a recent study, Liu et al³³ tracked longitudinal dynamics of the gut microbiota from stool samples of 106 COVID-19 patients at three hospitals in Hong Kong followed from admission until 6 months after recovery, and compared them with people who did not have COVID-19. COVID-related dysbiosis persisted after acute infection and showed correlations with PACS. At 3 months, 86 of the patients with COVID-19 had PACS- defined as at least one persistent, otherwise, unexplained symptom at 4 weeks after viral clearance. Stool analysis showed that microbial diversity and richness, were significantly lower at 6 months in those with PACS, compared with those without PACS and with controls. Patients with PACS had distinct gut microbiome dysbiosis, characterised by increased levels in the relative abundance of *R. gnavus*, *B. vulgatus* and reduced levels of *F. prausnitzii* when compared with those without PACS. Interestingly, a total of 81 bacterial species were associated with different categories of PACS and many of the bacteria species were associated with more than two categories of persistent symptoms. For instance, persistent respiratory symptoms were correlated with opportunistic gut pathogens, and neuropsychiatric symptoms and fatigue were correlated with nosocomial gut pathogens, including *Clostridium innocuum* and *Actinomyces naeslundii*. These data suggest that alterations of the gut microbiome induced by the initial infection or immune responses generated at admission or during the acute infection may reflect the susceptibility of the individual to long-term COVID-19 complications. Bacteria species including *Blautia wexlerae* and *B. longum* at admission were negatively correlated with PACS at six months, implying the putative protective role of these species in the recovery process³³. In contrast, species *Actinomyces_sp_S6_Spd3*, *Actinomyces johnsonii* and *Atopobium parvulum* were related with PACS at six months. The overlap of bacteria species such as *R. gnavus*, *C. innocuum*, and *Erysipelatous ramosum* that remained altered from baseline to follow-up and exhibited association with several PACS symptoms further highlighting the link between altered gut microbiome composition and the long-term complications in COVID-19 patients. These findings altogether support the intricate associations between the gut microbiome composition at baseline and the long-term sequelae after COVID-19 infection.

CONCLUSIONS AND FUTURE DIRECTIONS

The human gastrointestinal tract harbours a diverse microbial community that plays important roles in gastrointestinal and respiratory tract diseases, including preventing colonization of microbial pathogens and regulation of host immunity. Accumulating data suggest that the human gut microbiota plays a crucial role in the pathogenesis of COVID-19. This review summarized three major findings from data generated in the past year (Figure 1). First, numerous studies have confirmed changes in the composition of the gut microbiome characterised by overall lower microbial diversity in hospitalised COVID-19 patients. Secondly, specific gut bacteria species were associated with blood inflammatory markers and pro-inflammatory cytokines suggesting that the gut microbiome can impact COVID-19 severity potentially via modulating host immune

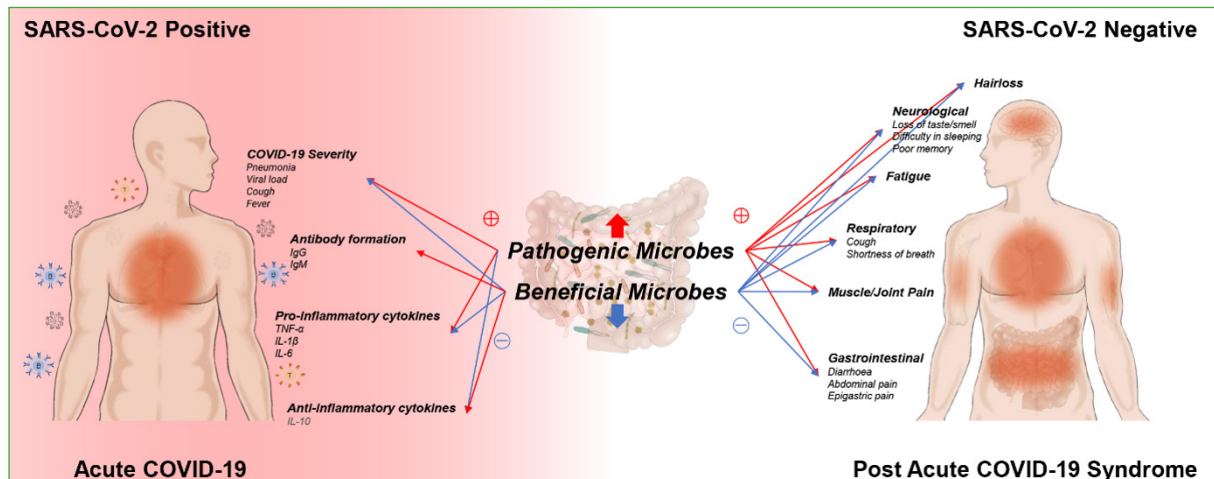


Figure 1. Schematic summary of persistent alterations in the fecal microbiome from acute infection phase to post-acute COVID-19. In acute COVID-19, specific gut bacteria species were associated with disease severity, blood inflammatory markers and pro-inflammatory cytokines suggesting that gut microbiome can potentially impact COVID-19 severity and pathophysiology via regulating host immune responses. Post-acute COVID-19 syndrome is defined as persistent symptoms beyond 4 weeks from the onset of symptoms. Distinct fecal opportunistic pathogenic species showed significant correlation with PACS, whereas some beneficial commensals showed an inverse correlation.

responses. Thirdly, after clearance of SARS-CoV-2, patients who developed PACS showed a lower microbial diversity and an increase in opportunistic pathogens in their gut compared with patients without PACS. These microbiota alterations included reduced abundances of species in the genus *Faecalibacterium*, *Eubacterium*, and *Roseburia*, and increased abundance of species in the genus *Actinomyces*. The associations between gut microbial composition with development of PACS have highlighted the potential of microbiome-based intervention in the treatment of COVID-19 and its complications. Further mechanistic studies are required to examine how specific bacteria species influence SARS-COV-2 pathogenesis and their impact on different variants including Omicron. A key of gut microbiota in immunity against SARS-CoV-2 infection and microbiota modulation to improve SARS-CoV-2 vaccine efficacy should be explored in clinical studies.

Conflict of Interest

S.C.N and F.K.L.C. are the scientific co-founders and sit on the board of Directors of GenieBiome Ltd. S.C.N has served as an advisory board member for Pfizer, Ferring, Janssen, and Abbvie and a speaker for Ferring, Tillotts, Menarini, Janssen, Abbvie, and Takeda. She has received research grants from Olympus, Ferring, Janssen and Abbvie. F.K.L.C. has served as an advisor and lecture speaker for Eisai Co. Ltd., AstraZeneca, Pfizer Inc., Takeda Pharmaceutical Co., and Takeda (China) Holdings Co. Ltd. Z.X. and W.T. are part-time employee of GenieBiome Ltd. All other co-authors have no conflict of interest.

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Informed Consent

Not applicable.

Authors' Contribution

QL and QS conceived and took responsibility for the preparation of the manuscript. IRL, FZ, organized the databases. FKLC contributed to the design. SCN contributed to the direction, guidance, and manuscript writing. All authors gave final approval for the version to be published.

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