Gut microbiota in the pathogenesis of COVID-19: viral replication and transmission (abridged secondary publication)

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KEY MESSAGES

1. Prolonged and active SARS-CoV-2 virus remained in the gut of patients with COVID-19, even after recovery.
2. Both gut bacterial and viral microbiota were disrupted in patients with COVID-19, persisting for up to 6 months after disease resolution.
3. Several gut commensal bacteria with known immunomodulatory potential—*Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Bifidobacteria*—and two RNA virus species derived from pepper plants were underrepresented in these patients.
4. Depletion of these bacterial and viral taxa was associated with more severe disease and higher levels of inflammatory cytokines and blood markers.

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Introduction

Although most COVID-19 cases are mild, the illness can result in hospitalisation, respiratory failure, or death.[1] The prevalence of patients exhibiting gastrointestinal symptoms, including diarrhoea, was 2% to 10% in early reports from Wuhan and was up to 20% in a meta-analysis.[2-5] SARS-CoV-2 was detected in anal swabs and stool samples of almost 50% of patients, suggesting that the digestive tract can be a site for viral replication and activity.[6,7] Moreover, faecal calprotectin was elevated in COVID-19 patients with diarrhoea,[8] indicating an inflammatory response in the gut. SARS-CoV-2 enters host cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is highly expressed in both the respiratory and gastrointestinal tracts.[9-11] ACE2 also plays important roles in controlling intestinal inflammation and gut microbial ecology.[12] The gut microbiome affects immune response and metabolism. The commensal microbiota is dynamic and can be modulated by invading viruses to elicit either stimulatory or suppressive responses.[13] Respiratory viral infections may alter the gut microbiome, increasing susceptibility to secondary bacterial infections.[14,15] Meta-transcriptomic analysis of bronchoalveolar lavage fluid from infected patients revealed dominance of pathogens or commensal bacteria in the oral and upper respiratory tracts.[16] Comorbidities in patients with severe COVID-19 have been linked to shifts in bacterial taxa from the phyla *Bacteroidetes* and *Firmicutes*,[17-20] which regulate *ACE2* expression in rodents.[21] Host microbial perturbations may impact response to infection and the efficacy of future immune interventions such as vaccines.[22] We hypothesised that SARS-CoV-2 replicates in the gastrointestinal tract and alters gut microbiota, leading to worsened clinical manifestations. This study aimed to explore the roles of the gastrointestinal tract and gut microbiota in the pathogenesis of COVID-19.

Methods

In total, 50 hospitalised patients with laboratory-confirmed SARS-CoV-2 infection, 30 hospitalised patients with community-acquired pneumonia (pneumonia controls), and 30 healthy controls were recruited. Stool and plasma samples were collected. Clinical data were collected prospectively using a standardised template developed by the International Severe Acute Respiratory and Emerging Infection Consortium.

Laboratory investigations included measurement of viral load in faecal samples, profiling of faecal microbes (including viruses and bacteria), inflammatory cytokine profiling (interleukin [IL]-1β, IL-6, IL-10, IL-12p70, tumour necrosis factor-α, C-X-C motif chemokine ligand [CXCL] 8, CXCL9, CXCL10, C-C motif chemokine ligand [CCL] 2, and CCL5), *ACE2* gene expression analysis, and bacterial co-culture studies of Caco-2 and HT-29 cells.

Results

SARS-CoV-2 nucleic acid was detected in the faeces

of 73.3% of patients during hospitalisation (median viral load, 3.86 $\times 10^3$ copies per mL inoculum), whereas active SARS-CoV-2 infection was confirmed in 46.7% of patients, with substantially higher genomic coverage at the 3′ end relative to the 5′ end of the SARS-CoV-2 genome in faecal viral metagenomes, even after clinical recovery. Patients with COVID-19 showed disrupted bacterial and viral microbiota, which persisted for up to 6 months after recovery. Several gut commensal bacteria with known immunomodulatory properties—*Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Bifidobacteria*—and two RNA virus species derived from pepper plants were underrepresented in patients. Decreased abundances of these bacterial and viral taxa were associated with more severe disease and elevated concentrations of inflammatory cytokines and blood markers including C-reactive protein, lactate dehydrogenase, aspartate aminotransferase, and gamma-glutamyl transferase. No significant correlations were observed between *ACE2* gene expression and disease severity, blood biomarkers, or gut microbiota composition. *Bacteroidetes* species (*Bacteroides dorei*, *Bacteroides thetaiotaomicron*, and *Bacteroides massiliensis*) did not downregulate *ACE2* expression in epithelial cells.

Discussion

Patients with COVID-19 had substantial active viral infection and replication in the gastrointestinal tract, which in some cases persisted after respiratory clearance of SARS-CoV-2. Clearance of gastrointestinal infection appeared to be delayed. The implications of this delayed clearance for viral transmission remain unclear.

The gut microbiome was disrupted in patients with COVID-19, characterised by enrichment of opportunistic pathogens and depletion of beneficial commensals. Loss of salutary species persisted in most patients for up to 6 months after clearance of SARS-CoV-2. Given that many recovered patients reported symptoms such as fatigue, dyspnoea, and joint pain, we speculate that a dysbiotic gut microbiome contributes to post-COVID-19 complications. Further investigations are needed to determine whether dysbiosis or specific microbial imbalances predispose individuals to future health issues.

SARS-CoV-2 infection may induce dysfunctional immune responses and cytokine storm syndrome in a subset of patients, resulting in more severe disease. The gut microbiota plays a key role in regulating the development and function of both the innate and adaptive immune systems. This study showed that depletion of several gut commensal bacteria—*F prausnitzii*, *E rectale*, and *Bifidobacteria*—and two RNA virus species derived from pepper plants was associated with more severe disease and elevated levels of inflammatory cytokines and blood markers. *F prausnitzii* has been shown to prime human colonic regulatory T cells to secrete the anti-inflammatory cytokine IL-10.[19] Higher relative abundances of *E rectale* in the gut have been linked to reduced inflammation in Alzheimer's disease,[10] and *Bifidobacterium adolescentis* is able to suppress the activation of nuclear factor-κB, a protein that promotes expression of pro-inflammatory cytokines.[21] The pepper-derived RNA virus is the most abundant and prevalent plant RNA virus found in human faeces; it has been proposed as an indicator of faecal contamination in aquatic environments and water treatment systems. SARS-CoV-2 may modulate host immunity and create an unfavourable environment for certain RNA viruses. The lysis or clearance of these viruses in the gut may lead to the release of nucleic acids, proteins, and lipids that act as pathogen-associated molecules that trigger inflammation. These gut microorganisms play a broader role in modulating systemic inflammation; their depletion in COVID-19 may contribute to severe disease and inflammatory symptoms via dysregulation of host immune responses.

Conclusion

Prolonged and active SARS-CoV-2 was detected in the gut of patients with COVID-19, even after recovery, highlighting the importance of long-term surveillance and the potential risk of faecal-oral transmission. Both gut bacterial and viral microbiota were disrupted in patients with COVID-19 and could persist for up to 6 months after recovery; the disruption was associated with disease severity and immune responses. These findings underscore the urgent need to elucidate the specific roles of gut microorganisms in immune regulation and systemic inflammation in the context of COVID-19.

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Disclosure

The results of this research have been previously published in:

1. Zuo T, Zhang F, Lui GC, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. Gastroenterology 2020;159:944-55.
2. Zuo T, Zhan H, Zhang F, et al. Alterations in fecal fungal microbiome of patients with COVID-19

during time of hospitalization until discharge. Gastroenterology 2020;159:1302-10.
3. Zuo T, Liu Q, Zhang F, Lui GCY, et al. Depicting SARSCoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. Gut 2021;70:276-84.
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