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Review

The gut microbiome: an under-recognised contributor to the COVID-19 pandemic?

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Abstract

The novel coronavirus infection (COVID-19) caused by the SARS-CoV-2 virus has spread rapidly across the globe, culminating in major global morbidity and mortality. As such, there has been a rapid escalation in scientific and clinical activity aimed at increasing our comprehension of this virus. This volume of work has led to early insights into risk factors associated with severity of disease, and mechanisms that underpin the virulence and dynamics involved in viral transmission. These insights ultimately may help guide potential therapeutics to reduce the human, economic and social impact of this pandemic. Importantly, the gastrointestinal (GI) tract has emerged as an important organ influencing propensity to, and potentially severity of, COVID-19 infection. Furthermore, the gut microbiome has been linked to a variety of risk factors for COVID-19 infection, and manipulation of the gut microbiome is an attractive potential therapeutic target for a number of diseases. While data profiling the gut microbiome in COVID-19 infection to date are limited, they support the possibility of several routes of interaction between COVID-19, the gut microbiome, angiotensin converting enzyme 2 (ACE-2) expression in the small bowel and colon and gut inflammation. This article will explore the evidence that implicates the gut microbiome as a contributing factor to the pathogenesis, severity and disease course of COVID-19, and speculate about the gut microbiome's capability as a therapeutic avenue against COVID-19.

Keywords: COVID-19, gastrointestinal disease, microbiota, SARS-CoV-2

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Lay summary

It has been noted that certain baseline gut profiles of COVID-19 patients are associated with a more severe disease course, and the gut microbiome impacts the disease course of several contributory risk factors to the severity of COVID-19. A protein called ACE-2, which is found in the small intestine among other sites, is a key receptor for COVID-19 virus entry; there is evidence that the gut microbiome influences ACE-2 receptor expression, and hence may play a role in influencing COVID-19 infectivity and disease severity. Furthermore, the gut microbiome plays a significant role in immune regulation, and hence may be pivotal in influencing the immune response to COVID-19. In terms of understanding COVID-19 treatments, the gut microbiome is known to interact with several drug classes being used to target COVID-19 and should be factored into our understanding of how patients respond to treatment. Importantly, our understanding of the role of the gut microbiome in COVID-19 infection remains in its infancy, but future research may potentially aid our mechanistic understanding of viral infection, and new ways in which we might approach treating it.

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Introduction

The severe global disease burden caused by COVID-19 infection has resulted in almost unprecedented levels of rapid activity by the international scientific community across various disciplines in an attempt to understand pathogenesis and develop treatment options for this novel virus, with a focus on slowing and preventing its further impact. One particular intriguing facet of COVID-19 infection is the marked heterogeneity of clinical presentation, with some infected people being asymptomatic whilst others progress to multi-organ failure and death.[1] Comparison of clinical, molecular and immunological data from a large Chinese cohort of COVID-19 patients demonstrated that viral genetic variation did not seem to be associated with disease severity, while host factors (including age and immune response) appeared to be much more important contributory factors.[2] As such, exploring further exactly what these host factors are is of key clinical importance.

A number of emerging clinical and scientific strands of research have implicated the gastrointestinal (GI) tract as an important organ for propensity to, and severity of, COVID-19infection. As one example, GI symptoms (including nausea, vomiting and diarrhoea) have been described consistently as common clinical features of infection in addition to typical respiratory and constitutional symptoms.[3] In addition, COVID-19 has been detected in tissues from throughout the GI tract,[4] and virus shedding in stool has been detected in a significant proportion of patients,[5] with such shedding often occurring for prolonged periods[6]; raised faecal calprotectin in association with infection has also been described.[7] Furthermore, emerging evidence using organoid models has shown that COVID-19 can infect the GI tract directly[5]; in particular, the SARS-CoV-2 virus uses angiotensin converting enzyme 2 (ACE-2), which is highly expressed on differentiated enterocytes, as a receptor for cell entry before inducing a viral response programme.[8,9]

In this article, we review the evidence that may implicate the gut microbiome as a contributory factor to the pathogenesis of COVID-19 infection, by reviewing what has been described specifically regarding the gut microbiome in COVID-19 patients to date. We also consider whether the gut microbiome may be relevant to our consideration for treatment of COVID-19, such as through its influence on anti-viral therapy or vaccine efficacy. Finally, we discuss practical difficulties in investigating the impact of the interaction between the SARS-CoV-2 virus and the gut microbiome and suggest key areas for future focus.

Profiling of the gut microbiome in patients with COVID-19 infection

As of October 2020, only very limited data have been described specifically on the gut microbiome profile in patients with COVID-19 infection. A cross-sectional study of 30 COVID-19 patients, 24 influenza A (H1N1) patients and 30 matched healthy controls (HC) attempted to identify differences in the gut microbiota by 16S ribosomal RNA (rRNA) gene V3–V4 region sequencing.[10] Compared with healthy controls, patients with COVID-19 had significantly reduced bacterial diversity, a significantly higher relative abundance of opportunistic pathogens (including *Streptococcus*, *Rothia*, *Veillonella* and *Actinomyces*) and a lower relative abundance of beneficial symbionts. Furthermore, it was noted that *Fusicatenibacter, Romboutsia, Intestinibacter, Actinomyces* and *Erysipelatoclostridium* could distinguish between the COVID-19 group and the healthy controls, with receiver operating characteristic (ROC)-plot area under the curve (AUC) value of 0.89 [95% confidence interval (CI), 0.8–0.97]. When comparison was made between patients with COVID-19 and influenza, it was also observed that patients with influenza had significantly lower alpha and beta diversities that those with COVID-19. There were further differences noted at the phylum level, with decreased abundances of Actinobacteria and Firmicutes in the influenza group compared with the COVID-19 group. There were further differences highlighted at the family level, including a decrease in putative aerobic butyrate-producing bacteria in the influenza group. This study suggests there may be differences in the gut microbiome between those with COVID-19 and those without, but these remain associative without clear evidence of causality.

Further supporting evidence regarding alterations in the gut microbiota in COVID-19 came from a study in Hong Kong that used shotgun metagenomics to analyse the serial composition of the stool microbiome of 15 patients during the course of COVID-19 infection, comparing this with the stool microbiome of patients with community-acquired bacterial pneumonia and HC.[11] Samples were collected two to three times per week until discharge, which was variable for each patient. Baseline stool (defined as the first stool donated

following hospital discharge) samples from COVID-19 patients were enriched in a range of pathobionts (associated with bloodstream infections) compared with controls, with baseline samples from COVID-19 patients treated with antibiotics ($n=8$) also being depleted of symbionts associated with host immunity, including *Faecalibacterium prausnitzii*.[11] Those bacterial species under-represented in the stool microbiome of COVID-19 patients compared with controls at baseline remained at low or undetectable levels throughout the disease course, even when SARS-CoV-2 was no longer detectable on nasopharyngeal or stool swab, and when respiratory symptoms had resolved.

Of further interest was the observation that members of the bacterial phylum Firmicutes (specifically, the genus *Coprobacillus*, and the two species *Clostridium ramosum* and *Clostridium hathewayi*) were associated with increased clinical severity of COVID-19 disease. Clinical severity was defined as mild, if there was no radiographic evidence of pneumonia; moderate, if pneumonia was present along with fever and respiratory tract symptoms; or severe, if respiratory rate $\geqslant$30/min, oxygen saturation $\leqslant$93% when breathing ambient air, or $PaO_2/FiO_2 \leqslant 300$ mmHg (1 mmHg = 0.133 kPa) or critical. This association is of particular interest, since *Coprobacillus* has been recognised to upregulate colonic ACE-2 strongly in the gut of mice.[12] One of the bacterial species most strongly negatively correlated with severity of COVID-19 was *Faecalibacterium prausnitzii*; this association is of note given the links that have hitherto been made between this bacterial species and anti-inflammatory activity within the gut.[13] An additional finding was a negative correlation between faecal SARS-CoV-2 load and the abundance of specific gut bacteria, particularly of a number of species from within the genus Bacteroides (*Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis* and *Bacteroides ovatus*). It has been noted previously that these species were associated with a reduction in ACE-2 expression within the mouse gut,[12] suggesting that they may limit the ability of COVID-19 to access enterocytes and cause infection *via* this mechanism. Further work from the same group noted higher abundance of short chain fatty acid (SCFA)-producing bacteria in stool samples from COVID-19 patients with low SARS-CoV-2 infectivity.[14]

A further study from the same group explored the alterations in the faecal fungal microbiome in patients with COVID-19. In this study, deep shotgun metagenomic sequencing analysis was performed on faecal samples from 30 patients with COVID-19 and compared with 9 subjects with community acquired bacterial pneumonia and 30 HC.[15] The same definitions were used as in the previous study. It was noted that patients with COVID-19 had significant alterations in their faecal mycobiomes compared with controls, characterized by enrichment of *Candida albicans* and a highly heterogeneous mycobiome configuration, at time of hospitalization. Furthermore, researchers found that samples collected at all timepoints from patients with COVID-19 had increased proportions of opportunistic fungal pathogens, *Candida albicans*, *Candida auris* and *Aspergillus flavus*, compared with controls. Importantly, there are likely confounders to this study, with the authors correctly identifying that determining whether these changes are a cause or an effect of COVID-19 needs to be explored further.

As such, overall, while data profiling the gut microbiome in COVID-19 infection to date are limited, they support the possibility of several routes of potential interaction between SARS-CoV-2, the gut microbiome, intestinal ACE-2 expression, and gut inflammation.[16]

Importantly, some of these associations have also been noted in critical illness and, hence, further studies are needed to explore changes in the gut microbiome and compare this with those with COVID-19.

Potential associations between COVID-19 risk factors and the gut microbiome

As clinical experience of COVID-19 infection grows, a range of different risk factors – both for initial acquisition of infection, and for outcome in infected patients – have emerged. In this section, we explore potential links between these risk factors and the gut microbiome. Importantly, many of these risk factors are likely to influence not only the gut microbiome but potentially also other mechanisms that alter the outcomes of COVID-19. Therefore, the changes in the gut microbiome are, at this stage, associative and are unlikely to be independent risk factors.

Old age

Across continents and multivariate analysis, age has been shown consistently to be a risk factor for

death from COVID-19.[17] This risk is likely to be multifactorial. The diversity of the gut microbiota in elderly individuals has been shown to be reduced compared with that of younger adults.[18–20] Frailty was associated negatively with alpha diversity of the gut microbiota.[21] The 'elderly' microbiome generally showed a shift away from Firmicutes, which dominated in younger adults, toward genera such as Alistipes and Parabacteroides.[22] It is believed that perturbations in the gut microbiota in the elderly trigger the innate immune response and may lead to chronic low-grade inflammation, leading to frailty and unhealthy aging.[23] Many patients with COVID-19 present with respiratory symptoms and one of the serious manifestations of COVID-19 is the development of pneumonia and acute respiratory distress syndrome. The gut–lung axis has been proposed in the pathogenesis of respiratory infections.[24] Patients with viral lung infections had an increase in the relative abundance of Bacteroidetes and a decrease in the relative abundance of Firmicutes in their gut, which was similar to the gut microbiota composition of elderly individuals.[25] Moreover, COVID-19 associated pneumonia and adverse outcomes from COVID-19 seen in elderly subjects may relate to the changes in the gut microbiome. Importantly, perturbations in the gut microbiome are common across a range of diseases with many confounders, such as medications, that are common in the elderly; therefore, this association needs to be evaluated further.

Diabetes mellitus

Diabetes mellitus (DM) has been shown to be a significant risk factor for mortality from COVID-19.[26] Patients with COVID-19 who have underlying diabetes had a two-fold increased risk of mortality compared with non-diabetic COVID-19 patients.[27] Gut microbiota composition has been shown to be altered in patients with diabetes.[28] Patients with diabetes had significantly reduced proportions of the phylum Firmicutes and the class Clostridia compared with non-diabetic subjects. Diabetic patients had significantly more Bacteroidetes and Proteobacteria.[29] The presence of Bifidobacterium, Bacteroides, Faecalibactrium, Akkermansia and Roseburia was suggested to be protective against type 2 DM, while Ruminococcus, Fusobacterium and Blautia were found to be positively associated with type 2 DM.[30] There have also been studies showing that the use of probiotics containing Lactobacillius and Bifidobacterium can delay the onset of glucose intolerance and lower fasting glucose in type 2 DM,[31,32] highlighting further a potential role of gut microbiota in the pathogenesis of DM. In this regard, perturbations in the gut microbiota in patients with DM might have a contributory role in the poor prognosis of COVID-19.

Hypertension

Hypertension is a common comorbidity associated with more severe course and mortality amongst patients with COVID-19.[33] Nearly 30% of patients hospitalised for COVID-19 had hypertension,[34] and the presence of hypertension was associated with a 2.5-fold increased risk of mortality due to COVID-19.[35] Hypertension is associated with increased gut wall permeability and microbial perturbations.[36] Patients with hypertension had less SCFA producers, including *Faecalibacterium* and *Roseburia*, while opportunistic pathogenic taxa including *Klebsiella* spp., *Streptococcus* spp. and *Parabacteroides merdae* were of increased abundance, compared with HC.[37] Bacterial diversity was associated negatively with hypertension and systolic blood pressure.[38] Both animal and human studies have shown that the administration of probiotics with Lactobacillus could effectively lower systolic and diastolic blood pressure.[38,39] The bioactive peptides produced from probiotics have ACE inhibitory properties during the fermentation process. ACE inhibition then lowers the synthesis of ACE-2, which results in attenuation of vasoconstriction and blood pressure. ACE-2 can modulate the gut microbiota.[40] There has been debate on whether the use of ACE inhibitors is associated with higher risk of COVID-19 infection or more severe disease and mortality associated with COVID-19. However, recent population-based and case control studies did not show that the use of renin-angiotensin-aldosterone system inhibitors was associated with an increased risk of COVID-19 infection or more severe disease or higher mortality.[41,42] One might speculate instead that it is the gut microbiota acting as a co-factor influencing worse outcomes in COVID-19 patients with hypertension.

Ethnicity

Ethnic minorities have had a disproportionately higher mortality from COVID-19,[43] but the underlying mechanisms remain poorly understood. Different ethnic groups have a broad range of different dietary, socioeconomic, genetic, lifestyle and environmental exposures. Importantly, it has been

demonstrated that there are significant variations in the gut microbiome of ethnic minorities.[26] In a study that explored geographical variations in the microbiota, it was found that microbial richness and evenness were highest in Hispanics, followed by Caucasians and Asian Pacific Islanders, with lowest richness found in south-Asian Surimanese and the highest distribution of uneveneess found in Moroccans.[44] Furthermore, it has been noted that geographical location showed the strongest associations with microbiota variations.[45] In a Dutch study that analysed 25,000 adults from various backgrounds it was found that ethnicity contributed to interindividual dissimilarities in gut microbiota composition.[44] An enrichment in *Prevotella* was noted in Moroccan, Turkish and Ghanaian ethnicities, as was an enrichment of Bacteroides in African Surinamese and South-Asian Surinamese, and an enrichment of Clostridiales in Dutch populations. The Dutch also exhibited the greatest gut microbiota α-diversity and the South-Asian Surinamese the smallest, with corresponding enrichment or depletion in numerous *operational* taxonomic units (OTU).[44] Further studies from India have demonstrated that the microbiome can vary even between two regions, with a greater abundance of *Prevotella* in Northern India, which has a carbohydrate-rich, plant-based diet, while the Southern Indian population, which has an omnivorous diet, had greater enrichment with *Bacteroides*.[46] Importantly, these remain associative studies; the impact on these alterations on COVID-19 will require further exploration. Significantly, as many facets contribute to the variations in the microbiome seen in different ethnic groups, there are many potential targets where we may be able to modify the gut microbiome and potentially reduce risk.

Potential mechanisms underpinning the role of the gut microbiome in COVID-19 infection

Entry of COVID-19 into human cells

It is known that COVID-19 enters cells using the ACE-2 receptor, which is expressed in tissues in a number of different organ systems. More specifically, recently, polymerase chain reaction (PCR) analysis has revealed that the ACE-2 receptor is also expressed in the lung, kidney and gastrointestinal tract. Importantly the microbiota and its interaction with the ACE-2 receptor has been elucidated in cardiovascular diseases,[40] intestinal inflammation, malnutrition,[47] immunity and energy metabolism,[48] suggesting a potential role in COVID-19 infection. Furthermore, in a murine model it has been shown that the gut microbiota regulates colonic ACE-2 receptors,[49] and thus may play a role in the infectivity and severity of SARS-CoV-2.

When considering specific sites of ACE-2 expression within the gastrointestinal tract, various mechanisms of entry have been implicated. Specifically, it has been demonstrated that the ACE-2 receptor increases with age in the duodenum, suggesting a potential entry mechanism through microbiome interactions.[50] Furthermore, in a single-cell transcriptome study, ACE-2 was found to be expressed highly in oesophageal upper and stratified epithelium, as well as in absorptive enterocytes derived from both the ileum and the colon.[51] Further areas of ACE-2 expression have been found in enterocytes of all parts of the small intestine, including the duodenum, jejunum and ileum, but not in colonic enterocytes.[52] Interestingly, ACE-2 activity was described in patients with *inflammatory bowel disease* (IBD).[53] We have started to appreciate the importance of the gut microbiome in the role of IBD[54]; hence, further studies may provide mechanistic insight into the role of the microbiome on ACE-2 expression and COVID-19.

Lung-microbiome changes in COVID-19

As COVID-19 is a disease affecting predominately the respiratory system in extreme disease, attempts have been made to understand microbiome changes in the lung. To date, two studies have explored this. One study highlighted that patients with COVID-19 had a microbiome that was similar to those with community-acquired bacterial pneumonia, with enrichment of pathogenic and commensal bacteria.[55] The other study explored post-mortem biopsies from 20 deceased COVID-19 patients. The most common bacterial genera were Acinetobacter, Chryseobacterium, Bukholderia, Brevundimonas, Sphingobium and Enterobacteriaceae. The most common fungal genera were Cutaneotrichosporon, followed by Issatchenkia, Wallemia, Cladosporium, Alternaria, Dipodascus, Mortierella, Aspergillus, Naganishia, Diutina and Candida.[56] These are, at present, associative findings, and evidence is again lacking of whether the changes in the lung microbiome are causative or associative. Furthermore, it remains unclear how the lung and the gut microbiomes interact.

Microbiome–immune interactions

It has been shown that the number of total T cells, CD4+ and CD8+ T cells are reduced dramatically in patients with COVID-19, and this phenomenon is more pronounced in patients requiring intensive care unit (ICU) care.[57] Furthermore, T cell numbers correlate negatively with serum interleukin (IL)-6, IL-10 and tumour necrosis factor (TNF)-α concentration, and patients in the disease resolution phase show reduced IL-6, IL-10 and TNF-α concentrations and restored T cell counts.[57] This observation was further supported by a study demonstrating that patients with severe COVID-19 had reduced circulating levels of CD8+ T cells.[58] When considering how these mechanisms may link to the gut microbiota, it is known that gut microbiota are key regulators of CD8+ T cell function.[59] The gut microbiota that produce SCFA can promote the memory potential of antigen-activated CD8+T cells.[60] In terms of mounting a response directly against the SARS-CoV-2 virus, it has been demonstrated that the SCFAs, butyrate and, to a lesser extent, propionate directly modulate gene expression of CD8+ cytotoxic T lymphocytes (CTLs) and Tc17 cells.[61] This study highlighted that butyrate increased IFN-γ and granzyme B expression on cytotoxic T lymphocytes as well as promoting the molecular switch of Tc17 cells towards a cytotoxic phenotype.[61] Extrapolating from these findings may implicate the products of gut microbiota metabolism in the regulation of the immune response against COVID-19 infection.

Importantly, intestinal antiviral immunity relies on lipopolysaccharides found on the surface of Gram-negative commensal-dependent nuclear factor kappa B (NF-κB) signalling, while enteric viral infection protects against intestinal damage and pathogenic bacteria.[62]

One of the key events that leads to severe respiratory distress syndrome appears to be the development of a cytokine storm. It has been demonstrated that gut microbiota are essential in the maintenance of a cytokine storm through Toll-like receptor (TLR)-mediated immune responses.[63] It is therefore plausible that the gut microbiota may play a significant role in the severity of COVID-19.

It has been speculated the gut virome is a missing link between the gut microbiome and diseases, including IBD.[64] Importantly, these cross-kingdom interactions can change the host (human) phenotype. Their role in the microbiome and viral defence is well established; one method of defence is through the production of mucus and synthesis of potential antiviral compounds.[65] There is also precedent for the gut microbiota initiating the inflammasome, which has induced dendritic cell migration to local lymph nodes to influence an influenza virus T cell response in the lung.[66,67] It is therefore possible that altering the gut virome, may alter the COVID-19 disease course (Figure1).

The SARS-CoV-2 virus enters cells through the ACE-2 receptor, which is found in both the gastrointestinal tract and the lung. The gut microbiota has been shown to modulate the ACE-2 receptor in an animal model, and interact with the lung microbiota to regulate pro-inflammatory and regulatory immune signals. In patients with severe COVID-19 infection, it has been shown that there is a dramatic decrease in the number of T cells, CD4+ T cells and CD8+ T cells. The gut microbiota produces SCFAs (butyrate and propionate), which can modulate the expression of CD8+ T cells. The gut microbiota is also an essential player in the maintenance of the cytokine storm through their interaction with TLRs. It is therefore possible that the gut microbiome may play a role in the regulation of our immune system, including regulating the immune response to the COVID-19 infection.

Vitamin D

In a study that explored the relationship between vitamin D level and incidence of COVID-19, the authors observed a negative correlation between levels of mean vitamin D [average 56.79 nmol/l, standard deviation (SD) 10.61] and number of cases of COVID-19/1 million population in each country.[68] This concept was further supported by an editorial that plotted mortality against latitude and noted that all countries that lie below 35 degrees north have relatively low mortality.[69] A study that explored Vitamin D levels in patients with COVID-19 was found that positive patients had a lower median serum 25(OH)D level of 27 nmol/l [interquartile range (IQR) = 20–47 nmol/l] compared with the COVID-19-negative arm, with median level of 52 nmol/l (IQR = 31.5–71.5 nmol/l) (p value = 0.0008).[70] To our knowledge, there is only one randomised controlled pilot study that explored the role of direct vitamin D treatment in COVID-19 patients. In this, the authors found that administration of high dose

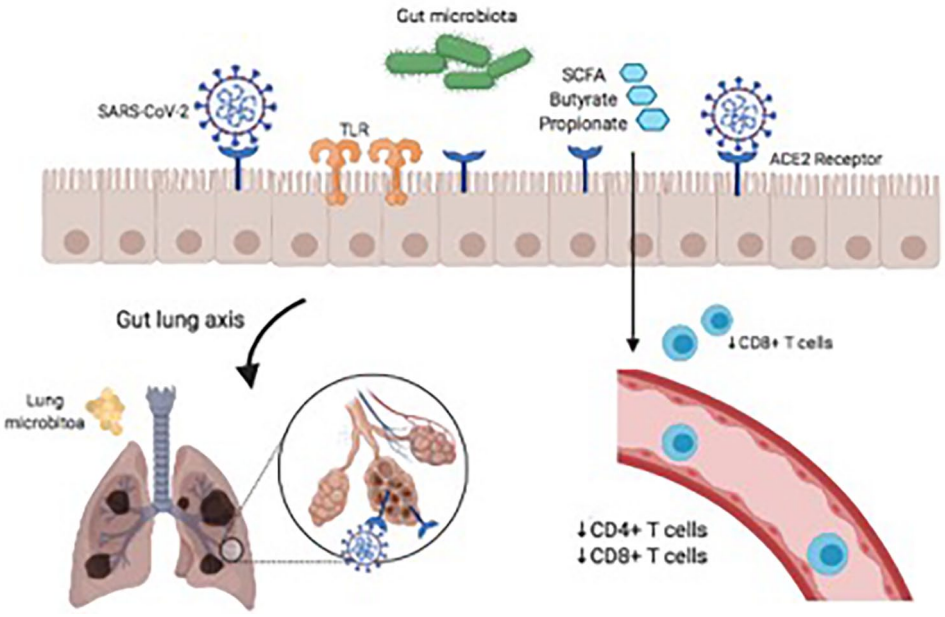

Figure 1. Potential mechanisms of interaction between the gut microbiome and SARS-CoV-2.
SCFA, short chain fatty acid; TLR, Toll-like receptor.

calcifedol or 25-hydroxyvitamin D significantly reduced the risk of ICU admission with proven COVID-19.[71]

Although this association is likely to be multifactorial,[72] vitamin D has been associated with the promotion of regulatory T cells (Tregs) inhibition of Th1 and Th17 cells, impairment of the development and function of B cells, and reducing monocyte activation.[73,74] It has been shown that the gut microbiome can be altered by vitamin D exposure.[75,76] Furthermore, it has been established that microbially derived bile acids, including lithocholic acid, are well established as ligands for the vitamin D receptor[77]. As such, if this association is valid, it may be possible that vitamin D-related manipulation of the gut microbiome may impact on the morbidity and mortality associated with COVID-19 infection.

Other factors

Perturbation of several other host pathways have also been linked to the pathogenesis of COVID-19 infection. For instance, one pathway that has received particular attention has been the apparent marked state of vitamin K deficiency that characterises COVID-19 infection in humans, and which correlates with poor outcome.[78] In particular, vitamin K deficiency may directly contribute to the coagulopathy and lung inflammation found in severe COVID-19 infection.[78] While there may be a number of contributory factors to this vitamin K deficient state, there has been increasing recognition recently of the key contribution of the gut microbiome and bile acids to normal vitamin K production and absorption.[79] This contribution may be of particular relevance given the high preponderance of antibiotic use in patients with COVID-19 infection, in the attempt to prevent or treat co-existing bacterial lung infections, which impacts the gut microbiota's contribution to the host's vitamin K pool.

Diet and COVID-19 infection

There is a multi-directional relationship between diet, the immune system, infection and nutrition, with changes in one of these components having a significant impact on the other.[80] Diet has been implicated in a broad range of diseases and it has been speculated that diet may be a key driver in determining the severity of COVID-19.[81] The World Health Organisation provides advice on nutrition during this pandemic to include plenty of fruit and vegetables.[82] There is a large evidence base on the impact of diet on the gut microbiota,[83] but as yet the relevance of diet-driven changes in the gut microbiome in the context of COVID-19 has yet to be established.

Interestingly, China's National Health commission and National Administration of Traditional Chinese medicines have recommended that patients with severe COVID-19 infection take probiotics as a means of preventing secondary bacterial infections.[84] Significantly, it was shown in two meta-analysis that critically ill patients who were given probiotics developed substantially less ventilator-associated pneumonia when compared with placebo.[85,86] However, the rationale for using probiotics in COVID-19 is based on indirect evidence only. Importantly, it has been suggested that conventional probiotics should not be recommended until the role of the microbiota on COVID-19 infection is understood.[87] Despite this advice, altering the gut microbiota to reduce the burden of COVID-19 infection may be an area that deserves further exploration. As of 22 October 2020, there are 11 clinical trials registered on clinicaltrials.gov on the use of probiotics/synbiotics for the treatment of COVID-19 infection.

The gut microbiome and proposed treatments for COVID-19 infection

In parallel with the efforts being made to understand the pathogenesis of COVID-19 disease, the pandemic has prompted the rapid development and roll out of an unprecedented number of investigational treatment studies. As of October 2020, over 3800 clinical trials targeting COVID-19 are registered across the globe.[88] The gut microbiome is well established as a modulator of a wide range of therapeutic compounds used in clinical practice,[89] including anti-virals,[90] anti-hypertensives,[89] and anti-diabetic medications,[91] and baseline gut microbiome profiles have been shown to predict responses to cancer therapies.[92] It is possible that inter-individual variation in gut microbiomes will have an impact on the success of proposed treatments for COVID-19.

A wide array of drugs are being trialled to combat COVID-19, with some showing evidence that they interact with the gut microbiome. Specifically, the macrolide antibiotic Azithromycin is being tested widely as a treatment for COVID-19, predominantly in combination with hydroxychloroquine. Azithromycin's action on gut bacteria is well recognised, hence its use in the treatment of *Campylobacter* infection in many parts of the world.[93] In a blinded, randomised, placebo-controlled study in young children, a 3-day course of Azithromycin (10 mg/kg) was shown to reduce alpha diversity of the gut microbiota characterised by loss of the genus *Bifidobacterium* at 14 days.[94] Furthermore, functional analysis of the metagenomes of African children treated with Azithromycin showed under-representation of metabolic pathways involved in immune regulation and inflammation.[95] The aforementioned studies concern children, and their applicability in the population severely affected by COVID-19, mainly older adults, is perhaps moot, although, in an adult population, azithromycin treatment has been associated with a diminishment of *Lachnospiraceae*.[96]

Antibiotic use in COVID-19 patients has been widespread, and there is growing concern that antimicrobial resistance will be exacerbated.[97] There is also evidence that antibiotic use prior to viral exposure may predispose individuals to more severe respiratory infections.[98] In a mouse model of influenza infection, animals given antibiotics had an abrogated interferon signature in lung stroma, which permitted early virus replication in the epithelia. Interestingly, faecal transplant following antibiotics restored the interferon signature,[98] suggesting that the gut microbiome plays an integral role in determining the integrity of barrier defences against infection at sites distant to the GI tract. If replicated in humans, this paradigm may be applicable to other viruses that enter *via* the respiratory epithelium, including SARS-CoV-2. As such, there has been a proposal for intervention studies with gut microbiota manipulation – with the aim of preventing or minimising the clinical extent of COVID-19 infection – including *via* probiotics and faecal transplant.[99,100]

The monoclonal anti-IL6 antibody tocilizumab is the focus of several studies attempting the counter the hyperinflammatory cytokine release storm observed in some patients with severe COVID-19.[101] Concern has been raised in some quarters that Tocilizumab, a drug used to treat rheumatological conditions such as rheumatoid arthritis, is known to cause lower intestinal perforation (2–3 per 1000 patients).[102,103] The mechanism is not known, although patients with diverticular disease are thought to be at higher risk. The presence of diverticular disease, regardless of symptoms, is associated with an altered microbiota.[104] It is noteworthy that IL-6 deficient mice have an impaired gut-epithelial

barrier with thinning of the mucus gel layer.[105] Interestingly, bacteria in the order Bacteroidales promote intra-epithelial lymphocytes in the colon which produce IL-6,[105] raising the possibility that the composition or function of the gut microbiota may be implicated in the aetiology of tocilizumab-related perforation.

Finally, there is clearly a major clinical need for a vaccine against COVID-19, and a number of trials are ongoing globally. It is well recognised that immune responses to vaccine administration against viral and other pathogens is notably variable between patients; this variability may represent the interplay of a number of different factors, but which may include the composition and/or functionality of the gut microbiome.[106] While there is much data to suggest immune response variability to vaccinations from animal studies, recent human data demonstrates that antibiotic-mediated gut microbiome disruption impairs the antibody response to influenza vaccination to patients with low pre-existing anti-influenza antibody titres.[107] As such, recent use of antibiotics (or other factors that may disrupt the gut microbiome) may be of relevance to consider when recruiting participants to COVID-19 vaccine studies.

Problems in assessing the microbiome

There are challenges with studying the microbiome during this pandemic. One of the main safety concerns is the prolonged presence of COVID-19 virus RNA in stools.[108] Prolonged viral shedding in the faeces leads to challenges in collecting stool for research purposes, and such experiments require a Category 2 laboratory to process samples. Retrieving tissue samples for microbiome analysis also represents a logistical challenge. Currently, there are restrictions regarding endoscopic practice during the COVID-19 pandemic,[109] meaning that the ease of acquiring tissue samples for research purposes is reduced. There is also a reduction in surgery during the COVID-19 pandemic.[110]

There has been a wider impact of the pandemic on other areas of research, with an overall reduction in academic activity outside of COVID-19 related studies.[111] Furthermore, from an infrastructure perspective, many academic and university staff have been redeployed to focus their efforts on other areas of COVID-19 research, including vaccine development.[112]

Conclusion

The gut microbiome plays a significant role in human health and disease states, and could play a significant role in the interplay between COVID-19 infection and the host. Microbiome studies may help our understanding of the pandemic and furthermore provide insights into preventative and therapeutic strategies.

The long-term implications of COVID-19 infection on a variety of organs are currently unknown, with the potential for long-lasting effects. Specifically it has been alluded to that the increased use of antimicrobials in this pandemic may result in the future burden of antibiotic resistance.[113] As we develop more knowledge regarding the virus, it will also be important to understand the effects of this pandemic on the gut microbiome and hence potential long-term implications.

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Author contributions

JPS, JWYM BHM, JLA SN, JM were responsible for conception, literature review, writing and revising the manuscript. SN, JM gave critical revisions and helped revised the manuscript. All authors agreed to the final version.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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