The gut microbiome: a missing link in understanding the gastrointestinal manifestations of COVID-19?

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Abstract Coronavirus disease 2019 (COVID-19), which is caused by infection with SARS-CoV-2, presents with a broad constellation of both respiratory and nonrespiratory symptoms, although it is primarily considered a respiratory disease. Gastrointestinal symptoms—including nausea, abdominal pain, vomiting, and diarrhea—rank chief among these. When coupled with the presence of viral RNA in fecal samples, the presence of gastrointestinal symptoms raises relevant questions regarding whether SARS-CoV-2 can productively infect the upper or lower gastrointestinal tract. Despite the well-documented prevalence of gastrointestinal symptoms and the high rate of SARS-CoV-2 fecal RNA shedding, the biological, clinical, and epidemiological relevance of these findings is unclear. Furthermore, the isolation of replication-competent virus from fecal samples has not been reproducibly and rigorously demonstrated. Although SARS-CoV-2 shedding likely occurs in a high proportion of patients, gastrointestinal symptoms affect only a subset of individuals. Herein, we summarize what is known about gastrointestinal symptoms and fecal viral shedding in COVID-19, explore the role of the gut microbiome in other respiratory diseases, speculate on the role of the gut microbiota in COVID-19, and discuss potential future directions. Taking these concepts together, we propose that studying gut microbiota perturbations in COVID-19 will enhance our understanding of the symptomology and pathophysiology of this novel devastating disease.

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GASTROINTESTINAL SYMPTOMS AND FECAL VIRAL SHEDDING IN COVID-19

Since its zoonotic transfer to humans, SARS-CoV-2 has spread across the globe with unprecedented speed. The associated coronavirus disease 2019 (COVID-19) is widely regarded as a respiratory disease because of the prevalence and severity of symptoms such as cough, sore throat, congestion, anosmia, and dyspnea. Interestingly, gastrointestinal (GI) symptoms, although initially overlooked, are now appreciated as a common manifestation of the disease. In a multicenter cohort study of 318 hospitalized COVID-19 patients in the United States, at admission, 107 (33.7%) patients reported diarrhea, 84 (26.4%) reported nausea, 49 (15.4%) reported vomiting, and 46 (14.5%) reported abdominal pain (Redd et al. 2020). In another cohort study of 254 people in China with COVID-19, 66 (26.0%) of patients reported at least one of the following GI symptoms: diarrhea, vomiting, nausea, or abdominal pain (Zhou et al. 2020b). Globally, a meta-analysis of 60 studies that have reported on GI symptoms on a total of 4243 unique patients found that the pooled prevalence of GI symptoms was 17.6% (95% confidence interval [CI], 12.3–24.5) (Cheung et al. 2020). The results of all

meta-analyses reporting on the prevalence of GI symptoms on PubMed prior to January 19, 2021 are summarized in Table 1. The true percentage of individuals experiencing GI symptoms could possibly be far higher given that these symptoms are less likely to merit testing or hospitalization and may be experienced in the absence of recognizable respiratory symptoms (Mao et al. 2020; Xu et al. 2020). In support of this postulated underappreciation of the prevalence of GI symptoms in COVID-19, one retrospective study of hospitalized patients reported that GI disturbances were the predominant presenting complaint and initial symptom in 103 (50.5%) of 204 individuals. Diarrhea was the most common individual GI symptom experienced by 38 (18.6%) of patients at admission (Pan et al. 2020), indicating that GI symptoms may not be secondary to respiratory disease, but rather due to a separate biological phenomenon. Perplexingly, the presence of GI symptoms does not appear to be consistently correlated with respiratory or overall disease severity. In some reports, patients who initially present with GI symptoms have a greater risk of disease progression, possibly due to delayed diagnosis and treatment (Mao et al. 2020), whereas other preliminary reports have found that GI symptoms are associated with milder courses of disease (Livanos et al. 2020).

In addition to GI symptoms, viral RNA has been detected in stool samples via quantitative real-time polymerase chain reaction (RT-qPCR) assay across multiple studies (Cheung et al. 2020; Parasa et al. 2020; Wang et al. 2020c). In the most recent and most comprehensive meta-analysis of fecal viral shedding to date, including 44 studies of at least 10 patients representing 1989 unique patients, van Doorn et al. (2020) found a pooled prevalence of positive fecal viral tests in 51.8% (95% CI, 43.8–59.7%) of stool samples or rectal swabs. Table 2 summarizes data on the prevalence and duration of fecal viral shedding compiled by meta-analyses available on PubMed prior to January 19, 2021. Other single-cohort studies have found that SARS-CoV-2 genomic viral RNA is shed in feces at viral loads that are comparable to respiratory specimens at their peak (Wölfel et al. 2020); and fecal shedding can persist for weeks after diagnosis, often long after respiratory specimens test negative (van Doorn et al. 2020; Wu et al. 2020). Interestingly, fecal viral shedding has also been reported in asymptomatic individuals, suggesting that a greater number of individuals may be shedding viral RNA in their feces than has been reported (Hua et al. 2020; Park et al. 2020). Although it is tempting to speculate that fecal RNA levels may be associated with the presence or absence of GI symptoms, to date, there does not appear to be a consistent significant correlation between the presence of fecal viral RNA and GI symptoms across 12 studies that reported on these statistics (van Doorn et al. 2020).

These reports naturally lead to the question of whether SARS-CoV-2 infection of the GI tract drives the underlying etiology of GI symptoms. Physiologically, there is evidence that this may be possible. Angiotensin-converting enzyme 2 (ACE2), the cellular receptor for SARS-CoV-2, is expressed by nonrespiratory tissues, including the heart and liver, with highest levels present in the small intestine, testes, kidney, and gallbladder (Hamming et al. 2004; Hikmet et al. 2020; Hoffmann et al. 2020; Li et al. 2020). Within the GI tract, ACE2 is expressed at threefold higher levels in the epithelial cells of the upper GI tract (ileum and jejunum) than in the lower GI tract (colon) (Hikmet et al. 2020) and is coexpressed with transmembrane serine protease 2 (TMPRSS2), a cell surface protein that facilitates viral entry into the cell (Ziegler et al. 2020). This supports the GI tract as a site of SARS-CoV-2 tropism. SARS-CoV-2 has further been shown to productively infect intestinal organoids derived from the upper GI tract (duodenum and ileum) (Lamers et al. 2020; Zang et al. 2020; Zhou et al. 2020a) and preliminary studies report culturing of infectious virus from stool samples of infected individuals (Xiao et al. 2020).

Although COVID-19 is not thought to be a predominantly GI disease and the direct evidence of viral infection of the GI tract is limited, framed within a historical context, the idea that SARS-CoV-2 has both GI and respiratory tropism is plausible. There is ample evidence of

Table 1. Prevalence of gastrointestinal (GI) symptoms in COVID-19 patients

Title	Authors	PMID	Total studies included	Total patients across all studies	Diarrhea, % (n if available)	Abdominal pain, % (n if available)	Vomiting and nausea, % (n if available)	All GI symptoms, % (n if available)	Dates for which data obtained
Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from a Hong Kong cohort: systematic review and meta-analysis	Cheung et al. 2020	32251668	60	4,243	12.5% [95% CI, 9.6%–16.0%] (326/3536)	9.2% [95% CI, 5.7%–14.5%] (52/751)	10.2% [95% CI, 6.6%–15.3%] (210/751)	17.6% [95% CI, 12.3%–24.5%]	Onset–March 11, 2020
Prevalence of gastrointestinal symptoms in coronavirus disease 2019: a meta-analysis	Merola et al. 2020	33321018	33	4,434	7.78% [95% CI, 5.05%–11.04%]	NR	3.57% [95% CI, 1.87%–5.80%]	11.51% [95% CI, 8.16%–15.35%] (537/4243)	Onset–March 25, 2020
AGA Institute rapid review of the gastrointestinal and liver manifestations of COVID-19, meta-analysis of international data, and recommendations for the consultative management of patients with COVID-19	Sultan et al. 2020	32407808	47	10,890	7.7% [95% CI, 7.2%–8.2%] (822/10,676)	2.7% [95% CI, 2.0%–3.4%] (108/4031)	7.8% [95% CI, 7.1%–8.5%] (465/5955)	NR	Onset–April 19, 2020
Prevalence of gastrointestinal symptoms and fecal viral shedding in patients with coronavirus disease 2019: a systematic review and meta-analysis	Parasa et al. 2020	32525549	29	4,805	7.8% [95% CI, 5.1%–11.9%]	NR	3.9% [95% CI, 2.1%–7.2%]	NR	Onset–March 30, 2020
Duration of respiratory and gastrointestinal viral shedding in children with SARS-CoV-2: a systematic review and synthesis of data	Xu et al. 2020	32618932	17	69 (pediatric)	NR	NR	NR	17.4% (12)	Onset–May 8, 2020
Gastrointestinal symptoms and fecal nucleic acid testing of children with 2019 coronavirus disease: a systematic review and meta-analysis	Wang et al. 2020b	33082472	38	3,028 (pediatric)	NR	NR	NR	17.7% [95% CI, 13.9%–21.5%] (536)	January 1–August 10, 2020

As reported on PubMed prior to January 19, 2021.
(PMID) PubMed Identifier, (CI) confidence interval, (NR) not reported.

Table 2. Prevalence and duration of fecal viral shedding in COVID-19 patients

Title	Authors	PMID	Studies included	Number of patients	Patients with a positive stool sample and/or rectal swab, % (n if available)	Stool samples positive for SARS-CoV-2	Duration of positivity, days (n if available)	Duration positive after negative respiratory swab, days	Maximum total duration observed, days	Dates for which data was extracted
Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from a Hong Kong cohort: systematic review and meta-analysis	Cheung et al. 2020	32251668	12	138	48.1% [95% CI, 38.3%–57.9%]	NR	NR	NR	33	Onset–March 11, 2020
Prevalence of gastrointestinal symptoms in coronavirus disease 2019: a meta-analysis	Merola et al. 2020	33321018	2	227	41.50% [95% CI, 17.70–67.65%]	NR	NR	NR	NR	Onset–March 25, 2020
Persistent viral shedding of SARS-CoV-2 in faeces—a rapid review	Gupta et al. 2020	32418307	26	824	53.9% [291/540]	NR	NR	1–33/47 max	NR	Onset–April 10, 2020
Detection of SARS-CoV-2 RNA in fecal specimens of patients with confirmed COVID-19: a meta-analysis	Wong et al. 2020	32535156	17	436	43.7% [95% CI, 32.6%–55.0%]	33.7% [95% CI, 20.1%–48.8%]	NR	NR	NR	Onset–March 25, 2020
Prevalence of gastrointestinal symptoms and fecal viral shedding in patients with coronavirus disease 2019: a systematic review and meta-analysis	Parasa et al. 2020	32525549	8	407	40.5% [95% CI, 27.4%–55.1%]	NR	NR	NR	NR	Onset–March 30, 2020
Incidence and persistence of viral shedding in COVID-19 postacute patients with negativized pharyngeal swab: a systematic review	Morone et al. 2020	32984389	50	1005	48.8% (490/1005)	NR	19 (226)	NR	NR	Onset–May 5, 2020
Duration of respiratory and GI viral shedding in children with SARS-CoV-2: a systematic review and synthesis of data	Xu et al. 2020	32618932	17	69 (pediatric)	85.7% (36/42)	NR	23.6 ± 8.8	28	37	Onset–May 8, 2020
Systematic review with meta-analysis: SARS-CoV-2 stool testing and the potential for faecal-oral transmission	van Doorn et al. 2020	32852082	44	2149	51.8% [95% CI, 43.8%–59.7%]	NR	NR	12.5 /33 max	NR	Onset–July 7, 2020
Gastrointestinal symptoms and fecal nucleic acid testing of children with 2019 coronavirus disease: a systematic review and meta-analysis	Wang et al. 2020b	33082472	13	106 (pediatric)	85.8% (91/106)	NR	NR	70	NR	January 1–August 10, 2020
Understanding viral shedding of severe acute respiratory coronavirus virus 2 (SARS-CoV-2): review of current literature	Fontana et al. 2020	33077007	5	226	NR	NR	22.1 [95% CI, 14.4%–29.8]	NR	50–55	Onset–Sept. 8, 2020

As compiled by meta-analyses available on PubMed prior to January 19, 2021.
(PMID) PubMed Identifier, (CI) confidence interval, (NR) not reported.

GI infectivity for the closely related SARS-CoV and MERS-CoV coronaviruses, which were responsible for epidemics in 2003 and 2012, respectively (Peiris et al. 2003b; Zaki et al. 2012). GI symptoms, such as diarrhea, were reported for both diseases in about 20% of SARS-CoV cases and up to 26% of MERS-CoV cases (Lee et al. 2003; Assiri et al. 2013). Additionally, viral genomic RNA was readily detected in the majority of fecal samples from SARS cases (Peiris et al. 2003a) and roughly one-third of MERS cases (Corman et al. 2016). Live virus could not be propagated from MERS fecal samples. However, SARS-CoV was isolated by culture from tissue biopsies from both the lung and small intestine from patients with SARS (Leung et al. 2003; To et al. 2004). Infection in the epithelial cells of the GI tract of SARS cases was further confirmed by immunofluorescence–fluorescence in situ hybridization (To et al. 2004).

Taken together, this body of evidence suggests SARS-CoV-2 may be capable of causing viral infection in the GI tract of affected individuals. However, even in absence of confirmed GI infection, the relevance of GI symptoms to overall disease severity or duration remains unclear. Several questions remain, particularly the question of which factors contribute to these symptoms and underlie the substantial heterogeneity observed among confirmed COVID-19 cases. One plausible explanation is that SARS-CoV-2 infection leads to perturbation of the gut microbiome toward dysbiosis, which may contribute to the underlying etiology of GI symptoms. Clinically, this may also be a sign of disease progression. SARS-CoV-2 infection is known to occur in two stages, with the initial phase marked by a robust innate immune response and the second phase marked by epithelial barrier injury and inflammation (Tay et al. 2020). Therefore, as a potent modulator of immunity and inflammation, the gut microbiome could potentially serve as a key mediator of COVID-19 symptoms and severity in both stages of disease and a potential target for new therapeutic interventions.

THE ROLE OF THE GUT MICROBIOME IN RESPIRATORY DISEASE

There is substantial evidence that the gut microbiome plays an influential role in respiratory health and disease. Immunological cross-talk between respiratory and enteric tissues has been well-described and is generally referred to as the gut–lung axis, highlighting the bidirectionality of the relationship. Inflammatory disease in either the lung or GI tract is sometimes linked to disturbances in the other organ (Budden et al. 2017). For example, chronic obstructive pulmonary disease and inflammatory bowel disease, which are inflammatory diseases affecting the lungs and GI tract, respectively, have both been documented to increase risk of coincident disease within the axis (Keely et al. 2012). It is hypothesized that increased cooccurrence of gut–lung inflammatory diseases may arise from a common mucosal immunological system (Bienenstock et al. 1978). Additionally, commensal gut microbe–derived products and metabolites can induce hematopoiesis, thus providing one mechanism by which gut microbiota may directly influence immune function and regulation in health (Dang and Marsland 2019). The current state of knowledge regarding the relationship between GI microbiota and respiratory tract in the settings of both health and disease are reviewed by Wypych et al. (2019).

In the setting of viral lung infections by noncoronavirus pathogens such as respiratory syncytial virus (RSV) or influenza, certain gut microbes have been implicated as being both altered in response to and protective against infection. In critically ill patients with acute viral pneumonia, both the lung and gut microbiome are significantly altered with a shift toward increased presence of opportunistic pathogens. In the lungs, increased bacterial load, increased alpha diversity, and the presence of gut-associated taxa such as *Lachnospiraceae* and *Enterobacteriaceae* are associated with worse prognoses (Kyo et al. 2019; Dickson et al. 2020). Conversely, in the gut, sublethal influenza A H5N1 respiratory infection was shown to deplete the microbiota and diminish community diversity in a mouse model,

thereby increasing susceptibility to gut invasion by pathogens (Yildiz et al. 2018). In mouse models of RSV and influenza infection, *Bacteroidetes*, particularly within the *Porphyromonadaceae* family, increased in relative abundance, whereas organisms in the *Lactobacillaceae* and *Lachnospiraceae* families decreased following lung infection but not following vaccination with live attenuated virus (Groves et al. 2018). Other studies in influenza mouse models have shown that type I interferon production in the lungs during respiratory viral infection is one mechanism that can drive gut microbial dysbiosis by depleting obligate anaerobes (Deriu et al. 2016). Some of the observed shifts in gut microbial composition, especially *Bacteroidetes* outgrowth, may be attributable to decreased dietary caloric intake owing to disease-induced inappetence, as suggested by a study in mouse models (Groves et al. 2020).

Additionally, a growing body of literature has provided mechanistic insight into the various roles that gut commensals play in enhancing innate immunity as well as modulating the balance between type I and type II adaptive immune response (Omenetti and Pizarro 2015). For example, prior to respiratory viral infection, studies in murine models have shown that lipooligosaccharides in the outer membrane of *Bacteroides sp.*, such as polysaccharide A produced by *Bacteroides fragilis*, prime immune resistance by stimulating interferon-ß signaling via activation of colonic dendritic cells (Stefan et al. 2020). In the setting of RSV or influenza infection, there is substantial evidence that specific commensal gut taxa, particularly *Lactobacillus*, can enhance immune resistance (Maeda et al. 2009; Park et al. 2013; Wu et al. 2013; Schuijt et al. 2016; Belkacem et al. 2018; Hanada et al. 2018). Specific microbiota may also play a role in regulating the inflammatory phase, which marks later stages of disease. For example, segmented filamentous bacteria have been shown to promote the development of T-helper 17 cells, thus inducing a pro-inflammatory immune state (Ivanov et al. 2009). Other avenues by which gut microbes prime and modulate immunity and inflammation have been reviewed by Belkaid and Hand (2014). Finally, in mouse models certain gut commensals, like *Lactobacillus*, boost innate immunity against bacterial respiratory infection. Specifically, these potent NOD-like receptor pattern recognition receptor stimulators enhance alveolar macrophage activity through the GM-CSF signaling pathway (Brown et al. 2017). This suggests that organisms like *Lactobacillus* may be a therapeutic strategy to prevent against secondary bacterial pneumonia, a common cause of mortality in patients with viral pneumonia. Thus, the existing body of literature provides clear mechanistic evidence that gut commensals are protective and influence prognosis following respiratory infection by pathogens other than SARS-CoV-2. Combined with the knowledge of gut dysbiosis in response to respiratory infection, this lends substantial support to the hypothesis that the gut microbiome may influence the severity of COVID-19 symptoms and outcomes related to the primary infection as well as secondary bacterial pneumonia.

THE ROLE OF THE GUT MICROBIOTA IN COVID-19

Since the start of the COVID-19 pandemic, many have proposed that the gut microbiome may be an influential factor in COVID-19 pathogenesis, but only a few have provided data in support of this claim. In their preprint, Gou et al. (2020) found correlative evidence to suggest that certain gut microbes may increase risk of more severe COVID-19 in healthy, noninfected individuals. Using a machine learning model, they identified operational taxonomic units in the gut microbiota of 301 healthy participants that explained up to 22% of the variability in a blood proteomic risk score predictive of COVID-19 disease severity. After testing these in a healthy independent cohort against a panel of pro-inflammatory cytokines, they found significant negative correlations for the *Bacteroides* and *Streptococcus* genera and positive associations for the *Ruminococcus*, *Blautia* and *Lactobacillus* genera, thus providing insight into

a possible mechanism by which certain gut microbes could promote COVID-19 progression after infection. Similarly, in a recent preprint, Ward et al. (2021) applied a random forest machine learning algorithm to identify gut microbial biomarkers of COVID-19 outcomes in a cohort of 62 COVID-19 patients. They found that a combination of features from the gut microbiota were able to predict COVID-19 severity with up to 92.05% accuracy. Of these, an enrichment of *Enterococcus faecalis* was one of the top predictors of COVID-19 severity, whereas enrichment for *B. fragilis* was strongly predictive of moderate disease. Together, these preliminary findings suggest that the gut microbiome may have prognostic value in assessing risk of severe COVID-19 outcomes.

Other studies have characterized the composition of the lung or gut microbiota in the setting of SARS-CoV-2 respiratory infection to identify features indicative of disease severity. An analysis of metatranscriptomic sequencing data from bronchoalveolar lavage fluid from eight patients with COVID-19 respiratory disease found similar microbiota compositions to those from patients with community acquired pneumonia—namely, an increase in the number of pathogenic organisms or an increase in commensals normally found in the oral cavity (Shen et al. 2020). A cross-sectional study of the gut microbiota of 30 patients with COVID-19 via 16S ribosomal RNA gene sequencing found significantly decreased Shannon diversity and an increased relative abundance of opportunistic pathogens, including *Streptococcus*, *Rothia*, and *Actinomyces*, as compared to 24 patients with influenza A (H1N1) and 30 healthy matched controls (Gu et al. 2020). Similarly, Zuo et al. (2020a) performed RNA metatranscriptomic sequencing of fecal samples from 15 hospitalized patients with COVID-19 and found a greater predominance of disease-associated microbiota in patients with higher sequencing coverage of the 3′ region of the SARS-CoV-2 viral genome as compared to the 5′ region, potentially indicative of active viral replication in the gut. In seven antibiotic naive patients within this same cohort, baseline gut microbiome shotgun metagenomic sequencing data revealed an enrichment for species including *Clostridium hathewayi* (reclassified in 2014 to *Hungatella hathewayi*) (Kaur et al. 2014), *Actinomyces viscosus*, and *Bacteroides nordii* and depletion of commensals, such as *Faecalibacterium prausnitzii*, as compared to six patients with community acquired pneumonia (Zuo et al. 2020b). The small number of subjects analyzed in these studies represents a significant limitation of these findings. However, in a recent study of an expanded patient cohort from the same authors, gut microbiome sampling data and paired blood cytokine profiling of 100 COVID-19 patients was compared with that of 78 healthy participants surveyed before the onset of the pandemic (Yeoh et al. 2021). Although they observed species level differences in the microbiome profile of COVID-19 and non-COVID-19 controls, these were only present at a few percent relative abundance. Interestingly, when they adjusted for antibiotic exposure and age, they found that *Faecalibacterium prausnitzii* and *Bifidobacterium bifidum* were negatively correlated with COVID-19 disease severity; *F. prausnitzii* was also found to be negatively correlated with pro-inflammatory cytokines.

Taken together, these findings begin to paint a picture for the key roles the gut microbiota may play at each stage of disease. First, prior to infection, the preliminary findings by Gu et al. (2020) suggest that microbes of the *Ruminococcus*, *Blautia* or *Lactobacillus* genus might indicate risk of more severe disease. Then immediately following infection, based on studies of RSV and influenza in mouse models, key gut microbes, such as *B. fragilis*, are capable of activating host antiviral immunity and determining the strength of the initial immune response. In later stages of disease, the loss of commensals such as *F. prausnitzii* and *B. bifidum* and enrichment for pathobionts such as *E. faecalis* may play a role in amplifying destructive inflammatory responses, as suggested by the recent findings from Ward et al. (2021) and Yeoh et al. (2021). Finally, on a community level, the studies by Zuo et al. (2020b) and Gu et al. (2020) indicate that gut dysbiosis is a common outcome following SARS-CoV-2 respiratory infection and is possibly a risk factor for more severe disease.

In the context of COVID-19, there is also evidence to suggest that the gut microbiome may undergo particular perturbations that are specific to SARS-CoV-2 infection. Besides being the receptor that mediates cellular viral entry, in vitro ACE2 is a chaperone for the neutral amino acid transporter B^0AT1 coexpressed in the small intestinal epithelium (Camargo et al. 2009). As a result, in mouse models, ACE2 deficiency in the GI tract leads to a decrease in neutral amino acid bioavailability and an increase in vulnerability to intestinal inflammation (Hashimoto et al. 2012; Singer et al. 2012; Liu et al. 2015). In particular, insufficient tryptophan bioavailability and the concomitant lack of host- and microbe-derived tryptophan metabolites is linked to defects in expression of α-defensins, a class of antimicrobial peptides that impact gut microbial composition and host–microbial immunological interactions as reviewed by Gao et al. (2018) and He et al. (2018). In this capacity, ACE2 is a key indirect regulator of gut microbial homeostasis. Infection by SARS-CoV, which similarly targets the ACE2 receptor for cellular entry, leads to decreased expression of ACE2 (Kuba et al. 2005). Therefore, it is plausible that SARS-CoV-2 mediates deficiency in enteric ACE2, thereby promoting gut microbial dysbiosis, which may drive the underlying etiology of GI symptoms in the setting of COVID-19.

OPEN QUESTIONS

Overall, the evidence presented here suggests that the GI system is an important site of disease in a significant proportion of SARS-CoV-2-infected individuals and provides evidence that certain gut microbes may play a role in disease pathogenesis. However, several major questions, which we outline below, remain to be addressed.

Although the finding that SARS-CoV-2 RNA is shed in the feces has clear epidemiological significance, for example, by enabling wastewater surveillance for community spread of disease, the clinical significance has yet to be enumerated. A recent meta-analysis suggests that about 50% of known COVID-19 positive individuals test positive by fecal viral RNA test (van Doorn et al. 2020). However, there is substantial variability in this number across individual studies, which may in part reflect inconsistencies in the clinical characteristics of the study population (e.g., disease severity, age, comorbidities), stool sampling frequencies, and methods for viral testing. Nevertheless, it is perplexing that only a subset of cases report GI symptoms or test positive by fecal testing. Moreover, it is unclear what underlying factors explain the observed protracted duration of fecal viral shedding. Across 49 studies, 282 out of 443 (64%) patients shed viral RNA in their stool for an average of 12.5 d after respiratory specimens tested negative (van Doorn et al. 2020). On one side, this could indicate delayed clearance of the virus from the upper respiratory tract. Alternatively, if viral RNA is shed from the upper or lower GI tract, then prolonged shedding could signal a protracted enteric infection after the resolution of respiratory disease.

An understanding of the underlying etiology of GI symptoms and fecal viral shedding in COVID-19 will hinge on a detailed analysis of SARS-CoV-2 infection from the perspective of the GI system. The idea of coronaviruses as respiratory viruses with enteric tropism is not novel. Several coronaviruses productively infect the GI tract in other animals (e.g., cats, dogs, pigs) and can cause both respiratory and diarrheal disease, as in the case of bovine coronavirus. Furthermore, the presence of GI symptoms, the weeks-long duration of fecal viral shedding after respiratory disease resolution, and the existence of the SARS-CoV-2 receptor on the intestinal epithelium support the hypothesis that SARS-CoV-2 could be causing protracted GI infection in COVID-19 cases.

In support of this hypothesis, Gaebler et al. (2021) found a more potent and broader range of SARS-CoV-2-neutralizing antibodies in a cohort of 87 COVID-19-convalescent individuals 6 mo after infection as compared to initial levels assessed at 1.3 mo postinfection.

Continuous antibody evolution, which requires selection for somatic mutants of anti-SARS-CoV-2 memory B cells and ongoing antigen exposure in germinal centers, suggests that SARS-CoV-2 antigen may persist in the body long after respiratory disease resolution. In five out of 14 individuals at an average of 4 mo post-COVID-19 infection, intestinal biopsies were found to have detectable SARS-CoV-2 N protein by immunostaining, and in two participants SARS-CoV-2 viral RNA was detected by in situ hybridization. These findings are highly suggestive of protracted SARS-CoV-2 GI infection in at least a subset of infected individuals, which may explain continued fecal viral detection long after the resolution of respiratory disease. However, there has yet to be conclusive evidence of SARS-CoV-2 GI infection in humans and the presence of infectious viral particles in fecal samples has not been robustly and reproducibly demonstrated.

Beyond this, the gut and/or lung microbiota may be a key indirect factor capable of protecting against or attenuating respiratory infection and possibly, although unproven, GI infection. The studies available to date have observed significant perturbations in the microbiota of COVID-19 patients as compared to both healthy controls and hospitalized patients with H1N1 or community acquired pneumonia. However, the underlying cause of this is unclear. SARS-CoV-2 could play an indirect role by inducing gut microbial dysbiosis via the gut–lung axis, or it is possible, although unproven, that it could directly infect the GI tract via ACE2 receptors highly expressed in the small intestine. Regardless of the mechanism, these studies lend support to the hypothesis that GI symptoms could be explained, in part, by gut microbial dysbiosis. Therefore, the gut microbiome may be a heretofore unexplored contributor to COVID-19 disease pathogenesis. If so, this would suggest that the gut microbiome might be an attractive therapeutic target for COVID-19 intervention. Unfortunately, it is difficult to draw concrete conclusions from these studies given that they are heavily confounded by the generally small cohort sizes and the inclusion of only hospitalized patients, who tend to have a greater degree of exposure to antimicrobials. Therefore, a significant remaining question is whether the observed perturbations in the gut microbiome are of prognostic value.

FUTURE DIRECTIONS

To shed light on these open questions, it is essential that future studies of the gut microbiota in individuals with COVID-19 associate findings with robust and comprehensive metadata in order to ensure that findings can reliably inform clinical decision-making. The gut microbiota of hospitalized patients with moderate to severe COVID-19 can be heavily perturbed by the antimicrobial regimens that tend to be initiated for possible secondary bacterial pneumonia in these settings. In a retrospective study of 138 hospitalized COVID-19 patients, it was reported that 90% received antiviral therapy and many received antibacterial therapies such as moxifloxacin (64%), ceftriaxone (24%), and azithromycin (18%) (Wang et al. 2020a). Another relevant concern is the prevalence of comorbid GI diseases that may confound microbiome sampling results in this patient population. However, this may be of lesser concern given that, in one report, out of 103 COVID-19 patients presenting with a GI symptom at hospitalization, only four had underlying comorbid GI disease (Pan et al. 2020). Finally, concomitant infections or superinfections can have a significant impact on gut microbiota composition and must be considered when evaluating microbiome sampling data. Among 140 hospitalized patients with RT-qPCR-confirmed SARS-CoV-2 infection, tests also found the co-occurrence of IgM antibodies for *Mycoplasma pneumoniae* (five patients), RSV (one patient), and Epstein–Barr virus (one patient). However, there was no radiological evidence of superinfection (Zhang et al. 2020). Capturing robust and comprehensive metadata in future studies will be absolutely essential for determining the degree to which the aforementioned factors confound microbiome sampling data.

Ideally, future studies that aim to illuminate the role of the gut microbiota in COVID-19 will meet the following criteria. First, they will include a large representative cohort of individuals from the population with mild to moderate disease not requiring hospitalization. Second, they will prospectively sample the gut microbiota longitudinally according to a fixed schedule that includes sampling in the early, late, and postconvalescent stages of disease. Finally, they will capture comprehensive data on all symptoms experienced and medications taken during the entire course of disease. Moreover, analysis of the gut microbiota should be paired with the results of fecal viral testing to inform our understanding of whether viral shedding is linked to gut dysbiosis. These features will be essential qualities of any study that seeks to clearly define the role of the gut microbiome in COVID-19 disease pathogenesis.

Aside from serving as a potential prognostic indicator, the gut microbiota could be therapeutically valuable as a target for new disease interventions. Given the relative dearth of effective interventions, even at this stage in the pandemic, probiotics, prebiotics, and other gut microbiota focused treatments could improve clinical management of disease. A meta-analysis of GI symptoms reported in 47 studies including 10,890 unique patients found that GI symptoms were less prevalent in reports from China (5.8% (95% CI, 5.3%–6.4%)) than in reports from countries outside China (18.3% (95% CI, 16.6%–20.1%)) (Sultan et al. 2020). Although speculative, this finding could hint at a possible role for diet in modulating GI symptoms. Given the fact that diet is one of the biggest factors shaping gut microbial community composition, dietary interventions are an example of just one avenue to modulate symptoms or severity of disease via the gut microbiome. Another avenue may be through the administration of probiotics. The finding from Yeoh et al. (2021) that *Bifidobacterium bifidum*, a commonly used probiotic, is associated with decreased disease severity suggests that there may be a role for probiotic agents in modifying patient outcomes. However, this hypothesis remains to be tested in a prospective manner and, until this is done, should be considered speculative. Thus, studies into the gut microbiota may serve as the key to understanding the perplexing heterogeneity in GI symptoms and fecal viral shedding in COVID-19, of crucial importance to fundamental knowledge of as well as clinical management of the disease.

ADDITIONAL INFORMATION

Referees

Matthew Kelly
Anonymous

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