Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

EoE, and the combined biomarker model Eot3 + major basic protein showed the greatest ability to assess mucosal eosinophilic inflammation. A follow-up survey of patients showed that large majority preferred the 1-hour EST over endoscopy.

This present study by Ackerman et al, therefore, demonstrates that an EST can capture biomarkers relevant to EoE in a clinically feasible time frame of 1 hour. This technique has a significant potential to impact on how we monitor and assess disease activity in EoE. The use of the EST would decrease the need to have repeat endoscopic studies, resulting in savings of costs, time, and decreasing potential procedural complications associated with endoscopy. For those patients who do not require dilation, this technique would allow for a minimally invasive approach to monitor dietary or steroid therapies. In particular, it may provide some patients and families who have been hesitant to pursue dietary therapies owing to the frequency of endoscopies with an alternative tool for identifying food triggers. This technique may also open the door to potential screening for EoE in low-risk children and would certainly be compelling as an in-office screening tool. In addition, it may be used as a disease monitoring tool in clinically asymptomatic EoE patients because there are no biomarkers currently that correlate with mucosal inflammation.

The barriers to repeated endoscopy include risk of procedural complications, financial burden and health care costs associated with these procedures (Ann Allergy Asthma Immunol 2019;123:166–172). These barriers may contribute toward delayed diagnosis and suboptimal monitoring, so less invasive techniques are critical. Other novel methods under development including transnasal endoscopy, Cytosponge, and tethered confocal microscopy (Clin Gastroenterol Hepatol 2019;17:2455–2462; J Gastroenterol Hepatol 2016;31:590–594; Am J Gastroenterol 2017;112:1538–1544; Sci Rep 2018;8:2631), but they either are not yet widely adopted in practice or are still in research. Similar to the 1-hour EST, transnasal endoscopy can be performed in an outpatient setting and does not require anesthesia or sedation (Gastrointest Endosc 2016;83:299–306). Barriers include the need for additional training and equipment. Biophotonic imaging (ie, confocal microscopy) provides real-time assessment of EoE activity; however, this procedure requires specialized equipment and is primarily still research tool. The Cytosponge also does not require anesthesia and collects esophageal surface epithelial cells to detect Barrett's esophagus or assess disease activity in EoE. However, this technique is also not yet widely available and may be more challenging in the pediatric population.

The study by Ackerman et al extends the prior data on the 16-hour EST (Gut 2013;62:1395–1405; PLoS One 2015;10:e0128346) to provide additional evidence that a shorter, more clinically applicable test has equal sensitivity and specificity to determine disease activity. Not only is the 1-hour EST more palatable to patients than the 16-hour EST, it captures mucosal inflammation and is also a proxy for surface inflammation. However, there are limitations to consider for this study. First, it did not track patients longitudinally, so it remains to be seen that over time the EST results can be used to successfully adjust treatments and monitor response. In addition, some patients were not able to tolerate the 1-hour EST (ie, unable to swallow the string capsule; had a known esophageal narrowing; had an allergy to the gelatin capsule), suggesting that this modality will not be applicable for all patients. Considering all of the minimally invasive techniques discussed elsewhere in this article, it will be important to identify the right candidate for the right technique.

Based on the results of this study, we are forced to ask a key question of whether it is time to move away from sedated endoscopic procedures to assess for esophageal inflammation. The novel EST technique has important implications for clinical practice in both pediatric and adult populations. Although this finding is very promising, longitudinal studies will be important to better understand the use of this technique over traditional endoscopy in long-term management, particularly in correlation with clinical symptoms, mucosal eosinophilic inflammation, endoscopic findings, and molecular changes. Ackerman et al have shown us that the 1-hour EST may be the clinical test that we have been waiting for, and all strings are attached.

RAJITHA D. VENKATESH
Division of Pediatric Gastroenterology
Department of Pediatrics
Nationwide Children's Hospital
Columbus, Ohio

EVAN S. DELLON
Center for Esophageal Diseases and Swallowing
Division of Gastroenterology and Hepatology
University of North Carolina School of Medicine
Chapel Hill, North Carolina

Novel Coronavirus Disease-2019 and the Gastrointestinal Tract: Lessons Learned from Human Organoids

Lamers MM, Beumer J, van der Vaart J, et al. SARS-CoV-2 productively infects human gut enterocytes. Science 2020;369:50–54.

Coronavirus disease 2019 (COVID-19), which is caused by a novel severe acute respiratory syndrome coronavirus (SARS-CoV-2), has become the worst public health crisis in the current century. Although initially described to be a mainly respiratory illness, patients can present a range of extrarespiratory symptoms, including gastrointestinal manifestations. In fact, the receptor used by the virus to enter host cells, angiotensin-converting enzyme 2 (ACE2), is highly expressed in intestinal cells.

In this study, the authors aimed to determine whether the SARS-CoV-2 virus is able to directly infect human intestinal cells and replicate there. To this end, they generated

human small intestinal organoids (hSIOs) from primary gut epithelial stem cells. These organoids can be expanded indefinitely in 3-dimensional culture, and contain all proliferative and differentiated cell types of the in vivo epithelium, thus providing a helpful tool for modeling disease. When grew in 4 different conditions, hSIOs displayed a different proportion of cell types expressing varying amount of ACE2.

After exposure to SARS-CoV-2, the virus readily infected hSIOs as assessed by quantitative reverse transcription polymerase chain reaction for viral sequences and by live virus titrations on a monkey kidney cell line known to permit SARS-CoV replication (ie, VeroE6 cells). Viral RNA and infectious virus particles increased over time for all conditions. In differentiated hSIOs, SARS-CoV-2 titers remained stable at 60 hours after infection.

Performing confocal analysis on hSIOs, comparable rates of viral infections were observed in all tested growing conditions. Organoids showed staining for viral components in rare single cells at 24 hours. Those small infection clusters substantially increased and spread through the whole organoid after 60 hours. The virus infected both mature and progenitor enterocytes, whereas infection of enteroendocrine cells or goblet cells was not observed across culture conditions. ACE2 protein was readily revealed as a bright and ubiquitous brush border marker in differentiated hSIOs. In contrast, ACE2 staining was much lower in organoids with a high content of progenitor cells.

By using transmission electron microscopy, they observed that viral particles of 80 to 20 nm occurred in the lumen of the organoid at the basolateral and apical side of enterocytes. They also identified double-membrane vesicles, which are the subcellular site of viral replication. Interestingly, the state of infection at 60 hours varied among organoids, with some of them showing intact cellular organization, whereas abundant disintegrated cells were present in others.

Finally, they also evaluated gene expression changes induced by SARS-CoV-2 infection of hSIOS by mRNA sequencing. Transcriptomic analysis revealed strong induction of a generic viral response program characterized by a broad signature of cytokines and interferon-stimulated genes attributed to type I and III interferon responses.

Comment. Although fever and respiratory symptoms dominate the clinical presentation of patients with COVID-19, gastrointestinal manifestations are not uncommon. Approximately one-third of patients experience gastrointestinal symptoms such as nausea, vomiting, or diarrhea, sometimes before developing fever and lower respiratory tract signs and symptoms (Gastroenterology 2020;159:373–375). Importantly, the highest expression of ACE2 in the human body occurs in the brush border of intestinal enterocytes (https://www.proteinatlas.org/ENSG00000130234-ACE2). Moreover, detection of viral RNA has been reported in anal swabs and stool samples of COVID-19 patients as well as in sewage sludge collected in different cities during the pandemic (J Med Virol 2020:10.1002/jmv.26007; JAMA 2020;323:1843–1844; Nature 2020;580:176–177). All this evidence suggests that the gastrointestinal tract might be a target organ of SARS-CoV-2.

It is also worth noting that several animal coronaviruses are natural enteric pathogens, cause gastrointestinal diseases, and spread by the fecal–oral route. In this sense, viral RNA can be found in stool samples and rectal swabs from COVID-19 patients long after the respiratory symptoms have been resolved, and nasopharyngeal testing has turned negative (Lancet Gastroenterol Hepatol 2020;5:434–435). The prolonged persistence of SARS-CoV-2 in those samples suggests replication of the virus in the gastrointestinal tract and a potential role of the fecal–oral transmission route in spreading the disease. However, only a few studies have been able to isolate infective (viable) virus from human stool, whereas most of them only report the presence of viral RNA assessed by quantitative reverse transcription polymerase chain reaction, which does not necessarily reflect the existence of viable virus (Nature 2020;581:465–469).

Understanding the biology of novel viruses rely largely on in vitro models that allow viral replication. Human and animal organoids are currently proving to be a valuable experimental platform in this setting. Organoids are in vitro-cultured 3-dimensional structures that retain the genotype and phenotype of the parental cells providing a faithful recapitulation of key aspects of in vivo tissue. They can be established from both pluripotent stem cells and adult stem cells. Organoids derived from adult stem cells can be established directly from the healthy or diseased epithelium of many organs. Importantly, organoids are amenable to any experimental approach that has been developed for cell lines and represent more physiologic in vitro models that mimic the gastrointestinal tract more accurately than any traditional culture system. Human organoids cultures also have many potential advantages over animal models: (i) to provide faster and potentially more robust outcomes, (ii) to be more readily accessible, (iii) to simulate more accurately human tissue, (iv) to supply a larger quantity of material to work with, and (v) to minimize the use of animals in research. Therefore, organoid technology has opened up new avenues for experimental research, which were previously not possible with immortalized cell lines, short-term cultures of primary intestinal cells or in vivo models. In this sense, applications in experimental biology involve the modeling of human tissue physiology and disease, including infectious diseases. Finally, this technology enables the creation of biobanks of patient-derived organoids that can be used for drug development research, thus holding promise for developing personalized medicine (Cell 2015;161:933–945).

To investigate the molecular mechanisms of SARS-CoV-2 infection in the intestine, Lamars et al used human small intestine organoids established from primary gut epithelial stem cells. These organoids recapitulate in vitro the cellular identity and molecular characteristics of the intestinal epithelium, thus providing a useful model for SARS-CoV-2 invasion and transmission studies, because they can be rapidly expanded and maintained and are amenable to a wide range of in vitro manipulations. The observations made in their study provide definite proof that SARS-CoV-2

can infect and multiply within cells of the gastrointestinal tract. In fact, the virus is able to readily infect not only mature enterocytes, which present high expression of ACE2, but also enterocyte progenitors with significantly lower levels of this protein. Moreover, almost simultaneously, two different studies using human organoids also reported that SARS-CoV-2 is able to infect human enterocytes, thus reinforcing that the gastrointestinal tract is a potential target of SARS-CoV-2, which permits viral replication and might play a role in disease spreading (Sci Immunol 2020;5:eabc3582; Nat Med 2020;26:1077–1083).

Despite the many advantages of organoids, these in vitro models still have some limitations. In this sense, gastrointestinal organoids derived from adult stem cells, as the ones used by Lamars et al, consist only of intestinal epithelial cells such as enterocytes, enteroendocrine cells, and goblet cells. Therefore, the tissue environment of these organoids differs from the actual intestine due to the absence of stromal cells such as fibroblasts, neural cells, immune cells, and endothelial cells. These cells are involved in key processes such as immune responses, nutrient supply, paracrine signaling, and extracellular matrix production. This results in differences in the environment between the native organ and the organoid, which prevents the latter from completely recapitulating the actual organ. Moreover, the gastrointestinal tract harbors a rich and complex resident microbiota. These resident microbes influence intestinal biology, such as epithelial turnover, physiologic processes, and immune homeostasis, and compete with other pathogens that enter through the diet. In fact, the intestinal microbiota affects drug pharmacokinetics and treatment outcomes, and its importance is becoming increasingly evident in recent years. Therefore, including stromal cells and incorporating microbiota into gastrointestinal organoids would increase their complexity and better simulate the in vivo gastrointestinal environment. To make organoid models more realistic, researchers are attempting to improve their culture using a variety of methods, such as 3-dimensional bioprinting, biomaterials, and coculture with multiple cell types and microbiota (Development 2019;146:dev166173). In any case, it is worth noting that gastrointestinal organoids derived from adult stem cells exhibit greater complexity and resemble the actual human gut more closely than any other in vitro culture approach.

The present study clearly demonstrates the ability of SARS-Cov-2 to infect the human intestine but unanswered questions and challenges still remain, such as how the virus reaches the gastrointestinal tract, the significance of virus detection in the stool or rectal swabs of asymptomatic subjects, and how the virus could survive to pass through the harsh gastrointestinal environment. Although we do not know yet whether SARS-CoV-2 present in the gastrointestinal tract of COVID-19 patients plays a significant role in transmission, the findings of this study support that this possibility deserves further evaluation. In the meantime, it would be advisable to pay special attention to those patients with gastrointestinal symptoms. Broader viral testing using not only nasal swabs but also serially collected rectal swabs or stool samples might be considered in the clinical practice.

In summary, the authors impressively demonstrate that SARS-CoV-2 readily infect and multiply within human enterocyte lineage cells in an organoid model. Similar infection rates were observed in mature and progenitor enterocytes, thus suggesting that low levels of ACE2 may be sufficient for viral entry. These results support clinical observations pointing to the gastrointestinal tract as a potential target involved in COVID-19. Finally, they provide a model system for studying the pathogenesis of SARS-CoV-2, both in a basic research context as well as during the development of therapies for the disease. Further research is required to fully elucidate the role of the gastrointestinal tract in COVID-19.

ANTONIO JURADO-GOMEZ
Institute of Biomedicine of Seville (IBiS)
Seville, Spain

MARIA D. GIRALDEZ
Institute of Biomedicine of Seville (IBiS) *and*
Digestive Diseases Unit
Virgen del Rocio University Hospital *and*
University of Seville
Seville, Spain