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Evidence for gastrointestinal infection of SARS-CoV-2

Fei Xiao[1,2,3,*], Meiwen Tang[4,*], Xiaobin Zheng[5,*], Ye Liu[6], Xiaofeng Li[7], and Hong Shan[2,3,8]

1. Department of Infectious Diseases, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
2. Guangdong Provincial Engineering Research Center of Molecular Imaging, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
3. Guangdong Provincial Key Laboratory of Biomedical Imaging, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
4. Department of Hematology, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
5. Department of Respiratory and Critical Care Medicine, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
6. Department of Pathology, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
7. Department of Gastroenterology, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
8. Department of Interventional Medicine, the Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China

*Authors share co-first authorship.

Reprint requests

Address reprint request to: Hong Shan MD, PhD, Guangdong Provincial Engineering Research Center of Molecular Imaging, Guangdong Provincial Key Laboratory of Biomedical Imaging, Department of Interventional Medicine, the Fifth Affiliated Hospital, Sun Yat-sen University, 52 East Meihua Road, Zhuhai 519000, Guangdong Province, China. e-mail: shanhong@mail.sysu.edu.cn; Xiaofeng Li, MD, PhD, Department of Gastroenterology, the Fifth Affiliated Hospital, Sun Yat-sen University, 52 East Meihua Road, Zhuhai 519000, Guangdong Province, China. e-mail: zdwylxf@163.com; Ye Liu, MD, PhD, Department of Pathology, the Fifth Affiliated Hospital, Sun Yat-sen University, 52 East Meihua Road, Zhuhai 519000, Guangdong Province, China. e-mail: ly77219@163.com.

Author Contributions

HS, FX design the study, analyzed the data and wrote the paper.

MT, XZ, YL, XL acquired, analyzed and interpreted the data. HS supervised the study. All authors have seen and approved the final draft.

Conflict of interest

The authors disclose no conflicts.

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Since the novel coronavirus (SARS-CoV-2) was identified in Wuhan, China, at the end of 2019, the virus has spread to 32 countries, infecting more than 80000 people and causing over 2600 deaths globally. The viral infection causes a series of respiratory illness including severe respiratory syndrome, indicating the virus most likely infects respiratory epithelial cells and spreads mainly via respiratory tract from human to human. However, viral target cells and organs haven't been fully determined, impeding our understanding of the pathogenesis of the viral infection and viral transmission routes. According to a recent case report, SARS-CoV-2 RNA was detected in a stool specimen[1], raising the question of viral gastrointestinal infection and fecal-oral transmission route. It has been proved that SARS-CoV-2 uses ACE2 as a viral receptor for entry process[2] . ACE2 mRNA is highly expressed and stabilized by B^0AT1 in gastrointestinal system[3, 4], providing a prerequisite for SARS-CoV-2 infection. To further investigate the clinical significance of SARS-CoV-2 RNA in feces, we examined the viral RNA in feces from 71 patients with SARS-CoV-2 infection during their hospitalization. The viral RNA and viral nucleocapsid protein were examined in gastrointestinal tissues from one of the patients.

Methods

From February 1 to 14, 2020, clinical specimens including serum, nasopharyngeal and oropharyngeal swabs, urine, stool and tissues from 73 SARS-CoV-2-infected hospitalized patients were obtained in accordance with China Disease Control and Prevention (CDC) guidelines and tested for detection of SARS-CoV-2 RNA using the China CDC-standardized quantitative polymerase chain reaction assay[5]. Clinical

characteristics of the 73 patients were shown in Supplementary Table 1. The esophageal, gastric, duodenal and rectal tissues were obtained from one of the patients using endoscopy. The patient's clinical information was described in Supplementary Case Clinical Information and Supplementary table 2. Histological staining (H&E) as well as viral receptor ACE2 and viral nucleocapsid (NP) staining were performed as described in Supplementary Methods. The images of fluorescent staining were obtained using a laser scanning confocal microscopy (LSM880, Carl Zeiss MicroImaging) and shown in Figure 1. This study was approved by the Ethics Committee of The Fifth Affiliated Hospital, Sun Yat-sen University, and all patients signed the informed consent.

Results

From February 1 to 14, 2020, among all the 73 SARS-CoV-2-infected hospitalized patients, 39 (53.42%) including 25 males and 14 females tested positive for SARS-CoV-2 RNA in stool as shown in Supplementary Table 1. The age of patients with positive SARS-CoV-2 RNA in stool ranged from 10 months to 78 years old. Duration time of positive stool ranged from 1 to 12 days. Furthermore, 17 (23.29%) patients remained positive in stool after showing negative in respiratory samples.

Gastrointestinal endoscopy was performed on a patient as described in Supplementary Case Clinical Information. As shown in Figure 1, the mucous epithelium of esophagus, stomach, duodenum and rectum showed no significant damage with H&E staining. Infiltrate of occasional lymphocytes was observed in esophageal squamous epithelium.

In lamina propria of stomach, duodenum and rectum, numerous infiltrating plasma cells and lymphocytes with interstitial edema were seen.

Importantly, viral host receptor ACE2 stained positive mainly in the cytoplasm of gastrointestinal epithelial cells (Figure 1). To note, we observed that ACE2 is rarely expressed in esophageal epithelium, but abundantly distributed in cilia of glandular epithelia. Staining of viral nucleocapsid protein (NP) was visualized in the cytoplasm of gastric, duodenal and rectum glandular epithelial cell, but not in esophageal epithelium. The positive staining of ACE2 and SARS-CoV-2 was also observed in gastrointestinal epithelium from other patients, who tested positive for SARS-CoV-2 RNA in feces (data not shown).

Discussion

In this manuscript, we provide evidence for gastrointestinal infection of SARS-CoV-2 and its possible fecal-oral transmission route. Since viruses spread from infected to uninfected cells[6], viral specific target cells or organs are determinants of viral transmission routes. Receptor-mediated viral entry into a host cell is the first step of viral infection. Our immunofluorescent data showed that ACE2 protein, which has been proved to be a cell receptor for SARS-CoV-2, is abundantly expressed in the glandular cells of gastric, duodenal and rectal epithelia, supporting the entry of SARS-CoV-2 into the host cells. ACE2 staining is rarely seen in esophageal mucosa probably because esophageal epithelium is mainly composed of squamous epithelial cells, which express less ACE2 than glandular epithelial cells.

Our results of SARS-CoV-2 RNA detection and intracellular staining of viral nucleocapsid protein in gastric, duodenal and rectal epithelia demonstrate that SARS-CoV-2 infects these gastrointestinal glandular epithelial cells. Although viral RNA was also detected in esophageal mucous tissue, absence of viral nucleocapsid protein staining in esophageal mucosa indicates low viral infection in esophageal mucosa.

After viral entry, virus-specific RNA and proteins are synthesized in the cytoplasm to assembly new virions[7], which can be released to gastrointestinal tract. The continuous positive detection of the viral RNA from feces suggests that the infectious virions are secreted from the virus-infected gastrointestinal cells. Recently, we and others have isolated infectious SARS-CoV-2 from stool (Manuscript under revision), confirming the release of the infectious virions to the gastrointestinal tract. Therefore, fecal-oral transmission could be an additional route for viral spread. Prevention of fecal-oral transmission should be taken into consideration to control the spread the virus.

Our results highlight the clinical significance of testing viral RNA in feces by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) since infectious virions released from gastrointestinal tract can be monitored by the test. According to the current CDC guidance for disposition of patients with SARS-CoV-2, the decision to discontinue Transmission-Based Precautions for hospitalized SARS-CoV-2 patients is based on negative results of rRT-PCR testing for SARS-CoV-2 from at least two sequential respiratory tract specimens collected ≥24 hours apart[8]. However, we

observed in more than 20% of SARS-CoV-2 patients that the viral RNA remained positive in feces even after negative conversion of the viral RNA in respiratory tract, indicating that the viral gastrointestinal infection and the potential fecal-oral transmission can last even after viral clearance in respiratory tract. Therefore, we strongly recommend that rRT-PCR testing for SARS-CoV-2 from feces should be performed routinely in SARS-CoV-2 patients, and Transmission-Based Precautions for hospitalized SARS-CoV-2 patients should continue if feces tests positive by rRT-PCR testing.

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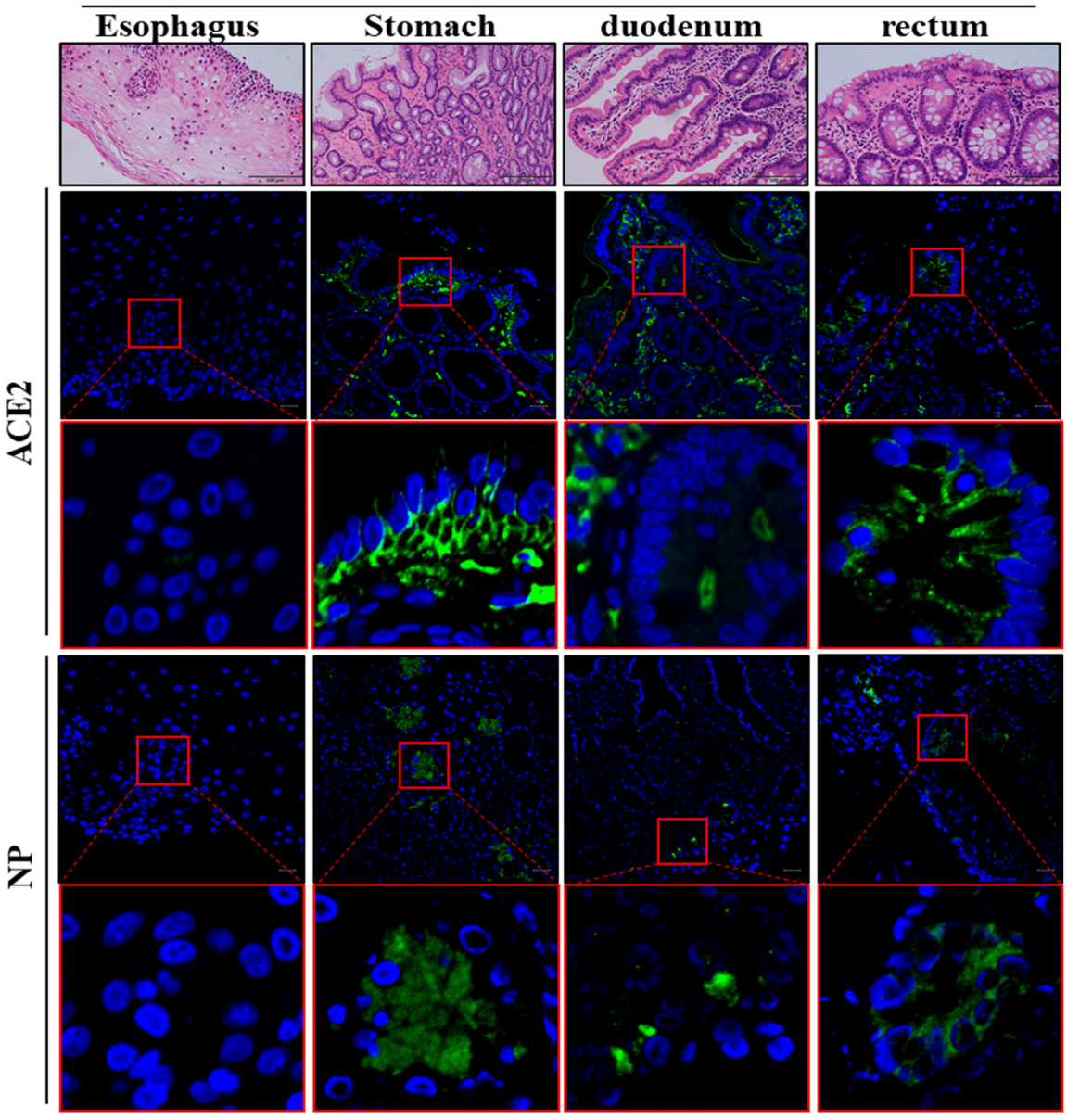
Figure Legend

Figure 1. Images of Histological and Immunofluorescent Staining of Gastrointestinal Tissues.

Shown are images of histological and immunofluorescent staining of esophagus, stomach, duodenum and rectum. The scale bar in the histological image represents 100 microns. The scale bar in the immunofluorescent image represents 20 microns.


Esophagus
Stomach
duodenum
rectum
ACE2
NP

Supplementary Material

Case Clinical Information

On January 17, 2020, a 78-year-old man, who came to visit his daughter together his wife from Wuhan six days ago, presented to the outpatient clinic at our hospital in Zhuhai, Guangdong Province, China, with 7-day cough and fever. He was admitted to the negative pressure isolation room in the Department of Infectious Diseases at our hospital as a suspected case of SARS-CoV-2 infection. On admission, the physical examination revealed a body temperature of 37.5□, blood pressure of 105/56 mmHg, pulse of 67 beats per minute and respiratory rate of 22 breaths per minute with oxygen saturation of 97%. On physical examination, auscultation revealed rhonchi and cracks on bilateral lungs. Initial arterial blood gas analysis showed the arterial partial pressure of oxygen (PaO_2) / fraction of inspiration oxygen (FiO_2) was 176. Nasopharyngeal and oropharyngeal swab specimens tested positive by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for SARS-CoV-2. Chest CT presented with multiple ground-glass opacities, coinciding with previous report[1,2] , showing evidence of pneumonia in both left and right lungs. His wife and daughter tested positive for SARS-CoV-2 RNA and admitted to the hospital on January 18, 2020.

On hospital days 1 through 3, the patient remained fever with stable vital signs. The oxygen saturation remained above 95% with high-flow oxygen therapy. Empiric antimicrobials with oseltamivir and moxifloxacin was given during this period of time.

On hospital day 4, the patient developed severe respiratory distress with the PaO_2/FiO_2 decreasing to 130 and was immediately transferred to the intensive care unit receiving an intubation and mechanical ventilation. Along with sedation, prone position mechanical ventilation was applied for 12 hours per day and low tidal volume was set. The PaO_2/FiO_2 increased to 350 immediately after intubation, but decreased gradually again in the following several days to the lowest level of 70 at 10 days after admission. Meanwhile, the chest X-ray showed extensive bilateral consolidation. And the emergent veno-venous extracorporeal membrane oxygenation (VV-ECMO) was applied at the same day. On day 10, coffee ground gastric contents were observed from the gastric drainage tube and fecal occult blood tested positive, indicating upper gastrointestinal bleed. Gastrointestinal endoscopy was performed to determine the exact location of bleeding. Mucosa damage in esophagus was observed under endoscopy. Biopsy samples were taken from esophagus, gastric, duodenum and colon for histopathological and immunofluorescent staining. 1 day after treatment with octreotide and esomeprazole, gastrointestinal bleeding stopped. As of February 12, 2020, the patient remained hospitalized. The vital signs are stable with mechanical ventilation, V-V ECMO, and low dose vasopressors. There is no obvious evidence of other organs dysfunction.

Methods

Histopathological and Immunofluorescent Staining

Esophageal, gastric, duodenal and rectal tissues were obtained using endoscopy on day 10. Samples were embedded with Paraffin and then stained with hematoxylin and

eosin. For immunofluorescent staining, 3 μm-thick sections were dewaxed in xylene, rehydrated in alcohol, and washed in distilled water 3 times before microwave repair. Following washing three times in phosphate-buffered saline with Tween (PBST), sections were incubated with 10% goat serum in PBST for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies (anti-ACE2, Sino Biological, 10108-T56, 1:500; anti-NP, Sino Biological, 40143-T62, 1:500). The slides were incubated with secondary antibodies (Alexa Fluor®647-conjugated goat anti-rabbit IgG, bs-0296G-AF647, Bioss, 1:100) for 1 h at room temperature followed by washing three times with PBST. Nuclei were then counterstained with 4', 6-diamidino-2-phenylindole (DAPI) after washing three times with PBST. Slides were imaged using a laser scanning confocal microscopy (LSM880, Carl Zeiss MicroImaging).

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Supplementary Table 1. Clinical characteristics of the 73 hospitalized SARS-CoV-2 patients.

	S+	R+S+	(R+S+/S+)%	~R+S+	(~R+S+/R+S+)%	~R-S+	(~R-S+/R+S+)%	~R-S-	(~R-S-/R+S+)%
	73	39	53.42%	6	15.38%	17	43.59%	16	41.03%
Sex									
F	32	14	43.75%	2	14.29%	5	35.71%	7	50.00%
M	41	25	69.98%	4	16.00%	12	48.00%	9	36.00%
Age	43 (0.83-7)	49 (0.83-78)		52.5 (3-78)		44 (0.83-69)		47 (19-75)	
Tumours	7	3	42.86%	1	33.00%	1	33.00%	1	33.00%
Surgical history	17	8	47.06%	1	12.50%	4	50.00%	3	37.50%
Ulcer	0	0		0		0		0	
Smoking	9	4	44%	0	0	2	50.00%	2	50.00%
Respiratory symptoms	53	30	56.60%	4	13.33%	13	43.33%	13	43.33%
Typical chest CT	66	36	54.55%	5	13.89%	16	44.44%	15	41.67%
Diarrhoea	26	17	65.38%	2	11.76%	6	35.29%	9	52.94%
Gastrointestinal bleedi	10	4	40%	1	25.00%	1	25.00%	2	50.00%
Use of corticosteroid	21	12	57.14%	2	16.67%	3	25.00%	7	58.33%
Antibiotic therapy	60	35	52.05%	6	17.14%	14	40.00%	15	42.86%
Antiviral therapy	73	38	49.32%	6	15.79%	16	42.11%	16	42.11%
PPIs therapy	51	24	47.06%	4	16.67%	6	25.00%	14	58.33%
NSAID	12	6	50.00%	1	16.67%	2	33.33%	3	50.00%
ICU	4	4	100%	1	25.00%	1	25.00%	2	50.00%

R: respiratory specimens, S+: tested positive in stool during hospitalization, CT: computerized tomography,

PPIs: proton pump inhibitors, ICU: Intensive care unit, NSAID= Non-steroidal anti-inflammatory drugs,

~R+S+: remained positive in both R and S till the date of writing the manuscript on February 14th, 2020,

~R-S+: tested negative in R but remained positive in stool till the date of writing the manuscript on February 14th, 2020.

Supplementary Table 2. Time line of detection of nCoV-2019 in different specimens of the SARS-CoV-2-patient.

Specimen	Day 1	Day 2	Day 3	Day 5	Day 7	Day 9	Day 10	Day 11	Day 13	Day 14	Day 16	Day 18	Day 20	Day 21	Day 22	Day 24	Day 26
Respiratory specimens	NT	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Stool	NT	NT	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive	Positive	NT	Positive	Positive	Positive	Positive	Positive
Serum	NT	NT	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	NT	NT	NT	Negative	NT	Negative
Urine	NT	NT	Negative	Negative	Negative	Negative	Negative	NT	Negative	NT	Positive	NT	NT	NT	NT	NT	NT
Esophagus	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Stomach	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Duodenum	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Rectum	NT	NT	NT	NT	NT	NT	Positive	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

NT denotes not tested.