Postoperative Onset and Detection of SARS-CoV-2 in Surgically Resected Specimens From Gastrointestinal Cancer Patients With Pre/Asymptomatic COVID-19

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Objective: To describe the epidemiologic features and clinical courses of gastrointestinal cancer patients with pre/asymptomatic COVID-19 and to explore evidence of SARS-CoV-2 in the surgically resected specimens.

Summary Background Data: The advisory of postponing or canceling elective surgeries escalated a worldwide debate regarding the safety and feasibility of performing elective surgical procedures during this pandemic. Limited data are available on gastrointestinal cancer patients with pre/asymptomatic COVID-19 undergoing surgery.

Methods: Clinical data were retrospectively collected and analyzed. Surgically resected specimens of the cases with confirmed COVID-19 were obtained to detect the expression of ACE2 and the presence of SARS-CoV-2.

Results: A total of 52 patients (male, 34) with a median age 62.5 years were enrolled. All the patients presented no respiratory symptoms or abnormalities on chest computed tomography before surgery. Six patients (11.5%) experienced symptom onset and were confirmed to be COVID-19. All were identified to be preoperatively pre/asymptomatic, as 5 were with SARS-CoV-2 presenting in cytoplasm of enterocytes or macrophages from the colorectal tissues and 1 had symptom onset immediately after surgery. The case fatality rate in patients with COVID-19 was 16.7%, much higher than those without COVID-19 (2.2%).

Conclusions: Gastrointestinal cancer patients with pre/asymptomatic COVID-19 were at high risk of postoperative onset and death. At current pandemic, elective surgery should be postponed or canceled. It highlights the need for investigating the full clinical spectrum and natural history of this infection. The early colorectal tropism of SARS-CoV-2 may have major implications on prevention, diagnosis, and treatment of COVID-19.

Keywords: asymptomatic, COVID-19, gastrointestinal cancer, SARS-CoV-2, surgery

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On December 31, 2019, an outbreak of pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), now known as 2019 novel coronavirus disease (COVID-19), was first reported in Wuhan, China.[1] On March 11, 2020, World Health Organization announced COVID-19 outbreak a pandemic. As of April 24, 2020, there were 2,626,321 confirmed cases and 181,938 deaths.[2] With the improvement of the emergency situations in some areas, ramping up to prepare for elective surgeries is becoming the present focus for an increasing number of facilities. However, it has been shown that estimated asymptomatic proportion was as high as 33.3%,[3] and SARS-CoV-2 could be transmitted by asymptomatic individuals.[4] Most importantly, a considerable number of pre/asymptomatic patients showed no abnormal signs in laboratory or radiological tests.[5,6] There were gastrointestinal cancer patients with pre/asymptomatic COVID-19 seeking surgical care during this pandemic, and it is foreseeable that there will be a significant number in postpandemic period. Hence, there is an urgent need to understand the epidemiologic features and clinical course of those patients.

During the early period of this pandemic in Wuhan, China, a small number of gastrointestinal cancer patients with pre/asymptomatic COVID-19 were sent to the operating room to undergo surgery. We presented here, for the first time, the epidemiologic features and clinical courses of a consecutive series of gastrointestinal cancer patients with pre/asymptomatic COVID-19 and evidence of SARS-CoV-2 in surgically resected specimens.

METHODS

Study Design and Patients

In this retrospective study, we enrolled a consecutive series of gastrointestinal cancer patients who underwent surgery between January 2 and January 21, 2020, and identified those who were confirmed to be SARS-CoV-2 infected after surgery at Renmin Hospital of Wuhan University in Wuhan, China. The diagnosis of COVID-19 was performed according to the Diagnosis and Treatment Program of 2019 New Coronavirus Pneumonia (6th edition) formulated by the National Health Commission of China, and all patients were followed up until April 23, 2020.

The study was approved by the Ethics Committee of Renmin Hospital of Wuhan University (approval number WDRY2020-K151). Before enrollment, the patients or their relatives were asked for verbal consent, where applicable, or such consent was waived.

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Data Collection

The epidemiological, demographic, and clinical information of patients was extracted from patients' electronic medical records. All data were reviewed independently by 2 physicians (Y.L.L. and J.R.).

Histologic Examination

All surgically resected specimens of the SARS-CoV-2 infected patients including stomach, appendix, colon, or rectum, if available, were sent for pathological evaluation. Formalin-fixed, paraffin-embedded (FFPE) sections of all specimens were made for routine histologic examination. For ultrastructural study, available tissues from those confirmed or suspected to be preoperative infected patients were fixed with 10% formaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, and processed for examination by electron microscopy. Surgically resected samples from age- and sex-matched patients without COVID-19 were used as controls in all experiments.

Multiplex Immunofluorescence Assay

FFPE sections underwent deparaffinization with xylene for 15 minutes twice, and then were dehydrated with 100%, 85%, and 75% ethanol, and water washes for 20 minutes at room temperature. Antigen retrieval was performed in pH 6.0 citrate buffer for 15 minutes at 100°C. After rinsing with phosphate-buffered saline (PBS, pH7.4), sections were blocked for 30 minutes at room temperature in 3% bovine serum albumin (BSA) and incubated with the primary antibody rabbit polyclonal antibody against angiotensin-converting enzyme-2 (ACE2; Servicebio, #GB11267) at dilution 1:3000 overnight at 4°C. Sections were rinsed with PBS and incubated with secondary goat IgG HRP conjugated goat antirabbit antibody (Servicebio, #GB23303) for 50 minutes at room temperature. After rinsing with PBS, sections were incubated for 10 minutes at room temperature in TSA. Slices were rinsed with TBST and antigen retrieval was performed in pH 6.0 citrate buffer for 15 minutes at 100°C and incubated with the primary antibody Anti-Coronavirus spike rabbit polyclonal mouse antibody (Sino Biological, #40150-T62-COV2) at dilution 1:200 overnight at 4°C. Secondary antibody goat IgG CY3 conjugated goat antirabbit antibody (Servicebio, #GB21303) was applied. Rinsing with PBS again, buffer containing DAPI (Servicebio, #G1012) were added for an additional incubation of 10 minutes. Sections were cover-slipped using antifade mounting medium (Servicebio, #G1401). Images were captured on a fluorescence microscopy (NIKON ECLIPSE C1, Tokyo, Japan) with an imaging system (NIKON DS-U3, Tokyo, Japan) and processed using ImageJ software (National Institutes of Health, Bethesda, MD).

Multiplex Immunofluorescence and Fluorescence In Situ Hybridization (ISH)

After deparaffinization, antigen retrieval was performed in pH 6.0 citrate buffer for 15 minutes at 100°C and digested by proteinase for 22 minutes at 37°C. After rinsing with PBS, prehybridization step was performed at 37°C for 1 hour. After removing prehybridization buffer, sections were exposed to 6 ng/μL SARS-CoV-2 target probe (5'-CY3-CCGUCUGCGGUAUGUGGAAAGGUUAUGG-3') and incubated at 37°C in a hybridization oven overnight. After washing the slides in 2× saline-sodium citrate (SSC), 1× SSC, and 0.5× SSC buffer for 30 minutes, antigen retrieval was performed in pH 6.0 citrate buffer for 15 minutes at 100°C. Sections were blocked for 30 minutes at room temperature in 3% BSA, then incubated with the primary antibody rabbit polyclonal antibody against angiotensin-converting enzyme-2 (ACE2; Servicebio, #GB11267) at dilution 1:200 overnight at 4°C. After rinsing in PBS (pH 7.4), sections were incubated with secondary goat IgG FITC conjugated goat antirabbit antibody (Servicebio, #GB22303) for 50 minutes at room temperature. Rinsing with PBS (pH 7.4) again, buffer containing DAPI (Servicebio, #G1012) were added for an additional incubation of 8 minutes. Sections were cover-slipped using antifade mounting medium (Servicebio, #G1401). All buffers were DEPC-treated.

Statistical Analysis

For statistical analyses, SPSS 20.0 (SPSS, Chicago, IL) was used. Continuous variables are presented as the median (interquartile range [IQR]), whereas categorical variables are described as proportions. Paired *t* tests were used for analysis of differences between groups. $P < 0.05$ was considered significant.

RESULTS

Clinical Features

A total of 52 patients (male, 34) with a median (IQR) age 62.5 (54.3–67.0) years were enrolled. Demographic and clinical features of these patients are summarized in Table 1. The median follow-up time were 101.5 (97.0–107.0) days. All the patients presented with no respiratory symptoms or abnormalities on chest computed tomography (CT) before surgery. The total case fatality rate was 3.8% (2/52). Among them, 6 (11.5%) patients experienced symptom onset and were confirmed to be COVID-19 after surgery. The median age of the 6 patients was 65.5 (IQR 54.5–73.0) years but ranged from 47 to 76 years (Table 2); 4 patients were female. Comorbidities amongst these patients included hypertension (3/6), diabetes, and past history of tuberculosis (1/6), and myelosuppression after neoadjuvant chemotherapy (1/6). The median time between surgery and symptom onset was 3.5 days (IQR 1.0–14.3 days) but ranged from 1 to 18 days. Unexplained fever was the most common presentation and seen in all patients; 3 patients had cough; 5 patients presented with dyspnea; 1 patient had both myalgia and diarrhea (from postoperative day [POD] 4 to POD 8 for diarrhea); 3 patients had fatigue; and none patient presented with abdominal distension and vomiting. The median time from first symptom onset to dyspnea was 9.0 days (IQR 8.0–10.5 days). One patient (Patient 4) required care in the intensive care unit and was ventilated for acute respiratory distress syndrome (ARDS). Unfortunately, this patient died on POD 22. As of April 23, the other 5 patients were all discharged.

Laboratory Findings

All patients had abnormal full blood counts at the time of symptom onset after surgery: Three patients had evidence of leucocytosis. Long-lasting lymphopenia ($<1.10 \times 10^9$/L), which lasted for more than 1 week, occurred in 5 patients. Three patients had an increased absolute neutrophil count. Abnormal liver function tests were also common and typically displayed decreased albumin (<40 g/L) in all patients, with no liver metastasis observed among them. Nasopharyngeal swabs were obtained from 5 patients after symptom onset and 1 patient (patient 5) before onset (regular screening for suspicious hospital-acquired infection) to test for SARS-CoV-2 with quantitative reverse-transcriptase-polymerase-chain-reaction assay by using a specific nucleic acid detection kit and all the patients were confirmed to be SARS-CoV-2 infected (Table 3).

When compared with preoperative levels, the 6 patients with COVID-19 showed significant decreases in total circulating lymphocytes on PODs 1, 3, 5, and 7 ($P = 0.005$, 0.007, 0.008, and 0.015, respectively) or on onset days 1, 3, 5, and 7 ($P = 0.013$, 0.008, 0.010, and 0.020, respectively); the 46 patients without COVID-19 had significant falls in lymphocyte counts on PODs 1, 3, and 5 ($P <$ 0.001, =0.002, and <0.001, respectively), and returned close to preoperative level on POD 7 ($P = 0.097$; Supplementary Fig. 1A and

TABLE 1. Demographic and Clinical Features of Gastrointestinal Cancer Patients With or Without COVID-19 Undergoing Surgery

	All patients (n = 52)	Patients without COVID-19 (n = 46)	Patients with COVID-19 (n = 6)
Median age (yrs old)	62.5 (54.3–67.0)	62.5 (53.8–67.0)	65.5 (54.5–73.0)
Sex			
Male	34 (65.4))	32 (69.6)	2 (33.3)
Female	18 (34.6)	14 (30.4)	4 (66.7)
Primary diagnosis			
Gastric cancer	16 (30.7)	15 (32.6))	1 (16.7)
Small intestinal cancer	3 (5.8)	3 (6.5)	0 (0)
Colorectal cancer	33 (63.5)	28 (60.9)	5 (83.3)
Comorbidities			
Diabetes mellitus	6 (11.5)	5 (10.9)	1 (16.7)
Cardiovascular disease	18 (34.6)	15 (32.6)	3 (50.0)
Pulmonary disease	7 (13.5)	6 (13.0)	1 (16.7)
Renal failure	2 (3.8)	2 (4.3)	0 (0)
Other	8 (15.4)	6 (13.0)	2 (33.3)
Outcomes			
Alive	50 (96.2)	45 (97.8)	5 (83.3)
Deceased	2 (3.8)	1 (2.2)	1 (16.7)

B, Supplemental Digital Content 1, http://links.lww.com/SLA/C490). The patients with COVID-19 presented more prolonged lymphopenia than those who without COVID-19. Both groups showed significant increases in absolute neutrophil counts on POD1 ($P = 0.029$ and <0.001 for the patients with COVID-19 and those without COVID-19, respectively), but this rise was short-lived for the patients with COVID-19 whose level returned to preoperative level on POD 3 ($P = 0.146$ and 0.012 for the patients with COVID-19 and those without COVID-19, respectively; Supplementary Fig. 1C, Supplemental Digital Content 1, http://links.lww.com/SLA/C490). Neutrophils also increased in the patients with COVID-19 because symptom onset and fell soon thereafter, but percentage increases or falls were not evident (all $P > 0.05$; Supplementary Fig. 1D, Supplemental Digital Content 1, http://links.lww.com/SLA/C490).

Radiological Findings

When compared with respiratory symptoms, postoperative chest radiological changes were disproportionally severe after symptom onset. Five patients showed typical findings on chest CT images

TABLE 2. Clinical Characteristics of the 6 Gastrointestinal Cancer Patients With Pre/Asymptomatic COVID-19 Undergoing Surgery

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (yrs old)	59	57	47	76	72	72
Sex	Female	Female	Male	Female	Female	Male
Primary diagnosis	Rectal cancer	Gastric cancer	Sigmoid colon cancer	Descending colon cancer	Rectal cancer	Sigmoid colon cancer
Comorbidities	None	Myelosuppression after neoadjuvant chemotherapy	Diabetes and past history of tuberculosis	Hypertension	Hypertension	Hypertension
Epidemiological history	Yes (contact with infected person)	Yes (contact with infected person)	Yes (exposure to relevant environment)	Yes (exposure to relevant environment)	Yes (exposure to relevant environment)	Yes (contact with infected person)
Date of surgery	Jan 7, 2020	Jan 13, 2020	Jan 17, 2020	Jan 17, 2020	Jan 19, 2020	Jan 21, 2020
Procedures	Laparoscopic abdominoperineal resection	Laparoscopic total gastrectomy	Laparoscopic sigmoidectomy	Left colectomy	Laparoscopic Hartmann's procedure	Laparoscopic sigmoidectomy
Complications	Perineal incision infection	None	None	ARDS	None	None
Onset day	POD 18	POD 1	POD 1	POD 5	POD 13	POD 2
First symptom	Fever	Fever	Fever	Fever	Fever	Fever
Fever	Yes	Yes	Yes	Yes	Yes	Yes
First day temp (°C)	37.4	38.5	37.6	37.7	37.5	37.3
Highest temp (°C)	37.8	40.0	38.1	39.1	37.5	38.1
Lasting days (d)	16	6	9	9	1	14
Cough	Yes	Yes	No	No	No	Yes
Dyspnea	Yes	Yes	No	Yes	Yes	Yes
From onset to dyspnea (d)	9	7	NA	10	9	11
Myalgia	No	Yes	No	No	No	No
Fatigue	No	Yes	No	No	Yes	Yes
Diarrhea	No	Yes	No	No	No	No
Abdominal distension	No	No	No	No	No	No
Vomiting	No	No	No	No	No	No
Outcomes	Discharged	Discharged	Discharged	Deceased	Discharged	Discharged

NA indicates not available.

TABLE 3. Laboratory Findings of the 6 Gastrointestinal Cancer Patients With Pre/Asymptomatic COVID-19 Undergoing Surgery

	Reference range	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6	
		Adm	Onset	Adm	Onset	Adm	Onset	Adm	Onset	Adm	Onset	Adm	Onset
White-cell count (10^9/L)	3.50–9.50	7.37	3.08	1.79	6.49	6.88	14.17	5.81	11.54	8.66	2.88	5.06	11.16
Red-cell count (10^{12}/L)	4.30–5.80	3.45	3.15	3.84	3.29	3.18	4.10	3.25	2.34	3.55	3.34	4.07	3.82
Absolute neutrophil count (10^9/L)	1.80–6.30	4.35	1.75	5.90	5.23	4.85	12.78	3.49	9.35	4.49	1.15	2.82	9.49
Absolute lymphocyte count (10^9/L)	1.10–3.20	2.48	1.07	0.83	0.53	1.29	0.92	1.84	0.99	1.68	1.37	1.64	0.79
Platelet count (10^9/L)	125-350	248	174	107	151	602	423	158	98	355	205	109	109
Hemoglobin (g/L)	130-175	115	103	119	102	70	99	112	79	108	106	129	121
Hematocrit (%)	0.350–0.450	0.327	0.300	0.360	0.313	0.247	0.326	0.324	0.233	0.338	0.323	0.385	0.353
Sodium (mmol/L)	137.0–147.0	137.0	143.0	143.1	143.0	139.4	136.0	114.6	145.0	140.1	141.0	139.4	137.7
Potassium (mmol/L)	3.50–5.30	3.70	3.05	3.95	3.44	3.99	4.81	4.22	3.67	3.72	4.25	3.85	3.75
Chloride (mmol/L)	99.0–110.0	102.2	106.5	111.2	114.3	105.8	103.6	111.3	109.0	105.4	109.0	107.2	105.8
Calcium (mmol/L)	2.11–2.52	2.31	2.01	2.13	1.70	2.16	2.02	2.18	1.99	2.00	1.97	1.90	1.87
Carbon dioxide (mmol/L)	22.0–33.0	28.4	29.4	23.2	20.9	25.1	25.0	23.5	25.4	24.4	28.6	29.5	23.8
Anion gap (mmol/L)	12.00–20.00	10.30	11.05	12.65	11.24	12.49	12.21	14.02	14.27	14.02	12.87	6.55	11.85
Glucose (mmol/L)	3.90–6.10	4.83	4.40	4.17	5.54	4.76	5.31	5.45	17.43	7.16	6.31	4.26	5.07
Blood urea nitrogen (mmol/L)	3.60–9.50	2.24	1.80	3.58	1.64	3.67	2.46	5.83	9.41	3.10	3.81	5.07	5.46
Creatinine (μmol/L)	57-111	46	35	50	56	53	63	113	137	38	35	73	76
Total protein (g/L)	65.0–85.0	69.0	55.8	64.1	49.4	60.8	52.4	67.4	47.7	57.4	55.1	56.4	51.9
Albumin (g/L)	40.0–55.0	45.0	33.4	38.6	26.8	38.9	32.2	40.6	30.1	34.9	30.9	34.3	31.4
Total bilirubin (μmol/L)	0–23.0	12.2	6.2	12.2	9.6	7.1	15.6	12.4	15.0	12.9	10.4	13.3	10.0
Alanine aminotransferase ALT(U/L)	9-50	8	29	26	35	20	12	9	10	16	25	15	18
Aspartate aminotransferase AST(U/L)	15-40	16	26	36	53	22	16	20	19	16	21	22	27
Alkaline phosphatase (U/liter)	15.0–125.0	46.0	46.8	111.4	93.7	51.9	44.1	58.0	47.5	79.0	69.0	55.5	56.9
COVID-19 laboratory confirmed		POD 18		POD 8		POD 7		POD 8		POD 5		POD 5	

Adm indicates at admission; Onset, on onset day.

with ground-glass opacity or consolidation (Fig. 1A–C, E, and F), and 1 severe patient showed bilateral stable streaky opacities in chest radiograph, a finding consistent with atypical pneumonia (Fig. 1D, patient 4).

Histologic and Virologic Findings

The surgical specimens were sent for pathological evaluation. FFPE sections with hematoxylin-eosin staining for all available specimens were reviewed by a pathologist (J.P.Y.). There was no evidence of bacterial invasion or viral inclusions in all patients. The sections of all patients showed no specific signs of viral infections in either cancer tissues or adjacent tissues. By using electron microscopy (reviewed by Y.G. and G.M.), coronavirus-like particles ranging in diameter from 50 to 90 nm within smooth walled envelopes were shown in the cytoplasm of epithelial cells from the adjacent normal appendix, colon or rectum tissues of 5 patients. Clusters of viral particles were also detected on cell surface microvilli (Fig. 2A–F).

ACE2 Expression and Presence of SARS-CoV-2 in Surgically Resected Specimens

Multiplex immunofluorescence assay or multiplex immunofluorescence and fluorescence ISH were used to detect ACE2, coronavirus spike protein, and SARS-CoV-2 RNA, respectively. ACE2 (green, Figs. 3 and 4) was considerably expressed in enterocytes or macrophages of the appendix, colon, and rectum, and

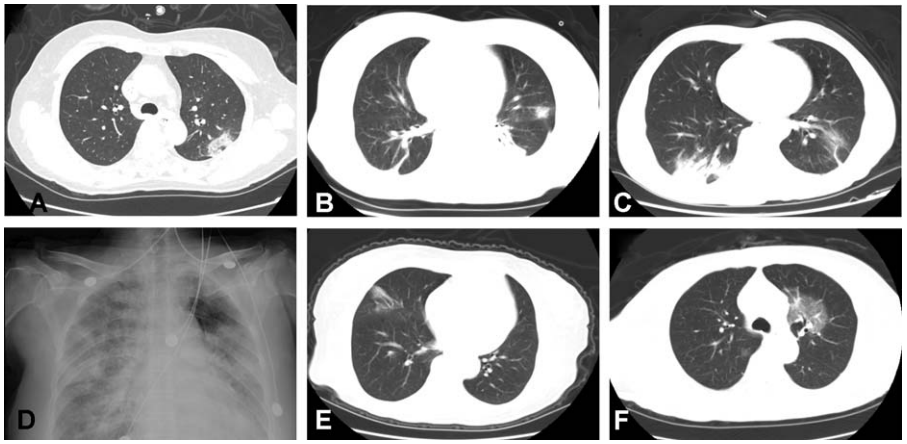

FIGURE 1. Postoperative chest radiological findings of the 6 gastrointestinal cancer patients with pre/asymptomatic COVID-19. Chest computed tomography images showed typical findings with ground-glass opacity or consolidation: A to C, E, and F, patient 1 to 3, 5, and 6; chest radiograph of 1 severe patient showed bilateral stable streaky opacities: D, patient 4.

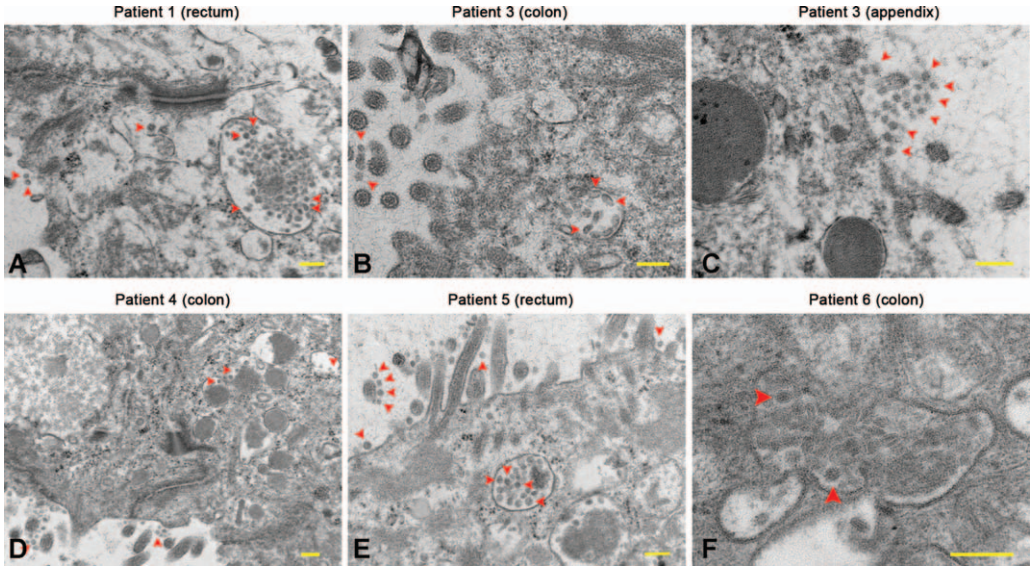

FIGURE 2. Ultrastructural study of 5 colorectal cancer patients with pre/asymptomatic COVID-19. Representative images of electron microscopy, viral particles ranging in diameter from 50 to 90 nm (indicated by red arrows) within smooth walled envelopes were shown in the cytoplasm of enterocytes from adjacent normal colorectal tissues. A number of viral particles were also seen on cell surface microvilli: A – F. Scale bar, 200 nm.

coronavirus spike protein (red, Fig. 3) and SARS-CoV-2 RNA (red, Fig. 4) presented in enterocytes or macrophages. Notably, most of the viral particles were colocalized with ACE2 (yellow; Figs. 3 and 4). Evidence of coronavirus was not found in tissue of 1 patient with gastric cancer (patient 2).

DISCUSSION

In this study, we enrolled 52 consecutive gastrointestinal cancer patients who underwent surgery in a single officially designated COVID-19 hospital in Wuhan, China during the early period of COVID-19 epidemic. We reported here, for the first time, the epidemiologic features, identification, diagnosis, clinical courses, and short-term outcomes of gastrointestinal cancer patients with preoperatively pre/asymptomatic COVID-19 and provided evidence of SARS-CoV-2 in surgically resected specimens.

It has been shown that more than 90% of confirmed COVID-19 cases reported no exposure to the Huanan Seafood Wholesale Market in Wuhan.[7] All patients in this study denied exposure to this market, but 3 patients admitted close contact with other asymptomatic or symptomatic persons, who were confirmed COVID-19 cases later, before surgery. Because all the patients were residents of Wuhan, China, they all had a history of epidemiological exposure to the relevant environment. It is now very clear that infections were spreading by human-to-human transmission.[6,8]

It may take 2 to 14 days for symptoms to appear after exposure to the virus.[9] In this study, 6 patients (11.5%) were confirmed to be preoperatively pre/asymptomatic latent COVID-19, as 5 of them were found to have SARS-CoV-2 presenting in cytoplasm of enterocytes or macrophages from the adjacent normal colorectal tissues and the left 1 had symptom onset on POD 1. However, 1 patient with presence of viral particles in the surgically resected tissue experienced symptom onset on POD 18, which implies a prolonged incubation period. Five out of 6 patients in this study suffered long-lasting lymphopenia, which confirms previous observations in 35% to 70% of the COVID-19 patients and affirms low absolute lymphocyte counts being used as a reference index in the diagnosis of COVID-19.[7,10,11] Lymphopenia is also commonly observed in postoperative patients, but it always exists for no more than 1 week after surgery as shown in this study.

The preoperative chest CT findings in all patients were normal, which added difficulties for the early detection of the infection. The results support previous observations in some patients who showed no ground-glass opacities or consolidations in initial chest CT, and some never did.[5] Even the quantitative reverse-transcriptase-polymerase-chain-reaction assays for COVID-19 were negative during the early stage in some cases.[6] All patients presented with their first symptom onset as fever after surgery. Their intermittent fever lasted 1 to 16 days before fully resolving, which is approximately coincident with 5 days reported in the first case of Canada and 9 days in the first case of the United States.[12,13] A median time of 8 days from first symptom onset to dyspnea was reported,[11] and it was 9.0 days in our patients.

Continuous diarrhea presented in 1 patient for 5 days, which was also reported in 3% to 10.1% of the patients.[7,11] A recent study showed that 6.5% (6 out of 62) of fecal samples tested positive for the virus.[14] The first case in the United States also showed positive results of the virus in stool.[13] Diarrhea, other gastrointestinal symptoms, and viruses in stool indicate that the digestive system might be one of the targets for COVID-19 destruction. Although there is still a lack of definite evidence, studies in SARS-CoV and Middle East respiratory syndrome-CoV (MERS-CoV) have proven that the human intestinal tract may serve as an alternative infection route for the virus.[15–17] These coronaviruses, including SARS-CoV-2, exhibit a predisposition for infecting the respiratory systems, but other organs may also be involved.[18–21] Recently, SARS-CoV-2 was reported to enter cells by using its spike protein bounding with ACE2 and bioinformatics analysis on available single-cell transcriptome data of normal gastrointestinal system revealed that ACE2 was highly expressed in stratified epithelial cells and absorptive enterocytes from ileum and colon.[22-24]

Our conventional histologic studies did not find evidence of viral inclusions in the surgically resected specimens, fortunately but

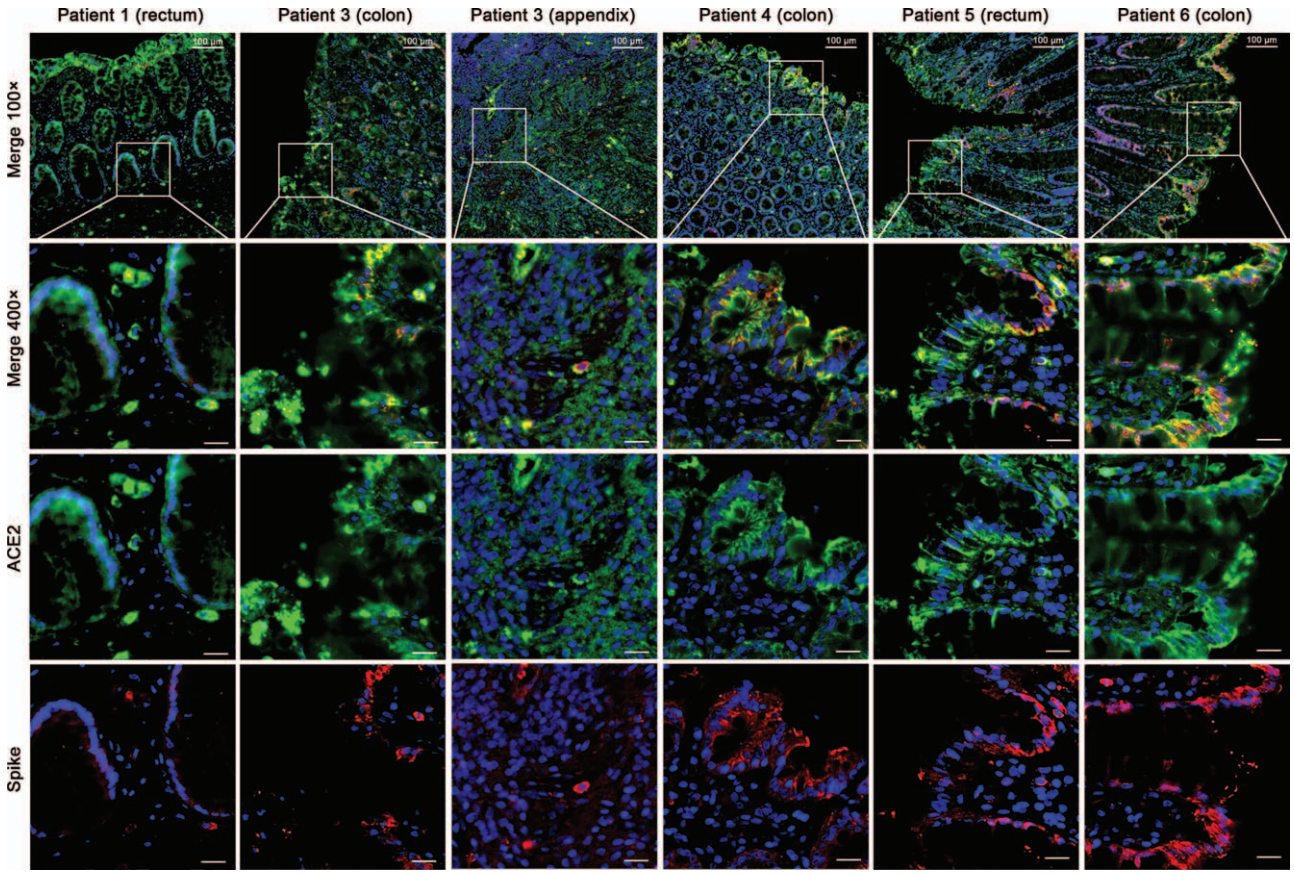

FIGURE 3. Multiplex immunofluorescence assay of 5 colorectal cancer patients with pre/asymptomatic COVID-19. Representative images of multiplex immunofluorescence assay showed positive signals for extensive expression of ACE2 (green) and coronavirus spike protein (red) in enterocytes or macrophages of appendix, colon, or rectum of the patients, and colocalization of ACE2 and spike (yellow, merge). Scale bar, 20 μm, otherwise indicated.

unfortunately, electron microscopy showed coronavirus-like particles in the cytoplasm or surface of enterocytes from 5 patients' appendix, colon or rectum. Diameter of the particles in this study ranged from 50 to 90 nm, somewhat smaller than a diameter of approximately 60 to 140 nm (average 100 nm) for SARS-CoV-2,[25] which may be due to the sample processing methods. Martines et al has found that SARS-CoV-2 particles measured in FFPE samples were smaller than those observed from fresh tissue.[26] Moreover, most particles shown in our study had uniform appearance viral particles with a membrane outer covering and dots inside indicating the nucleocapsid.[27] Most importantly, the immunofluorescence and fluorescence ISH confirmed considerable ACE2 expression and SARS-CoV-2 infection in the enterocytes or macrophages. This is the first report to show the presence of active SARS-CoV-2 in the appendix, colon and rectum of individuals alive with COVID-19, which is in line with a recent study showing this virus productively infects human gut enterocytes.[28] Most importantly, colocalization of most SARS-CoV-2 with ACE2 adds to the evidence of ACE2 as an entry receptor. Moreover, the histologic and virologic results in this study also showed that there was only minimal disruption of colon epithelial cells by the coronavirus, which may explain why some patients with evidence of the virus did not present with diarrhea. SARS-CoV-2 was not detected in the stomach of 1 patient with gastric cancer, which might be partially explained by low pH of gastric secretions.[29]

Unlike the high fatality rates of the earlier epidemics of MERS (35%) and SARS (10%) caused by a similar coronavirus strain, it appears that COVID-19 has a relatively lower case fatality rate so far.[1,30] However, because tumors, adjuvant therapy, or surgery can cause systemic immunosuppression, cancer patients undergoing surgery are particularly susceptible to various respiratory pathogens and pulmonary infections than individuals without cancer.[31,32] In a study including 2007 cases with laboratory-confirmed COVID-19, patients with cancer showed a higher risk of COVID-19 infection and poorer outcomes than those without cancer.[33] On March 13, 2020, the American College of Surgeons announced a formal recommendation to minimize, postpone, or cancel elective surgeries.[34] Though the advisory escalated a worldwide debate regarding the safety and feasibility of performing elective surgical procedures during this pandemic, most surgeons from the world followed this recommendation. In the current study, the case fatality rate of gastrointestinal cancer patients with pre/asymptomatic COVID-19 having undergone surgery is 16.7%, much higher than those without COVID-19. The worse short-term outcomes might be overstated due to small numbers of patients with pre/asymptomatic COVID-19 in this study. The long-term outcomes are still unknown. Since both cancer and surgery can cause systemic immunosuppression, gastrointestinal cancer patients with pre/asymptomatic COVID-19 are at high risk of postoperative onset and death. In the current pandemic, elective surgery should be postponed or canceled. Even during postpandemic period, repeated

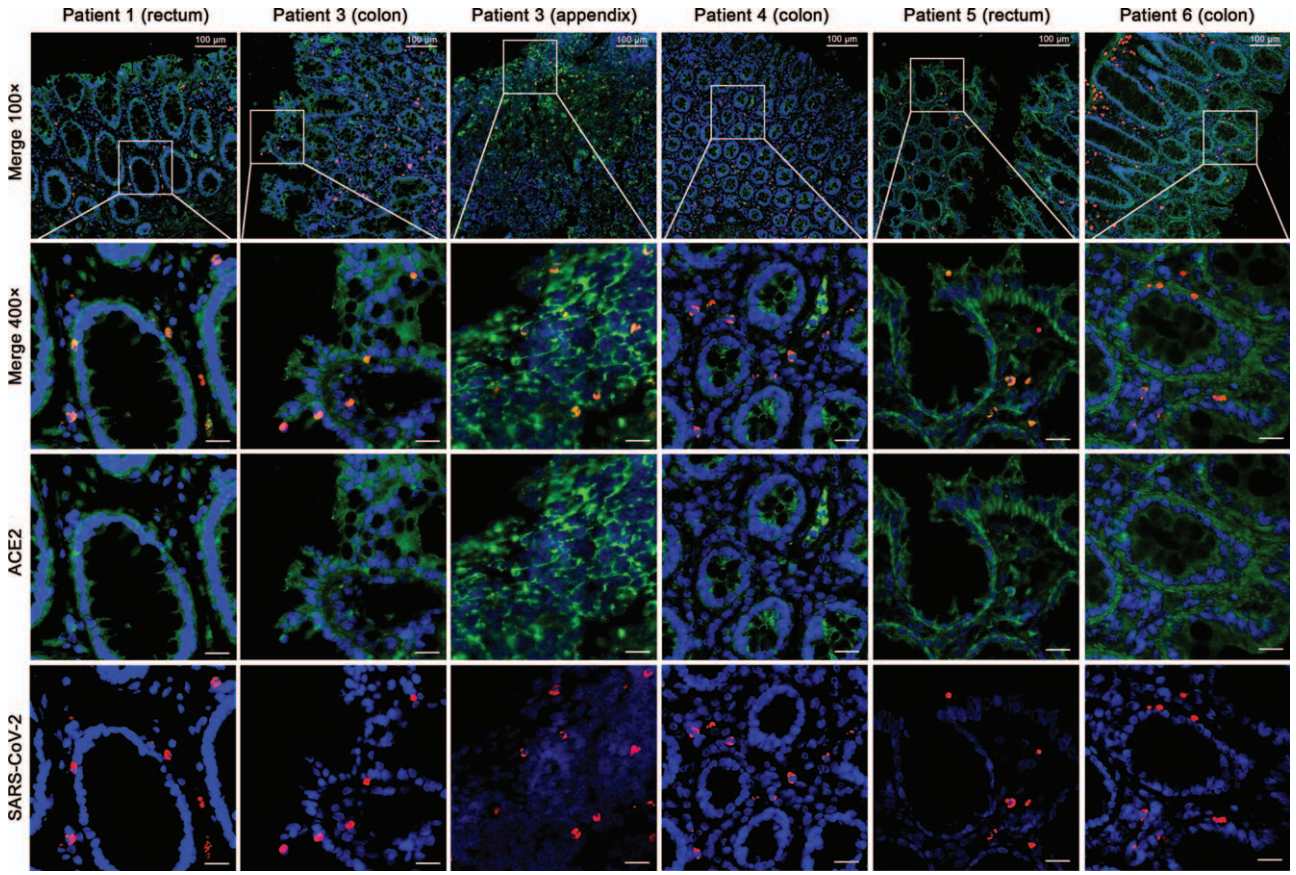

FIGURE 4. Multiplex immunofluorescence and fluorescence in situ hybridization of 5 colorectal cancer patients with pre/asymptomatic COVID-19. Representative images of multiplex immunofluorescence assay showed positive signals for extensive expression of ACE2 (green) and SARS-CoV-2 RNA (red) in enterocytes or macrophages of appendix, colon or rectum of the patients, and colocalizations of ACE2 and SARS-CoV-2 RNA (yellow, merge). Scale bar, 20 μm, otherwise indicated.

COVID-19 tests before undergoing surgery are suggested particularly in cancer patients who are theoretically at high risk for an infectious disease and present as pre/asymptomatic.

Our study has limitations. First, this is a single-center, retrospective study with a small number of patients. An extension of this line of work would be of great value in learning about the transmissibility, severity, and other features associated with this clinical illness in cancer patients. Second, all patients in this study did not undergo preoperative nasopharyngeal swabs for COVID-19 testing to exclude infection, as this test was not available in our hospital until January 21, 2020. Notably, most previous studies mainly focused on the more severe cases admitted to the hospital, and our clinical description pertains largely to relatively mild gastrointestinal cancer patients who experienced both virus infection and major surgery. This may add little information to the full clinical spectrum of emerging coronavirus infections.

Considering the existence of pre/asymptomatic latent infection cases, the prolonged incubation period, the difficulties in early diagnosis, the increased risk of severe infections, the lack of specific clinical signs of viral infection preceding severe complications, the potential to cause infection related complications including severe pneumonia, respiratory failure, ARDS, cardiac injury, and even fatal outcomes,[10,11,25] and limited data on the consequences of infection in gastrointestinal cancer patients, this study highlights the need for exploring more powerful, economical, and convenient tools for the early diagnosis of COVID-19 and investigating the full clinical spectrum and natural history of this infection in those patients. Last but not least, the colorectal tropism of the SARS-CoV-2 may have major implications on prevention, diagnosis, and treatment of this disease. Confronting the pandemic of COVID-19, surgeons and pathological specialists should not display a capacity of willful blindness that allowed the virus in surgical specimens to hide in plain sight, only in this way, we may potentially advance our medical knowledge of this disease and improve our clinical practice in future.

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