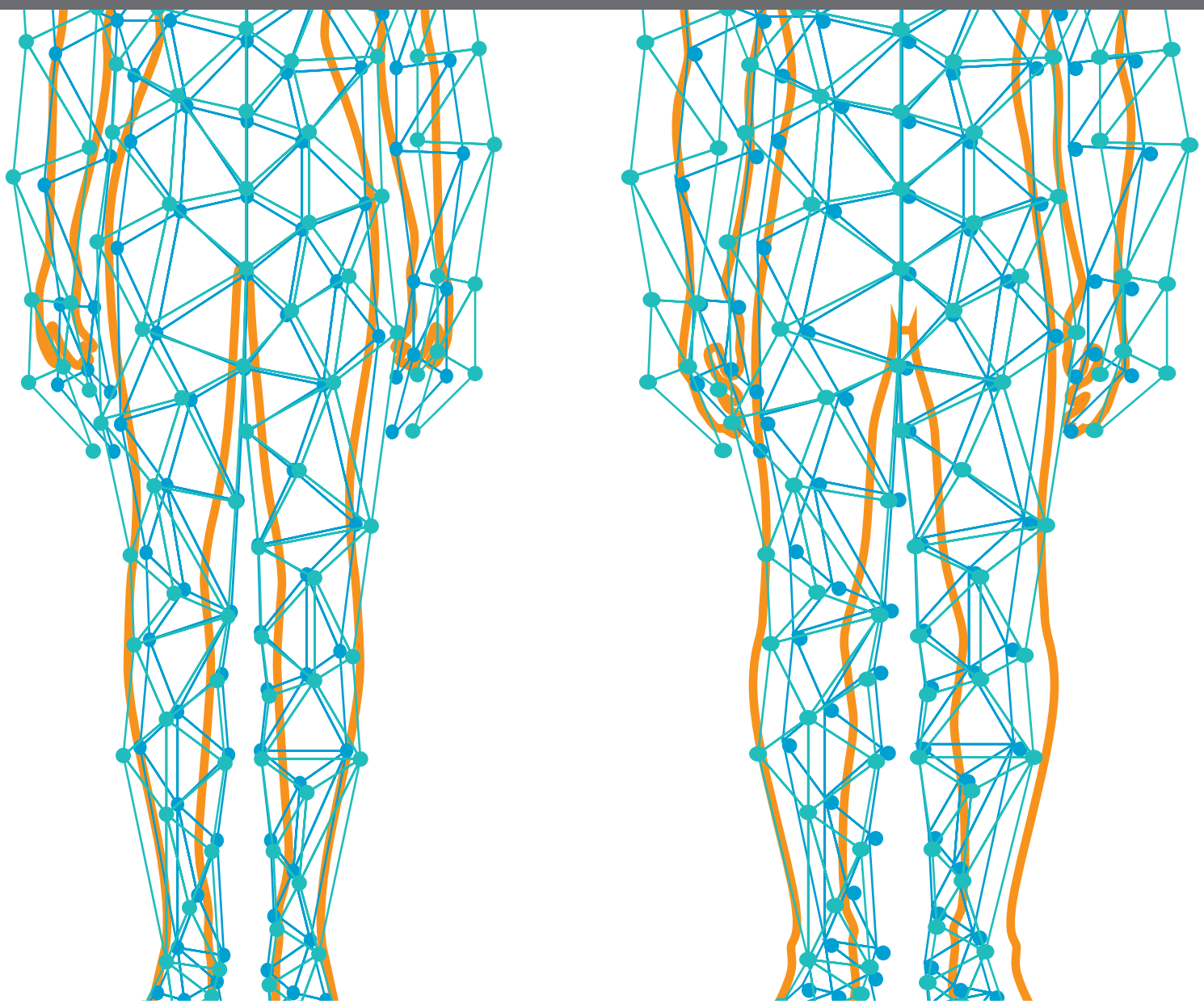
COVID-19 AND THE DIGESTIVE SYSTEM

EDITED BY: Hu Zhang, Bo Shen and Weiguo Dong

PUBLISHED IN: Frontiers in Medicine and Frontiers in Public Health



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ISSN 1664-8714
ISBN 978-2-88974-465-7
DOI 10.3389/978-2-88974-465-7

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COVID-19 AND THE DIGESTIVE SYSTEM

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Citation: Zhang, H., Shen, B., Dong, W., eds. (2022). COVID-19 and the Digestive System. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-465-7

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EDITORIAL
published: 30 March 2022
doi: 10.3389/fmed.2022.875063

Editorial: COVID-19 and the Digestive System

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Keywords: COVID-19, SARS-CoV-2, endoscopy, liver, pancreas, gastrointestinal tract

Editorial on the Research Topic

COVID-19 and the Digestive System

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), remains persistent worldwide. Gastrointestinal (GI) symptoms, such as nausea, vomiting, diarrhea, and abdominal pain, have been frequently reported in COVID-19 patients. Angiotensin-converting enzyme 2 (ACE2), the functional receptor of SARS-CoV-2, has also been detected in the digestive system, indicating that this system is an infection route of COVID-19 besides the respiratory system (1, 2). In this Research Topic, specialists probe the involvement of the digestive system in COVID-19 from mechanisms to clinical practice.

Perisetti, Goyal, Gajendran, et al. present the rates of various GI manifestations in COVID-19 patients. The study by Chen R et al., with 1,133 hospitalized COVID-19 patients, further shows that severe cases are more frequently accompanied by GI symptoms. Compared to those without GI symptoms, COVID-19 patients with GI symptoms were not only more likely to develop adult respiratory distress syndrome (ARDS) and required non-invasive mechanical ventilation (Chen R et al.), but also had significantly prolonged hospital stays and higher hospitalization costs (Zhang et al.). However, the correlation between GI symptoms and the progression of COVID-19 is still controversial (3), probably because of the difference in research methods, sample sizes, and epidemic prevention policies between regions among studies. Moreover, medications, such as glucocorticoids, may have varied effects on the GI tract in patients with COVID-19 (4).

The high expression of ACE2 on the GI tract may explain the existence of GI symptoms in COVID-19 patients (5, 6). ACE2 is specifically expressed in enterocytes which are mainly from the gastric mucosa of COVID-19 patients previously infected with *Helicobacter pylori* (*H. pylori*), suggesting that *H. pylori* infection may result in increased risks of COVID-19 infection (Zhang et al.). It is noteworthy that gut barrier integrity was found to be positively modulated by ACE2 through downregulation of stress-responsive pathways, so decreased expression of ACE2 in older patients with COVID-19 can attenuate their gut barrier defense, which provides a new insight into the mechanism of SARS-CoV-2 invasion (Moon et al.). Given the potential multiple roles of ACE2 in the GI tract, researchers are not sure whether the declined ACE2 in the GI tract with age is related to SARS-CoV-2 infection. More clinical and basic studies are needed to explore the multiple roles of ACE2 in the GI tract.

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *13 February 2022*
Accepted: *28 February 2022*
Published: *30 March 2022*

Citation:
Ma C, Dong W, Shen B and Zhang H (2022) Editorial: COVID-19 and the Digestive System. Front. Med. 9:875063. doi: 10.3389/fmed.2022.875063

Besides GI damages, liver injury has also been noted in COVID-19. The study by Lv et al. suggested that COVID-19 cases complicated with liver injury were more prone to becoming severe or critical, with a higher risk of death than those with normal liver function tests (LFTs). Jiang et al. also highlighted that SARS-CoV-2 infection may aggravate the hypercoagulability of pre-existing cirrhosis, which worsens the prognosis of COVID-19. In addition to D-dimer and total bilirubin (TBIL) (7), metabolic dysfunction-associated fatty liver disease (MAFLD) is also found as a risk factor of severe or critical COVID-19 (Hegyi et al.). Seow et al. also confirmed pre-existing liver diseases as a risk factor by analyzing the expression levels of ACE2 in five types of liver tissues *via* single-cell RNA-seq.

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic and relapse disorder in which immunosuppressive medications are frequently prescribed to induce and maintain remission. The COVID-19 pandemic has raised concerns about the management and therapy of IBD. On the one hand, as a series of biologic drugs widely used in IBD, anti-tumor necrosis factor α (anti-TNFα) agents are considered to increase the risk of virus infection including SARS-CoV-2 in IBD patients. However, Li et al. suggested that anti-TNFα treatment could potentially benefit IBD patients via downregulating the expression of colonic ACE2. On the other hand, COVID-19 has disrupted the management of IBD patients, posing a great challenge for gastroenterologists. A study by Qiu et al. demonstrated that the healthcare of IBD patients in epicentral areas is obviously impacted by COVID-19, including delayed lab tests/endoscopy procedures, delayed drug withdrawal, delayed biologics infusions, and postponed elective surgery. One way to counteract such a challenge is telemedicine (Qiu et al.), which in combination with virtual care, should be a promising future medical care paradigm in emergencies.

As a common examination in the GI department, GI endoscopy is a high-risk operation due to the potentially fecal-oral transmission of COVID-19, especially with its much higher transmissibility than influenza (8). Therefore, some precautions must be taken to contain virus transmission in this operation (Tian et al.). In addition, the psychological impacts of COVID-19 on GI endoscopists should not be overlooked (9), though they have adequate knowledge and awareness of occupational protection (Perisetti, Goyal, Sharma). Enough attention should be paid to the fear and anxiety of patients and medical staff for their psychological wellbeing during the COVID-19 pandemic (10).

We expect all the inspiring papers in this Research Topic "COVID-19 and the Digestive System" will contribute to improved prevention, diagnosis, and treatment for COVID-19.

AUTHOR CONTRIBUTIONS

CM and HZ wrote the manuscript. WD and BS edited the manuscript. HZ did the final checks of the manuscript and submitted it. All authors contributed to the article and approved the submitted version.

FUNDING

HZ is supported by Grants from the 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (Grant No. ZYJC18037).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
published: 04 September 2020
doi: 10.3389/fmed.2020.00564

Precautionary Measures: Performing ERCP on a Patient With Juxtapapillary Duodenal Diverticula (JPDD)-Related Biliary Stone After COVID-19 Lockdown Restriction Lifted in Wuhan, China

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 14 June 2020
Accepted: 11 August 2020
Published: 04 September 2020

Citation:
Chen Q, Yang M, Liao G, Zhao Y, Yue D, Yuan Y, Cheng B and Qin H (2020) Precautionary Measures: Performing ERCP on a Patient With Juxtapapillary Duodenal Diverticula (JPDD)-Related Biliary Stone After COVID-19 Lockdown Restriction Lifted in Wuhan, China. Front. Med. 7:564. doi: 10.3389/fmed.2020.00564

On April 8, 2020, after nearly 3 months of battling against the outbreak of COVID-19, Wuhan, where the pandemic began, began easing lockdown restrictions. However, given that asymptomatic carriers could continue to lead to transmission of COVID-19 during the very early stages, the endoscopists have taken precautions and conduct risk assessments to perform endoscopic intervention in this transition stage. Here, we have reported an urgent ERCP in a patient with acute pancreatitis secondary to JPDD-related biliary stone. Based on our experiences, the objective is to provide practical suggestions for the safe resumption of ERCP procedures in the setting of the COVID-19 pandemic with specific focus on patient risk assessment, personal protection equipment (PPE), and dress code modalities, all of which have been implemented in our hospital to reduce the risk of viral transmission.

Keywords: ERCP (endoscopic retrograde cholangiopancreatography), COVID-19, post-coronavirus outbreak, personal protection equipment (PPE), Healthcare workers (HCW)

BACKGROUND

The new strain of coronavirus, SARS-CoV-2, was first extracted in December 2019 from the lower respiratory tract samples of several pneumonia patients in our city, Wuhan, Hubei province, China (1–3). On March 11, 2020, the World Health Organization (WHO) declared the infection of SARS-CoV-2 with the official name COVID-19 (novel coronavirus disease-2019) a pandemic, highlighting the significance of its worldwide spread. The classic description of COVID-19 is as a respiratory illness that manifests with fever, dry cough, and dyspnea on exertion. However, fecal–oral transmission may be part of the COVID-19 clinical picture (4, 5). Accordingly, the endoscopy departments face a significant risk of diffusion of respiratory diseases that can be spread *via* an airborne route, including aspiration of oral and fecal material via endoscopes. Healthcare workers (HCW) have a high risk of infection; the infected HCW in Wuhan city consisted of 29% of COVID-19 patients at the beginning (6). Since then, the use of personal protective equipment (PPE), such as

gloves, mask, goggles, face shields, gowns, and hairnets, are strongly advocated among all medical societies for conducting physical examination and clinical procedures (7).

The gastroenterologists and the HCW in endoscopic fields have remarkable risk to be exposed to either respiratory or gastrointestinal fluids from patients during the endoscopy procedures (7, 8). To minimize human-to-human transmission and to best protect HCW, our hospital, which used to be a part of the coronavirus epicenter, has cut its ambulatory endoscopy practice and developed a screening system that only allows urgent endoscopies being performed during the COVID-19 outbreak. After lifting lockdown restrictions, the endoscopy services have resumed, and the hospitals and healthcare facilities take precautions for endoscopy procedures amid concerns over asymptomatic carriers who could potentially lead to second COVID-19 outbreak.

Diverticula located near the major duodenal papilla are termed juxtapapillary duodenal diverticula (JPDD) (9). Although JPDD is common and rarely give rise to severe complications, it tends to act as an independent risk factor for biliary stone formation. Furthermore, JPDD plays an etiological role in the development of acute pancreatitis, and the underlying pathophysiological mechanisms include biliary stone-induced obstruction, pressure changes in the sphincter of Oddi, and obstruction of pancreatic outflow directly caused by extraluminal diverticula compression, respectively (10). For patients with predicted severe acute biliary pancreatitis, whether or not cholangitis is present, urgent therapeutic endoscopic retrograde cholangiopancreatography (ERCP) within 72 h of admission has been recommended by several guidelines, as fewer complications tend to develop (11–14). Here we share our experience of conducting an urgent ERCP on a 73-year-old patient who developed acute pancreatitis secondary to JPDD-related biliary stone. Our experience may provide practical suggestions to minimize the transmission of COVID-19 during an ERCP procedure.

CASE PRESENTATION

A 73-year-old female presented at the outpatient department with a 2-day history of upper abdominal pain after a meal. She has no pre-existing conditions or major past medical history. Before admission, she went through the mandatory pre-screening assessment (**Figure 1**), which has been implemented at our hospital through the COVID-19 outbreak, including inquiry of potential contact history (whether contacted with a suspected or laboratory-confirmed COVID-19 patient in the last 2 weeks); patient's symptom check (body temperature $\geq$37.3°C, coughing or shortness of breath and/or other symptoms of acute respiratory symptom are highly suspected); laboratory test (a nasopharyngeal swab specimen for COVID-19 RNA test and serological tests for COVID-19 antibody) (6); and a chest computed tomography (CT) scan (a typical "ground glass opacity" image is highly suspected), respectively.

The patient was categorized as having a "low risk" of COVID-19 infection and was subsequently admitted to the GI unit. During routine physical examination, her vital signs were stable, whereas moderate rebound tenderness appeared at the upper abdominal region. The blood chemistry panel showed prominently elevated amylase (5,082 IU/L) and lipase (>3,000 IU/L) levels, suggesting pancreatitis. Bilirubin level and the lipid profile were normal, with the mild increase of gamma-glutamyl transpeptidase (GGT, 59 U/L), alamine amino transferase (ALT, 95U/L), and aspartate amino transferase (AST, 95U/L), respectively. In addition, complete blood count showed severe inflammation with increased white blood cell (20.5×10^9/L) and neutrophil (19.1×10^9/L) counts. An abdominal CT scan indicated inflammation and swelling of the pancreas, a mildly enlarged gallbladder, as well as a slightly dilated common biliary duct (CBD) (**Figure 2A**). Notably a diverticular pouch was present at the junction of second and third with no obvious stone identified at that time portions of duodenum. To further rule out the possible biliary or extrabiliary obstructive pathology, the patient was referred to MR cholangiopancreaticography (MRCP) examination. MRCP coronal haste thin slice image confirmed the presence of duodenal diverticular partially compressing the distal end of CBD and resulting in dilation of its proximal part (**Figure 2B**). The maximal diameter of the diverticula was 2.67 cm. An ERCP may be sufficient to identify the presence of small stones (and subsequently remove them) or, alternatively, to place a stent inside the duct to restore bile flow.

PATIENT PREPARATION AND DRESS CODE

The patient was informed of management options and agreed to endoscopic interventions. She was also acknowledged to have potential exposure risks to COVID-19 in the hospital environment. Surgical mask and gloves were provided to the patient and the relative who was responsible for transfer her to the ERCP unit. In addition, the patient was also provided with a disposable medical hair net and gown. They were further advised to minimize movement while waiting for the procedure to minimize the risk of contamination.

Prior to the ERCP procedure, the patient's status of COVID-19 was verified among the ERCP team. To limit the exposure risk for HCW, general anesthesia with tracheal intubation or deep sedation, which normally requires the anesthesia personnel to stay in the procedure room, was not applied in the current setting. Pre-ERCP screening was therefore critical to assess the patient's suitability to undergo conscious sedation. With regards to this, the patient had no cardio-pulmonary disease, no difficulties related to the airway, no morbid obesity, and, furthermore, no significant gastroesophageal reflux disease (GERD), which will probably cause an increased risk of developing complications during and after the ERCP procedure. Ten minutes before the procedure, intravenous administration of diazepam (5 mg) and dezocine (2.5 mg) was used to generate effects of anesthesia for the patient. In addition, antispasmodic (phloroglucinol, 20 mg) was given to reduce duodenal motility for the procedure. The vital parameters [heart rate (HR), blood pressure (BP),

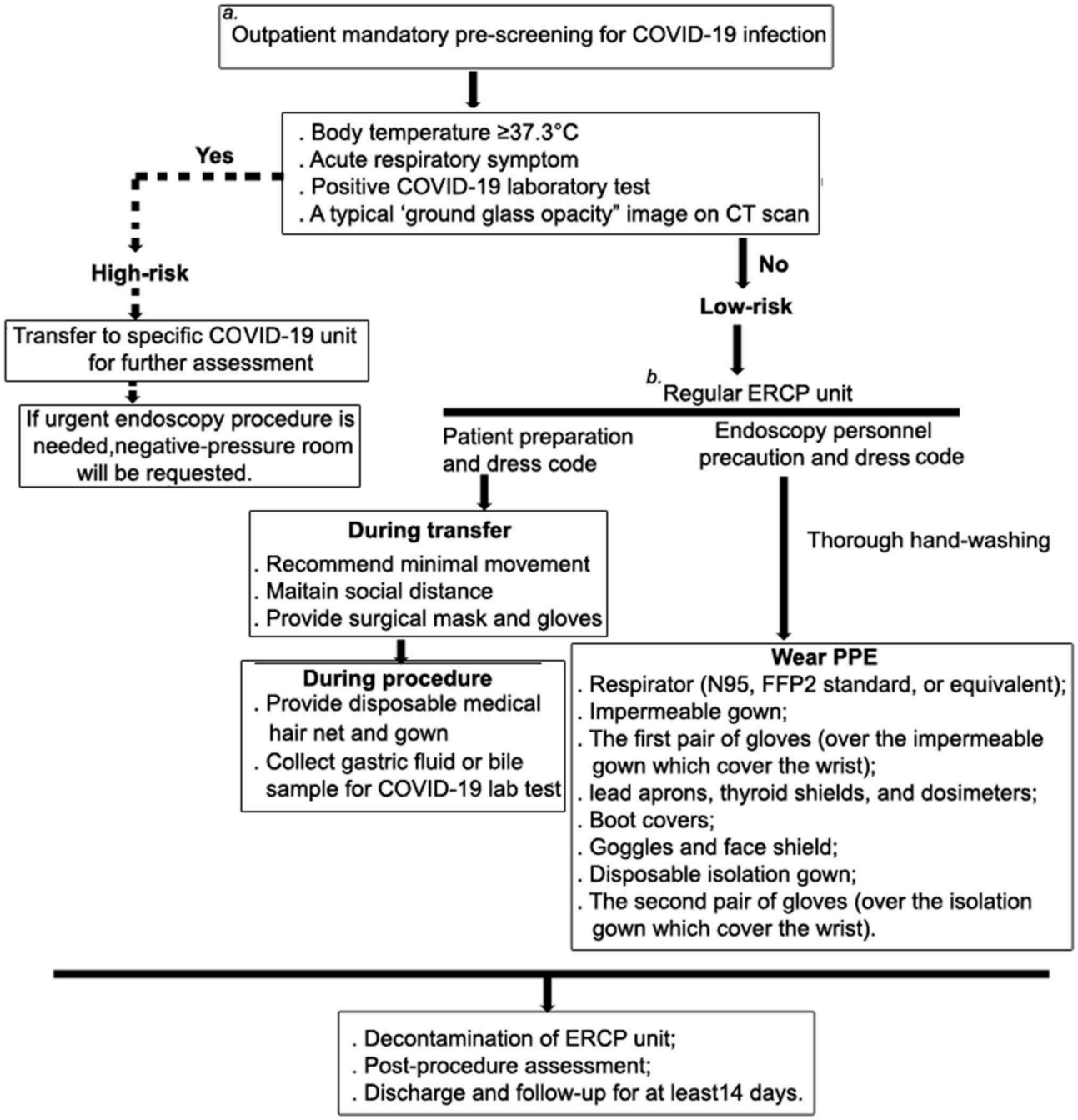

FIGURE 1 | Patient management and risk assessment and detailed workflow during ERCP procedure. To minimize human-to-human transmission and to best protect HCW, our hospital, which used to be the part of the coronavirus epicenter, has cut its ambulatory endoscopy practice and developed a screening system to only allow urgent endoscopy being performed during the COVID-19 outbreak. **(a)** Pre-assessment for COVID-19 is mandatory and conducted in the out-patient department, and it includes inquiries into potential contact history (whether contacted with a suspected or laboratory-confirmed COVID-19 patient in the last 2 weeks), a patient symptom check (body temperature ≥37.3°C, coughing or shortness of breath and/or other symptoms of acute respiratory symptom are highly suspected), laboratory test (a nasopharyngeal swab specimen for COVID-19 RNA test and serological tests for COVID-19 antibody), and a chest computed tomography (CT) scan (a typical "ground glass opacity" image is highly suspected). If the patient meets one of these criteria, they will be categorized as being at high risk of infection, and they will be administered at a separated unit for all patients with respiratory symptoms. The negative-pressure room will be requested for undergoing therapeutic procedure. **(b)** At the ERCP unit, patients are further assessed for suitability for conscious sedation. Those who have cardio-pulmonary disease, difficult airway, morbid obesity, and significant GERD are not eligible for conscious sedation.

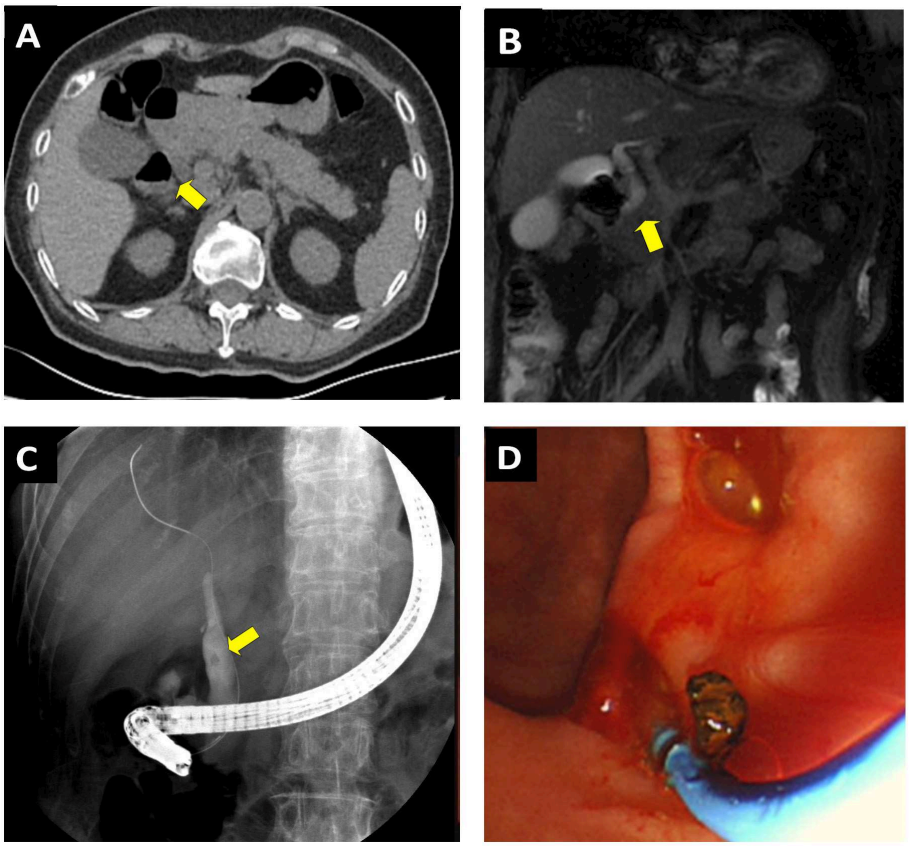

FIGURE 2 | Performing ERCP on a 73-year-old female patient presented with acute pancreatitis secondary to JPDD related CBD stone. **(A)** CT scan shows the inflammation and swelling of the pancreas and a mildly enlarged gallbladder. A diverticular pouch was present at the junction of second and third portions of the duodenum (yellow arrows). **(B)** MRCP coronal haste thin slice image confirmed shows the presence of periampullary diverticulum, which causes extrinsic compression upon the CBD (yellow arrow). Note the possible CBD stone associated with dilation of the distal end of CBD. **(C)** ERCP confirm the CBD stone and divericulum exerting compression upon the CBD outlet. **(D)** The stone was successfully removed though the ERCP procedure.

and respiration rate, (SpO2)] were monitored throughout the procedure.

ENDOSCOPY PERSONNEL PRECAUTIONS AND DRESS CODES DURING AN ERCP PROCEDURE

Although performing endoscopic procedures in a negative-pressure room during the COVID-19 outbreak was recommended among several gastroenterological endoscopy societies (7, 15, 16), this is not available in most endoscopy facilities around the world. We equipped an operative room with a negative-pressure system in a separate unit to be used for all patients with respiratory symptoms. Given that the current patient was categorized as being at a low risk of infection, she was not transferred to the negative-pressure room.

All HCW at the hospital have received appropriate training on hand hygiene and use of PPE prior to procedures (7, 8). The endoscopists and assistance wore PPE by reviewing the Asian Pacific Society for Digestive Endoscopy (APSDE) guidelines, American Society for Gastrointestinal Endoscopy (ASGE) guidelines, and CDC recommendations for ERCP (8, 15–17). Washing hands with soap and water or alcohol-based hand rub were mandatory before and after patient interaction, contact with potentially infectious sources, and before putting on and removing PPE. The step-by-step approach for wearing PPE is as follows (**Figures 3A–C**): wear a respirator [either N95, the US standards for respirator masks, or NK95, the Chinese standards for respirator masks, or the equivalent, which are rated to capture 95% of tiny particles (0.3 micron particles, to be exact)]; wear an impermeable gown; wear the first pair of gloves so they cover the impermeable gown, which cover the wrist; wear lead aprons, thyroid shields, and dosimeters; wear boot covers; wear goggles and a face shield; wear a disposable isolation gown; wear a second pair of gloves over the isolation gown so they cover the wrist.

At ERCP, JPDD was observed, and a duodenoscopy identified the papilla located on the edge of diverticular fundus (**Figure 2C**). ERCP was performed in the usual fashion with selective biliary cannulation and injection of contrast material into common bile. A small stone was visualized in the distal CBD where it was juxtaposed against the diverticulum. To remove the

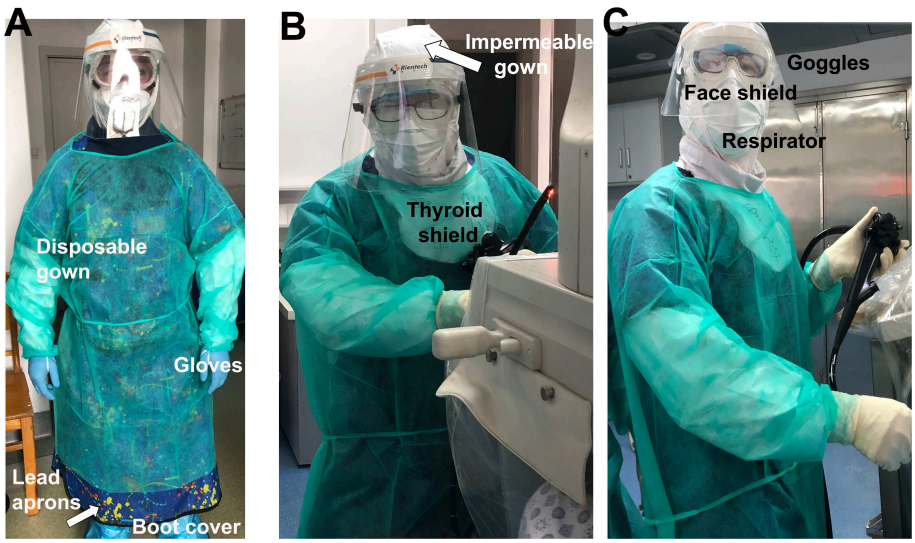

FIGURE 3 | (A–C) HCWs adhere to Level 2 biosafety requirement during the procedure. ERCP Endoscopy personnel precautions and dress code as follows: prior to ERCP procedure, the patient's status of COVID-19 was verified among the ERCP team. HCW wore PPE in the following order: respirator (N95, NK95, or the equivalent); impermeable gown; a first pair of gloves (over the impermeable gown that cover the wrist); lead aprons, thyroid shields, and dosimeters; boot covers; goggles and face shield; disposable isolation gown; and a second pair of gloves (over the isolation gown which cover the wrist). Washing hands with soap and water or alcohol-based hand rub were mandatory before and after patient interaction, contact with potentially infectious sources, and before putting on and upon removal of PPE.

stone, the endoscopist performed a small sphincterotomy in conjunction with balloon dilation, and subsequent removal of the stone was achieved with balloon extraction (**Figure 2D**). A 6F endoscopic nasobiliary drainage (ENBD) tube was placed to drain remnant stone. After the procedure, the patient's respiratory and cardiac signs were carefully monitored in the GI ward. Physical distancing was emphasized; we permitted only one relative or guardian per patient, and they were given masks and a separate room to other patients. All the nurses and healthcare providers were tested for COVID-19 and had to show negative results prior to returning work; we had mandatory education and training on infection measures, including hand hygiene and use of PPE. A total of 2 days later, the patient's amylase and lipase levels returned to normal, and the EBD tube was thus withdrawn. In addition, a sample of bile collected during ERCP procedure was tested for COVID-19 serology; it showed negative results. The possible ERCP-related complications, including pancreatitis, infection of the bile ducts or gallbladder, hemorrhage, and perforation in the bile or pancreatic ducts, were not observed. The patient was discharged afterwards and followed up 2 weeks later to assess whether she developed any respiratory symptoms and to further assess her progress after the procedure. A total of 2 months later, she was further followed up at an outpatient department. There was no retaining stone detected in CBD by CT scan, and no evidence of post-ERCP pancreatitis or other ERCP-related complications developed during the follow-up.

DISCUSSION

Duodenal diverticula are bulging pouch-like herniations in the duodenal wall, and those located near the major duodenal papilla are termed JPDD. JPDD are acquired lesions, and their presence rises with increasing age. Although they are usually asymptomatic, the association with biliary or pancreatic disease is not uncommon, and this includes choledocholithiasis, perforation, acute/chronic pancreatitis, bleeding, CBD obstruction, and rarely carcinoma (9). In particular, JPDD plays a major role in biliary stone disease in which the bile de-conjugation by diverticula's compression may likely act as the initial step leading to the precipitation of calcium bilirubinate and formation of pigment stones (18, 19). The further mechanical obstruction and sphincter of Oddis dysfunction subsequentially increase the risk to develop acute pancreatitis. Preforming ERCP and endoscopic sphincterotomy are widely accepted as the first-line therapy to remove bile duct stones and explicitly benefit those with the etiology of acute pancreatitis, and accordingly, urgent ERCP within 72 h is required to reduce the risk of developing acute pancreatitis-associated complications (11–14). JPDD has been previously considered as a risk factor not only for cannulation difficulty during ERCP, but it is also linked to developing complications upon endoscopic sphincterotomy, bile duct stone retention, as well as recurrence after an ERCP procedure. However, recent studies highlight the technical capability and suggest experienced endoscopists can overcome the anatomical difficulties to safely and successfully conduct cannulation and endoscopic sphincterotomy; furthermore, there is no increased risk of hemorrhage following sphincterotomy in patients with periampullary diverticulum compared to those without one (20). During the COVID-19 pandemic, acute biliary obstruction requiring stenting and acute cholangitis are the only hepato-pancreaticobiliary disorders commonly recommended by many international or national endoscopy

societies for demanding urgent ERCP (21), including the British Society of Gastroenterology (BSG) guidelines, APSDE guidelines, and ASGE guidelines (7, 15, 16). Here we presented the first case of acute pancreatitis secondary to JPDD related CBD stone at the post-outbreak stage of which COVID-19 is on the way to be fully controlled, whereas urgent ERCP was necessary to significantly improve the patient's outcomes. We hereby suggest the ERCP procedure for acute biliary pancreatitis should also be considered for urgent endoscopic intervention during the outbreak. Nevertheless, it is only conducted after risk stratification and careful pre-screening of the patient. Though the current case allows us to gain such experience, a lack of similar cases during the pandemic indeed prevents us from further assessing the additional challenges the HCW may face.

The current case was categorized as a low-risk COVID-19 patient. However, we adhered to Level 2 biosafety requirement when performing ERCP, and this is partly due to the aerosol-generating nature of the procedure. In addition, taking precautions to consider those asymptomatic carriers could lead to transmission of COVID-19 during very early stage (22), we emphasize the use of full PPE to protect our endoscopic personnel. We further highlight potential modifications as follows based on our experiences. First, before admission, the mandatory pre-screening assessment is implemented (which will last throughout the COVID-19 outbreak and after lockdown restriction are lifted). Second, the hospital has been reconstructed and divided into two sections, one for "low-risk" patients and the other for "high-risk" patients who will be administered at a separated section where all the patients with respiratory symptoms reside. Third, the patients with a "low risk" of COVID-19 are admitted to the GI unit prior to ERCP procedure. Furthermore, until now in Wuhan city, during the pandemic or in the post-COVID-19 period, pre-screening tests are required for both the patient and the patient's guardian or carer. The patient' relatives or guardian have to be at 'low risk' of COVID-19 to stay in the hospital. Fourth, HCW must adhere to Level 2 biosafety requirement during the procedure given the aerosol-generating nature of the virus and the precautions laid out for asymptomatic carriers. Fifth, to limit the exposure risk for HCW, general anesthesia with tracheal intubation or deep sedation, which normally requires the anesthesia personnel staying in the procedure room, are not currentl applied. Alternatively, conscious sedation is used after carefully pre-ERCP screening the patient's suitability.

We noted that one of the disadvantages related to usage of PPE was wearing goggles and a face shield together, as it can cause the lenses to fog up quickly. To prevent goggles from fogging, we used a small drop of a liquid soap to rub the lenses. Furthermore, the current design of face shields may not be able to cover the lower face region when endoscopists raise their heads (**Figure 3C**). This could be a potential risk for HCW while aerosolization appears. A modified design for the face shield could therefore be a solution.

Together, based on our practical experience and published guidelines, we strategically assigned HCW during an uncertain time to minimize concomitant exposure and applied the triage workflow throughout the urgent ERCP. Success in preventing COVID-19 transmission was achieved.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual (s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

GL and MY assisted endoscopic procedures in this study. YZ and YY collected and analyzed the clinical data. DY provided laboratory test and interpreted the results. HQ and BC supervised the study, performed the procedure and further gave valuable advice. The manuscript, images and their associated description were drafted by QC. All authors read and approved the manuscript.

FUNDING

All sources of funding received for the research has being submitted. This work was supported in part by grants of the National Natural Science Foundation of China (81974077). QC was the receiver of this grants no other funds has been received for open access publication fees, from my institution, library, or other grants.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 30 October 2020
doi: 10.3389/fmed.2020.588711

Prevalence, Mechanisms, and Implications of Gastrointestinal Symptoms in COVID-19

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Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *29 July 2020*
Accepted: *05 October 2020*
Published: *30 October 2020*

Citation:
Perisetti A, Goyal H, Gajendran M, Boregowda U, Mann R and Sharma N (2020) Prevalence, Mechanisms, and Implications of Gastrointestinal Symptoms in COVID-19. Front. Med. 7:588711. doi: 10.3389/fmed.2020.588711

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. The infection started as an outbreak of pneumonia-like symptoms in Wuhan, China. Within a few weeks, it spread across the entire globe resulting in millions of cases and thousands of deaths. While respiratory symptoms and complications are well-defined and can be severe, non-respiratory symptoms of COVID-19 are increasingly being recognized. Gastrointestinal manifestations such as nausea, vomiting, diarrhea, and abdominal pain have been added to the list of common COVID-19 symptoms. Their prevalence has been increasing, probably due to increased recognition and experience with the pandemic. Furthermore, diarrhea and stool testing may change prevalence and transmission rates due to suspicion for fecal-oral transmission of the COVID-19. Due to this risk, various countries have started testing wastewater and sewage systems to examine its role in the spread of SARS-CoV-2 among communities. In this review article, we describe the common gastrointestinal manifestations in COVID-19, their prevalence based upon the current literature, and highlight the importance of early recognition and prompt attention. We also note the role of fecal-oral transmission. Furthermore, the mechanisms of these symptoms, the role of medications, and potential contributing factors are also elaborated.

Keywords: COVID-19, SARS-CoV-2, endoscopy, gastrointestinal symptoms, diarrhea, fecal-oral transmission

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) manifesting mainly as pneumonia, acute respiratory distress syndrome, and multiorgan failure (1). Though originated in Wuhan, China, as a cluster of pneumonia-like presentation, soon it spread across the globe. As of July 28th, 2020, SARS-CoV-2 has affected all countries and territories in the World, with more than 17 million cases and 660,000 deaths (2). Non-respiratory manifestations are increasingly being recognized in COVID-19 (3, 4).

GI symptoms though less common compared to respiratory symptoms have gained increased significance lately (5). The Center for Disease Control (CDC) added multiple GI symptoms of COVID-19, which can coexist with respiratory symptoms, or they can be the only presentation of the disease (3). Anorexia, nausea, vomiting, diarrhea, and abdominal pain are some of the frequently observed gastrointestinal (GI) symptoms in COVID-19 patients (6). Additionally, GI bleeding, acute pancreatitis, and colitis have also been reported.

There is a potential of fecal-oral transmission due to the presence of the viral RNA in stools, especially in asymptomatic individuals. Due to this concern, sewage and the wastewater is being analyzed to detect the SARS-CoV-2 virus, examine the role of feco-oral transmission in the community, and identify mitigation strategies (7). Early identification and prompt attention to GI symptoms are critical because hospitalized COVID-19 patients with concomitant GI symptoms have been found to develop severe disease. Also, understanding the prevalence and mechanisms of GI manifestations can help to better characterization of these symptoms. In addition, educating patients about these symptoms can not only identify COVID-19 cases at an early stage but also can prevent potential spread to uninfected individuals.

ANOREXIA

Loss of appetite (anorexia) is one of the most common GI symptoms in COVID-19 patients (**Table 1**). The presence of anorexia in COVID19 is mostly underestimated and under-reported because of its non-specific nature. Prevalence among different studies ranged from 12.2 to 50.2% (8–10). A pooled analysis of multiple studies showed an overall prevalence of 26.8% (11). Anorexia is also frequently associated with other GI symptoms of vomiting, abdominal pain, and diarrhea. Pathophysiology of anorexia could be related to acute viral prodrome associated with COVID-19. Acute inflammation can increase the cytokine load such interleukins (IL-2, IL-7), tumor necrosis factors (TNF) which contribute to cytokine storm seen in COVID-19 (**Table 2**). In addition, altered or change in taste (dysgeusia) noted in these patients can further exacerbate loss of appetite (26).

NAUSEA AND VOMITING

COVID-19 patients can have nausea and vomiting as their only symptoms at presentation or as a combination with other GI symptoms, including anorexia, diarrhea, and rarely abdominal pain. A pooled prevalence of 7.8% (95% CI, 7.1–8.5%) was noted for nausea or vomiting from 26 studies spanning among different nations. Similar to other GI symptoms, the prevalence of nausea and vomiting among Chinese studies was lower (5.2%) compared to studies from other countries (14.9%). In one study' s Nobel et al. (27) noted that the presence of nausea or vomiting in as high as 22.7% patients (63 patients developed nausea among 278 patients, 95% CI, 17.9–28%). Similarly, Cholankeril et al. (28) and Hajifathalian et al. (29) reported the prevalence of symptoms of nausea and vomiting as 10.3 and 15.9%, respectively. While the precise reasons remain unclear, nausea, and vomiting could be related to a combination of effects on the gut and central nervous system (CNS) (13). After entry of SARS-CoV-2 into the GI tract, it can gain access to portal circulation and can affect the vagus nerve either through vascular or lymphatic routes. In addition, the cytopathic effect caused by SARS-CoV-2 combined with cytokine storm can stimulate central and peripheral (autonomic nervous) pathways, culminates into a sensation of nausea (with or without vomiting). Once neural pathways are stimulated, gastric dysrhythmia can occur, resulting in vomiting (30). Furthermore, antibiotics and antiviral agents are frequently used in COVID-19 patients, which further exacerbates their symptoms (31). If these factors contribute in an isolated fashion or combination is unknown.

DIARRHEA

Diarrhea is a commonly noted GI symptom in COVID-19 patients. It has significant public health importance given its potential for feco-oral transmission of disease. A pooled prevalence of multiple studies showed an overall prevalence of diarrhea of 5–10% (11, 32). There is a wide range of prevalence noted in multiple studies ranging from 2 to 50% (33). In a large cohort of 1,059 patients, 234 cases of diarrhea were noted with a prevalence of 22.1% (95% CI, 19.6–24.7%) (29). Similarly, among 355 cases in the Hubei province of China, 130 patients developed diarrhea with a prevalence of 36.6% (95% CI, 31.6–41.9%) (34). Several factors could be responsible for variation in the prevalence of diarrhea in these studies. Documentation of GI symptoms at the time of hospitalization, high suspicion, and early recognition, and if patients are treated either in an outpatient or inpatient basis, could be responsible for this variation.

Despite the high frequency of diarrhea, standardized criteria for diagnosis, and grading the severity of diarrhea are missing in most studies. Patients with a viral illness can present with a transient episode of loose stools with or without other GI symptoms. While persistent diarrhea (3 or more loose stools for more than 48 h) is significant, this definition is rarely used in the studies. Moreover, if diarrhea is not present at the admission, it becomes challenging to ascertain the cause of diarrhea. Several confounding variables, such as the use of enteral feeding (tube feeds), antiviral and antibiotics, altered gut flora, hyperinflammatory response, secondary bacterial infections, use of proton pump inhibitors (PPI) can potentially cause diarrhea in hospitalized COVID-19 patients (35). In order to determine if the persistent diarrhea is from SARS-CoV-2, evidence of direct viral-induced cytopathy (through histology or stool viral RNA positivity) should be documented.

ABDOMINAL PAIN

Patients with COVID-19 can present with abdominal pain, which is less frequent as compared to anorexia, nausea/vomiting, or diarrhea. The prevalence of abdominal pain ranges from 3.9 to 6.8% (10, 11, 28, 29, 36). There is no consensus regarding the

TABLE 1 | Typical and atypical GI symptoms in COVID-19.

Typical	Atypical
Loss of appetite (anorexia)	Altered taste (dysgeusia)
Nausea and vomiting	Gastrointestinal bleeding
Diarrhea	Secondary bacterial infection (*Clostridium difficile*)
Abdominal pain	

TABLE 2 | Mechanisms of GI symptoms.

Effect	Contributing factors
Viral cytopathic effect	• The entry of SARS-CoV-2 via ACE-2 receptors in GI glandular epithelium (12) • Isolated of viral RNA particles in stools of COVID-19 patients (13)
Altered gut microbiota	• Use of multiple antimicrobial agents • Change in gut microbial composition from viral proinflammatory mediators (14) • Abnormal mTOR activity and decreased antimicrobial activity (15) • Increased susceptibility for infections (*Clostridium difficile*) (16) • Hypochlorhydria induced by antisecretory agents (such as the use of proton pump inhibitors) (17) • Altered gut-lung axis (18)
Inflammation	• Increased cytokine release such as interleukins (IL-2, 7), tumor necrosis factor, granulocyte monocyte colony-stimulating factors (cytokine storm) (19) • The altered gut-brain axis (20) • Increased fecal calprotectin (21)
Worsening of prior GI conditions	• Overexpression of ACE-2 in the inflamed gut in inflammatory bowel disease (21) • Worsening of prior irritable bowel syndrome
Secondary infections	• Increased risk of *clostridium difficile* (16)
Others	• Intestinal ischemia (22, 23) • Viral colitis (24) • Altered GI epithelial integrity (25) • Altered enteric nervous system output (20)

SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; ACE-2, angiotensin-converting enzyme-2.

severity and duration of abdominal pain in COVID-19 patients. The location of the pain could be the right upper quadrant or epigastric or generalized. Few cases of COVID-19 presenting as acute abdomen has been reported (37). The precise mechanism for abdominal pain is unclear. Furthermore, any viral illness as a part of the prodrome can cause transient abdominal cramping and discomfort. Furthermore, abdominal pain can be combined with other GI symptoms of anorexia, nausea with or without vomiting. After the entry into GI tract, SARS-CoV-2 can exert its cytopathic/inflammatory changes, which can potentially lead to visceral pain. If this is a somatic due to the involvement of the peritoneum or a referred pain is unknown. Sporadic reports of pancreatitis have also been reported in COVID-19 patients (38). Additionally, high expression of ACE-2 receptors is noted in the pancreatic tissue, which makes it susceptible to its cytopathic effects. It can lead to leakage of pancreatic lipase and fatty acid oxidation. Few autopsy reports have shown ongoing pancreatic injury in COVID-19 patients without clinically evident acute pancreatitis (18). Hyperlipasemia has been identified in these patients in multiple studies (39, 40). It is unclear if a low level of elevated lipase is from viral pancreatic inflammation or as a part of viral gastroenteritis (38, 39).

ADDITIONAL DIGESTIVE SYMPTOMS

In addition to the above GI symptoms, other atypical manifestations such as changes or loss of smell and taste and GI bleeding have been documented in COVID-19 patients (17, 41). Aziz et al. noted that taste changes (ageusia/dysgeusia) are prevalent in up to 49.8% (95% CI: 8.2–91.5%, $I^2 = 99.6\%$) patients, although this meta-analysis had limited number of studies (26). Lin et al. reported a few cases of GI bleeding with viral RNA isolation from esophageal samples (12). Furthermore, endoscopically herpetic erosion was noted in these patients. If SARS-CoV-2 cytopathic effects lead to these erosions or if the virus is a bystander remains unclear. Secondary bacterial infections such as *Clostridium difficile* infections were noted in COVID-19 patients, probably due to the widespread use of antibiotics in these patients. Additionally, altered gut flora is documented in these patients, making them vulnerable to these infections (14).

PUTATIVE MECHANISMS OF GASTROINTESTINAL SYMPTOMS

SARS-CoV-2 enters the mucous membranes (nose, oral cavity) through its well-documented functional receptor angiotensin-converting enzyme-2 (ACE-2) (15, 26). While it can make its way to the gastric lumen via salivary secretions, it is subjected to the adverse effect of the acidic environment of the stomach. A pH of <2 significantly affects the life of the virus (16, 19). Patients with hypochlorhydria are susceptible to get a viral infection because of a higher viral load entering the small intestine (20). ACE-2 receptor concentrations differ among different GI tissues, with high expression noted in ileal enterocytes (21). Once SARS-CoV-2 enters the enterocytes, viral synthesis, replication can continue, and a cytopathic effect is noted (evidenced by intracellular staining of viral nucleocapsid) (22). The virus can continue its journey from here to other organs via the portal circulation. These changes can potentially lead to stool viral RNA positivity. If the presence of viral RNA in the stool is indicative of cytopathic changes or just a bystander needs further validation (**Table 2**).

Gut flora plays a significant role in maintaining GI homeostasis, and any perturbations can lead to diarrhea and various GI symptoms such as nausea and vomiting. COVID-19 patients are at high risk of microbiome alterations. We highlight 6 of the key factors for gut flora alteration in COVID patients. First, viral infections can increase the release of proinflammatory cytokines, which can alter gut flora (23). It is well-recognized that SARS-CoV-2 patients have elevated cytokines and markers of inflammation (24). Additionally, the

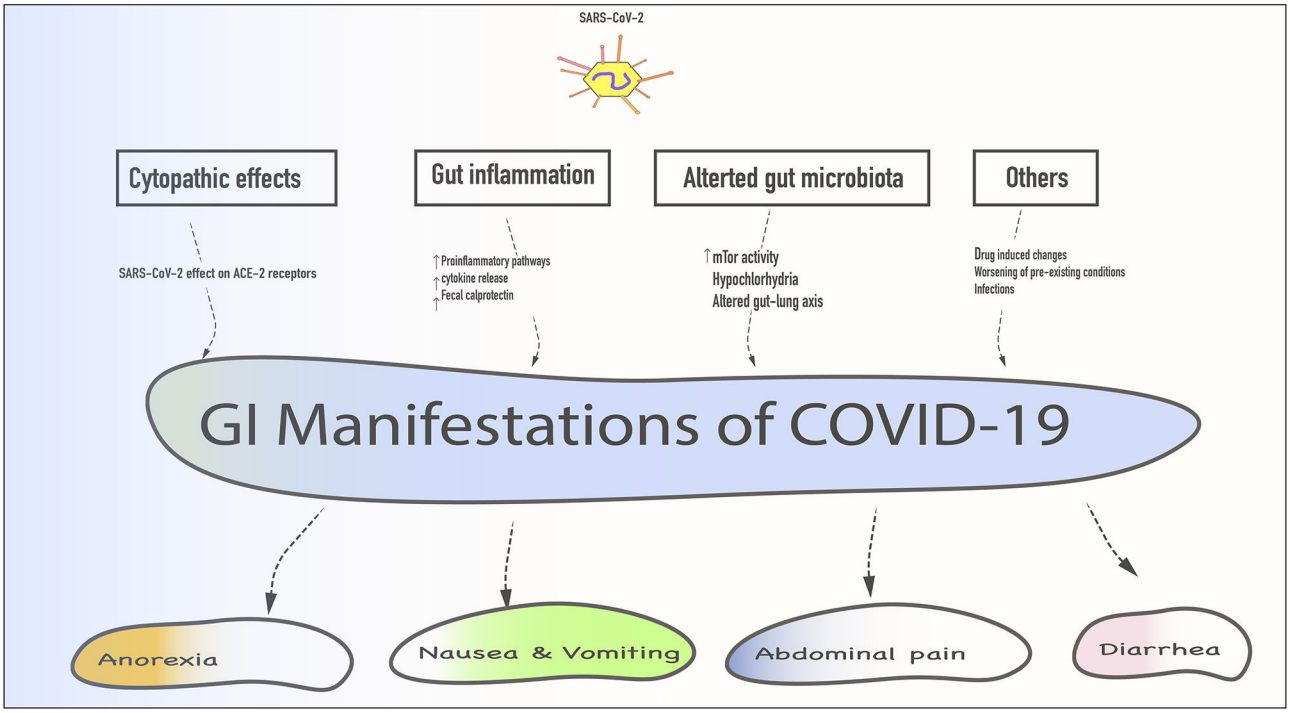

FIGURE 1 | Schematic representation of various gastrointestinal (GI) manifestations with their mechanisms. SARS-CoV-2 gains entry via mucous membranes of the oral cavity enters the stomach and small intestine to exert its cytopathic effect. Additionally, gut inflammation, altered gut flora, drug-induced changes, worsening of pre-existing GI condition, and secondary infections could contribute to these symptoms.

use of various antimicrobial medications (antibiotics, antivirals) can change the composition of flora, which can predispose individuals to GI adverse effects (31). A third factor, as respiratory symptoms are exceedingly common in COVID-19 patients, change in lung flora can contribute to the potential change in gut flora (14). This "gut-lung" axis is increasingly being recognized as a potential cause of GI symptoms in individuals with respiratory manifestations (14). A fourth factor is that altered flora can lead to a change in the ratio of pathogenic organisms, potentially leading to infections such as clostridium difficile. Recent reports of such infections were noted in COVID-19 patients (25). Fifth, the use of enteral nutrition, such as tube feeds, can further alter the gut microbiome already affected due to the aforementioned causes. Finally, ACE-2 receptor binding has been shown to alter flora by its aberrant mTOR activity (42).

All of the above mechanisms are only putative, as there is no reliable evidence if these mechanisms play a role independently or in combination in the development of GI symptoms in COVID-19 patients. Patients with pre-existing GI conditions such as inflammatory bowel disease (IBD) and malabsorption syndromes etc. are at risk of worsening GI symptoms if infected with SARS-CoV-2 (43, 44). While changes in the gut flora are universal in these populations, ACE-2 expression is elevated in IBD and inflammatory states (44). Fecal calprotectin, which is a marker for bowel inflammation, has been noted to be elevated in COVID-19 individuals with persistent diarrhea (45). If such increased expression predisposes these individuals to worsening symptoms needs to be studied. Furthermore, the enteric nervous system is integrally associated with GI motility, and any perturbation in these pathways can lead to worsening of GI symptoms (46) (**Figure 1**).

GI MANIFESTATIONS AND COVID-19 SEVERITY

As noted above, patients with COVID-19 frequently present with GI manifestations. However, data on the correlation between GI manifestations and severity of COVID-19 has been variable (6, 47–50). Ramachandran et al. studied 150 hospitalized patients with COVID-19 with and without GI symptoms and reported no difference in length of stay or need for mechanical ventilation or mortality (6). Pan et al. reported that as the severity of COVID-19 increased, GI symptoms were more pronounced (47). A pooled analysis of multiple studies evaluated the correlation between GI symptoms and COVID-19 severity. Abdominal pain was associated with 4 fold increased odds of severe disease, marginally increased odds with nausea/vomiting, and no correction with diarrhea (48). Further dedicated studies are needed to evaluate these correlations of GI symptoms and COVID-19 severity without potential confounders.

FECAL-ORAL SPREAD

Diarrhea can predispose the community to the fecal-oral spread of the disease. Previous outbreaks from other coronaviruses (SARS outbreak in 2003) showed that sewage could be a source of infection (51). Randazzo et al. noted that evaluating waste-water plant systems early in the outbreak can help in identifying the spread of infection even before patients exhibit clinical symptoms (7). These methods can potentially help in developing public health strategies and policies to interfere with the spread of the disease.

In patients with diarrhea, stool positivity is noted in almost half of the cases. A systematic review of multiple studies showed a pooled prevalence of 48.1% (95% CI, 38.3–57.9%) for stool positivity (11). It is debatable if this stool positivity can lead to infectivity and spread of disease to uninfected individuals. COVID-19 Patients can have viral RNA stools positivity for an extended period (up to 14 days) even after the resolution of respiratory symptoms (52, 53). Studies have shown a prevalence of 30 to 82% of stool positivity after viral respiratory clearance (22, 54, 55). Cheung et al. noted a higher median fecal viral load in patients with positive stool viral RNA as compared to individuals without diarrhea (5.1 $\log_{10}$ copies/ml vs. 3.9 $\log_{10}$ copies/ml; $p = 0.06$) (32). These factors play a significant role in the development of mitigation strategies and standard protocol before a patient could be deemed non-infective after discharge from the hospital.

COVID-19 AND GASTROINTESTINAL ENDOSCOPIES

Endoscopic procedures can increase the exposure of the endoscopy staff with spillage of GI secretions, especially with the use of multiple devices (56, 57). Due to the inherent nature of the procedures with proximity to oral-pharyngeal secretions, endoscopy staff can get exposed to increasing the risk of transmission (56). Furthermore, endoscopes such as duodenoscopes are at risk of microbial contamination due to their inherent design (elevator) (58). As endoscopy centers resume their workflow, significant changes in triaging have been implemented with pre-procedural testing and active screening for COVID-19 symptoms. In addition to classical symptoms of fever, cough, shortness of breath, altered taste, additional GI symptoms of nausea, vomiting, diarrhea, and abdominal pain should be a part of a pre-operative questionnaire for elective procedures for those centers in high prevalence areas (59). Multiple GI societies have recommended guidelines for the use of negative pressure rooms, especially for patients infected with SARS-CoV-2 (60, 61). During the procedure, endoscopists, and staff should take adequate precautions such as the use of personal protective equipment (PPE) to prevent transmission of infection. Additionally, if the disinfection of endoscopic equipment is inadequate, it can theoretically lead to contamination and spread (58, 62). Repici et al. reported data to assess the risk of COVID-19 transmission in GI endoscopy (63). A study composed of 851 patients from Northern Italy showed eight patients had symptoms of fever, cough of which only one patient turned COVID-19 positive. None of the patient required hospitalization, suggests a very low risk of endoscopic transmission SARS-CoV-2 for patients. Furthermore, Repici et al. assessed 968 health care workers (HCW) from 41 hospitals, 42 (4.3%) tested positive, and six (0.6%) were hospitalized (63). Of the 42 HCWs who tested positive, 85.7% occurred prior to the introduction of PPE or reduction of endoscopy volume. All of these point toward the low risk of transmission of SARS-CoV-2 during endoscopy (63). Nevertheless, endoscopy staff should adhere to strict protective measures to avoid any amount of transmission.

Multiple national and international societies recommended deferral of non-urgent and elective procedures during the "phase 1" of the pandemic. This led to significant changes in the functioning of endoscopy units. In countries like Brazil, endoscopy staff has been divided into COVID and non-COVID teams to facilitate the flow in the unit (64). Mask mandates have been issued for all the endoscopy staff (56, 57, 61). Layouts of endoscopy units were changed based on risk-based color-coding of the suite, waiting, and recovery rooms (65) Pre-procedure testing has been implemented across multiple endoscopy units (66). Studies showed a reduction of procedure volume up to 99% (67). Studies showed that these changes have led to a decrease in colon cancer screenings by almost 85% (68). Deferral of these procedures was predominately elective (such as screening, surveillance), resulting in potentially increased load during recovery or "phase 2." Although this is dependent on the rate of infectivity in the community and indication, it is likely expected that increased case volumes and backlogs will occur post-pandemic.

MEDICATIONS AND GI SYMPTOMS

Patients with COVID-19 are subjected to increased pharmacological interventions. Due to suspicion for secondary bacterial infections, they are empirically treated with antibiotics such as fluoroquinolones and cephalosporins. Antiviral agents such as ritonavir-lopinavir, hydroxychloroquine, remdesivir, and tocilizumab can potentially cause nausea, vomiting, and diarrhea (69–72). Other agents such as azithromycin, oseltamivir, favipiravir may be used in COVID-19 patients at different stages of the disease, which can all contribute to the GI symptoms. If these agents directly cause the GI symptoms or contribute to the cytopathic effects of the SARS-CoV-2 is unclear. Recently, the use of PPI and resulting hypochlorhydria being recognized as a potential for increased positivity of COVID-19. A recent retrospective study showed that pre-hospitalization PPI exposure in hospitalized COVID-19 patients associated with a higher risk of the need for mechanical ventilation and higher mortality (19, 20, 73, 74). Gastric acid has shown to have neutralizing effects on many bacteria and viruses. The similar effect of gastric acid is also proposed on its neutralizing effect on SAR-CoV-2 PPI ca cause profound hypochlorhydria, which could be the reason for the higher risk of COVID-19 in these patients. However, a similar effect was not observed for the H2blockers, which are weak acid-suppressing medications. Further studies are

required to discern if PPI use increases the viral stools shedding in COVID-19.

CONCLUSION

GI manifestations are increasingly being recognized in COVID-19 patients. Some studies have shown severe disease in these patients, which could be due to increased viral load and involvement of multiple organ systems. It is important to recognize that some patients with COVI-19 may have only GI symptoms either prior to or in the absence of subsequent respiratory symptoms. These symptoms can be varied in presentation–from loss of sense of taste and smell to severe GI upset with diarrhea and abdominal pain. Individuals with these symptoms working in healthcare or other higher-risk environments should be checked for Covi-19 and potentially isolates. A strict medical definition of diarrhea should be observed in these patients to differentiate if the virus itself directly causing diarrhea or it is due to the patients' overall sickness. Other potential causes of diarrhea, such as clostridium difficile and antibiotics-associated diarrhea, need to be ruled out in these patients. The role of viral stool positivity in the transmission of the COVID-19 needs to be further studied. Multiple studies have shown that endoscopy staff is at higher risk of acquiring SARS-CoV-2 infection, possibly because of aerosolization of the secretions during suctioning. As endoscopies procedures are being resumed, strict adherence to universal precautions and use of personal protective equipment is needed. The patient viral transmission during the endoscopic procedures has not been reported but is theoretically possible as there are reports viral transmission with other kinds of viruses.

There is an urgent need for the standardization of stool testing, disease severity, a strict definition of GI symptoms, and evaluation of potential confounders. Nevertheless, advances made so far have increased our understanding of the GI symptoms, and they will likely continue to evolve as this pandemic unfolds.

AUTHOR CONTRIBUTIONS

HG and AP: conception, design, and literature review. AP: first draft. All authors: critical revision, editing, and final approval.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 11 November 2020
doi: 10.3389/fmed.2020.608259

Gastrointestinal Symptoms Associated With Unfavorable Prognosis of COVID-19 Patients: A Retrospective Study

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *19 September 2020*
Accepted: *21 October 2020*
Published: *11 November 2020*

Citation:
Chen R, Yu Y-l, Li W, Liu Y, Lu J-x, Chen F, Zhou Q, Xia Z-y, Gao L, Meng Q-t and Ma D (2020) Gastrointestinal Symptoms Associated With Unfavorable Prognosis of COVID-19 Patients: A Retrospective Study. Front. Med. 7:608259. doi: 10.3389/fmed.2020.608259

Background and Aim: The global pandemic of COVID-19 has posed an enormous threat to the economy and people's lives across various countries. Patients with COVID-19 most commonly present with respiratory symptoms. However, gastrointestinal (GI) symptoms can also occur. We aimed to study the relationship between GI symptoms and disease prognosis in patients with COVID-19.

Methods: In a single-center and retrospective cohort study, the outcomes in COVID-19 patients with or without GI symptoms were compared. The propensity score is a conditional probability of having a particular exposure (COVID-19 patients with GI symptoms vs. without GI symptoms) given a set of baseline measured covariates. Survival was estimated using the Kaplan-Meier method, and any differences in survival were evaluated with a stratified log-rank-test. To explore the GI symptoms associated with ARDS, non-invasive ventilator treatment, tracheal intubation, tracheotomy, and CRRT, univariable and multivariable COX regression models were used.

Results: Among 1,113 eligible patients, 359 patients with GI symptoms and 718 without GI symptoms had similar propensity scores and were included in the analyses. Patients with GI symptoms, as compared with those without GI symptoms, were associated with a similar risk of death, but with higher risks of ARDS, non-invasive mechanical ventilation in COVID-19 patients, respectively.

Conclusions: The presence of GI symptoms was associated with a high risk of ARDS, non-invasive mechanical ventilation and tracheal intubation in patients with COVID-19 but not mortality.

Keywords: gastrointestinal symptoms, COVID-19, prognosis, SARS-CoV-2 (COVID-19), ARDS (acute respiratory distress syndrome)

INTRODUCTION

The global pandemic of COVID-19 has posed an enormous threat to the economy and people's lives across various countries (1, 2). The clinical spectrum of COVID-19 appears to be wide, ranging from asymptomatic infection, mild to critically-ill cases (3–6). Significant comorbidities such as type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVDs) were associated with developing severe and critical COVID-19 condition (7, 8). In severe cases, patients can rapidly develop acute respiratory distress syndrome (ARDS), septic shock, and multiple organ dysfunction syndromes (9). The most common symptoms of COVID-19 are fever, cough, fatigue, myalgia, and dyspnoea (10). Gastrointestinal (GI) symptoms were also observed in a significant proportion of patients (11–13), which were possibly due to the enrichment and infection of SARS-CoV-2 in the gastrointestinal tract.

Recent studies showed that angiotensin converting enzyme 2 (ACE2) plays a crucial role in the cellular infection with SARS-CoV-2 virus (14–16). Although ACE2 was found to be widely expressed across tissues, it was considered to be intestine-specific, and was enriched more than 4-fold in the epithelia of the intestinal tract compared with other tissues (17). SARS-CoV-2 disrupts ACE2 activity and infects the intestinal epithelium by inducing cytotoxicity (18), and then it is shed into feces, resulting in GI symptoms and/or positive SARS-CoV-2 viral load or RNA in stool (19). It is known that gastrointestinal problems in critically-ill patients were common and were associated with unfavorable outcomes (20). Trillions of diverse bacteria located in the intestinal tract and constituted the intestinal "microbiota" (21). Our previous studies found that bacteria and toxins enter into blood after intestinal mucosa injury caused by adverse stimulates, leading to damage of multiple remote organs (22). The impact of intestinal mucosa injured by SARS-CoV-2 infection and consequence on prognosis in patients with COVID-19 remains unknown. In this study, we investigated patients with GI symptoms, who were admitted to Renmin hospital of Wuhan University, Wuhan, China, associated with prognosis or outcome in patients with COVID-19.

MATERIALS AND METHODS

Study Design and Participants

This single-center, retrospective cohort study included two cohorts of inpatients from East Campus of Renmin Hospital of Wuhan University. It was approved by the Institutional Review Board at Renmin hospital of Wuhan University (No. WDRY2020-K111, March 12, 2020) and have been performed in accordance with the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013). Due to the urgency of this infectious disease, data analysis was performed anonymously and written informed consent was exempted. The East Campus of Renmin Hospital of Wuhan University is one of the major hospitals designated by the government to be responsible for patients with COVID-19 who are critically-ill, pregnant, or require surgery from January 25, 2020. This study included a total of 1,117 hospitalized patients with COVID-19 from January 25, 2020 to March 31, 2020. The diagnosis of COVID-19 according to the diagnostic criteria established by WHO and the New Coronavirus Pneumonia Prevention and Control Program (5th−7th edition) (23–25) issued by the National Health Commission of China. COVID-19 patients were diagnosed with clinical symptoms together with nasopharyngeal swabs tested positive for SARS-CoV-2 using real-time reverse transcription PCR (RT-PCR). All patients received chest radiography or chest CT scan on admission. Patients were divided into groups with gastrointestinal (GI) symptom or without GI symptom according to the presence or absence of GI symptoms.

Data Collection

All information including epidemiological, demographic, clinical, laboratory, treatment, and outcome data were extracted from the medical record system of the Renmin Hospital of Wuhan University, and were collected and reviewed by three investigators using a standardized data collection form. All data were collected including age, sex, exposure history, comorbidities (hypertension, diabetes, coronary heart disease, cerebrovascular disease, chronic heart failure, liver dysfunction, chronic kidney disease, and chronic pulmonary disease), GI symptoms (abdominal pain, acid reflux, nausea or vomiting, abdominal distension, diarrhea, tenesmus, and belching), common symptoms (fever, cough, chest tightness, chest pain, dyspnoea, myalgia, headache, and fatigue), laboratory values, and radiologic findings on admission, treatment [proton pump inhibitors (PPIs), antivirals, antibiotics, corticosteroids, and high-flow nasal oxygen therapy], as well as complications [ARDS, acute kidney injury (AKI), and acute liver injury] and mortality status. All data were double checked independently and further verifications were done wherever necessary.

Definition

The definition of patients with GI symptoms is that the patients had at least one of the GI symptoms of abdominal pain, acid reflux, nausea or vomiting, abdominal distension, diarrhea, tenesmus, and belching. Fever was defined as having an axillary temperature of $>37.3^{\circ}$C. Lymphocytopenia was defined as lymphocyte count $<0.8 \times 109$/L (26). The patients with COVID-19 were divided into four grades according to the degree of disease severity, based on the Chinese management guideline for COVID-19 (5th−7th edition) (23–25): Mild (slight clinical symptoms without CT imaging features of pneumonia); Moderate (fever and/or respiratory symptoms plus imaging features of COVID-19 pneumonia); Severe [respiratory distress (respiratory rate $\geq$30 breaths/min) together with the oxygen saturation $\leq$93% or arterial oxygen pressure (PaO_2)/fractional inspired oxygen (FiO_2) ratio $\leq$300 mmHg]; Critical [respiratory failure requiring mechanical ventilation or multiorgan failure requiring intensive care unit (ICU) admission]. Acute respiratory distress syndrome was diagnosed according to the Berlin definition (27). Acute kidney injury was identified on the basis of serum creatinine level according to the KDIGO clinical practice

guideline (28). The definition of liver damage was alanine aminotransferase (ALT) >50 U/L or aspartate aminotransferase (AST) >40 U/L (29).

Outcomes

The correlation of the GI symptoms of COVID-19 associated with mortality and other clinical features and interventions

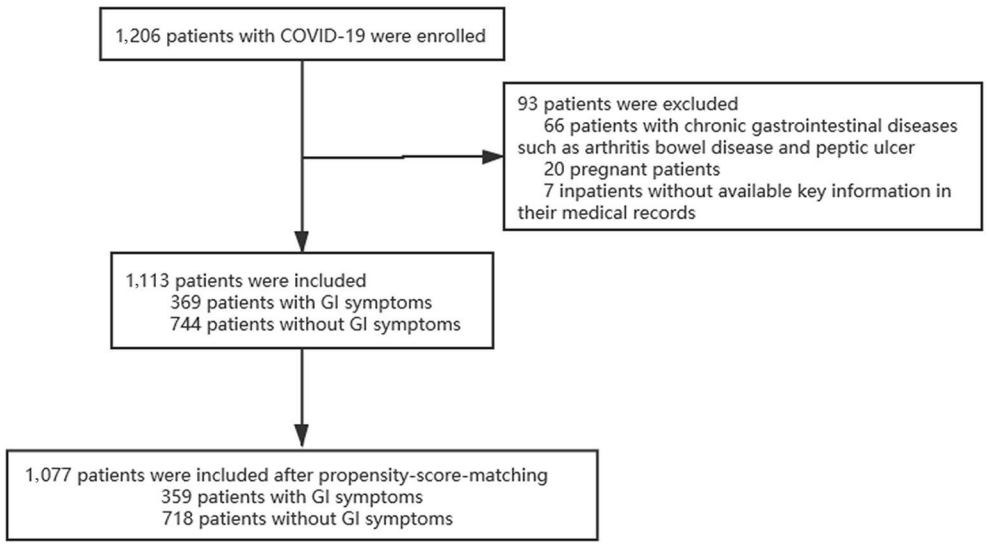

FIGURE 1 | Enrollment flowchart. GI, gastrointestinal injury.

TABLE 1 | Baseline characteristics of patients with COVID-19 before and after propensity score matching.

Characteristic	Before matching				After matching			
	All patients (n = 1,113)	Patients with GI symptoms (n = 369)	Patients without GI symptoms (n = 744)	p-value	All patients (n = 1,077)	Patients with GI symptoms (n = 359)	Patients without GI symptoms (n = 718)	p-value
Age, year								
Median (IQR)	59.0 (47.0–68.0)	61.0 (50.0–70.0)	57.5 (46.0–67.0)	<0.001	59.0 (47.0–68.0)	60.0 (48.0–70.0)	59.0 (47.0–67.0)	0.065
Distribution, n (%)								
<15	0	0	0		0	0	0	
15–44	238 (21.4)	65 (17.6)	173 (23.3)		219 (20.0)	65 (18.1)	154 (21.5)	
45–64	476 (42.8)	152 (41.2)	324 (43.5)		476 (42.7)	152 (42.3)	324 (45.1)	
≥65	399 (35.8)	152 (41.2)	247 (33.2)		382 (34.3)	142 (39.6)	240 (33.4)	
Sex, n (%)				0.496				0.763
Male	550 (49.4)	177 (48.0)	373 (50.1)		532 (49.4)	175 (48.7)	357 (49.7)	
Female	563 (50.6)	192 (52.0)	371 (49.9)		545 (50.6)	184 (51.3)	361 (50.3)	
Exposure history, n (%)	153 (13.7)	49 (13.3)	104 (14.0)	0.750	152 (14.1)	48 (13.4)	104 (14.5)	0.621
Comorbidity, n (%)	574 (51.6)	203 (55.0)	371 (49.9)	0.106	557 (51.7)	193 (53.8)	364 (50.7)	0.343
Hypertension	368 (33.1)	133 (36.0)	235 (31.6)	0.137	355 (33.0)	124 (34.5)	231 (32.2)	0.436
Diabetes	150 (13.5)	59 (16.0)	91 (12.2)	0.084	145 (13.5)	55 (15.3)	90 (12.5)	0.207
CHD	91 (8.2)	32 (8.7)	59 (7.9)	0.671	86 (8.0)	29 (8.1)	57 (7.9)	0.937
Cerebrovascular disease	31 (2.8)	12 (3.3)	19 (2.6)	0.505	27 (2.5)	10 (2.8)	17 (2.4)	0.683
Chronic heart failure	35 (3.1)	18 (4.9)	17 (2.3)	0.020	32 (3.0)	16 (4.5)	16 (2.2)	0.042
Liver dysfunction	89 (8.0)	29 (7.9)	60 (8.1)	0.905	87 (8.1)	28 (7.8)	59 (8.2)	0.812
CKD	57 (5.1)	29 (7.9)	28 (3.8)	0.004	52 (4.8)	25 (7.0)	27 (3.8)	0.056
Chronic pulmonary disease	59 (5.3)	27 (7.3)	32 (4.3)	0.035	58 (5.4)	26 (7.2)	32 (4.5)	0.174

Data are shown as median (IQR) or n (%). p-values were calculated using Mann-Whitney U-test, χ^2-test, or Fisher's exact-test, as appropriate. GI, gastrointestinal; CHD, Coronary heart disease; CKD, Chronic kidney disease.

including ARDS, non-invasive ventilator treatment, tracheal intubation, tracheotomy, and continuous renal replacement therapy (CRRT) were analyzed. Other outcomes including the rate of SARS-CoV-2-related AKI, acute liver injury and the proportion of patients requiring high-flow nasal oxygen therapy, non-invasive mechanical ventilation, tracheal intubation, tracheotomy, CRRT, and ICU admission were also analyzed.

Statistical Analyses

Given the differences in the baseline characteristics between eligible participants in the two groups, propensity-score matching was used to authenticate a cohort of patients with similar baseline characteristics. The propensity score is a conditional probability of having a particular exposure (COVID-19 patients with GI symptoms vs. without GI symptoms) given a set of baseline measured covariates. The propensity score was estimated, with COVID-19 patients with GI symptoms as the dependent variable, and age, sex, exposure history, comorbidities as covariates. Matching was performed with the use of a 1:2 matching protocol without replacement (greedy-matching algorithm), with a caliper width equal to 0.2 of the standard deviation of the logit of the propensity score. Standardized differences and *p*-values were estimated for all the baseline covariates before and after matching to assess pre-match imbalance and post-match balance. Standardized differences of <10.0% for a given covariate indicate a relatively small imbalance.

Continuous and categorical variables were presented as median (IQR) and *n* (%), respectively. We used the Mann-Whitney U-test, χ^2-test, or Fisher's exact-test to compare differences between patients with and without GI symptoms where appropriate. Survival was estimated using the Kaplan-Meier method, and any differences in survival were evaluated with a stratified log-rank-test. To explore the GI symptoms associated with ARDS, non-invasive ventilator treatment, tracheal intubation, tracheotomy, and CRRT, univariable and multivariable COX regression models were used. A two-sided α of <0.05 was considered statistically significant. Data were analyzed with the use of the statistical packages R (The R Foundation; http://www.r-project.org; version 3.4.3 2018–02-18) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc.).

RESULTS

Demographic and Epidemiological Characteristics

A total of 1,206 adult patients of COVID-19 were enrolled in our study from 25 January, 2020 to 31 March, 2020 in East Campus of Renmin hospital of Wuhan university; Of those, 93 were considered to be ineligible, including 66 patients with chronic gastrointestinal disease, 20 patients who were pregnant and 7 patients missing key information in their medical records. Final 1,077 patients were included in our study (**Figure 1**). There were differences between the two groups in several of the baseline variables before propensity score matching (PSM). After excluded 36 patients with PSM, 359 patients with GI symptoms were matched against 718 patients without GI symptoms. Their demographic data and other characteristics including comorbidities are presented in the **Table 1**.

TABLE 2 | Clinical features, disease classification of patients with COVID-19 with and without GI symptoms.

	All patient (*n* = 1077)	Patients with GI symptoms (*n* = 359)	Patients without GI symptoms (*n* = 718)	*p*-value
GI symptoms				
Abdominal pain	38 (3.5)	38 (10.6)	0 (0)	
Acid reflux	12 (1.1)	12 (3.3)	0 (0)	
Nausea or vomiting	71 (6.6)	71 (19.8)	0 (0)	
Abdominal distension	38 (3.5)	38 (10.6)	0 (0)	
Diarrhea	208 (19.3)	208 (57.9)	0 (0)	
Tenesmus	9 (0.8)	9 (2.5)	0 (0)	
Belching	6 (0.6)	6 (1.7)	0 (0)	
Other symptoms				
Fever	777 (72.1)	287 (79.9)	490 (68.2)	<0.001
Cough	267 (24.8)	168 (46.8)	99 (13.8)	<0.001
Chest tightness	143 (13.3)	60 (16.7)	83 (11.6)	0.019
Chest pain	20 (1.9)	6 (1.7)	14 (1.9)	0.816
Dyspnoea	105 (9.7)	52 (14.5)	53 (7.4)	<0.001
Myalgia	61 (5.7)	31 (8.6)	30 (4.2)	0.003
Headache	28 (2.6)	12 (3.3)	16 (2.2)	0.311
Fatigue	240 (22.3)	103 (28.7)	137 (19.1)	<0.001
Time of onset of GI symptoms				
On initial presentation	107 (9.9)	107 (29.8)	0 (0)	
During hospitalization	252 (23.4)	252 (70.2)	0 (0)	
Disease classification				<0.001*
Mild	29 (2.7)	2 (0.5)	27 (3.8)	
Moderate	485 (45.0)	118 (32.9)	367 (51.1)	
Severe	502 (46.6)	212 (59.1)	290 (40.4)	
Critical	61 (5.7)	27 (7.5)	34 (4.7)	

*Data are median (IQR) or n (%). p-values were calculated using Mann-Whitney U-test, χ^2-test, or Fisher's exact-test, as appropriate. GI, gastrointestinal. *χ^2-test comparing all subcategories.*

Clinical, Laboratory, and Radiographic Characteristics

Diarrhea (208, 57.9%), nausea or vomiting (71, 19.8%), abdominal pain (38, 10.6%) and abdominal distension (38, 10.6%) were the most frequently observed GI manifestations (**Table 2**). For those 359 patients with GI symptoms, 107 (29.8%) were present on initial presentation and 252 (70.2%) were present during hospitalization. Fever, cough, fatigue, chest tightness, and dyspnoea were the most common symptoms amongst all COVID-19 patients; Patients with GI symptoms had fever (287, 79.9%; $p < 0.001$), cough (168, 46.8%; <0.001), fatigue (103, 28.7%; <0.001), chest tightness (60, 16.7%; 0.019), and dyspnoea (52, 14.5%; <0.001), which were significantly higher than those

TABLE 3 | Laboratory and radiographic findings of patients with COVID-19 with and without GI symptoms.

	All patients ($n = 1,077$)	Patients with GI symptoms ($n = 359$)	Patients without GI symptoms ($n = 718$)	*p*-value
Laboratory findings				
White blood cell count, $\times 10^9$/L	5.6 (4.4–7.3)	5.5 (4.2–7.3)	5.7 (4.5–7.3)	0.091
Neutrophil count, $\times 10^9$/L	3.6 (2.5–5.3)	3.7 (2.5–5.6)	3.6 (2.6–5.3)	0.910
Lymphocyte count, $\times 10^9$/L	1.1 (0.8–1.6)	1.0 (0.7–1.4)	1.3 (0.9–1.7)	<0.001
Hemoglobin, g/L	125.0 (114.0–135.0)	125.0 (114.0–133.0)	125.0 (115.0–135.0)	0.531
Anemia	381 (35.4)	132 (36.8)	249 (34.7)	0.499
Platelet count, $\times 10^9$/L	214 (168.0–273.0)	207 (158.0–270.5)	219.0 (174.0–277.0)	0.006
Albumin, g/L	38.0 (34.4–41.1)	36.8 (33.7–40.0)	38.5 (34.7–41.4)	<0.001
ALT, U/L	25.0 (17.0–42.0)	26.0 (17.0–41.0)	24.0 (16.0–42.0)	0.607
AST, U/L	25.0 (19.0–38.0)	27.0 (20.0–40.0)	24.0 (18.0–38.0)	0.008
Urea, mmol/L	4.7 (3.7–6.2)	4.8 (3.7–6.5)	4.7 (3.7–6.1)	0.332
Creatinine, μmol/L	59.0 (49.0–71.0)	59.0 (49.0–73.0)	59.0 (50.0–70.0)	0.639
LDH, U/L	237.0 (188.0–325.0)	258.0 (200.0–355.0)	227.0 (181.0–309.0)	<0.001
PT, s	11.9 (11.2–12.6)	12.0 (11.3–12.7)	11.8 (11.2–12.5)	0.008
APTT, s	27.5 (25.6–29.9)	27.8 (25.7–30.8)	27.3 (29.4–25.5)	0.009
CRP, mg/L	16.8 (3.0–60.5)	30.8 (9.4–68.9)	9.4 (2.4–54.1)	<0.001
D-dimer, μg/mL	0.7 (0.4–1.9)	0.9 (0.4–2.5)	0.6 (0.3–1.6)	<0.001
Procalcitonin, ng/mL	0.05 (0.03–0.10)	0.06 (0.04–0.14)	0.05 (0.03–0.09)	<0.001
Glu, mmol/L	5.5 (4.8–6.9)	5.6 (4.9–7.1)	5.3 (4.8–6.7)	0.007
Na, mmol/L	141.0 (139.0–145.0)	141.0 (138.0–144.0)	142.0 (139.0–145.0)	0.009
K, mmol/L	4.0 (3.7–4.3)	4.0 (3.6–4.4)	4.0 (3.7–4.3)	0.376
Ca, mmol/L	2.1 (2.0–2.2)	2.1 (2.0–2.2)	2.2 (2.1–2.3)	<0.001
Radiologic findings				
Bilateral	843 (78.3)	301 (83.8)	542 (75.5)	0.002
Ground-glass opacity	656 (60.9)	225 (62.7)	431 (60.0)	0.401
Patchy shadows	612 (56.8)	165 (46.0)	447 (62.3)	<0.001
Diffuse interstitial infiltrations	14 (1.3)	8 (2.2)	6 (0.8)	0.083

Data are median (IQR) or n (%). p-values were calculated using Mann-Whitney U-test, χ^2-test, or Fisher's exact-test, as appropriate. GI, gastrointestinal; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; CRP, C-reactive protein.

in patients without GI symptoms (**Table 2**). Lymphocyte count in COVID-19 patients with GI symptoms was significantly lower than that in patients without GI symptoms [1.0 (0.7–1.4) vs. 1.3 (0.9–1.7), $p < 0.001$]; Lymphocytopenia occurred in 122 (34%) patients with GI symptoms. AST [27.0 (20.0–40.0) vs. 24.0 (18.0–38.0), $p = 0.008$], LDH [258.0 (200.0–355.0) vs. 227.0 (181.0–309.0)], CRP [30.8 (9.4–68.9) vs. 9.4 (2.4–54.1), $p < 0.001$] and procalcitonin [0.06 (0.04–0.14) vs. 0.05 (0.03–0.09), $p < 0.001$] were substantially higher in the COVID-19 patients with GI symptoms. Moreover, although most radiographic findings were similar between COVID-19 patients with and without GI symptoms, the rate of bilateral lung pneumonia in COVID-19 patients with GI symptoms was much higher than that in patients without GI symptoms [301 (83.8%) vs. 542 (75.5%), $p = 0.002$]. All these comparisons in the two groups are presented in the **Table 3**.

Treatment, Complications, and Clinical Outcomes

The number of patients receiving antivirals [342 (95.3%) vs. 648 (90.3%), $p = 0.004$], antibiotics [286 (79.7%) vs. 479 (66.7%), $p < 0.001$], and corticosteroids [148 (41.2) vs. 244 (34.0), $p = 0.020$] were significantly different between the COVID-19 patients with and without GI symptoms (**Table 4**). 298 (83.0%) COVID-19 patients with GI symptoms were treated with high-flow nasal oxygen therapy, 54 (15%) with non-invasive mechanical ventilation, 20 (5.6%) with tracheal intubation ventilation, 7 (1.9%) with CRRT, which were higher than those in patients without GI symptoms, respectively (**Table 4**). Acute respiratory distress syndrome was the most frequently observed complication, in addition to AKI and acute liver injury. The rate of ARDS in patients with GI symptoms was higher than that in patients without GI symptoms [72 (20.1%) vs. 61 (8.5), $p < 0.001$]. As of March 31, 785 (72.9%) patients with COVID-19 have been discharged from hospital, and 207 (19.2%) patients remained in hospital.

Correlation of Measures

Kaplan-Meier curves showed that there was no significant difference ($p = 0.479$) in mortality between COVID-19 patients with and without GI symptoms (**Figure 2**). The univariate regression analysis (**Table 5**) showed that the patients with GI symptoms was significantly associated with developing ARDS (HR 2.7, 95%CI 1.9–3.9, $p < 0.001$), requiring non-invasive

TABLE 4 | Treatment, complications and clinical outcomes of patients with COVID-19 with or without GI symptoms.

	All patients (n = 1,077)	Patients with GI symptoms (n = 359)	Patients without GI symptoms (n = 718)	p-value
Treatments				
PPIs	460 (42.7)	205 (57.1)	255 (35.5)	<0.001
Antivirals	990 (91.9)	342 (95.3)	648 (90.3)	0.004
Antibiotics	765 (71.0)	286 (79.7)	479 (66.7)	<0.001
Corticosteroids	392 (36.4)	148 (41.2)	244 (34.0)	0.020
High-flow nasal oxygen therapy	849 (78.8)	298 (83.0)	551 (76.7)	0.018
Non-invasive mechanical ventilation	99 (9.2)	54 (15.0)	45 (6.3)	<0.001
Tracheal intubation	33 (3.1)	20 (5.6)	13 (1.8)	<0.001
Tracheotomy	8 (0.7)	4 (1.1)	4 (0.6)	0.452
CRRT	9 (0.8)	7 (1.9)	2 (0.3)	0.008
ICU admission	52 (4.8)	22 (6.1)	30 (4.2)	0.159
ICU length of stay, days	14.0 (8.0–24.0)	15.5 (8.0–24.8)	14.0 (8.2–17.2)	0.498
Complications				
ARDS	133 (12.3)	72 (20.1)	61 (8.5)	<0.001
AKI	13 (1.5)	5 (1.4)	8 (1.1)	0.693
Acute liver injury	16 (1.5)	6 (1.7)	10 (1.4)	0.791
Hospital length of stay, days	16.0 (9.0–32.0)	24.8 (12.0–36.0)	14.0 (8.0–28.0)	<0.001
Clinical outcomes				
Discharge from hospital	785 (72.9)	254 (70.8)	531 (74.0)	0.265
Death	85 (7.9)	34 (9.5)	51 (7.1)	0.174
Staying in hospital	207 (19.2)	71 (19.7)	136 (19.9)	

Data are median (IQR) or n (%). p-values were calculated using Mann-Whitney U-test, χ^2-test, or Fisher's exact-test, as appropriate. GI, gastrointestinal; PPIs, proton pump inhibitors; CRRT, continuous renal replacement therapy; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; ICU, intensive care unit.

mechanical ventilation (HR 2.6, 95%CI 1.7–4.0, $p < 0.001$), tracheal intubation (HR 3.2, 95%CI 1.6–6.5, $p < 0.001$), and CRRT (HR 7.1, 95%CI 1.5–34.4, $P = 0.015$). In the multivariate analysis, after the adjusting with lymphocyte, PLT, Albumin, Urea, Creatinine, LDH, PT, APTT, D-dimer, CRP, and Procalcitonin, patients with GI symptoms independently associated with non-invasive mechanical ventilation (HR 3.1, 95%CI 1.8–5.4, $p < 0.001$), tracheal intubation (HR 2.4, 95%CI 1.1–5.5, $p = 0.037$) and ARDS (HR 2.8, 95%CI 1.7–4.6, $p < 0.001$). There was no association between patients with GI symptoms and the requirement for CRRT (HR 5.1, 95%CI 0.5–53.0, $P = 0.175$). After adjusted with variables in Adjust I model and antiviral treatment, antibiotics and corticosteroids, the presence of GI symptoms remained an independent predictor for ARDS (HR 2.9, 95%CI 1.8–5.0, $p < 0.001$), non-invasive mechanical ventilation (HR 3.3, 95%CI 1.9–5.7, $p < 0.001$), and tracheal intubation (HR 2.5, 95%CI 1.1–6.0, $p = 0.035$) in COVID-19 patients.

DISCUSSION

This retrospective cohort study of patients with COVID-19 showed that gastrointestinal (GI) symptoms were associated with a higher risk of ARDS, non-invasive mechanical ventilation, and tracheal intubation. The risk of death was similar amongst COVID-19 patients with or without GI symptoms. Many studies have confirmed that GI symptoms in COVID-19 patients are associated with the disease prognosis. Hajifathalian et al. (30) reported a lower mortality rate in patients with GI symptoms compared to those without any GI symptoms. Another study from Spain involving 2,226 patients with COVID-19 came to similar conclusions (31). In contrast, many studies have shown that GI symptoms are associated with poor prognosis. A meta-analysis reported that patients with GI symptoms had a higher rate of severe or critical COVID-19 infection compared to patients without any GI symptoms (32). To our knowledge, this is the first study examining the relationship between GI symptoms and prognosis in patients with COVID-19 with a relatively large sample size. In previously published studies of COVID-19 patients with GI symptoms, the patient numbers were too small to conclude the characteristics and mortality of these patients with SARS-CoV-2 pneumonia (29, 33, 34).

Although COVID-19 is characterized by respiratory tract manifestations, GI symptoms are not uncommon. In some cases, GI symptoms, particularly diarrhea, can be the initial presentation of COVID-19 in patients who may later (or never) present with respiratory symptoms (35). Moreover, another research from Wuhan showed that patients with GI symptoms risked not being promptly recognized, leading to a delayed diagnosis of COVID-19 (12). These patients were diagnosed as COVID-19 positive with SARS-CoV-2 nucleic acid in stool or rectal swabs (36). Among the total of 1,113 COVID-19 patients enrolled, the rate of patients with GI symptoms was 33.2%, which was higher than the data reported previously (3, 29). The reason of this discrepancy is unknown but may be related to the main task of East Campus of Renmin Hospital of Wuhan University in undertaking the treatment of critical COVID-19 patients.

In our study, the clinical characteristics of COVID-19 patients with GI symptoms were a significantly higher rate of fever, cough, chest tightness, dyspnoea, myalgia and fatigue, and had increased complication of ARDS and a higher tendency toward higher disease severity (rate of severe/critical type and mechanical ventilation) compared with COVID-19 patients without GI symptoms, which is consistent with a study reported previously (29). This may be related to bacterial translocation and electrolyte disturbances, as evidenced by significantly increased CRP and procalcitonin levels, decreased lymphocyte count and serum sodium levels. In addition, although the incidence of AKI and acute liver injury was similar between the COVID-19 patients with or without GI symptoms, the AST level and creatinine above 133 μmol/L in the COVID-19 patients with GI symptoms were higher than those without GI symptoms. These results highlighted the need to closely monitor liver and kidney functions during the course of the disease.

The functional host cell "receptor" for SARS-CoV-2 is angiotensin converting enzyme 2 (ACE2) (37, 38). The spike glycoprotein (S protein) on the virion surface mediates receptor

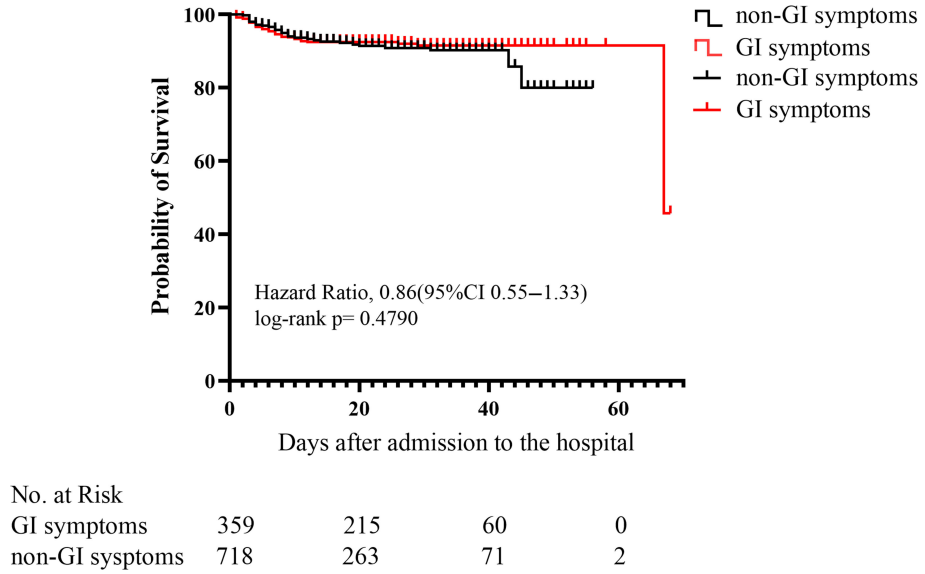

FIGURE 2 | Kaplan-Meier (KM) survival curve for COVID-19 patients with or without GI (gastrointestinal) symptoms. *p*-values were for differences by log-rank-test.

recognition and membrane fusion, thus exploiting ACE2 for host infection (39). ACE2 receptors are not only distributed in bronchial transient secretory cells (40) but also in various tissues and organs, such as kidneys, small intestine, (41) and testis (42). In the intestines, ACE2 is primarily distributed on the luminal surface of differentiated small intestinal epithelial cells, and is identified as a key regulator of dietary amino acid homeostasis, innate immunity, gut microbial ecology, and transmissible susceptibility to colitis (43). These may mediate the invasion of the virus, activation and amplification of gastrointestinal inflammation (44) and lead to GI symptoms in patients with COVID-19.

The gastrointestinal tract represents a large microbial ecosystem, housing several trillion microbiota. Under normal circumstances, the intestinal microbiota plays a critical role in the maturation of the host immune response (45), influences the regulation of intestinal endocrine functions (46) and maintains the homeostasis of gastrointestinal tract. An increase in gut permeability, bacterial translocation and local responses can be found in patients with critical illness of various causes (47). For example, in intestinal ischemia-reperfusion injury, it has been demonstrated that the reperfused gut can become a source of pro-inflammatory mediators (48) which can be delivered to remote organs and amplify the early systemic inflammatory response (22). Consistent with the results of previous animal studies (22), the presence of GI symptoms is associated with a higher rate of ARDS, non-invasive mechanical ventilation and tracheal intubation in patients with COVID-19. However, our study showed that GI symptoms did not appear to affect the mortality rate among COVID-19 patients but the sample size under power to detect any statistical significances of mortality can not be excluded. Furthermore, at the point of data analysis, some patients were still in the hospital and their long term outcomes are unknown whilst the retrospective nature and a single-center data of our study would call more studies into this during the disease pandemic.

In conclusion, this work is one of the largest cohort of COVID-19 patients with GI symptoms. COVID-19 patients with GI symptoms, as compared with absence of GI symptoms, were

TABLE 5 | Univariate and multivariate analysis for non-invasive mechanical ventilation, tracheal intubation, CRRT, and ARDS in COVID-19 patients with GI symptoms.

	Hazare ratio	95%CI	*p*-value
ARDS			
Unadjusted	2.7	1.9–3.9	<0.001
Adjusted I	2.8	1.7–4.6	<0.001
Adjusted II	2.9	1.8–5.0	<0.001
Non-invasive mechanical ventilation			
Unadjusted	2.6	1.7–4.0	<0.001
Adjusted I	3.1	1.8–5.4	<0.001
Adjusted II	3.3	1.9–5.7	<0.001
Tracheal intubation			
Unadjusted	3.2	1.6–6.5	0.001
Adjusted I	2.4	1.1–5.5	0.037
Adjusted II	2.5	1.1–6.0	0.035
CRRT			
Unadjusted	7.1	1.5–34.4	0.015
Adjusted I	5.1	0.5–53.0	0.175
Adjusted II	6.1	0.5–71.3	0.149

Adjust I model adjusting for Lymphocyte, PLT, Albumin, Urea, Creatinine, LDH, PT, APTT, D-dimer, CRP, and Procalcitonin; Adjust II model adjusting by variables in Adjust I model plus antiviral treatment, antibiotics, and corticosteroids. CRRT, continuous renal replacement therapy; ARDS, acute respiratory distress syndrome.

associated with high risks of ARDS, non-invasive mechanical ventilation, and tracheal intubation. Therefore, we should pay greater attention to COVID-19 patients with GI and other non-classical symptoms for better care of our patients and remain vigilant in the protection of healthcare providers.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board at Renmin hospital of Wuhan University (No. WDRY2020-K111, March 12, 2020). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LG, Q-tM, and DM designing research studies, reviewed, and edited the manuscript. YL and J-xL acquiring data. RC, Y-lY, and WL analyzing data and writing the paper. Illustrations and proofreading were performed by YL, FC, and QZ. All authors read and approved the manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (NSFC): 81671948.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

SYSTEMATIC REVIEW
published: 13 November 2020
doi: 10.3389/fmed.2020.572115

Pre-existing Liver Diseases and On-Admission Liver-Related Laboratory Tests in COVID-19: A Prognostic Accuracy Meta-Analysis With Systematic Review

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OPEN ACCESS

Edited by:
Angel Lanas,
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Reviewed by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *12 June 2020*
Accepted: *05 October 2020*
Published: *13 November 2020*

Citation:
Váncsa S, Hegyi PJ, Zádori N, Szakó L, Vörhendi N, Ocskay K, Földi M, Dembrovszky F, Dömötör ZR, Jánosi K, Rakonczay Z Jr, Hartmann P, Horváth T, Erőss B, Kiss S, Szakács Z, Németh D, Hegyi P and Pár G (2020) Pre-existing Liver Diseases and On-Admission Liver-Related Laboratory Tests in COVID-19: A Prognostic Accuracy Meta-Analysis With Systematic Review. Front. Med. 7:572115. doi: 10.3389/fmed.2020.572115

Background: We aimed to perform a systematic search and meta-analysis to evaluate the prognostic value of on-admission liver function tests and pre-existing liver diseases on the clinical course of coronavirus disease 2019 (COVID-19).

Methods: The study was registered on PROSPERO (CRD42020182902). We searched five databases between 01/01/2020 and 04/23/2020. Studies that reported on liver-related comorbidities and/or laboratory parameters in patients with COVID-19 were included. The main outcomes were COVID-19 severity, intensive care unit (ICU) admission, and in-hospital mortality. Analysis of predictive models hierarchical summary receiver-operating characteristic (HSROC) was conducted with a 95% confidence interval (CI).

Results: Fifty studies were included in the meta-analysis. High specificity was reached by acute liver failure associated by COVID-19 (0.94, 95% CI: 0.71–0.99) and platelet count (0.94, 95% CI: 0.71–0.99) in the case of mortality; chronic liver disease (CLD) (0.98, 95% CI: 0.96–0.99) and platelet count (0.82, 95% CI: 0.72–0.89) in the case of ICU requirement; and CLD (0.97, 95% CI: 0.95–0.98), chronic hepatitis B infection (0.97, 95% CI: 0.95–0.98), platelet count (0.86, 95% CI: 0.77–0.91), and alanine aminotransferase (ALT) (0.80, 95% CI: 0.66–0.89) and aspartate aminotransferase (AST) (0.84, 95% CI: 0.77–0.88) activities considering severe COVID-19. High sensitivity was found in the case of C-reactive protein (CRP) for ICU requirement (0.92, 95% CI: 0.80–0.97) and severe COVID-19 (0.91, 95% CI: 0.82–0.96).

Conclusion: On-admission platelet count, ALT and AST activities, CRP concentration, and the presence of acute and CLDs predicted the severe course of COVID-19. To

highlight, pre-existing liver diseases or acute liver injury associated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection plays an important role in the prediction of mortality.

Keywords: SARS—CoV-2, COVID-19, prognosis, hepatology, pandemic (COVID-19)

INTRODUCTION

In December 2019, a local outbreak of pneumonia caused by a novel coronavirus, namely, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was detected in Wuhan, China. In most cases, coronavirus disease 2019 (COVID-19) is an acute, self-limiting disease with a relatively brief period of symptoms and resolution within days. However, it can reach in-hospital mortality of 3–7% (1), which can result from massive alveolar damage, consequential acute respiratory distress syndrome (ARDS), respiratory failure, septic shock, or multiple organ dysfunction (2, 3).

It is important to explore the prognostic factors, which have a significant impact on the disease course, given the rapid spread of COVID-19 and its high mortality rate. The detrimental effects of hypertension, cardiovascular diseases, kidney disease, and diabetes mellitus on the disease course are already proven (4–6). Due to the limited number of reports on COVID-19 with underlying chronic liver disease (CLD) to date, the impact of pre-existing liver pathologies on COVID-19 progression and outcomes is unknown.

Although coronaviruses cause the worst damage on the lungs, studies suggest that other organs, such as the liver, intestines, heart, and central nervous system, could also be affected (7–11). In COVID-19, almost half of the hospitalized patients have various degrees of liver test abnormalities, and liver impairment was also observed in 14–53% of the patients (12).

We aimed to appraise the currently available literature of confirmed SARS-CoV-2 infections critically and to investigate the prognostic value of on-admission liver function and liver conditions on the clinical course of COVID-19.

MATERIALS AND METHODS

Our systematic review and meta-analysis was planned and reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 Statement (13) (**Supplementary Table 1**). This study was registered in advance on PROSPERO under registration number CRD42020182902 (see https://www.crd.york.ac.uk/prospero).

Search and Selection

A systematic search was conducted by two independent reviewers (LS and NZ) to identify all the relevant records on the prognostic value of liver impairment in COVID-19 patients published from January 1, 2020 to April 23, 2020. The search was performed in MEDLINE via PubMed, Embase, Scopus, Cochrane Library, and Web of Science with the terms ("covid 19") OR ("Wuhan virus") OR ("coronavirus") OR ("2019 nCoV") OR ("SARS-CoV-2") without language or other restrictions. References were managed by the EndNote X9 software (Clarivate Analytics, Philadelphia, PA, USA). Following the removal of duplicates, title and abstract screening were performed by two independent reviewers (PJH and NV) to identify potentially eligible articles. Disagreements were reviewed by a third review author (KJ) and resolved by consensus. The reference lists of the relevant articles were hand-searched, and additional eligible records were included.

We included studies without any restriction that reported on (*C*) liver diseases (as defined by eligible studies) and/or on-admission liver function tests in (*P*) patients with confirmed COVID-19. Concerning the laboratory parameters, cut-off values predefined by the individual studies were used for abnormal parameters (*O*). The assessed outcomes were as follows: in-hospital mortality, severe SARS-CoV-2 infection defined by eligible studies, and intensive care unit (ICU) requirement defined by eligible studies. Severity of COVID-19 was classified according to the guidelines on the Diagnosis and Treatment of COVID-19 issued by the National Health Commission of China (14). Details are presented in **Supplementary Table 2**. Studies with a sample size of fewer than 15 subjects were excluded because of the small effect size. When there were multiple publications using data with overlapping study populations, we included the one with a greater sample size.

Data Extraction and Outcomes

Relevant data were independently extracted from studies by review authors ZRD and FD. These included: first author, year of publication, country of origin, time interval and place of the study, study design, basic characteristics of the study population (age, percentage of females, and size of the study groups), the proportion of event (in-hospital mortality, severe SARS-CoV-2 infection, and need for ICU care) in patients with and without liver impairment, time of measurement for outcomes, and serum laboratory parameters [total bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), platelet count, international normalized ratio (INR), lactate dehydrogenase (LDH), and C-reactive protein (CRP)], predefined cut-off values, and information for risk of bias assessment. Extracted data were validated by MF and SK.

Statistical Analysis

Calculations were performed by Stata 15 data analysis and statistical software (StataCorp LLC, College Station, TX, USA). The first preference was the analysis of hierarchical summary receiver-operating characteristic (HSROC) predictive models with 95% confidence interval (CI) when at least five articles were available for the given outcome. The area under the curve (AUC) values and their 95% CIs for each prognostic factor and outcome were collected, and a meta-analysis using the random

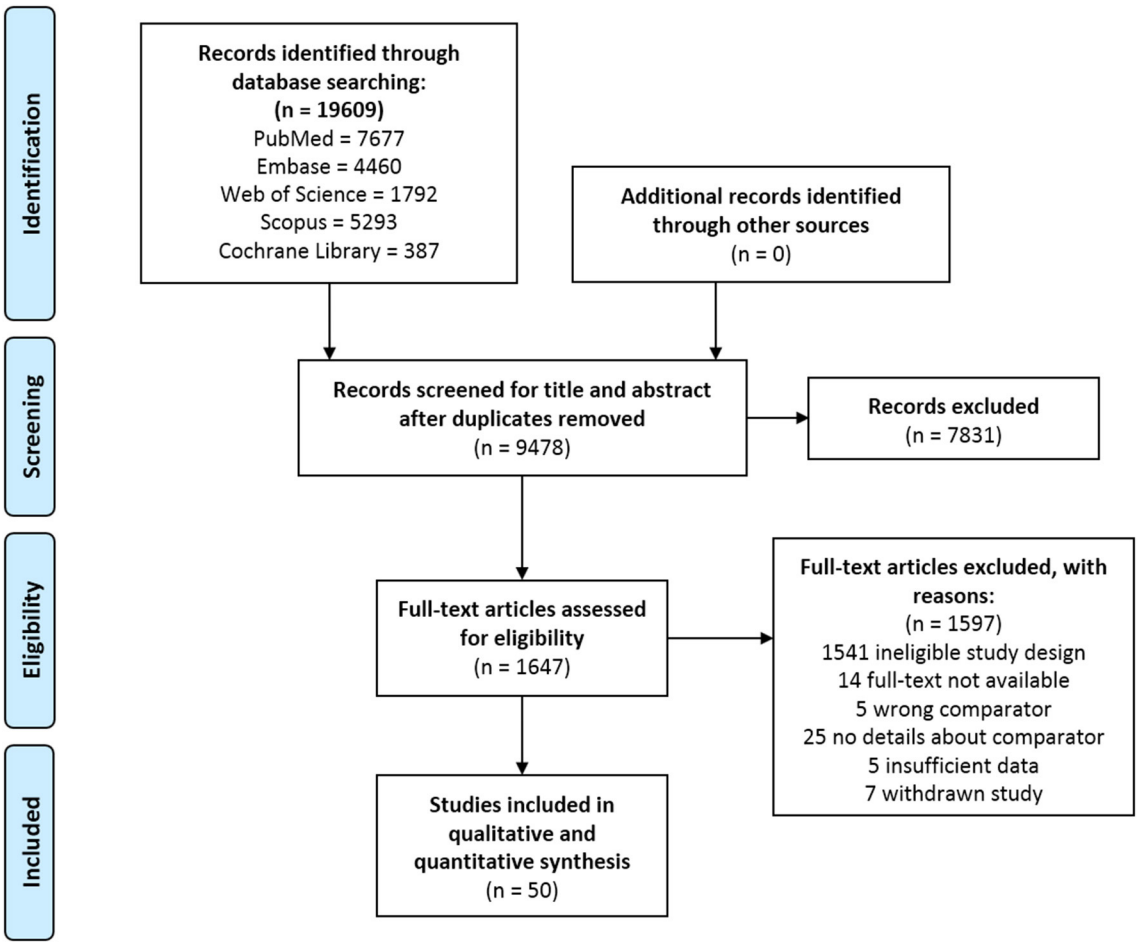

FIGURE 1 | PRISMA flowchart.

effect model to gain pooled AUC estimates with 95% CI was performed. Second preference in case of dichotomous variables (mortality, severe vs non-severe, and ICU vs. non-ICU) was the calculation of odds ratios (OR) with a 95% CI. A $p < 0.05$ was considered statistically significant.

Heterogeneity was tested with I^2 and χ^2 tests. As suggested by the Cochrane Handbook, I^2 values were interpreted as moderate (30–60%), substantial (50–90%), and considerable (75–100%) heterogeneity (15). A $p < 0.10$ was considered significant. Forest plots and HSROC curves were used to present the results of the meta-analyses. Publication bias was checked by Egger's test (alpha = 0.1) when at least 10 studies were available (16). A $p < 0.1$ was chosen because of the low number of studies included in our analyses, since it can determine a significant heterogeneity with greater certainty (17).

Assessment of Risk of Bias

Bias assessment was performed by two authors independently (PHa and TH) using the modified Quality In Prognosis Studies (QUIPS) assessment tool (18). Disagreements were resolved by a third investigator (GP). Details of the used QUIPS tool are shown in the footnote of **Supplementary Table 5**.

Protocol Deviation

We waived the need for data extraction and analysis regarding the continuous variables and Funnel plots after statistical consultation as it did not provide additional value.

RESULTS

Overall, 19,609 records were identified through the comprehensive search, from which 1,647 full texts were reviewed, and 50 studies were included in the qualitative and quantitative syntheses. The selection process is presented in **Figure 1**.

Basic characteristics of the included studies are shown in **Table 1** and **Supplementary Table 3**. Detailed eligibility criteria for each included study are presented in **Supplementary Table 4**.

TABLE 1 | Basic characteristics of the included studies.

Study	Country	Cohort type	Total number of patients (female %)	Age (year)‡	Outcome(s)	
					Definition	Event number (event rate %)
Cai et al. (19)	China	Retrospective	298 (51)	48	Severe COVID-19	58 (19)
Cai et al. (20)	China	Retrospective	318 (NR)	NR	Severe COVID-19	85 (27)
Cao et al. (21)	China	Prospective	102 (48)	54	Mortality	17 (17)
Chen et al. (22)	China	Retrospective	21 (19)	56	Severe COVID-19	11 (52)
Chen et al. (4)	China	Retrospective	1,590 (43)	NR	Mortality	50 (3)
Chen et al. (23)	China	Retrospective	274 (38)	62	Mortality	113 (41)
Chen et al. (24)	China	Retrospective	203 (38)	74	Mortality	19 (9)
Chen et al. (25)	China	Retrospective	48 (23)	65	ICU admission	17 (35)
Colombi et al. (26)	Italy	Retrospective	236 (25)	68	ICU admission	108 (46)
Du et al. (27)	China	Retrospective	109 (32)	71	ICU admission	51 (47)
Fan et al. (28)	Singapore	Retrospective	67 (45)	42	ICU admission	9 (13)
Fan et al. (29)	China	Retrospective	148 (NR)	NR	Mortality	1 (1)
					ICU admission	10 (7)
Feng et al. (30)	China	Retrospective	476 (43)	53	ICU admission	70 (15)
Goyal et al. (31)	USA	Retrospective	393 (39)	62	ICU admission	130 (33)
Grein et al. (32)	Multiple†	Retrospective	53 (25)	64	ICU admission	34 (64)
Guan et al. (5)	China	Retrospective	1,099 (42)	47	Severe COVID-19	173 (16)
Guan et al. (33)	China	Retrospective	1,590 (43)	49	ICU admission	99 (6)
					Severe COVID-19	254 (16)
Huang et al. (34)	China	Prospective	41 (27)	49	ICU admission	13 (32)
Ji et al. (35)	China	Retrospective	202 (44)	45	Severe COVID-19	39 (19)
Ji et al. (36)	China	Retrospective	208 (44)	44	Severe COVID-19	40 (19)
Li et al. (37)	China	Retrospective	548 (49)	60	Severe COVID-19	269 (49)
Liu et al. (38)	China	Retrospective	383 (58)	46	Mortality	49 (13)
Qi et al. (39)	China	Prospective	70 (NR)	NR	Severe COVID-19	3 (4)
Qian et al. (40)	China	Retrospective	324 (49)	51	Severe COVID-19	26 (8)
Qin et al. (41)	China	Retrospective	452 (48)	58	Severe COVID-19	286 (63)
Richardson et al. (42)	USA	Retrospective	2,634 (NR)	NR	Mortality	553 (21)
Ruan et al. (43)	China	Retrospective	150 (32)	NR	Mortality	68 (45)
Shen et al. (44)	China	Retrospective	119 (53)	49	Severe COVID-19	20 (17)
Shi et al. (45)	China	Retrospective	487 (47)	46	Severe COVID-19	49 (10)
To et al. (46)	China	Retrospective	23 (43)	62	Severe COVID-19	10 (43)
Tu et al. (47)	China	Retrospective	174 (55)	NR	Mortality	25 (14)
Wan et al. (48)	China	Retrospective	135 (47)	47	Severe COVID-19	40 (30)
Wan et al. (49)	China	Retrospective	123 (46)	NR	Severe COVID-19	21 (17)
Wang et al. (50)	China	Retrospective	339 (51)	69	Mortality	65 (19)
Wang et al. (51)	China	Retrospective	55 (60)	49	Severe COVID-19	2 (4)
Wang et al. (52)	China	Retrospective	69 (54)	42	ICU admission	14 (20)
Wu et al. (53)	China	Retrospective	280 (46)	43	ICU admission	83 (30)
Yang et al. (54)	China	Retrospective	93 (40)	46	Severe COVID-19	24 (26)
Yang et al. (55)	China	Retrospective	1,476 (47)	57	Mortality	238 (16)
Yang et al. (56)	China	Retrospective	52 (33)	60	Mortality	32 (62)
Zhang et al. (57)	China	Retrospective	221 (51)	55	Severe COVID-19	55 (25)
Zhang et al. (58)	China	Retrospective	663 (52)	56	Mortality	25 (4)
Zhang et al. (59)	China	Retrospective	140 (49)	57	Severe COVID-19	58 (41)
Zhang et al. (60)	China	Retrospective	120 (64)	45	Severe COVID-19	30 (25)
Zhang et al. (61)	China	Retrospective	115 (57)	50	Severe COVID-19	31 (27)

(Continued)

TABLE 1 | Continued

Study	Country	Cohort type	Total number of patients (female %)	Age (year)‡	Outcome(s)	
					Definition	Event number (event rate %)
Zheng et al. (62)	China	Retrospective	161 (50)	45	Severe COVID-19	30 (19)
Zheng et al. (63)	China	Retrospective	96 (40)	55	Severe COVID-19	74 (77)
Zhou et al. (64)	China	Retrospective	191 (38)	56	Mortality	54 (28)
Zhou et al. (65)	China	Retrospective	15 (33)	62	Mortality	7 (47)
Zhou et al. (66)	China	Retrospective	21 (38)	66	ICU admission	13 (62)

COVID-19, coronavirus disease 2019; ICU, intensive care unit admission; NR, not reported.
†Multiple countries (USA, Japan, Italy, Austria, France, Germany, Netherlands, Spain, and Canada); ‡mean or median.

TABLE 2 | Summary table of mortality, severe COVID-19, and intensive care unit requirement based on the HSROC analysis.

Prognostic factor	No. of studies (no. of cases)	AUC (95% CI)	Sensitivity (95% CI)	I^2 (%)	Chi^2	Specificity (95% CI)	I^2 (%)	Chi^2	PLR (95% CI)	NLR (95% CI)
Mortality										
Liver failure	5 (3,523)	0.67 (0.63–0.71)	0.31 (0.12–0.59)	99	0.001	0.94 (0.71–0.99)	99	0.001	5.5 (1.6–19.4)	0.73 (0.55–0.97)
Platelet count	5 (3,259)	0.71 (0.67–0.75)	0.40 (0.23–0.59)	95	0.001	0.89 (0.75–0.96)	99	0.001	3.7 (1.5–9)	0.68 (0.5–0.91)
ALT	5 (2,127)	0.76 (0.72–0.79)	0.41 (0.30–0.53)	71	0.01	0.77 (0.75–0.80)	0	0.63	1.8 (1.4–2.4)	0.76 (0.64–0.92)
LDH	5 (2,149)	0.81 (0.78–0.85)	0.87 (0.74–0.94)	71	0.01	0.58 (0.41–0.73)	95	0.001	2.1 (1.4–3.1)	0.22 (0.1–0.48)
Intensive care unit requirement										
Chronic liver disease	5 (831)	0.80 (0.77–0.84)	0.03 (0.01–0.06)	0	0.48	0.98 (0.96–0.99)	59	0.04	1.3 (0.5–3.3)	0.99 (0.97–1.02)
Platelet count	5 (628)	0.47 (0.43–0.52)	0.18 (0.11–0.28)	35	0.19	0.82 (0.72–0.89)	63	0.03	1 (0.6–1.6)	1 (0.9–1.12)
ALT	5 (1,190)	0.58 (0.54–0.62)	0.32 (0.25–0.41)	33	0.20	0.76 (0.70–0.81)	52	0.08	1.3 (1.1–1.7)	0.89 (0.81–0.98)
AST	6 (1,229)	0.65 (0.61–0.69)	0.55 (0.47–0.62)	37	0.16	0.69 (0.62–0.75)	78	0.001	1.7 (1.5–2.1)	0.66 (0.57–0.76)
CRP	6 (1,412)	0.75 (0.72–0.79)	0.92 (0.80–0.97)	88	0.001	0.31 (0.14–0.54)	95	0.001	1.3 (1.1–1.7)	0.27 (0.16–0.46)
Severe COVID-19										
Chronic liver disease	10 (2,182)	0.65 (0.60–0.69)	0.03 (0.02–0.07)	75	0.001	0.97 (0.95–0.98)	76	0.001	1.2 (0.6–2.1)	1 (0.97–1.02)
Chronic hepatitis B	7 (3,911)	0.71 (0.67–0.75)	0.03 (0.01–0.08)	84	0.001	0.97 (0.95–0.98)	85	0.001	1.2 (0.6–2.4)	1 (0.97–1.02)
Platelet count	7 (1,868)	0.66 (0.62–0.70)	0.26 (0.15–0.42)	88	0.001	0.86 (0.77–0.91)	92	0.001	1.8 (1.2–2.7)	0.86 (0.75–0.99)
ALT	8 (1,625)	0.60 (0.55–0.64)	0.31 (0.19–0.48)	94	0.001	0.80 (0.66–0.89)	96	0.001	1.6 (1.1–2.2)	0.86 (0.74–0.99)
AST	9 (2,780)	0.70 (0.65–0.74)	0.40 (0.30–0.50)	88	0.001	0.84 (0.77–0.88)	90	0.001	2.4 (1.8–3.2)	0.72 (0.63–0.83)
LDH	9 (2,500)	0.75 (0.71–0.79)	0.67 (0.57–0.77)	93	0.001	0.72 (0.62–0.80)	95	0.001	2.4 (1.8–3.1)	0.45 (0.35–0.58)
CRP	6 (2,253)	0.68 (0.64–0.72)	0.91 (0.82–0.96)	89	0.001	0.34 (0.23–0.47)	94	0.001	1.4 (1.2–1.5)	0.27 (0.18–0.42)

COVID-19, coronavirus disease 2019; HSROC, hierarchical summary receiver-operating characteristic; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; I^2 and Chi^2, heterogeneity; LDH, lactate dehydrogenase; NLR, negative likelihood ratio; PLR, positive likelihood ratio.

Diagnostic Metrics

For the prediction of mortality, a high specificity was reached by liver failure (specificity: 0.94, 95% CI: 0.71–0.99) and platelet count (specificity: 0.94, 95% CI: 0.71–0.99) and a moderate sensitivity by LDH (sensitivity: 0.81, 95% CI: 0.78–0.85).

For the prediction of possible ICU requirement, CLD (specificity: 0.98, 95% CI: 0.96–0.99) and platelet count (specificity: 0.82, 95% CI: 0.72–0.89) proved to be specific, whereas CRP was associated with high sensitivity (sensitivity: 0.92, 95% CI: 0.80–0.97).

For the prediction of severe disease course, CLD (specificity: 0.97, 95% CI: 0.95–0.98) and chronic hepatitis B infection (specificity: 0.97, 95% CI: 0.95–0.98) were highly specific, and platelet count (specificity: 0.86, 95% CI: 0.77–0.91), ALT (specificity: 0.80, 95% CI: 0.66–0.89), and AST (specificity: 0.84, 95% CI: 0.77–0.88) were moderately specific, whereas high sensitivity was reached by CRP (sensitivity: 0.91, 95% CI: 0.82–0.96).

CLD for mortality and total bilirubin in case of severe COVID-19 could not be analyzed because it was not feasible despite the number of included studies.

Detailed results about the AUC, sensitivity, specificity, likelihood ratios, and heterogeneity are shown in **Table 2**. The HSROC curves are summarized in **Supplementary Figures 1–3**.

Analysis of the Strength of the Association

Liver failure (OR: 7.59; 95% CI: 1.84–31.30), platelet count (OR: 5.36; 95% CI: 1.28–22.37), albumin level (OR: 6.32; 95% CI: 1.40–28.60), and ALT (OR: 2.49; 95% CI: 1.75–3.56), AST (OR: 5.39; 95% CI: 3.67–7.91), and LDH (OR: 9.23; 95% CI: 2.56–33.31) activities were related to a high rate of mortality. CLD, hepatitis B infection, and CRP concentration did not show significant difference, considering mortality.

Albumin (OR: 3.79; 95% CI: 2.08–6.93), ALT (OR: 1.56; 95% CI: 1.61–2.11), AST (OR: 2.53; 95% CI: 1.92–3.35), and LDH (OR: 7.95; 95% CI: 4.54–13.92) levels and CRP (OR: 4.72; 95% CI: 2.59–8.58) concentration were accompanied with high rate of ICU admission. A significant difference could not be stated regarding the need for ICU considering CLD, liver dysfunction, and platelet count.

Fatty liver disease (OR: 3.86; 95% CI: 1.20–12.47), liver failure (OR: 3.27; 95% CI: 1.20–8.87), total bilirubin (OR: 1.89; 95% CI: 1.35–2.63), platelet count (OR: 2.34; 95% CI: 1.53–3.58), albumin level (OR: 3.11; 95% CI: 1.61–6.01), ALT (OR: 1.82; 95% CI: 1.18–2.81), AST (OR: 3.34; 95% CI: 2.37–4.71), LDH (OR: 5.02; 95% CI: 3.41–7.40), CRP (OR: 4.52; 95% CI: 3.16–6.49), and GGT (OR: 3.03; 95% CI: 1.60–5.7) were accompanied with a higher risk for more severe course. CLD, hepatitis B infection, and elevated level of ALP did not show significant difference concerning severity.

Results of the analysis of association and heterogeneity are presented in **Table 3**. Forest plots for each analysis are shown in **Supplementary Figures 4–17**.

Risk of Bias Assessment

Results of the risk of bias assessment between studies are shown in **Supplementary Table 5**.

The assessment of publication bias could only be performed in the case of CLD on severe COVID-19. It did not suggest the presence of publication bias ($p = 0.764$).

DISCUSSION

This meta-analysis aimed to investigate the association between pre-existing liver diseases and on-admission liver functions and outcomes in COVID-19 infection, focusing on mortality, ICU admission, and severe disease course (**Figure 2**). Considering the prediction of mortality, liver failure and platelet count are highly specific, whereas LDH is moderately sensitive. For the prediction of ICU requirement, CLD was associated with high specificity, platelet count with moderate specificity, and CRP with high sensitivity. Regarding severe disease course, CLD and chronic hepatitis B infection were proven to be highly specific, and platelet count and ALT and AST activities were moderately specific, whereas CRP was highly sensitive.

In relation to the investigated factors and poorer outcomes, acute liver failure; platelet count; albumin level; ALT, AST, and LDH activities; and CRP concentration were associated with higher mortality. Albumin, ALT, AST, LDH, and CRP influenced the admission to the ICU. Fatty liver disease, liver injury, total bilirubin, ALT, AST, LDH, CRP, GGT, platelet count, and albumin level were associated with more severe disease course.

The knowledge about the impact of liver-related comorbidities in the clinical outcome of COVID-19 is limited. In line with our results, an earlier meta-analysis concluded that CLD is not associated with severity or mortality (67). However, clinicians should be skeptical about it, because these patients are more prone to infection due to cirrhosis-associated immune dysfunction and are more likely to have poor outcomes from ARDS (68, 69). This may account for the relatively low baseline prevalence of CLD in the included patients, as one previous meta-analysis suggests (70), or it was not well-reported. Further on, in a recently published letter on the involvement of the liver in COVID-19, the authors found an increased odds of severe infection and mortality in patients with liver injury (71). Another study analyzed the frequency of abnormal liver function derangements in severe COVID-19 and concluded that hypoalbuminemia followed by derangements in GGT and aminotransferases were more frequent in severe disease (72). On the other hand, another study highlights that digestive symptoms and liver injury are not uncommon in patients with SARS-CoV-2 infection (73).

Dysregulated hepatic immune responses caused by metabolic associated fatty liver disease (MAFLD) may contribute to cytokine storm in younger patients (74), whereas chronic low grade inflammation known to be associated with MAFLD may worsen outcome. Post-mortem liver biopsy showed overactivation of T cells in the liver, and liver injury is likely mediated by immune response rather than direct cytopathic damage (35).

Compared with previous results (12, 75, 76), our study reasserts that in severe forms of COVID-19, alterations of on-admission level of the liver enzymes can be observed, probably due to the virally induced cytotoxic T cells and the innate immune response against the virus. Another reason behind the liver test abnormalities in COVID-19 patients could be the cholangiocyte dysfunction due to direct infection of bile duct cells via angiotensin-converting enzyme 2 receptor (8). However, according to our results, ALP does not seem to be a significant predictive marker in COVID-19. Additionally, moderate microvesicular steatosis, mild lobular, and portal activity can be observed in the pathological samples of patients who died from COVID-19 (77).

Despite the lack of coagulation factors in liver diseases, a hypercoagulable state could also be present in COVID-19. A recent study concluded that COVID-19 disease has prominent manifestations from the hematopoietic system and is often associated with a major blood hypercoagulability (78). In histopathological findings, it was highlighted that extensive vascular portal and sinusoidal thrombosis could lead to abnormal high level of transaminases (79).

Considering the strengths of our meta-analysis, a rigorous methodology was followed. To our knowledge, this is the first study that addresses the prognostic value of on-admission liver parameters, underlying liver comorbidities, and COVID-19 induced hepatic failure on the level of sensitivity and specificity. On the other hand, our study has several limitations. We only included cohort studies that mostly originate from Asia, which might carry a high risk of bias. The definitions

TABLE 3 | Summary of findings.

Prognostic factor	Mortality				Intensive care unit requirement				Severe COVID-19			
	No. of studies (no. of pts)	Odds ratio (95% CI)	I^2 (%)	Chi^2	No. of studies (no. of pts)	Odds ratio (95% CI)	I^2 (%)	Chi^2	No. of studies (no. of pts)	Odds ratio (95% CI)	I^2 (%)	Chi^2
Chronic liver disease	4 (646)†	1.5 (0.42–5.41)	0	0.54	5 (831)	1.42 (0.56–3.63)	0	0.72	10 (2,182)	1.45 (0.87–2.42)	0	0.7
Liver dysfunction	2 (145)	1.13 (0.36–3.58)	0	0.33	2 (384)	1.77 (0.62–5.06)	0	0.98	2 (163)	1.11 (0.36–3.47)	0	0.56
Chronic hepatitis B	2 (1,864)	1.18 (0.42–3.34)	0	0.97	1 (1,590)	0.55 (0.07–4.11)	NR	NR	7 (3,911)	1,55 (0.85–2.83)	13	0.33
Fatty liver disease	NR	NR	NR	NR	NR	NR	NR	NR	4 (964)	3.86 (1.2–12.47)*	79	0
Liver failure	5 (3,523)	7.59 (1.84–31.30)*	91	0	1 (43)	1.88 (0.47–7.54)	NR	NR	4 (1,185)	3.27 (1.2–8.87)*	70	0.02
Total bilirubin	1 (975)	5 (2.48–10.07)*	NR	NR	2 (395)	1.66 (0.45–6.06)	33	0.22	6 (2,059)	1.89 (1.35–2.63)*	0	0.57
Platelet count	5 (3,259)	5.36 (1.28–22.37)*	95	0	5 (628)	0.95 (0.63–1.44)	0	0.79	7 (1,868)	2.34 (1.53–3.58)*	46	0.09
International normalized ratio	NR	NR	NR	NR	1 (20)	5 (0.18–139.17)	NR	NR	1 (115)	0.72 (0.31–1.66)	NR	NR
Albumin	3 (944)	6.32 (1.4–28.6)*	63	0.07	3 (744)	3.79 (2.08–6.93)*	0	0.81	4 (1,205)	3.11 (1.61–6.01)*	69	0.02
Alanine aminotransferase	5 (2,127)	2.49 (1.75–3.56)*	10	0.35	5 (1,190)	1.56 (1.16–2.11)*	0	0.99	8 (1,625)	1.82 (1.18–2.81)*	70	0
Aspartate aminotransferase	4 (1,966)	5.39 (3.67–7.91)*	0	0.63	6 (1,229)	2.53 (1.92–3.35)*	0	0.48	9 (2,780)	3.34 (2.37–4.71)*	60	0.01
Lactate dehydrogenase	5 (2,149)	9.23 (2.56–33.31)*	85	0	4 (748)	7.95 (4.54–13.92)*	0	0.75	9 (2,500)	5.02 (3.41–7.4)*	66	0
C-reactive protein	4 (1,846)	9.19 (0.84–100.63)	77	0	6 (1,412)	4.72 (2.59–8.58)*	35	0.17	6 (2,253)	f4.52 (3.16–6.49)*	31	0.21
Alkaline phosphatase	NR	NR	NR	NR	1 (19)	0.11 (0–2.73)	NR	NR	4 (623)	1.71 (0.66–4.46)	24	0.27
Gamma-glutamyl transferase	NR	NR	NR	NR	1 (19)	1.39 (0.22–8.92)	NR	NR	3 (635)	3.03 (1.6–5.72)*	50	0.14

CI, confidence interval; COVID-19, coronavirus disease 2019; I^2 and Chi^2, heterogeneity; NR, not reported.

**$p < 0.05$; † one study could not be included in the analysis, because there were no events.*

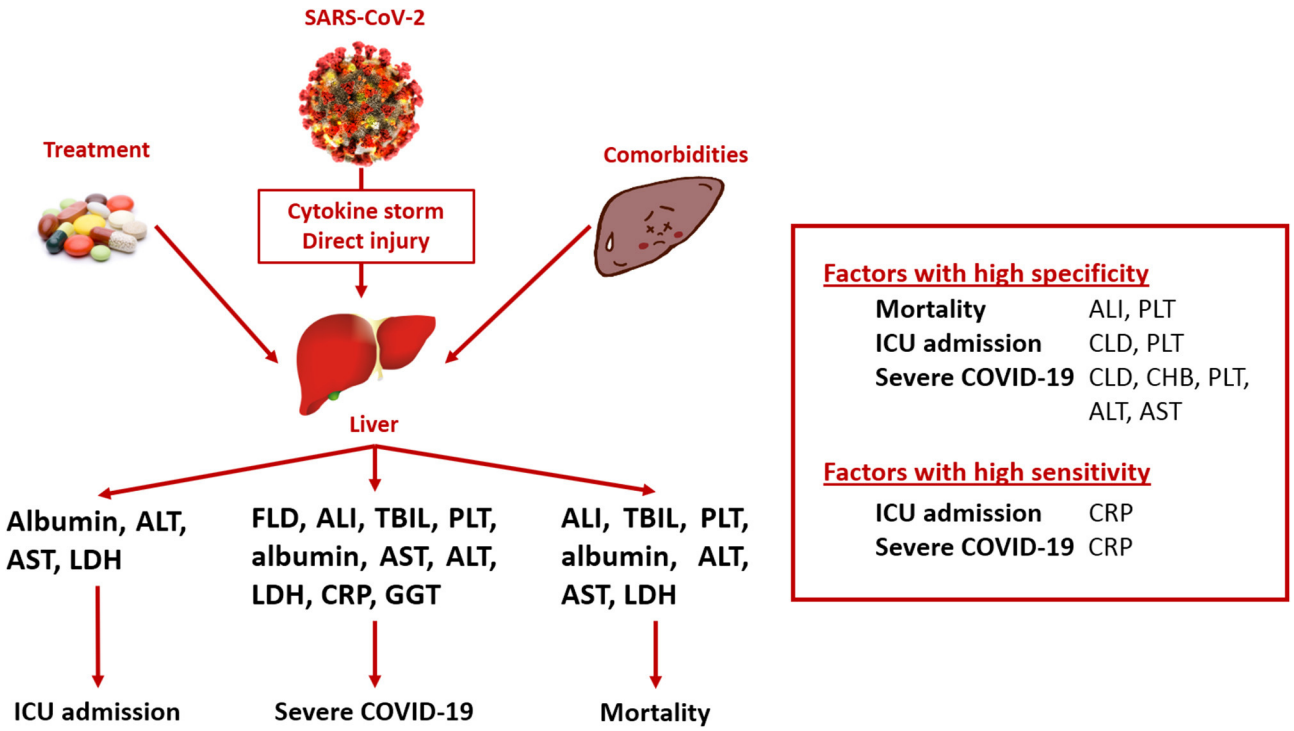

FIGURE 2 | Summary of findings. ALI, acute liver injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; FLD, fatty liver disease; GGT, gamma-glutamyl transferase; ICU, intensive care unit; LDH, lactate dehydrogenase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TBIL, total bilirubin.

of the investigated outcomes were not uniform among the included reports; to estimate this problem, we applied a modified QUIPS. The cut-off values of laboratory parameters and the definition of liver diseases (**Supplementary Tables 6**, **7**) were also slightly different among articles, causing probably significant heterogeneity in our analysis. However, the different laboratory methodologies among the centers might justify this difference. Furthermore, previous drug treatment before admission of COVID-19 was not investigated. Multivariate analysis was not applied; thus, the investigated prognostic factors should not be regarded as independent risk factors. This all could contribute to the significant heterogeneity in some of our results.

Implication for Practice

The establishment of a prognostic score assessing the possible outcomes of patients suffering from any liver pathology is needed. This meta-analysis succeeded to identify some factors, with high specificity, which might be a footstone for such a prognostic tool that might be completed by additionally recognized risk factors, for example, elevated absolute white blood cell count, decreased lymphocyte count, and elevated interleukin-6 and serum ferritin concentrations (80). Patients who are affected by the underlying liver pathology might need advanced therapy earlier to avoid undesired clinical outcomes.

Implication for Research

Based on our results and previously published analyses, further basic research is crucial for a better understanding of the liver injury caused by COVID-19, hepatic comorbidities, and treatment itself.

CONCLUSION

In conclusion, on-admission platelet count, ALT and AST activities, CRP concentration, and the presence of acute and CLDs predicted the severe course of COVID-19. To highlight, investigating hepatic injury associated by SARS-CoV-2 infection may play an important role in the prediction of mortality and may be used for the establishment of prognostic tools to identify patients with possible poorer outcomes.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SV, PJH, NZ, LS, and NV conceived the study. SV, PHe, and GP wrote the protocol. LS and NZ did the literature search. PJH, NV, ZD, and FD screened the records and extracted the data. KJ, MF,

and SK validated the extracted data. PHa and TH assessed the quality of included studies. DN did the statistical analysis. SV, ZS, ZR, and KO prepared the tables. NZ, LS, NV, SV, and PJH wrote the first draft of this manuscript. BE, ZS, GP, and PHe supervised the manuscript and approved the submitted draft. GP is the guarantor of this paper and, as a hepatologist, provided the team with an expert background. All authors provided critical conceptual input, interpreted the data analysis, and critically revised and approved the final version of the manuscript.

FUNDING

This study was supported by the Human Resources Development Operational Program Grant (EFOP-362-16-2017-00006), co-financed by the European Union (European Regional Development Fund) within the framework of the Széchenyi 2020 Program. The sponsor or the funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.572115/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 26 November 2020
doi: 10.3389/fmed.2020.587602

Gastrointestinal Endoscopy in the Era of COVID-19

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Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which led to a worldwide pandemic that started in early 2020. Healthcare systems across the world encountered an unprecedented surge of COVID-19 patients resulting in more than half a million deaths globally. COVID-19 has affected multiple sub-specialties and procedure-related fields, including gastroenterology. Gastrointestinal (GI) endoscopy centers are specialized units where thousands of endoscopies are performed annually. A significant proportion of these procedures are affected due to the national and regional lockdowns across the globe. To adapt to this rapidly evolving situation, endoscopy centers have undergone significant changes and have taken unprecedented precautions to avoid the transmission of the virus. However, endoscopy centers are going through financial strain due to a reduction in the number of procedures from lockdowns and fear of virus transmission. Theoretically, endoscopies could add to the disease transmission as SARS-CoV-2 has shown to be present in the GI secretions. Multiple precautions such as mandatory use of face masks, safe distancing, use of barriers between the endoscopists and patients, negative pressure rooms, extended use of personal protective equipment, and volume reduction have been taken to decrease the risk of disease transmission by these centers. Moreover, pre-endoscopy COVID-19 testing has now become the norm. In this review, we highlight the significant changes assumed by the endoscopy center. Furthermore, we discuss cost-related concerns of pre-endoscopy COVID-19 testing, the downtime and delays related to the procedures, and effects of rescheduling. As the pandemic progresses through multiple phases, endoscopy centers should use a dynamic approach to adapt and strive to provide the best patient care.

Keywords: coronavirus, coronavirus (2019-nCoV), SARS-CoV-2 infection, pandemic (COVID-19), endoscopy, gastrointestinal disease, fellowship and training

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Edited by:
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Sichuan University, China

Reviewed by:
Sara Regnér,
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *26 July 2020*
Accepted: *09 October 2020*
Published: *26 November 2020*

Citation:
Perisetti A, Goyal H and Sharma N (2020) Gastrointestinal Endoscopy in the Era of COVID-19. Front. Med. 7:587602. doi: 10.3389/fmed.2020.587602

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Within a few months, it has led to a pandemic of unprecedented levels affecting multiple countries with >16 million cases and >650,000 deaths as of July 26, 2020 (1). The pandemic has caused duress for medical systems and hospitals worldwide. Initially, it was believed that respiratory manifestations dominate the presentation of COVID-19. As the experience with the pandemic evolved, extrapulmonary manifestations are increasingly being

recognized (2–6). The Centers for Disease Control and Prevention (CDC) added multiple symptoms as a part of the COVID-19 presentation, which also includes gastrointestinal (GI) manifestations such as nausea, vomiting, dysgeusia, pancreatitis, hepatitis, colitis, etc. (6–10). Additionally, the virus has also been shown to be present in GI secretions. Specialties such as gastroenterology and surgery have been directly affected by COVID-19. This pandemic has had a disruptive effect on the workflow and safety of endoscopists, ancillary staff, and patients. Shortages of personal protective equipment (PPE), lack of testing kits, reduced patient volume, workforce furloughs, and lockdowns have forced these units to be innovative and have them prioritize the high-risk procedures and postpone or even cancel endoscopies in medium- to low-risk cases (11).

Outpatient endoscopy centers usually deal with high-volume and close-contact procedures, which could make them prone to become high-risk COVID-19 transmission areas if extreme precautions are not taken. Millions of colonoscopies are performed as part of the colorectal cancer screening program in the United States (US) (12, 13). A wide variety of other therapeutic endoscopic procedures are also performed on a regular basis (14). Furthermore, the staging and palliation of cancers to aid in managing these lesions are increasingly being performed (15). The COVID-19 pandemic has forced these endoscopy centers to drastically reduce the procedure volume for both elective and semi-urgent cases to reduce transmission risk and preserve PPE (16–19).

Furthermore, there are published data about a decrease in the non-variceal GI bleeding events in line with other disorders such as acute coronary syndrome admissions during the pandemic (20). Although the precise reason for these changes remains speculative, patients may have developed a fear of contracting the infection if they visit the medical centers. Additionally, there is an increased risk of exposure to the virus during outpatient endoscopy procedures due to exposure to GI secretions and respiratory secretions (21, 22). Furthermore, there is a potential for increased generation of infected droplets during coughing, retching, and suctioning, creating aerosolization and increased risk of transmission (23, 24).

METHODS

A search for published literature at the time of submission of the manuscript was performed from December 2019 to July 1, 2020. We performed a search of PubMed, Google Scholar, Embase, and Scopus databases to extract articles relevant to endoscopy in COVID-19 patients. The terms "endoscopy," "gastrointestinal endoscopy," "staffing," "barrier protection," pre-procedure testing," "COVID-19," "SARS-CoV-2," and "coronavirus" were performed. Due to the heterogeneity of the studies, a systemic review could not be performed.

CHANGES IN THE ENDOSCOPY SUITES

Changes in Endoscopy Suite Structure

Various operational changes have been proposed across endoscopy suits/centers to provide services while mitigating the risk of infection. While these changes depend on several factors (availability of resources locally, infection risk, the demographic profile of the patients, indication and hospital/ endoscopy unit policies), common goals of minimizing the risk of transmission of infection, conserving PPE, and achieving high efficiency remain. To achieve these, Cennamo et al. reported substantial changes in the layout of the endoscopy units with risk-based color-coding of the waiting room, endoscopy suites, and recovery room (25). Additionally, implementations of checkpoints, pathways, and processes based on the color-coding schema were implemented in this study (25). Post-procedure, patients are monitored in the recovery area, with no family available in the waiting room. Hospitals in the US have incorporated policies for not allowing family members, given the risk of exposure and transmission (26). Patients are transported to the hospital entrance to find their respective family member/driver who can further assist in discharge. Results of the procedures are discussed with the patient but are relayed via phone to the authorized person with face-to-face encounters (27). While these changes can potentially contribute to the reduction of endoscopy-related transmission, making it safer for the patients and the staff, they also decrease the in-person relay of information, which is critical at the discharge from the endoscopy units.

Changes in Staffing

COVID-19 first appeared in Wuhan, China, in December 2019. In some areas, the pandemic has overwhelmed the healthcare systems to the point that endoscopy units are potentially treated as COVID-19 units (27, 28). In Brazil, endoscopy staff has been divided into COVID treatment teams and non-COVID endoscopy teams (27). The use of PPE has been mandated by all healthcare systems to minimize the risk of transmission. Prior studies have shown that the use of PPE has not been universal among endoscopists (21, 23). However, with the current pandemic, the use of masks, N-95, gowns, and other PPE has drawn increased attention to avoid spread (24, 29, 30). Endoscopy staff with pre-existing conditions at higher risk of contracting COVID-19 have been assigned non-clinical duties without direct care to COVID-19 patients (27, 28).

Endoscopy staff performing procedures in the operating room should use strict precautions of properly donning and doffing in a separate room prior to entering the operating room. Endoscopy units and operating rooms should follow strict cleaning procedures. Advanced endoscopy procedures such as ERCP frequently need fluoroscopy, equipment trolley, worktable, and anesthesia equipment. The negative pressure rooms are highly recommended in these settings to avoid cross-contamination.

Procedure scheduling during the peak and post-peak has been an area of great challenge for hospitals (31). Increased endoscopy staff furloughs further complicated this challenge (32). Several patient procedures were deferred and put on hold due to decreased slots and uncertainty about when the restrictions will be lifted, and normalcy will be established. Post-procedure telephone follow-ups with patients could be utilized to inquire about developing any new COVID-19 related symptoms to take necessary precautions to individuals who were at potential risk

(24, 33). Patients should be informed about the risk of nosocomial infections and also should be informed to report back if they develop any *de novo* symptoms in the next few days after the procedure.

Change in the Endoscopy Indications

Multiple international societies have recommended restricting endoscopy procedures only to emergent and urgent indications (34). This essential step was taken to minimize the risk of transmission and reduce PPE utilization and use of resources. Studies from multiple countries have shown an endoscopy case-volume median reduction to as high as 99% (19, 35). The impact of COVID-19 varied based on the country, infection rate, initiation of stay-at-home orders, and timing of the pandemic. For example, endoscopy services in the United Kingdom (UK) reduced to 5% in March 2020 after the onset of the pandemic. These changes were noted across all UK regions and endoscopy procedures (36). Procedures were performed only when the benefit outweighs the risk of transmission among the staff.

While the indication for procedures varied, emergent procedures such as active GI bleeding, acute cholangitis, food impactions, and cancer diagnosis/staging/treatment were considered appropriate. A nationwide study in the UK showed that all endoscopic procedures reduced with the pandemic; however, the ERCP activity (performed for emergencies) remains well-preserved (36). Elective procedures, such as screening and surveillance, were deferred. However, the urgent indications of endoscopies remain a gray area based on the endoscopist, institutional guidelines, and available services. Deferring semi-urgent cases could delay the diagnosis of cancers (such as localized pancreatic cancer), and loss of window of therapeutic intervention (endoscopically resectable lesions can become unresectable due to spread). Studies showed that colorectal cancer (CRC) screening declined by 84.5% after the onset of the pandemic in the US (37). Similarly, a 72% reduction in CRC screening was noted in the UK (36). Although multiple GI societies have provided a road map, clinical judgment should prevail, and every case should be individualized with a multidisciplinary team-based approach (38).

Changes in Triaging

Endoscopy staff triage all patients who are undergoing non-urgent endoscopies. In the US, this triaging is done by the pre-procedural COVID-19 testing and a predetermined questionnaire about 2–3 days before the endoscopy date (39, 40). These patients are again triaged by this questionnaire at the time of presentation to the endoscopy suite. The patients are sent to the hospital to seek emergent medical attention in case they have any signs and symptoms of COVID-19 such as cough, shortness of breath, and persistent fever, along with a known history of contact with a COVID-19 patient or travel to high-risk areas (24, 41). Peri-procedural COVID testing involves coordination at multiple levels—contacting patients to undergo testing 24 to 72 h before the procedure and obtaining the results of the test (42). Patients who travel a long distance to get their procedure may cancel their procedure if their COVID-19 testing results are delayed. Also, the absence of family members before, during, and after the procedure can increase anxiety among the patients.

TABLE 1 | Pre-procedural universal testing.

Advantages	Disadvantages
Results can assist in planning the procedure based on risk and benefit analysis	Significant cost burden
Use of PPE accordingly to negative or positive cases	Risk of false-positives and false-negatives
Planning of the procedure with enhanced precautions and use of minimal personnel (in positive cases) and adequate personnel (in positive cases)	Delay in procedure during to processing times
Decreased transmission risk, reduced downtime and disinfection strategies	Additional trips to the endoscopy center/ testing sites

Pre-procedural Testing

COVID-19 testing of all patients before endoscopic procedures may help to identify infected patients and facilitate taking appropriate measures such as isolation precautions, high-risk PPE usage for positive PCR testing, and downtime after the procedure. A thorough analysis of the risks and benefits of widespread pre-procedural testing is needed (**Table 1**). There are some considerations needed before the development and implementation of the universal testing strategy. Testing for all patients incurs cost burden to the endoscopy units, which can be significant. Additionally, testing may delay procedures if test results are pending, and the possibility of false positivity and false negativity might alter decision-making, which can complicate the processes (1, 43).

Corral et al. reported that PCR testing could be used as an effective strategy to restart endoscopic procedures based on the phase of the pandemic (44). Testing individuals within 48 h of the procedure for semi-elective and elective cases can allow completion of 19.4% (if investing $22 per patient) and 95.3% (if investing $105 per patient) of baseline endoscopies. Implementing this strategy over 1 week in the US will return 165 million US dollars (for 13 million investing) and 767 million US dollars (for 64 million investing) (44). These numbers are promising and demonstrate the potential value of COVID-19 testing for all patients undergoing procedures. Expectedly, this modeling can change with the local prevalence of COVID-19, transmission rate (R_0), and accuracy of PCR results. Center for Medicaid and Medicare Services (CMS) and other insurance programs reimburse up to 36 USD to 51 USD per patient (45). These calculations may not apply in areas where testing is not rampant, and reimbursement for endoscopy is low. For example, Sundaram et al. noted that cost of SARS-CoV-2 PCR ($65) might exceed the reimbursement of an upper endoscopy ($30–$60) in countries like India (46). Additionally, the prevalence rate in some of the areas of the country might be too low to test all individuals undergoing endoscopy procedures (46). Testing of high-risk individuals only in specific hot spots is a matter of debate. Furthermore, pre-procedural testing is not

uniformly performed in Europe, and the decision is based on the pre-procedure questionnaire. As testing capabilities expand throughout the world, the availability of highly accurate point-of-care testing with rapid results can make this a possibility (47).

Barrier Protection

Safe distancing in the pre-operative area has decreased the number of patients the nursing staff can receive for pre-operative care. It has affected the efficiency of the endoscopy units severely. Only required and critical personnel (endoscopists, nurses, and anesthetists) should be allowed in the endoscopy units (42). Any instrument or device can potentially be a source of infection in aerosol-generating procedures (AGP). Staff should wear PPE as per the local institutional and national guidelines before starting the procedure. Appropriate donning and doffing of the PPE is essential to reduce the risk of infection (27). Belle et al. noted that gastroenterologists who performed procedures on COVID-19 patients have reported symptoms compatible with COVID-19 ranging from 0.6% (3/497 patients) in low prevalence areas compared to 6.1% (12/197) in high prevalence areas (16). Similarly, Chen et al. reported that 5.7% (8/141 patients) reported that gastroenterologists or their colleagues developed work-related COVID-19 infections (17). It led to the development of multiple barrier devices between endoscopists and patients to reduce the risk of exposure to GI secretions (48–53) (**Table 2**).

Given the inherent nature of the procedures (upper or lower endoscopies), endoscopists are almost always in a "high-transmission zone" (within 3 feet of the patients). Campos et al. introduced a transparent aerosol box (endoprotector) to reduce contact with droplets (49). In this technique, a barrier is used during upper endoscopy, which is made of acrylic plastic to shield the respiratory droplets and potential aerosolization during the procedure (49). A similar barrier is used to decrease the exposure to patients' respiratory droplets during endotracheal intubation (ETI) (50). Traina et al. reported the use of an endoscopic COVID Cube (C-Cube), which is a protective box with access to anesthesiologist's hands and another port for endoscope access (51). Liu et al. reported using a unique disposable device with a combined bite block and oxygen mask for upper GI endoscopic procedures (48). Furthermore, a closed chamber ear, nose, and throat (ENT) examination unit was developed for AGP endoscopic examinations of COVID-19 patients (54). While ETI is usually a one-time event to secure the airway, the use of an endoscope through an endoprotector might make the procedure challenging due to the repeated hand movement of the endoscopists. Nevertheless, the use of these barriers has a significant role in reducing the disease transmission, especially in high-risk or COVID-19 patients.

Endoscopic Transmission

Among the endoscopy procedures, duodenoscopes and echoendoscopes carry a high risk of nosocomial infections (55). While single-use duodenoscopes might be of value in COVID-19-positive patients, they are not universally available and have cost-related constraints (56). Multiple societies have recommended using negative pressure rooms, especially for patients who are suspected of COVID-19 or when the endoscopy is being performed emergently without COVID-19 testing results (30). Intraprocedural changes such as minimal verbal communication, avoiding spill of GI contents via biopsy channel, and avoiding procedures in patients with inadequate bowel preparation should be done (27). Franzini et al. reported the use of a "double gauze technique" where the endoscopists use one gauze and the other by the technician in a controlled fashion to avoid the "whip" effect of accessories and spillage of GI secretions (27). Institutional policies have been developed for minimal personnel to be present for the procedure (57). This is to minimize the risk of exposure among the endoscopy staff. Procedures performed with moderate sedation without the need for anesthesia providers (endoscopist guided sedation) can further minimize the risk of transmission. However, for procedures requiring general anesthesia, societies currently recommend using ETI to reduce the risk of aerosolization with suspected or confirmed COVID-19 (58).

Enhanced cleaning procedures have been implemented by most endoscopy units (24). Strict adherence to local and national policies should be followed while cleaning the endoscopy suites (59). This includes cleaning all horizontal surfaces, frequently touched surfaces with particular emphasis on areas within a few feet of the patient. Multiple studies showed that SARS-CoV-2 can involve any segment of the GI tract. Intestinal autopsy in COVID-19 patients showed stenosis and dilatation of the small intestine (60). Mucosal damage was noted in multiple areas such as esophagus, stomach, duodenum, colon, and rectum (61, 62). Endoscopic procedures, due to their inherent nature of coming in contact with GI secretions, could potentially get contaminated with virus. Although there is a theoretical risk of endoscopes acting as potential vectors for viral infections, so far, there is no published report of SARS-CoV-2 transmission via endoscopes (39). Nevertheless, the reprocessing process should include high-level disinfection (HLD). Traditionally, testing for leakage is performed before washing of the endoscope. However, suggestions have been made for performing this after washing the endoscope. Whether this can affect the proper functioning of the scope remains to be studied (48).

Procedural Downtime

Patients undergoing endoscopy have the potential risk of aerosol generation. All rooms after the procedure should be deemed contaminated after the procedure. During the induction of anesthesia, only essential personnel for securing the airway should be present, which requires endoscopy staff to wait outside the procedure room. After completing the endoscopic procedure, the endoscopist and non-essential staff should exit the room before extubating the patient.

The time needed to allow for dispersion of the virus-laden aerosols to clear will depend on the rate of air changes/hour (ACH). If a rate of 25–30 cycles/hour is used, 3 min are needed to wait before a procedure could be started after intubation. The precise time needed for closure of the room depends on the use of negative pressure and air-exchange rate (63). While this is dependent on transmission dynamics, multiple other factors such as air-exchange rate, duration of aerosolized droplets suspended in the air, viral load in the droplets, the viability of the virus (can

TABLE 2 | Barriers to prevent transmission during endoscopy.

Name	Material	Description
Endoprotector (40)	Acrylic plastic	Composed of four faces of the box. Face A (for endoscope insertion), B (for anesthetist), C (air aspiration and creation of negative pressure), D (for patients' neck and shoulders)
C-Cube (42)	Plexiglas	Multiple entryways (endoscopists and anesthesiologists' access) for procedures involving oral cavity
Aerosol box (41)	Plastic	Predominately used for endotracheal intubation. Two circular ports provided for the clinician hands to perform airway procedure
ORIGAMI (43)	Coated cardboard and polypropylene film	Disposable face-protective shield to protect surgical mask and N-95 respiratory mask from aerosols
Endoscopic shield (44)	Plastic cube	Two small holes for endoscopist access to the oral cavity
Chamber unit (45)	Multiple structures	For Ear, Nose, Throat exams- Composed of air inlet, ultraviolent lamps, exhaust system with vents, speaker and additional screen

TABLE 3 | Factors* predicting downtime between endoscopic procedures.

Increased downtime (increased delay between procedures)	Decreased downtime (decreased delay between procedures)
High viral load in the droplet secretions (contaminant concentration)	High air changes per hour (ACH)
Heavy environmental contamination	Efficient vent system (removal efficiency)
Air stagnation	Negative pressure room availability
Large room volume	Good mixing of the air within the space

**Final factors determining the downtime is dependent on transmission dynamics, manufacturer recommendations and contaminant concentrations.*

be up to 3 h), and environmental contamination could play a role (**Table 3**).

A cautious approach is recommended until further data emerge (19). Per the CDC (63), airborne contaminant removal is dependent on ACH and duration, which determines the efficiency of removal. For example, a room with a minimum ACH of 12–15 cycles per hour, at a duration of 28 min to 35 min, is needed to achieve a contaminant removal efficiency of 99.9% (63). This efficiency comes down if the ACH is low and if viral contamination in the air is high. Societies have recommended adequately ventilated rooms (with at least 12 ACH and controlled direction of airflow with mechanical ventilation should be used). For rooms without negative pressure capability, a minimum of 60 min delay (downtime) is recommended compared to 30-min downtime for negative pressure room (24).

Endoscopy Trainee Involvement

Endoscopy trainees' (gastroenterology fellows and surgery residents) involvement in GI procedures have been affected significantly during the pandemic (64, 65). A multinational survey study spanning 63 countries reported a reduction of endoscopy volume by up to 93.8%, with colonoscopy being affected the most, which is a core endoscopy skill (35). Furthermore, an increased degree of anxiety and burnout were noted among endoscopists. This concern not only was restricted to trainees but also affected entire endoscopy staff for the risk of acquiring COVID-19, especially after the resumption of elective endoscopies (66). However, in areas with low risk of infection transmission, trainees continue to be involved in the procedures.

During the initial phase of the pandemic, most endoscopy units implemented policies to have only essential fully trained staff to avoid exposure and reduce the turnover time (30). Multiple survey studies have demonstrated adverse effects on endoscopy training and an unexpectedly significant fear and anxiety during pandemic (35, 67, 68). Multiple GI and surgical societies have increased the availability of electronic resources to fill this gap in the training (69, 70). Additionally, programs have implemented various mechanisms to mitigate the loss of procedure volume with video recording, simulation labs, and increasing involvement in low-risk procedures.

FUTURE OUTLOOK OF ENDOSCOPY UNITS

In the future, endoscopy units will likely incorporate some of the changes during the pandemic for increased safety of the patients and endoscopy staff. It remains speculative to predict the end of this pandemic, but localized outbreaks may continue to occur even when we see a pandemic downtrend (71). Important questions remain open if endoscopy staff and patients should continue to be screened and tested regularly. It is only presumptive to say about the effect of cancer burden due to delayed screening, surveillance, and handling the increased backlog cases. Multiple strategies can be adopted to decrease or ease endoscopy demand. Patients who are eligible for the screening should be provided with options of CRC screening, including stool-based testing, which do not need patients to present to the healthcare facilities. Home-based stool testing has the advantage of testing without contact with hospitals or clinics (72). For patients who test positive, there is a significant risk of advanced adenomas on the endoscopy, and hence a triage system should be developed to prioritize the procedures (72). Because of these, there will be increased demand in the recovery phase

that likely needs to be phased appropriately to avoid significant waiting times for procedures that need to happen in a timely fashion. It involves careful evaluation of patient demographics (comorbidities) and environmental factors (staff availability, local resources, community spread, and infection rate) (73). As the recovery phase starts, the real effect on the delay in cancer screening will emerge.

Patients should be communicated about the importance of screenings in the recovery phase to avoid delays and to keep the appointments. There should be an effective use of electronic health record communication strategies to provide updates to patients about COVID-19-related changes in endoscopy units. Virtual tools such as increased telehealth visits to discuss and engage patients about cancer screening programs will increase the endoscopy show rates (74). A triage system to review all the posted case by qualified medical personnel and reschedule the procedures in a tiered fashion can make this process less stressful (42). Furthermore, endoscopy staff should communicate with schedulers about the patient's concerns, which can be directly addressed. Finally, a higher threshold should be adopted for endoscopy procedures, which will less likely change the outcomes in patients (75). Despite these changes, as this pandemic unfolds with localized outbreaks, endoscopy units remain at a threat of temporary closures and need for enhanced disinfection protocols. Preparing for future pandemics should be a part of the operation of the endoscopy units' stress response. Nevertheless, endoscopy units should continue to adapt and navigate to provide high-quality patient care with equal emphasis on patient and staff safety.

LIMITATIONS

Due to the rapidly evolving nature of the COVID-19 pandemic, endoscopy units continue to adapt, and the above recommendation can change. Due to the heterogeneity of the published literature, we could not perform a systematic review. Pre-procedural testing, triaging, and trainee involvement in the procedures are dependent on infection risk, local endoscopy unit, and hospital policies. As countries are starting the recovery phase of the COVID-19 pandemic, these measures are constantly being updated.

CONCLUSION

Endoscopy units are on the verge of significant changes and evolution with the unfolding of the COVID-19 pandemic. The current pandemic calls for multiple changes at different levels not only to perform procedures in a safe environment for patients but also to prevent infection to the endoscopy staff. It appears likely that COVID-19 will be an integral part of our lives, like other viruses such as influenza. Similar to other procedures' predominant specialties, endoscopy units are incorporating operational changes in order to provide care in these unprecedented times. The use of enhanced protocols with particular emphasis on assessing the risk status of the patient, proper use of PPE, and perioperative procedural changes incorporated during this pandemic should be a lesson for the future. While some of these changes can gain permanent stance in the future, adapting to the future outbreaks is critical to provide excellent care.

AUTHOR CONTRIBUTIONS

HG and AP: conception and design and literature review. AP: first draft. All authors: critical revision, editing, and final approval. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 30 November 2020
doi: 10.3389/fmed.2020.576891

Impact of COVID-19 on the Healthcare of Patients With Inflammatory Bowel Disease: A Comparison Between Epicenter vs. Non-epicenter Areas

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Background and Aims: The COVID-19 pandemic poses a great challenge to healthcare. We aimed to investigate the impact of COVID-19 on the healthcare of patients with inflammatory bowel disease (IBD) in epicenter and non-epicenter areas.

Methods: Patients with IBD from Hubei province (the epicenter of COVID-19) and Guangdong province (a non-epicenter area), China were surveyed during the pandemic. The questionnaire included change of medications (steroids, immunomodulators, and biologics), procedures (lab tests, endoscopy, and elective surgery), and healthcare mode (standard healthcare vs. telemedicine) during 1 month before and after the outbreak of COVID-19.

Results: In total, 324 IBD patients from Guangdong province (non-epicenter) and 149 from Hubei province (epicenter) completed the questionnaire with comparable demographic characteristics. Compared to patients in Guangdong province (non-epicenter), significantly more patients in Hubei (epicenter) had delayed lab tests/endoscopy procedures [61.1% (91/149) vs. 25.3% (82/324), $p < 0.001$], drug withdrawal [28.6% (43/149) vs. 9.3% (30/324), $p < 0.001$], delayed biologics infusions [60.4% (90/149) vs. 19.1% (62/324), $p < 0.001$], and postponed elective surgery [16.1% (24/149) vs. 3.7% (12/324), $p < 0.001$]. There was an increased use of telemedicine after the outbreak compared to before the outbreak in Hubei province [38.9% (58/149) vs. 15.4% (23/149), $p < 0.001$], while such a significant increase was not observed in Guangdong province [21.9% (71/324) vs. 18.8% (61/324), $p = 0.38$]. Approximately two-thirds of IBD patients from both sites agreed that telemedicine should be increasingly used in future medical care.

OPEN ACCESS

Edited by:
Angel Lanas,
University of Zaragoza, Spain

Reviewed by:
Hakan Akin,
Marmara University, Turkey
Daniel Carpio,
Complejo Hospitalario de Pontevedra, Spain

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 27 June 2020
Accepted: 23 October 2020
Published: 30 November 2020

Citation:
Qiu Y, Zhang Y-F, Zhu L-R, He J-S, Tan J-Y, Tan N-D, Lin S-N, Lin X-Q, Ghosh S, Chen M-H and Mao R (2020) Impact of COVID-19 on the Healthcare of Patients With Inflammatory Bowel Disease: A Comparison Between Epicenter vs. Non-epicenter Areas. Front. Med. 7:576891. doi: 10.3389/fmed.2020.576891

Conclusions: Our patient-based survey study in a real-world setting showed that COVID-19 resulted in a great impact on the healthcare of patients with IBD, and such an impact was more obvious in the epicenter compared to the non-epicenter area of COVID-19. Telemedicine offers a good solution to counteract the challenges in an unprecedented situation such as COVID-19.

Keywords: COVID-19, inflammatory bowel disease, medical care, telemedicine, epicenter, non-epicenter

INTRODUCTION

The pandemic of COVID-19 has tremendously impacted the entire world. This pandemic poses a great challenge to the healthcare of patients with many chronic diseases including inflammatory bowel disease (IBD). It is now clear that IBD is increasing worldwide and has become a global emergence disease in industrial-urbanized societies (1). Characterized by a relapsing and remitting course, patients with IBD need close monitoring and therapy adjustment in order to avoid acute flares. Thus, optimal management of IBD patients requires large healthcare resource utilization (2) which becomes a big challenge for hospitals, especially in the epicenter of disease, who are completely occupied by critical COVID-19 patients and have no room for "general" patients.

Telemedicine might be a virtual solution to counteract such a challenge. In two recently published articles (3, 4), the influence of COVID-19 on the treatment of immune-mediated inflammatory diseases was reported, and telemedicine was proposed as a solution to counteract challenges in healthcare delivery posed by COVID-19. However, the impact of COVID-19 on the healthcare of patients with such diseases, and the role of telemedicine in such a situation has seldom been investigated in a real-world setting.

The aim of this study was to investigate the impact of COVID-19 on the healthcare of patients with IBD using a patient-based survey, and to compare the data before and after the outbreak of COVID-19, both in Hubei province (epidemic) and Guangdong province (non-epidemic) in China.

METHODS

Survey Design

Electronic questionnaire surveys were carried out to compare IBD patients in Hubei province (epicenter of COVID-19) and Guangdong province (non-epicenter), China. The questionnaire was focused on the change of medications (steroids, immunomodulators, and biologics), procedures (lab tests, endoscopy, and elective surgery), and healthcare mode (standard healthcare vs. telemedicine) during 1 month before and after the outbreak of COVID-19. We also investigated the impact of COVID-19 on attitudes of patients toward telemedicine.

All questions were closed with multiple choice answers. The Chinese questionnaire (**Supplementary Material**) was piloted for comprehensibility among 10 patient-volunteers from the center of Guangzhou.

Statistical Analysis

Answers were summarized based on the total number of respondents to each question, and missing data for a question were excluded from that particular analysis. Categorical variables were expressed in frequencies and percentages. Continuous variables were expressed as mean and standard deviation (SD) or median and range. Two independent samples were tested by the Student T-test; the analysis of variance or Kruskal-Wallis rank-sum test was used for comparison between multiple groups. The χ^2 test was performed to compare count data, and a 2-tailed value of $P < 0.05$ was considered statistically significant.

Statistical analysis was performed using GraphPad Prism (5.03, GraphPad Soft- ware, Inc., San Diego, USA).

RESULTS

In total, 324 IBD patients from Guangdong province (non-epicenter) and 149 from Hubei province (epicenter) completed the questionnaire, and the demographic characteristics were comparable between patients from these two provinces (**Table 1**).

TABLE 1 | The baseline of survey IBD patients from Guangdong and Hubei.

	Guangdong ($n = 324$)	Hubei ($n = 149$)	*P*
Diagnosis			
CD:UC:IBD-U	235:75:14	94:48:7	0.102
Gender			
M:F	207:117	88:61	0.314
Age, *n* (%)			0.043
<16 y	14 (4.3)	4 (2.7)	
16–40 y	217 (67)	93 (62.4)	
>40 y	93 (28.7)	49 (32.9)	
>65 y	0	3 (2)	
Disease duration, *n* (%)			<0.001
≤2 y	66 (20.4)	48 (32.2)	
2–5 y	106 (32.7)	68 (45.6)	
5–10 y	92 (28.4)	27 (18.1)	
>10 y	60 (18.5)	6 (4)	

IFX, infliximab; ADA, adalimumab; MTX, methotrexate; SASP, salazosulfapyridine; 5-ASA, 5-aminosalicylic acid; CD, Crohn's disease; UC, ulcerative colitis; IBD-U, inflammatory bowel disease unclassified; M, male; F, female; y, year.

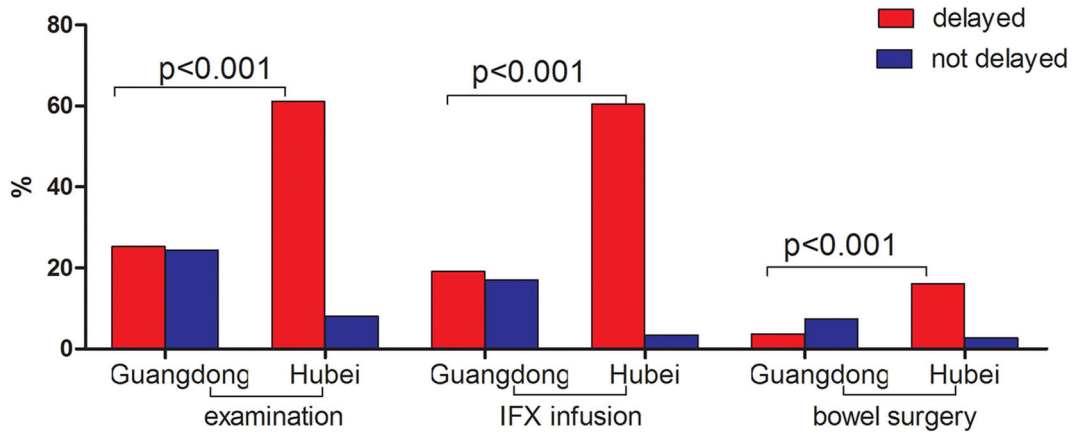

FIGURE 1 | Comparison of medications and procedures between pre-and post-pandemic in Guangdong (non-epicenter) and Hubei (epicenter) province.

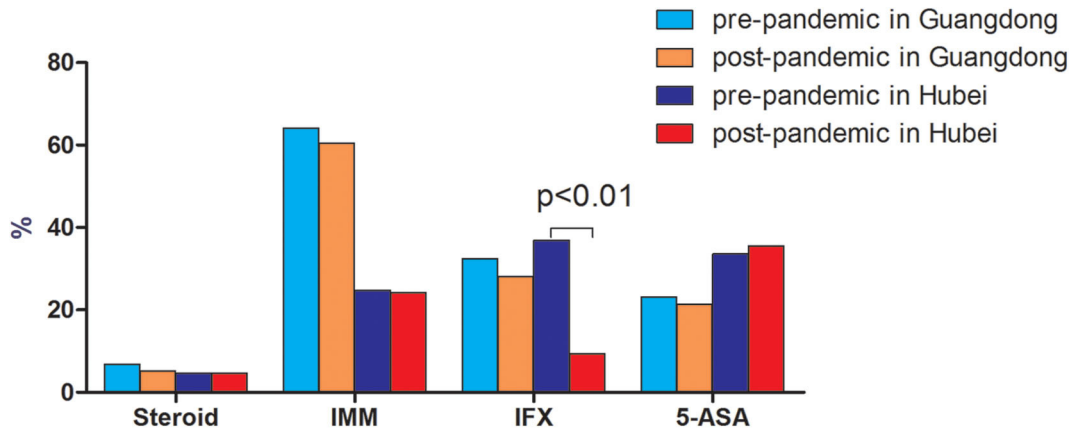

FIGURE 2 | Comparison of medication use between pre-and post-pandemic in Guangdong (non-epicenter) and Hubei (epicenter) province.

Change in Medications and Procedures During the COVID-19 Outbreak

Compared to patients in Guangdong province (non-epicenter), significantly more patients in Hubei (epicenter) had delayed lab tests/endoscopy procedures [61.1% (91/149) vs. 25.3% (82/324), $p < 0.001$], drug withdrawal [28.6% (43/149) vs. 9.3% (30/324), $p < 0.001$], and postponed elective surgery [16.1% (24/149) vs. 3.7% (12/324), $p < 0.001$] (**Figure 1**). There was no significant change in use of steroids, thiopurines, and aminosalicylates before and after the pandemic outbreak in both areas. However, there were significantly more patients with delayed biologics infusions in the epicenter compared to the non-epicenter area [60.4% (90/149) vs. 19.1% (62/324), $p < 0.001$, **Figure 2**].

Change in the Healthcare Mode Before and After the Outbreak of COVID-19

The outbreak of COVID-19 resulted in a substantial decrease of patients participating in standard face-to-face visits. The number of patients who attended standard face-to-face visits reduced more dramatically in Hubei province [59.1% (88/149) vs.12.1% (18/149), $p < 0.001$] than that in Guangdong province [66.4% (215/324) vs. 37.7% (124/324), $p < 0.001$] (**Figure 3**). There was an increased use of telemedicine after the outbreak compared to before the outbreak in Hubei province [38.9% (58/149) vs.15.4% (23/149), $p < 0.001$], while such a significant increase was not observed in Guangdong province [21.9% (71/324) vs. 18.8% (61/324), $p = 0.38$]. Regarding the frequency of telemedicine use, there was a trend toward, though not significantly, a higher percentage of patients using telemedicine (≥3 times) in Hubei province (26/149, 17.5%) compared to that in Guangdong province (39/324, 12.1%) ($p = 0.082$) (**Table 2**). Among all kinds of telemedicine, hospital-based online clinics and WeChat consultations were the two most used both in Guangdong province and Hubei province. Approximately two-thirds of IBD patients from both sites agreed that telemedicine should be increasingly used in future medical care (**Table 2**).

DISCUSSION

The current unprecedented pandemic poses a great challenge to public health resource as well as patients with IBD. A lot

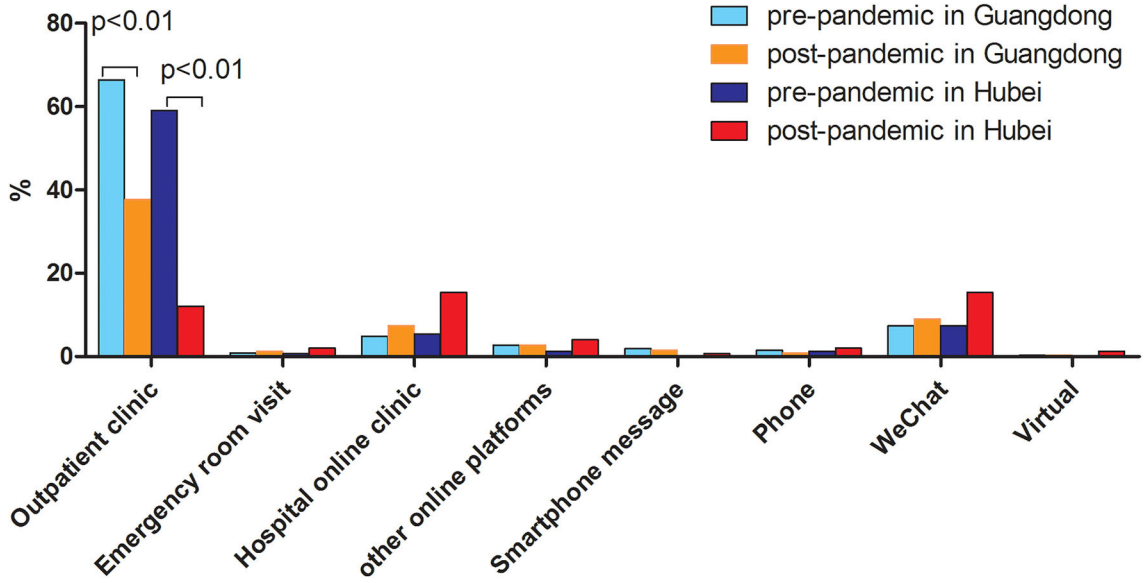

FIGURE 3 | Comparison of mode of medical care between pre-and post-pandemic in Guangdong (non-epicenter) and Hubei (epicenter) province.

of focus has been put on the outcomes of IBD patients with COVID-19. However, attention should be also paid to the impact of COVID-19 on regular IBD patients (non COVID-19 infection) who are the majority of the IBD population. Indeed, a recent survey of members of the European Crohn's and Colitis Organization (ECCO) showed that COVID-19 has disrupted and revolutionized the management of IBD patients, forcing physicians to face new problems (5). The present study using a patient-based survey explored the difference in the impact of COVID-19 on the medical care of IBD patients in the epidemic compared to the non-epidemic area.

As demonstrated in our survey, patients both in the epicenter and non-epicenter areas had limited access to healthcare evident by the decreased number of standard fact-to-fact visits, and delayed examinations, biologics infusion, and selective surgery. The situation was more serious in the epidemic area of Hubei province due to the lockdown of the whole province and the fact that many gastroenterologists were reassigned and directly involved in the care of COVID-19 patients.

Not only is providing adequate follow-up for IBD complicated during the COVID-19 outbreak, but ensuring adequate care of patients with acute conditions is complicated as well (6). Although COVID-19 is principally defined by its respiratory symptoms, it is now clear that the virus can also affect the digestive system (7). Patients with COVID-19 may present with gastroenterology symptoms, such as diarrhea, nausea and/or vomiting, and abdominal pain with no respiratory symptoms (8). As such, COVID-19 may mimic IBD relapse symptoms, adding a diagnostic challenge to this group of patients. Moreover, patients' fear to visit the hospital in addition to the shortage of medical resources may cause diagnosis and treatment delay, consequently leading to treatment failure or even to the need of urgent surgical intervention (e.g., in patients with severe ulcerative colitis or complications). According to our survey, there was a rise, though not significantly, in the number of patients who paid a visit to the emergency room for medical care in Hubei province [1 (0.7%) vs. 3 (2%)].

As for today, the current guidelines from the main medical societies suggests maintaining current medication (e.g., immunosuppressive and biological agents) as a preventive strategy in IBD patients without symptoms suggestive of COVID-19 (5). Whether patients who stopped IBD drugs experienced IBD flares leading to hospitalizations and surgeries needs to be further addressed. According to a recent study by Bezzio et al. (9) which presented the characteristics and outcomes of IBD patients with COVID-19, active disease, old age, and comorbidities were risk factors of a negative outcome of COVID-19, whereas IBD medication was not. Global data from the SECURE-IBD registry (https://covidibd.org/) show that older age and health conditions are the major drivers of more severe COVID-19 and death. Steroid use continues to be the strongest medication-associated risk factor. Other IBD medications including anti-TNF biologics appear to be safe. According to our survey, there was no significant change in use of steroids, thiopurines, and aminosalicylates before and after the pandemic outbreak in both sites. However, there were significantly more patients with delayed biologics infusions in the epicenter compared to the non-epicenter area [60.4% (90/149) vs. 19.1% (62/324), $p < 0.001$], which implies that intravenous infusions in the hospital were affected more in the epicenter than that in the non-epicenter area.

According to the web-survey conducted by ECCO (5), physical contact with other people was feared by about half of respondents (45.1%), and most specialists (73.2%) canceled or rescheduled consultations due to the COVID-19 outbreak. In this way, telemedicine may serve as a perfect solution to counteract

TABLE 2 | Comparison of use of medication, source to get medication, and way of seeking medical care in Guangdong and Hubei pre- and post-pandemic.

	Guangdong ($n = 324$)		*P*	Hubei ($n = 149$)		*P*
Medication, *n* (%)	Pre	Post	0.118	Pre	Post	<0.001
Steroids	22	17		7	7	
Thiopurine	128	122		28	28	
Thalidomide	61	57		8	6	
Oral MTX	6	7		1	2	
MTX im	13	10		0	0	
IFX	105	91		55	14	
ADA	0	0		1	0	
On trials	1	0		1	0	
SASP	5	3		4	4	
5-ASA	70	66		46	49	
None	10	24		13	42	
Access to medications, *n* (%)	Pre	Post	<0.001	Pre	Post	<0.001
Outpatient clinic	200 (61.7)	124 (38.3)		60 (40.3)	16 (10.7)	
Emergency	6 (1.9)	3 (0.9)		3 (2)	1 (0.7)	
Pharmacy	40 (12.3)	30 (9.3)		42 (28.2)	35 (23.5)	
Online	76 (23.5)	113 (34.9)		26 (17.4)	35 (23.5)	
Way of seeking medical care, *n* (%)	Pre	Post	<0.001	Pre	Post	<0.001
Outpatient clinic	215 (66.4)	122 (37.7)		88 (59.1)	18 (12.1)	
Emergency	3 (0.9)	4 (1.2)		1 (0.7)	3 (2)	
Hospital online clinic	16 (4.9)	24 (7.4)		8 (5.4)	23 (15.4)	
Other online platform	9 (2.8)	9 (2.8)		2 (1.3)	6 (4)	
Message	6 (1.9)	5 (1.5)		0 (0)	1 (0.7)	
Phone	5 (1.5)	3 (0.9)		2 (1.3)	3 (2)	
WeChat	24 (7.4)	29 (9)		11 (7.4)	23 (15.4)	
Video	1 (0.3)	1 (0.3)		0 (0)	2 (1.3)	
No visit	74 (22.8)	150 (46.3)		42 (28.2)	89 (59.7)	

IFX, infliximab; ADA, adalimumab; MTX, methotrexate; SASP, salazosulfapyridine; 5-ASA, 5-aminosalicylic acid; CD, Crohn's disease; UC, ulcerative colitis; IBD-U, inflammatory bowel disease unclassified; M, male; F, female; y, year.

these challenges. As demonstrated in our survey, patients turned to telemedicine including hospital-based online clinics and Wechat consultations as an alternative way of seeking medical advice. There was an increased use and need of telemedicine after the COVID-19 outbreak especially in Hubei province, the epicenter area. According to our survey, two-thirds of IBD patients from both sites equally support the notion of increasing telemedicine in future medical care ($p = 0.39$). The rapidly developing technological advances in artificial intelligence and virtual reality provide a solid foundation for delivering the right care to the right patient at the right time. It is time to look beyond the traditional role of telemedicine as a connectivity only tool.

The study may be limited in some way. The major limitation of this study is that the survey returns may have been affected by the disaster conditions within the provinces, especially for severely ill patients, patients with a low education level, and patients with other chronic diseases. Some of these patients may have been hospitalized COVID-19 patients who were not able to answer the survey. This may be a bias that would be difficult to overcome due to the conditions during the pandemic.

In summary, our patient-based survey study in a real-world setting showed that COVID-19 resulted in a great impact on the healthcare of patients with IBD, and that such an impact was more obvious in the epicenter compared to the non-epicenter area of COVID-19. Telemedicine which transcends geography offers a good solution to counteract the challenges in such an unprecedented situation such as COVID-19, and there is support for more widespread adoption of telemedicine among patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RM and M-HC conceived the study and supervised the overall study. RM, YQ, and Y-FZ wrote the manuscript. L-RZ, J-SH, N-DT, J-YT, S-NL, X-QL, and SG critically revised the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank all patients that completed the survey.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.576891/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

HYPOTHESIS AND THEORY
published: 22 December 2020
doi: 10.3389/fmed.2020.606991

Public Database-Driven Insights Into Aging Stress-Associated Defective Gut Barrier With Low SARS-CoV-2 Receptors

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The novel coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a global pandemic, and resulted in high case-fatality rate in the elderly. In addition to typical respiratory responses, ∼50% of clinical cases include gastrointestinal symptoms such as diarrhea, vomiting, abdominal pain, and persistent fecal shedding of the virus even after its clearance from the pulmonary system. In the present study, we assessed aging-associated gut transcriptomic responses considering the gastrointestinal symptoms contributing to COVID-19 severity. Intestinal expression of SARS-CoV-2 receptors and defense biomarkers decreased with increasing age. Moreover, aging-associated integrated stress responses (ISR) and mTOR-linked cell metabolic stress signals counteracted gut defense biomarkers. However, SARS-CoV-2 receptor expression was positively associated with gut barrier integrity potently via downregulation of the two stress-responsive signals. Gut transcriptome-based mechanistic prediction implicates that high susceptibility to COVID-19 in the elderly with low SARS-CoV-2 receptors is due to aging stress-associated defective gut defense, providing a new avenue for viral entry receptor-independent interventions.

Keywords: SARS-CoV-2, gut barrier, integrated stress responses, metabolic stress, aging

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *16 September 2020*
Accepted: *30 November 2020*
Published: *22 December 2020*

Citation:
Moon Y (2020) Public Database-Driven Insights Into Aging Stress-Associated Defective Gut Barrier With Low SARS-CoV-2 Receptors. Front. Med. 7:606991. doi: 10.3389/fmed.2020.606991

INTRODUCTION

Since the first report on an unknown pneumonia-like disorder caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in Wuhan area, the coronavirus disease (COVID-19) has become a worldwide pandemic, and is majorly attributed to zoonotic sources (1–3). Although the common symptoms include fever, cough, dyspnea, fatigue, and sputum production, fatal cases present lymphopenia and severe inflammatory distress such as organ failure in addition to airway dysfunction (4). Such severe complications are prominent in subjects with underlying health conditions including cardiovascular diseases, diabetes, or obesity, requiring hospitalization and intensive care (4–7). Moreover, based on the population-based studies, the elderly group (particularly aged 70 years or older) among the patients with COVID-19 presented high case-fatality rate with severe complications in Italy and China (3, 8, 9). A quantitative systemic review demonstrated that ∼25 and 71% of the elderly subjects developed renal injuries and required supplementary oxygen, respectively (8). Although the complications of COVID-19 with aging

are evident, its mechanistic assessments are required for developing precise interventions for the susceptible population.

During cellular infection by SARS-CoV-2, the viral spike (S) protein recognizes angiotensin converting enzyme 2 (ACE2) as a viral receptor to enter the host cells. Moreover, this entry requires S protein priming by cellular proteases, which entails S protein cleavage and allows fusion of viral and cellular membranes. SARS-CoV-2 employs the cellular serine protease, transmembrane protease serine 2 (TMPRSS2), which cleaves the S protein of human coronaviruses on the cell membrane for priming (10). Successful viral entry depends on ACE2 and TMPRSS2, which are not only coexpressed in the airway epithelia but also highly expressed in gut cells such as esophageal, ileal, and colonic epithelial cells (11), indicating that the gastrointestinal tract acts as an alternative route for SARS-CoV-2 invasion. Furthermore, for ∼50% of COVID-19 clinical cases, SARS-CoV-2 can be detected in fecal samples and gut mucosa of the infected hosts (12–14). In addition, half of infected patients display prolonged fecal shedding of SARS-CoV-2 even after viral clearance from the respiratory tract (15), thereby suggesting the transmission of coronavirus via fecal–oral route. In particular, persistent inflammatory distress in the insulted gut during viral infection may contribute to COVID-19 severity.

In response to viral infection, human cells activate a common adaptive pathway, known as the integrated stress response (ISR), to restore cellular integrity. The core biochemical event in ISR is the phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) by the eIF2α kinase family, leading to global translational arrest, and the induction of specific stress-responsive genes to achieve biological homeostasis in the insulted hosts (16, 17). In the present study, assuming age to be a crucial risk factor of COVID-19 severity, we investigated the transcriptomic features of human gut with aging stress. In particular, the aging stress in association with ISR and other stress signaling was evaluated to predict the defective responses to SARS-CoV-2 in the elderly subjects.

METHODS

Age-Linked Transcriptome Data

RNA-seq raw counts and normalized TPM matrices (Illumina paired-end, 76 bp) were downloaded from the Genotype-Tissue Expression (GTEx) Portal (version 8, 17,382 samples from 30 tissue types). All accessed data used in this study are publicly available on the web portal (https://gtexportal.org/home/index.html) and have been deidentified, except for patient age range and gender. Non-diseased transverse colon tissues ($n = 937$) containing the mucosal parts from the different age groups were selected for the transcriptomic analysis (**Supplementary Figure 1A**). Samples from the sigmoid colon without the mucosa were excluded.

Genomic Analysis Using Colon Cancer Datasets

Clinical sources of transcriptomic data from colon cancer tissue samples of patients are listed in the dataset (GEO ID: gse39582, $n = 566$). Among a large series of colon cancer data collected for the Cartes dIdentité des Tumeurs (CIT) program from the French Ligue Nationale Contre le Cancer (http://cit.ligue-cancer.net), 566 were analyzed for mRNA expression profiles using Affymetrix U133plus2 chip and, among these, 463 were analyzed for DNA alteration profiles using the CGH Array (CIT-CGHarray V6). Survival analysis was performed in three datasets of patients with colorectal cancer (gse39582 [$n = 566$], gse24551 [$n = 333$], and gse14333 [$n = 290$]). Dataset gse24551 was derived from genome-wide expression at exon level for two independent series of colorectal cancer tissue biopsies using the Affymetrix Human Exon 1.0 ST platform. Dataset gse14333 was from the expression profiles of surgically resected specimens in 290 patients with colorectal cancer using Affymetrix Human Genome U133Plus 2.0 arrays.

Genomic Analysis Using IBD Datasets

Human intestinal tissue datasets were obtained from the gene expression arrays of patients with IBD (gse117993, $n = 190$). These experiments tested the differential gene expression in these three types of IBD relative to healthy control samples. RNA was isolated from biopsies from 190 pediatric patients undergoing diagnostic colonoscopy for inflammatory bowel diseases, including Crohn's disease (CD) and ulcerative colitis (UC). Single-end, 75-bp sequencing was performed, and raw reads were aligned to the human genome using Gencode v 24 as a reference. We included 14,085 protein-coding mRNA genes in downstream analyses. For clinical dataset, the three major clinical subsets of IBD included only UC, colon-only CD (cCD), and ileocolonic CD (iCD) (**Supplementary Figure 1B**).

Statistics

Statistical analyses were performed using GraphPad Prism v. 5.01 (La Jolla, CA, USA). For comparative analysis of two groups of data, Student's *t*-test was performed. For comparative analysis of multiple groups, data were subjected to analysis of variance (ANOVA) with *Newman–Keuls* method as a *post-hoc* ANOVA assessment.

RESULTS

Aging Attenuates Expression of SARS-CoV-2 Receptors and Gut Defense Biomarkers

We analyzed aging-associated patterns mainly using the transcriptome dataset of non-diseased tissue from the Genotype-Tissue Expression (GTEx) project. Since the gut is a persistent source of fecal SARS-CoV-2 production, we specifically analyzed colonic RNA-seq transcriptomes from donors of varying ages (aged 20–79 years) (**Figures 1A,B**). Expression of two SARS-CoV-2 receptors was assessed in different age groups. Compared to the levels in young age group (aged 20–29 years), expression of *ACE2* and *TMPRSS2* tended to decrease with age, which was prominent in elderly groups (aged 60–79 years) (**Figure 1A**). For successful viral entry by directly binding to ACE2, other accessary components such as TMPRSS2 and cathepsin L (CTSL) can facilitate S protein priming for receptor binding on the host cell surface (10); however, CTSL without proteolysis activity on

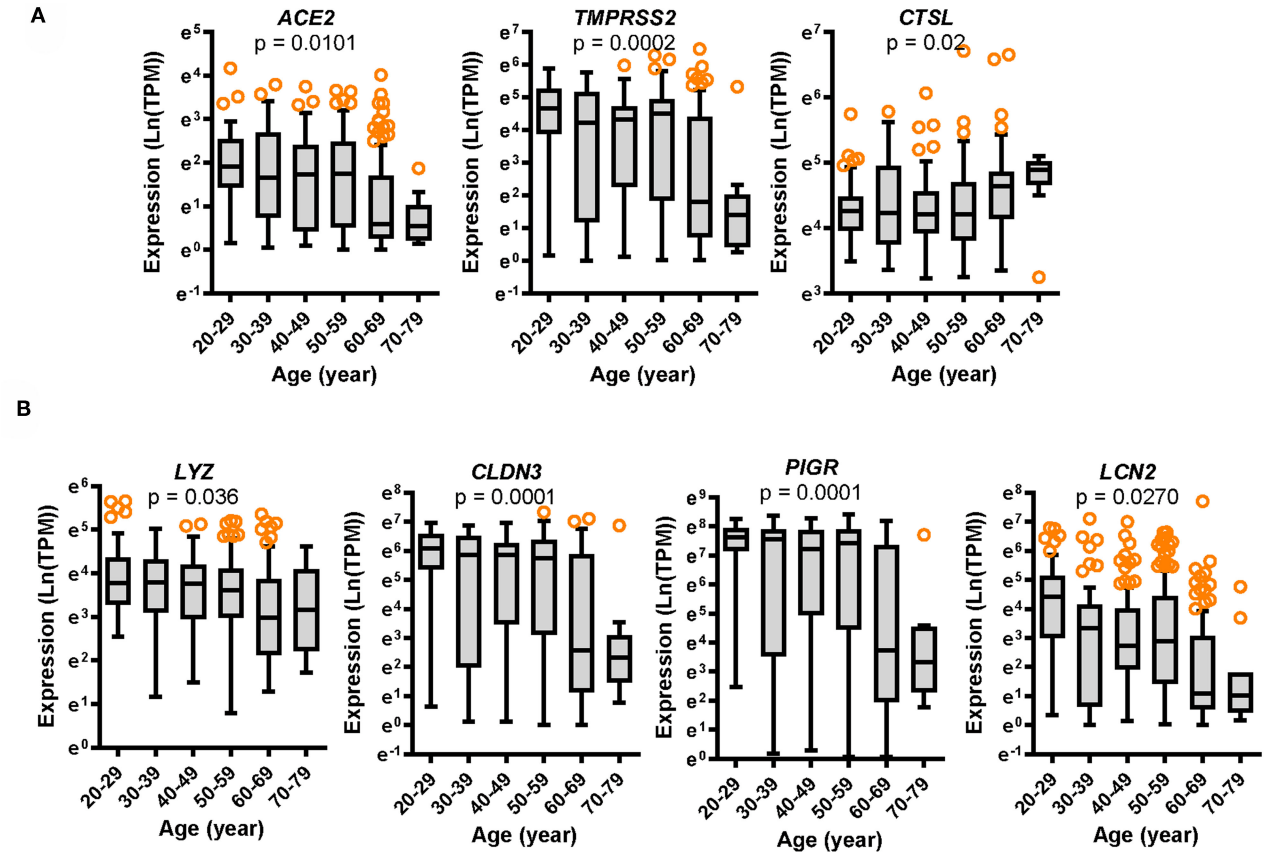

FIGURE 1 | Expression of SARS-CoV-2 receptors and gut defense biomarkers with age. Results are depicted as box-and-whisker plots (Turkey) for the expression of SARS-CoV-2 receptors *ACE2, TMPRSS2*, and *CTSL* **(A)** or gut defense makers *LYZ, CLDN3, PIGR*, and *LCN2* **(B)** in normal mucosal intestinal tissues (GTEx dataset v8). Values are presented as natural logarithm of transcripts per million (TPM). Statistical significance of the expression variation with age is illustrated on the top of each plot (Kruskal–Wallis test).

the cell surface is dispensable for host cell entry of SARS-CoV-2 (10, 18). In contrast with the levels of *ACE2* and *TMPRSS2*, *CTSL* expression tended to increase with age in the colon tissue (**Figure 1A**).

SARS-CoV-2 Receptors Are Positively Associated With Gut Defense During Aging or Chronic Disease Progression

In response to viral entry, the host epithelial defense is a deterministic factor of the pathogenic outcomes in infected patients. We analyzed the expression of gut barrier defense biomarkers such as lysozyme (*LYZ*), claudin 3 (*CLDN3*), polymeric immunoglobulin receptor (*PIGR*), and lipocalin 2 (*LCN2*) in the colon. Expression of the corresponding genes *LYZ, CLDN3, PIGR*, and *LCN2* was modestly associated with age ($p = 0.036$, $p = 0.0001$, $p = 0.0001$, and $p = 0.0270$, respectively) and tended to decrease with increasing age (**Figure 1B**). Notably, levels of gut defense biomarkers were significantly attenuated in the elderly subjects (aged 60–79 years). Moreover, expression of key SARS-CoV-2 receptors was positively associated with levels of gut defense biomarkers (**Figures 2A,B**). From the GTEx-based dataset, subjects with high expression of *ACE2* or TMPRSS2 presented high levels of *LYZ*, *CLDN3*, *PIGR*, and *LCN2* in the intestine, indicating a protective action of SARS-CoV-2 receptors against gut infection.

In addition to the analyses of the non-diseased tissues from GTEx project, gene expression in biopsies from patients with chronic intestinal distress [colon cancer and inflammatory bowel disease (IBD)] was also evaluated. In patients with colon cancer, *ACE2* expression tended to increase with disease progression (**Figure 3A**), whereas *TMPRSS2* levels were not significantly altered in the lesions (**Supplementary Figure 2A**); however, patients with high levels of *ACE2* displayed good prognosis compared to those with low expression (**Figure 3B**). Moreover, high expression levels of *TMPRSS2* are positively associated with good prognoses for CRC patients (**Supplementary Figure 2B**). Results of survival analyses demonstrate the protective roles of SARS-CoV-2 receptors in oncological disease progress, which were in accordance with the results in non-diseased colonic tissues of the GTEx dataset. Furthermore, the patterns were also verified in the tissue expression from patients with

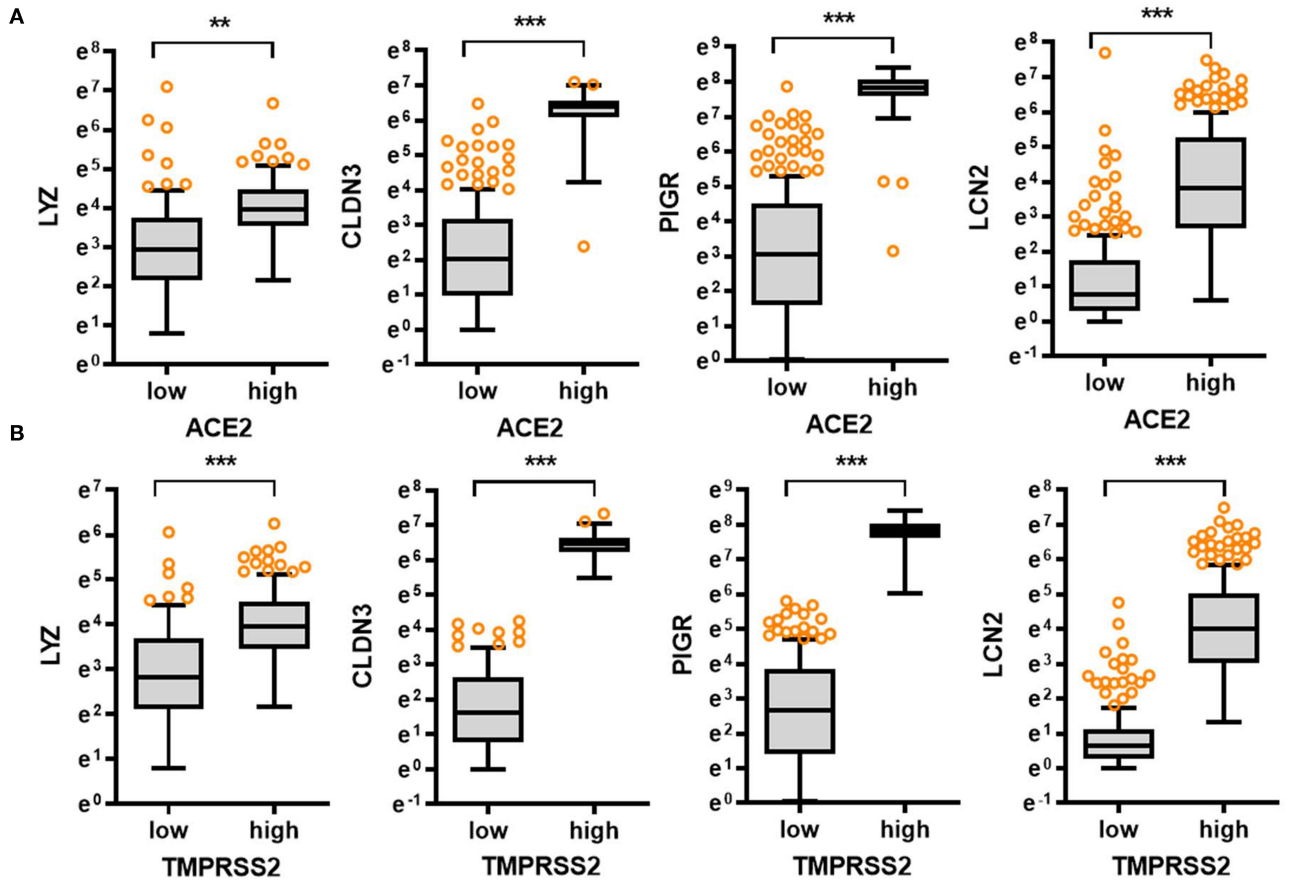

FIGURE 2 | Comparative expression of barrier defense biomarkers with SARS-CoV-2 receptor levels. Based on *ACE2* **(A)** or *TMPRSS2* **(B)** levels in the normal mucosal intestinal tissues (GTEx dataset v8), we selected the 150 highest and 150 lowest level samples, which were further evaluated based on *LYZ, CLDN3, PIGR*, and *LCN2* levels. Values are presented as natural logarithm of transcripts per million (TPM). Asterisks (*) indicate significant differences from the low expression group (**$p < 0.01$, ***$p < 0.001$).

IBD (**Figures 3C,D**). Expression of *ACE2* and *TMPRSS2* was markedly elevated in patients with UC and CD, including colon-only CD (cCD) and ileocolonic CD (iCD), when compared to that in the control group (**Figure 3A** and **Supplementary Figure 2C**, respectively). Patients with high expression of *ACE2* or *TMPRSS2* displayed high levels of *LYZ* and *LCN2* in the intestine (**Figure 3D**), indicating a protective action of SARS-CoV-2 receptors against gut barrier disruption.

Aging-Associated ISR Potently Counteracts Levels of Gut Defense Biomarkers

To elucidate the molecular mechanisms of gastrointestinal distress, eIF2α kinase-mediated ISR was evaluated as the common adaptive pathway in response to the external insults including viral infection. The alpha subunit of eIF2 is targeted by four different stress-related mammalian protein kinases, namely, heme-regulated eIF2α kinase (HRI, EIF2AK1), double-stranded RNA-dependent protein kinase R (PKR, EIF2AK2), RNA-dependent protein kinase-like ER kinase (PERK, EIF2AK3), and eIF2α kinase general control non-repressed 2 (GCN2, EIF2AK4) (16, 17). In particular, SARS-CoV-2-infected cells display a PKR-linked pathogenesis including specific 28S rRNA cleavage (19–21). Expression of four eIF2α kinases was assessed in different age groups. Expression of *EIF2AK2* and *EIF2AK4* was significantly associated with age ($p = 0.0012$, and $p = 0.0003$, respectively) and tended to increase with age (**Figure 4A**). Notably, the levels of *EIF2AK2* and *EIF2AK4* were significantly elevated in elderly subjects (aged 60–79 years) when compared to those in the young group (aged 20–29 years). Furthermore, we evaluated whether eIF2α kinases are involved in regulation of gut barrier integrity. Expression of *EIF2AK2* or *EIF2AK4* was positively associated with the levels of gut defense biomarkers (**Figures 4B,C**). Subjects with high expression of *EIF2AK2* or *EIF2AK4* displayed low levels of *LYZ, CLDN3, PIGR*, and *LCN2* in the intestine, thereby suggesting a negative regulation of gut defense by eIF2α kinase-linked signaling.

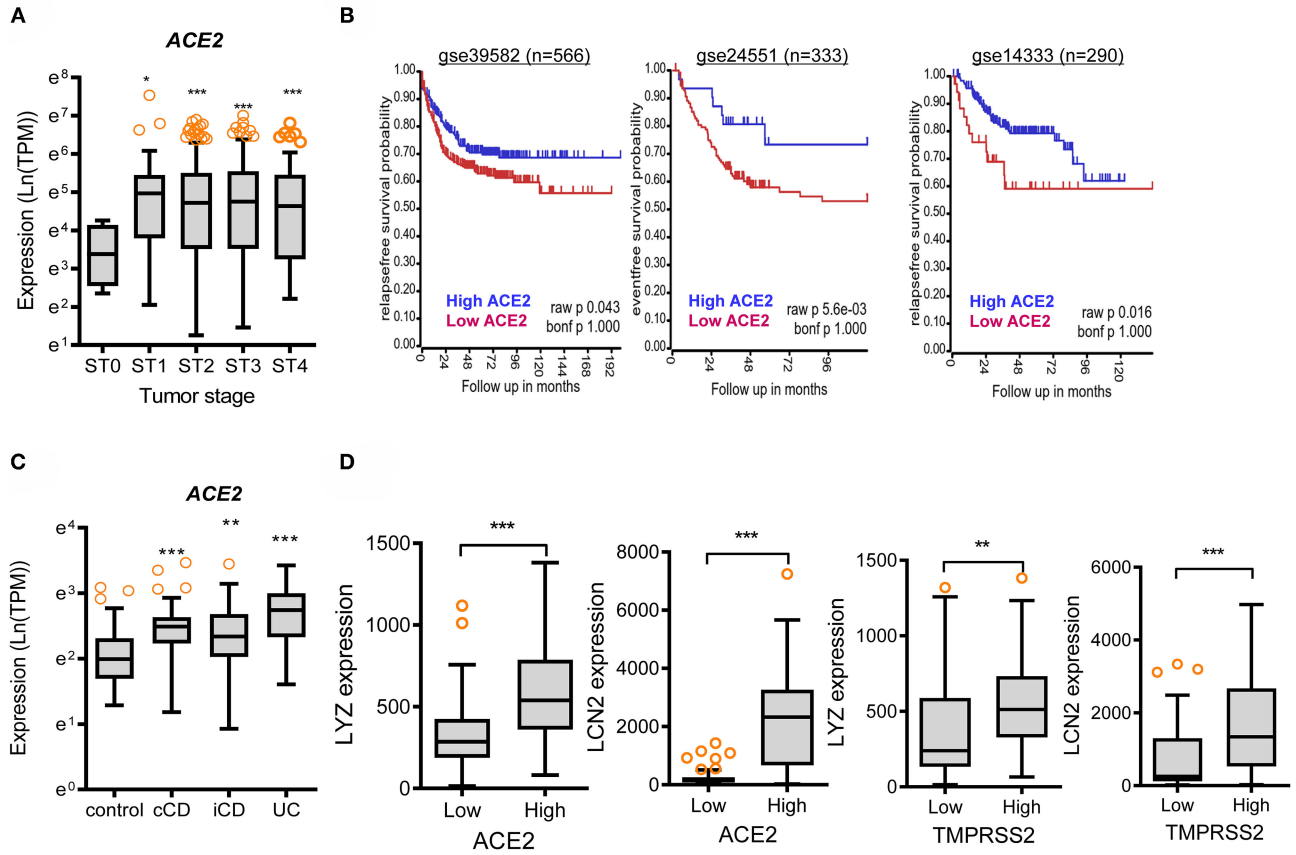

FIGURE 3 | Involvement of SARS-CoV-2 receptors in chronic intestinal diseases. **(A)** *ACE2* expression in different tumor stages from the transcriptome dataset in patients with colon cancer (GEO ID: gse39582, $n = 566$). Values are presented as natural logarithm of transcripts per million (TPM). Asterisks (*) indicate significant differences from levels at Stage 0 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ using two-tailed unpaired Student's *t*-test). **(B)** Kaplan–Meier plot of survival analysis based on tissue *ACE2* transcript levels in patients with CRC from three datasets (gse39582 [$n = 566$, expression cutoff 150.5], gse24551 [$n = 333$, expression cutoff 444.6], and gse14333 [$n = 290$, expression cutoff 44.5]). **(C)** Intestinal expression of *ACE2* was compared in patients with different IBD types from datasets gse117993 ($n = 190$). UC, ulcerative colitis; cCD, colon-only CD; iCD, ileocolonic CD. Values are presented as natural logarithm of transcripts per million (TPM). Results are depicted as box-and-whisker plots (Turkey). Asterisks (*) indicate significant differences from the control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ using two-tailed unpaired Student's *t*-test). **(D)** Based on *ACE2* or *TMPRSS2* levels from datasets gse117993 ($n = 190$), we selected the 150 highest and 150 lowest level samples, which were further evaluated based on *LYZ* and *LCN2* levels. Values are presented as natural logarithm of TPM. Asterisks (*) indicate significant differences from the low expression group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Aging-Associated Cell Metabolic Stress Downregulates Levels of Gut Defense Biomarkers

The mammalian target of rapamycin (mTOR) is a central sentinel component of cellular metabolism that regulates the key aging processes including nutrient availability, energy homeostasis, cellular senescence, cell stemness, and proteostasis (22, 23). Although the expression of mTOR was not significantly associated with age ($p = 0.353$), there was an association between age and levels of ribosomal protein S6 kinase beta 1 (RPS6KB1) as a hallmark of activation by mTOR ($p = 0.0001$), which tended to increase with age (**Figure 5A**). Notably, expression of *RPS6KB1* was significantly elevated in the elderly subjects (60–79 years) when compared to those in the young group (aged 20–29 years). Furthermore, we verified whether mTOR-S6 kinase signaling module as the key aging-regulator is involved in gut barrier defense by analyzing the GTEx dataset. Expression of *mTOR* or *RPS6KB1* was associated with levels of gut defense biomarkers (**Figures 5B,C**). Subjects with high expression of *mTOR* or *RPS6KB1* presented low levels of *LYZ, CLDN3, PIGR*, and *LCN2* in the intestine, thereby indicating a negative regulation of gut defense by mTOR-S6 kinase signaling module. Since mTOR-S6 kinase signaling facilitates processes that fuel cell growth and proliferation, the signaling module counteracts cell differentiation to polarized enterocytes and other specialized intestinal epithelial cells such as goblet cells and Paneth cells, which is crucial for maintaining the gut epithelial barrier integrity (24). As a key intestinal differentiation factor, Krüppel-like factor 4 (*KLF4*) expression tended to decrease with age (**Supplementary Figure 2D**). Moreover, subjects with high expression of *mTOR* or *RPS6KB1* displayed low levels of *KLF4* in the intestine (**Figures 5B,C**), indicating insufficient

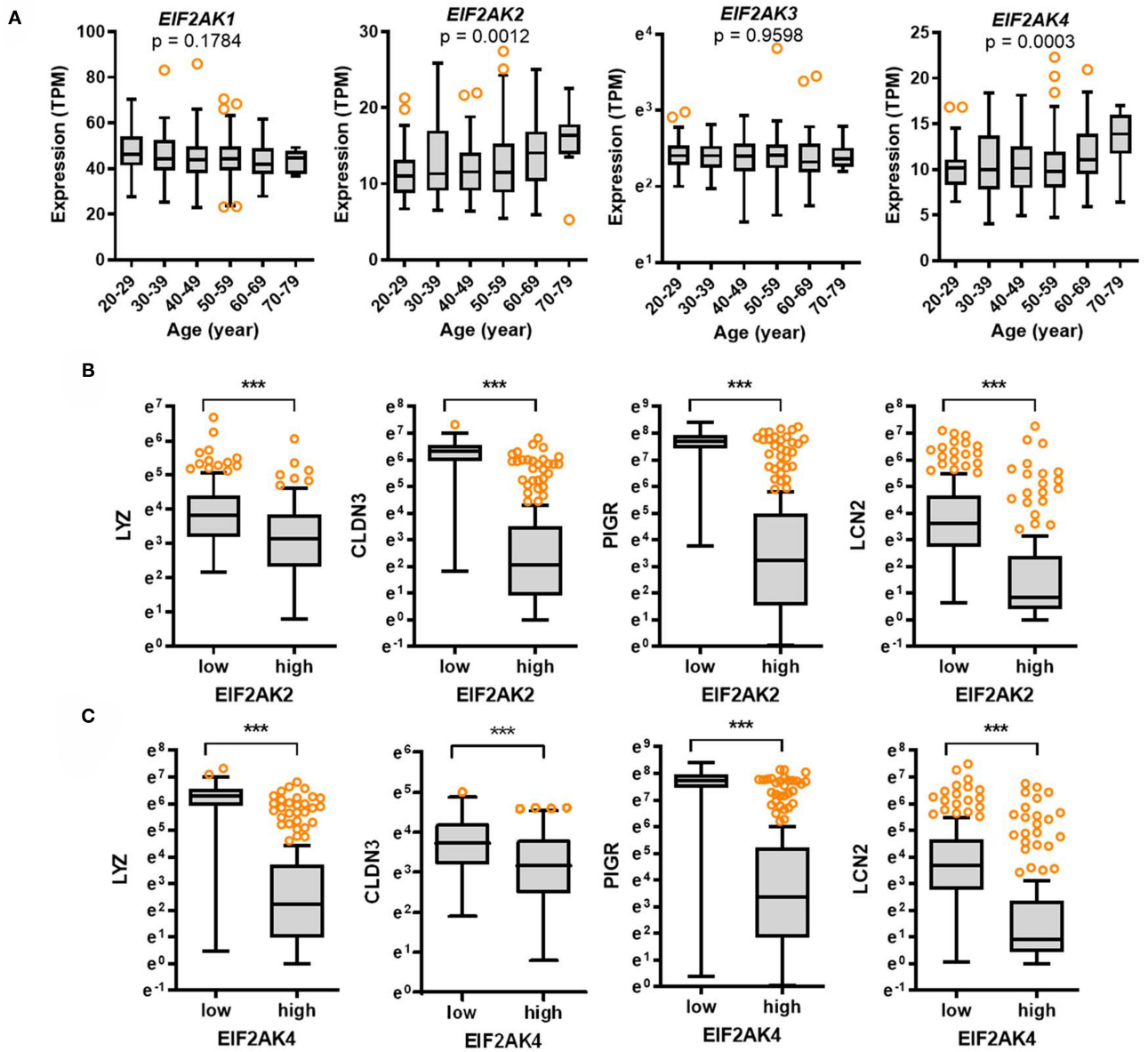

FIGURE 4 | Expression of eIF2α kinases and their association with gut defense biomarkers with age. **(A)** Results are depicted as box-and-whisker plots (Turkey) for expression of eIF2α kinases (*EIF2AK1, EIF2AK2, EIF2AK3, or EIF2AK4*) in normal mucosal intestinal tissues (GTEx dataset v8). Values are presented as transcripts per million (TPM). Statistical significance of the expression variation with age is illustrated on the top of each plot (Kruskal–Wallis test). **(B,C)** Considering the *EIF2AK2* **(B)** or *EIF2AK4* **(C)** levels, we selected the 150 highest and 150 lowest level samples, which were further evaluated based on *LYZ, CLDN3, PIGR*, and *LCN2* levels. Values are presented as natural logarithm of TPM. Asterisks (*) indicate significant differences from the low expression group (***$p < 0.001$).

differentiation and immature gut barrier by mTOR-S6 kinase signaling activation with age.

We studied two stress signaling modules (eIF2α kinase and mTOR-S6 kinase) counteracting gut barrier integrity via clinical transcriptome analysis. Moreover, eIF2α kinase and mTOR-S6 kinase signaling modules were assessed for their association with levels of SARS-CoV-2 receptors. Subjects with high expression of *ACE2* or *TMPRSS2* displayed low levels of *EIF2AK2, EIF2AK4, mTOR*, or *RPS6KB1* (**Figures 6A,B**), thereby supporting negative regulation of eIF2α kinase and mTOR-S6 kinase signaling by SARS-CoV-2 receptors in the gastrointestinal tract.

DISCUSSION

As a prediction model from the results, SARS-CoV-2-responsive receptors positively contribute to maintenance of gut barrier defense via attenuation of two stress signaling pathways of

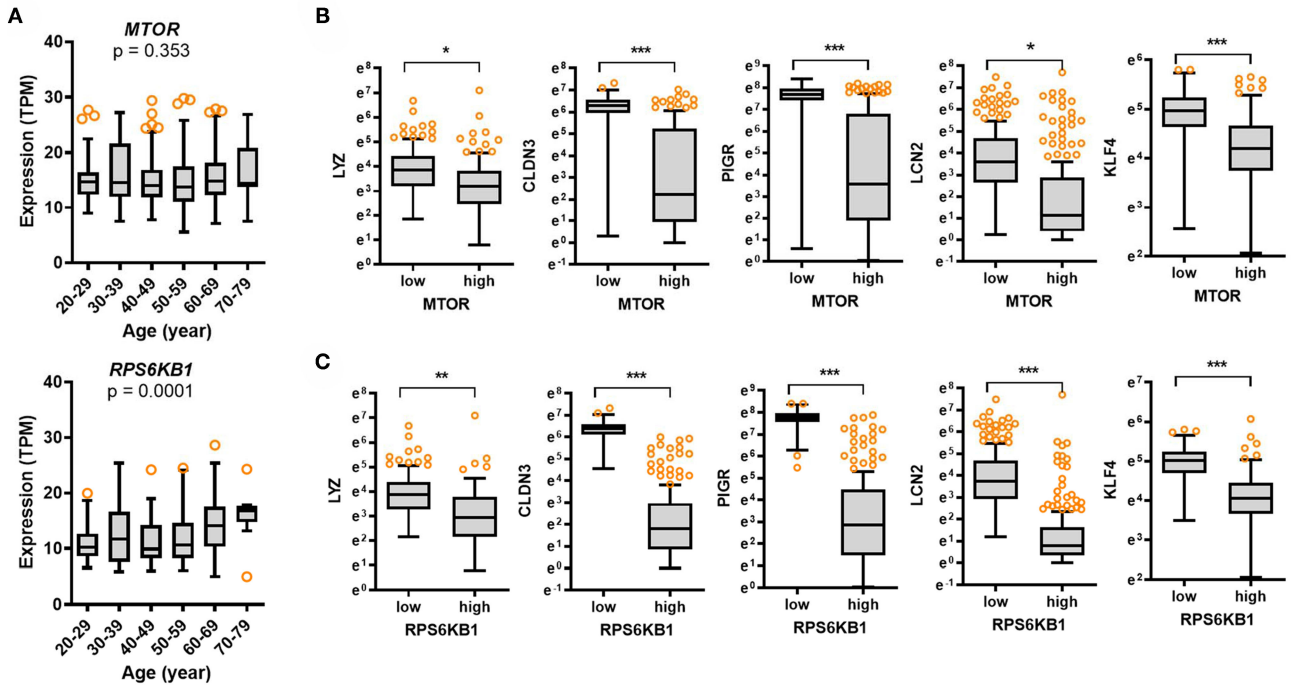

FIGURE 5 | Expression of cell metabolic stress markers and their association with gut defense biomarkers with age. **(A)** Results are depicted as box-and-whisker plots (Turkey) for expression of cellular metabolic stress markers (*mTOR or RPS6KB1*) in normal mucosal intestinal tissues (GTEx dataset v8). Values are presented as transcripts per million (TPM). Statistical significance of the expression variation with age is illustrated on the top of each plot (Kruskal–Wallis test). **(B,C)** Considering the *mTOR* **(B)** or *RPS6KB1* **(C)** levels, we selected the 150 highest and 150 lowest level samples, which were further evaluated based on *LYZ, CLDN3, PIGR,* and *LCN2* levels. Values are presented as natural logarithm of TPM. Asterisks (*) indicate significant differences from the low expression group ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

eIF2α kinase and mTOR-S6 kinase (**Figure 6C**). Nevertheless, the expression of viral receptors diminishes with age, thereby elevating two stress signaling modules and subsequently weakening the gut barrier defense in elderly subjects. The gut acts as an alternative source of SARS-CoV-2 infection, leading to symptoms such as diarrhea and prolonged fecal shedding of the virus, which potently occurs due to high levels of SARS-CoV-2 receptors in the gastrointestinal tract. High expression of *ACE2* in the intestinal epithelial cells implicates two potent routes of infection into the gastrointestinal tract. First, the well-known airway infection via human-to-human transmission presumably spreads via circulation to the rest of the body including gut and liver. The second route of gastrointestinal infection is airway-bypassing fecal–oral transmission from infected water or food. In particular, ACE2 acts as a coreceptor for nutrient uptake and particularly amino acid absorption from food (25), thereby indicating that SARS-CoV-2 in the contaminated food utilizes the receptor for its entry into the human body. Based on recent clinical evidences, ∼50% of the COVID-19 patients present detectable levels of fecal SARS-CoV-2 RNA even after its clearance from the respiratory tract (11–13, 15, 25, 26), indicating that the digestive tract may act as a major site of viral replication and activity. Moreover, the infected gastrointestinal tract can be a crucial source of proinflammatory mediators such as bacterial products, metabolites, and gut-derived immune components which reversely aggravate the disease severity in the respiratory tract and other organs in infected hosts. This gut-to-airway infection supports the recent experimental evidence that intragastric inoculation of SARS-CoV-2 causes productive infection and leads to pulmonary pathological changes (27). Collectively, the enteric entry and replication of SARS-CoV-2 can be one of pivotal pathogenic pathways in addition to the airway infection.

Expression of the SARS-CoV-2 receptor is high in the gut; however, it decreases with age according to our transcriptomic analysis of the clinical dataset (**Figure 1A**). Nevertheless, the elderly subjects are more susceptible to COVID-19 than the younger groups in the recent global pandemic. In the present study, we propose mechanistic links of high disease severity in the elderly patients with low level of SARS-CoV-2 receptors. In addition to the SARS-CoV-2 receptors, the virus can impact host physiology via ISR. In case of SARS-CoV-2 infection, cells display EIF2AK2 (PKR)-linked pathogenesis including the ribosomal stress response via specific cleavage of 28S rRNA (19–21). Even though levels of the virus entry receptors decrease with age, the viral RNA triggers ribosomal stress leading to PKR activation and ISR via pattern recognition receptors, which can contribute to SARS-CoV-2-induced mucosal pathogenesis. Epithelial PKR activation plays a pivotal role in gut barrier disruption by regulating the lipid raft including caveolae (28).

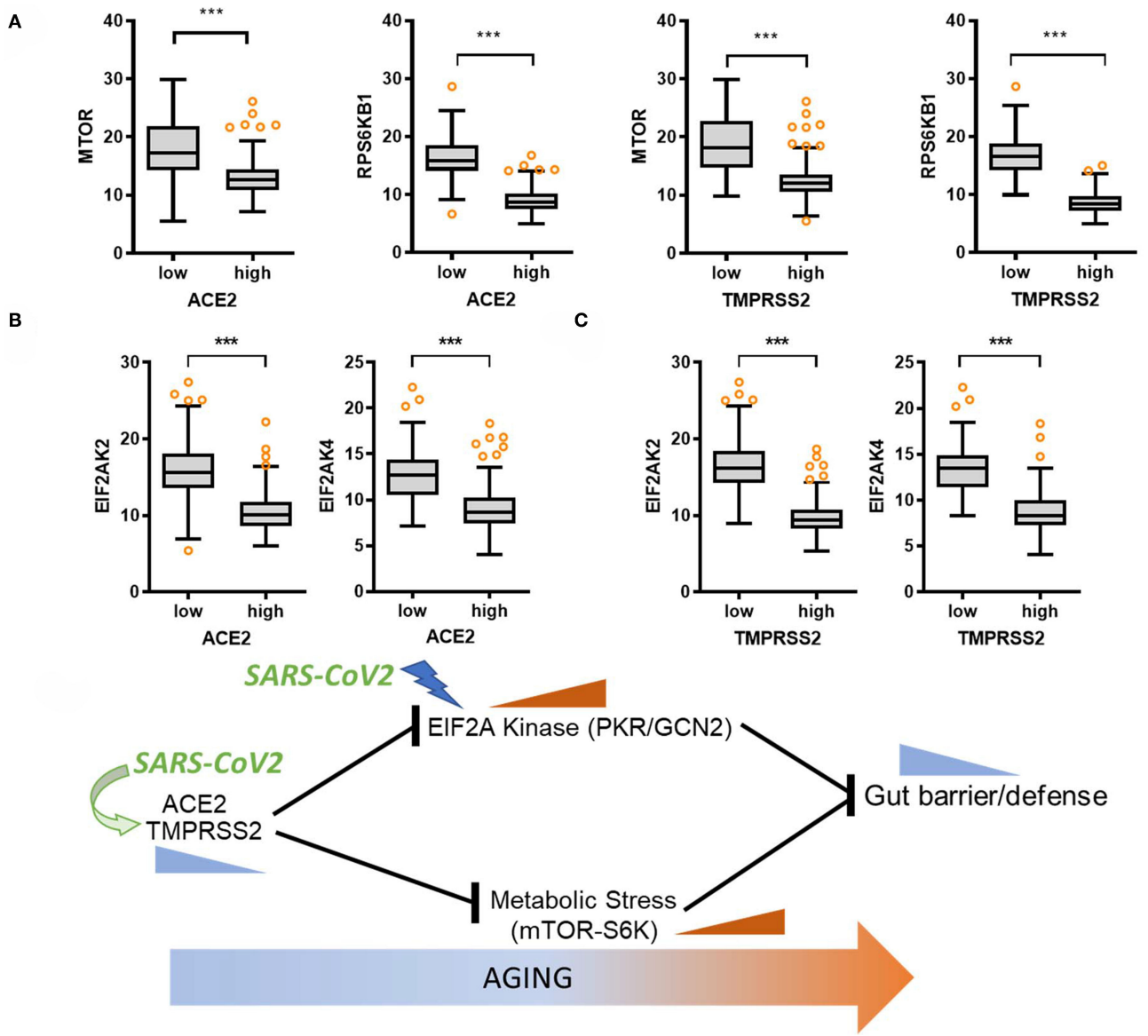

FIGURE 6 | Regulation of stress responsive biomarkers by SARS-CoV-2 receptor. Considering the *ACE2* or *TMPRSS2* levels in normal mucosal intestinal tissues (GTEx dataset v8), we selected the 150 highest and 150 lowest level samples, which were further evaluated based on the expression of cell metabolic stress markers (**A**, *mTOR* or *RPS6KB1*) or eIF2α kinases (**B**, *EIF2AK2 or EIF2AK4*). Values are presented as transcripts per million (TPM). Asterisks (*) indicate significant differences from the low expression group (***$p < 0.001$). **(C)** A putative scheme for SARS-CoV-2 receptor-mediated modulation of gut barrier defense with age. In response to SARS-CoV-2 infection, SARS-CoV-2-responsive receptors positively contribute to maintenance of gut barrier defense via suppressing two stress signaling pathways including eIF2α kinase and mTOR-S6 kinase.

Moreover, lipid rafts contribute to SARS-CoV-2 infection in the early replication process (29, 30). Notably, ACE2 is located in the lipid rafts, which potently plays a pivotal role in the initial step of the virus entry-triggered signaling. PKR activation-induced structural alterations in lipid rafts facilitate caveolae-mediated degradation of epidermal growth factor receptor that is a crucial signaling mediator for maintaining the gut epithelial barrier integrity (28). PKR-linked molecular events during virus entry are well consistent with the patterns in clinical transcriptome analyses in the present study. Elevated levels of PKR signaling were associated with deterioration of gut defense with age despite attenuated *ACE2* expression in the elderly subjects. Therefore, ISR-linked disruption of gut defense may be an important mechanism of COVID-19 severity in elderly groups with low level of SARS-CoV-2 receptors.

We propose that mTOR-S6 kinase signaling is inversely associated with the expression of *KLF4*, a key intestinal differentiation factor. Due to insufficient cues for differentiation,

formation of goblet and Paneth cells can be retarded in the gut barrier, which results in deficiencies in mucus and lysozyme secretion (24). Therefore, aging-associated increase of mTOR-S6 kinase signaling potently counteracts KLF4-mediated differentiation of the gut barrier cells, which can account for reduced mucosal defense against SARS-CoV-2 infection in the elderly population. Moreover, mTOR-S6 kinase signaling directly inhibits adenosine monophosphate-activated protein kinase (AMPK), the key regulator of energy metabolism, to promote cell proliferation under nutrient stress (31). Since AMPK improves gut epithelial differentiation and barrier function (32), mTOR may downregulate gut defense via attenuation of AMPK pathway. Mechanistically, AMPK inactivation is associated with reduced expression of caudal type homeobox 2 (*CDX2*), the key transcription factor for intestinal epithelium maturity and Paneth cell development (33). Detailed molecular epigenetic machinery of *CDX2* expression can be associated with polycomb repressive complex 2-regulated enrichment of H3K27me3 and lysine-specific histone demethylase-1-mediated reduction of H3K4me3 (32). Collectively, cell metabolic stress signaling of mTOR-S6 kinase potently attenuates AMPK activation, thus contributing to immature epithelial barrier via insufficient cellular differentiation with age. Although the expression of SARS-CoV-2 receptors is inversely associated with two stress signaling modules (eIF2α kinase and mTOR-S6 kinase), the levels of SARS-CoV-2 receptors diminish with age. Instead, elevated two stress signaling modules were positively involved in defective gut defense in the elderly subjects. Furthermore, disrupted gut barrier may increase the exposure to infectious agents and subsequently excessive inflammatory responses, which could be a crucial step of COVID-19 severity associated with age; however, extensive experimental evidences are still warranted to support the clinical transcriptome-based predictions of age-associated responses to COVID-19 and future interventions with the planet disorder.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Project design and hypotheses were developed by YM. YM analyzed the data, prepared the manuscript, and supervised the overall project.

FUNDING

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A3B05041889).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.606991/full#supplementary-material

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 12 January 2021
doi: 10.3389/fmed.2020.613475

Down-Regulation of Colonic ACE2 Expression in Patients With Inflammatory Bowel Disease Responding to Anti-TNF Therapy: Implications for COVID-19

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 02 October 2020
Accepted: 11 December 2020
Published: 12 January 2021

Citation:
Li X-Z, Qiu Y, Jeffery L, Liu F, Feng R, He J-S, Tan J-Y, Ye Z-Y, Lin S-N, Ghosh S, Iacucci M, Chen M-H and Mao R (2021) Down-Regulation of Colonic ACE2 Expression in Patients With Inflammatory Bowel Disease Responding to Anti-TNF Therapy: Implications for COVID-19. Front. Med. 7:613475. doi: 10.3389/fmed.2020.613475

Background and Aims: Angiotensin-converting enzyme II (ACE2) is the key molecule for understanding the pathophysiology of COVID-19. The risk of COVID-19 and impact of immunosuppressive treatment on disease course in patients with inflammatory bowel disease (IBD) remain controversial. We aimed to determine the change of intestinal ACE2 expression before and after biologics treatment including anti-tumor necrosis factor α (anti-TNFα), anti-integrin, and anti-interleukin (IL)12/23 in IBD patients.

Methods: We analyzed the ACE2 expression through the public database of paired intestinal biopsies from IBD patients before and after biologic therapy. Change of ACE2 RNA and protein expression were validated in two independent cohorts (Birmingham cohort and Guangzhou cohort). The correlation between ACE2 expression and disease activity was also analyzed.

Results: Mining information from the GEO database showed that compared with healthy control, intestinal ACE2 expression was downregulated in ileum of CD patients, while upregulated in colon of both CD and UC patients. Colonic ACE2 RNA expression was decreased significantly in patients responding to anti-TNFα but not anti-integrin and anti-IL12/23, which was validated in the Birmingham cohort. Using the Guangzhou cohort including 53 patients matched by pre- and post-anti-TNFα therapy, colonic ACE2 protein expression was significantly downregulated after anti-TNFα treatment in responders ($P < 0.001$) rather than non-responders. Colonic ACE2 expression was significantly higher in patients with severe histologically active disease compared with those with moderate ($P < 0.0001$) and mild ($P = 0.0002$) histologically active disease.

Conclusion: Intestinal inflammation influences the expression of intestinal ACE2 in IBD patients, with different alterations in the ileum and colon. Colonic ACE2 expression was

downregulated after anti-TNFα therapy in IBD patients responding to treatment. This might provide new clues regarding the risk of SARS-CoV-2 infection and the potential benefit of sustaining anti-TNFα treatment in patients with IBD.

Keywords: COVID-19, ACE2, inflammatory bowel disease, intestine, anti-TNFα

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has widely spread around the world. Angiotensin-converting enzyme II (ACE2) has emerged as a key molecule in the pathophysiology of COVID-19 (1). ACE2 is expressed in the respiratory tract as well as gastrointestinal tract (2, 3). Emerging data (4–7) showed that the SARS-CoV-2 may actively infect and replicate in the human gut enterocytes or organoids and might be transmitted via fecal-oral transmission.

Given the use of immunosuppressive agents as well as malnutrition status, patients with inflammatory bowel disease (IBD) are generally at increased risk of infection. Unexpectedly, current epidemiology of SARS-CoV-2 infection in IBD patients, including published reports from China (8), Spain (9), Italy (10, 11), and the global data from the SECURE-IBD registry (https://covidibd.org/) (12), did not support a higher risk of COVID-19 in IBD patients compared to that in the general population.

Similar immune signatures in IBD and COVID-19 indicate that medications of IBD may play a potential role in the treatment of COVID-19. Some studies supported that infliximab downregulated ACE2 expression in colon tissue of IBD (13, 14). Another recent study has showed that biologics and steroids are linked to the significantly lower expression of ACE2 in intestinal lamina propria CD11b-enriched cells (15). However, to our knowledge, few studies have investigated the influence of biologics on ACE2 expression in gut enterocytes which are directly exposed to the virus. Our study identified the ACE2 expression with the public database and then validated RNA and protein expression using clinical samples from two independent cohorts including China and the UK aiming to determine the alter expression of intestinal ACE2 especially in enterocytes before and after biologic therapy including anti-tumor necrosis factor α (anti-TNFα), anti-integrin and anti-interleukin (IL) 12/23 in IBD patients.

METHODS

Transcriptomic Change of Intestinal ACE2 Pre- and Post-biologic Therapy From the Gene Expression Omnibus (GEO) Database

We searched the gene expression data sets regarding biologics in IBD in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) with the key words "Inflammatory bowel disease/Crohn's disease/Ulcerative colitis/IBD/CD/UC," "intestine/tissue" and "Homo sapiens." The inclusion criteria from the data sets were: (1) intestinal tissue from patients with IBD; (2) paired samples were collected before and after various biologics and small molecule inhibitors therapy including but not limited to anti-TNFα, anti-integrin, and anti-IL12/23; (3) therapeutic efficacy (i.e., response or not) of each patient was described; (4) the number of samples per group was not <12 (16). We extracted the expression value of ACE2 and used two-class paired or unpaired analyses according to experimental design. Value distributions were evaluated.

Validation of Intestinal ACE2 RNA Expression Change by Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Pre- and Post-anti-TNFα Therapy

This validation was conducted in a cohort at the University of Birmingham, UK. Colonoscopy-confirmed active UC and CD patients were recruited prior to initiation of anti-TNFα (Infliximab [IFX] or adalimumab [ADA]) therapy. Institutional research ethics approval was obtained for the study and all patients had signed informed consent.

Colonoscopic biopsies were taken from the inflamed segments of IBD patients (UC and CD) before and 12–16 weeks after starting treatment with biologics. The endoscopic response was judged by Mayo endoscopic score 0–1 or Simple Endoscopic Score-CD decrease of 50% or greater at week 12–16 compared to baseline (17). Biopsies were transferred immediately to "RNA later" upon collection and stored at 4°C prior to lysis and gentleMACS homogenization (Miltenyi Biotec) followed by RNeasy on-column RNA extraction and purification (Qiagen). RNA was quantified by Qubit (Life Technologies) and 1.5 μg reverse transcribed using iScript reagents (Bio-Rad). Expression of ACE2 receptor relative to 18SrRNA was measured by qPCR using Taqman reverse transcription gene-assays (18SrRNA: 4319413E. ACE2: Hs01085333_m1) (Life Technologies). Reactions were performed in triplicate as singleplex assays and expression of ACE2 relative to 18SrRNA calculated by $10^6(2^{-dCt})$. The expression change between pre- and post-treatment was tested.

Validation of Intestinal ACE2 Protein Expression Change Using Immunohistochemistry (IHC) Assays Pre- and Post-anti-TNFα Treatment

To determine the protein expression of ACE2 in the intestinal epithelial cells, patients with CD receiving anti-TNFα treatment were included from the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. All patients underwent colonoscopy by gastroenterologists with more than 5 years of experience in IBD before and 12–14 weeks after anti-TNFα

treatment, and all biopsies were taken from inflamed gut segments. Institutional research ethics approval was obtained for the study.

IHC was performed using paraffin-embedded tissues from intestinal mucosal biopsies obtained during endoscopy from the IBD patients mentioned above. Tissue sections were deparaffinized in xylene and hydrated through a graded series of alcohol to tap water. Antigen was retrieved in citrate buffer for 20 min and cooled to room temperature. The sections were incubated with 3% H_2O_2 in distilled water for 15 min. After being rinsed three times with phosphate-buffered saline (PBS), the sections were incubated with rabbit polyclonal IgG primary antibodies against ACE2 (1:500 dilution, ab15348; Abcam; USA) overnight at 4°C. Then, the sections were incubated with the secondary antibody (1:50000, ab205718; Abcam; USA) for 30 min at room temperature, followed by two times 5 min washing with PBS. Finally, the sections were stained with hematoxylin. The protein expression of ACE2 was evaluated in a random and blinded fashion and was assigned an IHC score, which was based on the approximate percentage of positively stained cells over overall intestinal epithelial cells (ranging from 0 to 100%), as described in previously published methods (18).

Assessment of Disease Activity and Definition of Outcome

The disease activity of the CD patients from the China cohort was analyzed using endoscopic and histologic assessment. The Crohn's Disease Endoscopic Index of Severity (CDEIS) score was used for endoscopic scoring. Consistent with the STRIDE guidelines (17), a decrease by >5 or at least 50% from baseline in CDEIS demonstrates endoscopic response. A semiquantitative evaluation for endoscopic disease activity was given as follows: CDEIS <3 suggested inactive, 3–8 mildly active, 9–12 moderately active, and >12 severely active (19).

Histological disease activity was assessed by a blinded IBD experienced pathologist in random order. The modified Global Histologic Disease Activity Score (mGHAS) (20, 21) was used and the histologic response was defined as modified GHAS $\leq$4 in those patients with baseline score >4. A semiquantitative evaluation for histological disease activity was given as follows: inactive, 0; mildly active, 1–5; moderately active, 6 −10; and severely active, 11–14.

Statistical Analysis

The original expression data was collected and then plotted by GraphPad Prism 8 (GraphPad Software, La Jolla, CA). All statistical analyses were performed IBM SPSS Statistics 25.0 software package (IBM, Armonk, NY, USA). Continuous variables were summarized as medians and interquartile ranges (IQRs). The Student's *t*-test or analysis of variance (ANOVA) test was used for parametric tests, while the Wilcoxon signed-rank test or Kruskal-Wallis test was performed for non-parametric tests. The Spearman correlation was used to evaluate the relationship of IHC score and endoscopic or histological disease activity score. All statistical testing was two-sided, and $P < 0.05$ was considered significant and indicated as follows: ns, not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; $^{****}P \leq 0.0001$.

RESULTS

Intestinal ACE2 Expression in GEO Database

Five GEO datasets [GSE16879 (22), GSE23597 (23), GSE92415 (24), GSE73661 (25), and GSE112366 (26)] were included in the final analysis. Detailed information for datasets included was summarized in **Table 1**. Compared with healthy control, intestinal ACE2 expression was downregulated in ileum of CD patients (GSE16879), while upregulated in colon of both CD and UC patients (GSE16879, GSE92415, and GSE73661), significantly.

As shown in **Figures 1A–C**, ACE2 expression in colon tissue was decreased significantly in patients responding to anti-TNFα (except GSE23597). On the contrary, ACE2 in ileum tissue was upregulated significantly in CD patients using anti-TNFα regardless of the response status (GSE16879). Intestinal ACE2 expression did not decrease after VDZ or UST treatment (**Figures 1D,E**).

Colonic ACE2 RNA Expression Was Downregulated in IBD Patients Responding to anti-TNFα

In the UK cohort, we studied 24 IBD patients (11 CD, 13 UC) initiating biologic therapies (CD 8 ADA, 2 IFX, 1 UST; UC 5 ADA, 4 IFX, 4 VDZ). The baseline characteristics of patients are shown in **Table 2**. **Figure 1F** shows a statistically significant decrease in colonic expression of ACE2 in responders ($n = 11$) to anti-TNFα ($P = 0.0250$). Non-responders to anti-TNFα (4 UC, 1 CD) did not exhibit any significant decrease in ACE2 expression. Patients treated with VDZ did not show any significant decrease in ACE2 expression.

Colonic ACE2 Protein Expression Was Downregulated in CD Patients Responding to anti-TNFα

In the China cohort, we included 66 CD patients for IHC validation and found 53 patients matched by pre- and post-anti-TNFα therapy (**Supplementary Table 1**). The baseline characteristics of patients are shown in **Table 2**. As demonstrated in **Figure 2A**, in both endoscopic and histologic assessments, ACE2 expression was significantly downregulated in colonic biopsy after anti-TNFα treatment in responders ($P < 0.001$) rather than non-responders (the representative IHC images are shown in **Figure 2D**). Besides, ACE2 protein expression in ileum increased after anti-TNFα treatment in responders ($n = 3$). There was no difference in ACE2 protein expression in colon or ileum of non-responders.

Colonic ACE2 Protein Expression Positively Correlated With Disease Activity

We studied 147 specimens with different disease activity from total CD patients of the China cohort. Colonic ACE2 expression was significantly higher in patients with severe histologically active disease compared with those with moderate ($P < 0.0001$) and mild ($P = 0.0002$) histologically active disease. Ileal ACE2

TABLE 1 | Summary of included GEO datasets.

Patients Cohort	Healthy Control	IBD Type				Response or not		Matched Comparison (Yes/No)	Sample Source	Study Time Point[c]	Definition of Outcome[d]	Treatment	GEO Dataset
		CD[a]			UC	Responders Pair[b]	Non-responders Pair						
		Ileal CD (L1)	Colonic CD (L2)	Ileocolonic CD (L3)									
Leuven, Belgium (20)	12	18	19	0	24	28 L1: 8 L2: 12 UC: 8	33 L1: 10 L2: 7 UC: 16	Yes	ileum (L1), colon (L2)	weeks 0, 4/6	Endoscopic and Histological score	IFX (5mg/kg)	GSE16879
ACT1 study (21)	0	0	0	0	48	49 IFX/PBO w0-8: 18/3 IFX/PBO w0-30: 14/4 IFX/PBO w0-8-30: 9/1	28 IFX/PBO w0-8: 7/5 IFX/PBO w0-30: 5/6 IFX/PBO w0-8-30: 3/2	Yes	colon	weeks 0, 8, 30	Endoscopic score	IFX (5/10 mg/kg) PBO	GSE23597
PURSUIT-SC study (22)	21	0	0	0	162	82 (unpaired) GLM w0/6: 32/29 PBO w0/6: 11/10	80 (unpaired) GLM w0/6: 27/21 PBO w0/6: 17/15	No	colon	weeks 0, 6	Endoscopic score	GLM	GSE92415
GEMINI study (23)	12	0	0	0	67	29 IFX w0-4/6: 8 VDZ w0-6: 6 VDZ w0-12: 5 VDZ w0-52: 10	51 IFX w0-4/6: 15 VDZ w0-6: 21 VDZ w0-12: 10 VDZ w0-52: 5	Yes	colon	IFX weeks 0, 4/6 VDZ weeks 0, 6, 12, 52	Endoscopic score	IFX, VDZ, PBO	GSE73661
UNITI study (24)	26	26	18	54	0	56 w0-8: 35 w0-44: 21	42 w0-8: 29 w0-44: 13	Yes	ileum	weeks 0, 8, 44	CDAI score	UST	GSE112366

[a]Disease subtypes are classified according to Montreal classification; [b]Responders/non-responders pair, one pair means that the patient has biopsy samples before and after treatment; [c]Study time point, time to definite response status; [d]Definition of outcome, Method to definite response/non-response after treatment.

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; IFX, infliximab; GLM, golimumab; VDZ, vedolizumab; UST, ustekinumab; PBO, placebo; CDAI, Crohn's Disease Activity Index.

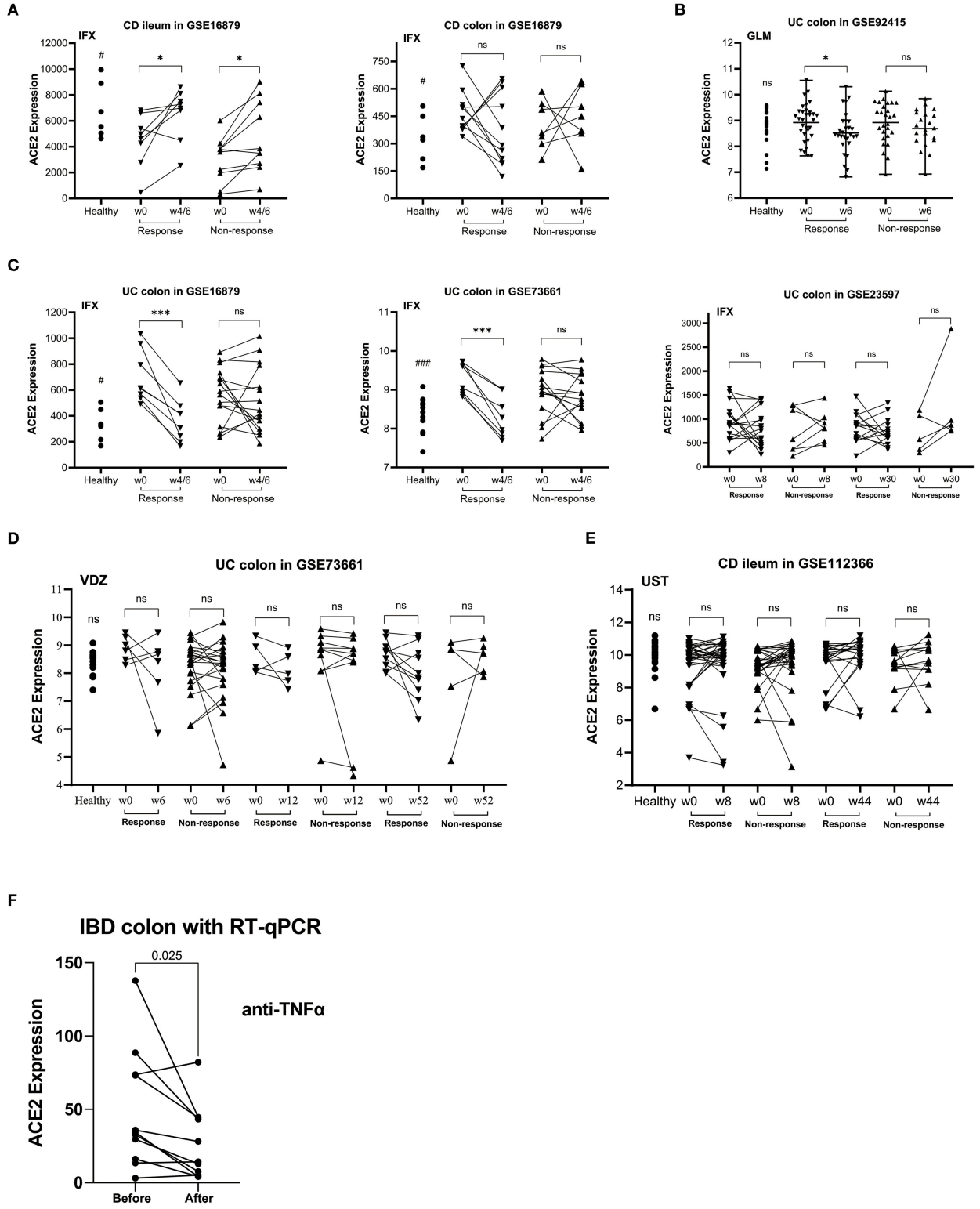

FIGURE 1 | The relative ACE2 mRNA expression level of intestinal mucosal biopsy specimens before and after biologic therapy with anti-TNFα (infliximab/IFX, **A,C**; golimumab/GLM, **B**), vedolizumab/VDZ **(D)** or ustekinumab/UST **(E)** in patients with CD **(A,E)** or UC **(B–D)** from GEO data sets. **(F)** RT-qPCR data of the intestinal mucosal ACE2 expression in IBD responders before and after anti-TNFα therapy. In the matched comparison **(A,C–F)**, lines between two samples represent the change in ACE2 expression before and after treatment for one patient. In the unpaired comparison **(B)**, mean and range are shown in the scatterplot. There was not statistical difference between the IBD patients before and after therapy. ns, not significant; $^{*}P < 0.05$; $^{***}P < 0.001$ (in patients after vs. before therapy). $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$ (in healthy controls vs. patients before therapy).

TABLE 2 | Baseline characteristics of patients with inflammatory bowel disease enrolled in validation cohorts.

Characteristics	Total Patients (China cohort)	Total Patients (UK cohort)
No. of patients	66	24
Male, *N* (%)	43 (65.2%)	12 (50%)
Age at time of collection (years)		
Mean (SD)	23.4 (8.9)	42.0 (12.6)
Range	10–46	24–63
Duration of disease (months)		
Mean	44.7 (55.8)	121.0 (106.0)
Range	1–366	6–372
Crohn's Disease (CD), *N*	66	11
Location, *N*		
L1, Ileal	5	4
L2, Colonic	3	5
L3, Ileocolonic	58	2
Disease behavior, *N*		
B1, Non-stricturing, non-penetrating	39	6
B2, Stricturing	19	3
B3, Penetrating	8	2
Ulcerative Colitis (UC), *N*	0	13
Proctitis (E1)	0	1
Left sided (E2)	0	5
Extensive (E3)	0	7
Biologics commenced, *N*		
Anti-TNF	66 (all CD)	19 (10 CD, 9 UC)
Vedolizumab	0	4 (all UC)
Ustekinumab	0	1 (CD)

expression was comparable among different disease activity groups. When stratifying the disease activity by endoscopic score, no significant difference existed among disease activity groups (**Figure 2B**).

As is shown in **Figure 2C**, ACE2 expression positively but weakly correlated with histological disease activity ($\rho = 0.3357$, 95% confidence interval [CI] 0.1502 to 0.4983, $P = 0.0004$) and endoscopic disease activity ($\rho = 0.1881$, 95% CI −0.0105–0.3723, $P = 0.0559$) in colon, while no correlation existed between ileal ACE2 expression and histological or endoscopic disease activity.

DISCUSSION

There are controversies about the risk of SARS-CoV-2 infection in patients with IBD (27). It was reported that the soluble form of ACE2, which acts as a competitive binding partner for SARS-CoV-2, is up-regulated in the peripheral blood of IBD patients and then limits SARS-CoV-2 infection (28, 29). Several studies showed that IBD medications especially biologics could regulate the intestinal ACE2 expression of IBD (13–15). However, few studies have directly investigated the influence of biologics on ACE2 expression in gut enterocytes which are directly exposed to the virus. The two recent landmark studies (6, 7) have confirmed that SARS-CoV-2 could productively infect human gut enterocytes and intestinal organoids. In our study, we found that IBD patients had a higher expression of ACE2 in colon tissue while lower in ileum tissue vs. healthy control, which was consistent with the published data (13). Additionally, our result showed that the expression of colonic epithelial ACE2 was downregulated in IBD patients responding to anti-TNFα therapy, using GEO data analysis and then validated with qPCR and IHC assays. These results might provide new evidence and knowledge to the risk of SARS-CoV-2 infection in patients with IBD using different medications and the potential role of anti-TNFα in the treatment of COVID 19.

Our study demonstrated that intestinal epithelial ACE2 expression increased with more severe disease activity, which may be due to the higher inflammatory cytokines. Previous studies (30, 31) showed that ACE2 is increased in human bronchial epithelial cells infected by SARS-CoV-2 as a response to inflammatory cytokine stimulation including interferon (IFN)-γ. Several inflammatory cytokines like IFN-γ, TNFα, IL-1, and IL-6 could be upregulated in active IBD patients (28). In addition, ACE2 expression was downregulated after anti-TNFα therapy only in responders rather than non-responders. In the registered IBD patients with COVID-19 from SECURE-IBD (12), there were 762 patients with anti-TNFα therapy alone, 651 (85%) of whom recovered without hospital admission and four patients died in total. On the contrary, 65% of 773 patients with treatment of sulfasalazine/mesalamine recovered without hospital admission and 37 patients died. These data indicated that IBD patients with anti-TNFα treatment might have a better outcome of COVID than other medications (32). The potential explanations may have three points: (1) anti-TNFα treatment downregulated IFN-γ which would induced the expression of ACE2 through downregulating IFN-γ (32); (2) anti-TNFα treatment also downregulated other proinflammatory cytokines in "TNF dependent cytokine cascade," such as IL-1, IL-6 and IFN-γ which also play important roles in cytokine storm syndrome in COVID-19 (32); (3) anti-TNFα could induce a reduction in leucocyte trafficking due to reduction of adhesion molecules, vascular endothelial growth factor and chemokines in both IBD and COVID-19 (33, 34). Indeed, an urgent demand for clinical trials of anti-TNFα therapy for COVID-19 has been proposed recently (35). Moreover, the clinical trial of anti-TNFα in treating COVID-19 (ChiCTR2000030089) is ongoing. Future studies investigating the protective role of anti-TNFα for IBD or COVID 19 patients during the COVID-19 pandemic are warranted.

Except for the regulation of inflammatory cytokines, ACE2 may participate in intestinal stem cell proliferation, mucosal healing and crypt pathology in the pathogenesis of IBD. ACE2 plays an important role in the endothelial repair in acute lung injury (36) and the healing of gastric ulcers (37), potentially through reducing Angiotensin (Ang) II and increasing the production of Ang 1–7. A recent study (38) proposed that ACE2 contributed to the proliferation of intestinal stem cells and the maintenance of epithelial barrier function in DSS-induced colitis mice. ACE2-deficient mice developed increased intestinal epithelial injury associated with crypt damage compared to the

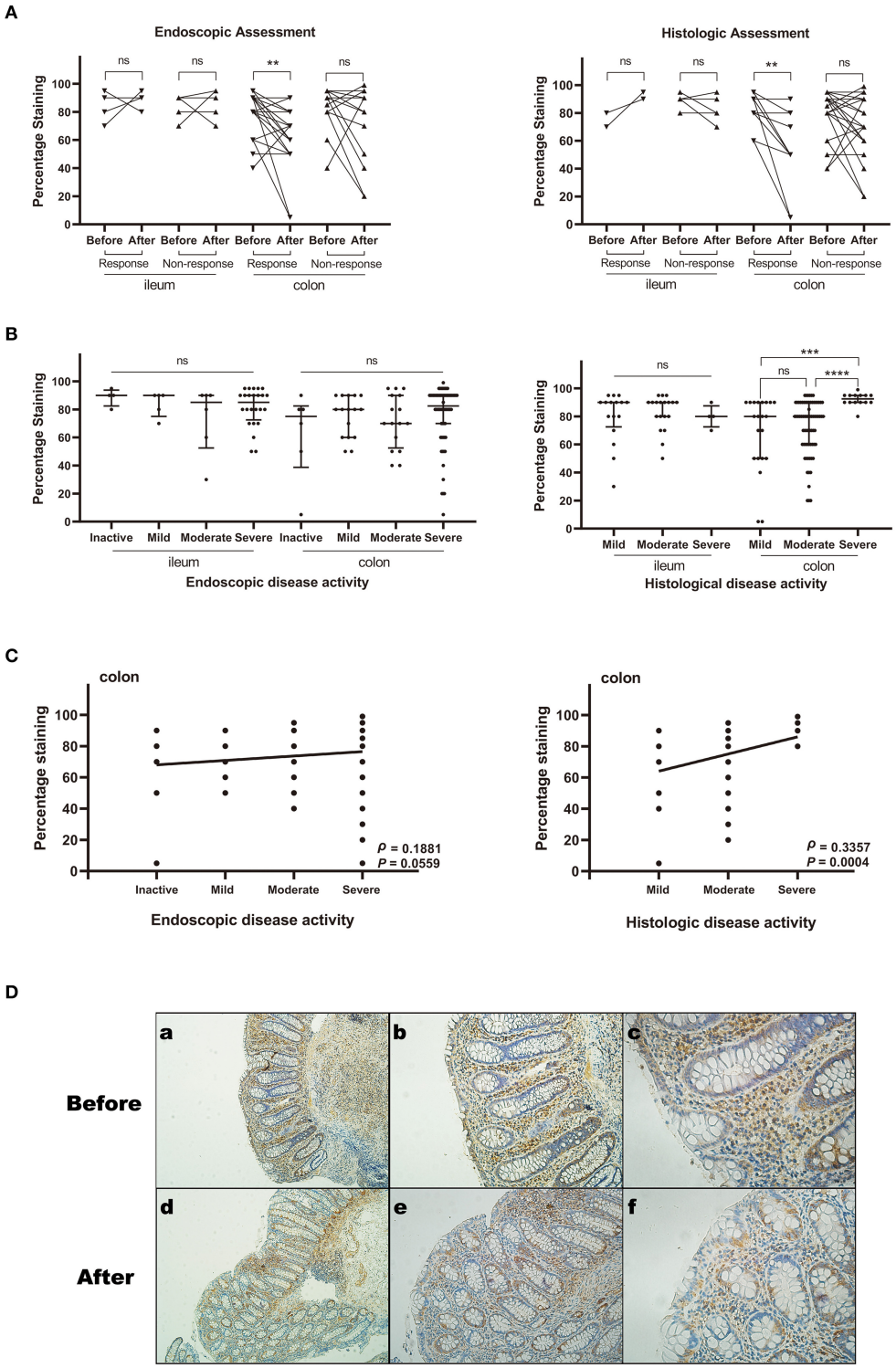

FIGURE 2 | The relative ACE2 protein expression in intestinal mucosal biopsy specimens from patients with CD by Immunohistochemistry assays. The expression level was measured by percentage of positively stained cells. The sample sizes of each group are shown in **Supplementary Table 1**. **(A)** ACE2 expression before and after anti-TNFα treatment (matched comparison). **(B)** ACE2 expression among different disease activity groups defined by endoscopic and histological assessment. Median and interquartile range are shown in the scatterplot. **(C)** Spearman rank correlation analysis between ACE2 expression of colonic epithelial cells and endoscopic or histological disease activity. **(D)** Representative images (immunohistochemical staining for ACE2) of the colonic biopsy specimens before and after anti-TNFα treatment. (a–c) Biopsy before treatment. The histological score is 6. The percentage staining of ACE2 in colonic epithelial cells is 80%. (d–f) Biopsy after treatment. The histological score is 1. The percentage staining of ACE2 in colonic epithelial cells is 20%. All scale bars are 100 μm. ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$, $^{****}P < 0.0001$.

wild-type mice (39). However, there have been debates on the role of ACE2 in IBD. It is also reported that an ACE2 inhibitor may have an anti-inflammatory effect in DSS-induced colitis mice (40). Considering the dual role of ACE2 in the development of colitis, it warrants further study.

In the current study, a significant difference of ACE2 expression was found in responders rather than non-responders to anti-TNFα in IBD patients with colonic involvement, which was validated with IHC assays of CD patients in China cohort (**Figure 2A**). There are two patients with significant changes of ACE2, whose endoscopic and histological scores post-treatment are both close to zero. It demonstrated that the ACE2 may play an important role in the anti-TNFα mediated anti-inflammatory pathways in colonic CD. The difference was still statistically significant when taking out these two patients. Anti-TNFα is the mainstay of CD treatment. Nonetheless, around one-third of CD patients experience a loss of response (41). Besides, ACE2 or renin-angiotensin system (RAS) has been demonstrated to influence the inflammation and fibrosis in IBD (18). Thus, whether ACE2 or RAS could help for predicting response to anti-TNFα treatment deserves more research. It is unclear whether the concomitant medication influence ACE2 expression. In the present study, of 53 patients with intestinal biopsies pre- and post-anti-TNF therapy, none of them were on concomitant steroid use, and only one patient was on recent methylprednisolone use before anti-TNF therapy. There were 45 patients who received combination therapy of anti-TNF with azathioprine (**Supplementary Table 2**), most of whom had treatment failure of azathioprine before accelerating anti-TNF therapy. We further performed a subgroup analysis of patients on anti-TNF and azathioprine therapy and came to the same conclusion that colonic ACE2 was decreased significantly in patients responding to anti-TNFα (endoscopic response, $P = 0.0096$; histologic response, $P = 0.0039$). In recent studies (14, 32), international data from SECURE-IBD highlighted the association of corticosteroids with adverse COVID-19 outcomes and the probable safety of anti-TNF. The association between monotherapy or combination therapies and the risk of COVID-19 has been explored in some observational studies (14, 32, 42). Our study used paired samples before and after anti-TNF therapy, which could minimize the inter-individual differences such as concomitant medication. However, some confounding factors are inevitable in our current retrospective study. Further prospective well-designed studies are needed to validate our results.

Our study also showed that anti-TNFα could downregulate the ACE2 expression level in colon of patients with IBD rather than in ileum. ACE2 in ileum tissue was upregulated significantly in CD patients using anti-TNFα regardless of the response status (GSE16879, **Figure 1A**), which demonstrated that anti-TNFα may not influence the ileal ACE2 expression. Numerous previous evidence (43) supported that colonic CD is a different phenotype from ileal CD at the level of genetics, macroscopic, cellular immunology, microbiota, and treatment. It is worth mentioning that isolated ileal disease location has been observed to be a negative predictor of responses to anti-TNFα therapy in several cohort studies and there was no significant difference in efficacy of VDZ treatment in different locations (44). Therefore, we speculated that the RAS may be an important factor in the TNF-pathway of colonic CD and UC.

There was a positive correlation between epithelial ACE2 expression and disease activity, and the association was stronger using histological score compared to endoscopic score. Endoscopic and clinical measurements are predominately used to determine response to therapy in IBD. There has been growing interest in using histological score as measuring disease activity and treatment outcome. Previous studies (45) have shown that endoscopic assessment and clinical measures may not adequately reflect disease activity, whereas histologic measurement is more sensitive to detect disease activity and predict response to therapy.

One strength of the present study was that we included the data of matched intestinal mucosal biopsies from IBD patients before and after biologic therapy, so participant variables (i.e., individual differences) are reduced. Besides, we provided three sets of data to support our ACE2 expression changes after biologics use especially the down-regulation after anti-TNFα treatment and validated in IBD cohorts from different countries. More importantly, we not only assessed the disease activity and response by endoscopic score but also histological score which was better to illustrate the association between intestinal epithelial ACE2 expression and inflammatory activity.

Several certain limitations also existed. Firstly, because of the inconvenience of collecting biopsies from patients during the COVID19 pandemic, the validation of ACE2 mRNA and protein was conducted in two separate cohorts and a small amount of ileum tissue was included, which limited the assessment of the difference in ACE2 expression between terminal ileum and colon. Besides, we did not include samples for validation of ACE2 protein expression before and after VDZ/UST treatment, given these two biologics were not available in China before 2020. Finally, the validation of ACE2 protein expression did not included UC patients, because there were insufficient numbers of specimens of UC patients with anti-TNFα treatment to conduct statistical analysis. Further research is needed to confirm these findings.

In conclusion, our study showed that colonic ACE2 expression was downregulated after anti-TNFα therapy in IBD patients responding to treatment. This might provide new clues regarding the risk of SARS-CoV-2 infection and the potential benefit of maintaining anti-TNFα treatment in patients with IBD.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University. University of Birmingham

Human Biomaterials Resource Centre. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RM and M-HC conceived and supervised the overall study. X-ZL, MI, and RM wrote the manuscript. FL, RF, Z-YY, J-SH, J-YT, S-NL, SG, MI, M-HC, and RM critically revised the manuscript. X-ZL, YQ, and LJ performed the experiment and analyzed the data. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (81970483, 81700482), Guangdong Natural Science Foundation (2017A030310211), and Guangdong Medical Research Foundation (A2017292).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.613475/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 13 January 2021
doi: 10.3389/fmed.2020.587350

COVID-19 With Preexisting Hypercoagulability Digestive Disease

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The outbreak of coronavirus disease of 2019 (COVID-19) has become a global public health and economic crisis. The advent of hypercoagulability and thrombotic complications can substantially influence the prognosis of COVID-19 patients. In this review, we elaborate on the clinical findings, potential underlying pathogenesis, and therapeutic strategy of hypercoagulability and thromboembolism in COVID-19, particularly focusing on the COVID-19 patients with preexisting digestive hypercoagulability disease.

Keywords: coronavirus disease 2019, hypercoagulability, thromboembolism, SARS-CoV-2, inflammatory bowel disease

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *25 July 2020*
Accepted: *11 December 2020*
Published: *13 January 2021*

Citation:
Jiang M, Mu J, Shen S and Zhang H (2021) COVID-19 With Preexisting Hypercoagulability Digestive Disease. Front. Med. 7:587350. doi: 10.3389/fmed.2020.587350

INTRODUCTION

The coronavirus disease of 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was initially reported in Wuhan, China and then rapidly spread throughout China and even all over the world within a few months, resulting in a global public health and economic crisis (1, 2). As of June 22, 2020, the total number of Coronavirus cases had already risen to 9,060,780, with 470,939 deaths according to the data published on WHO (https://www.worldometers.info/coronavirus/). SARS-CoV-2 is a positive strand RNA coronavirus which belongs to the family *Coronaviridae*. To date, seven human coronaviruses (HCoVs) have been identified, including SARS-CoV-2, respiratory syndrome coronavirus (SARS-CoV), CoV-229E (alpha coronavirus), CoV-NL63 (alpha coronavirus), CoV-OC43 (beta coronavirus), CoV-HKU1 (beta coronavirus) and Severe Acute Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 transmission is mainly via respiratory transmission and direct contact infection. Angiotensin converting enzyme-2 (ACE2) protein is the functional receptor of SARS-CoV-2, which is widely distributed in lung, heart, blood vessels, kidney, and gastrointestinal tract (3–5). Therefore, SARS-CoV-2 not only affects the respiratory system but also affects the gastrointestinal tract, cardiovascular system, and central nervous system. COVID-19 is mainly characterized by symptoms of fever, dyspnea and dry cough. The severe complications reported so far are respiratory failure which is the main reason of inpatient death, acute respiratory distress syndrome (ARDS), heart failure, secondary infections, and multiple organ failure (6, 7).

Thromboembolism-related complication is common in severe COVID-19 patients. SARS-CoV-2 could directly infect the endothelial cell and diffuse endothelial inflammation through ACE2 receptor. However, the detailed mechanism of hypercoagulability and thromboembolism in COVID-19 disease remains unknown. Thromboembolism is associated with vascular endothelial injury, hypercoagulability and blood stasis. Malfunctioning endothelium could contribute to thromboembolism in arteries or veins. Recent evidence has indicated that the incidence of venous thromboembolism (VTE) including deep venous thrombosis (DVT) and pulmonary

embolism (PE) were higher in COVID-19 patients in intensive care units (ICU). VTE was difficult to diagnose in intubated patients and strict thrombosis prophylaxis should be recommended to ICU COVID-19 patient (8). Moreover, exudative diffuse alveolar damage with massive capillary microthrombi was the primary cause of death in COVID-19 related respiratory failure (9). In addition, hypercoagulability may contribute to a poor prognosis for patients with COVID-19. D-dimer could be used both for the prediction of thromboembolism and as a prognostic tool for risk stratification in COVID-19 patients.

Therefore, more attention should be given to COVID-19 patients with potential hypercoagulability or thromboembolism disease. Cirrhosis and Inflammatory bowel disease (IBD) are two major digestive diseases at a high risk of thromboembolism. Both cirrhosis and IBD patients are in a status of hypercoagulability. Thromboembolism related complications may affect the prognosis of these two patients. Thus, it is vital to investigate the mechanism of hypercoagulability, the progression and outcomes of thromboembolism in COVID-19 patients with preexisting cirrhosis and IBD.

HYPERCOAGULABILITY AND THROMBOEMBOLISM IN COVID-19

To date, accumulating autopsy evidence has demonstrated that abnormal coagulation activation and thromboembolism may be associated with a severe disease course, containing admission to the ICU and death. An autopsy study from Sigurd indicated that segmental and subsegmental pulmonary arterial thrombosis may be the cause of COVID-19-related death (10). A prospective study by Lax et al. indicated that 11 deceased patients had thrombosis in small and mid-sized pulmonary arteries, of whom eight were associated with infarction and six were associated with bronchopneumonia. Moreover, thrombosis of central vein in liver was also been found in these patients. The fact that the pulmonary embolism was the direct cause of death was further illustrated by Dominic and colleagues, who validated and compared clinical features with data from medical autopsy (11). They also revealed that DVT was not suspected in seven of 12 patients until death. In addition, a case report of an autopsy revealed that thrombi could be present in the veins and microcirculation of multiple organs, including lungs, spleen, pancreas, kidneys, adrenal glands, and mesenteric lymph nodes (12). Thus, the existence of microvascular thrombosis is vital to predict the deterioration of COVID-19, and this finding is valuable to develop suitable therapeutic strategies for clinical physicians.

Consistent with this, several clinical studies have also established that the VTE and abnormal coagulation parameters in COVID-19 patients were related to a severe disease course and negative prognosis. Wang et al. also indicated that COVID-19 patients with a high risk (Sequential Organ Failure Assessment, SOFA score $\geq$ 4) of VTE had poorer outcomes than patients with a lower risk (13). A study form Saskia et al. compared the incidence of VTE between ICU patients and non-ICU hospitalized patients (14). One hundred ninety-eight hospitalized patients with COVID-19 were involved in the study, 75 (38%) patients were admitted to the ICU eventually. The incidence of VTE was higher in the ICU patients (26 and 59% at 7 and 21 days) than regular ward patients (5.8 and 9.2% at 7 and 21 days). It is suggested that VTE is associated with a high mortality risk, particularly in ICU patients. A study including 150 COVID-19 patients showed that 64 (42.7%) patients developed clinically thrombotic complications, including pulmonary embolisms (15). Among them, COVID-19 ARDS patients developed significantly critically life-threatening thrombotic complications than non-COVID-19 ARDS patients. Therefore, abnormal coagulation parameters are essential to prognosticate the risk of VTE in COVID-19. D-dimer and fibrinogen were found to be elevated in COVID-19 patients with VTE. A retrospective cohort study from China indicated that the older age, a higher SOFA score and D-dimer more than 1 μg/mL at the time of hospital admission had remarkable relationship with in-hospital death (16). Similarly, another research from China showed that the incidence of VTE was 25% (20/81) in severe COVID-19 patients and the D-dimer $>1.5\,\mu$g/mL was value to predicting VTE within the sensitivity of 85% and specificity of 88.5% (17). Interestingly, another early publication revealed that elevated creatinine on admission and hospital length of stay were related to VTE diagnosis (18). Hence, it is noteworthy that more attention should be given to patients who present with elevated D-dimer, high SOFA score or at high risk of VTE.

Recent observations suggested that adequate thromboprophylaxis should be taken into consideration. In a study of 82 COVID-19 patients, 30 ICU patients and 48 non-ICU patients received different dosages of enoxaparin as anticoagulant therapy, only 4 (13%) ICU patients and 2 (4%) Non-ICU patients developed VTE at the end of the study. The incidence of VTE was significantly lower in this study (19). In an analysis from Maatman, 109 severe COVID-19 patients were recruited and all the patients received routine anticoagulation prophylaxis. VTE was diagnosed in 31 patients (28%) within a median 8 ± 7 inpatient days, suggesting that a routine chemical VTE prophylaxis may be inadequate in preventing thrombotic complications in critically ill COVID-19 patients. Thus, a more aggressive prophylactic anticoagulation strategy might be considered in COVID-19 patients, specifically in severe COVID-19 patients.

The pathogenesis of hypercoagulability and thromboembolism in COVID-19 patients remains unknown yet. Previous studies demonstrated that coagulation is activated by the host inflammatory response through several procoagulant pathways (19). SARS-CoV-2 infection could initiate complex systemic inflammatory reaction, which has been emphasized as "cytokine storm" (20, 21). This hyperinflammatory stats cause severe inflammatory response syndrome (SIRS) and cytokine dysregulation, which makes a great contribution to the activation of coagulation factors in COVID-19 disease. Compared with mild COVID-19 patients, the **SIRS** which contributes to ARDS is more active in severe COVID-19 patients, and eventually progresses to multiple organ failure. Accumulating evidence demonstrates that the "cytokine storm"

TABLE 1 | The incidence of VTE in COVID-19 patients.

References	The incidence of VTE	Significant laboratory parameter
Middeldorp et al. (14)	ICU patients (26% and 59% at 7 and 21 days) Regular ward patients (5.8% and 9.2% at 7 and 21 days)	D-dimer
Helms et al. (15)	COVID-19-ARDS patients 16.7% Non-COVID-19-ARDS patients 1.3%	D-dimer Fibrinogen
Cui et al. (17)	Severe COVID-19 patients 25%	D-dimer
Lodigiani et al. (24)	ICU patients 27.6% Regular ward patients 7.7%	Not stated
Trimaille et al. (25)	Regular ward patients 17.0% Transfer to ICU (VTE vs. non-VTE, 43.8% vs. 21.33%)	Not stated
Nopp et al. (26)	ICU patients 22.7% Non-ICU patients 7.9%	Not stated
Klok et al. (27)	ICU patients 37%	Not stated
Hippensteel et al. (18)	All the hospitalization patients 26.1%	Not stated
Llitjos et al. (28)	Severe COVID-19 patients 69% Prophylactic Anticoagulation vs. Therapeutic Anticoagulation (100% vs. 56%)	Not stated
Poissy et al. (29)	ICU patients 20.6%	Not stated
Thomas et al. (30)	ICU patients 27%	Not stated

syndrome developed in almost all the subgroup of patients with severe COVID-19 (22). The levels of proinflammatory cytokines and chemokines including interleukin IL-1β, IL-6, interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), inducible protein 10 (IP-10), caspase-1 and monocyte chemotactic protein 1(MCP-1) were high in both circulatory system and bronchoalveolar lavage fluid of COVID-19 ARDS patients (1, 23). These activated inflammasomes were important causes of "cytokine storm" in severe COVID-19 patients. Besides, severe COVID-19 infection is generally associated with old age and chronic illness including chronic obstructive respiratory disease, chronic liver disease, chronic cardiovascular disease and so on (22) (**Table 1**) However, the concept that the SARS-CoV-2 directly or indirectly interferes with coagulation pathways causing systemic VTE has become a hot research topic. ACE2, the receptor of SARS-CoV-2, is highly expressed on the membrane of endothelial cells. Endothelial cell dysfunction/inflammation and hypercoagulability could cause thromboembolism. Thus, more investigations should be focused on pathophysiological and molecular mechanism of hypercoagulability and thromboembolism in COVID-19 disease.

SARS-CoV-2 INFECTION IN CIRRHOSIS

Cirrhosis is a chronic liver disease which is in a status of hypercoagulability and tends to develop venous thromboembolism. The incidence of VTE in cirrhosis is 0.33–26% (31). Portal vein thrombosis (PVT) is one of the major complications in liver cirrhosis patients, which may lead to poor prognosis (7, 32–34). The pathogenesis of VTE in cirrhosis consists of systemic disorder, inherited or acquired thrombophilia, systemic risk factors and local factors (35). Systemic disorder includes an advanced portal hypertension which could reduce the flow velocity of portal vein, large spontaneous portosystemic shunts, transjugular intrahepatic portosystemic shunt (TIPS) and malignancy. The role of inherited and acquired thrombophilia in VTE is still controversial. It has been reported that the mutation of factor V Leiden and prothrombin G20210A gene, the deficiency of antithrombin, protein C and S are all associated with PVT (36). However, a meta-analysis from Anstee et al. suggested that proteins C and S are not significantly associated with the progression of PVT in cirrhosis. Hypercoagulation could even aggravate hepatic fibrosis (37).

Available evidence has certified that liver could be infected with SARS-CoV-2. ACE2 is the receptor of SARS-CoV-2. The expression of ACE2 is low in normal liver tissue. However, ACE2 has been detected in most hepatocytes and cholangiocytes of cirrhosis (38). Additionally, the mRNA expression of ACE2 in hepatocytes could increase significantly under hypoxic condition. Almost all the COVID-19 patients could suffer from severe hypoxia. Hence, it seems that patients with cirrhosis are at a great risk of SARS-CoV-2 infection. Given that SARS-CoV-2 infection could lead to liver injury, cirrhosis patients with COVID-19 may develop acute-on-chronic liver failure. The portal system of liver is vital. Vascular endothelial cell of portal veins is susceptible to injury under the "cytokine storm" status. It is worth noting that hypercoagulability could be severer in COVID-19 patients with preexisting cirrhosis than in non-cirrhosis COVID-19 patients. A study from Iavarone et al. indicated that respiratory support and heparin were necessary in 71 and 80% cirrhosis COVID-19 patients, respectively. Mortality was significantly higher in cirrhosis COVID-19 patients (39). Bajaj et al. (40) also indicated that cirrhosis COVID-19 patients needed a higher BiPAP/ventilation. A multicenter study from South Korea demonstrated that the incidence of ARDS was higher in cirrhosis COVID-19 patients (41). Therefore, a close monitoring of coagulation function and diagnostic imaging for VTE should be early implemented in COVID-19 patients with preexisting cirrhosis. Unfortunately, the data of SARS-CoV-2 infection in liver cirrhosis and investigations which focused on coagulation

activation or portal vein thrombosis progression in COVID-19 patients with preexisting cirrhosis is insufficient. Theoretically, anticoagulant therapy (such as vitamin K antagonist, Factor Xa inhibitor or direct thrombin IIa inhibitor) should be taken into consideration. However, gastrointestinal hemorrhage is a common complication of cirrhosis, which is also one of the main causes of death. Thus, the anticoagulant therapy timing, preferred type, dose, and duration of treatment are an enormous challenge of clinicians. More basic and clinical prospective studies should be focused on theses aspects.

SARS-CoV-2 INFECTION IN INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a chronic intestinal disorder characterized by severe gastrointestinal mucosal inflammation, which is also in a status of hypercoagulability. A meta-analysis has demonstrated that IBD is associated with an ∼2-fold increase in the risk of VTE compared with individuals without IBD (42). VTE is considered as an extraintestinal manifestation of IBD with a significant morbidity and mortality (43). Moreover, mucosal capillary thrombi have also been found in both Crohn's disease (CD) and Ulcerative colitis (UC) rectal biopsies, suggesting that mucosal microvascular system could also be involved in IBD patients (44). Pro-inflammatory cytokines associated with endothelial injury have an effect on coagulation and fibrinolysis pathways. Evidence from several studies has found that blood coagulation factors (Va, VIIa, VIIIa, Xa, Xia, XIIa), plasminogen activator inhibitor type 1 (PAI-1), thrombin-activated fibrinolytic inhibitor (TAFI), α2 plasmin inhibitor (α2-PI) and were elevated in IBD patients, while the level of antithrombin and the activity of tissue type plasminogen activator (t-PA) was reduced in IBD patients (45, 46). Moreover, A study consisted of 175 IBD patients revealed that there was a statistically significant decrease in mean platelet volume (MPV), platelet distribution width (PDW) levels and increase in platelet-crit (PCT) levels when compared to healthy controls, suggesting that the change of platelet indices in IBD is noteworthy (47). Thus, the imbalance between prothrombotic factors and antithrombotic factors may be the underlying cause of thrombosis in IBD.

The fact that SARS-CoV-2 could infect gastrointestinal tract was proved by several studies (48). On the one hand, autopsy, biopsy, and feces have detected live SARS-CoV-2 in digestive tract. On the other hand, the expression of ACE2 is increased in the inflamed mucosa of patients with IBD (49). Besides, the expression of ACE2 is significantly higher in CD patients. A research from Italy including 79 COVID-19 patients with IBD demonstrated that active IBD, old age and comorbidities were significantly related to a negative COVID-19 prognosis, whereas concomitant IBD treatments were not (50). The evidence that COVID-19 occurs more frequently in IBD patients than in the general population is unclear yet. A large study which contained 1,918 IBD patients found that only 12 patients were diagnosed with COVID-19, indicating that IBD patients do not have an increased risk of COVID-19. Besides, the study also revealed that the COVID-19 associated mortality did not increase in IBD patients compared with the general population (51). Another study from Norsa demonstrated that none of the 522 patients with IBD in their cohort was admitted to the hospital due to SARS-CoV-2 infection (52). Currently, some physicians are concerned that immunosuppressants or biologics which IBD patients are taking may increase the risk of COVID-19 infection. Hence, the true incidence of COVID-19 infection in IBD deserves to be further explored in future related studies. However, the alteration of coagulation activation or vein thrombosis progression in COVID-19 patients with preexisting IBD remains uncertain. On the basis of recent studies, SARS-CoV-2 infection may not exacerbate thromboembolism complications in IBD patients. One proposed explanation is that the immunosuppressor IBD patients are taking has an effect on suppressing cytokine driven-inflammatory response which could be beneficial for preventing COVID-19-driven pneumonia and COVID-19-driven thromboembolism complications. Immunosuppressor, such as azathioprine and methotrexate may serve as an additional choice for the treatment of COVID-19. Hospitalization is an independent risk factor for VTE in IBD patients, which are at a remarkable risk (10–40%) of developing DVT, according to the guidelines of VTE prevention from American College of Chest Physicians (ACCP) and Canadian Association of Gastroenterology guidelines (43, 53, 54). Therefore, it is reasonable to perform prophylactic anticoagulation strategy in COVID-19 patients with preexisting IBD. Thus, more efforts should be made toward future studies about the mechanism and outcomes of hypercoagulability and thromboembolism in COVID-19 patients with IBD.

CONCLUSION

Abnormal coagulation activation and VTE should be cautiously considered for COVID-19 patients, and anticoagulation therapy should be performed when COVID-19 patients are at the high risk of thrombotic complications. Furthermore, the anticoagulation therapy of COVID-19 patients with preexisting hypercoagulability disease should be more cautious to maintain the balance between the hemorrhage and coagulation. The prognosis of COVID-19 with preexisting hypercoagulability disease deserves to be further explored by prospective researches.

AUTHOR CONTRIBUTIONS

HZ and MJ outlined the overall manuscript. MJ, JM, and SS drafted the manuscript. HZ supervised the preparation of the draft and edited it. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by National Natural Science Foundation of China (Grant Number: 81570502) and by 1.3.5 Project for disciplines of excellence, West China Hospital, Sichuan University (Grant Number: ZYJC18037).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 14 January 2021
doi: 10.3389/fmed.2020.597967

Susceptibility Factors of Stomach for SARS-CoV-2 and Treatment Implication of Mucosal Protective Agent in COVID-19

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Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 23 August 2020
Accepted: 07 December 2020
Published: 14 January 2021

Citation:
Zhang M, Feng C, Zhang X, Hu S, Zhang Y, Min M, Liu B, Ying X and Liu Y (2021) Susceptibility Factors of Stomach for SARS-CoV-2 and Treatment Implication of Mucosal Protective Agent in COVID-19. Front. Med. 7:597967. doi: 10.3389/fmed.2020.597967

Objectives: This work aims to study the gastrointestinal (GI) symptoms in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients and the susceptibility factors of the stomach for SARS-CoV-2.

Materials and Methods: We investigated the SARS-CoV-2 susceptibility by analyzing the expression distribution of viral entry-associated genes, *ACE2* and *TMPRSS2*, in single-cell RNA sequencing data derived from 12 gastric mucosa samples. We also analyzed the epidemiological, demographic, clinical, and laboratory data of 420 cases with SARS-CoV-2-caused coronavirus disease 2019 (COVID-19).

Results: *ACE2* and *TMPRSS2* are specifically expressed in enterocytes which are mainly from gastric mucosa samples with *Helicobacter pylori* (*H. pylori*) infection history and intestinal metaplasia (IM). A total of 420 patients were surveyed, of which 62 were with and 358 were without GI symptoms. There is a significant difference in average hospital stay ($p < 0.001$) and cost ($p < 0.001$) between the two groups. Among 23 hospitalized patients including seven with upper GI symptoms and 16 with lower GI symptoms, six (85.7%) and five (31.3%) had *H. pylori* infection history, respectively ($p = 0.03$). Of 18 hospitalized patients with initial upper GI symptoms, none of the eight patients with mucosal protective agent therapy (e.g., sucralfate suspension gel, hydrotalcite tablets) had diarrhea subsequently, whereas six out of 10 patients without mucosal protective agent therapy had diarrhea subsequently ($p = 0.01$).

Conclusion: IM and *H. pylori* infection history may be susceptibility factors of SARS-CoV-2, and the mucosal protective agent may be useful for the blockade of SARS-CoV-2 transmission from the stomach to the intestine.

Keywords: single-cell RNA sequencing, COVID-19, SARS-CoV-2, H pylori infection, intestinal metaplasia

INTRODUCTION

The current SARS-CoV-2-caused coronavirus disease 2019 (COVID-19) pandemic is an ongoing global health crisis (1, 2). COVID-19 patients generally exhibited initial symptoms such as fever, fatigue, myalgia, dyspnea, and cough. Recent studies (3–5) showed that 20–50% patients had gastrointestinal symptoms as initial symptoms, and a large number of patients would have GI symptoms during hospitalization. SARS-CoV-2 RNA has also been detected in the patients' stools and will last a long time, suggesting that SARS-CoV-2 could be transmitted via the fecal–oral route (6, 7). SARS-CoV-2 transmission through the GI tract requires extensive attention.

The distribution of SARS-CoV-2 entry receptor may be highly associated with the route of infection, which is essential for understanding the pathogenesis mechanism (7–9). Recent studies (10, 11) reported that the viral host receptor ACE2 and the viral nucleocapsid were mainly in the cytoplasm of gastrointestinal epithelial cells. The scRNA-seq findings also uncovered that the SARS-CoV-2 entry receptor ACE2 and TMPRSS2 were specifically expressed in gastrointestinal epithelial cells such as enterocytes (7, 12). SARS-CoV-2 can invade the enterocytes and result in diarrhea. However, there are still a number of patients with non-diarrhea GI symptoms clinically, and it is still unknown whether these symptoms are due to stomach infection. As stomach is the upstream target organ in the fecal–oral route, a systematic survey of the distribution of SARS-CoV-2 entry receptor in the stomach and its susceptibility factors for SARS-CoV-2 infection will benefit our understanding of the mechanism of non-diarrhea GI symptoms and further guide effective prevention and treatment.

In this study, we aim to explore the susceptibility factors affecting gastrointestinal infections and the possible preventive or therapeutic measures using scRNA-seq data and the admission data of 420 laboratory-confirmed SARS-CoV-2 infection cases.

METHODS

Analysis of Single-Cell and Bulk RNA Expression Matrices

The single-cell RNA expression matrices derived from 12 gastric mucosal samples were downloaded from the Gene Expression Omnibus [GEO, number GSE134520 (13)]. The bulk RNA sequencing expression matrices for human normal lung, colon, rectum esophagus, and stomach tissues were downloaded from the UCSC Xena website (https://xenabrowser.net/). We used the Seurat (14) package for scRNA-seq data analysis, including data integration, identification of highly variable genes, unsupervised graph-based clustering, differentially expressed genes, and dimension reduction using principal component analysis and Uniform Manifold Approximation and Projection. We also analyzed the expression of SARS-CoV-2 entry receptors in human normal lung, colon, rectum, and stomach tissues. We further performed Pearson correlation analysis between the expression levels of SARS-CoV-2 entry receptors with the average expression level of enterocyte markers (defined as enterocyte score) to validate the scRNA-seq findings.

Study Design and Participants

In this retrospective, single-center study in Wuhan Central Hospital, we reviewed the admission data, including clinical records and laboratory test results, of 420 laboratory-confirmed SARS-CoV-2 infection cases from January 20 to April 30, 2020. According to the World Health Organization diagnostic guidelines and Chinese expert consensus of new coronavirus pneumonia prevention and treatment (15), the patients were divided into suspected cases and clinically diagnosed case. If the suspected case has the CT imaging features of COVID-19 pneumonia, it is classified as a clinically diagnosed case. The laboratory confirmed COVID-19 patients were diagnosed as positive for SARS-CoV-2 by real-time reverse transcription PCR. The patients with suspected and clinical diagnosis that have not been verified by laboratory examination are not included in this study.

The symptoms of COVID-19 are divided into four groups: mild, ordinary, serious, and critical groups according to the standard previously reported in Lin et al. (4), and the patients were further divided into non-severe (mild and ordinary) and severe (serious and critically) cases. The GI symptoms are divided into two parts: initial presentation group (IPG) and hospitalized presentation group (HPG) according to the occurrence time. The upper GI symptoms (UGIS) are defined with nausea/vomiting but without diarrhea, while diarrhea is defined as a lower GI symptom (LGIS). We also counted the occurrence rate of other non-specific GI symptoms such as anorexia and abdominal pain/abdominal discomfort. For patients with a co-occurrence of nausea/vomiting and diarrhea, we record the order in which the symptoms occur. We also investigated the admission examination and treatment of patients to record information such as gastric surgery history, upper gastrointestinal ulcer history, and *Helicobacter pylori* infection history. For those who had not been examined and recorded during the course of the disease, we followed up the information of *H. pylori* infection history and treatment within the last year.

Statistical Analysis

All statistical tests were implemented with R statistical programming language (V.3.62). The continuous variables denoted as mean ± SD were compared by Wilcoxon test. The categorical data presented as percentage (%) were compared by χ^2-test or Fisher's exact test. A two-sided $p < 0.05$ was considered as statistically significant.

RESULTS

ScRNA-seq Analysis Reveals the Specific Expression of ACE2 and TMPRSS2 in Gastric Mucosa With *H. pylori* Infection and Intestinal Metaplasia

We analyzed 34,541 individual cells (**Figure 1A**) derived from 12 gastric mucosa samples (six intestinal metaplasia, IM; three non-atrophic gastritis, NAG; three chronic atrophic gastritis, CAG) of nine patients (two with and seven without *H. pylori* infection history). Unsupervised graph-based clustering revealed 12 cell

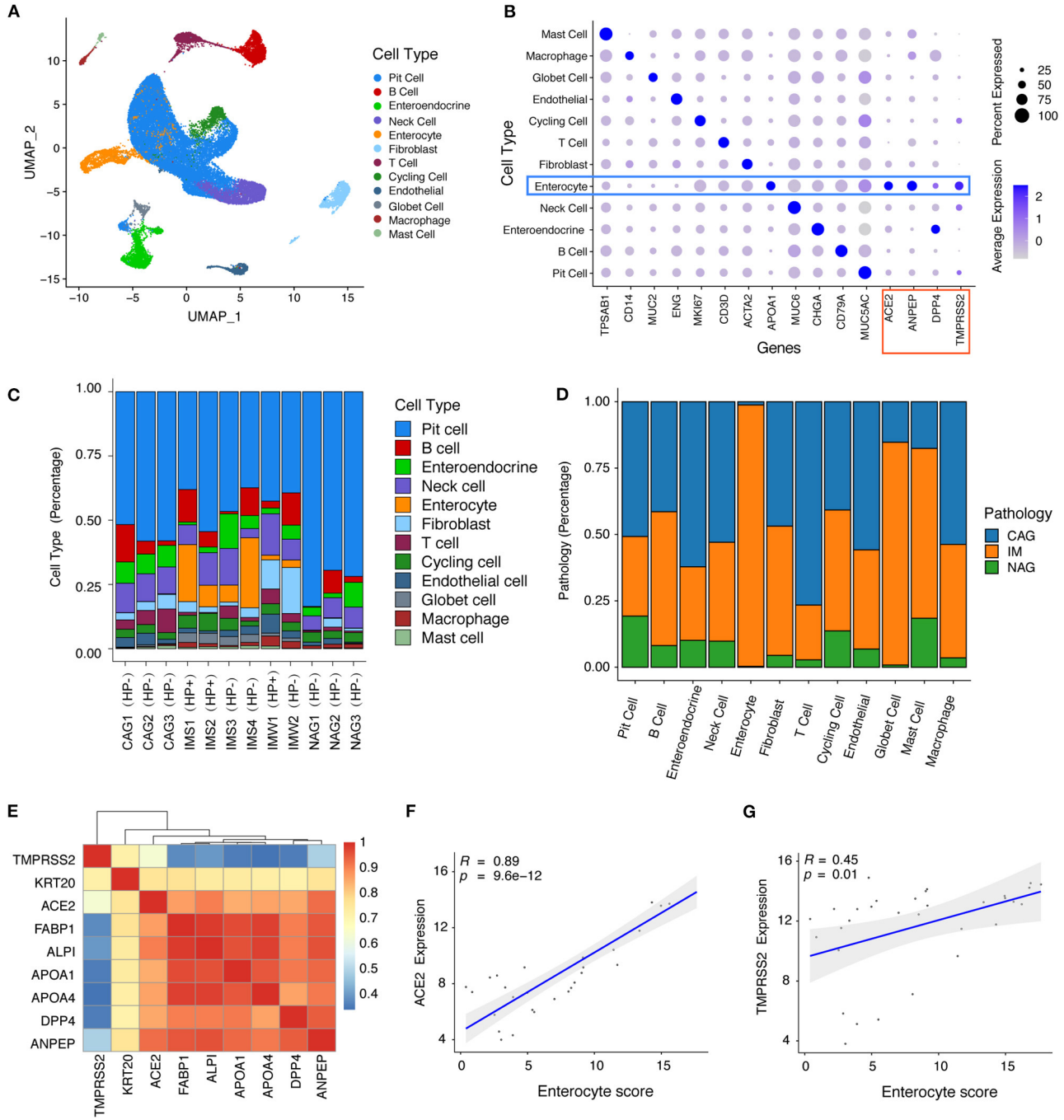

FIGURE 1 | ScRNA-seq and bulk RNA-seq profiles reveal that ACE2 and TMPRSS2 were specifically expressed in gastric mucosa with *Helicobacter pylori* infection and intestinal metaplasia. **(A)** Unsupervised graph-based clustering revealed 12 cell types of 34,541 cells shown in Uniform Manifold Approximation and Projection plot. **(B)** Dot plot showing the expression of cell type markers and SARS-CoV-2 entry receptor ACE2 and TMPRSS2 and another two virus receptors (ANPEP and DPP4). **(C)** Bar plot showing the fraction of different cell types per sample. **(D)** Bar plot showing the fraction of different sample pathology per cell type. **(E)** Heat map showing the correlation coefficient between SARS-CoV-2 cell entry receptor ACE2 and TMPRSS2 with enterocyte genes. **(F-G)** Scatter plot showing the correlation coefficient of SARS-CoV-2 cell entry receptors **(F)** ACE2 and **(G)** TMPRSS2 with enterocyte gene score.

types (**Figure 1A**), and the enterocytes specifically expressed SARS-CoV-2 entry receptor (9) ACE2 and TMPRSS2 and another two virus receptors (ANPEP receptor for HCoV-229E virus and DPP4 receptor for MERS-CoV virus) (**Figure 1B**). Interestingly, the vast majority of enterocytes were derived from gastric mucosa samples with *H. pylori* infection and IM

TABLE 1 | The demographics, baseline features, and clinical outcomes of 420 patients infected with SARS-CoV-2.

	All patients (*n* = 420)	**Patients with gastrointestinal (GI) symptoms (*n* = 62)**	**Patients without GI symptoms (*n* = 358)**	***P*-value**
Age (year)	51.7 ± 17.5	53.0 ± 19.0	51.5 ± 17.3	0.62
Age groups				0.47
15–60	253 (60.2)	36 (58.1)	225 (62.8)	
>60	167 (39.8)	26 (41.9)	133 (37.2)	
Sex				0.10
Female	246 (58.6)	42 (67.7)	204 (57.0)	
Male	174 (41.4)	20 (32.3)	154 (43.0)	
Smoking history	9 (2.1)	0	9 (2.1)	0.37
Alcoholism history	2 (0.5)	0	2 (0.5)	1.00
Disease class				0.99
Non-severe	366 (87.1)	54 (87.1)	312 (87.2)	
Severe	54 (12.9)	8 (12.9)	46 (12.8)	
Coexisting illness				
Hypertension	114 (27.1)	11 (17.7)	103 (28.8)	0.07
Diabetes mellitus	52 (12.4)	7 (11.3)	45 (12.6)	0.78
Cardio-cerebrovascular disease	37 (8.8)	10 (16.1)	27 (7.5)	0.03
Malignant tumor	7 (1.7)	1 (1.6)	6 (1.7)	1.00
Chronic lung disease	18 (4.3)	4 (6.5)	14 (3.9)	0.57
Chronic kidney	9 (2.1)	1 (1.6)	8 (2.2)	1.00
Clinical outcome				0.63
Discharged	379 (90.0)	57 (91.9)	322 (90.0)	
Died	41 (10.0)	5 (8.1)	36 (10.0)	
Average hospital stay (day)	17.8 ± 9.4	24.2 ± 8.6	16.71 ± 9.1	7e-10
Average hospitalization cost (CNY)	21,658.0 ± 19,051.2	32,949.6 ± 22,542.1	19,702.5 ± 17,696.8	5e-7

(**Figures 1C,D**). The bulk RNA-seq profiles revealed that the expression levels of ACE2 and TMPRSS2 had a high correlation with the average expression levels of the enterocyte marker genes (**Figures 1E,F**), indicating that ACE2 and TMPRSS2 were specifically expressed in enterocytes. We further investigated the expression of ACE2 and TMPRSS2 in human lung, colon, rectum, and stomach. We found that ACE2 and TMPRSS2 have higher expression levels in intestinal-phenotype stomach (paired adjacent normal tissues of intestinal-phenotype gastric cancer) than those of gastric-phenotype stomach (paired adjacent normal tissues of diffuse-phenotype gastric cancer) (**Supplementary Figures 1A,B**). As we know, human IM stomachs are characterized by the emergence of intestine-specific cell types such as enterocytes (16), and *H. pylori* infection history is an important factor leading to IM (17). We speculate that SARS-Cov-2 can infect the stomach with *H. pylori* infection history and IM, thereby resulting in upper GI symptoms. These results also revealed that *H. pylori* infection history and IM might be susceptibility factors of SARS-Cov-2.

A Systematic Survey of the Clinical Data of 420 Patients With COVID-19

A total of 420 COVID-19 patients (246 women and 174 men) were included in this study (**Table 1**). Most of the patients were non-severe (87.1%), and a few patients had smoking (2.1%) or alcoholism (0.07%) history. More than half of the patients had coexisting basic illnesses, and the most common illnesses are hypertension (27.1%), diabetes mellitus (12.3%), and cardio-cerebrovascular disease (8.8%) (**Table 1**). Among the 420 patients, 62 (14.8%) occurred with GI symptoms and 358 (85.2%) without GI symptoms. There was no statistically significant difference in the general demographics or clinical outcomes between the patients with and without GI symptoms. The patients with GI symptoms had a higher percentage of coexisting cardio-cerebrovascular disease than those without GI symptoms ($p = 0.03$). Interestingly, we found that the patients with GI symptoms had a significantly longer hospital stay ($p < 0.001$) and higher hospitalization costs ($p < 0.001$) than those without GI symptoms.

The 62 patients with GI symptoms are classified into three groups: UGIS group (12 patients), LGIS group (30 patients), and non-specific GI symptoms group (20 patients). We compared the manifestations of patients in the UGIS group and the LGIS group (**Table 2**). No statistically significant differences are found in most general demographics, manifestations, or clinical outcomes between the UGIS and the LGIS groups except *H. pylori* infection and time from hospital admission to cardinal symptom onset. The average age of the patients with simple UGIS was 54.0 ± 17.0, higher than that of the patients with simple LGIS (50.1

TABLE 2 | Comparison of upper and lower gastrointestinal (GI) manifestations of 42 patients with SARS-CoV-2 infection.

	Patients with upper GI symptoms ($n = 12$)	Patients with lower GI symptoms ($n = 30$)	*P*-value
Age (year)	54.0 ± 17.0	50.1 ± 18.8	0.57
Sex			1.00
Female	7/12 (58.4)	19/30 (63.3)	
Male	5/12 (41.7)	11/30 (36.7)	
Disease classification			0.67
Non-severe	9/12 (75.0)	25/30 (83.3)	
Severe	3/12 (25.0)	5/30 (16.7)	
Coexisting illness			
Hypertension	4/12 (33.3)	7/30 (23.3)	0.70
Diabetes mellitus	4/12 (33.3)	3/30 (10.0)	0.09
Cardio-cerebrovascular disease	4/12 (33.3)	6/30 (20.0)	0.43
Malignant tumor	0	1/30 (3.3)	1.00
Chronic lung disease	2/12 (16.7)	2/30 (6.7)	0.56
Chronic kidney disease	0	1/30 (3.3)	1.00
Stomach diseases history			
HP infection	6/7 (85.7)	5/16 (31.3)	0.03
Operation history	0	1/30 (3.3)	1.00
Ulcer	2/12 (16.7)	1/30 (3.3)	1.00
Died	1/12 (8.3)	2/30 (6.7)	1.00
Average hospital stay (day)	25.1 ± 9.0	23.47 ± 9.0	0.77
Average hospitalization cost (CNY)	32,113.4 ± 17,406.5	28,715.9 ± 20,360.5	0.40
On initial presentation (IPG)			
Cardinal symptoms	Nausea and vomiting (8)	Diarrhea (23)	
Concomitant symptoms	Inappetence (3) hematemesis (1)	Nausea (10) Inappetence (11)	
Duration of cardinal symptoms (day)	7.9 ± 4.6*	8.4 ± 3.2#	0.87
During hospitalization (HPG)			
Cardinal symptoms	Nausea and vomiting (4)	Diarrhea (7)	
Concomitant symptoms	Inappetence (3)	Inappetence (4)	
Time from hospital admission to symptom onset (day)	7.0 ± 2.9	2.4 ± 1.3	0.02
Duration of cardinal symptoms (day)	5.3 ± 3.6	3.4 ± 1.7	0.56
Imaging examination			
Not obvious	1/12 (8.3)	0	0.29
Patchy shadows involving both Lungs	9/12 (75.0)	26/30 (86.7)	0.39
Pulmonary consolidation/pleural effusion	2/12 (16.7)	4/30 (13.3)	1.00
Laboratory examination			
Fecal RNA test	3/8 (37.5)	13/28 (46.4)	0.70
WBC ($<3.5 \times 10^9$/L)	4/12 (33.3)	14/30 (46.7)	0.51
LYM ($<1.1 \times 10^9$/L)	6/12 (50.0)	17/30 (56.7)	0.74
NEUT ($<1.8 \times 10^9$/L)	1/12 (8.3)	6/30 (20.0)	0.65
MONO ($>0.6 \times 10^9$/L)	1/12 (8.3)	1/30 (3.3)	0.49
TBIL (>20 umol/L)	0	0	1.00
ALT (>40 U/L)	1/12 (8.3)	1/30 (3.3)	0.49
AST (>35 U/L)	1/12 (8.3)	4/30 (13.3)	1.00
CRP (> 3 mg/L)	4/12 (33.3)	12/30 (40.0)	0.74

**Duration of cardinal symptoms (upper GI symptoms) between IPG vs. HPG. p = 0.49.*
#Duration of cardinal symptoms (lower GI symptoms) between IPG vs. HPG. p = 6.0e-4.

TABLE 3 | The clinical outcome of drug treatment involvement of COVID-19 patients with upper GI symptoms.

		Upper GI symptoms (+) Subsequent diarrhea (+) (*n* = 6)	Upper GI symptoms (+) Subsequent diarrhea (–) (*n* = 12)	*P*-value
Mucosal protective agent*	Treated with	0	8	0.01
	Treated without	6	4	
Probiotics	Treated with	3	2	0.27
	Treated without	3	10	
Montmorillonite powder	Treated with	2	2	0.57
	Treated without	4	10	
Proton pump inhibitors	Treated with	6	12	1.00
	Treated without	0	0	
Prokinetic agents	Treated with	4	2	0.11
	Treated without	2	10	

**Any drug that protects the mucosal lining of the stomach from acidic gastric juices, including sucralfate suspension gel, hydrotalcite tablets.*

± 18.8), although the *p*-value is not significant ($p = 0.57$). We also investigated the presence of SARS-CoV-2 in feces for 36 hospitalized patients, including eight with UGIS and 28 with LGIS, of which three (37.5%) and 13 (46.4%) were positive for SARS-CoV-2, respectively (**Table 2**).

Lymphopenia is the most common abnormal biochemical indicator in patients with COVID-19. In this study, 50% of patients in both UGIS and LGIS groups exhibited lymphopenia (**Supplementary Table 1**). We compared the lymphocyte counts of each patient at the time of hospitalization and recovery from discharge and found that nine (81.8%) and 24 (85.7%) had a lymphocyte count increase in the UGIS and LGIS groups, respectively. These results indicated that lymphocyte count is an important prognostic factor (18).

We also explored the association of *H. pylori* infection with GI symptoms for 23 hospitalized patients, including seven with UGIS and 16 with LGIS, of which six (85.7%) and five (31.3%) had *H. pylori* infection, respectively ($p = 0.03$, **Table 2**). These results indicate that *H. pylori* infection is associated with the presence of GI symptoms, especially for UGIS, which also supports our scRNA-seq findings that *H. pylori* infection might be a susceptibility factor of SARS-CoV-2.

According to the occurrence time of GI symptoms, patients from the UGIS and the LGIS groups were then further divided into two groups: IPG and HPG. We mainly focused on the difference of GI symptom duration between IPG and HPG and found that IPG had longer durations in both nausea/vomiting (7.9 ± 4.6 vs. 5.3 ± 3.6, $p = 0.49$) and diarrhea (8.4 ± 3.2 vs. 3.4 ± 1.7, $p < 0.001$) than HPG (**Table 2**). The results indicated that patients with timely clinical therapeutic intervention may help to accelerate the recovery process.

Based on this finding, we further investigated the correlation of timely clinical therapeutic intervention with clinical outcome. Among the 18 hospitalized patients with initial UGIS, eight with and 10 without mucosal protectant therapy (e.g., sucralfate suspension gel, hydrotalcite tablets), zero and six (60%) had subsequent diarrhea, respectively ($p = 0.01$, **Table 3**). We speculate that timely clinical therapeutic intervention may help to reduce virus load and to blockade SARS-Cov-2 transmission into the intestine.

DISCUSSION

Several recent studies have shown that SARS-Cov-2 needs to bind with ACE2 in order to invade human cells (10, 19, 20). The gastrointestinal epithelial cells express SARS-CoV-2 entry receptors (8); therefore, the GI tract (6, 7) may be a potential transmission route and target organ of SARS-CoV-2. Diarrhea is the most common GI symptom because SARS-CoV-2 could invade enterocytes. However, a number of patients had simple upper GI symptoms, i.e., nausea/vomiting but without diarrhea. Whether the stomach infection is related to UGIS and the susceptibility factors of the stomach for SARS-CoV-2 remain poorly investigated.

As we know, gastric IM is characterized by the emergence of intestine-specific cell types, including enterocytes. In China and many other countries, the incidence of gastric IM increases with age (21), and *H. pylori* infection is an important factor resulting in IM (16). In addition, we found that the stomach with *H. pylori* infection history and IM was enriched with enterocytes, and these cells specifically expressed SARS-CoV-2 entry receptor genes *ACE2* and *TMPRSS2*. The gastric mucosa with IM usually occurs alongside parietal cell loss and then leads to gastric juice PH elevation; thus, the SARS-CoV-2 virus is not inactivated by stomach acid (8). The normal gastric mucosa normally secrete gastric juice, and the PH is usually below 3. SARS-Cov-2 can be inactivated by gastric juice; thereby, it may not be infecting the normal stomach. Therefore, we speculate that the stomach with *H. pylori* infection history and IM may be susceptible to SARS-CoV-2.

Since it is unrealistic and difficult to check the stomach pathology, especially the IM status, in so many COVID-19 patients with GI symptoms, we conducted a systematic survey of the clinical data of 420 patients with COVID-19 to investigate the correlation of *H. pylori* infection history with GI symptoms. Interestingly, we found that most of the patients (six of seven)

with UGIS and only five (31.25%) cases with LGIS had *H. pylori* infection history. This result, derived from 420 COVID-19 patients' clinical data, together with our findings on scRNA-seq data further provide evidence that *H. pylori* infection history and IM may be susceptibility factors of SARS-CoV-2 in the stomach.

In addition, our results revealed that the duration of GI symptoms in the HPG was shorter than that of IPG, suggesting the necessity of timely clinical therapeutic intervention. We further compared the clinical outcome of COVID-19 patients with UGIS with or without usage of mucosal protective agent. We found that the usage of mucosal protective agent reduced the occurrence of subsequent diarrhea. These results suggested that timely GI management, e.g., the usage of mucosal protective agent (e.g., sucralfate suspension gel, hydrotalcite tablets), will help to prevent further transmission from the stomach to the intestine through fecal–oral infection.

This study has limits since a small cohort of patients were enrolled; secondly, we did not perform a stomach biopsy examination in COVID-19 patients, especially the IM status. However, our scRNA-seq findings and the survey of 420 patients' data provided evidence that IM and *H. pylori* infection history may be susceptibility factors of SARS-CoV-2, and a mucosal protective agent may benefit to prevent further SARS-CoV-2 transmission.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/geo/, GSE134520.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Central Hospital of Wuhan affiliated to Tongji Medical College of Huazhong University of Science and Technology (2020-112). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MZ designed the experiments, analyzed the data, and wrote the manuscript. XZ, MM, and YZ collected the clinical data. CF and SH were responsible for data acquisition, analysis, and interpretation and writing of the manuscript. YL, XY, and BL were responsible for the study concept, design, and interpretation and revision of the manuscript. All the authors participated in the discussion.

FUNDING

This work was supported by the National Science and Technology Major Project (grant no. 2018ZX10201-001), Natural Science Foundation of Beijing Municipality (grant no. 7192201), and Outstanding Youth Training Fund of the Chinese PLA General Hospital (grant no. 2019-JQPY-001).

ACKNOWLEDGMENTS

We thank the following agencies and foundations for their financial support: National Science and Technology Major Project (grant no. 2018ZX10201-001), Natural Science Foundation of Beijing Municipality (grant no. 7192201), and 2019 Outstanding Youth Training Fund of the PLA General Hospital (grant no. 2019-JQPY-001).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.597967/full#supplementary-material

Supplementary Figure 1 | Bulk RNA-seq profiles showing the expression of ACE2 **(A)** and TMPRSS2 **(B)** in normal human lung, colon, rectum, and stomach. NOS, not otherwise specified.

Supplementary Table 1 | The clinical laboratory results alteration trend at the time of hospitalization and recovery from discharge.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

SYSTEMATIC REVIEW
published: 12 March 2021
doi: 10.3389/fmed.2021.626425

Metabolic Associated Fatty Liver Disease Is Associated With an Increased Risk of Severe COVID-19: A Systematic Review With Meta-Analysis

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *05 November 2020*
Accepted: *22 February 2021*
Published: *12 March 2021*

Citation:
Hegyi PJ, Váncsa S, Ocskay K, Dembrovszky F, Kiss S, Farkas N, Erőss B, Szakács Z, Hegyi P and Pár G (2021) Metabolic Associated Fatty Liver Disease Is Associated With an Increased Risk of Severe COVID-19: A Systematic Review With Meta-Analysis. Front. Med. 8:626425. doi: 10.3389/fmed.2021.626425

Background: The most common pre-existing liver disease, the metabolic dysfunction-associated fatty liver disease (MAFLD) formerly named as non-alcoholic fatty liver disease (NAFLD), may have a negative impact on the severity of COVID-19. This meta-analysis aimed to evaluate if MAFLD or NAFLD are associated with a more severe disease course of COVID-19.

Methods: A systematic search was performed in five databases for studies comparing severity, the rate of intensive care unit (ICU) admission, and mortality of COVID-19 patients with and without MAFLD or NAFLD. In meta-analysis, pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results: Altogether, we included nine studies in our quantitative and qualitative synthesis. MAFLD was associated with an increased risk of severe COVID-19 compared to the non-MAFLD group (28 vs. 13%, respectively; OR = 2.61, CI: 1.75–3.91). Similarly, in the NAFLD vs. non-NAFLD comparison, NAFLD proved to be a risk factor as well (36 vs. 12%, respectively; OR = 5.22, CI: 1.94–14.03). On the other hand, NAFLD was not associated with an increased risk of ICU admission (24 vs. 7%, respectively; OR = 2.29, CI: 0.79–6.63). We were unable to perform meta-analysis to investigate the association of MAFLD with the rate of ICU admission and with mortality.

Conclusion: In conclusion, patients with MAFLD and NAFLD showed a more severe clinical picture in COVID-19. Our results support the importance of close monitoring of COVID-19 patients with MAFLD. Further research is needed to explore the cause of increased severity of COVID-19 in MAFLD.

Keywords: SARS-CoV-2, COVID-19, pandemic, prognosis, non-alcoholic fatty liver disease, metabolic associated fatty liver disease

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents a global health challenge. Coronavirus disease 2019 (COVID-19) is mostly a self-limiting disease; however, in some cases, mortality can reach 3–7% (1). The high mortality has been mainly linked to the excessive production of pro-inflammatory cytokines that lead to organ failure, most importantly, acute respiratory distress syndrome (ARDS) (2).

Advanced age and comorbidities, such as hypertension, chronic obstructive pulmonary disease, or cardiovascular diseases are proved risk factors in COVID-19 (1, 3). Patients with elements of metabolic syndrome (MS), such as diabetes, obesity, or hyperlipidemia are more susceptible to infection and also have worse outcomes in COVID-19 (4, 5). MS was found to be associated with chronic low-grade inflammation that compromises the immune system and causes microvascular endothelial dysfunction, which may contribute to poor outcomes in COVID-19 (6, 7).

Metabolic-associated fatty liver disease (MAFLD) and non-alcoholic fatty liver disease (NAFLD) are the most common chronic liver diseases (CLD), which affect about a quarter of the world's adult population (8). Pre-existing liver diseases such as NAFLD or the recently defined MAFLD, as the hepatic manifestations of MS (8), might also be significant risk factors of hospitalization and severity in COVID-19 (9, 10). The MAFLD criteria are based on evidence of hepatic steatosis in addition to one of the following three criteria: overweight/obesity, presence of type 2 diabetes mellitus, and proof of metabolic dysregulation (8).

According to recent publications, the presence of MAFLD and NAFLD may exacerbate the virus-induced inflammatory "storm" possibly through the hepatic release of pro-inflammatory cytokines and by increased reactive oxygen production in COVID-19 patients (11–13).

There are still limited reports on how MAFLD and NAFLD influence clinical outcomes in patients with COVID-19, and there are no meta-analytical reports of the available evidence. This meta-analysis aimed to evaluate if MAFLD or NAFLD are associated with a more severe disease course of COVID-19.

METHODS AND MATERIALS

We report our systematic review and meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 Statement (**Supplementary Table 1**) (14). We registered the protocol of this study onto the International Prospective Register of Systematic Reviews (CRD42020210923) and adhered to it during the course, except for including mortality in our outcomes (see https://www.crd.york.ac.uk/prospero).

Search and Selection

A systematic search was performed in five databases, namely Scopus, Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Embase, and MEDLINE (via PubMed) without any search restrictions from inception to 15th Sept, 2020. The following search key was used: (NASH OR steatohepatitis OR "metabolic associated fatty liver disease" or "Non-alcoholic Fatty Liver Disease" OR "Nonalcoholic Fatty Liver Disease" OR MAFLD OR NAFLD) AND ("COVID 19" OR "Wuhan virus" OR "coronavirus" OR "2019 nCoV" OR "SARS-Cov-2").

After the removal of duplicates with a reference manager software (EndNote X9, Clarivate Analytics, Philadelphia, PA, USA), papers for title, abstract, and full-text were screened by two independent authors separately according to a predetermined set of rules. In the case of any disagreement, a consensus was reached after discussion with a third author.

Eligible studies reported on (P) patients with confirmed SARS-CoV-2 infection and compared the outcomes of patients (I and C) with and without MAFLD or NAFLD to each other. The outcomes (O) were severe COVID-19, ICU admission, and in-hospital mortality. Studies with cohort or case-control design (>5 participants) were considered eligible. The severity of COVID-19 was classified according to the guidelines on the Diagnosis and Treatment of COVID-19 issued by the National Health Commission of China (**Supplementary Table 2**) (15). When there were multiple publications using data with overlapping study populations, we included the one with greater sample size.

Data Extraction

Two independent review authors performed data extraction from eligible studies into a standardized data collection form. A third independent author resolved disagreements.

The following information was extracted from each study: first author, year of publication, digital object identifier, study design, study period, the number of centers, study site (country), demographic characteristics of the study population, the number of patients, the number of participants with and without MAFLD or NAFLD separately, the number of patients with event (severe COVID-19, ICU admission, mortality) with and without MAFLD or NAFLD separately, and, if available, odds ratios for COVID-19 severity, ICU admission, and mortality regarding MAFLD or NAFLD, and parameters included in multivariate adjustments.

Statistical Analysis

All calculations were performed by Stata 15 data analysis and statistical software (Stata Corp LLC, College Station, TX, USA). All outcomes were handled as dichotomous variables, and odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated (reference groups: patients without NAFLD or MAFLD). Random effects model was used to calculate the pooled estimates using the DerSimonian-Laird method (16). A p-value of <0.05 was considered statistically significant. Forest plots were used to present the results of the meta-analyses.

Heterogeneity was tested with I^2 and χ^2 tests. As suggested by the Cochrane Handbook (17), I^2 values were interpreted as "might not be important" (0–40%), "moderate" (30–60%), "substantial" (50–90%), and "considerable" (75–100%) heterogeneity, with a $p < 0.1$ considered significant (18).

We were unable to assess the presence of publication bias because of the low number of studies included in each analysis.

Assessment of Risk of Bias

Two independent review authors carried out the assessment. Discrepancies were resolved by third-party arbitration. We used the modified version of the Quality in Prognostic Studies (QUIPS) tool (19) as per the recommendations of the Cochrane Prognosis Methods Group (20). Methodological details of the assessment are summarized in **Supplementary Appendix 1**.

RESULTS

Search and Selection

The selection process is detailed in **Figure 1**. We identified 319 records in five databases for evaluation. After the removal of duplicates and careful selection, 25 articles were eligible for full-text assessment. Altogether, 10 papers were eligible for qualitative and quantitative synthesis (9, 10, 21–27), however, we excluded one due to overlapping study population (12).

Characteristics of the studies included

The main characteristics of the studies are summarized in **Table 1**. Two articles recruited subjects from the USA, one from Israel, and another six from China. Except for two prospective study, all were retrospective cohort studies. MAFLD was defined in all studies based on the consensus by Eslam et al. (8); NAFLD was defined by the presence of hepatic steatosis on imaging. The proportion of patients with MAFLD and NAFLD ranged from 28 to 50%, and from 6 to 38%, respectively, across studies. Eligibility criteria of the studies included are presented in **Supplementary Table 3**.

Quantitative Syntheses

In our meta-analysis, we included a total of six studies with 7,284 patients evaluating the severity of COVID-19, the proportion of severe COVID-19 ranged from 10 to 19%. Three articles with 7,433 patients reported on the need for ICU admission, the proportion of ICU admission ranged from 6 to 38%.

MAFLD was associated with an increased risk of severe COVID-19 compared to the non-MAFLD group [28 vs. 13%, respectively; OR = 2.61, CI: 1.75–3.91 in a homogenous dataset ($I^2 = 0.0\%$ with $p = 0.483$)] (**Figure 2A**). Similarly, in the NAFLD vs. non-NAFLD comparison, NAFLD proved to be a risk factor as well [36 vs. 12%, respectively; OR = 5.22, CI: 1.94–14.03 in a heterogenous dataset ($I^2 = 85.1\%$ with $p = 0.001$)] (**Figure 2B**).

Although patients with NAFLD were more likely to be admitted to ICU compared to those without NAFLD, the difference did not reach the level of statistical significance [24 vs. 7%, respectively; OR = 2.29, CI: 0.79–6.63 in a heterogenous dataset ($I^2 = 85.1\%$ with $p = 0.001$)] (**Figure 2C**).

Qualitative Syntheses

We were not able to make a meta-analytical analysis for the MAFLD vs. non-MAFLD comparison on the rate of ICU admission, however, two studies (10, 27) reported on ICU admission. Gao et al. (27) in non-diabetic MAFLD patients found an increased risk of intensive care requirement in those with critical illness compared to non-MAFLD patients ($p = 0.003$, 4.6 vs. 0.0%, respectively). Zhou et al. (10), in a matched cohort of MAFLD and non-MAFLD patients, found a significantly increased risk of the composite outcome of severe and critical COVID-19 in MAFLD patients compared to the non-MAFLD group (OR = 3.65, CI: 1.31–10.16).

Regarding in-hospital mortality, Hashemi et al. (23) found similar rates in COVID-19 patients with NAFLD compared to those without NAFLD ($p = 0.54$, 16.4 vs. 13.2%).

A summary of multivariate logistic regression analyses from each study included can be found in **Supplementary Table 4**. Most of the studies adjusted for age, sex, and underlying conditions in multivariate analysis. In the study of Ji et al. (24), NAFLD was associated with COVID-19 progression (adjusted OR = 6.4, CI: 1.5–31.2). Bramante et al. (9) found an increased odds of hospital admission in COVID-19 patients with NAFLD (adjusted OR = 2.04, CI: 1.55–2.69). Based on two studies, ICU admission (adjusted OR = 1.70, CI: 1.20–2.40; adjusted OR = 2.3, CI: 1.27–4.17, respectively) and need for mechanical ventilation (adjusted OR = 1.98, CI: 1.28–3.06; adjusted OR = 2.15, CI: 1.18–3.91, respectively) were also increased with NAFLD (9, 23). Finally, NAFLD was not found to increase in-hospital mortality in COVID-19 (adjusted OR = 0.99, CI: 0.54–1.77) (9).

On the other hand in COVID-19 patients with MAFLD, Mahamid et al. (25) found that MAFLD was associated with severe COVID-19 in both sexes (adjusted OR = 3.29, CI: 3.28–3.58 for men, adjusted OR = 3.25, CI: 3.09–3.47 for women), independently of MS. In the study of Zhou et al. (21), an association between the presence of MAFLD and COVID-19 severity was observed in patients younger than 60 years (adjusted OR = 2.67, CI: 1.13–6.34), but not in those above 60 years (adjusted OR = 0.61, CI: 0.18–2.03). In non-diabetic patients, Gao et al. (27) found an increased risk of severe COVID-19 only in MAFLD patients with both obesity and metabolic dysregulation (adjusted OR = 5.25, CI: 1.23–22.33), but the difference was non-significant if only one of the criteria was present (OR = 2.60, CI: 0.47–14.42).

Risk of Bias Assessment

Among the included studies, three were of moderate overall risk of bias. All the other studies were rated to carry high overall risk of bias. The summary of risk of bias assessment is shown in **Supplementary Figures 1–5**.

DISCUSSION

In our meta-analysis, we aimed to analyse the association between MAFLD or NAFLD and COVID-19 outcomes. Based on our results, we identified that MAFLD is associated with 2.6 times higher risk of severe COVID-19 compared to the non-MAFLD group. In the NAFLD vs. non-NAFLD comparison, we found a five-times increased risk of severe COVID-19. The rate of the ICU admission was higher in NAFLD patients compared to those without NAFLD; however, the difference was statistically non-significant. Finally, we did not find any difference regarding in-hospital mortality in COVID-19 patients with MAFLD or NAFLD in qualitative synthesis.

Previous reviews have assessed the effect of MAFLD or NAFLD in COVID-19 patients, however, to our knowledge, this

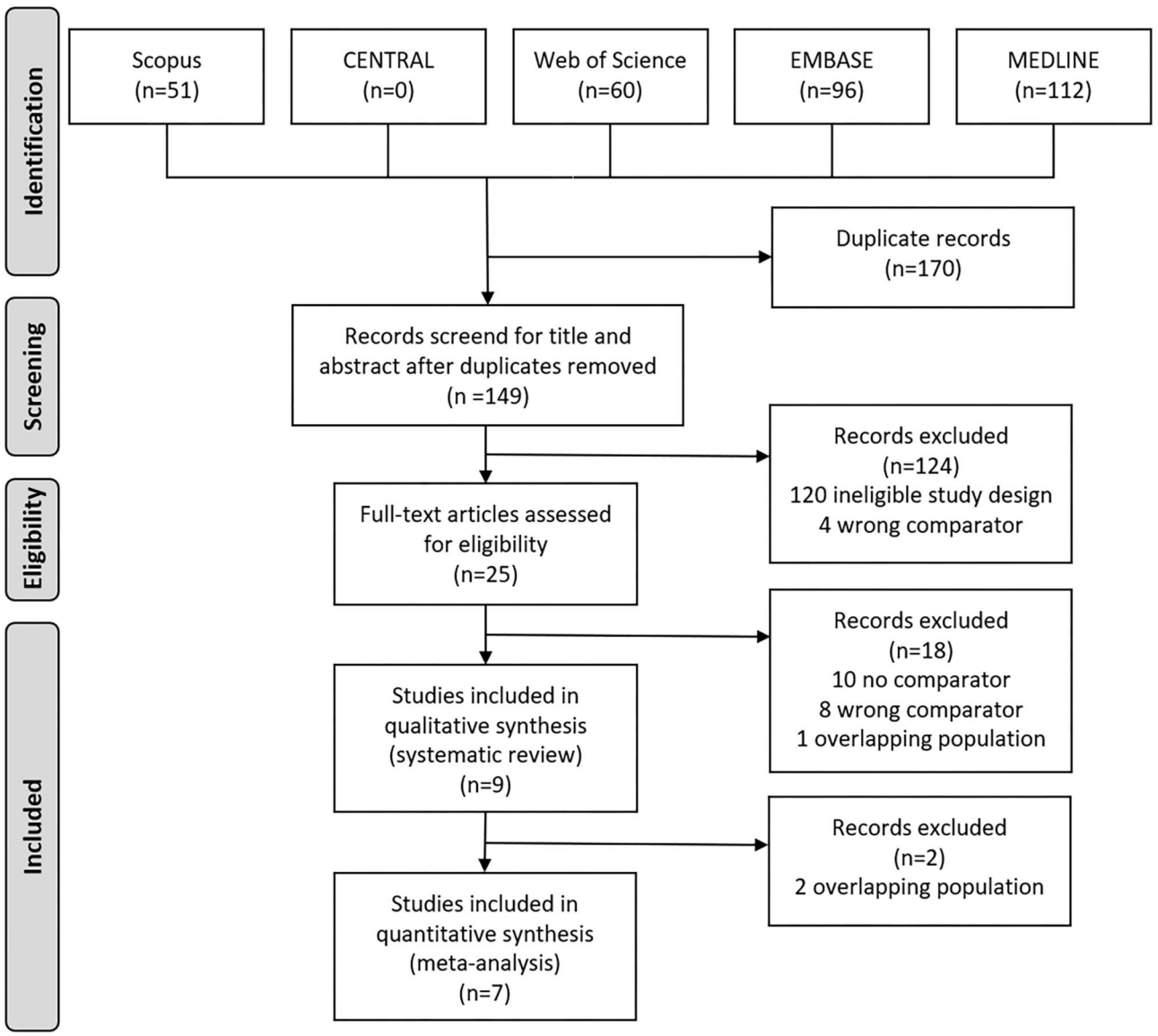

FIGURE 1 | Preferred Reporting in Systematic Reviews and Meta-analyses (PRISMA) flowchart showing the selection process.

is the first systematic review and meta-analysis in this topic (6, 11, 28).

Six of the included articles reported on covariate adjusted results (9, 21, 23–25, 27), most of them supporting our conclusion on the impact of MAFLD and NAFLD in COVID-19. We could not perform a meta-analytical evaluation of these results, as there were different outcomes assessed and covariates adjusted for. Based on these results, MAFLD and NAFLD are associated with a higher risk of severe COVID-19 and ICU admission both in uni- and multi-variate analyses.

Previously several comorbidities such as hypertension, diabetes, extreme obesity, and cardiovascular disease were reported to be associated with worse prognosis in COVID-19 patients (3, 5). Several meta-analyses reported on the role of CLD in COVID-19 (29, 30). Based on our previous paper (31), pre-existing liver diseases and on-admission liver-related laboratory results predicted a more severe outcome in SARS-CoV-2 infection. However, none of the articles performed subgroup analysis based on the underlying liver condition.

The association between MAFLD or NAFLD and COVID-19 severity is certainly multifactorial. MS and elements of it have been already linked to untoward outcomes in COVID-19 (32). In type 2 diabetes, the second most common comorbidity in COVID-19, the poor prognosis is likely the consequence of the whole clinical picture: poor glucose control, advanced age, and diabetes-associated comorbidities (33). Obesity is associated with chronic inflammation compromising the immune response resulting in an increased risk of more severe infections (34, 35),

TABLE 1 | Basic characteristics of included studies in the systematic review and meta-analysis.

Author	Country	Total N⁰ of patients	Female%	Age (year)†	N⁰ of patients with MAFLD or NAFLD (% of total)	Outcome(s)	
						Definition	Event N⁰ (% of total)
NAFLD vs. no-NAFLD comparison							
(9)	USA	6,802	44	46	373 (5.5)	Severe COVID-19/ ICU admission	930 (13.67)
							428 (6.3)
(23)	USA	351	45	63.4	57 (16)	ICU admission/ In-hospital mortality	132 (37.6)
							55 (15.67)
(22)	China	280	48	43	86 (31)	Severe COVID-19/ ICU admission	28 (10)
							18 (6.43)
(24)	China	202	44	44.5	76 (38)	Severe COVID-19	39 (19.31)
MAFLD vs. no-MAFLD comparison							
(27)	China	130	37	46	65 (50)	ICU admission	3 (2.31)
(25)	Israel	71	73	51	22 (31)	Severe COVID-19	13 (18.31)
(26)	China	310	52	47	94 (30)	Severe COVID-19	50 (16.13)
(21)‡	China	327	ND	ND	93 (28)	Severe COVID-19	59 (18)
(10)‡	China	110	26	42	55 (50)	ICU admission	3 (2.73)

†*mean or median,* ‡*prospective study.*

COVID-19, coronavirus disease 2019; ICU, intensive care unit; NAFLD, non-alcoholic fatty liver disease; ND, not defined; MAFLD, metabolic associated fatty liver disease.

on the other hand, obesity is also a significant risk factor for ICU admission and invasive mechanical ventilation (5). In patients with diabetes, hyperinflammatory response, microvascular endothelial dysfunction, and microthrombi formation may contribute to the poorer outcomes in COVID-19 (6).

Similarly, based on previous reports (26), in patients with MAFLD, a pro-inflammatory state could exacerbate the SARS-CoV-2 induced cytokine storm. Ji et al. (24) found in a retrospective study that COVID-19 patients with MAFLD had a poorer prognosis, two-fold higher prevalence of severe disease course, and also higher viral shedding time, and more liver failure during hospitalization.

In the included studies several differences between study populations were highlighted. Increased liver fat content was associated with a higher risk of symptomatic COVID-19 in univariate analysis (OR = 1.85, 95% OR: 1.05–3.25) (36). Moreover, the authors found that obesity and concomitant >10% liver fat content exposed an increased risk of severe COVID-19 (OR = 2.96, 95% CI: 1.12–7.78); those obese patients with normal liver fat content (<5%) showed no elevation of risk (OR = 0.36, 95% CI: 0.1–1.26). The importance of the liver fat content has been pointed out in the study by Bramante et al. (9) as well.

On the other hand, the presence of fibrosis in MAFLD patients is another risk factor for severity of COVID-19, independently of metabolic comorbidities. Based on Targher et al. (12), the severity of COVID-19 significantly increased with the extent of liver fibrosis; those with a FIB-4 score higher than 2.67 had the highest risk of developing severe COVID-19 (OR = 5.73, 95% CI: 1.84–17.9). After adjustment for sex, obesity, and diabetes, this considerable association persisted (adjusted OR = 2.91, 95% CI: 1.20–7.06).

The same authors demonstrated that the presence of MAFLD together with a neutrophil-to-lymphocyte ratio (NLR) higher than 2.8 is associated with a higher risk of severe COVID-19 compared to patients without MAFLD and with normal NLR (26). NLR was previously highlighted to be a useful, widely available prognostic factor in the early phase of SARS-CoV-2 infection (37).

Another interesting point was reported by Zhou et al. (21). In COVID-19 patients with MAFLD under 60 years, a more than 4-fold risk of severe COVID-19 was observed compared to those without MAFLD (OR = 3.97, 95% CI: 1.89–8.35); after adjusting for covariates (adjusted OR = 2.67, 95% CI: 1.13–6.34) the risk remained significantly higher. In contrast, in multivariate analysis in elderly patients, MAFLD was not associated with severity of COVID-19. These results need to be supported by further cohort analysis.

None of the studies reported on long-term outcomes in COVID-19.

Strengths and Limitations

Considering the strengths of our meta-analysis, a rigorous methodology was followed, and we did not deviate from the pre-study protocol, except for including mortality in our investigated outcomes. Several limitations must be considered when interpreting our results. First of all, we could not analyse in-hospital mortality in our meta-analysis. Secondly, our study involved data from only nine articles. It must be noted that,

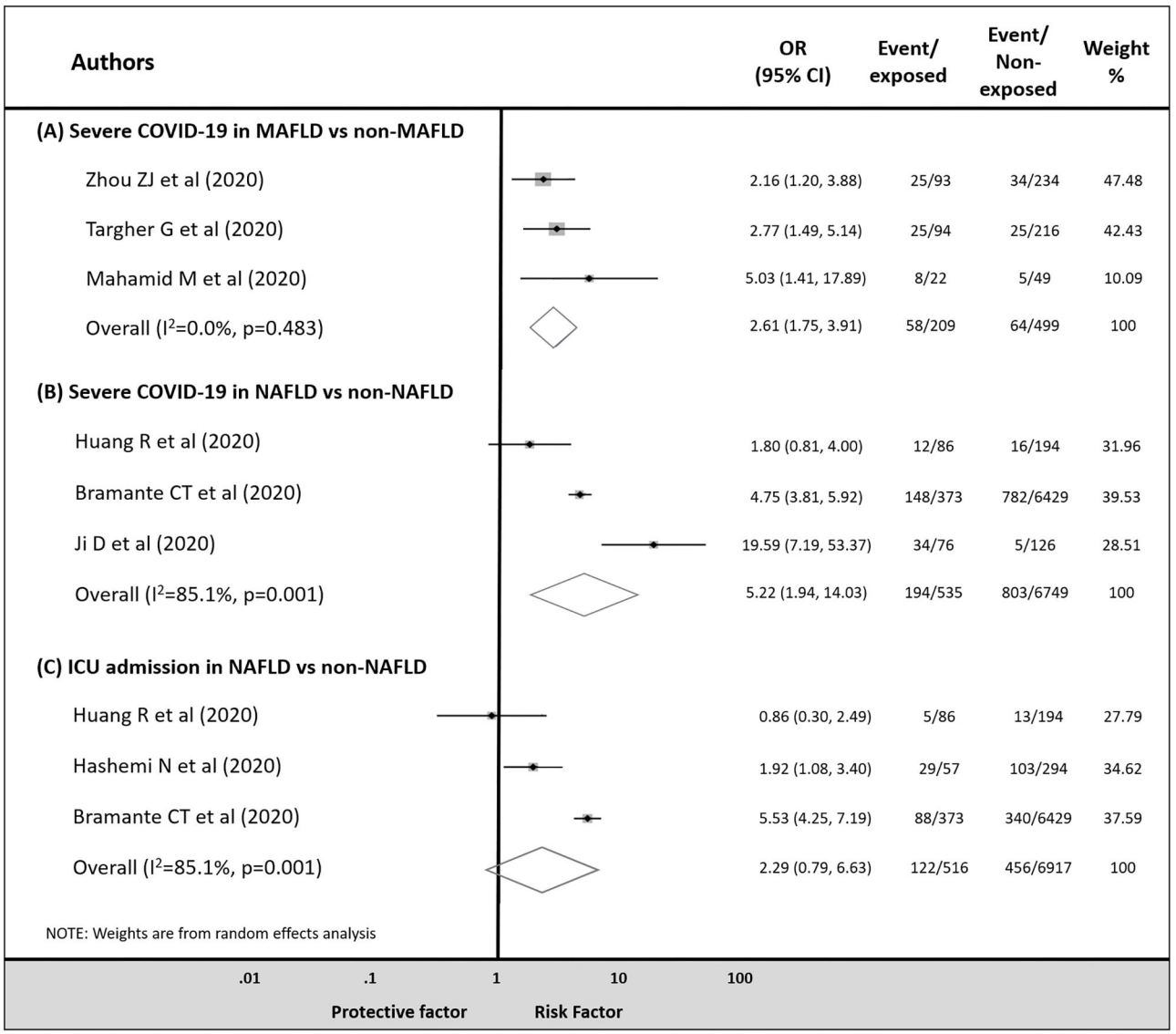

FIGURE 2 | Odds ratio for COVID-19 severity in patients **(A)** with MAFLD vs. non-MAFLD, **(B)** with NAFLD vs. non-NAFLD, and odds ratio for ICU admission in patients **(C)** with NAFLD vs. non-NAFLD.

we detected significant differences despite the limited study populations, however, with considerable statistical heterogeneity in some of our results. Most of the studies included a low number of patients. The number of studies prevented us from analyzing publication bias (<10 articles). Most of the articles were published from Asian countries; therefore, it is difficult to generalize these results. Also the rate of MAFLD and NAFLD in the study populations differed from the rate reported in the general population. The definition of MAFLD was homogenous, however, NAFLD was diagnosed using different methods across studies. Finally, data came mostly from retrospective studies, with most of them carrying high risk of bias.

CONCLUSION

Implication for Practice

In conclusion, the presence of MAFLD or NAFLD is associated with a more severe COVID-19. The presence of further metabolic dysfunction may have additional negative impact on the course of COVID-19. Based on this, health-care providers should follow MAFLD patients cautiously and preventive measures should be taken in these high-risk populations. Therefore, weight loss and regular physical activity should be encouraged in MAFLD patients.

Implication for Research

The underlying mechanisms behind our results are still poorly understood. Further research is needed to understand the effect of the pro-inflammatory state associated with MAFLD on the cytokine storm caused by SARS-CoV-2 infection. The severity of COVID-19 should be further stratified based on the severity of MAFLD to explore further high-risk patient groups. Further research is needed to support our results as well as other outcomes, such as mortality, should be analyzed.

DATA AVAILABILITY STATEMENT

The original contributions generated for the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

GP and PJH designed the research and the study concept. ZS and SV performed the data extraction. NF analyzed and interpreted the data. FD and KO performed the quality and risk assessment, PJH, BE, SV, SK, PH, and GP wrote the article. BE, PH, and GP conducted a critical revision of the manuscript for important intellectual content. All of the co-authors granted final approval of the version of the article to be published.

FUNDING

Study costs are covered by an Economic Development and Innovation Operative Program Grant (GINOP 2.3.2-15-2016-00048) and by a Human Resources Development Operational Program Grant (EFOP-3.6.2-16-2017-00006), both co-financed by the European Union (European Regional Development Fund) within the framework of the Széchenyi 2020 Program. Sponsors had no role in the design, data collection, analysis, interpretation, and preparations of the manuscript.

ACKNOWLEDGMENTS

The analysis was conducted on behalf of the Translational Action and Research Group against Coronavirus (KETLAK) Study Group. Future study costs will be covered by Economic Development and Innovation Operative Program Grant (GINOP-2.3.4-15-2020-00010).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.626425/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Public Health

ORIGINAL RESEARCH
published: 22 March 2021
doi: 10.3389/fpubh.2021.632608

The Status of Occupational Protection During COVID-19 Pandemic: Knowledge, Attitudes, and Practice of Healthcare Workers in Endoscopy Units, China

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Background: SARS-CoV-2 spreads rapidly around the world, and some patients present gastrointestinal symptoms. The existence of the virus in the gastrointestinal tract makes digestive endoscopy a high-risk operation, which associated with an increased risk of infection rate in healthcare workers. This study aimed at exploring current knowledge, practice and attitudes of healthcare workers in endoscopy units in China regarding the status of occupational protection during COVID-19 pandemic.

Methods: A cross-sectional study of a national online survey involving 717 healthcare workers in endoscopy units from 94 medical structures in 24 provinces and municipalities around China was conducted online via a questionnaire platform called Wenjuanxing (wjx.cn). The data were analyzed using correlation approaches, Kruskal-Wallis test for independent samples, and linear regression models.

Results: Most Chinese healthcare workers in endoscopy units had a good knowledge of COVID-19 (median: 10; range: 7–12), showed a strikingly positive attitude (median: 65; range: 39–65), and carried out good practice (median: 47; range: 14–50) in strengthening the protection, disinfection and management of COVID-19. In terms of attitudes, female staff was more concerned about protection against COVID-19 than male staff (KW = 8.146, $P = 0.004$). Nurses performed better in both attitude (KW = 2.600, $P = 0.009$) and practice (KW = 6.358, $P < 0.001$) than endoscopic physicians when carrying out personal protection, patient care and environmental disinfection against SARS-CoV-2 infection. More positive attitudes in protection were related to better protective behavior in endoscopic daily medical work ($r = 0.312$; $P < 0.001$).

Conclusion: The findings of this study suggest that Chinese endoscopy healthcare workers have an excellent mastery of knowledge about COVID-19, which is transformed into positive beliefs and attitudes, contributing to good practice during daily endoscopic procedures. Medical staff may benefit from further education. With the gradual normalization amid the ongoing COVID-19 pandemic, protection and management in endoscopy units may be changed accordingly.

Keywords: occupational protection, COVID-19, endoscopy, knowledge, attitudes, practice

OPEN ACCESS

Edited by:
Omur Cinar Elci, Asian University for Women, Bangladesh

Reviewed by:
Andrea Spinazzè, University of Insubria, Italy
Siti Munira Yasin, MARA University of Technology, Malaysia

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Specialty section:
This article was submitted to Occupational Health and Safety, a section of the journal Frontiers in Public Health

Received: *24 November 2020*
Accepted: *22 February 2021*
Published: *22 March 2021*

Citation:
Tian Y, Nian B, Ma Y, Guo X, Wang F and Rong L (2021) The Status of Occupational Protection During COVID-19 Pandemic: Knowledge, Attitudes, and Practice of Healthcare Workers in Endoscopy Units, China. Front. Public Health 9:632608. doi: 10.3389/fpubh.2021.632608

INTRODUCTION

The severe acute respiratory syndrome caused by new coronavirus (SAS-CoV-2) was first cluster in December 2019 and reported from China (1). This disease was spread into global pandemic rapidly, and a total of 93,194,922 confirmed cases and more than 2 million deaths were reported in January 2021 (2). The pandemic of coronavirus disease 2019 (COVID-19) in China at stable status, while a "second wave" of contagion was outbreak outside of China (3). As a highly contagious disease, the risk of infection among healthcare workers is significant. Twenty nine percentage of patients (40 out of 138) were healthcare workers in one of the earliest studies in Wuhan (4). A report of American Center for Disease Control and Prevention (CDC) of US stated that from February 12 to April 9, a total of 9,282 healthcare workers were diagnosed with COVID-19, including 27 deaths. Eleven to nineteen percentage of COVID-19 cases were identified as medical staffs (5). Studies have already illustrated the virus transmission, and found physical distancing of 1 m or more, and use of face masks, respirators, and eye protection could prevent the transmission of COVID-19 (6–13) while the current knowledge, practice and attitudes of healthcare workers in endoscopy units remains unclear.

Digestive symptoms are increasingly recognized among patients with COVID-19, including anorexia, diarrhea, nausea, vomit, and abdominal pain (14). Several studies pointed out that some patients presented only GI symptoms and no typical symptoms throughout the course of the disease (15). Viral RNA was detected in the feces of COVID-19 patients, and active virus particles were isolated (16). Most atypical patients with GI symptoms did not visit the Pulmonary Department, Emergency Department or Fever Clinic, but the Gastroenterology Department, which resulted in healthcare providers being exposed to either respiratory and gastrointestinal droplets or body fluids from patients when performing endoscopy. Aerosols generated from coughing in upper endoscopy and flatus produced in colonoscopy played an important role in endoscopist exposure to the virus (17). Endoscopy therefore was a potential route of infection according to the characteristics and transmission of the virus. These preliminary findings highlight that adequate protection of healthcare workers is critical.

The theory of knowledge, attitude/belief and practice (KAP) model on PHEIC may distinguish from general issues (18, 19). At the early stage of SARS-CoV-2 epidemic in China, National Health Commission of the PRC and Chinese CDC conducted public education and took prevention measures quickly in the whole society as responses to COVID-19 (20). In addition, the Chinese Society of Digestive Endoscopy also made special regulations on endoscopic work (21). With the joint efforts, people's knowledge reserve for epidemic prevention and control reached a high and stable level, which partially accounted for the negative results from knowledge. It is easier for endoscopic healthcare workers who have received medical education for years to master the knowledge of COVID-19. For instance, endoscopy physicians who believe low population density can reduce the transmission of SARS-CoV-2 may limit the daily number of patients examined. Given the adequate protective knowledge, different attitudes lead to different practice. This cross-sectional study was performed using an online questionnaire to evaluate the occupational protection status of healthcare workers in endoscopy units of different hospital scale in different regions in China. The level of knowledge and awareness of healthcare workers about COVID-19 occupational protection during the pandemic, or the behavior of participants with respect to personal protective equipment and disinfection management were assessed in this study, so as to give advice and suggestions to endoscopic units in other regions.

MATERIALS AND METHODS

Study Subjects

Endoscopic healthcare workers, including endoscopy physicians, nurses, and cleaning workers from general hospitals, specialized hospitals and community medical institutions from 94 medical structures in 24 provinces and municipalities around China were enrolled and invited to complete the questionnaire in this study. Ten times the number of questionnaire entries with extra 10% invalid questionnaires, 389 was regarded as the minimum sample size for this study. This study was approved by the Peking University First Hospital Biomedical Research Ethics Committee (No. 2020-124). All subjects finally enrolled in this study were considered to have signed informed consent agreement prior to answering the questionnaire.

Questionnaire Design

Based on the guidance issued by Chinese Medical Association on the endoscopic diagnosis and treatment during the prevention and control of new coronavirus infection, the questionnaire items were designed and screened by a group of specialists who had experience in the fields of endoscopic diagnosis and treatment, epidemic prevention and control, and public health research. This questionnaire was applied to the evaluation of endoscopic healthcare workers from three aspects, namely, knowledge, attitudes, and behavior toward COVID-19. More details are shown in **Table 1** and Appendix 1 (**Supplementary Material**). The response for each item of knowledge part was scored 0–1. A five-grade scoring method was used to indicate the level for attitude part: 5, strongly agree; 4, agree; 3, neutral; 2, disagree; 1, strongly disagree. Moreover, the five-grade scoring method was applied to indicate the level for practice part: 5. Always; 4. Often; 3. Sometimes; 2. Occasionally; 1. Hardly ever. The scoring system for knowledge ranged from 0 to 12, and the good knowledge score was defined as >7.2 (above 60%), and poor knowledge was defined as below 60%. Similarly, the scoring system for attitude and practice ranged from 13 to 65, and 10 to 50, respectively, and the good attitude and good practice were defined as > 52 (attitude scores above 80% were defined as good attitude) and > 40 (scores >80% were classified as having good practice), respectively (22).

Questionnaire Evaluation

The quality of the present questionnaire was evaluated from two aspects, namely, validity and reliability. For content validity, the

TABLE 1 | Demographic characteristics of subjects.

Items	No. (n)	Ratio (%)
Gender		
Male	206	28.7%
Female	511	71.3%
Age		
20–35	219	30.54%
36–50	439	61.23%
51–65	59	8.23%
Occupational identity		
Endoscopic physicians	329	45.9%
Nurses	378	52.7%
Cleaning workers	10	1.4%
Length of service		
<5 years	200	27.9%
5–10 years	277	38.6%
>10 years	240	33.5%
Education		
Bachelor degree or below	614	85.6%
Master degree or above	103	14.4%
Hospital grade		
Primary	14	2.0%
Secondary	237	33.0%
Tertiary	466	65.0%

consistency of the contents to be tested with questionnaire items was assessed by five experts from related fields using a four-level scoring method, in which score 1 represented "irrelevant," 2 "a little bit relevant," 3 "relevant," and 4 "very relevant." Content validity index (CVI) was served as the measurement, and an index value of >0.8 indicated an acceptable content validity. External reliability, also known as test-retest reliability, was also examined in this study.

Investigation Method

Electronic questionnaire was adopted in this study to investigate current situations of endoscopic healthcare workers during COVID-19 pandemic. The questionnaire entries were imported to the online platform Wenjuanxing (wjx.cn), and distributed to endoscopic healthcare workers around China via WeChat. All the subjects were invited to finish the survey before April 4th, 2020. The data were subsequently downloaded and sorted by specialists. Investigators were blinded to the identity information of the subjects.

Statistical Analysis

Descriptive statistics was used to summarize demographic data, and internal reliability was measured by Cronbach's α. The questionnaire scores according to demographic data were compared by using independent sample *t*-test, Mann-Whitney U test, one-way analysis of variance, rank-sum test and Pearson/Spearman correlation analysis separately based on the data distribution. A $P < 0.05$ was considered to be significant, and the results of all tests noted above were analyzed using SPSS 24.0 software.

RESULTS

Demographic Characteristics of Subjects

A total of 717 valid questionnaires were collected before April 4th. The questionnaire was completed by healthcare workers from 94 medical structures in 24 provinces and municipalities. More demographic details are shown in **Table 1**. The average rating index of this questionnaire was defined as CVI, which was 0.924, indicating an acceptable content validity.

Level of Knowledge

The distribution of responses to the statements that examined the level of knowledge with respect to COVID-19 is presented below (**Table 2**). The variable ranged from 0 to 12. Overall, medical staff in endoscopy units had a good knowledge, with the median total score of 10 (total score range: 7–12), and 83.33% of accuracy. The good knowledge rate was 99.4% (713/717). There were no significant differences between other demographic characteristics and the level of knowledge about COVID-19.

Level of Attitudes

The distribution of responses to statements that examined attitudes is shown in **Table 3**. The variable in attitudes ranged from 13 to 65, and medical staff had a strikingly positive attitude toward strengthening the protection, disinfection and management of COVID-19, with the median score of 65 (score range: 39–65). 99.3% (712/717) of participants supported limited daily endoscopy services or service suspension, and 92.9% (666/717) had a positive attitude toward risk-based screening before the endoscopy procedure and appropriate occupational protection during the outbreak. The good attitude rate was 99.3% (712/717). Female staff were more concerned about COVID-19 than male staff (KW = 8.146, $P = 0.004$), and the same phenomenon was observed between nurses and physicians. Nurses had a more positive attitude than physicians (KW = 2.600, $P = 0.009$, Adj. $P = 0.028$).

Level of Practice

Table 4 shows the distribution of responses to statements that examined personal protection, patient care and disinfection management practice or behavior. The variable in behavior ranged from 10 to 50. The median score of the survey was 47 (score range: 14–50), which showed that medical staff had good practice in COVID-19. The good practice rate was 87.2% (625/717). The comparison of attitudes showed that 93.8% (673/717) of the subjects provided limited daily endoscopy services, the risk-based visit process was implemented in the endoscopy units of 88.1% (632/717) of the subjects, and 1.4% (10/717) believed that their hospitals needed to increase the supply of personal protective equipment.

Similar to the above findings about healthcare workers' attitudes, female staff were more active than male staff in carrying out personal protection, patient care, and environmental disinfection practice against SARS-CoV-2 infection (KW =

TABLE 2 | Distribution of responses to the knowledge questionnaire.

	Score distribution *n* (%)				*P*-value
	Median (range)	7–8	9–10	11–12	
Sex					
Male	10 (7–12)	13 (6.31)	109 (52.91)	84 (40.78)	0.991
Female	10 (7–12)	17 (3.33)	295 (57.73)	199 (38.94)	
Age					
<40	10 (7–12)	17 (4.97)	190 (55.56)	135 (39.47)	0.810
≥40	10 (7–12)	13 (3.47)	214 (57.07)	148 (39.47)	
Occupational identity					
Endoscopic physicians	10 (7–12)	21 (6.38)	183 (55.62)	125 (37.99)	0.345
Nurses	10 (7–12)	9 (2.38)	214 (56.61)	155 (41.01)	
Cleaning workers	10 (10–11)	0 (0)	7 (70.00)	3 (30.00)	
Length of service					
<5 years	10 (7–12)	11 (5.50)	115 (57.50)	74 (37.00)	0.551
5–10 years	10 (7–12)	10 (3.61)	157 (56.68)	110 (39.71)	
>10 years	10 (7–12)	9 (3.75)	132 (55.00)	99 (41.25)	
Education					
Bachelor degree or below	10 (7–12)	23 (3.75)	340 (55.37)	251 (40.88)	0.036
Master degree or above	10 (8–12)	7 (6.80)	64 (62.14)	32 (31.07)	
Hospital Grade					
Primary	10 (8–11)	1 (7.14)	8 (57.14)	5 (35.71)	0.729
Secondary	10 (7–12)	11 (4.64)	127 (53.59)	99 (41.77)	
Tertiary	10 (7–12)	18 (3.86)	269 (57.72)	179 (38.41)	

18.564, $P < 0.001$). Nurses (KW = 6.358, $P < 0.001$, Adj. $P < 0.001$) and cleaning workers (KW = −2.585, $P = 0.010$, Adj. $P = 0.029$) had a higher score than physicians. Medical staff in tertiary hospitals performed better in practice than those in secondary hospitals (KW = −3.591, $P < 0.001$, Adj. $P = 0.001$).

The Relationships Among Knowledge, Attitudes, and Practice

The relationships among three dimensions were explored via Spearman's rank correlation analysis. As a result, there was no significant correlation either between knowledge and practice ($r = 0.014$; $P = 0.710$) or between knowledge and attitudes ($r = 0.038$; $P = 0.314$). However, a positive correlation between the level of attitudes and practice was found in the subjects ($r = 0.312$; $P < 0.001$). More positive attitudes in protection were related to better protective behavior in endoscopic daily medical work (**Figure 1**).

DISCUSSION

The KAP proposed in the last century has been applied to explaining how personal knowledge and attitudes affected practice in various fields (23–25). In general, knowledge is the basis of behavior formation, and only when knowledge rises to the level of belief can an individual be possible to adopt a positive attitude to change practice. During the COVID-19 pandemic, Chinese health departments have organized various forms of learning activities about SARS-CoV-2, including the virus characteristics, transmission routes, personal protection, quarantine policies, and so on. All the Chinese citizens had access to the knowledge, which was transformed into beliefs. Positive beliefs and attitudes were the motivation for the protective behavior. The medical staff have close contact with patients, and the risks was high, and the KAP theory was more important for medical staff. Therefore, we designed the present questionnaire and enrolled staffs from different institutions to investigate the application of KAP theory by endoscopic healthcare workers in COVID-19 pandemic in China (26–29).

It was found that a high proportion of participants had a good knowledge of COVID-19, which could be possibly attributed to the effective continuing medical education and training going on across the country. Endoscopy-related continuing medical education has an important part to play in preparing for and responding to this situation. Li et al. (30) underscored the importance of continuous medical education and training in this pandemic. Chinese National Health Commission has held online lectures, requiring all medical staff to learn the characteristics and protection requirements of COVID-19.

Moreover, the Endoscopic Society delivered a course of recommended operating procedures in endoscopy units, especially about personal protection and endoscope decontamination, to related healthcare workers, and related questions were required to answer after the course. SARS-CoV-2 is a newly emerged virus, whose virological and disease characteristics are gradually explored and may change at any time. Therefore, continuing education courses for medical staff

TABLE 3 | Distribution of responses to the attitude questionnaire.

	Score distribution *n* (%)							*P*-value
	Median (range)	<50	51–53	54–56	57–59	60–62	63–65	
Sex								
Male	65 (39–65)	4 (1.94)	7 (3.40)	5 (2.43)	18 (8.74)	20 (9.71)	152 (73.79)	0.004
Female	65 (51–65)	0 (0)	9 (1.76)	6 (1.17)	18 (3.52)	59 (11.55)	419 (82.00)	
Age								
<40	65 (39–65)	2 (0.58)	9 (2.63)	4 (1.17)	17 (4.97)	33 (9.65)	277 (80.99)	0.447
≥40	65 (44–65)	2 (0.53)	7 (1.87)	7 (1.87)	19 (5.07)	46 (12.27)	294 (78.40)	
Occupational identity								
Endoscopic physicians	65 (39–65)	4 (1.22)	7 (2.13)	8 (2.43)	26 (7.90)	35 (10.64)	249 (75.68)	0.023
Nurses	65 (51–65)	0 (0)	9 (2.38)	3 (0.79)	10 (2.65)	43 (11.38)	313 (82.80)	
Cleaning workers	65 (62–65)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10.00)	9 (90.00)	
Length of service								
<5 years	65 (39–65)	2 (1.00)	6 (3.00)	2 (1.00)	8 (4.00)	21 (10.50)	161 (80.50)	0.708
5–10 years	65 (52–65)	0 (0)	8 (2.89)	4 (1.44)	16 (5.78)	25 (9.03)	224 (80.87)	
>10 years	65 (44–65)	2 (0.83)	2 (0.83)	5 (2.08)	12 (5.00)	33 (13.75)	186 (77.50)	
Education								
Bachelor degree or below	65 (39–65)	2 (0.33)	15 (2.44)	10 (1.63)	26 (4.23)	65 (10.59)	496 (80.78)	0.063
Master degree or above	65 (44–65)	2 (1.94)	1 (0.97)	1 (0.97)	10 (9.71)	14 (13.59)	75 (72.82)	
Hospital Grade								
Primary	65 (59–65)	0 (0)	0 (0)	0 (0)	1 (7.14)	1 (7.14)	12 (85.71)	0.102
Secondary	65 (39–65)	2 (0.84)	2 (0.84)	6 (2.53)	19 (8.02)	30 (12.66)	178 (75.11)	
Tertiary	65 (44–65)	2 (0.43)	14 (3.00)	5 (1.07)	16 (3.43)	48 (10.30)	381 (81.76)	

are also regularly updated in order to enable them to better cope with COVID-19.

Healthcare workers had an extremely positive attitude and carried out favorable practice overall in COVID-19 pandemic. We found that women tended to be more concerned about strengthening the occupational protection, disinfection and management than men, and they did better than men in protective behavior as well. There was a similar phenomenon between nurses and doctors. However, ∼87% of men were endoscopy physicians, whereas over 70% of women were nurses in endoscopy units. The results above couldn't distinguish whether the differences in attitudes and behavior were due to gender, occupation, or both of them. We further analyzed the differences between male/female endoscopy physicians and male/female nurses, and noticed that there was a statistical difference between male doctors and female nurses in attitudes. The distinctions in behavior were mainly caused by occupation, not gender. The causes might be as follows. Firstly, nurses spend more time with patients than endoscopy physicians. Endoscopy nurses need to not only assess patients, answer patients' questions and address their concerns before the procedure but also assist doctors throughout the procedure, help patients recover, and complete all necessary documentation including patient notes and discharge documents after the procedure. Secondly, nurses may be more aware of the disinfection because they are responsible for preparing the instruments, equipment and supplies for the procedure as well as cleaning and sterilizing equipment before and after use.

Additionally, medical staff in tertiary hospitals had better protective behavior than those in secondary hospitals. Tertiary hospitals are comprehensive or general hospitals at the city, provincial or national level with a bed capacity exceeding 500. One possible explanation of the phenomenon above is as follows. During the outbreak of COVID-19, it was recommended to defer the elective endoscopies and only perform the urgent endoscopies by strategically assigned staff to minimize concomitant exposure. Endoscopic examinations on patients who were suspected or confirmed with COVID-19 should be performed in a negative pressure room with strict isolation precautions when available (31). Therefore, it was more in line with the protection requirements to complete the urgent endoscopies in a tertiary hospital setting, where the medical staff was more experienced in protective measures and environmental treatment.

The present study investigated the relationships among knowledge, attitudes, and practice of healthcare workers during the prevention and control of new coronavirus infection. The attitudes of endoscopic healthcare workers were positively related to their actual behaviors. In addition, according to theories of mediation effects and KAP, people acquire protection-related knowledge through learning, when their beliefs and attitudes gradually form, which contribute to the emergence of corresponding behavior (32, 33). In this study, we attempted to explore this pattern through mediation effect analysis, but failed to reach a statistical result.

TABLE 4 | Distribution of responses to the practice questionnaire.

	Score distribution *n* (%)								*P*-value
	Median (range)	<20	21–25	26–30	31–35	36–40	41–45	46–50	
Sex									
Male	46 (18–50)	1 (0.49)	0 (0)	6 (2.91)	12 (5.83)	32 (15.53)	47 (22.82)	108 (52.43)	0.000
Female	48 (14–50)	1 (0.20)	4 (0.78)	7 (1.37)	16 (3.13)	33 (6.46)	102 (19.96)	348 (68.10)	
Age									
<40	48 (18–50)	1 (0.29)	3 (0.88)	9 (2.63)	13 (3.80)	37 (10.82)	62 (18.13)	217 (63.45)	0.545
≥40	47 (14–50)	1 (0.27)	1 (0.27)	4 (1.07)	15 (4.00)	28 (7.47)	87 (23.20)	239 (63.73)	
Occupational identity									
Endoscopic physicians	46 (18–50)	1 (0.30)	0 (0)	9 (2.74)	18 (5.47)	48 (14.59)	85 (25.84)	168 (51.06)	0.000
Nurses	48 (14–50)	1 (0.26)	4 (1.06)	4 (1.06)	10 (2.65)	17 (4.50)	63 (16.67)	279 (73.81)	
Cleaning workers	50 (41–50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (10.00)	9 (90.00)	
Length of service									
<5 years	48 (14–50)	1 (0.50)	3 (1.50)	5 (2.50)	10 (5.00)	20 (10.00)	34 (17.00)	127 (63.50)	0.582
5–10 years	48 (18–50)	1 (0.36)	1 (0.36)	5 (1.81)	11 (3.97)	28 (10.11)	46 (16.61)	185 (66.79)	
>10 years	46.5 (30–50)	0 (0)	0 (0)	3 (1.25)	7 (2.92)	17 (7.08)	69 (28.75)	144 (60.00)	
Education									
Bachelor degree or below	48 (14–50)	2 (0.33)	4 (0.65)	10 (1.63)	20 (3.26)	58 (9.45)	122 (19.87)	398 (64.82)	0.109
Master degree or above	46 (28–50)	0 (0)	0 (0)	3 (2.91)	8 (7.77)	7 (6.80)	27 (26.21)	58 (56.31)	
Hospital Grade									
Primary	48 (41–50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (21.43)	11 (78.57)	0.001
Secondary	46 (14–50)	2 (0.84)	4 (1.69)	4 (1.69)	14 (5.91)	30 (12.66)	52 (21.94)	131 (55.27)	
Tertiary	48 (26–50)	0 (0)	0 (0)	9 (1.93)	14 (3.00)	35 (7.51)	94 (20.17)	314 (67.38)	

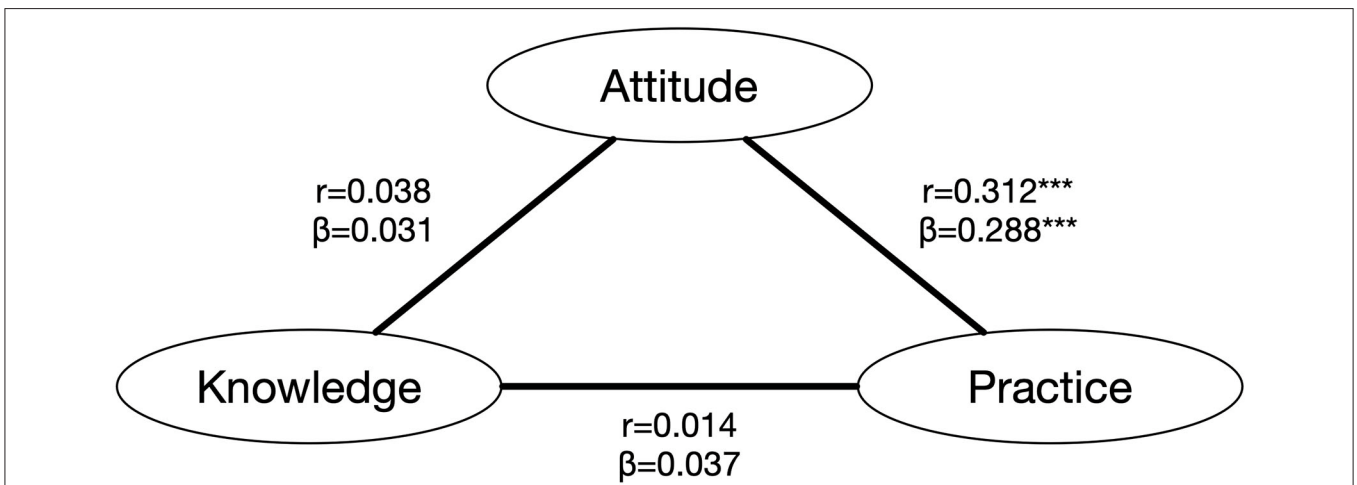

FIGURE 1 | The relationships among knowledge, attitudes, and practice. ***$P < 0.001$.

The present study also has some limitations. We only received 10 questionnaires from the cleaning workers, which might be too small to present the real world accurately, thus affecting the comparison among different occupational identities. A larger sample of research is required to be conducted in the future. In addition, our study has geographical bias, to some extent. Most of the questionnaires collected came from non-epidemic areas, while there were fewer questionnaires from areas with severe epidemics. There were particularities in the questionnaire during the epidemic. In the early stage of the epidemic, the country issued corresponding policies that required all organizations to learn the knowledge of the COVID-19, which led to the skewed results of the questionnaire and a narrow gap of knowledge among different occupational identities, thereby concealing some statistical differences.

CONCLUSIONS

In conclusion, most Chinese healthcare workers in endoscopy units are well-trained for protection against COVID-19 infection. Given the adequate protective knowledge, more positive attitudes

lead to more effective practice. Female staff has a more positive attitude than male staff, and nurses perform better in both attitudes and practice than endoscopic physicians. Medical staff in tertiary hospitals is more experienced in practice than those in secondary hospitals.

The outbreak of COVID-19 has exposed human vulnerability to unknown diseases, and new viruses have caught us off guard. Future campaigns on medical education should emphasize medical staff's knowledge about the virus and the corresponding protective measures they should take to respond to such sudden public health incidents, especially the protective practice for medical operations, such as endoscopy and endotracheal intubation, which have a high risk of exposing the staff to respiratory infectious diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Peking University First Hospital Biomedical Research Ethics Committee (No. 2020-124). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LR had the idea for and took responsibility for the integrity of the data and the accuracy of the data analysis. XG, YT, and YM designed the questionnaire. XG and BN collected the questionnaire and provided the analysis. YT, YM, BN, and XG contributed to the statistical analysis. YT, YM, and BN drafted the manuscript. LR revised the manuscript. FW participated in the literature search and discussion. All authors read and approved the final manuscript.

FUNDING

This research was funded by Youth Clinical Research Project of Peking University First Hospital (2018CR28).

ACKNOWLEDGMENTS

We acknowledge all healthcare workers participate in the survey; we thank Prof. Weidong Nian, Prof. Jianxiang Liu, Ass. Prof. Nan Li, Ass. Prof. Liping Liu, and Ass. Prof. Xi Yao for their professional questionnaire evaluation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2021.632608/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 22 April 2021
doi: 10.3389/fmed.2021.603374

Single-Cell RNA-seq Reveals Angiotensin-Converting Enzyme 2 and Transmembrane Serine Protease 2 Expression in TROP2^{+} Liver Progenitor Cells: Implications in Coronavirus Disease 2019-Associated Liver Dysfunction

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OPEN ACCESS

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *06 September 2020*
Accepted: *01 March 2021*
Published: *22 April 2021*

Citation:
Seow JJW, Pai R, Mishra A, Shepherdson E, Lim TKH, Goh BKP, Chan JKY, Chow PKH, Ginhoux F, DasGupta R and Sharma A (2021) Single-Cell RNA-seq Reveals Angiotensin-Converting Enzyme 2 and Transmembrane Serine Protease 2 Expression in TROP2^{+} Liver Progenitor Cells: Implications in Coronavirus Disease 2019-Associated Liver Dysfunction. Front. Med. 8:603374. doi: 10.3389/fmed.2021.603374

The recent coronavirus disease 2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2. COVID-19 was first reported in China (December 2019) and is now prevalent across the globe. Entry of severe acute respiratory syndrome coronavirus 2 into mammalian cells requires the binding of viral Spike (S) proteins to the angiotensin-converting enzyme 2 receptor. Once entered, the S protein is primed by a specialized serine protease, transmembrane serine protease 2 in the host cell. Importantly, besides the respiratory symptoms that are consistent with other common respiratory virus infections when patients become viremic, a significant number of COVID-19 patients also develop liver comorbidities. We explored whether a specific target cell-type in the mammalian liver could be implicated in disease pathophysiology other than the general deleterious response to cytokine storms. Here, we used single-cell RNA-seq to survey the human liver and identified potentially implicated liver cell-type for viral ingress. We analyzed ∼300,000 single cells across five different (i.e., human fetal, healthy, cirrhotic, tumor, and adjacent normal) liver tissue types. This study reports on the co-expression of angiotensin-converting enzyme 2 and transmembrane serine protease 2 in a TROP2^{+} liver progenitor population. Importantly, we detected enrichment

of this cell population in the cirrhotic liver when compared with tumor tissue. These results indicated that in COVID-19-associated liver dysfunction and cell death, a viral infection of TROP2^{+} progenitors in the liver might significantly impair liver regeneration in patients with liver cirrhosis.

Keywords: SARS-CoV-2, COVID-19, ACE2, tmprss2, Trop2, liver, ScRNA-seq

INTRODUCTION

Since December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has impacted millions of lives worldwide. As of August 23, 2020, more than 23 million people are reported to be infected, with ∼5% mortalities (https://coronavirus.jhu.edu/map.html). The SARS-CoV-2 is a single-stranded RNA virus belonging to the Coronaviridae family of zoonotic viruses that infect mammals and birds (1). The novel SARS-CoV-2 was first isolated from the lung airway epithelial cells of a patient with pneumonia (2). Since then, it has been reported that SARS-CoV-2 uses receptor angiotensin-converting enzyme 2 (ACE2) for entry into human cells and utilizes transmembrane serine protease 2 (TMPRSS2) for Spike (S) Protein priming (3). SARS-CoV-2 shares ∼80% sequence similarity with SARS-CoV and ∼50% with Middle East respiratory syndrome coronavirus, all of which cause severe respiratory symptoms (3). Moreover, in addition to respiratory disease, SARS and MERS are known to cause liver impairments (4–6).

Importantly, SARS-CoV-2 RNA was discovered in the stool sample of the first patient in the United States, indicating gastrointestinal (GI) tract infection (7). Laboratory results of the patients in this study showed an increase in the levels of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase, indicating that the hepatic function is affected. A recent study reported 14–53% cases with higher levels of alanine aminotransferase and aspartate aminotransferase in the liver of coronavirus disease 2019 (COVID-19) patients (6, 8). Moreover, these symptoms were elevated in patients admitted to intensive care units compared with those who did not require treatment in the intensive care unit (8). Recent studies have shown how elevated alkaline phosphatase or bilirubin are indicators of SARS-CoV-2-induced liver injury. A liver function pattern in infected patients with abnormal liver function has been studied by observing the levels of alkaline phosphatase or bilirubin (9, 10). A retrospective study done on 105 patients comparing severe with mild cases concluded that patients with severe cases are more likely to have an abnormal liver function (11). It remains to be investigated whether SARS-CoV-2 directly infects liver cells. In addition, concerns have been raised on the effect of SARS-CoV-2 infection on preexisting liver conditions (6, 12–14).

Since it was reported that ACE2 and TMPRSS2 are required for the entry of SARS-CoV-2 into human epithelial cells, there have been several papers that have shown the expression of these markers in different organs. In a study consisting of human, primate, and mouse samples, ACE2 and TMPRSS2 expression was observed in the lung, gut, and nasal mucosa (15). These target markers were also expressed in human and mouse ocular cells concluding that the cornea can be potentially infected by the virus (16).

Because SARS-CoV-2 binds to ACE2 and requires TMPRSS2 for activation and previous reports have shown that the liver is one of the organs that is affected by the virus, we surveyed the human liver (from tumor and adjacent normal regions of hepatocellular carcinoma patients) by single-cell RNA-seq (scRNA-seq) to identify which cell type co-express these two genes.

Here, we report that ACE2 and TMPRSS2 are co-expressed in only one subpopulation in the human liver. Based on the expression of cell type-specific markers ALB (Albumin), KRT (Keratin), and EPCAM and the unique expression pattern of TROP2 (TACSTD2) and SOX9 (SRY-box 9), we annotated this population as liver progenitors. The results of the study suggest that the SARS-CoV-2-binding receptor ACE2 is only expressed on TROP2^{high} cholangiocyte-biased progenitors, whereas TROP2^{high} and TROP2^{int} populations express serine protease TMPRSS2. These results indicate that SARS-CoV-2 infection might preferentially infect the TROP2^{high} cholangiocyte-biased progenitor pool, thereby compromising the regenerative abilities of an infected liver and/or contributing to liver pathology (17).

RESULTS

Expression of Angiotensin-Converting Enzyme 2 and Transmembrane Serine Protease 2 in Human Liver Single-Cell RNA-seq Atlas

We performed scRNA-seq on the human liver tissue obtained from the tumors and adjacent normal tissue of hepatocellular carcinoma patients (18). In total, we analyzed ∼74,000 cells and additional ∼60,000 cells from the human fetal liver. Furthermore, we integrated these data with healthy (19) and cirrhotic (20) human liver scRNA-seq data. We identified ∼45 clusters based on the expression of cell type-specific genes (**Figure 1A**). We observed integration of multiple tissue types in similar clusters indicating conservation of cell types across tissue (**Figure 1B**). Next, we investigated which cell types in the human liver express hepatocyte marker ALB (**Figure 1C**), SARS-CoV-2-binding receptor ACE2 (**Figure 1D**), and the priming enzyme TMPRSS2 (**Figure 1E**). Our analysis revealed the specific expression of ACE2 and TMPRSS2 in the ALB negative epithelial cluster. More importantly, this cluster also expresses TROP2, a gene associated with the liver epithelial progenitor population (21) (**Figure 1F**). This suggests that a subpopulation of liver

epithelial cells expresses machinery for both SARS-CoV-2 entry (ACE2) and priming (TMPRSS2) and might be susceptible to viral infection leading to liver dysfunction.

TROP2+ Liver Epithelial Progenitors Express Angiotensin-Converting Enzyme 2 and Transmembrane Serine Protease 2

A recent scRNA-seq study has suggested heterogeneity in liver epithelial progenitors (21). Therefore, we further sub-clustered the epithelial cells (hepatocytes and progenitors) to understand the nature of ACE2 expressing liver progenitors (**Figure 2A**). Sub-clustering also showed the predominant presence of normal and cirrhotic liver cells in the progenitor cluster (cl. 9) (**Figure 2B**). Furthermore, we detected the absence of ALB in these cells, indicating a lack of differentiated cells in this cluster (**Figure 2C**). Interestingly, the lower abundance of these cells in the human liver is in concordance with the rare stem-like or progenitor population in epithelial tissues. We also detected the highest expression of ACE2, TMPRSS2, and TROP2 in cluster-9 (**Figures 2D–F**). Furthermore, we analyzed the proportion of cells from different tissue types in cluster-9 and observed the higher number of cells from adjacent normal and cirrhotic liver tissue (**Figure 3A**). Importantly, this is the only cell type that co-express ACE2 and TMPRSS2 in human liver single-cell atlas (**Figures 3B,C**). Finally, we analyzed the co-expression of ACE2, TMPRSS2, and TROP2 in human liver epithelial clusters and identified a higher proportion of $ACE2^{+}/TMPRSS2^{+}/TROP2^{+}$ cells in cluster-9 and, more importantly, cirrhotic liver (**Figures 3D,E**).

We then analyzed the expression of hepatocyte, cholangiocyte, and bi-potent markers in these clusters (**Supplementary Figure 1**). The progenitor cluster specifically expressed EPCAM (progenitor marker) as well as KRT19 and cystic fibrosis transmembrane conductance regulator, which are known to be expressed in progenitors with a cholangiocyte fate bias (**Supplementary Figure 1**) (22). Importantly, we failed to detect the expression of hepatocyte fate bias genes, asialoglycoprotein receptor 1, and ALB in this cluster. As this progenitor cluster demonstrated bias for cholangiocyte fate, we further investigated the expression of the TROP2 gene. TROP2 expression is known to mark the fate of liver epithelial progenitors, where lower TROP2 expression is linked with hepatocyte fate and $TROP2^{high}$ cells with cholangiocyte fate (21).

Recently, Aizarani et al. demonstrated the progenitor-like properties of $TROP2^{+}$ cells, where $TROP2^{Int}$ cells demonstrated the highest organoid-forming efficiency followed by $TROP2^{high}$ cells, whereas $TROP2^{low}$ cells failed to generate organoids (21). Therefore, we investigated whether any of the epithelial clusters co-expressed ACE2, TMPRSS2, and TROP2. Notably, we observed that only the $EPCAM^{+}$ progenitor cluster expressed all three genes (**Supplementary Figure 1E**). We then subdivided this cluster into TROP2 low, intermediate, and high cells and investigated the expression of ACE2, TMPRSS2, and other cell fate markers. Remarkably, we observed that $TROP2^{high}$ cells expressed the highest levels of ACE2 and TMPRSS2, followed by $TROP2^{Int}$ and $TROP2^{low}$ cells (**Supplementary Figure 1F**). Our analysis revealed that $TROP2^{Int}$ (bi-potent) cells also express MUC6 and SOX9, whereas $TROP2^{high}$ (cholangiocyte fate bias) cells express makers such as cystic fibrosis transmembrane conductance regulator, CXCL8, HES1, and KRT19. Our results suggest that SARS-CoV-2 can infect $TROP2^{high}$ cells *via* ACE2 and TMPRSS2, thereby contributing to liver dysfunction by compromising the ability of the human liver to regenerate cholangiocytes.

Enrichment of Angiotensin-Converting Enzyme 2 and Transmembrane Serine Protease 2 Co-expressing Cells in Cirrhotic Liver

As scRNA-seq analysis indicated a higher number of $ACE2^{+}/TMPRSS2^{+}/TROP2^{+}$ co-expressing cells in cirrhotic liver, we used the RNA-FISH approach to validate these results in formalin-fixed paraffin-embedded (FFPE) tissues from the fatty (cirrhotic) liver, tumor, and adjacent normal sectors of hepatocellular carcinoma (**Figures 4A–F**). We probed the expression of ACE2, TMPRSS2, and Epcam in an RNA-FISH experiment and detected the higher number of $Epcam^{+}/ACE2^{+}/TMPRSS2^{+}$ cells in fatty liver tissue when compared with adjacent normal and tumors (**Figure 4G**). Taken together, our results suggest that inflamed tissues such as the cirrhotic liver harbored a higher number of $ACE2^{+}/TMPRSS2^{+}$ epithelial progenitors when compared with normal and tumor tissues. These results indicate that patients with liver cirrhosis may have a higher probability of SARS-CoV-2 infection in the liver when compared with other individuals; this might worsen their regenerative abilities, leading to long COVID phenotypes.

DISCUSSION

In recent reports of the SARS-CoV-2 pandemic in the human population, the presence of viral messenger RNA in an infected patient's stool suggests a potential GI tract infection in COVID-19 patients. SARS-CoV-2 can reach the liver either through the general circulation once the patient has become viremic or through transmigration through the GI tract. We surveyed human liver scRNA-seq data to understand the expression pattern of the ACE2 and TMPRSS2 gene, which are essential for SARS-CoV-2 entry into human cells. Our analysis reveals that in the human liver, only $EPCAM^{+}$ progenitors co-express genes for viral entry (ACE2) and S-protein priming (TMPRSS2). Further analyses revealed the specific expression of ACE2 and TMPRSS2 in $TROP2^{high}$ cells. These results indicate that ACE2 and TMPRSS2 are specifically present in liver progenitors with a cholangiocyte fate bias, suggesting SARS-CoV-2 may be affecting cholangiocyte precursors, thereby potentially impeding the homeostasis of the cholangiocyte pool. Recent studies have reported the expression of ACE2 in cholangiocytes, however, they do not reflect on the heterogeneity of the $ACE2^{+}$ population (23). The present study explored the heterogeneity of $ACE2^{+}$ cells and systematically characterized ACE2 and TMPRSS2 co-expression as hallmarks of $TROP2^{+}$ epithelial progenitors.

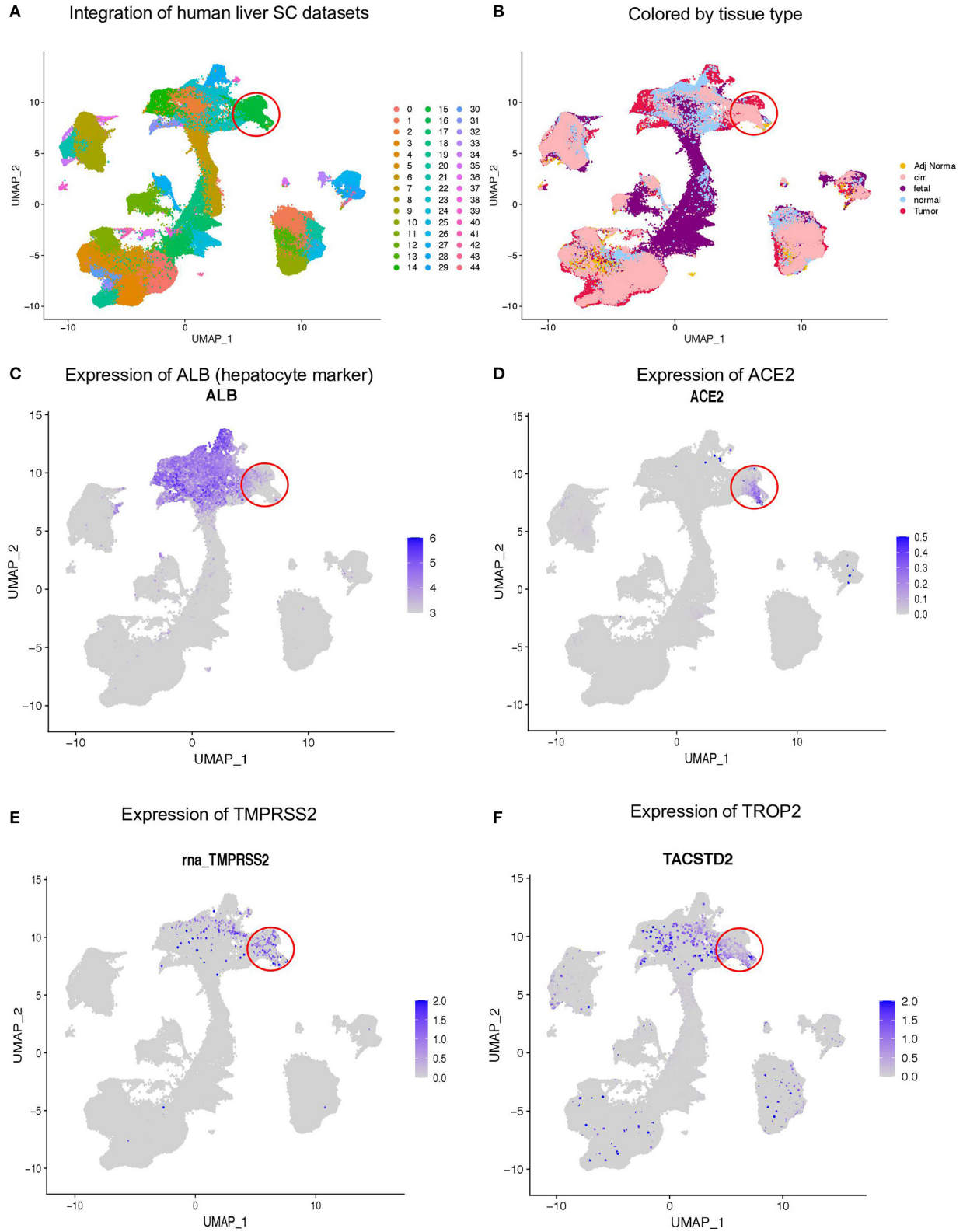

FIGURE 1 | Expression of ACE2 and TMPRSS2 in human liver. **(A)** Integration of ~300,000 sc-RNA-seq libraries from fetal, adult normal, cirrhotic, and HCC patients, which identified 45 clusters in the human liver. **(B)** Louvain clusters, colored by tissue types. Expression of **(C)** ALB, **(D)** ACE2, **(E)** TMPRSS2, and **(F)** TROP2.

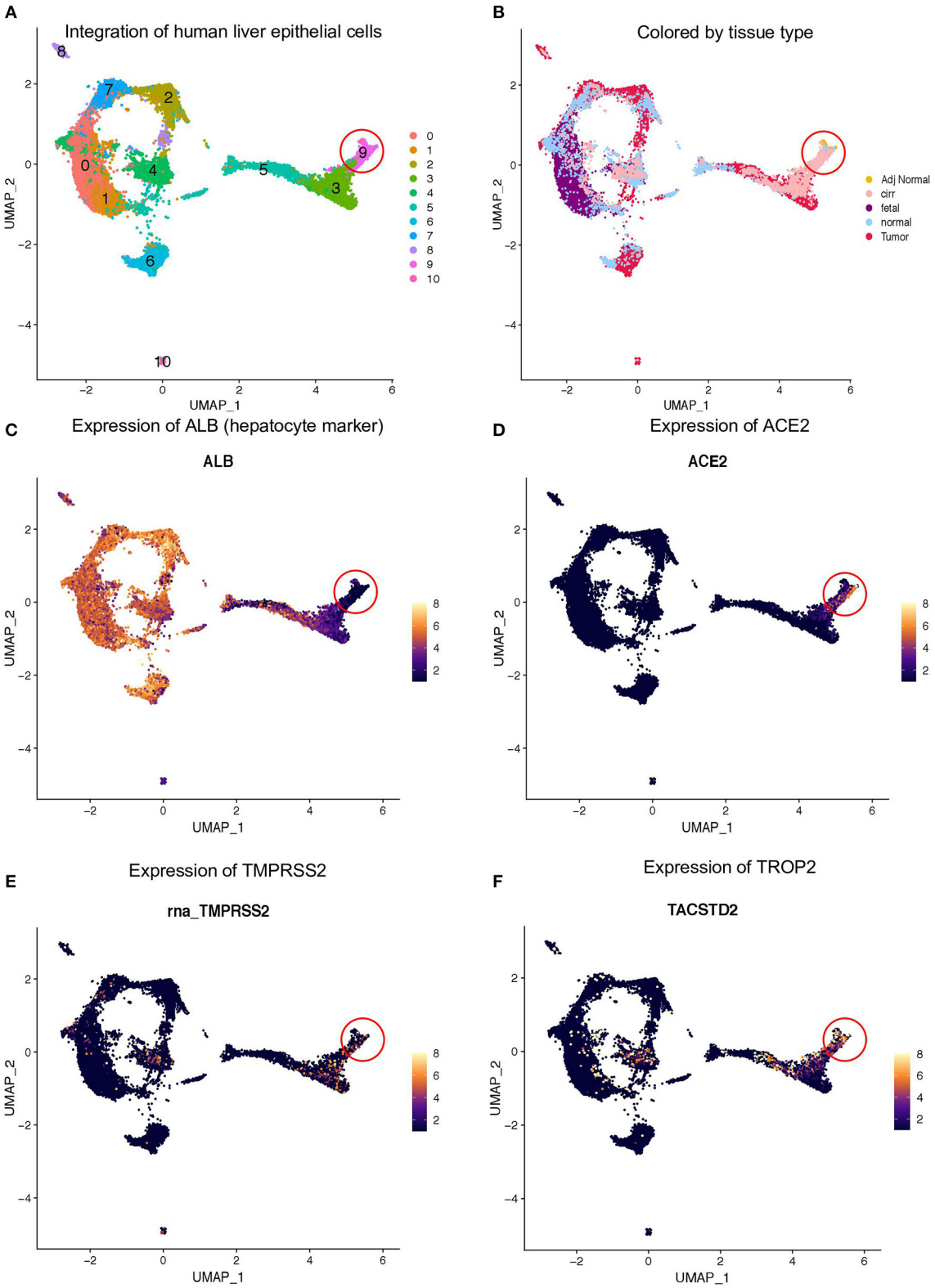

FIGURE 2 | Expression of ACE2 and TMPRSS2 in liver epithelial cells. **(A)** Sub-clustering of epithelial cells from fetal, adult normal, cirrhotic, and HCC patients, which identified 11 clusters in the human liver. **(B)** Louvain clusters, colored by tissue types. Expression of **(C)** ALB, **(D)** ACE2, **(E)** TMPRSS2, and **(F)** TROP2.

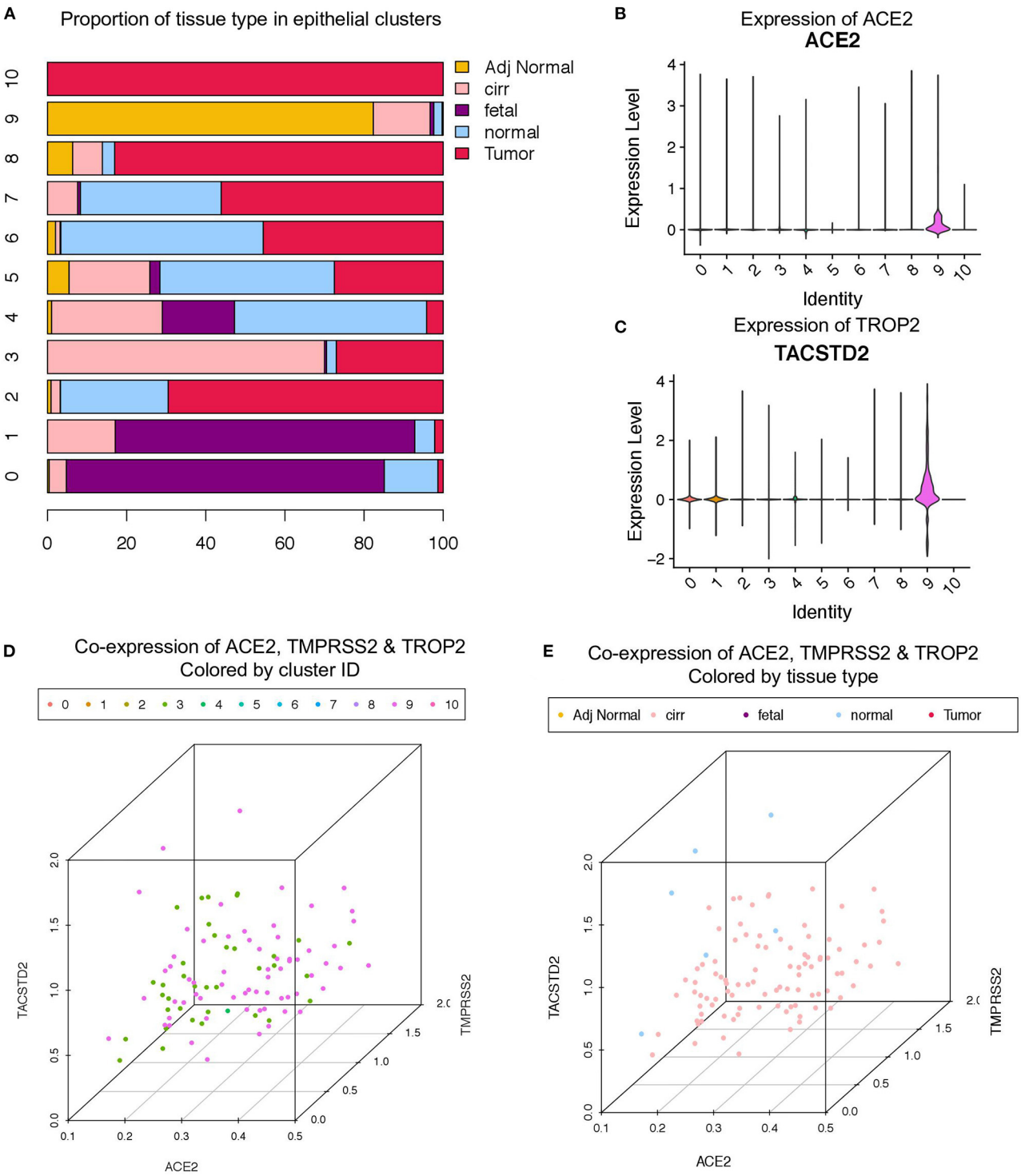

FIGURE 3 | Co-expression of ACE2, TMPRSS2, and TROP2 in liver epithelial cells. **(A)** Bar plot depicting the proportion of different tissue types in liver epithelial clusters. Expression of **(B)** ACE2 and **(C)** TROP2 in Louvain clusters. Three-dimensional plots of ACE2, TMPRSS2, and TROP2 co-expression **(D)** colored by Louvain cluster ID and **(E)** colored by tissue type.

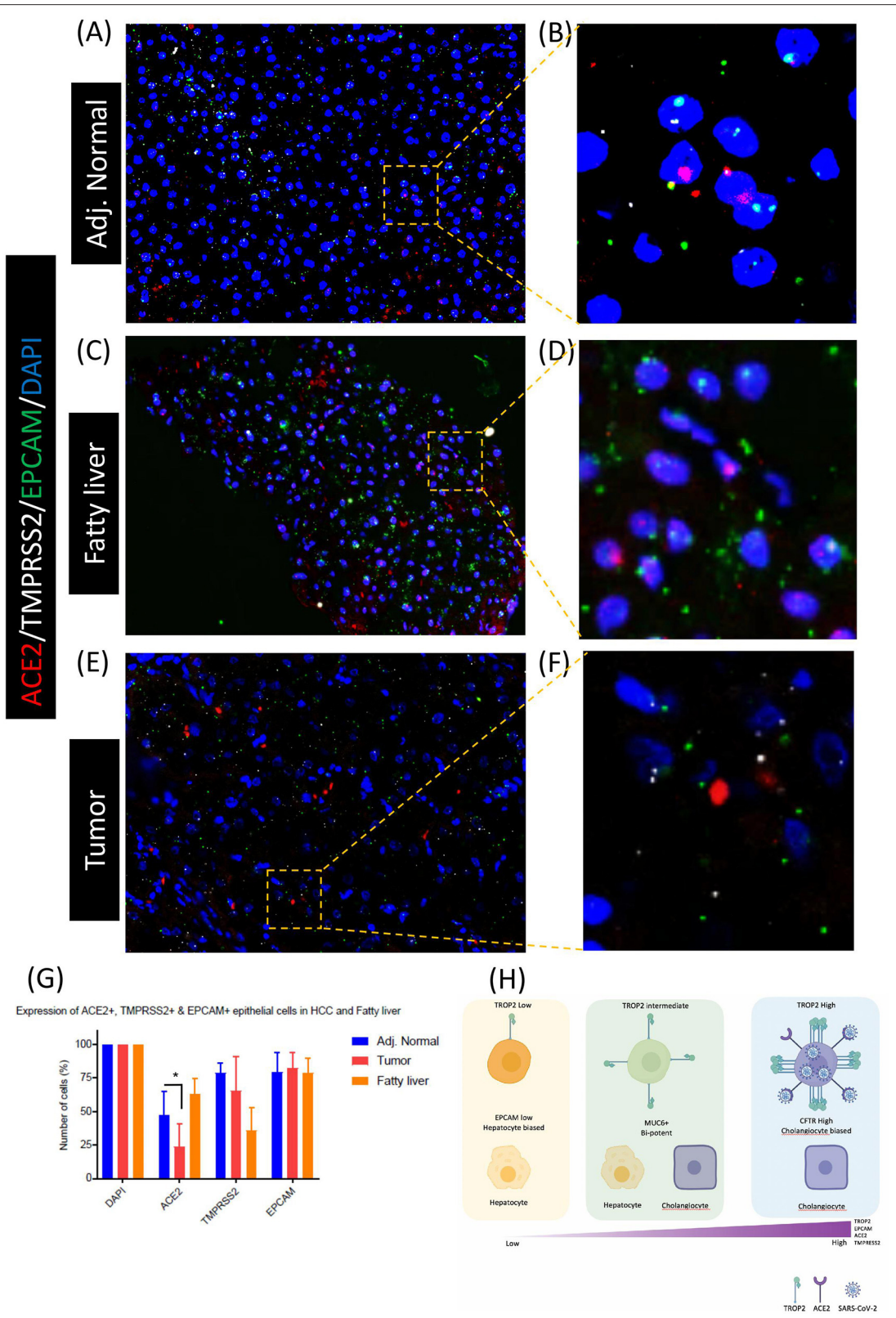

FIGURE 4 | Co-enrichment of ACE2, TMPRSS2, and EPCAM in liver epithelial cells. RNA-FISH-based detection of ACE2, TMPRSS2, and EPCAM in **(A,B)** adjacent normal, **(C,D)** fatty liver, and **(E,F)** tumor tissues. **(G)** Quantification of RNA-FISH images. **(H)** Schematic of TROP2 expression level and cell fate choices in adult human liver progenitor cells. TROP2^{high} cells express genes exhibiting cholangiocyte fate bias. TROP2^{high} cells also express a higher level of ACE2 and TMPRESS2, making these cells more susceptible for SARS-CoV-2 infection, indicating implications in COVID-19-associated liver dysfunctions. *means statistical significance of $P < 0.05$.

Our study reveals the potential of SARS-CoV-2 to infect TROP2$^+$ progenitor-like cells in the cirrhotic liver. It is important to note that TROP2 is expressed in multiple epithelial progenitors (24–26). In the future, it will be important to survey other GI tract tissues at the single-cell level for the expression of ACE2 and TMPRSS2 and their associated transcriptomes. Given the GI tract infection and multi-organ failure in COVID-19, it is important to understand whether other progenitor-like cells are also susceptible to SARS-CoV-2 infection. Moreover, TROP2 expression has been associated with amplifying progenitor cells in the partial hepatectomy mouse model (24), indicating the important role of TROP2$^+$ cells in liver regeneration. Taken together, our analysis suggests that cirrhotic human liver TROP2$^+$ progenitors could be a prime target of SARS-CoV-2 (**Figure 4H**).

General hepatocyte cell damage from cytokine storms in ill patients with viremia from a respiratory viral infection is not uncommon. Such hepatocyte damage is usually transient, and the resulting liver regeneration usually restores liver function efficiently. In the case of COVID-19, however, the predilection of the SARS-CoV-2 virus for cholangiocyte precursor cells may significantly impair liver regeneration. Clinicians looking after patients with COVID-19 should be alerted to the possibility of progressive liver deterioration in patients with serious SARS-Cov-2 viremia. This study demonstrates the power of scRNA-seq to understand the pathobiology of COVID-19 and pave the way for similar studies to understand the effect of SARS-CoV-2 on different tissue and cell types.

EXPERIMENTAL METHODS

Tissue Acquisition

Fresh tissue samples were obtained from Singapore General Hospital and National University with written consent and approval from the SingHealth Centralized Institutional Review Board (CIRB2012/669/B) to study liver cancer. The samples were delivered with MACS Tissue Storage Solution (Miltenyi, Cat#:130-100-008).

Human Fetal Liver Samples

The donation of fetal liver tissues for research was approved by the Centralized Institutional Research Board of the Singapore Health Services in Singapore followed by proper international ethical guidelines and in accordance with a favorable ethical opinion from Singapore SingHealth and National Health Care Group Research Ethics Committees. Women gave written informed consent for the donation of fetal tissue to research nurses who were not directly involved in the research or the clinical treatments of women participating in the study, as per the Polkinghorne guidelines. This protocol was reviewed on an annual basis by the Centralized Institutional Research Board (IRB2013/837/D), including annual monitoring of any adverse events, for which there had been none. All fetal liver tissues were obtained from the second trimester (16 and 21 weeks estimated gestational age) elective pregnancy terminations carried out for sociopsychological reasons. All fetuses were considered structurally normal on ultrasound examination before termination and by gross morphological examination after termination. In total, 2 fetuses of 16 and 21 weeks estimated gestational age were used for this study.

Tissue Processing

Tissues were transferred immediately and transferred to a sterile 10-mm^2 tissue culture dish and cut into very small fragments. The dissociation buffer consisted of 0.43 mg/ml of collagenase IV (Thermofisher, Cat#: 17104019) and 0.172 mg/ul of DNAse1 (Worthington, Cat#: LS002147) dissolved in phosphate-buffered saline (PBS) (Thermofisher, Cat#: 20012-043). The tissue was digested in a dissociation buffer for 30–40 min depending on sample size at 37°C with constant shaking at 220 rpm while keeping the falcon tube in a slanted position. The solution was resuspended with a 10-ml pipette followed by an 18-g needle. The 1% bovine serum albumin (BSA) PBS solution was added to the digested tissue, and then, the solution was passed through a 70-um filter before centrifuging at 800 $\times g$ for 6 min at 4°C. Cells were treated with 5 ml of 1× RBC lysis buffer (Biolegend, Cat#: 420301) on ice for 10–15 min. One percent BSA PBS solution was then added, and the cells were passed through a 40-um filter. Cells were dissolved in 1% BSA PBS solution before counting.

RNA *in situ* Hybridization

FFPE slides of HCC and fatty liver samples were used in this experiment. Slides were stained using the RNAscope® Multiplex Fluorescent Reagent Kit v2 Assay Kit (Advanced Cell Diagnostics) following the manufacturer's protocol. The slides were baked in an oven at 60°C for 1 h. The slides were deparaffinized and dehydrated using fresh xylene (two washes for 5 min each) and fresh 100% ethanol (two washes for 2 min each). These deparaffinized slides were treated to RNAscope® Hydrogen Peroxide at room temperature for 10 min. The slides were placed in a slide holder with 200 ml of RNAscope® 1X Target Retrieval Reagent at 99°C for 15 min for target retrieval. The slides were allowed to cool down, and then, a hydrophobic barrier was drawn around the tissue with the ImmEdge™ hydrophobic barrier pen and was left to dry for around 5–10 min. Four to six drops of RNAscope® Protease Plus used for FFPE slides were added onto the slides and incubated at 40°C for 30 min. RNAscope probes from ACDbio were used in this experiment: ACE2 (Cat# 848151) in the C1 channel, TMPRSS2 (Cat# 470341) in the C2 channel, and EPCAM (Cat# 310281) in the C3 channel. Of the probe mix, 150–200 µl was added onto the slide, and probe hybridization was performed at 40°C for 2 h. The slides were then stored in 5× SSC overnight, and amplification steps were performed the following day. For fluorescence, 1:500 dilution of Opal dyes (Perkin Elmer, Cat# NEL821001KT) was used, and the slides were mounted using a drop of ProLong Diamond Antifade Mountant (Thermofisher, Cat#: P36970). Imaging of slides was performed using Vectra® Polaris™ Automated Quantitative Pathology Imaging System.

Image quantification was done using ImageJ and Cell Profiler software. Using the ImageJ software, the images were split into single-channel grayscale images. These grayscale images were added onto Cell Profiler software and analyzed using a published pipeline (27).

Data Processing Using Cell Ranger Software

Sequenced fastq files are aligned, filtered, barcoded and UMI counted using Cell Ranger Chromium Single Cell RNA-seq version 2.0.2, by 10× Genomics with Cell Ranger, GRCh38 database (version 1.2.0) as the human genome reference. All 62 sectors are aggregated using *cellranger aggr* by normalizing all runs to the same sequencing depth.

Clustering and Downstream Analysis

Downstream analysis was performed using Scanpy, a scalable Python-based package (version 1.4) designed for single-cell gene expression datasets. Scanpy implements numerous functions from preprocessing to visualization, clustering, differential gene expression, and trajectory inference analysis on Jupyter Notebooks. Parameters used in each function are manually curated to portray the best clustering of cells. In preprocessing, cells are filtered based on the criteria of expressing a minimum of 200 genes and a gene that is expressed by a minimum of 30 cells. Dying cells with a mitochondrial percentage of more than 5% are excluded. Cell count was normalized using *scanpy.api.pp.normalize_per_cell* with a scaling factor of 10,000, whereas gene expression was scaled to unit variance and mean value of 0 using *scanpy.api.pp.scale*. Dimension reduction starts with PCA using *scanpy.api.tl.pca*; the number of PCs used in each clustering exercise varies depending on the importance of embeddings to be included. In the interest of crisp clustering, we first calculated the neighborhood graph (*scanpy.api.pp.neighbors*) of cells. Best matched k-Nearest Neighbor is automatically weighted by the algorithm to compute the best UMAP topology (*scanpy.api.tl.umap*, minimum distance between 0.3 and 0.5) which is consistently used throughout this paper. Louvain method (*scanpy.api.tl.louvain*) is then used to detect a community of similar cells. By default, Louvain's resolution parameter is set to the maximum value of 1.0; this, in theory, finds more and smaller clusters. In our experiments, the value is set between 0.6 and 1. Genes are then ranked using *scanpy.api.tl.rank_genes_groups* (Benjamini–Hochberg, *t*-test overestimated variance with adjusted *p*-value). Cell types were manually and iteratively assigned based on overlaps of literature curated and statistically ranked genes. To leverage the heterogeneity of this dataset, we used partition-based graph abstraction (*scanpy.api.tl.paga*) to reconstruct lineage between cell types. This lineage trajectory provides a continuous cell type transition from the assigned discrete cell types. The thickness of the edges represents connectivity scores, an entropy-based measure provided by partition-based graph abstraction indicating the relatedness between clusters; spurious connections are discarded while tuning thresholds.

Statistical Analysis

The statistical analysis for the image quantification was performed in Prism 7 (GraphPad). Data are expressed as mean ± SEM.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, GSE156337.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved on an annual basis by the Centralized Institutional Research Board (IRB2013/837/D), including annual monitoring of any adverse events, for which there had been none. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AS, PC, FG, and RD: conceptualization. AS and JS: methodology, formal analysis, and data curation. AS, JS, and AM: investigation. AS: writing—original draft and supervision. PC, FG, and RD: writing—review and editing. PC, FG, AS, and RD: funding acquisition. JC, TL, BG, PC, FG, and RD: resources. All authors contributed to the article and approved the submitted version.

FUNDING

This work is supported by National Medical Research Council (Singapore) grant TCR15Jun006, Agency for Science, Technology and Research (A*STAR) core funds to RD and FG. AS is supported by the National Medical Research Council young investigator grant (OFYIRF18nov-0056).

ACKNOWLEDGMENTS

We thank all patients and families involved in this study. We thank members of GIS, SIgN, Singapore General Hospital, NCCS and KKH teams, and Liver TCR group for useful discussions. We thank Sin Chi Chew, the GIS sequencing core, and the SIgN FACS core platforms for their help and support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.603374/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 09 June 2021
doi: 10.3389/fmed.2021.639855

Abnormal Liver Function Tests Were Associated With Adverse Clinical Outcomes: An Observational Cohort Study of 2,912 Patients With COVID-19

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: 10 December 2020
Accepted: 17 May 2021
Published: 09 June 2021

Citation:
Lv Y, Zhao X, Wang Y, Zhu J, Ma C, Feng X, Ma Y, Zheng Y, Yang L, Han G and Xie H (2021) Abnormal Liver Function Tests Were Associated With Adverse Clinical Outcomes: An Observational Cohort Study of 2,912 Patients With COVID-19. Front. Med. 8:639855. doi: 10.3389/fmed.2021.639855

Background and Aim: The impact of liver function test (LFTs) abnormality on adverse clinical outcomes in coronavirus disease 2019 (COVID-19) patients remains controversial. The aim of this study was to assess the impact of abnormal LFTs on clinical outcomes in a large cohort of hospitalized patients with COVID-19.

Methods: We retrospectively collected data on 2,912 consecutive patients with COVID-19 who were admitted to a makeshift hospital in China between 5 February and 23 March 2020. The association between LFTs abnormalities (baseline and peak values) and clinical outcomes was measured by using Cox regression models.

Results: On admission 1,414 patients (48.6%) had abnormal LFTs, with alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) elevation in 662 (22.7%), 221 (7.6%), 52 (1.8%), 135 (4.6%), and 536 (18.5%) patients, respectively, and hypoalbuminemia in 737 (25.3%) patients. During a median 13 (IQR: 8–19) days of hospitalization, 61 patients (2.1%) died, 106 patients (3.6%) admitted to intensive care unit (ICU), and 75 patients (2.6%) required mechanical ventilation. After adjustment for confounders, baseline abnormal LFTs were independently associated with increased risks of mortality (adjusted HR 3.66, 95%CI 1.64–8.19, $p = 0.002$), ICU admission (adjusted HR 3.12 95%CI 1.86–5.23, $p < 0.001$), and mechanical ventilation (adjusted HR 3.00, 95%CI 1.63–5.52, $p < 0.001$), which was homogeneous across the severity of COVID-19 infection. Among the parameters of LTFs, the associations with the outcomes were more pronounced for AST and albumin abnormality. In contrast, ALT elevation was not significantly associated with those outcomes. Similar results were observed for peak values of LFTs during hospitalization.

Conclusions: Abnormality of AST, albumin, TBIL, ALP, and GGT but not ALT were independently associated with adverse outcomes.

Keywords: coronavirus disease-2019, liver function test abnormality, mortality, severe acute respiratory syndrome coronavirus 2, mechanical ventilation

INTRODUCTION

The current coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), has become a serious threat to global public health (1–4). Although initially reported in Wuhan, China, it has rapidly spread around the world (5). Outcomes of COVID-19 range from asymptomatic infection to death (6, 7). Older age; male gender; and comorbid conditions, such as hypertension and diabetes, have been identified as risk factors for severe outcomes (7, 8). While COVID-19 is typically characterized by symptoms of viral pneumonia, SARS-CoV-2 causes a systemic disease, with possible involvement of the heart, liver, pancreas, and kidneys, as well as alterations in circulating lymphocytes and the immune system, because of the ubiquitous distribution of the main viral entry receptor, namely angiotensin converting enzyme 2 (ACE2) (2, 9, 10).

Liver impairment has been reported as a common manifestation, with a derangement of liver function tests (LFTs) ranging from 14 to 75% (11–27). Nevertheless, the clinical relevance of LFTs abnormalities remains controversial, with some studies suggesting its association with the severity of COVID-19 pneumonia and adverse outcomes, while others not. Most of those reports were small-sized and the parameters of LFTs, the diagnostic time point (i.e., on admission or during disease progression) and cut-off values of abnormal LFTs varies among studies (28, 29). Furthermore, composite outcomes combining admission to intensive care unit (ICU), mechanical ventilation, and/or death, are used in a majority of studies, thus it is difficult to determine whether LFTs abnormalities are equally predictive of all the outcomes evaluated. In addition, due to LFTs were categorized in almost all previous studies, the actual relationship between the LFTs and outcomes (liner, dose-response, threshold/saturation effect pattern, or others) remains unknown. It is also yet unclear whether the effect of LFTs on the outcomes equal or differ among patients with different severity of COVID-19 infection.

Thus, the aim of this study was to assess the clinical features and the impact of abnormal LFTs on the outcomes (mortality, ICU admission, and mechanical ventilation) in a large cohort of hospitalized patients with COVID-19.

Abbreviations: ACE2, angiotensin converting enzyme 2; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; COVID-19, coronavirus disease 2019; GGT, gamma-glutamyltransferase; HR, hazard ratio; ICU, intensive care unit; LFTs, liver function tests; SARSCoV-2, severe acute respiratory syndrome coronavirus 2; TBIL, total bilirubin; ULN, upper limit of normal.

METHODS

Study Design and Participants

We retrospectively extracted the data from the electronic charts of consecutive patients with confirmed COVID-19 at Huoshenshan hospital (Wuhan, China) from 5 February to 23 March 2020. The Huoshenshan hospital, a makeshift hospital with 1,000 beds, was opened by the government on 5 February 2020, and assigned to treat exclusively COVID-19 patients. This study was approved by the National Health Commission of China and the institutional review board at Huoshenshan hospital. Written informed consent was waived by the ethics committee of the Huoshenshan hospital for patients with emerging infectious diseases.

Inclusion criteria for the study were (i) hospitalized patients with confirmed COVID-19 infection; (ii) age >18 years old. Patients with no data on LFTs were excluded from the study. COVID-19 was diagnosed by clinical manifestations, chest computed tomography (CT), and confirmed by real-time polymerase chain reaction (RT-PCR) according to World Health Organization (WHO) interim guidance (30), and the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China (31). The severity of COVID-19 was categorized as mild, severe, or critical (31, 32). Mild type was defined as having slight clinical symptoms without signs of pneumonia or with mild pneumonia (multiple small patchy shadows and interstitial changes, mainly in the outer zone of the lung and under the pleura) by radiography (31, 32). Severe cases were characterized by dyspnoea, respiratory frequency ≥30/min, blood oxygen saturation ≤93%, PaO_2/FiO_2 ratio <300 mmHg, and/or lung infiltrates >50% within 24–48 h (31, 32). Such patients were considered as critical case if they developed respiratory failure requiring mechanic ventilation, septic shock, and/or multiple organ dysfunction/failure (31, 32).

Data Collection

Baseline data collected within 24 h after admission include patient demographics, clinical features at inclusion, clinical history, comorbidities, initial blood pressure, and heart rate, laboratory values (peripheral white blood cell, neutrophil, lymphocyte, hemoglobin, platelet count, creatinine, blood urea nitrogen, potassium, sodium, D-dimer, prothrombin time, activated partial thromboplastin time, international normalized ratio, creatine kinase, lactate dehydrogenase, procalcitonin, and c-reactive protein), and radiological reports. Data regarding the specific drug therapy provided during the hospitalization also were collected. Liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total

bilirubin, (TBIL), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT)] from the time of hospital admission until discharge or death were obtained. The performing of LFT was determined by the attending physicians based on the demand of clinical decision. LFTs were considered as abnormal when at least one among AST, ALT, albumin, TBIL, ALP, and GGT were above the upper limit of normal (ULN) of laboratory reference range standards (i.e., AST >40 U/L, ALT >45 U/L, albumin <35 g/L, TBIL >26 μmol/L, ALP >125 U/L, GGT >60 U/L). All data were reviewed and confirmed by two certified investigators (Yong Lv and Huahong Xie) to ensure accuracy.

Outcome and Definitions

The primary endpoint was all-cause mortality during hospitalization. Secondary endpoints included ICU admission and use of mechanical ventilation. All clinical outcomes were obtained from clinical charts and assessed on April 15, 2020, when all survived patients were discharged and the Huoshenshan Hospital was shut down. The criteria for discharge are: (i) throat swab specimens collected 24 h apart were negative for tests of SARS-CoV-2; (ii) body temperature was normal for three consecutive days; (iii) symptoms of COVID-19 were resolved; (iv) the radiographic findings of COVID-19 significantly improved (31).

Statistical Analysis

For all analyses, missing data of the covariates were imputed with multiple imputations methods (detailed in **Supplementary Materials and Methods**). Data are presented as frequencies (percentage), mean ± standard deviation (SD), or medians with interquartile range (IQR) as appropriate. Comparisons of variables between groups were performed using Student *t*-test, non-parametric Mann-Whitney U-test, chi-squared test, or Fisher's exact test as appropriate. Dynamic changes in liver function were presented using locally weighted scatterplot smoothing (LOESS). The cumulative probability model [an ordinal regression model for continuous outcomes (33)] was used to evaluate the association of baseline characteristics and treatment before peaking of FLTs with the peak levels of LFTs in hospital, where the liver function markers were treated as continuous response variables. The non-linear relationships between liver function markers and the risk of the evaluated outcomes were visualized using restricted cubic splines by entering the liver function markers as a continuous variable into the logistic regression analysis. Cumulative risks of death was assessed with Kaplan-Meier curves and compared using the log-rank test. Cumulative incidences of ICU admission or mechanical ventilation were estimated in a competing risks setting, where the death competed with the event of interest. The contribution of each variable to the risk of developing the endpoint was reported as a hazard ratio (HR) with 95% confidence interval (CI). We assessed the unadjusted and confounder-adjusted effects of LFTs on the evaluated outcomes using Cox regression models. Age, gender, severity of COVID-19 (severe/critical vs. mild), comorbidities (include hypertension, cardiovascular disease, diabetes, chronic pulmonary diseases, cerebrovascular disease, malignancy, and autoimmune disease) and chronic liver diseases (include hepatitis B virus infection, hepatitis C virus infection, and autoimmune liver disease) were considered as potential confounders. We assessed the heterogeneity in the effect of LFTs across the severity of COVID-19 by including a LFTs-by-COVID-19-severity interaction term in the Cox regression models. A significant interaction would indicate that the effect of LFTs was different across the severity of COVID-19. Significance was established at $p < 0.05$. All statistical calculations were performed using R 3.6.1(http://www.R-project.org/) with the add-on packages *Hmisc, rms, riskRegression, pec, prodlim*, and *cmprsk*.

RESULTS

Baseline Clinical Features of Patients With COVID-19

During the study period, 2,922 patients with confirmed COVID-19 were admitted to the Huoshenshan hospital, and 10 patients were excluded because of incomplete relevant data. Ultimately, 2,912 patients with COVID-19 were included in the study. In the entire cohort, the mean age was 58.4 ± 14.4 years, and 1,512 (51.9%) were female. On admission, the severity of COVID-19 was mild in 2,160 (74.2%) patients, severe in 714 (24.5%) and critical in 38 (1.3%). Among the 752 serious and critically ill patients, 54 (7.2%) patients had multiple organ dysfunction syndromes. A total of 1236 (42.4%) patients had comorbidities, with hypertension (910 patients [31.2%]) being the most common one, followed by diabetes (392 patients [13.5%]). Sixty-eight patients (2.3%) had chronic liver disease, among which 58 had hepatitis B virus infection, 8 had hepatitis C virus infection, and 2 autoimmune liver disease. No patients had cirrhosis. The most common symptoms of COVID-19 were fever (2,057 patients [70.6%]), followed by cough (2,001 patients [68.7%]), fatigue (1,461 patients [50.2%]), dyspnea (1,394 patients [47.9%]), myalgia (774 patients [26.6%]), anorexia (523 patients [18.0%]), and expectoration (420 patients [14.4%]). Nausea, vomiting, abdominal pain, diarrhea, headache, dizziness disorders of consciousness were rare.

On admission 1,414 patients (48.6%) had abnormal LFTs, with ALT, AST, TBIL, ALP, and GGT above ULN in 662 (22.7%), 221 (7.6%), 52 (1.8%), 135 (4.6%), and 536 (18.5%) patients, respectively, and hypoalbuminemia (<35g/L) in 737 (25.3%) patients. The baseline characteristics of the study population according to normal and abnormal LFTs on admission are summarized in **Table 1**. Compared with patients with normal LFTs, patients with abnormal LFTs were older, with more severe COVID-19 disease and more likely to have symptoms of fever, cough, expectoration, dyspnea, fatigue, myalgia, anorexia, and nausea. The mean values of white blood cell count, neutrophil count, lymphocyte count, hemoglobin, platelet count, creatinine, D-dime, activated partial thromboplastin time, creatine kinase, lactate dehydrogenase, procalcitonin, and C-reactive protein were also higher in patients with abnormal LFTs. Mover, patients with abnormal LFTs had a higher likelihood of receiving antiviral therapy, antibiotics, immunoglobin, glucocorticoid therapy, high

TABLE 1 | Baseline characteristics of patients according to normal vs. abnormal liver function test on admission.

Variable	All (n = 2,912)	Normal LFTs (n = 1,498)	Abnormal LFTs (n = 1,414)	*P*-value
Age (years)	58.4 ± 14.4	56.6 ± 14.2	60.3 ± 14.4	<0.001
Female gender, *n* (%)	1,512 (51.9%)	897 (59.9%)	615 (43.5%)	<0.001
Smoking history, *n* (%)	217 (7.5%)	110 (7.3%)	107 (7.6%)	0.873
Drinking history, *n* (%)	130 (4.5%)	70 (4.7%)	60 (4.2%)	0.637
Severity of COVID19, *n* (%)				<0.001
Mild	2,160 (74.2%)	1,174 (78.4%)	986 (69.7%)	
Severe	714 (24.5%)	319 (21.3%)	395 (27.9%)	
Critical	38 (1.3%)	5 (0.3%)	33 (2.3%)	
Comorbidities on admission	1,236 (42.4%)	616 (41.1%)	620 (43.8%)	0.147
Hypertension, *n* (%)	910 (31.2%)	461 (30.8%)	449 (31.8%)	0.596
Cardiovascular disease, *n* (%)	219 (7.5%)	107 (7.1%)	112 (7.9%)	0.468
Diabetes, *n* (%)	392 (13.5%)	205 (13.7%)	187 (13.2%)	0.757
Chronic pulmonary diseases, *n* (%)	141 (4.8%)	63 (4.2%)	78 (5.5%)	0.119
Cerebrovascular disease, *n* (%)	125 (4.3%)	55 (3.7%)	70 (5.0%)	0.107
Malignancy, *n* (%)	63 (2.2%)	25 (1.7%)	38 (2.7%)	0.078
Gastrointestinal diseases, *n* (%)	53 (1.8%)	22 (1.5%)	31 (2.2%)	0.186
Autoimmune disease, *n* (%)	20 (0.7%)	7 (0.5%)	13 (0.9%)	0.211
Chronic liver diseases, *n* (%)	68 (2.3%)	31 (2.1%)	37 (2.6%)	0.390
Hepatitis B virus infection, *n* (%)	58 (2.0%)	26 (1.7%)	32 (2.3%)	0.376
Hepatitis C virus infection, *n* (%)	8 (0.3%)	5 (0.3%)	3 (0.2%)	0.785
Autoimmune liver disease, *n* (%)	2 (0.1%)	0 (0.0%)	2 (0.1%)	0.454
Clinical characteristics on admission				
Fever (>37.5°C), *n* (%)	2,057 (70.6%)	1,029 (68.7%)	1,028 (72.7%)	0.020
Cough, *n* (%)	2,001 (68.7%)	998 (66.6%)	1,003 (70.9%)	0.014
Expectoration, *n* (%)	420 (14.4%)	186 (12.4%)	234 (16.5%)	<0.001
Dyspnea, *n* (%)	1,394 (47.9%)	651 (43.5%)	743 (52.5%)	<0.001
Fatigue, *n* (%)	1,461 (50.2%)	697 (46.5%)	764 (54.0%)	<0.001
Myalgia, *n* (%)	774 (26.6%)	357 (23.8%)	417 (29.5%)	<0.001
Anorexia, *n* (%)	523 (18.0%)	228 (15.2%)	295 (20.9%)	<0.001
Nausea, *n* (%)	63 (2.2%)	38 (2.5%)	25 (1.8%)	0.194
Vomiting, *n* (%)	47 (1.6%)	31 (2.1%)	16 (1.1%)	0.063
Abdominal pain, *n* (%)	31 (1.1%)	17 (1.1%)	14 (1.0%)	0.842
Diarrhea, *n* (%)	126 (4.3%)	56 (3.7%)	70 (5.0%)	0.130
Headache, *n* (%)	54 (1.9%)	30 (2.0%)	24 (1.7%)	0.636
Dizziness, *n* (%)	36 (1.2%)	24 (1.6%)	12 (0.8%)	0.095
Disorders of consciousness, *n* (%)	19 (0.7%)	5 (0.3%)	14 (1.0%)	0.049
Systolic blood pressure (mmHg)	129.7 ± 16.2	130.3 ± 16.6	129.1 ± 15.7	0.059
Diastolic blood pressure (mmHg)	80.8 ± 11.6	81.5 ± 11.3	80.2 ± 11.9	<0.001
Heart rate (beat per minute)	86.8 ± 13.4	87.2 ± 13.3	86.3 ± 13.5	0.091
Respiratory rate (breaths per minute)	20.4 ± 3.0	20.1 ± 2.7	20.6 ± 3.3	<0.001
Chest radiography or CT on admission, *n* (%)				<0.001
Normal	50 (1.7%)	33 (2.2%)	17 (1.2%)	
Interstitial pneumonia	1,389 (47.7%)	707 (47.2%)	682 (48.2%)	
Ground glass opacity	1,362 (46.8%)	719 (48.0%)	643 (45.5%)	
Local consolidation	69 (2.4%)	29 (1.9%)	40 (2.8%)	
Bilateral consolidation	42 (1.4%)	10 (0.7%)	32 (2.3%)	
Laboratory examination on admission				
White blood cell count ($\times 10^9$/L)	6.2 ± 2.8	5.9 ± 2.1	6.5 ± 3.4	<0.001
Neutrophil count ($\times 10^9$/L)	4 ± 2.7	3.6 ± 1.6	4.5 ± 3.5	<0.001

(Continued)

TABLE 1 | Continued

Variable	All (n = 2,912)	Normal LFTs (n = 1,498)	Abnormal LFTs (n = 1,414)	*P*-value
Lymphocyte count ($\times 10^9$/L)	0.7 ± 2.6	0.6 ± 1.2	0.8 ± 3.6	0.062
Hemoglobin (g/L)	124.2 ± 18.4	124.9 ± 16.2	123.4 ± 20.4	0.025
Platelet count ($\times 10^9$/L)	232.1 ± 82.4	225.3 ± 69.9	239.3 ± 93.3	<0.001
Alanine aminotransferase (ALT), U/L	33.0 ± 34.8	19.6 ± 8.4	47.3 ± 45.0	<0.001
ALT <40 U/L, *n* (%)	2,250 (77.3%)	1,498 (100.0%)	752 (53.2%)	
ALT 40–120 U/L, *n* (%)	584 (20.0%)	0 (0.0%)	584 (41.3%)	
ALT >120 U/L, *n* (%)	78 (2.7%)	0 (0.0%)	78 (5.5%)	
Aspartate aminotransferase (AST), U/L	25.7 ± 41.3	18.2 ± 5.2	33.6 ± 58.1	<0.001
AST <45 U/L, *n* (%)	2,691 (92.4%)	1,498 (100.0%)	1,193 (84.4%)	
AST 45–135 U/L, *n* (%)	200 (6.9%)	0 (0.0%)	200 (14.1%)	
AST >135 U/L, *n* (%)	21 (0.7%)	0 (0.0%)	21 (1.5%)	
Albumin (g/L)	37.8 ± 9.4	39.7 ± 9.4	35.8 ± 9.1	<0.001
Albumin >40 g/L, *n* (%)	826 (28.4%)	563 (37.6%)	263 (18.6%)	
Albumin 30–40 g/L, *n* (%)	1,925 (66.1%)	935 (62.4%)	990 (70.0%)	
Albumin <30 g/L, *n* (%)	161 (5.5%)	0 (0.0%)	161 (11.4%)	
Total bilirubin (TBIL), μmol/L	10.3 ± 6.6	9.6 ± 4.2	10.9 ± 8.3	<0.001
TBIL ≥26 μmol/L	52 (1.8%)	0 (0.0%)	52 (3.7%)	<0.001
Alkaline phosphatase (ALP), U/L	75.6 (32.8)	68.8 (17.6)	82.8 (42.3)	<0.001
ALP ≥125 U/L, *n* (%)	135 (4.6%)	0 (0.0%)	135 (9.5%)	<0.001
γ-Glutamyl transpeptidase (GGT), U/L	45.3 ± 49.3	26.9 ± 12.3	64.9 ± 64.0	<0.001
GGT <60 U/L, *n* (%)	2,376 (81.5%)	1,498 (99.8%)	878 (62.1%)	
GGT 60–180 U/L, *n* (%)	456 (15.8%)	0 (0.0%)	456 (32.2%)	
GGT >180 U/L, *n* (%)	80 (2.7%)	0 (0.0%)	80 (5.7%)	
Creatinine (μmol/L)	70.5 ± 48.8	67.4 ± 48.3	73.9 ± 49.1	<0.001
Blood urea nitrogen (mmol/L)	5.4 ± 12.1	5.2 ± 15.2	5.5 ± 7.4	0.496
Potassium (mmol/L)	4.4 ± 2.9	4.3 ± 1.5	4.4 ± 3.8	0.326
Sodium (mmol/L)	141.7 ± 24.3	141.7 ± 4.3	141.7 ± 34.5	0.93
D-dimer (μg/ml)	1.1 ± 4.4	0.7 ± 2.5	1.5 ± 5.7	<0.001
Prothrombin time (s)	10.8 ± 6	10.8 ± 5.3	10.8 ± 6.7	0.786
Activated partial thromboplastin time (s)	28.1 ± 6.8	27.8 ± 3.9	28.4 ± 8.8	<0.001
International normalized ratio	1.2 ± 3.1	1.2 ± 3.8	1.2 ± 2.1	0.838
Creatine kinase (U/L)	62.1 ± 64.2	59.1 ± 42.8	65.3 ± 80.8	<0.001
Lactate dehydrogenase (U/L)	198.5 ± 90	175.8 ± 64.2	222.5 ± 105.8	<0.001
Procalcitonin (ng/ml)	0.2 ± 0.7	0.1 ± 0.7	0.2 ± 0.7	<0.001
C-reactive protein (mg/L)	13.6 ± 30.4	5.5 ± 15.8	22.2 ± 38.6	<0.001
Liver function tests during hospitalization				
Peak aspartate aminotransferase (ALT), U/L	41.4 ± 60.3	31.6 ± 56.7	51.7 ± 62.3	<0.001
ALT <40 U/L, *n* (%)	2,057 (70.6%)	1,303 (87.0%)	754 (53.3%)	
ALT 40–120 U/L, *n* (%)	721 (24.8%)	156 (10.4%)	565 (40.0%)	
ALT >120 U/L, *n* (%)	134 (4.6%)	39 (2.6%)	95 (6.7%)	<0.001
Peak aspartate aminotransferase (AST), U/L	31.4 ± 50.1	27.2 ± 51.6	35.8 ± 48.1	<0.001
AST <45 U/L, *n* (%)	2,591 (89.0%)	1,409 (94.1%)	1,182 (83.6%)	
AST 45–135 U/L, *n* (%)	270 (9.2%)	67 (4.5%)	203 (14.4%)	
AST >135 U/L, *n* (%)	51 (1.8%)	22 (1.5%)	29 (2.1%)	<0.001
Nadir albumin, g/L	35.9 ± 5.5	37.6 ± 4.8	34.1 ± 5.5	<0.001
Albumin >40 g/L, *n* (%)	567 (19.5%)	391 (26.1%)	176 (12.4%)	
Albumin 30–40 g/L, *n* (%)	2,000 (68.7%)	1,015 (67.8%)	985 (69.7%)	
Albumin <30 g/L, *n* (%)	345 (11.8%)	92 (6.1%)	253 (17.9%)	<0.001
Peak total bilirubin (TBIL), μmol/L	11.8 ± 16.8	10.9 ± 8.0	12.7 ± 22.6	<0.001

(Continued)

TABLE 1 | Continued

Variable	All (*n* = 2,912)	Normal LFTs (*n* = 1,498)	Abnormal LFTs (*n* = 1,414)	*P*-value
TBIL ≥ 26 μmol/L, *n* (%)	103 (3.5%)	34 (2.3%)	69 (4.9%)	<0.001
Peak alkaline phosphatase (ALP), U/L	79.4 ± 42.8	73.1 ± 28.8	86.1 ± 53.0	<0.001
ALP ≥125 U/L, *n* (%)	166 (5.7%)	34 (2.3%)	132 (9.3%)	<0.001
Peak γ-glutamyl transpeptidase (GGT), U/L	49.1 ± 54.3	32.9 ± 30.9	66.3 ± 66.9	<0.001
GGT <60 U/L, *n* (%)	2,270 (78.0%)	1,396 (93.2%)	874 (61.8%)	
GGT 60–180 U/L, *n* (%)	560 (19.2%)	92 (6.1%)	468 (33.1%)	
GGT >180 U/L, *n* (%)	82 (2.8%)	10 (0.7%)	72 (5.1%)	<0.001

COVID-19, coronavirus disease 2019, CT, computed tomography; LFTs, liver function tests. Plus-minus values are means ± standard deviation.

TABLE 2 | In-hospital treatment and outcomes according to normal vs. abnormal liver function on admission.

Variable	All (*n* = 2,912)	Normal LFTs (*n* = 1,498)	Abnormal LFTs (*n* = 1,414)	*P*-value
Antiviral therapy, *n* (%)	1,338 (45.9%)	594 (39.7%)	744 (52.6%)	<0.001
Include abidor, *n* (%)	1,191 (40.9%)	556 (37.1%)	635 (44.9%)	<0.001
Include ribavirin, *n* (%)	89 (3.1%)	22 (1.5%)	67 (4.7%)	<0.001
Include oseltamivir, *n* (%)	223 (7.7%)	63 (4.2%)	160 (11.3%)	<0.001
Include interferon, *n* (%)	235 (8.1%)	113 (7.5%)	122 (8.6%)	0.314
Antibiotics, *n* (%)	964 (33.1%)	372 (24.8%)	592 (41.9%)	<0.001
Quinolones, *n* (%)	699 (24.0%)	265 (17.7%)	434 (30.7%)	<0.001
Cephalosporins, *n* (%)	87 (3.0%)	14 (0.9%)	73 (5.2%)	<0.001
Macrolides, *n* (%)	31 (1.1%)	12 (0.8%)	19 (1.3%)	0.213
Traditional Chinese medicine, *n* (%)	2,627 (90.2%)	1,362 (90.9%)	1,265 (89.5%)	0.207
Immunoglobin, *n* (%)	134 (4.6%)	35 (2.3%)	99 (7.0%)	<0.001
Glucocorticoid therapy, *n* (%)	414 (14.2%)	116 (7.7%)	298 (21.1%)	<0.001
High flow nasal cannula, *n* (%)	1,771 (60.8%)	848 (56.6%)	923 (65.3%)	<0.001
Continuous renal replacement therapy, *n* (%)	10 (0.3%)	0 (0.0%)	10 (0.7%)	<0.001
Extracorporeal membrane oxygenation, *n* (%)	3 (0.1%)	0 (0.0%)	3 (0.2%)	0.228
Mechanical ventilation, *n* (%)	75 (2.6%)	13 (0.9%)	62 (4.4%)	<0.001
Non-invasive	28 (1.0%)	7 (0.5%)	21 (1.5%)	
Invasive	12 (0.4%)	3 (0.2%)	9 (0.6%)	
Non-invasive + Invasive	35 (1.2%)	3 (0.2%)	32 (2.3%)	
Admission or transfer to ICU, *n* (%)	106 (3.6%)	18 (1.2%)	88 (6.2%)	<0.001
Death, *n* (%)	61 (2.1%)	7 (0.5%)	54 (3.8%)	<0.001
Length of hospital stay (days)	14.9 ± 9.0	12.8 ± 7.4	17.2 ± 9.9	<0.001
Composite endpoin†	121 (4.2%)	22 (1.5%)	99 (7.0%)	<0.001

Plus-minus values are means ± standard deviation. ICU, intensive care unit, LFTs, Liver function tests.
† The composite end-points consist of admission to intensive care unit, mechanical ventilation, and/or death.

flow nasal cannula, and continuous renal replacement therapy during hospitalization (**Table 2**).

When stratified according to the severity of COVID-19 infection, patients with severe or critical COVID-19 had higher median values of AST, TBIL, ALP, and GGT and lower median value of albumin. The proportion of patients with abnormal AST, albumin, TBIL, ALP, and GGT were higher in severe or critical cases (**Figure 1** and **Supplementary Tables 1**, **2**). Nevertheless, the median value of ALT and the proportion of patients with abnormal ALT were not significantly across the severity of COVID-19.

Dynamic Changes of Liver Functions

Figure 2 depicts the dynamic trajectories of ALT, AST, albumin, TBIL, ALB, and GGT according to normal or abnormal LFTs on admission. The ALT, AST, TBIL, ALP, and GGT values in the abnormal LFTs group increased slightly within the first 5 days after admission and trended downwards thereafter, while those values in the normal LFTs group tended upwards for the entire in-hospital duration. The albumin values trended downwards in both groups within the first 5 days of hospitalization and then fluctuated slightly for the entire duration of follow-up.

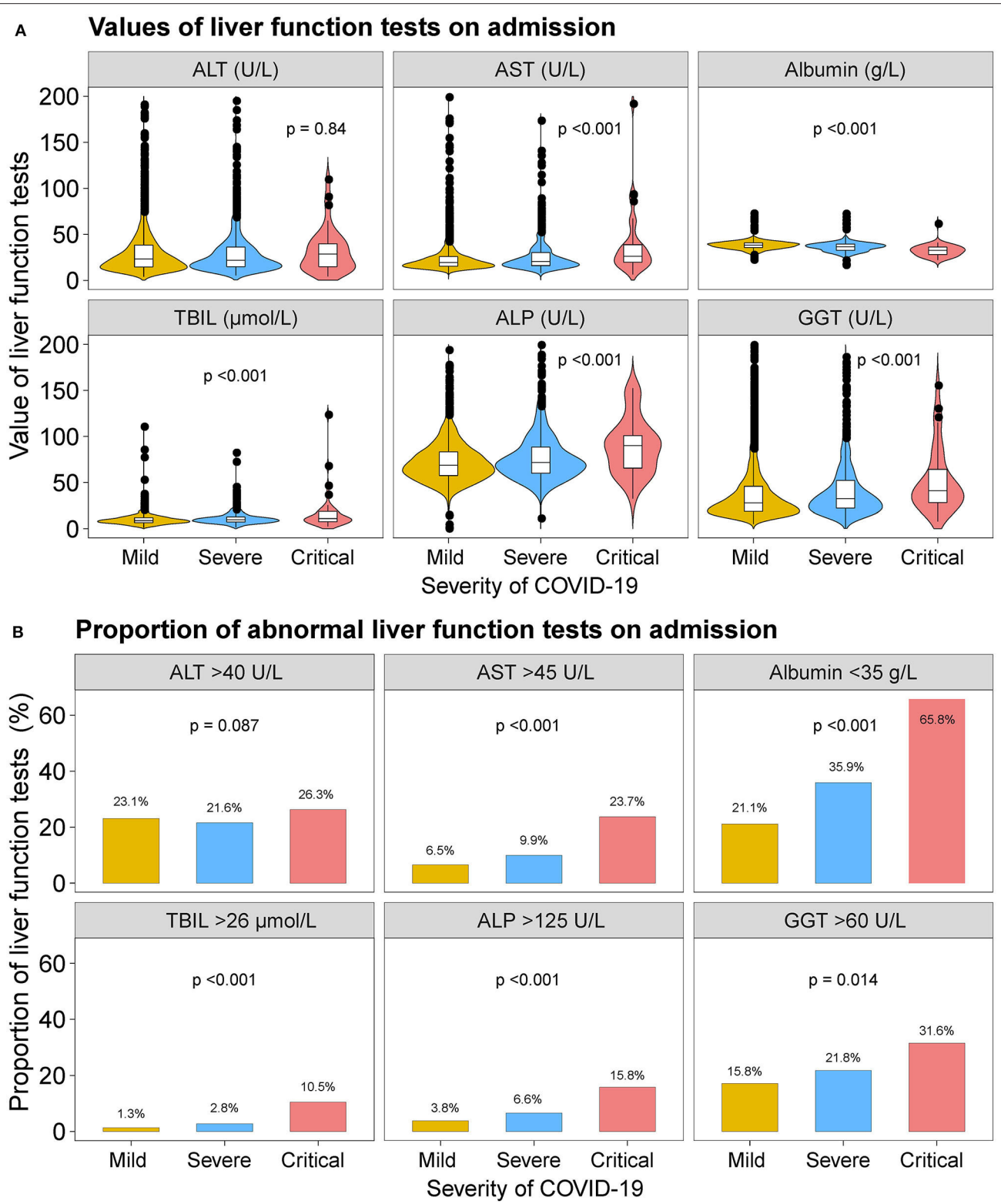


FIGURE 1 | Liver function tests on admission. **(A)** Violin and box plots showing the median values of ALT, AST, albumin, TBIL, ALP, and GGT by severity of the COVID-19 disease. **(B)** Bar plots showing the proportion of abnormal ALT, AST, albumin, TBIL, ALP, and GGT tests on admission by severity of the COVID-19 disease. ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TBIL, total bilirubin abnormal.

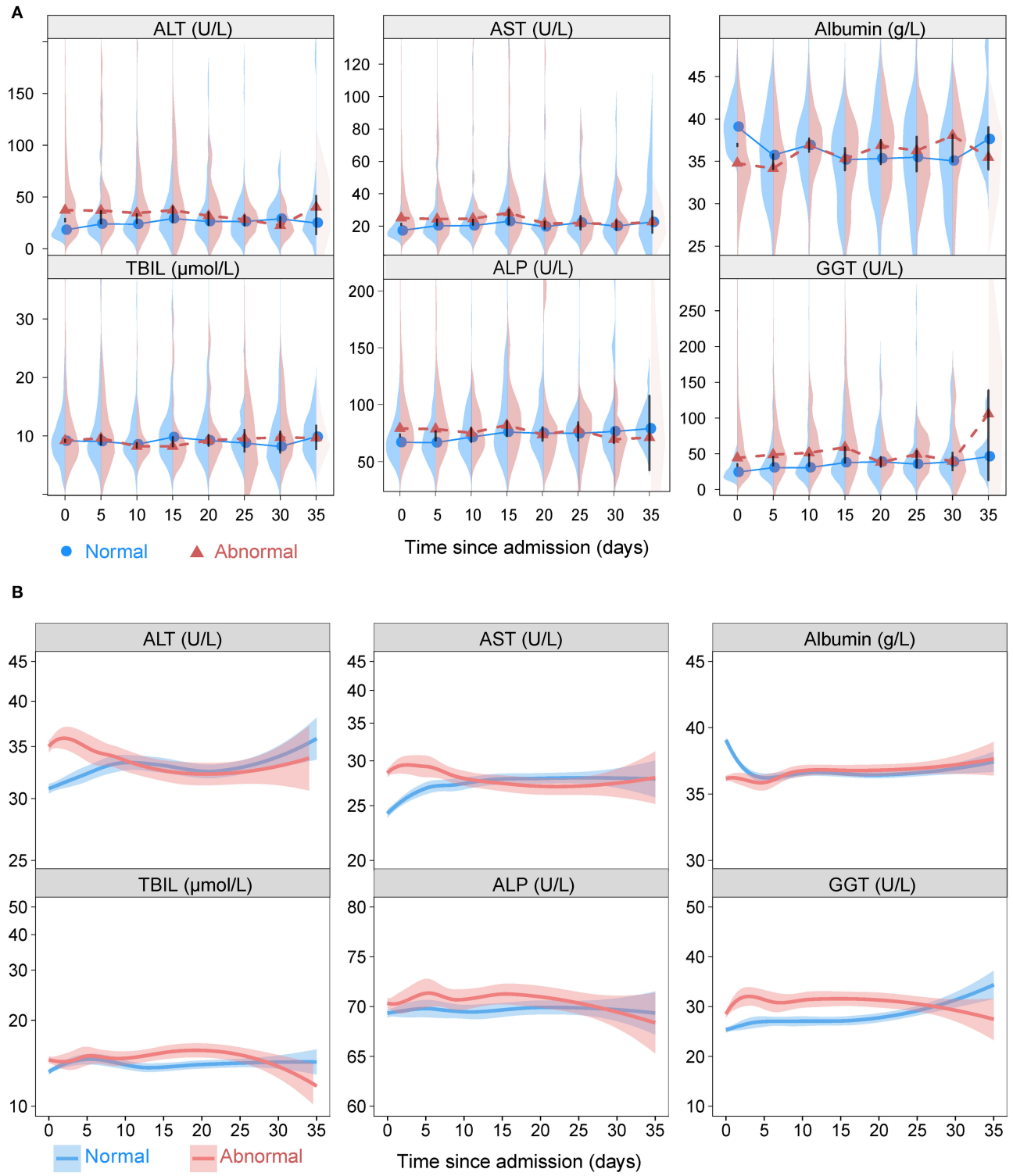


FIGURE 2 | The liver function variations. **(A)** Longitudinal back-to-back violin plots showing the variations of ALT, AST, albumin, TBIL, ALP, and GGT during follow-up stratified by presence or absence of abnormal liver function tests on admission. Circles and triangles indicate medians. The black vertical bars have lengths equal to one-half the length of the 95% confidence interval for the difference in medians. When this bar does not touch the circles and triangles, there is a significant difference in medians at the 0.05 level. **(B)** Smooth trajectories of mean ALT, AST, albumin, TBIL, ALP, and GGT by disease severity with 95% confidence band based on locally weighted scatterplot smoothing stratified by presence or absence of abnormal liver function tests on admission. ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TBIL, total bilirubin abnormal.

When stratified according to the severity of COVID-19 (mild vs. severe/critical), the dynamic curves of LFTs showed downward trends of ALT, AST, TBIL, ALP, and GGT and a upward trend of albumin in both mild and severe/critical groups (**Supplementary Figure 2**). Furthermore, the values of ALT, AST, TBIL, ALP, and GGT were higher and albumin was lower in patents with the outcomes of death, ICU admission, and mechanical ventilation compared those without in most time-points (**Supplementary Figures 3–5**).

Predictors of Peak (Nadir) Value of Liver Function Test During Hospitalization in COVID-19

The cumulative probability model revealed the association between baseline characteristics and hospital treatment on peak ALT, AST, TBIL, ALP, GGT levels, and nadir albumin levels in the entire cohort (**Supplementary Figure 6** and **Figures 3**, **4**). Younger age, male gender, use of antibiotics, increased hemoglobin, increased C-reactive protein, and increased lactate dehydrogenase were factors positively associated with elevated ALT levels. Male gender, diabetes, higher C-reactive protein, and increased lactate dehydrogenase were the leading factors positively associated with elevated AST levels. Total bilirubin levels rise were tightly associated with male gender, decreased creatinine, decreased platelet count, increased C-reactive protein, and increased lactate dehydrogenase. Older age, male gender, antiviral, antibiotics, systemic corticosteroids use, hemoglobin reduction, C-reactive protein, and lactate dehydrogenase elevation were main factors positively correlated with decreased albumin levels. Alkaline phosphatase levels were closely linked with older age, male gender, platelet count, C-reactive protein, and lactate dehydrogenase elevation. Male gender, white blood cell, platelet count, hemoglobin, C-reactive protein, and lactate dehydrogenase increase were identified as factors positively associated with elevated GGT levels. C-reactive protein, lactate dehydrogenase platelet count, hemoglobin, and male gender were common factors positively associated with ALT, AST, TBIL, ALP, GGT elevation, and albumin reduction during hospitalization. To predict the peak (nadir) value of these LFTs, nomograms that incorporated the significant risk factors were established.

Associations Between Abnormal Liver Function Test on Admission and Clinic Outcomes

During a median 13 (IQR: 8–19) days of hospitalization, 61 patients (2.1%) died, 106 patients (3.6%) admitted or transfer to ICU, and 75 patients (2.6%) required mechanical ventilation (**Table 2** and **Supplementary Figure 7**). The 30-day cumulative incidences of death was significantly higher in patients with abnormal LFTs on admission compared with those with normal LFTs (abnormal vs. normal: 3.3 vs. 0.47%; HR 8.32, [95%CI 3.79 −18.26]; $p < 0.001$, **Figure 5A**). Similarly, patients with abnormal LFTs on admission had a higher 30-day cumulative incidences of ICU admission (5.9 vs. 1.2%; HR 5.18 [95%CI 3.12–8.60]; $p < 0.001$; **Figure 5C**) and mechanical ventilation requirement (4.2 vs. 0.8%; HR 5.14 [95%CI 2.82–9.34]; $p < 0.001$, **Figure 5E**). This pattern persisted after adjusting for potential confounders, with the adjusted HRs of abnormal LFTs were 3.66 (95%CI 1.64–8.19, $p = 0.002$, **Figure 5B**) for death, 3.12 (95%CI 1.86–5.23, $p < 0.001$, **Figure 5D**) for ICU admission, and 3.00 (95%CI 1.63–5.52, $p < 0.001$, **Figure 5F**) for mechanical ventilation requirement. Furthermore, these effects were homogeneous across the severity of COVID-19 ($P_{\text{interaction}} > 0.1$ for all comparisons, **Figure 6** and **Supplementary Figures 8**, **9**). Notably, chronic liver disease was not associated with an increased risk of either of these adverse outcomes (**Figures 5**, **6** and **Supplementary Figures 8**, **9**).

The relationship between the baseline ALT, AST, albumin TBIL, ALP as well as GGT and death rate during hospitalization was depicted in **Figure 7**. The increased ALT, AST, TBIL, ALP, GGT, and decreased albumin on admission had a non-linear positive association with the risk of death, which was homogeneous across the severity of COVID-19. Similar results were observed for the secondary endpoint of ICU admission (**Supplementary Figure 10**) as well as mechanical ventilation requirement (**Supplementary Figure 11**).

When stratified according to different levels of LFTs, abnormal levels of baseline AST, albumin, TBIL, ALP, and GGT were significantly associated with the risk of death, ICU admission, and mechanical ventilation (**Figure 8** and **Supplementary Figures 12**, **13**). Among them, AST over three-fold ULN and albumin <30 g/L had the highest risks of death, ICU admission, and mechanical ventilation. The elevation of ALT tended to be associated with increased risks of those outcomes. Nevertheless, the difference did not reach significance.

Associations Between *De novo* Abnormal Liver Function Test During Hospitalization and Clinic Outcomes

Among the 1,498 patients with normal LFTs upon admission, 368 patients (24.6%) developed *de novo* abnormalities of LFTs (**Supplementary Table 3**). Univariable and multivariable logistic regression analysis showed that lymphocyte count (OR 1.13, 95%CI: 1.03–1.25, $p = 0.007$), use of quinolones (OR 1.48, 95%CI: 1.10–1.98, $p = 0.010$), and cephalosporins (OR 4.80, 95%CI: 1.58–14.59, $p = 0.006$) were independently were associated with *de novo* abnormalities of LFTs (**Supplementary Figure 14** and **Figure 9**). Compared with those without *de novo* abnormal LFTs, patients with *de novo* abnormal LFTs had higher risk of death, ICU admission as well as the mechanical ventilation requirement. The trends persisted after adjusting for potential confounders, but the differences were not significant (**Supplementary Table 4** and **Figure 10**).

Associations Between Peak (Nadir) Liver Function Test During Hospitalization and Clinic Outcomes

Overall, 1,782 patients (61.2%) had abnormal LFTs during hospitalization in the entire cohort. Peak ALT, AST, TBIL, ALP, and GGT above ULN was observed in 855 (29.4%), 321 (11.0%), 103 (3.5%), 166 (5.7%), and 642

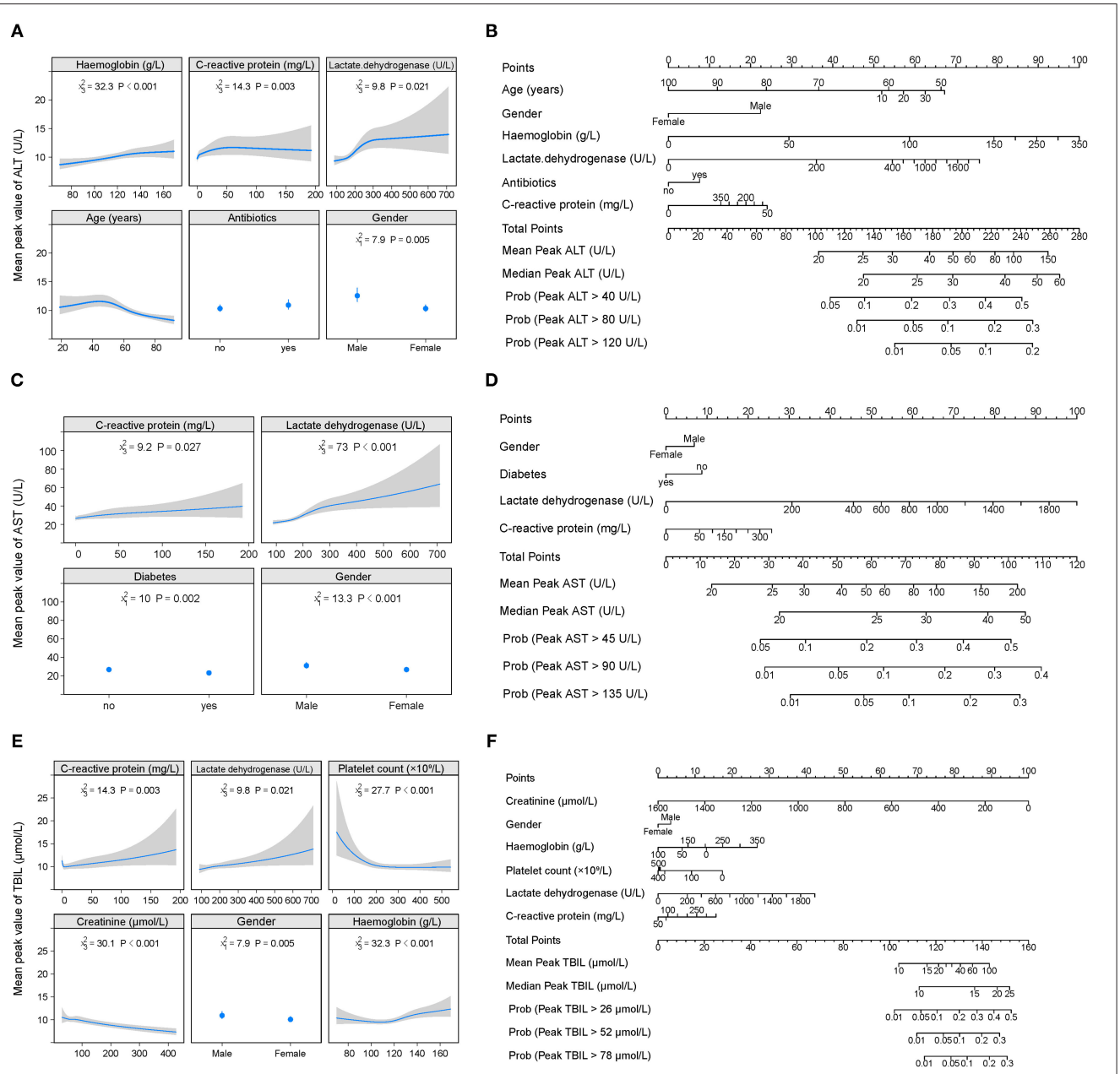


FIGURE 3 | Factors and nomogram for predicting the peak values of ALT, AST, and TBIL during hospitalization. **(A, C, E)** Multivariable analysis of factors associated with the peak values of ALT, AST, and TBIL during hospitalization. The non-linearity of continuous variables were considered and analyzed with restricted cubic splines. **(B, D, F)** Nomogram for predicting the peak values of ALT, AST, and TBIL during hospitalization. To use the nomogram, first draw a vertical line to the top points row to assign points for each variable; then, add the points from each variable together and drop a vertical line from the total points row to obtain the median, mean values of peak ALT, AST, and TBIL during hospitalization as well as the probability of above the 1-, 2-, 3-time upper limit of normal of these parameters.

(22.0%) patients, respectively. Nadir albumin <35 g/L was observed in 1,114 (38.3%) patients during hospitalization. The baseline characteristics of patients grouped according to LFTs abnormalities during hospitalization are shown in **Supplementary Table 5**. Compared with those with normal liver function impairment during hospitalization, patients with abnormal LFTs had severer COVID-19 disease and more common of respiratory and digestive symptoms. Similar to baseline LFTs abnormality, abnormal LFTs during hospitalization, peak AST, TBIL, ALP, GGT, and nadir albumin but not peak ALT were significantly (or a trend toward) associated with adverse outcomes of COVID-19 (**Figures 11–13**, **Supplementary Tables 6**, **7**, and **Supplementary Figures 15–19**).

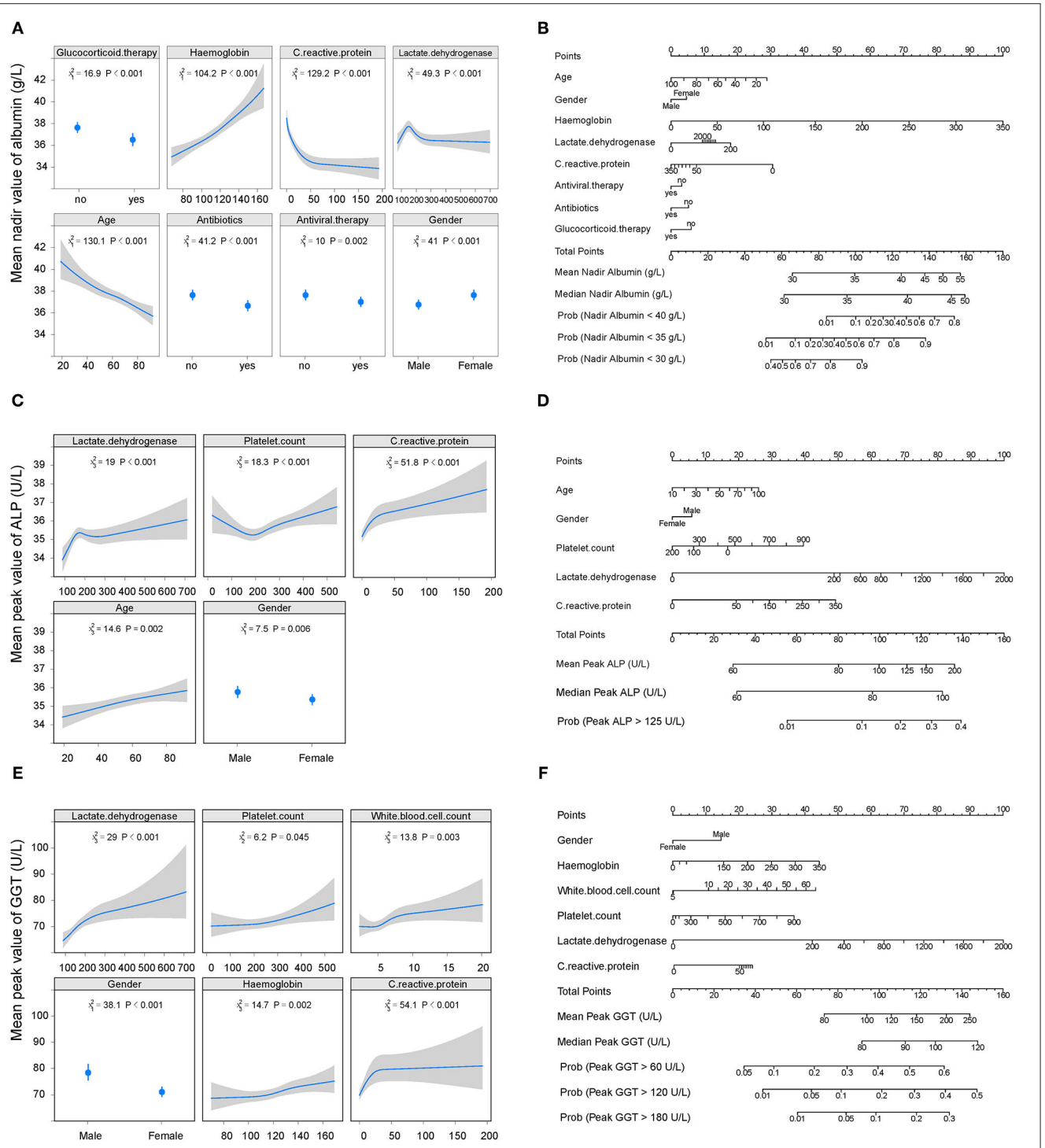


FIGURE 4 | Factors and nomogram for predicting the nadir albumin and peak ALP, GGT during hospitalization. **(A, C, E)** Multivariable analysis of factors associated with nadir albumin and peak ALP, GGT during hospitalization. The non-linearity of continuous variables were considered and analyzed with restricted cubic splines. **(B, D, F)** Nomogram for predicting the peak values of nadir albumin and peak ALP, GGT during hospitalization. To use the nomogram, first draw a vertical line to the top points row to assign points for each variable; then, add the points from each variable together and drop a vertical line from the total points row to obtain the median, mean values of nadir albumin and peak ALP, GGT during hospitalization as well as the probability of above the 1-, 2-, 3-time upper limit of normal of these parameters. ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase.

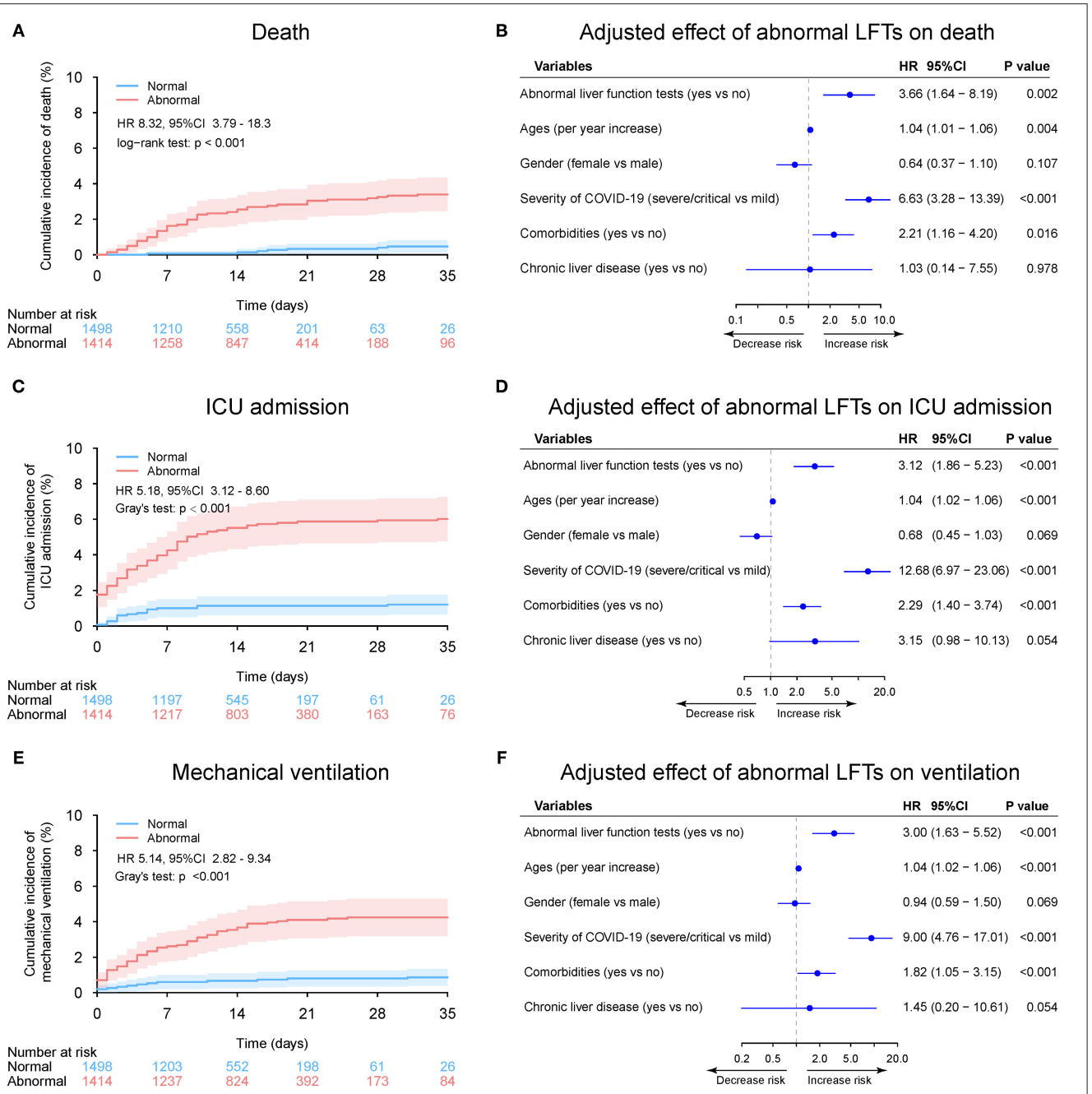


FIGURE 5 | Outcome analysis according to admission abnormal vs. normal liver function tests (LFTs). **(A)** Cumulative incidence of death in patients with abnormal vs. normal LFTs on admission. **(B)** Independent effect (hazard ratio with 95% confidence intervals) of admission abnormal vs. normal LFTs on all-cause mortality adjusted for potential confounders using the Cox multivariable regression models. **(C)** Cumulative incidence of ICU admission in patients with abnormal vs. normal LFTs on admission based on competing risk approach (the Fine and Gray method) with death being the competing events. **(D)** Independent effect (hazard ratio with 95% confidence intervals) of admission abnormal vs. normal LFTs on ICU admission adjusted for potential confounders using the Cox multivariable regression models. **(E)** Cumulative incidence of mechanical ventilation in patients with abnormal vs. normal LFTs on admission based on competing risk approach (the Fine and Gray method) with death being the competing events. **(F)** Independent effect (hazard ratio with 95% confidence intervals) of admission abnormal vs. normal LFTs on mechanical ventilation adjusted for potential confounders using the Cox multivariable regression models. Comorbidities include hypertension, cardiovascular disease, diabetes, chronic pulmonary diseases, cerebrovascular disease, malignancy, and autoimmune disease. Chronic liver diseases include hepatitis B virus infection, hepatitis C virus infection, and autoimmune liver disease. LFTs, liver function tests; ICU, intensive care unit.

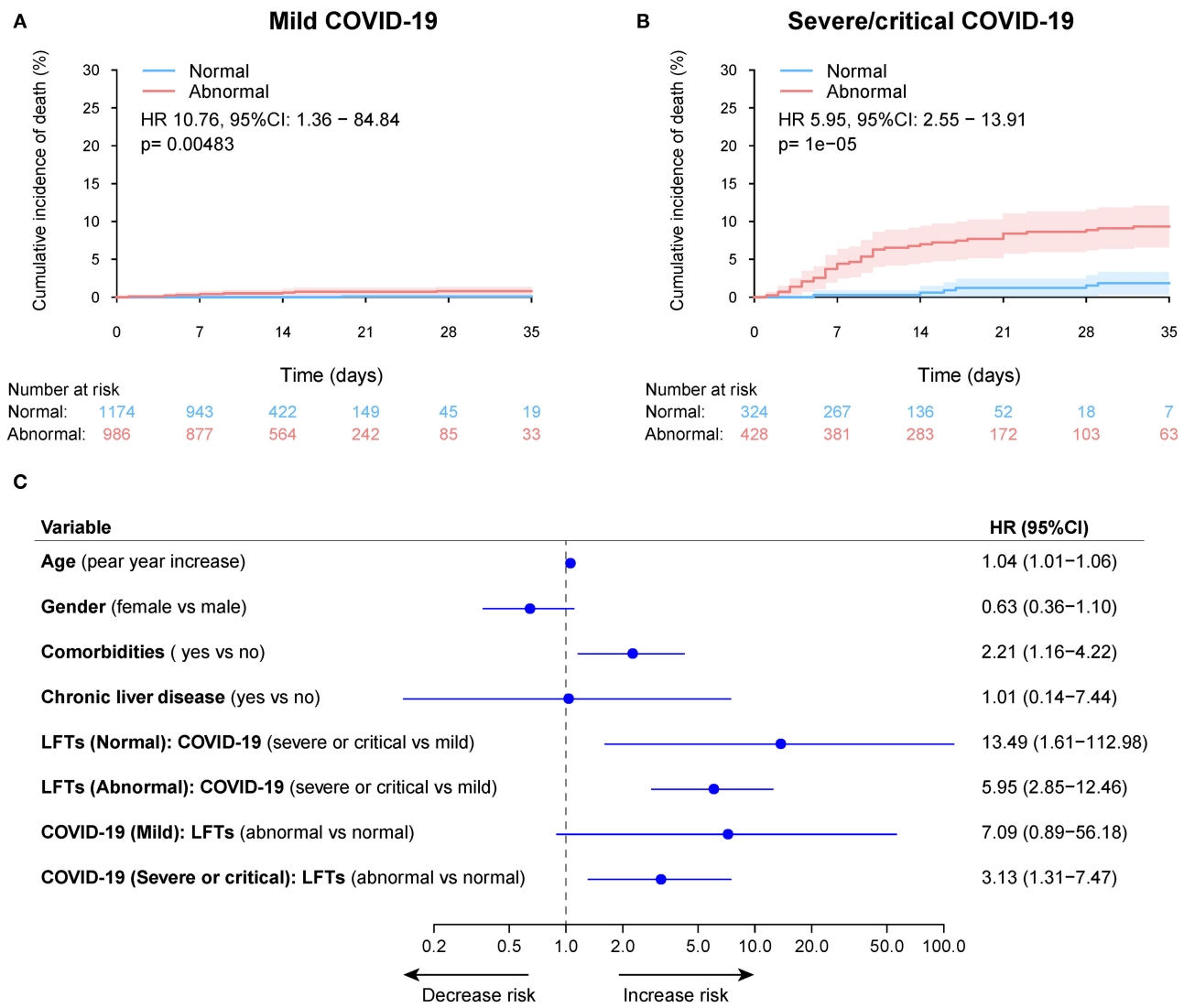


FIGURE 6 | Cumulative incidence of death according to admission abnormal vs. normal liver function tests (LFTs) and severity of COVID-19 infection. **(A)** Cumulative incidence of death in patients with abnormal vs. normal LFTs on admission and mild COVID-19 infection. **(B)** Cumulative incidence of death in patients with abnormal vs. normal LFTs on admission and severe/critical COVID-19 infection. **(C)** Forest plot showing the interaction test of the LFTs (normal vs. abnormal) and severity of COVID-19 infection (mild vs. severe/critical) on death after adjustment for potential confounders using the Cox multivariable regression models. $P_{interaction} = 0.521$, showing a homogeneous effect of LFTs on death across the severity of COVID-19 infection. Comorbidities include hypertension, cardiovascular disease, diabetes, chronic pulmonary diseases, cerebrovascular disease, malignancy, and autoimmune disease. Chronic liver diseases include hepatitis B virus infection, hepatitis C virus infection, and autoimmune liver disease. COVID-19, coronavirus disease 2019; LFTs, liver function tests.

DISCUSSION

In this observational study of 2,912 hospitalized patients with COVID-19, we present the patterns and trajectories of LFTs as well as depict their clinical significance. The major findings were: (i) the derangement of liver function was generally mild (1–2 time of ULN) in non-severe patients but more frequent and to a greater extent in patients with severe/critical COVID-19 infection; (ii) Pattern of LFTs abnormality is predominantly hepatocellular rather than cholestatic; (iii) abnormality of LFTs was transient and tended to resolve over time; (iv) common factors associated with the peak (nadir) LFTs were C-reactive protein, lactate dehydrogenase, platelet count, hemoglobin, and male gender; (v) abnormal LFTs (AST, albumin, TBIL, ALP, and GGT but not ALT) were independently associated with increased risks of mortality, ICU admission, and mechanical ventilation requirement, which was homogeneous across the severity of COVID-19 infection. The strengths and novelties of the current study lie in: (i) use of a death-based primary endpoint, which is an objectively assessed and clinically relevant endpoint; (ii) a large sample size which allow providing estimates with narrow CIs; (iii)

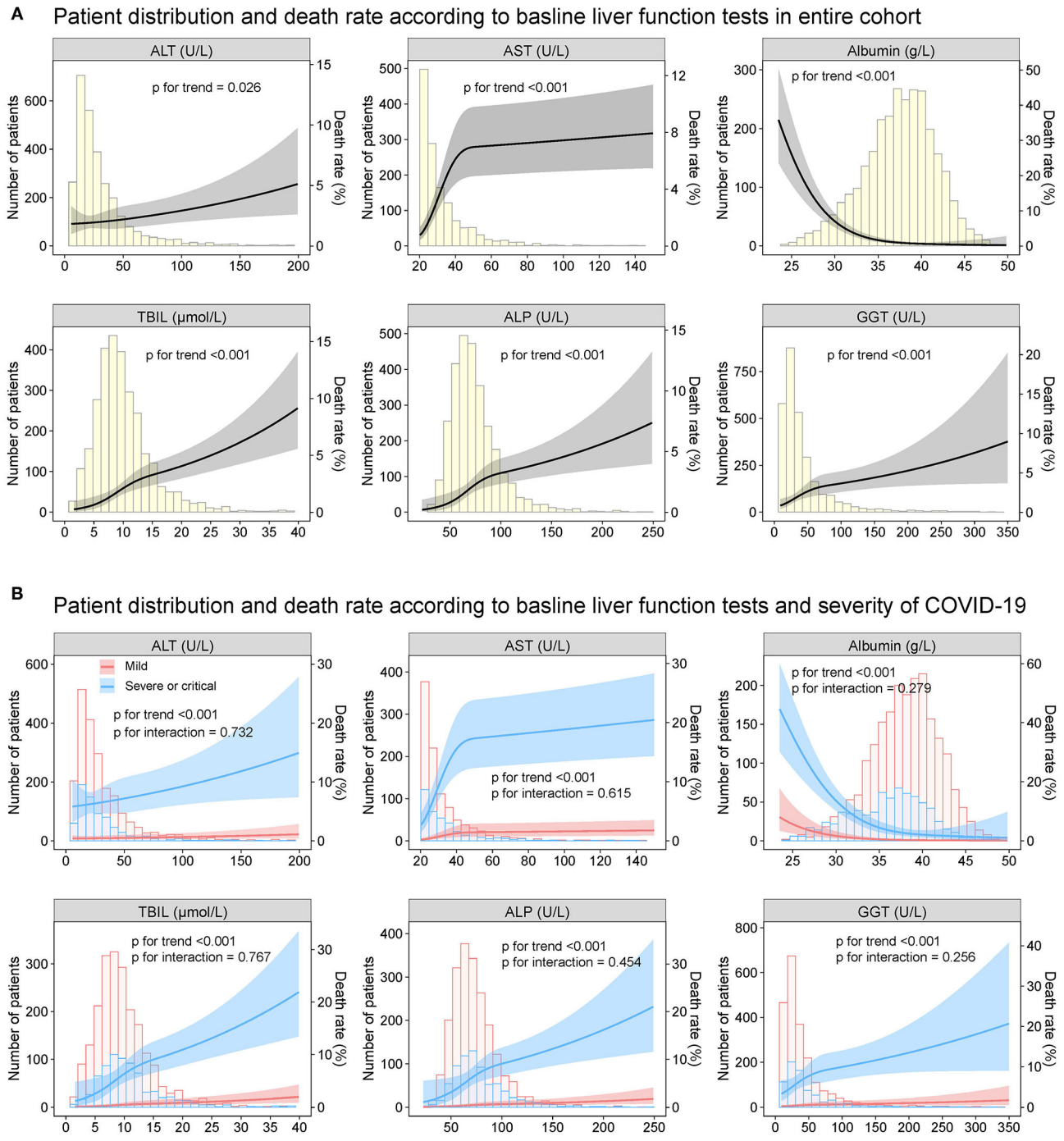
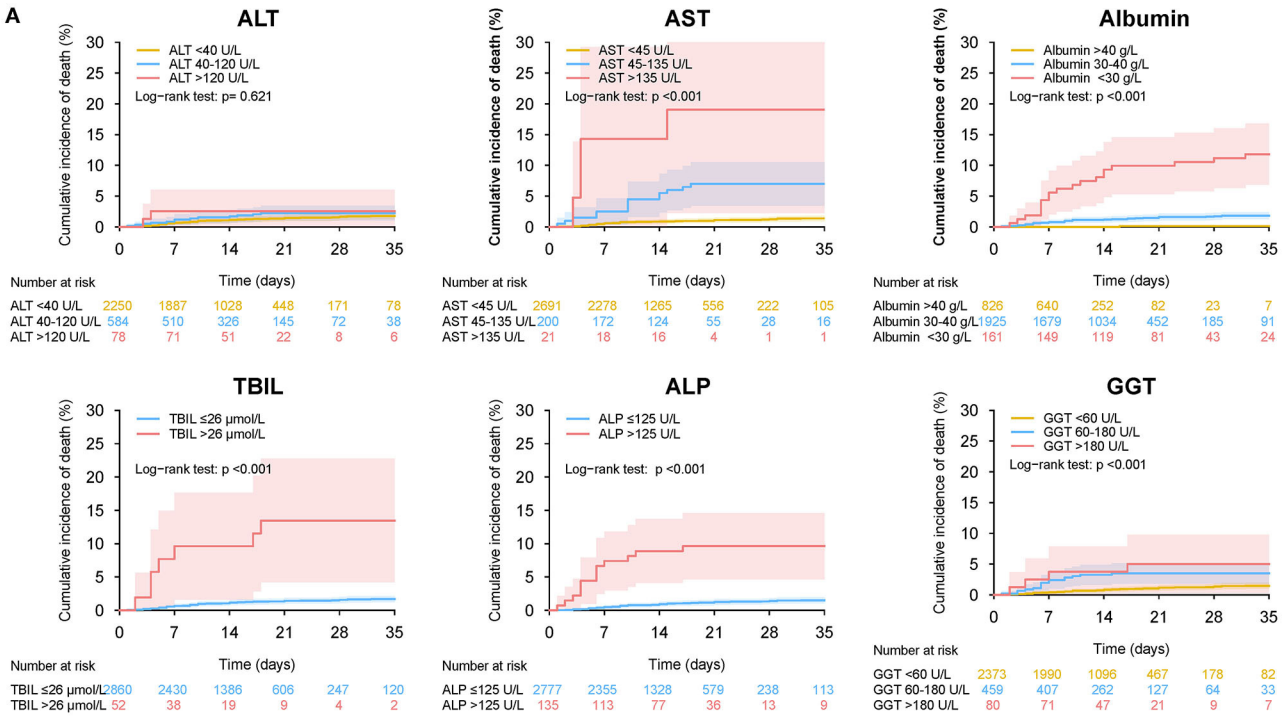


FIGURE 7 | Patient distribution and death rate according to baseline liver function tests Patient distribution and death rate according to baseline ALT, AST, albumin, TBIL, ALP, and GGT **(A)** in entire cohort and **(B)** by severity of COVID19 infection (mild vs. severe/critical). Restricted cubic splines were generated using logistic regression models. ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TBIL, total bilirubin abnormal.

multivariate and subgroup analysis, which permitted adjustment for potential confounding factors and explore the effect homogeneity; (iv) adopting not only categorized but continuous LFT analysis; (v) comprehensive liver function parameters and outcomes analyses; (vi) differentiation between baseline and in-hospital elevations of liver enzymes; (vii) significant amount of data on pre-existing liver disease and therapies used during hospitalization.



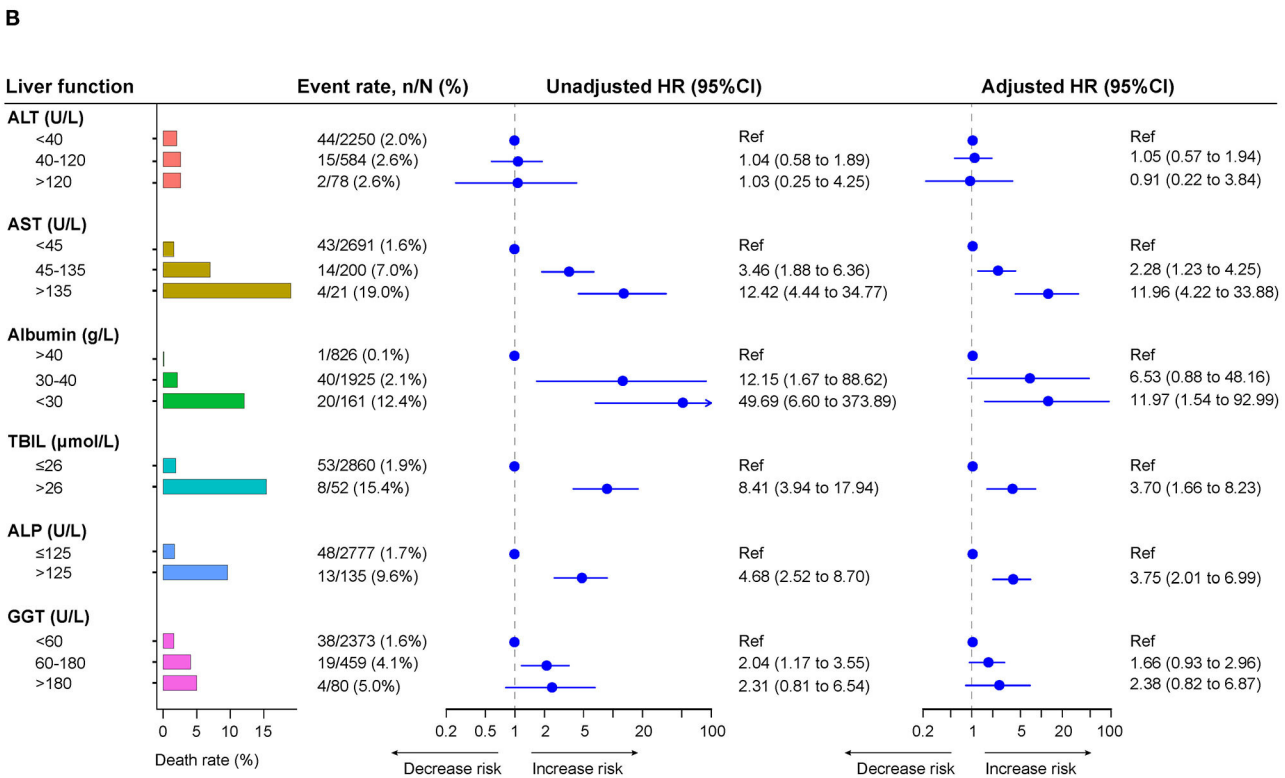


FIGURE 8 | Mortality during hospitalization in patients with different level of baseline liver function tests. **(A)** Cumulative incidence of death in patients with different level of liver function test on admission. **(B)** Death rate in patients with different level of liver function test on admission, the unadjusted adjusted effect of liver function test at different level on the mortality during hospitalization. Adjusted HRs are derived from multivariate Cox regression models, adjusted for age, gender, comorbidities (hypertension, cardiovascular disease, diabetes, chronic pulmonary diseases, cerebrovascular disease, malignancy, autoimmune disease) and chronic liver diseases (hepatitis B virus infection, hepatitis C virus infection, autoimmune liver disease). ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; GGT, gamma-glutamyltransferase; HR, hazard ratio; TBIL, total bilirubin.

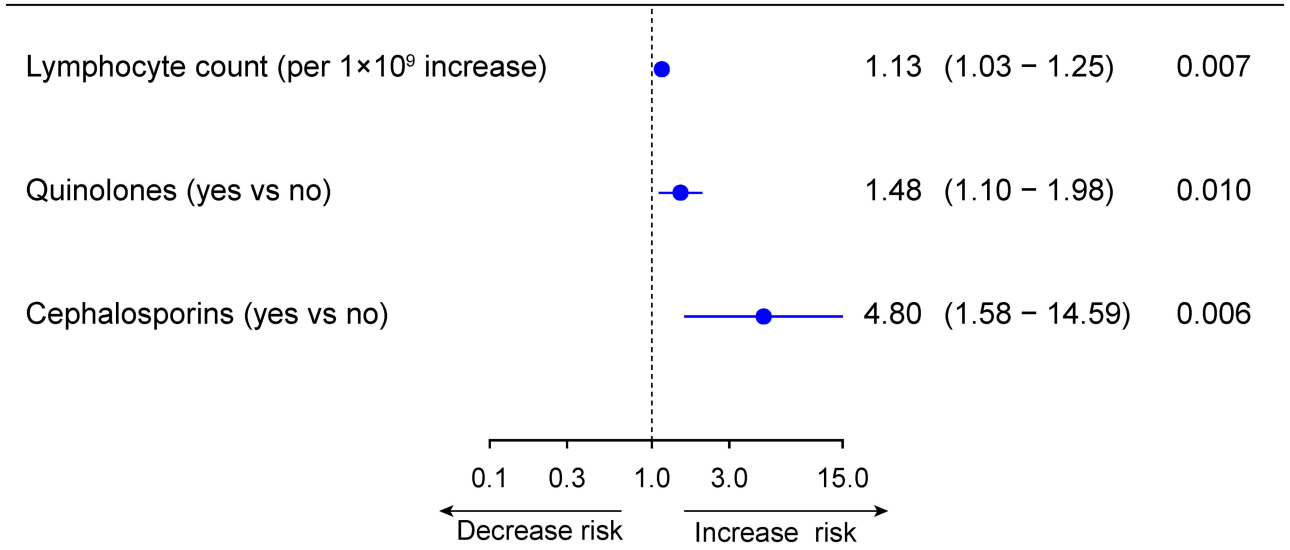


FIGURE 9 | Multivariable analysis of factors associated with *de novo* abnormal vs. normal liver function tests (LFTs) during hospitalization.

In our cohort, 48.6% had abnormal liver biochemistries at admission and 61.2% had liver biochemistries derangement during hospitalization, which was slightly higher than what has been reported in the literature (34, 35). Disparity may be attributed to the diverse definition of abnormality of LFTs. Indeed, the liver enzymes (ALT, AST, ALP, and GGT) elevation observed here is similar to those in previous cohorts (11–27). Nevertheless, the hypoalbuminemia was considered as part of abnormal LFTs in our definition while it was not included in most previous studies. As in other reports (11–27), liver enzyme elevations in COVID-19, even in the severe COVID-19 category, are mild-to-moderate in most of the cases, and the pattern of abnormal liver biochemistries was characterized by slight increases in hepatocyte-related enzymes, including ALT and AST, with accompanying GGT elevation. Pure cholestatic alterations characterized by ALP elevation were rare, and an increase in TBIL was less commonly observed (36, 37). However, significant hypoalbuminemia was observed, particularly among patients with severe COVID-19 disease. The possible explanation might be that albumin is a negative acute phase reactant rather than a manifestation of a hepatic synthetic dysfunction.

Furthermore, when stratifying according to disease severity of COVID-19 infection, we found that the AST, TBIL, ALP, and GGT were elevated more frequently and to a greater extent in patients with severe COVID-19 compared to those with mild disease. However, ALT elevation was not significantly higher in the severe/critical patients. This observation may be related to the mechanism of LFTs abnormality. Available evidence suggests that hepatic involvement in COVID-19 could be related to the direct cytopathic effect of the virus, an uncontrolled immune reaction, sepsis, or drug-induced liver injury (2, 11, 37, 38). The postulated mechanism of viral entry is through the host angiotensin-converting enzyme 2 (ACE2) receptors (39, 40). However, the ACE2 receptor is much more heavily expressed in cholangiocytes than in hepatocytes. Furthermore, the concentrations of serum ALP was normal in most patients with COVID-19, suggest the most common mechanism of liver damage is not due to a direct cytopathic effect of the SARS-CoV-2 virus. Our analysis showed that peak (nadir) liver function markers were commonly correlated with the direct or indirect markers of inflammation (C-reactive protein, lactate dehydrogenase, platelet count, hemoglobin at baseline), which support the point that most cases of liver derangement may reflect sepsis related cholestasis and inflammatory changes, or hepatotoxicity from concomitant medications (41, 42). Furthermore, studies have confirmed increased NETosis, a form of non-apoptotic and highly immunogenic cell death causing bystander damage and coagulation changes, accompanies disease severity (42, 43). It can be imagined that the alteration of immune balance occurs with increased severity of COVID-19, thus explaining why increases in serum AST, ALP, and TBIL levels but not ALT tend to parallel the severity of pulmonary disease, in an analogous fashion to patterns seen in sepsis (44). Lymphocyte count, use of quinolones and cephalosporins were independently were associated with *de novo* abnormalities of LFTs during hospitalization, suggesting drug-induced liver injury should not be overlooked in patients with COVID-19. With further analysis of longitudinal patterns, we found that the abnormality of LFTs manifested as transient elevation in most cases and liver involvement tended to resolve during prolonged disease course, indicating that supportive care alone might be sufficient to achieve liver recovery. Therefore, we advise checking baseline LFTs in all patients on admission and monitoring of LFTs throughout the hospitalization, particularly

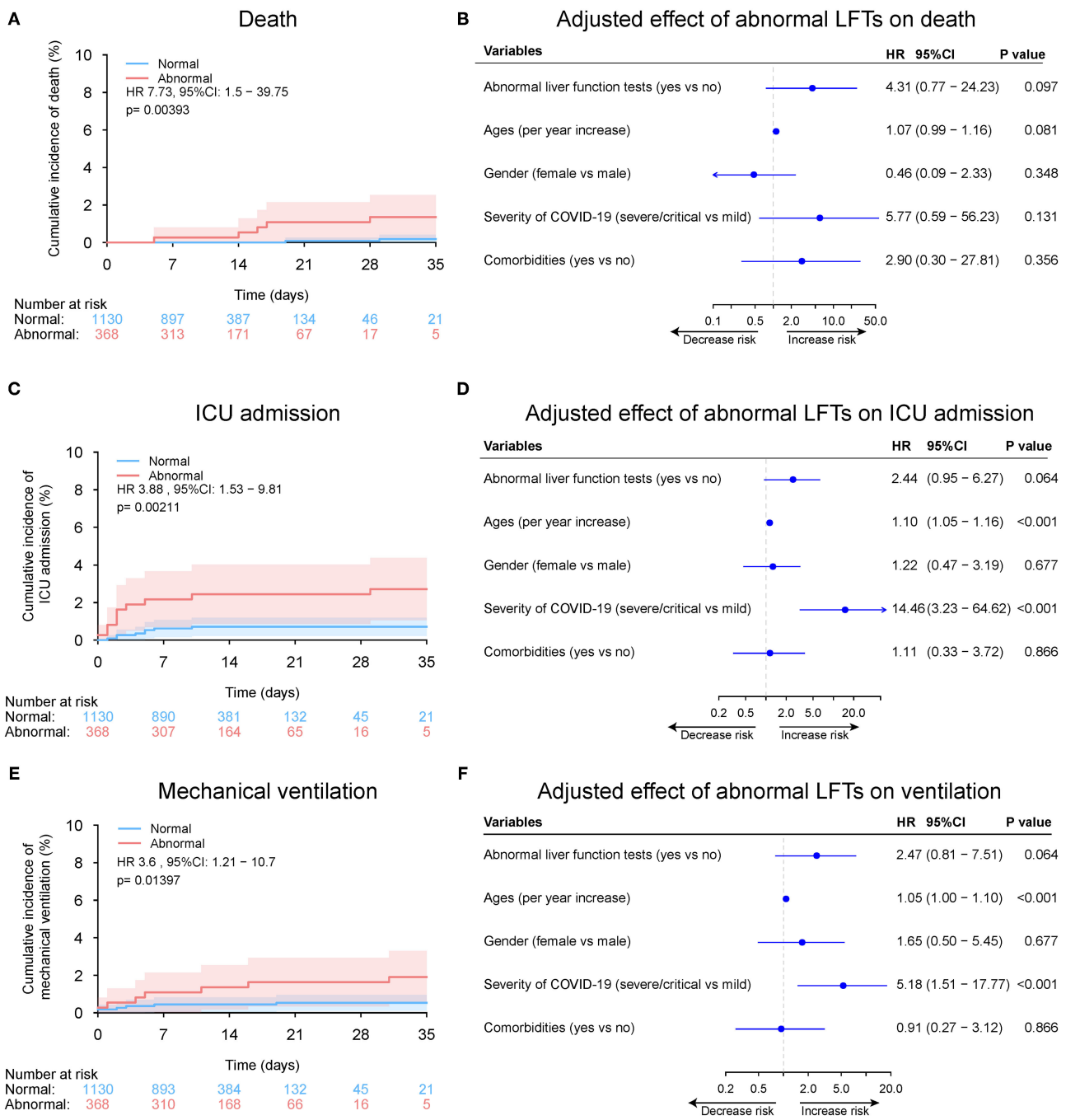


FIGURE 10 | Outcome analysis according to *de novo* abnormal vs. normal liver function tests (LFTs) during hospitalization. **(A)** Cumulative incidence of death in patients with *de novo* abnormal vs. normal LFTs during hospitalization. **(B)** Independent effect (hazard ratio with 95% confidence intervals) of *de novo* abnormal vs. normal LFTs during hospitalization on all-cause mortality adjusted for potential confounders using the Cox multivariable regression models. **(C)** Cumulative incidence of ICU admission in patients with *de novo* abnormal vs. normal LFTs during hospitalization based on competing risk approach (the Fine and Gray method) with death being the competing events. **(D)** Independent effect (hazard ratio with 95% confidence intervals) of *de novo* abnormal vs. normal LFTs during hospitalization on ICU admission adjusted for potential confounders using the Cox multivariable regression models. **(E)** Cumulative incidence of mechanical ventilation in patients with *de novo* abnormal vs. normal LFTs during hospitalization based on competing risk approach (the Fine and Gray method) with death being the competing events. **(F)** Independent effect (hazard ratio with 95% confidence intervals) of *de novo* abnormal vs. normal LFTs during hospitalization on mechanical ventilation adjusted for potential confounders using the Cox multivariable regression models. Comorbidities include hypertension, cardiovascular disease, diabetes, chronic pulmonary diseases, cerebrovascular disease, malignancy, and autoimmune disease. Chronic liver diseases include hepatitis B virus infection, hepatitis C virus infection, and autoimmune liver disease. LFTs, liver function tests; ICU, intensive care unit.

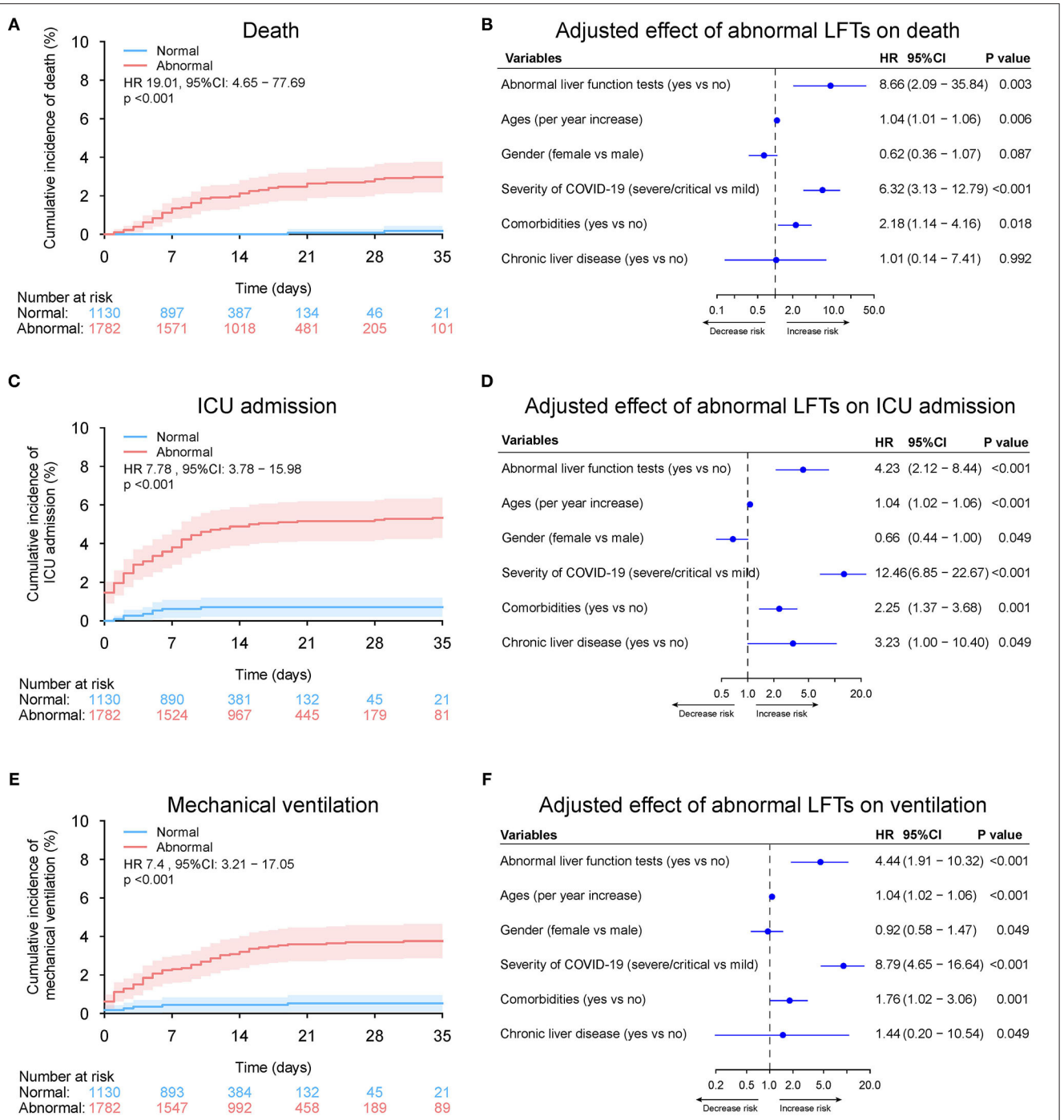


FIGURE 11 | Outcome analysis according to abnormal vs. normal liver function tests (LFTs) during hospitalization in entire cohort. **(A)** Cumulative incidence of death in patients with abnormal vs. normal LFTs during hospitalization in entire cohort. **(B)** Independent effect (hazard ratio with 95% confidence intervals) of abnormal vs. normal LFTs during hospitalization on all-cause mortality adjusted for potential confounders using the Cox multivariable regression models. **(C)** Cumulative incidence of ICU admission in patients with abnormal vs. normal LFTs during hospitalization based on competing risk approach (the Fine and Gray method) with death being the competing events. **(D)** Independent effect (hazard ratio with 95% confidence intervals) of abnormal vs. normal LFTs during hospitalization on ICU admission adjusted for potential confounders using the Cox multivariable regression models. **(E)** Cumulative incidence of mechanical ventilation in patients with abnormal vs. normal LFTs during hospitalization based on competing risk approach (the Fine and Gray method) with death being the competing events. **(F)** Independent effect (hazard ratio with 95% confidence intervals) of abnormal vs. normal LFTs during hospitalization on mechanical ventilation adjusted for potential confounders using the Cox multivariable regression models. Comorbidities include hypertension, cardiovascular disease, diabetes, chronic pulmonary diseases, cerebrovascular disease, malignancy, and autoimmune disease. Chronic liver diseases include hepatitis B virus infection, hepatitis C virus infection, and autoimmune liver disease. LFTs, liver function tests; ICU, intensive care unit.

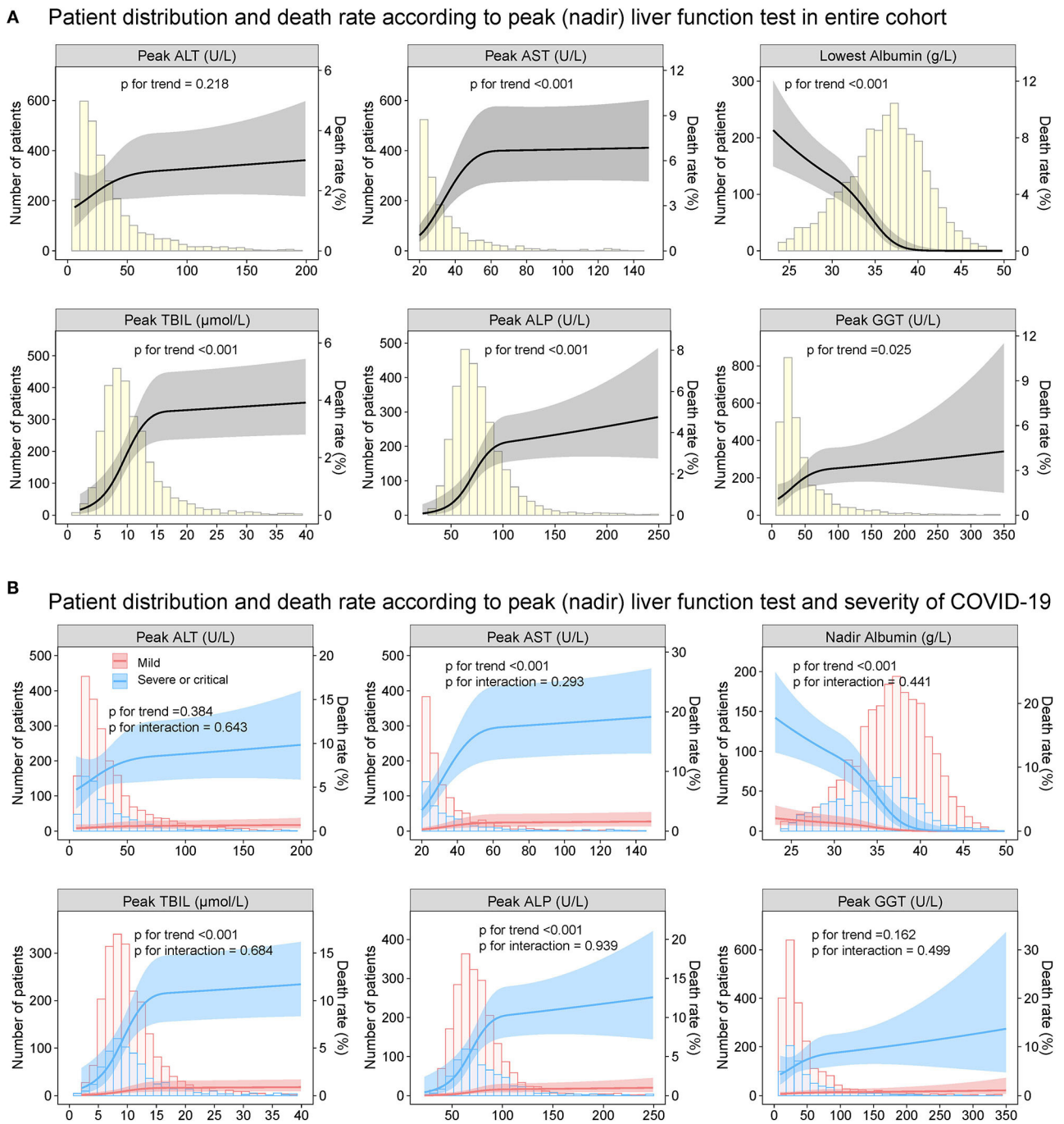


FIGURE 12 | Patient distribution and death rates according to peak (nadir) liver function test in entire cohort. Patient distribution and death rate according to peak ALT, peak AST, nadir albumin, peak TBIL, peak ALP, and peak GGT during hospitalization **(A)** in entire cohort **(B)** by severity of COVID-19 infection (mild vs. severe/critical). Restricted cubic splines were generated using logistic regression models. ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; COVID-19, coronavirus disease 2019; GGT, gamma-glutamyltransferase; TBIL, total bilirubin.

in patients undergoing drug therapy for COVID-19 with potential hepatotoxicity.

Our results showed that abnormalities of LFTs on admission as well during hospitalization were associated with death, ICU admission and mechanical ventilation requirement in COVID-19 patients. More importantly, these associations were independent from the most commonly described predictors of the evaluated outcomes in multivariable analysis. Furthermore, the effects

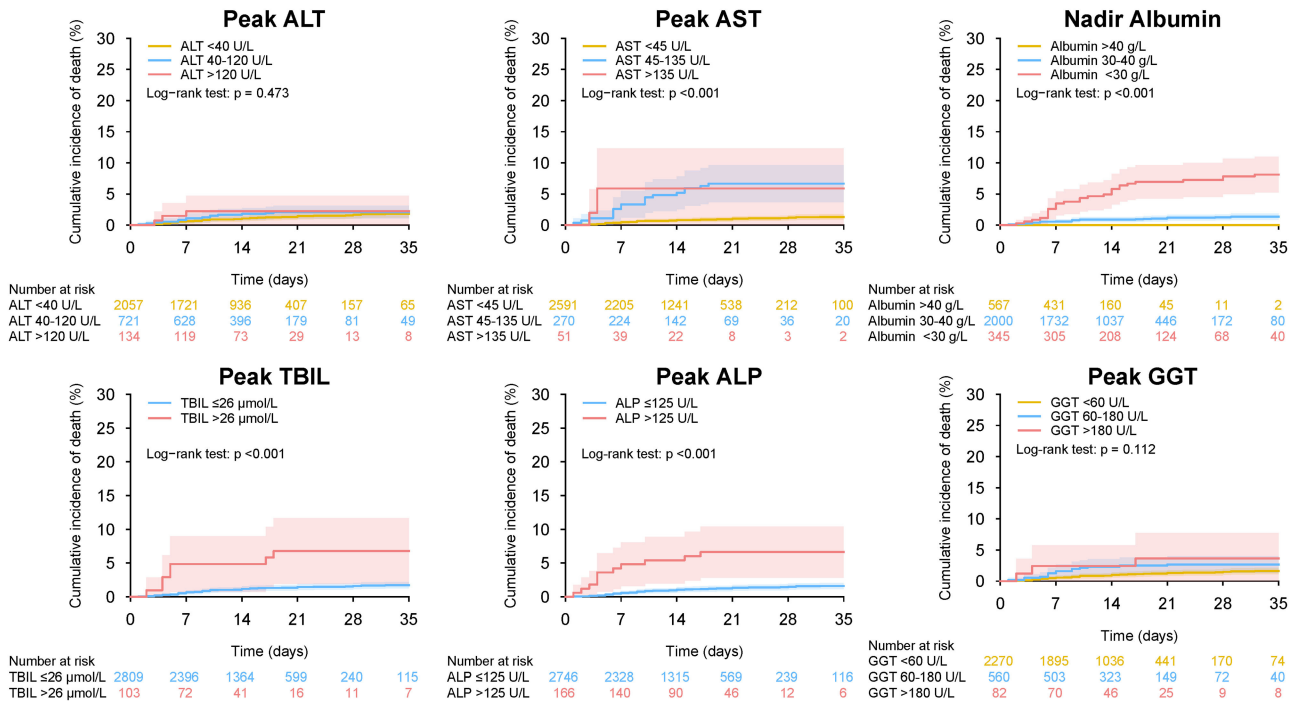


FIGURE 13 | Mortality during hospitalization in patients with different level of peak (nadir) liver function test in entire cohort. Cumulative incidence of death during hospitalization in patients with different level of peak ALT, peak AST, nadir albumin, peak TBIL, peak ALP, and peak GGT during hospitalization. ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TBIL, total bilirubin abnormal.

of LFTs on the evaluated outcomes were homogeneous across the severity of COVID-19, suggesting the impact of LFTs on the evaluated outcomes were not modified by the severity of COVID-19. Several studies have reported on the association between the abnormal LFTs and severity of disease or outcomes, with conflicting results (11–27). Most of them reported the results of univariate analyses without appropriately adjust for potential confounders. Thus, it is unclear whether the influence of abnormal LFTs on the prognosis was real or mediated by its association with other co-existing diseases. A large multicenter study of 5,771 Chinese individuals showed that peak liver biochemistries (AST, ALT, ALP, and TBIL) predicted mortality, after adjusting for age, gender, and comorbidities in Cox regression model (18). Similarly, an Italian study with 565 inpatients showed that abnormality of LFTs (ALT, AST, ALP, GGT, and TBIL) observed at admission was independently associated to a composite endpoint of transfer to the ICU or death (24). In contrast, another Italian study by Ponziani et al. (22) suggested baseline liver test (AST, ALT, and GGT) abnormalities were associated with increased risk of ICU admission but not with mortality. The discrepancy might be due to the somewhat low incidence of death in the latter study, which may reduce the likelihood of association between LFTS and mortality of COVID-19, with a wide CI of the HR. Thus, patients with abnormal LFTs should be closely followed up due to the potential worse outcomes.

In our study, while AST, albumin, TBIL, ALP, and GGT were significantly associated with adverse outcomes, no such an association were observed in ALT. This was in agreement with the study by Hao et al. (27) showing no differences in the severity, discharge rate, and median hospitalization time between patients with and without ALT elevation. However, this finding is in contrast with two previous studies where higher peak ALT values were significantly associated with increased risk of mortality or discharge to hospice (OR = 1.14 or 1.43) (18, 19). The main reason for the discrepancy was not clear. Nevertheless, it should be noted that the association between ALT and death was not so strong (18, 19), and our patients were generally healthier compared with previously published cohorts.

The prevalence of chronic liver disease in our cohort was 2.3%, which is within the range (2–11%) reported in recent data from other cohorts (45–48). Previous studies showed that those with chronic liver disease are more likely to have more adverse outcomes and mortality when compared to those without (49–52). In our study, however, the presence of chronic liver disease was not significantly associated with disease progression and mortality, which may be due in part to the overall low numbers of patients with these disease entities. Another possible explanation may be that the severity of chronic liver disease in our patients is generally mild, with no patient having cirrhosis. Indeed, the term "chronic liver disease" constitutes a spectrum of patients with varying prognosis ranging from chronic hepatitis, cirrhosis, decompensated cirrhosis to acute-on-chronic liver failure that may differentially affect outcomes (53).

Our study has several limitations. First, the single-center nature may limit its representativeness. However, quality

control was ensured because all the diagnostic and therapeutic algorithms were uniform. Second, potential bias in the selection of samples is inherent to its retrospective design. Nevertheless, we included all consecutive patients with confirmed COVID-19 admitted to the hospital, which minimizes the risk of selection bias. Third, although multivariate regression analyses were conducted to adjust for potential confounders, our findings may be biased due to unidentified confounding. Fourth, liver biochemistries and other important laboratory markers were not assessed daily on every patient because this was not required for clinical decision making. Fifth, our study patients represent an exclusively inpatient population. Therefore, this information may not be generalizable to outpatients. Sixth, this is an observational study. Thus, the association should not be regarded as causal effect. Seventh, alcohol abuse and hepatotoxic drug intake prior to development of COVID-19 have not been considered. Finally, with only a few cases of incompletely characterized chronic liver disease in this cohort, we cannot draw conclusions about hepatic impairment and other outcomes for those patients.

In conclusion, abnormal liver function was common and associated with adverse clinical outcomes in COVID-19 patients. Thus, clinicians should keep close monitoring of liver biochemistries and cautiously use appropriate medications with least hepatotoxicity in such patients. Due to the nature of such retrospective study, these results should be interpreted with caution and are needed to be confirmed in future large prospective studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Health Commission of China and the institutional review board at Huoshenshan Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Study concept and design: YL and HX, acquisition of data: YL, XZ, YW, JZ, CM, XF, YM, YZ, LY, GH, and HX, analysis and interpretation of data, drafting of the manuscript, and statistical analysis: YL, critical revision of the manuscript for important intellectual content: HX and GH. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported in part by grants from Boost program of Xijing Hospital (XJZT19ML15), Clinical Applied Research Subject of Military Medicine (XJGX15Y0) for HX, Boost Program of Xijing Hospital (XJZT18H02) for GH, and China Postdoctoral Science Foundation (2019TQ0134) for YL.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.639855/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 11 August 2021
doi: 10.3389/fmed.2021.644751

Gut Microbiota Dysbiosis Is a Crucial Player for the Poor Outcomes for COVID-19 in Elderly, Diabetic and Hypertensive Patients

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A new infectious disease, named COVID-19, caused by the coronavirus associated to severe acute respiratory syndrome (SARS-CoV-2) has become pandemic in 2020. The three most common pre-existing comorbidities associated with COVID-19-related death are elderly, diabetic, and hypertensive people. A common factor among these risk groups for the outcome of death in patients infected with SARS-CoV-2 is dysbiosis, with an increase in the proportion of bacteria with a pro-inflammatory profile. Due to this dysbiosis, elderly, diabetic, and hypertensive people present a higher propensity to mount an inflammatory environment in the gut with poor immune editing, culminating in a weakness of the intestinal permeability barrier and high bacterial product translocation to the bloodstream. This scenario culminates in a low-grade, persistent, and systemic inflammation. In this context, we propose here that high circulating levels of bacterial products, like lipopolysaccharide (LPS), can potentiate the SARS-CoV-2-induced cytokines, including IL-6, being crucial for development of the cytokine storm in the severe form of the disease. A better understanding on the possible correlation between gut dysbiosis and poor outcomes observed in elderly, diabetic, and hypertensive people can be useful for the development of new therapeutic strategies based on modulation of the gut microbiota.

Keywords: COVID19, aging, diabetes, gut microbiota, hypertension, SARS-CoV-2

OPEN ACCESS

Edited by:
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Reviewed by:
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Kyoto Prefectural University of Medicine, Japan
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Rey Juan Carlos University, Spain

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *21 December 2020*
Accepted: *20 July 2021*
Published: *11 August 2021*

Citation:
Magalhães NS, Savino W, Silva PMR, Martins MA and Carvalho VF (2021) Gut Microbiota Dysbiosis Is a Crucial Player for the Poor Outcomes for COVID-19 in Elderly, Diabetic and Hypertensive Patients. Front. Med. 8:644751. doi: 10.3389/fmed.2021.644751

INTRODUCTION

In early December 2019, a new infectious disease, caused by the coronavirus associated to severe acute respiratory syndrome (SARS-CoV-2), emerged in Wuhan, China (1). The disease caused by this infection, COVID-19, spread very rapidly in many other countries reaching pandemic proportions (2, 3). By 24 May 2021, there were 166,814,851 individuals diagnosed with COVID-19, including 3,458,905 fatal cases, as shown in the WHO data center (4). In severe COVID-19 patients, 93% of deaths result from respiratory failure caused by acute respiratory distress syndrome (ARDS). Besides, the storm of cytokines and symptoms of sepsis, with failure of some vital organs, including

heart and kidney, derived by the primary viral infection and/or secondary infections were observed in 70% of fatal cases (5). No specific effective therapeutics are so far available for COVID-19 and the management of the disease includes physical distancing, mask wearing, supportive medical care, and vaccines (4). Herein, we propose a role of gut dysbiosis in the worse prognosis of COVID-19 in elderly people and in patients with *Diabetes mellitus* (DM) or hypertension.

COVID-19 AND GUT MICROBIOTA

The human microbiota is made up of microorganisms, including bacteria, fungi, archaea, viruses, and protozoa, that colonize particular locations of the human body such as skin, as well as respiratory and gastrointestinal tracts (6, 7). The gut microbiota refers specifically to a complex bacterial community situated in the gastrointestinal tract (8). Although approximately 40% of patients infected with SARS-CoV-2 showed a high concentration of viral genetic material in the anal swab, and various patients reported nausea, vomiting, and diarrhea (9, 10) little has been so far discussed on the role of the gut in the pathophysiology of COVID-19, especially envisioning microbiota as being responsible for the greatest risk factor to develop the severe form of the disease.

It is well known that the membrane angiotensin I converting enzyme 2 (ACE2) is the pathway of entry into the target cells (11). Human mature enterocytes located in the small intestine express membrane ACE2, and SARS-CoV-2 is able to infect those cells in a process facilitated by TMPRSS2 and TMPRSS4 proteases (12). The infection of enterocytes with SARS-CoV-2 may promote a significant reduction of enteric ACE2 integrity/functionality. The decrease of ACE2 expression leads to an upregulation of other renin-angiotensin system components, including angiotensin (Ang) II (13). Remarkably, increased Ang II levels can modify gut microbial composition and metabolomics in a sex-specific manner (14). In addition, the SARS-CoV-2 infection-induced reduction of ACE2 function may also culminate in gut dysbiosis through a decrease in the mTOR-mediated synthesis of AMPs independently of RAS (15).

The possibility that SARS-CoV-2 infection of enterocytes modify gut microbiota is supported by the fact that some patients with COVID-19 present intestinal dysbiosis (16, 17). There is evidence that hospitalized COVID-19 patients exhibit a significant reduction in gut microbiome diversity with depletion of beneficial bacterial symbionts and enrichment of opportunistic pathogens, including *Actinomyces*, *Rothia*, and *Streptococcus* (17, 18). Patients infected with SARS-CoV-2 also showed a decrease in the relative abundance of *Faecalibacterium prausnitzii* and *Bifidobacterium bifidum*, which are bacteria responsible for the production of butyrate (17, 19). Butyrate is a short-chain fatty acid (SCFA) that influences both the proliferation and differentiation of epithelial intestinal cells, by enhancing the renewal and integrity of the epithelial barrier function (20). Moreover, patients undergoing allogeneic hematopoietic cell transplantation showing greater abundance of butyrate-producing bacteria have five-fold protection against the development of viral lower respiratory tract infection (21).

Interestingly, there are several pathologies in which the gut microbiota is modified and in some of them a direct relationship has been found with the severity of COVID-19, including elderly, diabetes, hypertension, obesity, periodontitis, and kidney diseases, as summarized in **Table 1**. Among these conditions, aging, diabetes, and hypertension stand out, since they are the main cause of COVID-19-related death (95–99). Yet, before getting into this point, it seemed worthwhile to discuss basic aspects of the gut microbiota, as well as the dysbiosis seen in aging and disease, particularly diabetes and hypertension.

AGING AND GUT MICROBIOTA

Aging is usually accompanied by a progressive decline of physiological functions determined by (epi) genetic, stochastic, and environmental processes (100). The elderly population has an increasing tendency to multimorbidity, fragility and disability. One of the biological systems most compromised by senility is the gastrointestinal tract (101). Along with aging, there is a degeneration of the enteric nervous system (ENS), alteration of intestinal motility, and changes in the intestinal mucous barrier, decreasing the defense function and favoring the development of gastrointestinal disorders (101, 102).

A mutual characteristic of aging in tissues and aging-related diseases is the *inflammaging*, which is the low-grade, persistent and systemic inflammation, even in the absence of infection, culminating in tissue degeneration and chronic diseases (101, 103). In addition, other hallmarks of immunosenescence are represented by a decrease in the capacity to respond to new antigens and the accumulation of memory T cells (103, 104). In aging, the gut dysbiosis leads, at least partly, to immune dysfunction, culminating in a more inflammatory environment with poor immune editing (29, 105). It is important to know that although the gut microbiota does not age its profile changes during aging. Furthermore, the maintenance of a "youthful" or "healthy" gut microbiota architecture throughout aging may postpone or limit immunosenescence (22).

During aging, the gut microbiota is characterized by an increase in the expression of proteolytic genes and a decrease in saccharolytic ones leading to the growth of pathogens, which in turn intensify inflammation (29). The most striking change in the microbiota of elderly individuals is the change in the relative proportions of Firmicutes and Bacteroidetes; the elderly having

Abbreviations: ACE2, angiotensin I converting enzyme 2; ALI, acute lung injury; AMP, anti-microbial peptides; ARDS, acute respiratory distress syndrome; BP, blood pressure; CD3, cluster of differentiation 3; COV, coronavirus; DAMP, danger-associated molecular pattern; DM, diabetes mellitus; ENS, enteric nervous system; GF, germ-free; HMGB1, high-mobility group box 1; IFN-γ; interferon gamma γ; IgA, immunoglobulin A; IL-10, interleukin 10; IL-17, interleukin 17; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-7, interleukin 7; IL-8, interleukin 8; LADA, Latent Autoimmune Diabetes in Adults; Lcp2, lymphocyte cytosolic protein 2; LPS, lipopolysaccharide; mRNA, messenger ribonucleic acid; OxPAPC, oxidized 1-palmitoyl-2-arachidonoyl-phosphatidylcholine; PBMC, peripheral blood mononuclear cells; SARS, severe acute respiratory syndrome; SCFA, short-chain fatty acids; SHR, hypertensive rats; TGF-β1, Transforming growth factor beta 1; Th17, T helper 17; TLR4, toll-like receptor 4; TMA, Trimethylamine; TNF, Tumor necrosis factor; WKY, Wistar Kyoto; WT, wild type.

TABLE 1 | Summary of the alterations in the gut microbiota, gut immune cells, blood and gut cytokine profiles in main groups at risk for COVID-19.

Condition	Species	Gut microbiota	Gut imune cells	Gut cytokines	Blood cytokines	Ref
Aging	Murine model	↑ *Prevotella sp.* ↓ *Lachnospiraceae* ↓ *Akkermansia sp.* ↓ *Lactobacillus sp.*	↓ Th1 ↑ Th17 ↑ Treg	↑ IL-4 ↓ IL-10 ↓TGF-β	↑ IL-1β ↓ IL-2 ↑ IL-6 ↑ IL-8 ↑ IL-13 ↑ IL-17 ↑ TNF	(22–28)
	Human	↑ *Clostridium difficile* ↑ *Enterobacter spp.* ↑ *Enterobacteriaceae.* ↑ *Eubacterium sp.* ↑ *Staphylococcus spp* ↑*Streptococcus spp.* ↓ *Akkermansia sp.* ↓ *Bifidobacterium sp.* ↓ *Faecalibacterium sp.* ↓ *Lactobacillus spp*	↓ Th17	↑ IL-6	↑ IL-1β ↓ IL-2 ↓ IL-4 ↑ IL-6 ↑ IL10 ↑ IL-17 ↑ IL-18 ↑ TGF-β ↑ TNFα	(23, 29–43)
Diabetes	Murine model	↓ *Faecalibacterium sp.* ↓ *Akkermansia muciniphila*	↓ Th2 ↑ Th17 ↓ Treg	↑ IL-10 ↓ IL-18 ↑ IL-17 ↑ IL-23	↑ IL-1β ↑ IL-6	(44–50)
	Human	↑ *Bacteroides* ↑ *Clostridium sp.* ↓ *Akkermansia muciniphila* ↓ *Eubacterium rectale* ↓ *Faecalibacterium sp.* ↓ *Roseburia sp.*	-	-	↑ IL-10 ↑ IL-17 ↓ IL-18 ↑ IL-23	(51–58)
Hypertension	Murine model	↑ *Prevotella* ↑ *Streptococcus spp.* ↓ *Lactobacillus spp* ↓ *Bifidobacterium sp.* ↓ *Roseburia*	↑ Th17	↑ IL-1β ↓ IL-6 ↓ IL-7 ↓TGF-β1 ↑ TNF-α	↑ IL-1β ↑ IL-6 ↑ IL8 ↑ IL-17 ↑ TNF-α	(59–67)
	Human	↑ *Klebsiella,* ↑ *Desulfovibrio* ↑ *Prevotella* ↓ *Blautia,* ↓ *Butyrivibrio* ↓ *Clostridium* ↓ *Enterococcus* ↓ *Faecalibacterium* ↓ *Oscillbacter* ↓ *Roseburia* ↓*Bifidobacterium* ↓*Lactobacillus*	-	-	↑ IL-6 ↑ TNF	(59, 60, 66, 68)
Obesity	Murine model	↑ *Mollicutes* ↓ *Akkermansia muciniphila* ↓ *Bacteroides* ↓ *Bacteroides thetaiotaomicron* ↓ *Bifidobacterium* ↓ *Enterobacteriale* ↓ *Lactobacillus* ↓ *Prevotella*	↑ Th1 ↑ Th17 ↓ Treg	↑ IL-1β ↓ IL-10 ↓ IL-17 ↑ IL-18 ↓ IL-22 ↑ TNFα	↑ IL-1β ↑ IL-6 ↑ TNF α	(69–72)
	Human	↑ *Clostridium sp.* ↑ *Eubacterium* ↓ *Bifidobacteria* ↓ *Faecalibacterium sp.* ↓ *Bacteroides* ↓ *Lactobacillus sp.* ↓ *Akkermansia muciniphila*	↑ Th1 ↓ Treg	- ↑ IFN-γ ↑ TNF-α	↑ IL-1 ↑ IL-5 ↑ IL-6 ↑ IL-10 ↑ IL-12 ↑ IL-13 ↑ IL-23 ↑ IL-36	(69, 72–77)

(Continued)

TABLE 1 | Continued

Condition	Species	Gut microbiota	Gut imune cells	Gut cytokines	Blood cytokines	Ref
Periodontitis	Murine model	↑ *Bacteroidetes* ↑ *Prevotella* ↓ *Lactobacillus spp*	↑ IL-1β ↑ Th17	↑ IL-1β ↑ IL-6 ↑ IL-12b ↑ IL-17c ↑ TNFα ↑ TGF-β	↑ IL-1β ↑ IL-6 ↑ TNFα	(78–82)
	Human	↑ *Enterobacteriaceae* ↑*Eubacteriaceae* ↓ *Faecalibacterium sp.*	↑ Th17	↑ IL-17 ↑ IFNγ	↑ IL-1 ↑ IL-6 ↑ IL-17 ↑ IL-22 ↑ INFγ ↑ TNFα	(81, 83, 84)
Kidney Disease	Murine model	↑ *Bifidobacterium* ↓ *Lactobacillaceae* ↓ Prevotellaceae	-	↑ IL-1β ↑ IL-6 ↑ IL-12b ↑ IL-17a ↑ TNFα ↑ IFNγ	↑ IL-1β ↑ IL-5 ↑ IL-6 ↑ IL-10 ↑ IL-12 ↑ IFNγ ↑ TNFα	(85–89)
	Human	↑ *Clostridium* ↑ *Enterobacteriaceae* ↑ *Streptococcaceae* ↑ *Streptococcus* ↓ *Roseburia* ↓ *Faecalibacterium sp.* ↓ *Lactobacillus* ↓ *Prevotellaceae*	-	-	↑ IL-1β ↑ IL-6 ↑ TNFα	(90–94)

a higher proportion of Bacteroidetes, while in young adults the Firmicutes prevail (30). Moreover, the production of anti-inflammatory factors by the microbiota of elderly individuals is reduced, including butyrate (29). All these alterations observed in the gut microbiota during aging enhance a more pro-inflammatory environment, contributing to *inflammaging*.

Aging-associated gut dysbiosis induces a weakening of the intestinal barrier (102). Therefore, it is possible to observe high levels of bacterial products in the bloodstream such as LPS (31, 103), which could lead to an increase in the production of pro-inflammatory mediators. Indeed, elderly people have a rise in the amount of circulating cytokines as well as a decrease in the lymphocyte response, natural killer cells, and phagocytic activity (32, 103). Furthermore, aging animals have increased inflammatory cytokines in the plasma and an augmentation in the intestinal permeability compared to young animals (33). This pro-inflammatory status seems to be related causally to the microbiota profile, since aged GF animals do not present *inflammaging* status. In addition, when both aged and young GF animals received the microbiota from aging wild type (WT) animals, they exhibited an increase in the circulating contents of inflammatory cytokines and intestinal permeability. Aging animals also showed an increase in the LPS-induced inflammatory cell infiltration and IL-6 levels compared to young animals, indicating the development of ARDS that is one of the most prevalent morbidities associated with aging. Nevertheless, old GF mice presented less LPS-evoked inflammatory infiltrates in the lungs compared to WT animals (33). Therefore, the microbiota of aging animals is important to the development of *inflammaging*.

DIABETES AND GUT MICROBIOTA

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia. Usually, DM is classified as type 1 and type 2 and related to low production and failure of insulin action, respectively (106). Nevertheless, this simple subdivision is not accurate, because it does not take into account the intermediate forms of DM with overlapping features. The "double diabetes" or type 1.5 diabetes is a disease with metabolic characteristics of type 2 DM with autoantibodies for β-cells typical of type 1 DM (107). Another intermediate form of DM is the Latent Autoimmune Diabetes in Adults (LADA), which shares autoimmune destruction of β-cells and insulin resistance, although to a lesser extent than type 1 DM (108). The hyperglycemia noted in diabetic patients is accompanied by the presence of cytokines such as IL-1β, IL-6, and TNF-α, characterizing a low-grade inflammation status (109).

A common change in all types of DM patients is the dysbiosis (110, 111). Although there is a controversy about which bacterial phyla is altered in the gut microbiota of diabetic patients, it is a consensus that the relationship between Firmicutes and Bacteroidetes is unbalanced in these patients (51, 112, 113). Besides, diabetic animals treated with probiotics

containing the *Lactobacillus rhamnosus* NCDC17 improved the parameters regarding oral glucose tolerance test and led to an increase in plasma insulin, together with decreased the inflammatory cytokines IL-6 and TNF in the epididymal fat (114). Therefore, the absence or excessive proliferation of some bacteria could be one of the mechanisms of intestinal barrier dysfunction observed in diabetic models, leading to increased permeability of bacterial content to the bloodstream, as LPS (110). Replacement with *Faecalibacterium sp.* in diabetic animals improved the intestinal barrier integrity and circulating LPS levels (115).

Interestingly, the gut microbiota of non-obese diabetic mice changed before the onset of diabetes (52). Alterations observed included reduction of bacteria abundance and diversity, and one of the most affected groups was the butyrate-producing bacteria (53). Butyrate regulates the permeability of the intestinal barrier by inducing mucin production and decreasing the transit of bacteria, oxidative stress, as well as local and systemic inflammation (54). Accordingly, the increased permeability of the intestinal barrier observed in diabetic patients can be attributed, at least partly, to the reduction of butyrate-producing bacteria (55). Thus, it is plausible to think that butyrate replacement in diabetic patients, through direct administration or ingestion of prebiotics, may reduce intestinal permeability and low-grade inflammation triggered by gut microbiota products translocated into the bloodstream.

HYPERTENSION AND GUT MICROBIOTA

Hypertension is a progressive cardiovascular syndrome whose early markers are usually present even before the sustained increase of blood pressure (BP). The progression of hypertension may be represented as stages 1, 2, and 3. In stage 1, patients present occasional or intermittent BP elevations, early cardiovascular disease, and no target organ disease. In stage 2, patients exhibit sustained BP elevations or progressive cardiovascular disease and early signs of target organ disease. In stage 3, the patients show marked and sustained BP elevations or advanced cardiovascular disease and overtly present target organ disease (116). Unfortunately, despite advances in awareness about lifestyle improvements, new therapies, and intensive medical interventions, around a third of hypertensive patients do not obtain control of BP when prescribed three or more antihypertensive drugs, presenting the so-called "treatment-resistant" hypertension (59).

Although the etiology of hypertension seems to depend on both genetic and environmental factors, the exact cause remains unknown. Several pieces of evidence suggest that hypertension can result from intestinal dysbiosis. For instance, treatment with antibiotics produces an increase in BP, indicating the participation of gut microbiota in the control of BP (60). Furthermore, GF mice showed lower BP as compared to conventional ones and present attenuation of BP increase in response to infused angiotensin II (61). Also, metabolites of gut microbiota are involved in the control of BP, including trimethylamine N-oxide, hydrogen sulfate, and SCFAs (117).

Causative evidence for the role of gut dysbiosis in the genesis of hypertension came since transfection of dysbiotic fecal samples from hypertensive patients to GF mice raised BP in the recipients (22). A study carried out in pre-hypertensive and hypertensive patients detected a lower richness and diversity of the intestinal microbiota as compared to healthy individuals. Hypertensive patients presented an increase of gram-negative groups and an elevation of the ratio between Firmicutes and Bacteroidetes (22, 34, 35).

Gut microbiota and their metabolites reduce the epithelium barrier integrity during hypertension, and this is linked to the downregulation of tight junction protein expression (118, 119). Hypertensive rats also presented a higher intestinal permeability to trimethylamine (TMA), a microbiota metabolite precursor of trimethylamine N-oxide, which is a marker of cardiovascular mortality. Furthermore, spontaneously hypertensive rats (SHR) showed suppression of components of T cell receptor signaling cascade in the colonic epithelium compared to Wistar Kyoto (WKY) normotensive rats, including glycoprotein CD3 gamma chain and lymphocyte cytosolic protein 2 (Lcp2). SHR animals also presented a decrease in the expression of IL-6, IL-7, and TGF-β1 in the colonic epithelium, related to marked lower production of alkaline phosphatase in the intestinal epithelial cells (120). Together, these alterations in the colonic epithelium of SHRs characterize changes in the gut immune response and epithelial layer in hypertension.

It is well known that one of the major triggers of hypertension is the imbalanced diet with high salt content (121, 122). Such high salt environment induces Th17 cells (62, 123), which are pro-inflammatory; being also involved with the development of hypertension (63, 68). Mice and humans exposed to a high salt challenge showed depletion of *Lactobacillus spp.* in the gut microbiome along with the rise of Th17 cells and BP (35), indicating an association of Th17 cells produced by gut microbiota and the generation of hypertension. Of note, an increase in pro-inflammatory cytokines was also reported in hypertensive rats (64). In particular, IL-6 is a central cytokine in the regulation of BP, since it is responsive to angiotensin II to raise BP regardless of baseline values (65). Furthermore, a study carried out in hypertensive patients found an increase in pro-inflammatory cytokines in peripheral blood samples associated with changes in the profile of intestinal microbiota (124).

CAN GUT MICROBIOTA DYSBIOSIS BE IMPORTANT TO SARS-CoV-2-INDUCED IMMUNE HYPERRESPONSIVENESS AND SARS DEVELOPMENT IN ELDERLY, DIABETIC, AND HYPERTENSIVE INDIVIDUALS?

The main groups at risk for the COVID-19-related death are aging, DM, and hypertension. These conditions have a key

point in common, which is dysbiosis that results in high intestinal permeability, translocation of bacterial contents to the bloodstream, and the development of basal inflammation. Therefore, a central question arises from this observation: can dysbiosis and the consequent pro-inflammatory status be critical for development of COVID-19 severity in aging, DM, and hypertensive individuals, similar to SARS and hyper-immune response also referred as a cytokine storm? Likely yes is the answer.

Some TLR4-activated danger-associated molecular pattern (DAMP) signals, including oxidized 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (OxPAPC) and high-mobility group box 1 (HMGB1), are increased in the acute lung injury (ALI) caused by respiratory viruses such as the influenza virus (125, 126). It is important to note that influenza-triggered ALI seems to occur secondary to the cytokine storm induced by the activation of TLR4 by host-derived DAMPs such as OxPAPC and HMGB1 (125, 127). Notably, $TLR4^{-/-}$ mice have been protected against influenza A virus-provoked lethality, and the therapeutic treatment with TLR4 antagonists, Eritoran and FP7, inhibited influenza virus-induced cytokine production, ALI, and mortality in wild-type mice (127–129).

Interestingly, low doses of LPS exacerbate the TLR3 activation-induced inflammatory response in human monocytes *in vitro* (130). Furthermore, macrophages infected with Influenza A and stimulated with low concentrations of LPS showed increased levels of cytokines compared to macrophages that were infected only with the virus. The authors proposed that LPS enhances the release of bioactive cytokines by infected macrophages, which can lead to a decompensated increase in inflammatory metabolites (131, 132). These data reinforce the idea that weakness of intestinal permeability and consequent translocation of LPS in the elderly, diabetic and hypertensive individuals can be relevant to the severity of COVID-19 in these populations.

In a clinical setting involving 48 subjects, the expression of TLR4 and its downstream signaling molecules as well as S100A9 (TLR4 ligand) were significantly upregulated in PBMCs from severe COVID-19 patients as compared to those from healthy controls. Furthermore, S100A9 amplified the recombinant S2 protein of SARS-CoV-2-induced IL-1β mRNA expression in PBMCs *in vitro* (133), suggesting that activation of TLR4 by LPS from the gut microbiota of elderly, diabetic, and hypertensive individuals may be related to the severity of COVID-19. In keeping with these results, respiratory syncytial virus infection induced an increase of TLR4 expression in the airway epithelial cells *in vitro*, and activation of these cells with LPS potentiated the release of IL-6 and IL-8 induced by the virus (134).

Since severe COVID-19 patients show high expression of TLR4 in PBMCs (133), we can speculate that the activation of

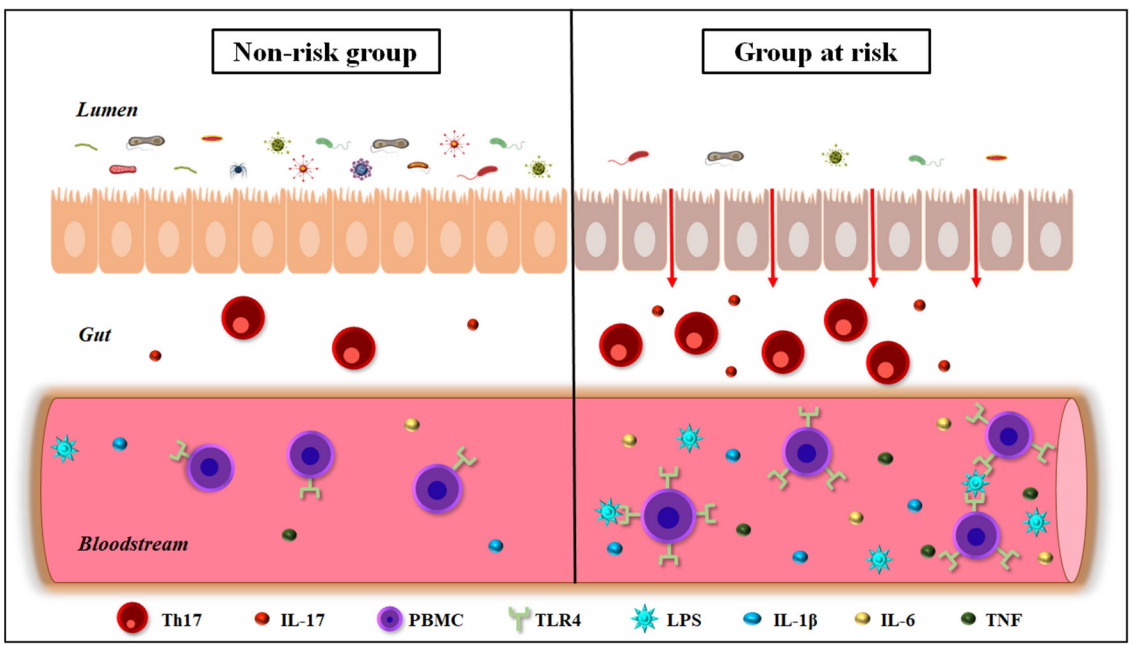

FIGURE 1 | Gut-immune interactions in elderly, diabetic, and hypertensive individuals. These conditions are the three most COVID-19-related death risk factors, and show a decrease in the diversity of the gut microbiota, leading to dysbiosis and weakness of the intestinal barrier permeability. In addition, people belonging to risk groups for COVID-19-related death show hyperimmune activation in the intestine, increasing $Th17^{+}$ T cells and IL-17 production. These individuals also exhibit a rise in the circulating levels of bacterial endotoxins such as LPS, as well as pro-inflammatory cytokines, as IL-1β, IL-6, and TNF. Furthermore, the elderly, diabetic, and hypertensive individuals show an increase in the expression of TLR4 in peripheral blood mononuclear cells (PBMCs). IL-17, interleukin-17; IL-1 β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; Th17, T helper 17; TLR4, Toll-like receptor 4; TNF, Tumor necrosis factor.

this receptor by LPS derived from the gut microbiota of elderly, diabetic, and hypertensive individuals would also potentiate the production of IL-6 induced by SARS-CoV-2. In this respect, it should be pointed out that, among all increased cytokines, the rise of IL-6 circulating levels predicted mechanical ventilation, intensive care unit admission, shock, and death in severe patients with COVID-19 (18, 135, 136). Furthermore, a follow-up with 21 individuals with several or critical COVID-19 revealed that a single dose of tocilizumab, an anti-IL-6 receptor drug, recovered 90% of patients (137).

CONCLUSION

In conclusion, we postulate that the gut dysbiosis may be responsible for COVID-19-related death in elderly individuals as well as diabetic and hypertensive patients, since these subjects show a change in the profile of gut microbiota followed by low-grade inflammation, especially with high circulating levels of IL-6. The possibility does exist that augmentation of pro-inflammatory bacteria in the gut may alter the intestinal immune repertoire with consequent weakness of epithelium-intestinal permeability and increased LPS translocation into the bloodstream. We believe that the hyperactivation of TLR4 induced by gut microbiota products, translocated into the circulation, strongly contributes to the cytokine storm, worsening the prognosis of COVID-19 in the elderly, diabetic and hypertensive individuals (**Figure 1**). In this respect, new therapeutic strategies based on prebiotics or bacterial metabolites, as butyrate, appear as potentially practical approaches for adjuvant treatment of these patients.

AUTHOR CONTRIBUTIONS

NM contributed to the conception and design of the study, wrote the manuscript, discussed the content and contributed to the manuscript revision. PS, MM, and WS discussed the content and contributed to the manuscript revision. VC contributed to the conception and design of the study, wrote the manuscript, discussed the content and contributed to the manuscript revision. All authors reviewed and/or edited the manuscript prior submission.

FUNDING

This work was supported by Oswaldo Cruz Institute, Oswaldo Cruz Foundation (Fiocruz), Ministry of Health, Brazil.

ACKNOWLEDGMENTS

We would like to thank the PrInt Fiocruz-CAPES Program; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Programa Fiocruz de Fomento a Inovação (INOVA-FIOCRUZ) and Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ); Mercosur Fund for Structural Convergence (FOCEM/Mercosur) for the financial support. This work was developed in the context of the Brazilian National Institute of Science and Technology on Neuroimmunomodulation and the Rio de Janeiro Research Network on Neuroinflammation. This article is dedicated to Juliana de Meis, young researcher in the Laboratory on Thymus Research (Fiocruz, Rio de Janeiro), who passed way on July 16th 2021, due to COVID-19.

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frontiers in Medicine

ORIGINAL RESEARCH
published: 17 August 2021
doi: 10.3389/fmed.2021.663646

Elevated Pancreatic Enzymes in ICU Patients With COVID-19 in Wuhan, China: A Retrospective Study

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OPEN ACCESS

Edited by:
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Sichuan University, China

Reviewed by:
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Zhejiang University, China
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *03 February 2021*
Accepted: *23 July 2021*
Published: *17 August 2021*

Citation:
Ding P, Song B, Liu X, Fang X, Cai H, Zhang D and Zheng X (2021) Elevated Pancreatic Enzymes in ICU Patients With COVID-19 in Wuhan, China: A Retrospective Study. Front. Med. 8:663646. doi: 10.3389/fmed.2021.663646

Background: Pancreatic enzyme elevation has been reported in patients with COVID-19 during the pandemic. However, with the shortage of medical resources and information, several challenges are faced in the examination and treatment of this condition in COVID-19 patients. There is little information on whether such condition is caused by pancreatic injury, and if this is a warning sign of life threatening complications like multiple organ failure in patients. The objective of this study is to explore the relationship between elevated pancreatic enzymes and the underlying risk factors during the management of COVID-19 patients.

Method: A total of 55 COVID-19 patients admitted to the intensive care unit (ICU) of Wuhan Jinyintan hospital from January 1 to March 30, 2020 were enrolled in this study. All participants underwent transabdominal ultrasound imaging to assess their pancreas.

Results: Out of the 55 patients, three patients had pancreatitis, 29 (52.7%) with elevated pancreatic enzymes, and 23 (41.8%) without. The most common symptoms of patients with COVID-19 were fever and cough. There was no statistical difference in most baseline characteristics except myalgia on admission. Compared with those having normal enzyme levels, patients with elevated pancreatic enzymes had higher rates of mortality (79.3 vs. 52.2%; $P = 0.038$), and lower rates of discharge (20.7 vs. 47.8%; $P = 0.038$). Patients with elevated enzymes had higher incidence of mechanical ventilation ($P = 0.004$) and kidney injury ($P = 0.042$) than patients without elevated pancreatic enzymes. The results of multivariable logistic analysis showed that the odds ratio were 10.202 ($P = 0.002$) for mechanical ventilation and 7.673 ($P = 0.014$) for kidney injury with the elevated enzymes vs. the normal conditions.

Conclusions: The findings show that the incidences of pancreatic enzymes elevation are not low in critical COVID-19 patients and only a few of them progressed to acute pancreatitis (AP). Increased pancreatic enzymes levels is associated with poor prognosis in COVID-19 patients. In addition, the kidney injury and oxygenation degradation are associated with the pancreatic enzymes elevation in COVID-19 patients.

Keywords: pancreatic enzymes, amylase, lipase, pancreatitis, COVID-19

INTRODUCTION

There was reported outbreak of a typical pneumonia-like respiratory disease in Wuhan, Hubei, China, that quickly spread all over the country and the world. The outbreak was described as a pandemic on March 11, 2020 by the World Health Organization. Through deep sequencing of respiratory specimens, it was later confirmed as an acute respiratory infectious disease caused by a novel coronavirus 2019 (SARS-coronavirus 2) (1). SARS-coronavirus 2 (SARS-CoV-2) belong to the β coronavirus genes, similar to the severe acute respiratory syndrome coronavirus (SARS-COV) and Middle East respiratory syndrome coronavirus (MERS-COV). Similar to SARS-COV and MERS-COV, SARS-CoV-2 also enters the human body cells through spike protein to combine with the angiotensin-converting enzyme-2 (ACE-2) receptor (2–4).

Both SARS-CoV-2 and SRAS-COV have spike proteins sharing a high degree of homology in sequences and a number of amino acids (5, 6). However, their genetic characteristic is different in some aspects and their nucleic acid homology is <80% (2). The SARS-CoV-2 has a higher rate of spreading from one person to another than SRAS-COV. According to previous studies, it is suspected that SARS-CoV-2 has higher and more efficient ability to identify human ACE2 receptor than SARS-COV. It binds with ACE2 receptors more strongly and this facilitates its quick entry to human cells (7). Human alveolar epithelial type-II cells express abundant ACE-2 receptors to facilitate the virus enter the lung. This makes the lung to be the most vulnerable target organ to the virus (8, 9).

The ACE2 is not only abundantly expressed in lung and small intestine tissues but also in endothelial cells and smooth muscle cells of almost all human organs (10). In 2003, the infectious pneumonia SARS-COV virus was found in several human organs including lung, kidney, intestine, and pancreas (10). Irina et al. demonstrated the prominent expression of ACE-2 in the pancreatic ductal and microvascular epithelium. This makes the tissues to be a more potential targets of the coronavirus and subsequent pancreatic injury (11). Amer-Hadi et al. reported the presentation of acute pancreatitis as a complication caused by SARS-CoV-2 in two of the three members of the same family with the coronavirus disease 2019 (COVID-19) (12).

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2; SARS-COV, severe acute respiratory syndrome-related coronavirus; MERS-COV, Middle East respiratory syndrome-related coronavirus; ACE-2, angiotensin-converting enzyme-2; ICU, intensive care unit; ULN, upper limit of normal; RT-PCR, real-time reverse transcription-polymerase chain reaction; KDIGO, Kidney Disease: Improving Global Outcomes; CT, computed tomography; MRI, magnetic resonance imaging; NLR, rate of neutrophils and lymphatic; PLR, rate of platelets and lymphatic; CRP, C-reactive protein; PCT, procalcitonin; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; γ-GT, γ-glutamyltranspeptidase; Cr, creatinine; LDH, dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase-MB; TnT, troponin T; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; CRRT, continuous renal replacement therapy; SD, standard deviation; Cmax, maximum concentration; Max, maximum; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; B, regression coefficient; OR, odds ratio; CI, confidence interval.

Two studies (13, 14) have reported different cases of the COVID-19 which developed into severe acute pancreatitis (AP). Interestingly, several patients had extremely high lipase levels but not diagnosed with pancreatitis. This was confirmed by abdominal imaging in our ICU clinical work during the COVID-19 epidemic in Wuhan, China. The interesting phenomenon in these studies have been puzzling: Did it also occurred on other COVID-19 patients? Was the incidence high or just casual? Was this a warning sign of multiple organ failure? Were there several risk factors for the pancreatic enzymes elevation?

There are several research studies on the complication of elevated pancreatic enzymes in ICU COVID-19 patients. Wang et al., *for example*, reviewed lipase levels and described the incidence of pancreas injuries in 52 patients with COVID-19 (15). However, they did not perform abdominal imaging on the patients that would be important for pancreas assessment. They also did not analyze the possible risk factors of the pancreatic enzymes elevation. To understand the relationship between the SARS-CoV-2 and the clinical phenomenon of elevated pancreatic enzymes, this study reviewed relevant clinical data to explore the phenomenon and the possible risk factors behind it.

METHOD

Data Collection

In our retrospective research, the inclusion criteria were all critical patients (age ≥18 years) with positive SARS-CoV-2 and in the intensive care unit (ICU) of Wuhan Jinyintan hospital from January 1 to March 30, 2020 ($n = 328$). Detailed laboratory data of 290 patients was available. Pancreatic lipase (normal range between 8 and 78 U/L) or amylase (normal range between 35 and 135 U/L) were tested in 277 patients. Transabdominal ultrasound imaging was conducted on 55 patients (**Figure 1**). This research study was approved by the Medical ethics committee of the Wuhan Jinyintan hospital and the patients were followed up to discharge or death.

Research Object

The respiratory tract or blood samples from patients were tested positive for the new coronavirus nucleic acid by real-time reverse transcription-polymerase chain reaction (RT-PCR), or specimen viral gene sequencing was highly homologous to the known new coronavirus (16). The seventh edition (17) of the COVID-19 diagnosis and treatment plan was used to classify the clinical severity of the new coronavirus pneumonia. The specific classification criteria were as follows: ordinary type (with fever, respiratory symptoms, and pneumonia manifestations on imaging); severe type [met any of the following: (1) breathing rate ≥30/min; (2) oxygen saturation ≤93% at rest; (3) oxygenation index ≤300 mmHg; (4) lung imaging shows that the lesion has progressed significantly more than 50%]; critical type [met any of the following: (1) respiratory of failure requires mechanical ventilation; (2) shock; (3) other organ failure requires ICU monitoring and treatment]. According to the revised Atlanta Classification, pancreatitis was defined as at least 2 of the following 3 items: (1) abdominal pain; (2) serum lipase and/or amylase at least 3 times more than the upper limit

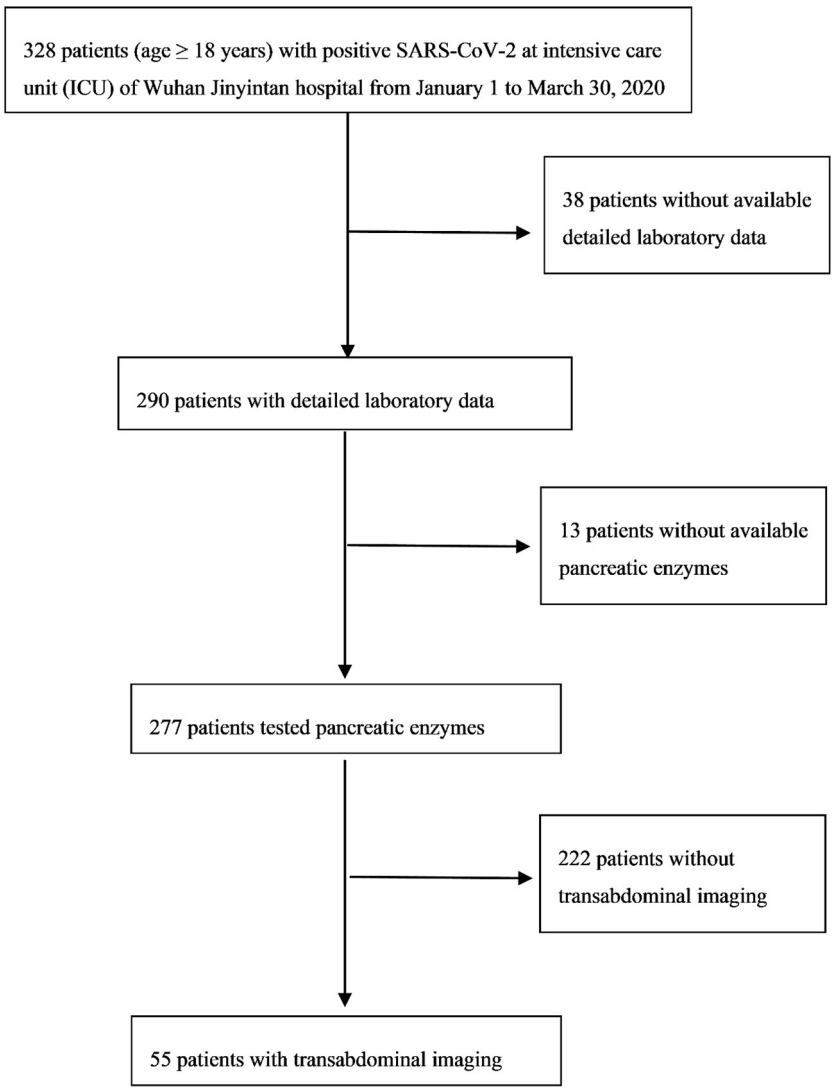

FIGURE 1 | Recruitment flowchart.

of normal (>3×ULN); (3) imaging characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CT), transabdominal ultrasonography, or magnetic resonance imaging (MRI) (18). Kidney injury was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) (19). Shock was defined as life-threatening acute circulatory failure accompanied by inadequate cellular oxygen utilization (20, 21). It was noted that the oxygenation deterioration and the judgment of whether to change the mode of oxygen therapy or the need for invasive endotracheal intubation were at the discretion of the ICU clinicians.

Statistical Method

The continuous data of this study were described as mean ± S.E.M (standard error of mean), whereas the categorical data were described as percentages. Comparisons of the categorical data were appropriately conducted using Chi-square tests or Fisher's exact tests. Single-factor analysis of variance or Kruskal-Wallis H tests were performed for the appropriate comparisons of the continuous data. Univariate logistic analysis and multivariate logistic analysis were used to quantify the associations between pancreatic enzymes elevation with relevant risk factors. The significant difference was reported at $P < 0.05$.

RESULTS

Baseline Clinical Characters

A total of 55 patients were enrolled in the study. Out of the 55 critically ill COVID-19 patients, there were three patients with pancreatitis, 29 with elevated pancreatic enzymes, and 23 within the normal range of pancreatic enzymes. The enrolled patients were aged between 29 and 79 years. Eighteen (32.7%)

TABLE 1 | Clinical characteristics of patients with COVID-19.

Variable	Patients of COVID-19 with pancreatitis				Patients of COVID-19 with elevated pancreatic enzymes (*N* = 29)	Patients of COVID-19 without elevated enzymes (*N* = 23)	*P*
	P1	P2	P3	Overall			
Age (mean)	55	45	29	43 ± 13	63 ± 12	61 ± 11	0.18
Sex, male n (%)	N	Y	N	2(66.7)	18(62.1)	17(73.9)	0.72
Epidemiology	N	N	N	0(0)	0(0)	0(0)	–
Comorbidities, *n* (%)							
Hypertension	N	N	N	0(0)	13(44.8)	9(39.1)	0.41
Diabetes	N	N	N	0(0)	6(20.7)	4(17.4)	1
COPD	N	N	N	0(0)	0(0)	0(0)	–
Cardiac disease	Y	N	N	1(33.3)	2(6.9)	2(8.7)	0.37
Chronic Renal disease	N	N	N	0(0)	1(3.4)	2(8.7)	0.64
Carcinoma	N	N	N	0(0)	1(3.4)	3(13)	0.46
HCV	N	N	N	0(0)	0(0)	0(0)	–
HIV	N	N	N	0(0)	0(0)	0(0)	–
Hyperlipidemia	Y	N	N	1(33.3)	0(0)	0(0)	0.05

COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; HCV, hepatitis C virus. The enrolled patients were aged between 29 and 79 years. Eighteen (32.7%) were women, the median age was 63 years and the average age was 61 years.

TABLE 2 | Chief complaints of patients with COVID-19.

Variable	Patients of COVID-19 with pancreatitis				Patients of COVID-19 with elevated pancreatic enzymes (*N* = 29)	Patients of COVID-19 without elevated enzymes (*N* = 23)	*P*
	P1	P2	P3	Overall			
Chief complaints on admission, *n* (%)							
Fever	Y	Y	Y	3(100)	27(93.1)	20(87)	0.73
Cough	N	Y	Y	2(66.7)	21(72.4)	19(82.6)	0.50
Expectoration	N	Y	Y	2(66.7)	10(34.5)	12(52.2)	0.33
Fatigue	N	N	N	0(0)	15(51.7)	9(39.1)	0.24
Nausea	N	N	N	0(0)	2(6.9)	2(8.7)	1
Vomit	N	N	N	0(0)	1(3.4)	0(0)	1
Diarrhea	N	N	N	0(0)	2(6.9)	0(0)	0.55
Stomachache	N	N	N	0(0)	0(0)	0(0)	–
Myalgia	Y	N	Y	2(66.7)	3(10.3)	1(4.3)	0.02
Headache	N	Y	N	1(33.3)	1(3.4)	0(0)	0.11
Previous history							
Smoking history	N	N	N	0(0)	2(6.9)	7(30.4)	0.09
Drinking history	N	N	N	0(0)	1(3.4)	4(17.4)	0.28

were women, the median age was 63 years and the average age was 61 years. The age of the three patients with pancreatitis was 55, 45, and 29 years (mean, 43 years). The average age of patients with and without elevated pancreatic enzymes was 63, 61 years, respectively (**Table 1**). It was observed that the most common clinical symptoms were fever and cough (**Table 2**). It was reported that among the patients with pancreatitis, one patient had hyperlipidemia before the study. None of the patients in all the three groups had stomachache. Comparative analysis show that the patients with elevated pancreatic enzymes had a higher incidence of diarrhea, myalgia, vomit, and previous history including hypertension and diabetes on admission than those without elevated enzymes. The patients in the three groups show significant differences in myalgia after admission ($P = 0.02$).

Baseline Laboratory Results

We collected laboratory test results of 55 COVID-19 patients on admission (**Table 3**). According to the results, the patients with pancreatitis had increased leukocytes, neutrophils, NLR, and PLR. Further, there were no significant differences in the inflammatory indicators, such as CRP, PCT, ESR, and IL-6, blood coagulation functions, as well as blood biochemistry among the 55 patients with COVID-19.

TABLE 3 | Laboratory results of patients with COVID-19.

Variable	Patients of COVID-19 with pancreatitis				Patients of COVID-19 with elevated Pancreatic enzymes ($N = 29$)	Patients of COVID-19 without elevated enzymes ($N = 23$)	*P*
	P1	P2	P3	Overall			
Blood cytology(10^9/L)							
Mean ± SD							
Leukocytes (3.5–9.5)	33.48	12.64	7.84	18.0 ± 13.6	11.0 ± 5.5	8.40 ± 3.6	0.09
Neutrophils (1.8–6.3)	32.55	11.89	7.23	17.2 ± 13.5	12.7 ± 15.3	7.30 ± 3.5	0.17
Lymphocyte (1.1–3.2)	0.4	0.44	0.47	0.44 ± 0.04	0.72 ± 0.6	0.70 ± 0.2	0.55
Platelets (125–350)	234	220	114	189.3 ± 65.6	201 ± 116.9	190.20 ± 77.3	0.92
NLR	81.4	27.0	15.4	41.3 ± 35.2	28.7 ± 40.5	12.40 ± 7.5	0.06
PLR	585	500	242.3	442.5 ± 178.3	437.8 ± 493.2	320.00 ± 182.0	0.53
Inflammatory indicators							
Mean ± SD							
CRP (0–5 mg/L)	122.5	160	43.8	108.8 ± 59.3	103.4 ± 53.5	95.3 ± 57.2	0.13
PCT (<0.5 ng/ml)	0.24	0.2	0.05	0.16 ± 0.1	0.81 ± 2.5	0.32 ± 0.55	0.66
ESR (0–20 mm/h)	37	115	65	72.3 ± 39.5	54.2 ± 22.0	62.3 ± 26.4	0.22
IL-6 (0–7)	13.7	12.8	9.2	11.9 ± 2.4	12.9 ± 8.3	10.10 ± 4.3	0.88
Blood biochemistry							
Mean ± SD							
ALB (40–55 G/L)	30.8	26.7	27	28.2 ± 2.3	30.9 ± 9.1	28.90 ± 4.8	0.59
ALT (7–40 U/L)	129	48	25	67.3 ± 54.6	66.00 ± 107.9	38.30 ± 40.2	0.49
AST (13–35 U/L)	65	35	32	44 ± 18.2	60.9 ± 50.3	41.70 ± 18.1	0.21
γ-GT (7–45 U/L)	159	112	50	107 ± 54.7	92.40 ± 84.4	89.70 ± 138.6	0.97
ALP (50–135 U/L)	108	122	44	91.3 ± 41.6	112.70 ± 56.0	104.3 ± 58.5	0.76
TB (0–21 umol/L)	12	24.6	9.8	15.5 ± 8.0	22.10 ± 18.7	13.20 ± 7.3	0.162
Cr (41–81 umol/L)	31.8	39	81.7	50.8 ± 27.0	145.40 ± 262.7	184.50 ± 276.5	0.68
LDH (120–250 U/L)	672	209	337	406 ± 239.1	697.10 ± 635.46	483.00 ± 213.6	0.24
CK (40–200 U/L)	123	258	35	138.7 ± 112.3	234.0690 ± 384.8	208 ± 185.1	0.86
CK-MB (0–24 U/L)	17	12	7	12 ± 5	19.60 ± 22.1	19.80 ± 15.0	0.79
TnT (0–28 Pg/ml)	127.8	7.7	5.2	46.9 ± 70.1	1418.9 ± 5599.1	76.00 ± 179.9	0.48
Coagulation functions							
Mean ± SD							
PT (10.5–13.5S)	10.1	13.4	9.5	11 ± 21	13.70 ± 5.3	17.70 ± 19.3	0.47
APTT (21–37S)	19.1	27.3	17.5	21.3 ± 5.3	30.30 ± 7.8	34.30 ± 18.4	0.23
INR (0.8–1.2)	0.87	1.18	0.82	0.96 ± 0.2	1.20 ± 0.6	1.43 ± 1.6	0.63
Fibrinogen (2–4 g/l)	4	7.2	1.1	4.1 ± 3.1	4.30 ± 1.8	5.10 ± 2.1	0.3

NLR, rate of neutrophils and lymphatic; PLR, rate of platelets and lymphatic; CRP, C-reactive protein; PCT, procalcitonin; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; γ-GT, γ-glutamyltranspeptidase; Cr, creatinine; LDH, dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase-MB; TnT, troponin T; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio.

Corticosteroid and immunoglobulin therapy was given to two of the three patients with pancreatitis. Less than half of the patients received corticosteroid and immunoglobulin therapy in the other two groups (**Table 4**). Almost all the patients received antibiotics because of the secondary infection in the ICU. It was noted that there were statistical differences in organ supports therapy (continuous renal replacement therapy $P = 0.003$ and mechanical ventilation $P = 0.002$) among the 55 patients with COVID-19.

Three Critical COVID-19 Patients With Pancreatitis

Among the 55 critically ill COVID-19 patients, three patients were diagnosed with acute pancreatitis. The trends of amylase and lipase in the three patients were plotted during hospitalization until discharge or death (**Figure 2**). The peak of amylase was 547 U/L in the first patient, 554 U/L in the second patient, 943 U/L in the third patient. The peak of lipase was 1,049 U/L in the first patient, 955 U/L in the second patient, >1,200 U/L in the third patient. The three patients with acute pancreatitis showed similar upward trends of amylase and lipase. The time to the peak of pancreatic enzymes was 11 days in the first patient, 17 days in the second patient, and 17 days in the third patient. Two of three patients died of severe multiple organ failure during hospitalization.

Pancreatic Enzymes Elevation Was Associated With Several Influence Factors in Our Study

The results found that 136 out of the 277 cases had pancreatic enzymes elevation (**Figure 1**). The incidence of mild elevation (1–3 ULN) was 39.1%, and >3×ULN was 10.9%. There were

TABLE 4 | Treatments of patients with COVID-19.

Variable	Patients of COVID-19 with pancreatitis				Patients of COVID-19 with elevated pancreatic enzymes ($N = 29$)	Patients of COVID-19 without elevated enzymes ($N = 23$)	*P*
	P1	P2	P3	Overall			
Hospital treatment, *n* (%)							
Corticosteroid	N	Y	Y	2(66.7)	14(48.3)	11(47.8)	1
Immunoglobulin	Y	N	Y	2(66.7)	10(34.5)	5(21.7)	0.23
Antibiotics	Y	Y	Y	3(100)	29(100)	23(100)	-
Mechanical Ventilation	N	Y	Y	3(100)	21(72.4)	7(30.4)	0.002
CRRT	N	Y	Y	2(66.7)	13(44.8)	2(8.7)	0.003

CRRT, continuous renal replacement therapy.

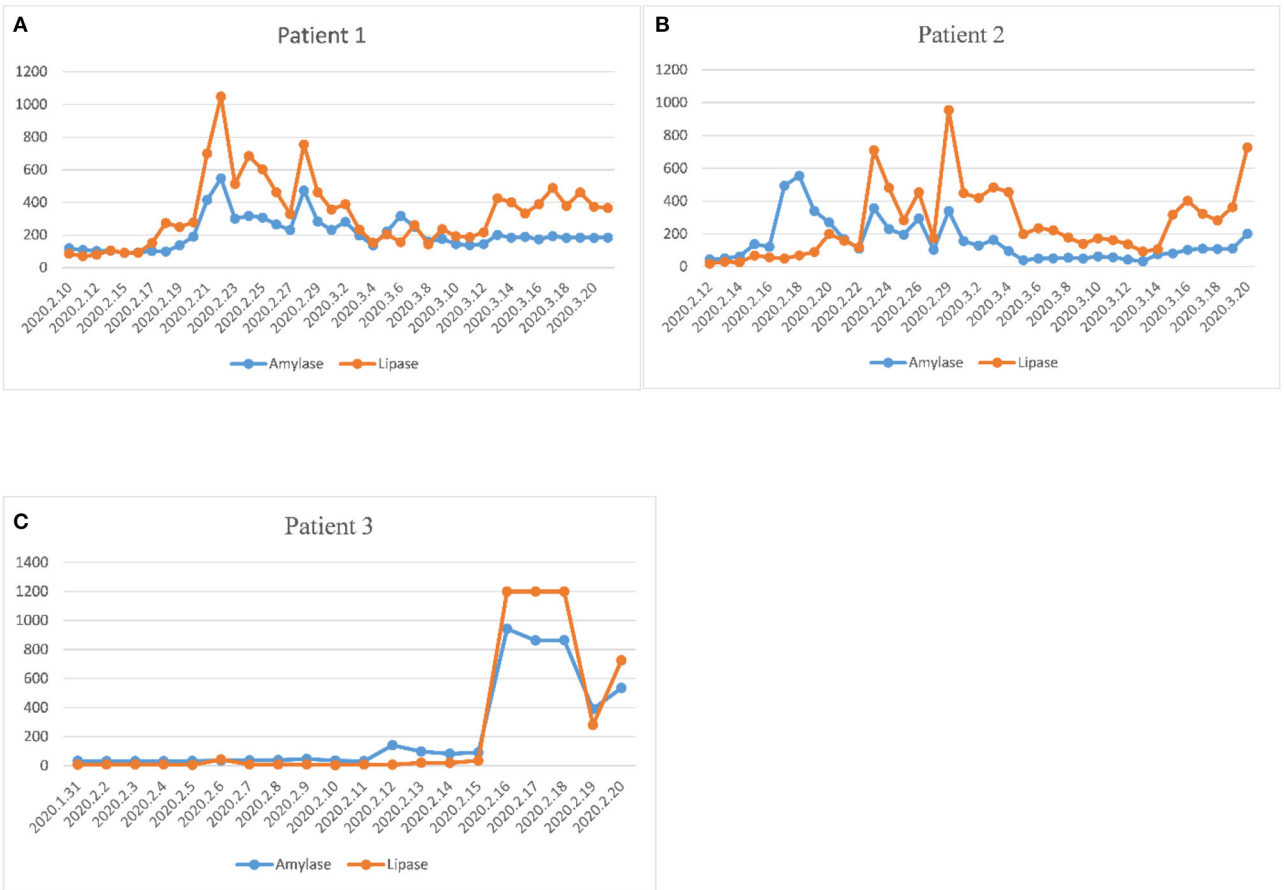

FIGURE 2 | Evolution of plasma amylase and lipase during hospitalization in three pancreatitis patients. **(A)** The dynamic change of pancreatic enzymes in the first patient. **(B)** The dynamic change of pancreatic enzymes in the second patient. **(C)** The dynamic change of pancreatic enzymes in the third patient. The three patients with acute pancreatitis showed similar upward trends of amylase and lipase. The time to the peak of pancreatic enzymes was 11 days in the first patient, 17 days in the second patient, and 17 days in the third patient.

32 patients with elevated pancreatic enzymes among the total recruited patients ($n = 55$). Twenty-nine patients with elevated pancreatic enzymes did not develop pancreatitis. This was confirmed by repeated transabdominal ultrasonography during hospitalization. Elevated pancreatic enzymes were seen in 58.2% of critically ill COVID-19 patients, and >3×ULN in 40%. The median time to the amylase and lipase peaks (>3×ULN) was 12 and 13 days, respectively. The peak value of amylase and lipase (>3×ULN) was 819.2 ± 334.5 U/L and 355.8 ± 169.5 U/L, respectively (**Table 5**).

The outcomes of patients with or without elevated pancreatic enzymes were shown as **Table 6**. Patients with elevated pancreatic

TABLE 5 | Elevated amylase or lipase of 29 patients with COVID-19.

Enzymes	Amylase			Lipase		
	Normal(10)	1–3 ULN(8)	>3 ULN(11)	Normal(1)	1–3 ULN(12)	>3 ULN(16)
Cmax (Mean ± SD)	92.3 ± 23.0	220.6 ± 39.3	819.2 ± 334.5	46	121.2 ± 30.0	355.8 ± 169.5
Median (U/L)	94.5	222.5	690	46	123	295
Time to Max (d)	6.2 ± 5.0	14.9 ± 12.3	15.0 ± 11.0	20	9.9 ± 12.0	17.5 ± 10.6
Median (d)	6.5	16.5	12	20	7.5	13

ULN, upper limit of normal; SD, standard deviation; Cmax, maximum concentration; Max, maximum. Twenty-nine patients with elevated pancreatic enzymes did not develop pancreatitis. Elevated pancreatic enzymes were seen in 58.2% of critically ill COVID-19 patients, and >3×ULN in 40%.

TABLE 6 | Association of elevated pancreatic enzymes with outcomes.

Outcomes	Patients with elevated ($n = 29$)	Patients without elevated ($n = 23$)	*P*
Death (*n*, %)	23(79.3)	12(52.2)	0.038
Discharge (*n*, %)	6(20.7)	11(47.8)	0.038

enzymes had higher rates of mortality (79.3 vs. 52.2%; $P = 0.038$), and lower rates of discharge (20.7 vs. 47.8%; $P = 0.038$) than the patients without elevated pancreatic enzymes.

Although abnormally high pancreatic enzymes (>3×ULN) are sensitive for the diagnosis of pancreatitis, there were several exceptions in our study. It is essential for clinicians to find the risk factors for increased pancreatic enzymes. This study analyzed the relevant possible influencing factors of patients during hospitalization. Patients with elevated pancreatic enzymes had a higher incidence of mechanical ventilation ($P = 0.004$) and kidney injury ($P = 0.042$) than patients without elevated pancreatic enzymes (**Table 7**). Multivariable logistic analysis show that pancreatic enzymes elevation was associated with mechanical ventilation (odds ratio = 10.202, $P = 0.002$) and acute kidney injury (odds ratio = 7.673, $P = 0.014$) (**Table 8**).

DISCUSSION

SRAS-Cov-2 uses ACE2 receptors to invade the human body tissue cells (2). The pancreas can be a target of SARS-CoV-2 virus because it also expresses the ACE2 receptors (11). Several reports have shown that pancreatitis is one of the serious possible complications of COVID-19 disease (12–14). Furthermore, pancreatic enzymes elevation in COVID-19 patients has been reported in recent studies. Julia et al. reported that 2 of 71 patients (2.8%) had lipase elevation of >3×ULN but none of the patients had acute pancreatitis (22). According to a study by Usman (23), 16.8% of patients have elevated levels of lipase enzyme (>3×ULN). However, the two studies did not assess pancreas injury and relevant risk factors of elevated enzymes in COVID-19 patients were also not addressed. The present study was aimed to show the baseline characteristics and investigate the association of enzymes elevation with the outcomes and relevant risk factors in the first Chinese patients reported with critical COVID-19 disease.

In our study, elevated pancreatic enzymes were seen in 58.2% of critical-ill COVID-19 patients whereas >3×ULN was reported in 40% of the patients. These results suggested that pancreatic enzyme elevation was common in critical COVID-19 patients. On the other hand, acute pancreatitis (AP) was rare. The development of pancreatitis is multifactorial consisting of susceptibility factors and associated injuries. The common causes of acute pancreatitis are alcohol, biliary obstruction, gall stones, and hypertriglyceridemia. It was found that one of the three patients with pancreatitis had gall stone and hypertriglyceridemia in our study. In the light of our clinical and review evidence, pancreatitis in the other two patients might be associated with the SARS-CoV-2 virus. Unfortunately, we did not carry out a postmortem to confirm if the SARS-CoV-2 virus actually existed in pancreas tissue. Therefore, further studies are needed to investigate the causes of AP in COVID-19.

A previous cohort study reported that COVID-19 patients with elevated pancreatic enzymes (>3×ULN) have higher rates of ICU admission and intubation as compared with lower lipase levels (23). However, the study also lacked abdominal imaging to evaluate the pancreatic injury as a source of elevated enzymes. A higher incidence of intubation was also found in our patients. There are some factors that affect the prognosis of the COVID-19 patients, for example, male gender, older age, chronic kidney disease (24), hypercoagulability, and thrombotic complications (25). What's more, our study found that the elevation of pancreatic enzymes in critically ill COVID-19 patients have higher rate of mortality and lower incidence of discharge. This indicates that pancreatic enzymes elevation is also associated with adverse outcomes.

Serum pancreatic enzymes elevations can occur in many conditions not accused by pancreatitis, such as obstruction in gastroenteritis (26, 27), post-cholangiopancreatography (28), diabetes (29), several related drugs (dipeptidyl peptidase-4 inhibitors, alcohol) (30, 31), infection (HCV, HIV) (32, 33), multi-trauma (especially with head injury, blunt abdominal or pelvic trauma, liver injury) (34), biliary or gastrointestinal tumor, hepatocellular cancer, bowel cancer with liver metastases, renal injury (35, 36), and some critical-ill patients with mechanical ventilation or shock in ICU (35, 36).

TABLE 7 | Univariate analysis of elevated pancreatic enzymes in COVID-19 patients ($n = 52$).

Influence factors	Group	Elevated ($n = 29$)	Without elevated ($n = 23$)	*P*
Age	≥60	19	15	0.174
	<60	10	8	
Sex	1	18	17	0.368
	0	11	6	
Smoking history	1	2	7	0.079
	0	27	16	
Drinking history	1	1	4	0.125
	0	28	19	
Hypertension	1	13	9	0.68
	0	16	14	
Diabetes	1	6	4	0.785
	0	23	19	
COPD	1	0	0	-
	0	29	23	
Chronic nephrosis	1	1	2	0.436
	0	28	21	
Carcinoma	1	1	3	0.228
	0	28	20	
Hyperlipidemia	1	0	0	-
	0	29	23	
HCV	1	0	0	-
	0	29	23	
HBV	1	0	0	-
	0	29	23	
Fatty liver	1	7	7	0.612
	0	22	16	
Gallstone	1	4	6	0.271
	0	25	17	
Cholestasis	1	6	1	0.119
	0	23	22	
Mechanical ventilation	1	21	7	0.004
	0	8	16	
Shock	1	5	1	0.180
	0	24	22	
Kidney injury	1	13	4	0.042
	0	16	19	

HBV, hepatitis B virus; HCV, hepatitis C virus.

To understand the relevant possible risk factors, we performed univariate analysis and multivariate analysis on the critical cases of COVID-19 with elevated pancreas enzymes and excluded pancreatic injury (**Tables 7**, **8**). There were associations among pancreatic enzymes elevation, mechanical ventilation ($P = 0.004$) and kidney injury ($P = 0.042$). Subsequently, a multivariable logistic regression model was fit for pancreatic enzymes elevation among these variables showed signicant differences. Multivariable analysis confirmed that pancreatic enzymes elevation was associated with mechanical ventilation (odds ratio = 10.202, $P = 0.002$) and kidney injury (odds ratio = 7.673, $P = 0.014$). Therefore, in critically ill COVID-19 patients, oxygenation degration and kidney injury may be associated with abnormal pancreatic enzymes levels.

Glomerular filtration is primarily responsible for the clearance of serum amylase and lipase (37). However, some research studies pointed out that there was no much correlation between the raised amylase with acute kidney injury (38–40). Otherwise, Chen et al. found that the incidence of amylase and lipase elevation more than the normal upper limits were 35.7 and 26.2% in chronic renal failure, respectively (41). The finding of this study also shows that renal failure may be one of the risk factors in the occurrence of pancreatic enzymes elevation.

Inflammation caused by immune-medicated β-cell may have destroyed and caused the spill out of pancreatic enzymes

TABLE 8 | Multivariate analysis of elevated pancreatic enzymes in COVID-19 patients ($n = 52$).

Variable	*B*	OR	95%CI	*P*
Mechanical ventilation	2.323	10.202	2.358–44.133	0.002
Kidney injury	2.038	7.673	1.521–38.714	0.014

B, regression coefficient; OR, odds ratio; CI, confidence interval.

through the exocrine pancreas in insulin-dependent diabetic conditions (42). This retrospective study showed that there was no association between preexisting diabetes and pancreatic enzymes elevation ($P = 0.785$). The same results were seen in other influencing factors including gallstone, fatty liver, cholestasis, related carcinoma, hypertriglyceridemia, HCV, and HBV infection (**Table 7**). Some scientific studies have pointed that elevations of pancreatic enzymes in ICU were related to septic shock and respiratory failure. Further, pancreatic hypoperfusion can also be responsible for enzymes elevation. Critical illness may cause the pancreatic enzymes in the gut to enter into the submucosa and subsequently to the circulation as gut ischemia (35). Data from our study also demonstrated that oxygenation deterioration was associated with the elevated pancreatic enzymes in COVID-19 patients.

The limitation of our study included a relatively small sample size. The subjects were the first to be reported with critical COVID-19 disease in China at the beginning of 2020. However, the disease has been properly controlled at the later stage of the pandemic. Therefore, the results of this study were the initial state of critical COVID-19 disease at that time. Due to the shortage of medical resources, abdominal imaging could not be performed on every patient with elevated pancreatic enzymes. Additionally, due to the shortage of medical information, the examination was at the discretion of the ICU clinicians based on the patients' specific illness. In addition, the mortality could have been overestimated because of the lack of proper medical resources and information on COVID-19 during the early stage of the epidemic. It is recommended that further large-scale studies should be carried out to investigate the meaning of elevated pancreatic enzymes in critically ill patients.

In conclusion, it was found that, although the incidence of pancreatic enzymes elevation was more in critically ill COVID-19 patients, only a few progressed to acute pancreatitis (AP). It was also noted that critically ill COVID-19 patients with increased pancreatic enzymes could have developed poor clinical outcomes. Further, renal injury and oxygenation degradation could be associated with the elevation of the pancreatic enzymes. Therefore, this study analyzed relevant clinical data and articles retrospectively to provide the clinicians with a more comprehensive understanding for better clinical decisions for COVID-19 patients with elevated pancreatic enzymes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the research ethics board of Wuhan Jinyintan Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

PD wrote the main manuscript text. PD and BS collected the data. XL, XF, and HC analyzed the data. XZ and DZ revised the manuscript and gave final approval for the version to be published. All authors had contributed to the research conception and designed for the study. All authors have read and approved the manuscript.

FUNDING

The work was supported by Scientific Research Fund of National Health Commission of China - Zhejiang Health Major Science and Technology Plan Project (WKJ-ZJ-2110).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

BRIEF RESEARCH REPORT
published: 20 August 2021
doi: 10.3389/fmed.2021.637885

Acid pH Increases SARS-CoV-2 Infection and the Risk of Death by COVID-19

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Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *04 December 2020*
Accepted: *26 July 2021*
Published: *20 August 2021*

Citation:
Jimenez L, Campos Codo A, Sampaio VdS, Oliveira AER, Ferreira LKK, Davanzo GG, Brito Monteiro Ld, Victor Virgilio-da-Silva J, Borba MGS, Fabiano de Souza G, Zini N, Andrade Gandolfi Fd, Muraro SP, Luiz Proença-Modena J, Val FA, Cardoso Melo G, Monteiro WM, Nogueira ML, Lacerda MVG, Moraes-Vieira PM and Nakaya HI (2021) Acid pH Increases SARS-CoV-2 Infection and the Risk of Death by COVID-19. Front. Med. 8:637885. doi: 10.3389/fmed.2021.637885

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can infect a broad range of human tissues by using the host receptor angiotensin-converting enzyme 2 (ACE2). Individuals with comorbidities associated with severe COVID-19 display higher levels of *ACE2* in the lungs compared to those without comorbidities, and conditions such as cell stress, elevated glucose levels and hypoxia may also increase the expression of *ACE2*. Here, we showed that patients with Barrett's esophagus (BE) have a higher expression of *ACE2* in BE tissues compared to normal squamous esophagus, and that the lower pH associated with BE may drive this increase in expression. Human primary monocytes cultured in reduced pH displayed increased *ACE2* expression and higher viral load upon SARS-CoV-2 infection. We also showed in two independent cohorts of 1,357 COVID-19 patients that previous use of proton pump inhibitors is associated with 2- to 3-fold higher risk of death compared to those not using the drugs. Our work suggests that pH has a great influence on SARS-CoV-2 Infection and COVID-19 severity.

Keywords: COVID-19, pH, SARS-CoV-2, proton pump inhibitors, Barrett's esophagus

INTRODUCTION

As of August 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected over 20 million people worldwide (World Health Organization). The new coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 is characterized by a broad range of symptoms, from respiratory to neurological and digestive disorders (1, 2). Although a small fraction of patients develops highly lethal pneumonia, at least 20% of COVID-19 patients may display one or more gastrointestinal (GI) symptoms (1), such as diarrhea, vomiting, and abdominal pain (2, 3).

SARS-CoV-2 tissue tropism can be directly linked to the diverse clinical manifestations of COVID-19. The main receptor utilized by the virus to enter the cells is the angiotensin-converting enzyme 2 (ACE2), which is found in several tissues, including the GI epithelial cells and liver cells (4, 5). SARS-CoV-2 was detected in biopsies of several tissues, including esophagus, stomach, duodenum and rectum, and endoscopy of hospitalized patients revealed esophageal bleeding with erosions and ulcers (2, 6).

Higher levels of ACE2 in the tissues may explain in part some of the comorbidities associated with severe COVID-19. Recently, we showed that *ACE2* was highly expressed in the lungs of people with pulmonary arterial hypertension and chronic obstructive pulmonary diseases (7). Since the expression of *ACE2* changes under conditions of cell stress, elevated glucose levels and hypoxia (8, 9), other comorbidities related to the GI tract can be associated with different forms of COVID-19.

Here, we suggest that gastroesophageal reflux disease (GERD) and Barrett's esophagus (BE) may represent novel comorbidities associated with COVID-19. In the United States, it has been estimated that 5.6% of adults have BE, a disease where GERD damages the esophageal squamous mucosa (10). We demonstrated that *ACE2* is highly expressed in the esophagus of patients with BE, and that the acid pH associated with this condition is a key inducer of *ACE2* expression. Human primary monocytes cultured in reduced pH display increased expression of ACE2, and higher viral load upon SARS-CoV-2 infection. We also showed that patients taking proton pump inhibitors, which are recommended for GERD treatment, have a higher risk of developing severe COVID-19, observed by an increased risk of ICU admittance and death.

METHODS

Acidosis and Barrett's Esophagus Meta-Analysis

We manually curated the Gene Expression Omnibus (GEO) repository (https://www.ncbi.nlm.nih.gov/geo/) to find esophagus transcriptome datasets related to "Barrett's esophagus" and cell line transcriptome datasets related to "acidosis" and "pH reduction." Author-normalized expression values and metadata from these datasets were downloaded using the GEOquery package (11). We performed differential expression analyses using the limma package (12). The GEO study ID and the groups of samples compared are listed in **Supplementary Table 1**. The MetaVolcanoR package (13) was used to combine the *P* values using the Fisher's method. To adjust for multiple comparisons, we calculated the false discovery rate (FDR) using the Benjamini-Hochberg procedure. For enrichment analyses, we utilized the EnrichR tool (14) and fgsea R package (15) with gene sets from the Gene Ontology Biological Process database. We then selected pathways with a *P* value adjusted for multiple comparisons lower than 0.10.

Single Cell Transcriptomic Analysis of Barrett's Esophagus

The single cell RNA-seq (scRNA-seq) data from esophagus, Barrett's esophagus, gastric and duodenum cells from patients with BE were acquired from Owen et al. (16). Cells with <1,000 genes were excluded from analysis using Seurat v3 (17). Raw UMI counts were log transformed and variable genes called on each dataset independently based on the VST method. The *AddModuleScore* function was used to remove batch effects between samples and based on *C1orf43, CHMP2A*, *EMC7*, *GPI*, *PSMB2*, *PSMB4*, *RAB7A*, *REEP5*, *SNRPD3*, *VCP*, *VPS29* genes. We assigned scores for S and G2/M cell cycle phases based on previously defined gene sets using the *CellCycleScoring* function. Scaled z-scores for each gene were calculated using the *ScaleData* function and regressed against the number of UMIs per cell, mitochondrial RNA content, S phase score, G2/M phase score, and housekeeping score. Scaled data was used as an input into PCA based on variable genes. These PCA components were used to generate the UMAP reduction visualization. To identify the number of clusters, UMI log counts were used as input to SC3 (18). Technical variation was tested using BEARscc (19), which models technical noise from ERCC spike-in measurements. The clusters were then annotated based on genes previously characterized (16).

Peripheral Blood Mononuclear Cells Isolation

Buffy coats provided by the Hematology and Hemotherapy Center of the University of Campinas (SP-Campinas, Brazil) were used for PBMC isolation as described (9). The study was approved by the Brazilian Committee for Ethics in Human Studies (CAAE: 31622420.0.0000.5404). Briefly, buffy coats were mixed and then diluted in Phosphate Buffer Saline (PBS) (1:1) and carefully to 50 mL tube containing Ficoll (Sigma-Aldrich) and centrifuged. PBMCs were cultured in RPMI 1640 for 2–3 h to allow cell adhesion. Next, cells were washed twice with PBS and adherent cells, enriched in monocytes, were further incubated until infection in RPMI 1640 containing 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (Pen-Strep) at 37°C with 5% CO2. Monocytes were maintained in different pH levels (6, 6.5, and 7.4) during 24 h and subsequently infected with SARS-CoV-2, as described below.

Viruses and Infection

HIAE-02 SARS-CoV-2/SP02/human/2020/BRA (GenBank MT126808.1) virus was isolated as described (9). Stocks of Sars-CoV-2 were prepared in the Vero cell line. The supernatant was harvested at 2–3 dpi. Viral titers were obtained by plaque assays on Vero cells. Monocytes were infected with SARS-CoV-2 at MOI 0.1 under continuous agitation at 15 rpm for 1 h. Next, monocytes were washed twice and incubated in RPMI with 10% FBS and 1% Pen-Strep for 24 h at 37°C with 5% CO2 for 24 h.

Viral Load and Gene Expression Analyses

Total RNA extraction was performed using TRIzol Reagent (Sigma-Aldrich). RNA concentration was measured with

NanoDrop 2000 spectrophotometer (Thermo Scientific). RNA was reverse-transcribed using GoScript™ Reverse Transcriptase cDNA synthesis kit following manufacturer's instructions. SARS-CoV-2 viral load was determined with primers targeting the N1 region and a standard curve was generated as described (20). Viral load and gene expression were made using SYBR Green Supermix in BIO-RAD CFX394 Touch Real-Time PCR Detection System. Fold change was calculated as $2^{-\Delta\Delta}$Ct. Primer sequences used: 18S (Forward: 5′-CCCAACTTCTT AGAGGGACAAG-3′; Reverse: 5′-CATCTAAGGGCATCAC AGACC-3′); ACE2 (Forward: 5′-GGACCCAGGAAATGTT CAGA-3′; Reverse: 5′-GGCTGCAGAAAGTGACATGA-3′); SARS-CoV-2_IBS_N1 (Forward: 5′-CAATGCTGCAATCGTGC TAC-3′; Reverse: 5′-GTTGCGACTACGTGATGAGG-3′).

Clinical Data Analysis

We retrieved clinical data from two independent cohorts of 551 and 806 RT-qPCR confirmed COVID-19 patients aged 18 years or older that went to reference hospitals for COVID-19 in Manaus, Amazonas, Brazil (North region cohort) and in São José do Rio Preto city, São Paulo, Brazil (Southeast region cohort), respectively. They were followed for at least 28 days (North region cohort) or 120 days (Southeast region cohort) after recruitment. Information about the previous history of proton pump inhibitors use (e.g., omeprazole and pantoprazole), a surrogate evidence of low gastric pH-related diseases, time of hospitalization, ICU admittance, and time to death, as well as demographics, previous use of other drugs, clinical, laboratory, and outcome variables were collected. The protocol was approved

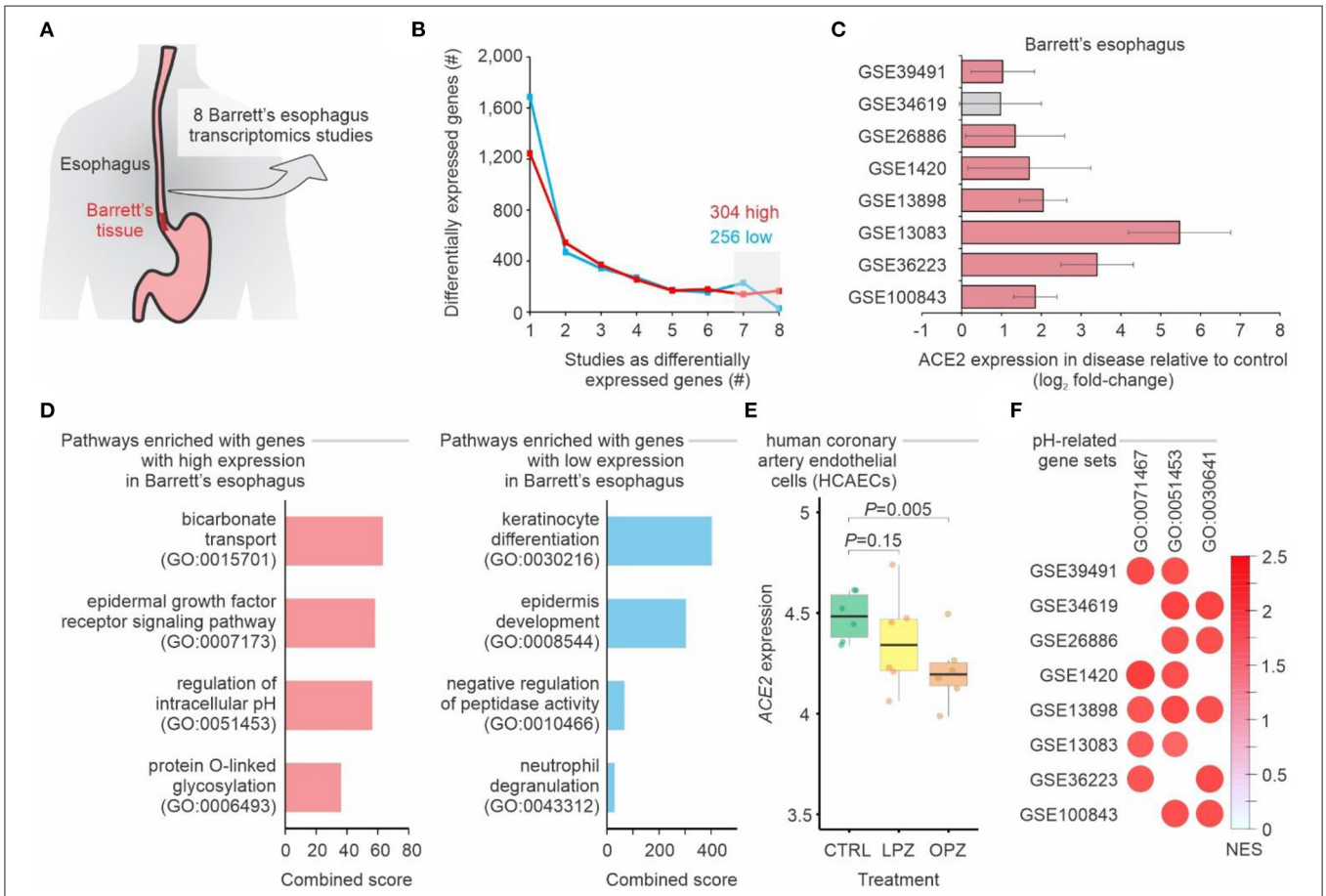

FIGURE 1 | Meta-analysis of gastroesophageal junction transcriptomes of patients with Barrett's esophagus. **(A)** Meta-analysis of 8 studies of Barrett's esophagus transcriptomes. **(B)** Number of differentially expressed genes in Barrett's esophagus compared with non-Barrett's esophagus. The lines show the number of genes (y-axis) considered up-regulated (red lines) or down-regulated (blue lines) in Barrett's esophagus (P-value < 0.05; log2 fold-change > 1; combined FDR < 0.01) in one or more datasets (x-axis). The numbers of up-regulated and down-regulated genes in at least seven studies are indicated. **(C)** *ACE2* is upregulated in patients with Barrett's esophagus. Each bar represents the log2 expression fold-change between patients and control individuals. The error bars indicate the 95% confidence interval. Bars in red represent a P-value < 0.05 and in gray a non-significant P-value. **(D)** Pathway enrichment analysis using the up-regulated and down-regulated genes in at least seven studies. The bars represent the combined score (x axis) calculated by Enrichr tool for selected Gene Ontology gene sets (y axis). **(E)** ACE2 expression in cells treated with proton pump inhibitors. Each boxplot represents the log2 expression of untreated (CTRL) cells and cells treated with either omeprazole (OPZ) or lansoprazole (LPZ). **(F)** Gene Set Enrichment Analysis (GSEA) of the 8 studies of Barrett's esophagus transcriptomes using pH-related gene sets. The size and color of the circles are proportional to the normalized enrichment score (NES) of the gene sets (columns) on each study (rows). The Gene Ontology IDs are indicated at the top.

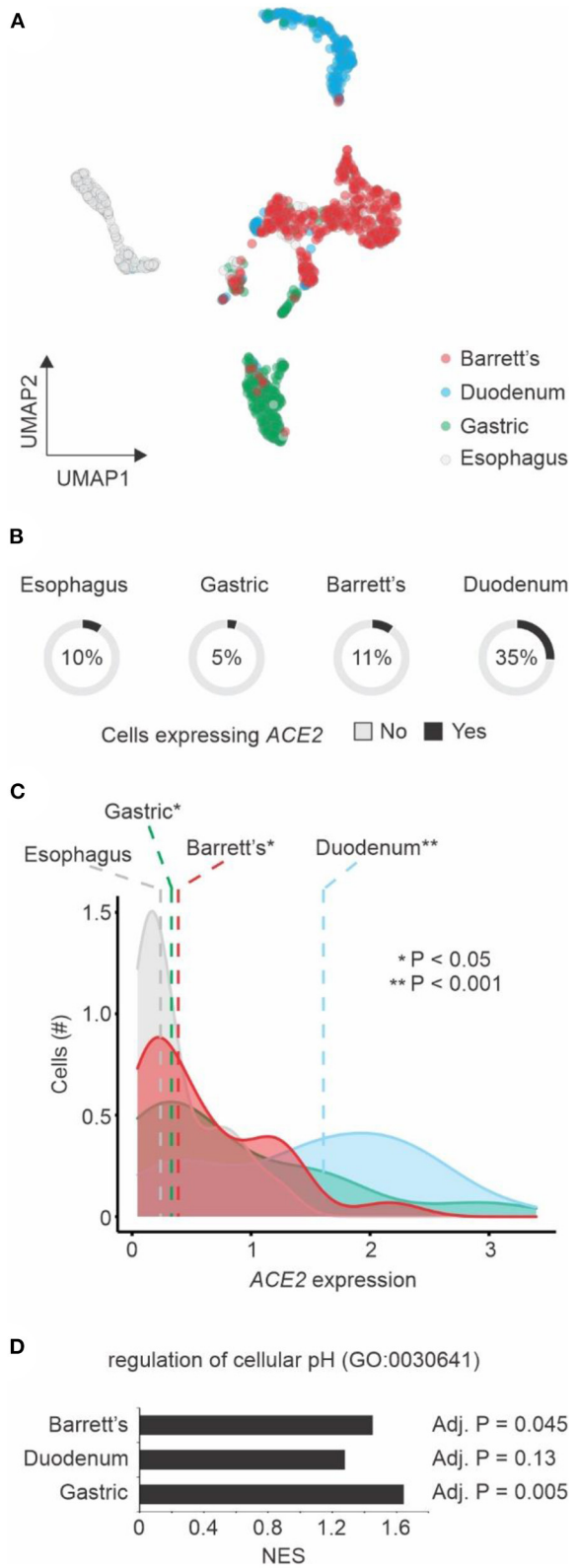

FIGURE 2 | Single cell transcriptomics of Barrett's esophagus. **(A)** Dimension reduction of single cells using Uniform Manifold Approximation and Projection (UMAP). Cells from four patients with Barrett's esophagus ($n = 1,168$) are shown. The colors represent the tissue types. **(B)** ACE2 expression by tissue type. The pie charts show the number of single cells with (black) or without (gray) ACE2 expression (expression values > 0). The fractions of ACE2-expressing cells are indicated. **(C)** Distribution of ACE2 expression by cells from different tissue types. The colors of histograms represent the tissue types. The dashed vertical line shows the median values of each tissue type. Student's *t*-test *P*-value between tissue types vs. esophagus is indicated. **(D)** Gene Set Enrichment Analysis (GSEA) of the three tissue types compared to esophagus using the regulation of cellular pH gene set. The normalized enrichment score (NES) are shown in the x-axis for each one of the tissue types. The adjusted *P*-value of the enrichment is displayed right next to the corresponding bar.

(Continued)

by the Brazilian Committee of Ethics in Human Research (CAAE: 30152620.1.0000.0005 and 30615920.2.0000.0005 for North region cohort, and 31588920.0.0000.5415 for Southeast region cohort). Data were collected and managed using REDCap (v. 10.2.1) electronic data capture tools hosted at *Fundação de Medicina Tropical Dr. Heitor Vieira Dourado*.

Adjusted hazard ratios and risk ratios with respective 95% confidence intervals (CI) were estimated for time to death and ICU admittance, respectively by Cox regression and log-binomial generalized linear model models. To adjust for confounders, ages higher than 60 years old and obesity, defined by both BMI and fat percentage, were used as covariables in the multivariable analyses. Wilcoxon Rank-Sum analysis was used to test differences in the days of hospitalization. A 2-tailed $P < 0.05$ was considered significant. The statistical analyses were carried out using Stata v. 13.0 (StataCorp LP, College Station, TX).

RESULTS

To evaluate whether people with BE may have higher chances of being infected with SARS-CoV-2 when compared to people without the disease, we performed a meta-analysis of eight transcriptomic studies of BE (**Figure 1A**, **Supplementary Table 1**). A total of 304 and 256 genes displayed, respectively, higher and lower expression in BE when compared to normal esophagus tissue in at least 7 of these studies (**Figure 1B**). *ACE2* was among the genes consistently up-regulated in the BE compared to normal esophagus (**Figure 1C**). While pathways related to keratinocyte differentiation and epidermis development were enriched with down-regulated genes, we found that bicarbonate transport and regulation of intracellular pH pathways were enriched with up-regulated genes (**Figure 1D**), suggesting that pH may influence ACE2 expression. In fact, when human coronary artery endothelial cells were treated with proton pump inhibitors—omeprazole or lansoprazole—the expression of ACE2 decreased in comparison to untreated cells (**Figure 1E**). Gene set enrichment analysis (GSEA) confirmed that Barrett's esophagus tissues have higher expression of genes related to pH alterations (**Figure 1F**).

We also investigated *ACE2* expression in Barrett's esophagus at single-cell level. Our analysis showed that single cells from Barrett's esophagus patients were distinct from normal esophagus cells, as well as cells from duodenum and gastric tissues

(**Figure 2A**). While a large fraction of duodenum cells expresses *ACE2* (21), only 11% of the single cells from Barrett's samples have *ACE2* expression above 0 (**Figure 2B**). However, among the cells expressing *ACE2*, higher levels of this gene were found in gastric, Barrett's, and duodenum cells when compared to esophagus cells (**Figure 2C**). Using GSEA approach, we found that genes associated with regulation of cellular pH were enriched among the up-regulated genes in gastric, Barrett's and duodenum cells when compared to esophagus cells (**Figure 2D**).

To further evaluate whether pH may influence the expression of *ACE2*, we analyzed publicly available transcriptomic studies of cells under experimentally-induced acidosis. Cells cultured at lower pH displayed higher expression levels of *ACE2* when compared to those cultured under higher pH (**Figures 3A,B**). We validated this finding with human primary monocytes cultured at pH 7.4, 6.5, and 6.0 under normoxia. ACE2 expression was significantly increased at pH 6.5 and 6.0 compared to pH 7.4 (**Figure 3C**). The reduction of pH alone also significantly increased SARS-CoV-2 infection of human monocytes (**Figure 3D**), indicating that pH plays a role in ACE2-mediated SARS-CoV-2 infection.

Proton pump inhibitors (PPI) decrease the amount of acid produced in the stomach and are often utilized to treat subjects with GERD symptoms (22). The use of PPIs prior to COVID-19 may serve as a proxy for identifying subjects with tissue irritation and inflammation caused by stomach acid. In two independent cohorts of 551 and 806 RT-qPCR confirmed COVID-19 patients from North and Southeast regions of Brazil, respectively, we investigated the effects of gastrointestinal discomfort and COVID-19 severity. Survival curve analysis showed that people that were taking PPIs had a 2- to 3-fold increased risk of death compared to those not using the drug (**Figure 4A**). When controlling for potential confounders (i.e., age above 60 years old, diabetes, and hypertension), the adjusted hazard ratio was 2.183 (95CI: 1.635–2.914; $P < 0.0001$) for the North region cohort and 2.332 (95CI: 1.661–3.274; $P < 0.0001$) for the Southeast cohort (**Figure 4B**). These clinical findings indicate that the reduction of physiological pH (caused by stomach acid) may play a significant role in SARS-CoV-2 infection and COVID-19 severity.

DISCUSSION

Our findings suggest that acid pH increases SARS-CoV-2 infection by up-regulating the ACE2 receptor, and this may have clinical implications for patients with GERD or Barrett's esophagus. No clear mechanism exists linking pH alterations and *ACE2* expression. Although evidence indicates that hypoxic conditions can increase the expression of *ACE2* (8, 9), the expression of neither SIRT1 nor HIF1A seem to be associated with Barrett's esophagus (**Supplementary Table 2**). We found that known regulators of ACE2—HNF1B (23) and FOXA2 (24)—were up-regulated in 6 out of 8 Barrett's esophagus

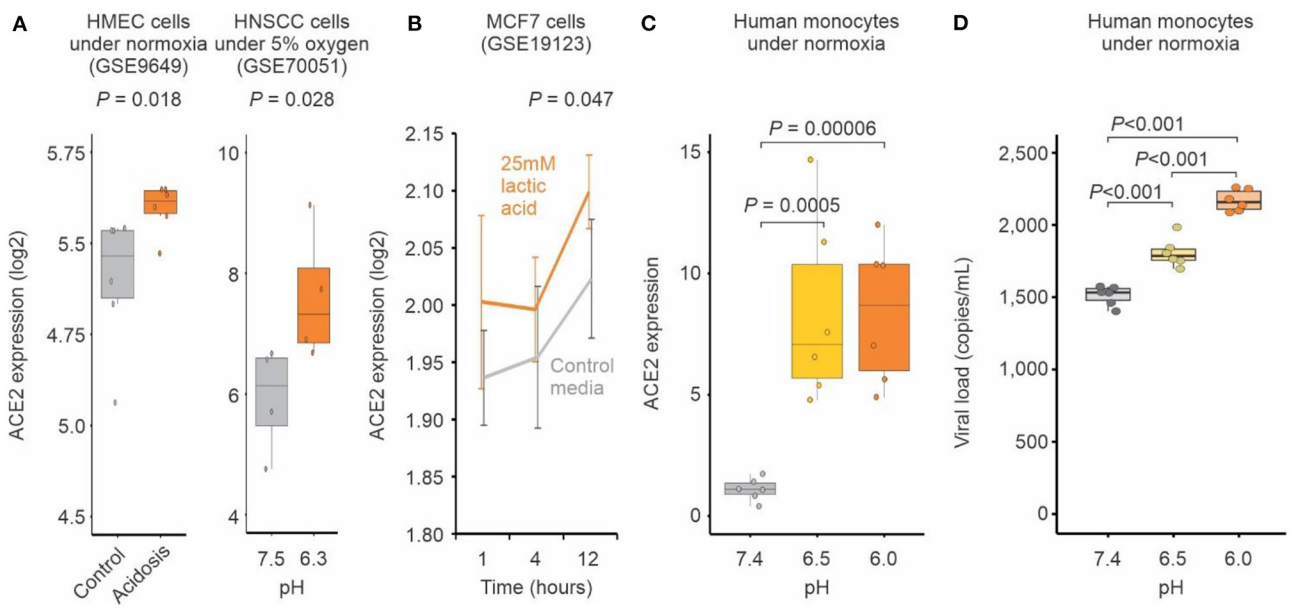

FIGURE 3 | Acidosis increases ACE2 expression and SARS-CoV-2 infection. **(A)** Human cells exposed to acidosis. Each boxplot represents the log2 expression of samples untreated (gray) or treated with lactic acidosis (brown) for two microarray studies (GSE9649 and GSE70051). Student's *t*-test *P*-values are indicated. **(B)** MCF7 cells exposed to pH reduction increases ACE2 expression. Gray and brown lines represent, respectively cells treated with control media or with 25 mM lactic acid for 1, 4, and 12 h (x-axis). Each point represents the mean log2 expression and the error bars the standard deviation of biological replicates. **(C)** Acid pH increases ACE2 expression in monocytes. Human peripheral blood monocytes were incubated in medium at three different pH (6, 6.5, 7.4) for 24 h. Each boxplot represents the fold change ACE2 expression. **(D)** Acid pH increases SARS-CoV-2 viral load. Human peripheral blood monocytes were incubated in medium at three different pH (6, 6.5, 7.4) for 24 h. The cells were infected with CoV-2 (MOI 0.1) for 1 h under continuous agitation. The RNA viral load was measured by qPCR.

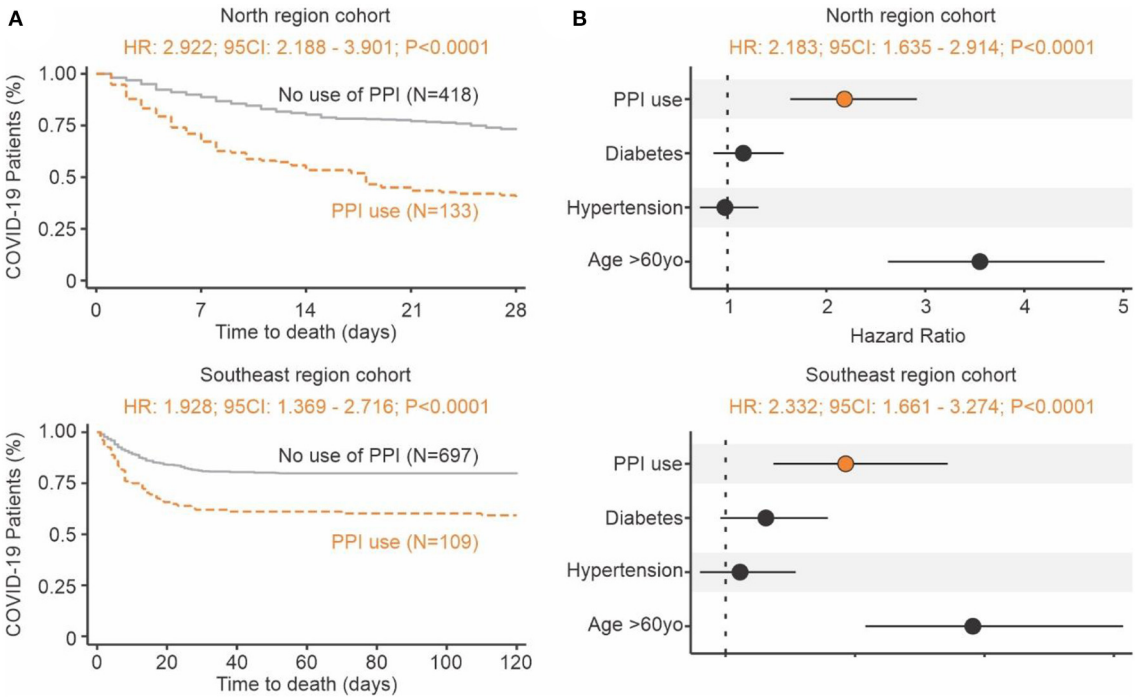

FIGURE 4 | Increase risk of death in individuals with COVID-19 using proton pump inhibitors prior infection. **(A)** Time to death. Kaplan-Meier survival curves showing a higher risk of death for the group of patients that used PPIs (brown) prior to admittance when compared to those not using them (gray). The North region cohort result is shown at the top and Southeast region cohort result is shown at the bottom. **(B)** Risk of death. The forest plot presents the hazard ratios and respective 95CI for the main explanatory variable (brown), as well as the potential confounders (black) used in the multivariate model. The North region cohort result is shown at the top and Southeast region cohort result is shown at the bottom.

transcriptomic studies (**Supplementary Table 2**), suggesting that they may be involved with the pH-induced ACE2 expression in Barrett's esophagus.

Pulmonary damage, one of the main features of severe COVID-19, may lead to acute hypoxia and further respiratory acidosis. It is possible that the acidosis in the blood of some patients with severe COVID-19 (25) worsen the disease by increasing the levels of ACE2 and facilitating the entry of SARS-CoV-2 into human cells. Hypoxia itself may contribute to the regulation of ACE2 (9, 26). In addition, elevated levels of the enzyme lactate dehydrogenase (which converts lactate from pyruvate) has been associated with worse outcomes in patients with COVID-19 (27). The excess of lactate may directly alter the extracellular and intracellular pH which in turn can impact ACE2 expression. The extent to which acute systemic acidosis contributes to COVID-19 severity is poorly known and deserves further research.

The drug famotidine suppresses gastric acid production by blocking the histamine 2 receptor in the stomach. Recently, Freedberg et al. (28) have shown that early treatment of patients tested positive for SARS-CoV-2 significantly improved clinical outcomes among the hospitalized patients. However, a meta-analysis of 5 COVID-19 studies performed by Chenyu Sun et al. (29) have shown that famotidine treatment was not associated with reduced risk of progression to severe disease or death. Although famotidine may have antiviral effects, it is possible that pH itself can play an important role in regulating ACE2 expression and limiting SARS-CoV-2 infection in patients.

We showed here that the previous use of PPIs is associated with increased risk of death from COVID-19. Such association is supported by a meta-analysis of eight studies (30) that showed that previous use of PPIs increases the risk of progression to severe COVID-19. Almario et al. (31) recently described that individuals taking PPIs had greater chances for testing positive for COVID-19 when compared to those not using PPIs. Their hypothesis is that PPIs might increase the risk for COVID-19 by undermining the gastric barrier to SARS-CoV-2 and thus reducing the microbial diversity in the gut (31). Rather, we believe that PPIs are important markers of hidden comorbidities that involve the damage caused by the excess stomach acid in GI tissues.

By going from disease (Barrett's esophagus) to molecule (ACE2) to cells (*in vitro* experiments) and back to clinical findings (COVID-19 patients), we showed that pH may have a great influence on SARS-CoV-2 infection and COVID-19 severity. Additional studies should be performed to not only confirm the clinical findings on a larger scale but also to assess the molecular mechanism related to pH-induced ACE2 expression.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Brazilian Committee of Ethics in Human Research (CAAE: 30152620.1.0000.0005 and 30615920.2.0000.0005 for North region cohort, and 31588920.0.0000.5415 for Southeast region cohort). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LJ, AO, LF, and HN performed the transcriptome analyses. AC, GD, LB, JV, GF, SM, JL, and PM-V performed the experimental work. VS, MB, NZ, FA, MN, FV, GC, WM, and ML performed the clinical analysis. HN coordinated the study. LJ and HN wrote the manuscript with inputs from all of the co-authors. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Brazilian National Council for Scientific and Technological Development (grant number 313662/2017-7), the São Paulo Research Foundation (grant numbers 2018/14933-2; 2020/04836-0), and CAPES.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.637885/full#supplementary-material

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frontiers in Medicine

CASE REPORT
published: 26 November 2021
doi: 10.3389/fmed.2021.678227

Case Report: Clinical Features of a COVID-19 Patient With Cirrhosis

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Coronavirus disease 2019 (COVID-19) was first reported in Wuhan, Hubei Province, China in December 2019. At present, COVID-19 has emerged as a global pandemic. The clinical features of this disease are not fully understood, especially the interaction of COVID-19 and preexisting comorbidities and how these together further impair the immune system. In this case study, we report a COVID-19 patient with cirrhosis. A 73-year-old woman with cirrhosis reported a fever and cough on February 6, 2020. CT of the chest indicated an infection in her bilateral lungs. She tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The woman was treated with lopinavir and ritonavir tablets and interferon alpha-2b injection, but there was no obvious effect. Although this patient was basically asymptomatic after 2 days in the hospital, the inflammation of the bilateral lungs was slow to subside as shown in CT of the chest. In addition, the white blood cell count (WBC), absolute neutrophil count, and absolute lymphocyte count remained decreased and the result of real-time reverse transcription polymerase chain reaction (PCR) (rRT-PCR) assay was still positive for SARS-CoV-2 on hospital day 28. After infusion of plasma from a recovered COVID-19 patient four times, the patient tested negative for SARS-CoV-2. She was discharged on March 13, 2020. This patient tested negative for SARS-CoV-2 after infusion of plasma from a recovered COVID-19 patient four times. Cirrhosis could impair the homeostatic role of the liver in the systemic immune response, which may affect the removal of SARS-CoV-2. This could lead to a diminished therapeutic effect of COVID-19. Thus, clinicians should pay more attention to COVID-19 patients with cirrhosis.

Keywords: COVID-19, cirrhosis, SARS-CoV-2, treatment, cured patient

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *09 March 2021*
Accepted: *01 November 2021*
Published: *26 November 2021*

Citation:
Zhou J, Jiang D, Wang W, Huang K, Zheng F, Xie Y, Zhou Z and Sun J (2021) Case Report: Clinical Features of a COVID-19 Patient With Cirrhosis. Front. Med. 8:678227. doi: 10.3389/fmed.2021.678227

INTRODUCTION

At present, many studies have indicated the epidemiological and clinical characteristics of coronavirus disease 2019 (COVID-19) (1–4). However, there are many diseases that may affect the immune system, such as AIDS, cirrhosis, and advanced malignant tumors, which may affect the removal of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), further affecting the treatment of COVID-19 patients. A nationwide analysis in China analyzed the major strategies for patients with cancer in this COVID-19 crisis (5). The process of advanced cirrhosis is complicated with cirrhosis-associated immune dysfunction. Cirrhosis has the potential to injure the homeostatic role of the liver in the immune system (6, 7). In this case study, we report a case of a COVID-19

patient with cirrhosis. We describe the symptoms, diagnosis, treatment, and management of this patient, which may provide more information for the treatment of COVID-19 patients with cirrhosis.

CASE REPORT

On February 11, 2020, a 73-year-old woman came to the Fever Clinic of the First Hospital of Changsha, China. Ten minutes later, she was taken to the examination room and evaluated by a clinic doctor. The chief complaint of the patient was a fever—her body temperature peaked at 39°C—with cough, expectoration, shortness of breath, and general weakness that started prior 5 days. She developed mild diarrhea (3–4 stools/day) 2 days prior to coming to the hospital. Her daughter was diagnosed with COVID-19. Given her symptoms and recent close contact with a COVID-19-positive patient, she decided to go to a healthcare provider. The patient had a history of cirrhosis and type 2 diabetes, but no history of smoking or drinking. Physical examination indicated a body temperature of 38.8°C, a pulse of 100 beats/min, a respiratory rate of 22 breaths/min, an oxygen saturation of 85%, and bowel sounds at four times/min. She presented with a characteristic feature of chronic liver disease, hepatic facies, and liver palms, but no spider nevus. In addition, she had thick breathing sounds on both sides of the lungs and audible wet murmurs in both the lungs. The abdomen of the patient was soft and had no lumps. No pain was found in the liver without mobile dullness.

Considering the possibility of SARS-COV-2 infection, we performed a chest CT examination and found bilateral pneumonia (**Figure 1**). The results of a nucleic acid amplification test (NAAT) for influenza A and B were negative. Her blood tests demonstrated simultaneous reduction of the ternary systems (red blood cells: 2.83×10^{12} cells/l; peripheral blood hemoglobin: 83 g/l; white blood cells: 0.78×10^{9} cells/l; lymphocytes: 0.11×10^{9} cells/l; lym%: 14.5%; platelets: 41×10^{9} cells/l) and an elevated percentage of neutrophils (0.65×10^{9}/L; n%: 82.8%), C-reactive protein (62.5 mg/l), and erythrocyte sedimentation rate (129 mm/h) (**Table 1**). In view of the close contact history and clinical examination results of the patient, we carried out COVID-19 test for the patient. Specimens were collected following the Chinese Center for Disease Control and Prevention (CCDC) guidance. The results showed that she tested positive for SARS-COV-2. Therefore, she was admitted to the isolation ward for further treatment.

On day 1 of the hospital stay (illness day 5), the patient was administered lopinavir and ritonavir tablets (2 pills BID peros), which were recommended by *the Diagnosis and Treatment of Pneumonitis with COVID-19 Infection* (DTPI) published by the National Health Commission of the People's Republic of China (PRC) and interferon alpha-2b injection (5 million IU added into 2 ml of sterile water, inhalation BID). To inhibit inflammation in the lungs, she was treated with methylprednisolone sodium succinate (40 mg QD, intravenously). Yellow-green expectoration predicted the presence of a bacterial infection and, as such, moxifloxacin hydrochloride and sodium chloride injection (0.4 g QD) were given intravenously to the patient as treatment. Moreover, other supportive treatments included human immunoglobulin (10 g QD, intravenously) for improving immunity, *Bifidobacterium lactobacillus* trifecta orally for regulating the intestinal flora, recombinant human granulocyte colony-stimulating factor for promoting cell proliferation, and ampeptide elemente tablets for promoting the formation of platelets.

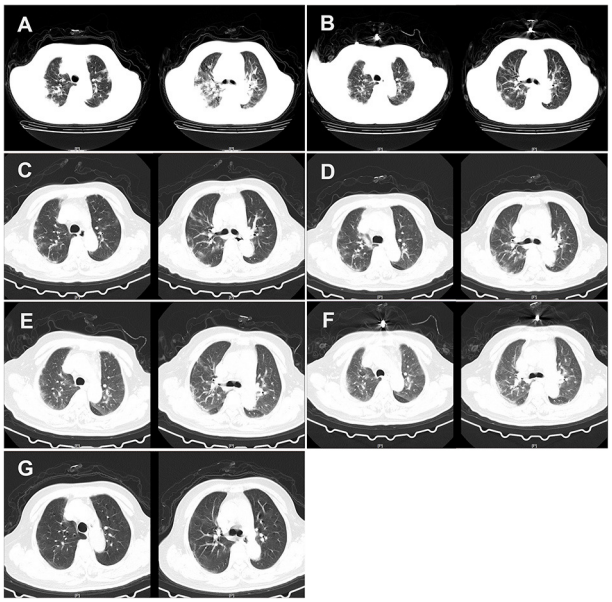

FIGURE 1 | CT of the chest of the patient. **(A)** CT of the chest was obtained on February 12, 2020 (hospital day 2, illness day 6). The major morphogenesis of her bilateral lungs took on increased bronchovascular shadows and multiple patchy and maculas shadows, with cord-like ground-glass opacity (GGO) in the middle and lower regions of the lung. CT scan of the chest also showed increased lung markings. The texture of the trachea and blood vessels in both the lungs became thicker. **(B)** CT of the chest was obtained on February 16, 2020 (hospital day 6, illness day 10). The patchy lesions and maculas in both the lungs were partially absorbed. Increased lung markings were observed in the bilateral lungs. **(C)** CT of the chest was obtained on February 20, 2020 (hospital day 10, illness day 14). Decreased density of the patchy lesions in both the lungs was observed. The texture of the trachea and blood vessels in both the lungs became thicker. **(D)** CT of the chest was obtained on February 24, 2020 (hospital day 14, illness day 18). The pulmonary lesions remained unchanged. **(E)** CT of the chest was obtained on February 28, 2020 (hospital day 18, illness day 22). There was no obvious change in the patchy lesions in both the lungs. GGO was slightly increased. **(F)** CT of the chest was obtained on March 3, 2020 (hospital day 22, illness day 26). The major lesions of the bilateral lungs were not absorbed. **(G)** CT of the chest was obtained on March 10, 2020 (hospital day 29, illness day 33). The multiple patchy and maculas shadows of the bilateral lungs were further absorbed and the bronchovascular shadows were reduced.

On day 2 of the hospital stay (illness day 6), she was asymptomatic apart from a cough, expectoration, chest tightness, and shortness of breath. Additionally, her temperature dropped to 36.9°C, but she reported that diarrhea still existed, approximately four times/day (**Table 2**). CT scans showed that the patchy infiltration was scattered as a small range of ground-glass opacity effusion and strip lesions in the bilateral lungs, which was similar to day 1 in the hospital (**Figure 1**).

TABLE 1 | Clinical laboratory results.

Measure	Reference range	F11/H1	F12/H2	F15/H5	F17/H7	F19/H9	F20/H10	F22/H12	F25/H15	F28/H18	M2/H21	M6/H25	M10/H29
qRT-PCR	N	P	-	P	-	-	P	-	P	N	P	P	N^T
White cell count (10^9/L)	4–10	0.78*	4.09	1.65*	4.47	3.40*	2.52*	1.86*	0.99*	1.01*	1.01*	0.78*	0.94*
Red cell count (10^{12}/L)	3.5–5.5	2.83*	3.05*	3.02*	3.00*	3.54	3.19*	2.93*	2.68*	2.41*	2.38*	2.68*	2.78*
Absolute neutrophil count (10^9/L)	2-7	0.65*	3.64*	1.36*	4.13	3.01	2.04	1.58*	0.73*	0.66*	0.66*	0.41*	0.56*
Absolute lymphocyte count (10^9/L)	0.8–4	0.11*	0.22*	0.19*	0.13*	0.16*	0.29*	0.14*	0.17*	0.20*	0.23*	0.31*	0.28*
Monocyte (10^9/L)	0.12–1.2	0.02*	0.18	0.09*	0.20	0.13	0.18	0.14	0.09*	0.07*	0.10*	0.06*	0.09*
Basophil (10^9/L)	0.00-0.10	0.00	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01
Eosinophil (10^9/L)	0.02–0.5	0.00*	0.03	0.00*	0.00*	0.09	0.01*	0.00*	0.00*	0.00*	0.01*	0.00*	0.00*
Procalcitonin (ng/ml)	0–0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Platelets transfusion (10^9/L)	100–300	41*	47*	44*	46*	60*	55*	45*	40*	31*	26*	35*	50*
Erythrocyte sedimentation rate (mm/h)	0-15	129#	-	-	103#	-	-	81#	82#	83#	100#	75#	71#
C-reactive protein (mg/L)	0–8	62.50#	-	9.50#	-	3.40	2.90	3.90	2.70	1.60	2.10	0.80	1.10
Albumin (g/L)	35-55	26.20*	-	32.90*	-	29.30*	31.90*	30.70*	31.30*	32.60*	32.90*	34.1*	36.7
PaO_2 (mmHg)	80–100	62*	89	83.3	66.4*	102	63*	85.2	66.3*	-	70*	83	87
PaO_2/FiO_2 (mmHg)	400–500	124*	178*	208*	201*	309*	300*	293*	315*	-	333*	321*	338*
Alanine aminotransferase (U/L)	0-42	8.20	-	14.50	-	21.70	35.80	27.70	23.10	18.10	12.30	10.6	12.7
Aspartate aminotransferase (U/L)	0–37	31.10	-	19.50	-	17.00	40.30	28.40	29.10	25.40	20.30	26.2	24.3
Total bilirubin (μmol/L)	3.4–20.5	14.20	-	26.70#	-	12.20	14.90	22.70#	13.50	11.20	12.00	11.7	10.9
D-Dimer (μg/mL)	0–1	0.36	-	6.45#	5.87#	-	10.70#	8.29#	6.43#	5.46#	6.77#	7.24#	5.03#
Prothrombin time (s)	0–15	13.8	-	14.2	-	16.2#	13.5	12.3	11.7	-	15.9#	15.7#	12.9
International normalized ratio	0.92–1.38	1.27	-	1.31	-	1.49#	1.25	1.14	1.08	-	1.47#	1.45#	1.19
Fibrinogen (g/L)	2.0–4.0	2.59	-	1.63*	-	0.96*	1.19*	2.14	2.21	-	1.97*	1.76*	1.89*
Activated partial thromboplastin time (s)	26.2–46.0	32.4	-	25.1*	-	22.7*	23.8*	28.0	31.6	-	34.3	35.1	35.4
Thrombin time (s)	8–15	15.3#	-	14.4	-	26.1#	20.7#	17.7#	15.6#	-	19.0#	17.6#	16.4#

** The value in the patient was below normal.*

 # The value in the patient was above normal.

T Tested negative for three times (M10, M11, and M12) by qRT-PCR.

F, February; M, March; H, hospital day; qRT-PCT, quantificational Real-Time Polymerase Chain Reaction.

Otherwise, the laboratory results reflected that there was still a reduction in the tertiary system and hypoproteinemia due to liver dysfunction. Human serum albumin (50 ml BID) was then given intravenously. To prevent of episodes of hepatic encephalopathy, which is a chronically debilitating complication of hepatic cirrhosis, lactulose was added to the therapeutic regimen of the patient and nutritious meals were supplied to improve her anemia. The CCDC repeatedly confirmed that the oropharyngeal swabs of this patient tested positive for SARS-CoV-2 by real-time reverse transcription PCR (rRT-PCR) assay.

On day 3 of the hospital stay (illness day 7), the patient reported she felt better. Her pulse oxygen saturation increased significantly, up to 100%, at an oxygen flow rate of 2 l/min. Since that she still had diarrhea symptoms and lactulose was stopped to avoid the occurrence of imbalance of water and electrolytes. On day 4 of her hospital stay (illness day 8), a gastroenterologist was contacted to evaluate the persistent diarrhea of the patient. According to the suggestion of the gastroenterologist, the patient was treated with pantoprazole enteric-coated tablets (40 mg QD orally) for acid suppression. In addition, reduced glutathione (0.6 g QD) was given intravenously to protect her liver from subsequent damage.

On days 5–10 of the hospital day (illness days 9–14), the patient reported that her diarrhea improved to a degree and her clinical condition improved with supportive care. On hospital day 6 of the hospital stay, CT scans showed that the partial patchy lesions in the bilateral lungs were absorbed compared with the CT images obtained previously (**Figure 1**). Given the clinical presentation of the patient, treatment with human serum albumin was stopped on day 6 of the hospital stay. Lopinavir and ritonavir tablets, methylprednisolone sodium succinate, moxifloxacin, ampeptide elemente tablets, pantoprazole enteric-coated tablets, and human immunoglobulin were stopped on day 8 of the hospital stay of the patient (**Table 3**). However, the clinical course of the patient continued to deteriorate in terms of her respiratory symptoms, who typically presented with a cough and shortness of breath. Thymosin (0.1 g QD) and plasma (200 ml) from recovered COVID-19 patients plasma were then given intravenously to boost the immunity of the patient. On day 9 of the hospital stay (illness day 13), the C-reactive protein of this patient dropped to 3.4 mg/l. Nevertheless, CT scans of the chest indicated that the symptoms of the bilateral lungs of the patient did not improve on day 10 of the hospital stay (**Figure 1**). Moreover, the oropharyngeal swabs of this patient retested positive. Therefore, chloroquine phosphate (0.5 g BID) was administered orally instead. Additionally, the treatments did not improve the level of blood cells because of liver dysfunction and hypersplenism caused by cirrhosis.

On days 11–18 of the hospital stay (illness days 15–22), she was in good clinical condition, except for a persistent cough and intermittent diarrhea. In order to further alleviate the diarrhea of the patient, montmorillonite powder (3 g QD) and loperamide hydrochloride (2 mg QD) were administrated orally. Moreover, interferon alpha-2b injections were stopped due to its limited effect on the clearance of the virus and the plasma from recovered COVID-19 patients was infused again on day 15 of the hospital stay (illness day 19). As the diarrhea of the patient improved,

TABLE 2 | Body temperatures and symptoms from February 6 to March 13, 2020.

Date	F6–8	F9–10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27	F28	F29–M13
Illness day	Home	Home	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23–36
Hospital day			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19–32
Fever (°C)	Fever	Fever	38.8	36.9	36.0	36.0	36.6	36.0	36.1	36.2	36.4	36.4	36.4	37.7	37.0	37.0	36.7	36.7	36.0	36.0	36.1–36.9
Cough	✓	✓	✓								✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Shortness of breath	✓	✓	✓								✓										
Chest distress																					
Fatigue	✓	✓	✓																		
Headache																					
Sore throat																					
Chest pain																					
Diarrhea		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓				

F, February; M, March.

TABLE 3 | Order sheet of the physician.

Drug	Date	Hospital day	Dose	Usage
Lopinavir and ritonavir tablets	F11–F18	H1–H8	2 pills BID	po
Interferon alfa-2b injection	F11–F25	H1–H15	5 million IU BID	inh
Methylprednisolone sodium succinate	F11–F18	H1–H8	40 mg QD	ivgtt
Bifidobacterium lactobacillus trifecta	F11–F11	H1–H1	2 g BID	po
Human immunoglobulin	F11–F18	H1–H8	10 g QD	ivgtt
Ampeptide elemente tablets	F11–F18	H1–H8	0.4 g TID	po
Human serum albumin	F12–F16	H2–H6	50 ml BID	ivgtt
Pantoprazole enteric-coated tablets	F14–F18	H4–H8	40 mg QD	po
Reduced glutathione for injection	F14–F29	H4–H19	0.6 g QD	ivgtt
Moxifloxacin hydrochloride and sodium chloride injection	F15–F18	H5–H8	0.4 g QD	ivgtt
Thymosin	F19–F19	H9–H9	0.1 g QD	ivgtt
Chloroquine	F20–F27	H10–H17	0.5 g BID	po
Montmorillonite powder	F25–F28	H15–H18	3 g QD	po
loperamide hydrochloride	F26–F28	H16–H18	2 mg QD	po

F, February; H, hospital day; ivgtt, intravenously guttae; po, per os; inh, inhalation.

antidiarrheal drugs were discontinued on day 18 of the hospital stay (illness day 22).

On days 19–29 of the hospital stay (illness days 23–33), the vital signs of the patient were largely stable. The patient reported that her cough and diarrhea had abated and her clinical condition improved. Given these good clinical conditions, a reduction in glutathione injections was initiated on day 19 of her hospital stay. However, since the oropharyngeal swabs of this patient tested positive again, she was treated with plasma from a recovered COVID-19 patient for the third time. On day 29 of the hospital stay (illness day 33), CT scans showed that the patchy lesions in the bilateral lungs of the patient had absorbed compared with the CT images obtained previously (**Figure 1**). On the same day, the patient tested negative for COVID-19 infection (**Table 1**). On day 30 of the hospital stay (illness day 34), the patient was once again treated with the plasma from a recovered COVID-19 patient in order to ensure that the virus was completely cleared. On days 30–31 of the hospital stay, the patient tested negative for COVID-19 by an rRT-PCR assay for two times. She was discharged on March 13, 2020 (day 32 of the hospital stay, illness day 36).

DISCUSSION

Cirrhosis affects the cellular and humoral immune response of the entire body and the immune system of the liver (6, 8). The proportion of $CD4^+/CD8^+$ cells in the liver of patients with cirrhosis decreases and the distribution of lymphocytes varies within different lesions. $CD8^+$ cells predominate in the necrotic area, while $CD4^+$ cells increase in the manifold area. T-helper type 1 (Th1) cells dominate during the early stages of cirrhosis and then gradually drift toward Th2 cells. In order to understand the impact of cirrhosis on the treatment of COVID-19, we report the symptoms, diagnosis, treatment, and management of a COVID-19 patient with cirrhosis.

In this case study, the patient tested positive for SARS-CoV-2, which was supported by CT scan of the chest and she was admitted to the isolation ward at the First Hospital of Changsha City, China. Lopinavir and ritonavir tablets combined with interferon alpha-2b injections were given to her on her first day in the hospital. Though she was basically asymptomatic on day 2 of her hospital stay and her body temperature also returned to a normal range, the inflammation of her bilateral lungs was difficult to subside, suggesting that clinicians should be aware of COVID-19 patients with diseases affecting the immune system. These patients may show mild or even no symptoms, while the inflammation of lungs may be progressing. Therefore, if a person with basic diseases that impair the immune system was exposed to confirmed COVID-19 cases, they should immediately come to the hospital even if they have no symptoms. Also, doctors need to be aware of the progression of inflammation in the lungs.

Previous reports showed that COVID-19 patients with cirrhosis had lower albumin than patients with COVID-19 (9), which was consistent with the results of this case study. Moreover, Qi et al. discovered that leukopenia, lymphopenia, and thrombocytopenia occurred in COVID-19 patients with cirrhosis (10), which were similar to the results we obtained. Additionally, increasing evidence indicated that patients with COVID-19 exhibited a hypercoagulability in the lung (11). In this case study, the D-dimers of COVID-19 patient with cirrhosis were elevated, suggesting hypercoagulability of the patient. The liver synthesizes a variety of coagulation factors. When cirrhosis causes liver insufficiency, the production of coagulation factors is reduced, which leads to prolonged prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT), and a decrease of fibrinogen. Therefore, the PT, APTT, and TT of COVID-19 patient with cirrhosis were prolonged and fibrinogen was decreased, which was similar to the previous study (12). In addition, venous thromboembolism (VTE) including deep venous thrombosis (DVT) is common in cirrohsis patients. Additionally, the patient in this case study was treated with antiviral drugs, which had no obvious effect on her symptoms. Previous study indicated that 96% cirrhotic patients with confirmed SARS-CoV-2 infection needed

hospitalization or prolonged an ongoing one (13). In this case study, we observed similar results. This COVID-19 patient with cirrhosis was hospitalized for 32 days. She was tested positive for COVID-19 on day 25 of her hospital stay. Moreover, the numbers of WBC and the absolute neutrophil count and absolute lymphocyte count remained reduced in this patient. The process of advanced cirrhosis is complicated with cirrhosis-associated immune dysfunction. Cirrhosis has the potential to injure the homeostatic role of the liver in the immune system, which may be associated with the process of COVID-19. Additionally, although the mortality of COVID-19 was mediated by pulmonary involvement, cirrhosis is assumed to be a high-risk factor for severe COVID-19 because of an altered gut-liver axis and inherent immune dysfunction. Cirrhosis can impair the cellular and humoral immune system of the entire body, which may impair the removal of SARS-CoV-2. Thus, physicians may need to monitor immune indicators in COVID-19-positive patients with comorbidities that impair the immune system.

The patient in this case study was administered the plasma (200 ml) from recovered COVID-19 patients four times. After the last administration of plasma on day 30 of the hospital stay, the patient tested negative for SARS-CoV-2 three consecutive times and then she was discharged on day 32 of her hospital stay. This suggested that the treatment for COVID-19 is passive immunotherapy. Cirrhosis can impair the homeostatic role of the liver in the systemic immune response; thus, passive immunotherapy, such as plasma administration from recovered COVID-19 patients, may be an option for treatment. However, this case study has a limitation that needs to be cautious. These findings have only been observed in one patient. Further multicenter with large sample studies are needed to perform to verify the results.

CONCLUSION

This case study described the symptoms, diagnosis, treatment, and management of a COVID-19 patient with cirrhosis, emphasizing the need to pay attention to underlying diseases in COVID-19-positive patients. More information about this disease is still needed in order to successfully explore its clinical management.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The First Hospital of Changsha City Committee for Clinical Research. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JZ and JS conceived and designed the study and also critically revised the manuscript. JZ and WW conducted the experiments and drafted the manuscript. DJ, KH, FZ, YX, and ZZ contributed to the revision of the manuscript. All authors have read and approved the final manuscript.

FUNDING

This study was funded by the Innovative Major Emergency Project Funding against the New Coronavirus Pneumonia in Hunan Province (Grant Nos. 2020SK3014 and 2020SK3013), the Key Research & Developmental Program of Hunan Province (2022SK2047), Chinese Public Health Union (GWLM202039), Health and Family Planning Commission Fund Project in Hunan Province (Grant No. B2017209), Natural Science Foundation of Hunan Province (Grant No. 2018JJ2452), New Coronavirus Pneumonia Emergency Project of Changsha Science and Technology Bureau (Grant Nos. kq2001010 and kq2001008), the Mittal Innovation Project of Central South University (Grant No. GCX20190879Y) and the Fundamental Research Funds for the Central Universities of Central South University (Grant No. 2018zzts930). The study funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

ACKNOWLEDGMENTS

The authors would like to thank all the co-investigators and colleagues who made this study possible. The authors would like to thank the Changsha CDC, Hunan CDC, and CCDC for their assistance with laboratory testing. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this revised manuscript.

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frontiers in Medicine

ORIGINAL RESEARCH
published: 10 December 2021
doi: 10.3389/fmed.2021.649759

Impact of the Lockdown Due to the COVID-19 Pandemic on Patients With Inflammatory Bowel Disease

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Background: The government of Japan declared a state of emergency on April 16, 2020, owing to the coronavirus disease 2019 (COVID-19) pandemic. The subsequent lockdown altered lifestyles and worsened mental illnesses. Inflammatory bowel disease (IBD) is an intestinal disorder that is affected by environmental factors. Therefore, we aimed to assess the effects of COVID-19 and the state of emergency on the lifestyle and disease activity of patients with IBD.

Methods: We conducted a questionnaire survey on patients with IBD from June 16 to August 21, 2020 during their regular follow-up at our hospital, 2 months after the state of emergency was declared.

Results: Overall, 241 patients with ulcerative colitis (UC) and 210 with Crohn's disease (CD) completed the survey, of which 82 (34%) and 97 (46%) patients, respectively, reported disease exacerbation within 2 months after the lockdown. Multivariate logistic regression analysis identified age at enrollment (odds ratio, OR 0.98, 95% CI 0.96–0.99; $P < 0.05$), sleep hours (OR, 0.74; 95% CI, 0.57–0.97; $P < 0.05$), and increased stress due to the COVID-19 pandemic (OR, 6.06; 95% CI, 1.79–20.50; $P < 0.01$) as independent factors associated with UC exacerbation. Patients with exacerbated CD were younger at CD onset and had higher patient-reported outcome 2 scores before the state of emergency than patients with non-exacerbated CD. On multivariate analysis, age (OR, 0.97; 95% CI, 0.95–0.99; $P < 0.01$) and active disease before the state of emergency (OR, 2.20; 95% CI, 1.23–3.95; $P < 0.01$) were independently associated with CD exacerbation.

Conclusions: Improving sleep quality and preventing psychological stress may be crucial in IBD management during a pandemic, especially in young patients.

Keywords: COVID-19, inflammatory bowel disease, lockdown, ulcerative colitis, Crohn's disease

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *05 January 2021*
Accepted: *23 November 2021*
Published: *10 December 2021*

Citation:
Nishida Y, Hosomi S, Fujimoto K, Nakata R, Sugita N, Itani S, Nadatani Y, Fukunaga S, Otani K, Tanaka F, Nagami Y, Taira K, Kamata N, Watanabe T, Ohfuji S and Fujiwara Y (2021) Impact of the Lockdown Due to the COVID-19 Pandemic on Patients With Inflammatory Bowel Disease. Front. Med. 8:649759. doi: 10.3389/fmed.2021.649759

INTRODUCTION

In December 2019, coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, and the outbreak rapidly spread worldwide (1). It was considered a global health emergency by the World Health Organization. One measure that was adopted by the governments of many countries, especially

those more affected by the pandemic, was the lockdown of cities. Consistent with other countries' policies, the government of Japan declared a state of emergency on April 16, 2020, which continued until May 25, 2020. Central and local governments could request or instruct behaviors to prevent the spread of infection, such as school closure, social distancing, and quarantine. Although this approach was partially successful in temporarily preventing the spread (2), concerns were raised regarding the negative impact of these measures not only in terms of economics but also for mental and physical health (3, 4). The lockdown altered sleep, exercise, and nutrition patterns; compromised treatment compliance; increased childcare and work burden (owing to the lack of a workforce); and worsened mental illnesses, such as anxiety and depression (5–8).

Inflammatory bowel diseases (IBDs), comprising ulcerative colitis (UC) and Crohn's disease (CD), are intestinal disorders affected by environmental factors, such as sleep, stress, diet, and smoking (9–13). However, few studies have evaluated the relationship between lockdown measures to control the COVID-19 pandemic and IBD exacerbation. Therefore, this study aimed to assess the effects of the COVID-19 pandemic and state of emergency on the lifestyle and disease activity of patients with IBD.

MATERIALS AND METHODS

Study Design and Participants

This study was conducted through a questionnaire survey among patients with IBD during their regular follow-up at a hospital in Japan, 2 months after the initiation of the state of emergency (from June 16 to August 21, 2020). We asked all patients with IBD who visited the hospital during this period to complete the questionnaire. Patients with repeated visits were investigated only once. The exclusion criteria were a diagnosis of IBD in the last 3 months, inability to complete the questionnaire despite assistance, presence of colostomy or ileostomy, and history of total proctocolectomy with ileal pouch-anal anastomosis.

Questionnaire Design

The questionnaire included questions regarding the patient's epidemiological history of COVID-19, demographic data (sex, age at recruitment, and age at disease diagnosis), gastrointestinal symptoms, lifestyle (sleeping time, working time, walking time, exercise time, and number of meals) before and after the declaration of the state of emergency, stress related to the state of emergency (due to childcare burden), COVID-19, family budget, inability to exercise, staying indoors, IBD, and worsening of diet and nutritional status), and current medication use.

Evaluation

Gastrointestinal symptoms were assessed before and after the state of emergency (from April 16 to May 15) using the 6-point Mayo score (14, 15) and patient-reported outcome 2 (PRO2) score (16) for UC and CD, respectively. Severe active UC, moderate active UC, mild active UC, and UC remission were defined as a 6-point Mayo score of $\geq$ 5, 3–4, 2 and 0–1, respectively (15). Severe active CD, moderate active CD, mild active CD, and CD remission were defined as a PRO2 score of $\geq$34, 14–33, 8–13, and 0–7, respectively. Patients with mild, moderate, or severe UC were defined as having active disease (16). The primary endpoint was disease exacerbation defined as an increase in the 6-point Mayo or PRO2 scores. "Stress related to the state of emergency" was defined as newly emerging stress during the state of emergency. Deterioration of adherence was defined as an increase in the number of times a patient forgot to take a prescribed medication within a week after the state of emergency.

Statistical Analysis

Continuous variables are summarized as medians and interquartile ranges. The differences in clinical characteristics were compared using either the chi-square test or Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. Multivariate logistic regression analyses were performed to identify factors associated with exacerbation. Variables in the multivariate analysis were selected among those showing significant differences in a comparison between exacerbated and non-exacerbated patients, and based on known risk factors for exacerbation.

A P-value of < 0.05 was considered significant. All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6-3), which includes statistical functions frequently used in biostatistics.

RESULTS

Patients

A total of 511 questionnaires were returned, of which 60 were excluded owing to missing items. Overall, 451 patients completed the survey. Two participants had come into close contact with confirmed cases of COVID-19, and one of these had undergone isolation; however, no cases of COVID-19 were enrolled in the study.

Disease-Related Variables

Regarding specific diagnosis and disease activity before lockdown, 241 patients had UC (remission, 213 [88.4%]; mild activity, 14 [5.8%]; moderate activity, 11 [4.6%]; severe activity, 3 [1.2%]) and 210 patients had CD (remission, 123 [58.6%]; mild activity, 46 [21.9%]; moderate activity, 39 [18.6%]; severe activity, 2 [1.0%]). The median age at enrollment was 50 years (IQR 39–63) for both patients with UC and CD. The median age at diagnosis was 31 years (IQR 24–42) in patients with UC and 25 years (IQR 19–33) in patients with CD. The median disease duration was 13 years for both patients with UC (IQR 7–23) and CD (IQR 6–24). The detailed characteristics of the patients are shown in **Table 1**.

Abbreviations: COVID-19, coronavirus disease; CD, Crohn's disease; IBD, inflammatory bowel disease; OR, odds ratio; PRO2, patient-reported outcome 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UC, ulcerative colitis.

TABLE 1 | Demographic data and disease-related variables of participants.

		Ulcerative colitis	Crohn's disease
Demographics	Number of patients	241	210
	Sex (male/female)	129/112	158/52
	Age at enrollment (years), median (IQR)	50 (39–63)	44 (34–50)
	Age at diagnosis (years), median (IQR)	31 (24–42)	25 (19–33)
	Disease duration (years), median (IQR)	13 (7–23)	13 (6–24)
	6-point Mayo score before the declaration of the state of emergency	0 (0–1)	
	6-point Mayo score during the state of emergency	0 (0–1)	
	PRO2 score before the declaration of the state of emergency		6 (0–11)
	PRO2 score during the state of emergency		9 (4–15)
Lifestyle during the state of emergency	Sleeping time (hours/day), mean (IQR)	6 (6–7)	6 (6–7)
	Working time (hours/week), median (IQR)	12 (0–40)	8 (0–8.75)
	Walking time (hours/day), median (IQR)	1 (0–1)	1 (0–1)
	Exercise time (minutes/week), median (IQR)	0 (0–120)	10 (0–40)
	Number of meals per day, median (IQR)	3 (3–3)	3 (2–3)
	Increased smoking	1 (0.4%)	14 (6.7%)
	Increased alcohol intake	29 (12.0%)	23 (11.0%)
	Deterioration of drug-adherence	3 (1.2%)	2 (1.0%)
Stress related to the state of emergency†	Stress due to childcare burden	2 (0.8%)	0 (0%)
	Stress due to COVID-19	14 (5.8%)	7 (3.3%)
	Stress due to family budget	10 (4.1%)	3 (1.4%)
	Stress due to inability to exercise	21 (8.7%)	10 (4.8%)
	Stress due to staying indoors	25 (10.4%)	18 (8.6%)
	Stress due to inflammatory bowel disease	7 (2.9%)	3 (1.4%)
	Stress due to worsening of diet and nutritional status	5 (2.1%)	2 (1.0%)
Medication	Mesalamine	214 (88.8%)	123 (58.6%)
	Enteral nutrition	0 (0%)	66 (31.4%)
	Corticosteroids	8 (3.3%)	8 (3.8%)
	Immunomodulators (azathioprine or 6-mercaptopurine)	64 (26.6%)	70 (33.3%)
	Anti-TNF therapy	31 (12.9%)	109 (51.9%)
	Ustekinumab	0 (0%)	26 (12.4%)
	Vedolizumab	11 (4.6%)	7 (3.3%)
	Tofacitinib	6 (2.5%)	not approved in Japan*
	Molecularly targeted therapies**	48 (19.9%)	141 (67.1%)

† *"Stress related to the state of emergency" was defined as newly emerging stress during the state of emergency. *Tofacitinib is not approved for the treatment of Crohn's disease in Japan. **"Molecularly targeted therapies" include anti-TNF therapy, ustekinumab, vedolizumab, and tofacitinib. IQR, interquartile range; COVID-19, coronavirus disease; PRO2, patient-reported outcome 2; TNF, tumor necrosis factor.*

Impact of the Lockdown on Disease Activity, Lifestyle, and Psychological Stress

Within 2 months after the declaration of the state of emergency, gastrointestinal symptoms worsened in 34.0% and 46.2% of patients with UC and CD, respectively. **Figure 1** shows a comparison of disease activity before and during lockdown. UC and CD activity after lockdown were as follows: UC (remission, 180 [74.7%]; mild activity, 29 [12.0%]; moderate activity, 22 [9.1%]; severe activity, 10 [4.1%]) and CD (remission, 96 [45.7%]; mild activity, 53 [25.2%]; moderate activity, 54 [25.7%]; severe activity, 2 [1.0%]). Additional treatment was only required for 14.6% and 12.4% of patients with exacerbated UC and CD, respectively. Among 213 patients with UC and 123 patients with CD who were in remission before lockdown, gastrointestinal symptoms worsened in 71 (33.3%) and 48 patients (39.0%), respectively. The rate of disease exacerbation did not significantly differ between all participants and those in remission for UC ($P = 0.921$) and CD ($P = 0.21$). In contrast, among 170 and 148 patients with UC and CD, respectively, who did not receive additional treatment due to disease exacerbation within 1 year before the state of emergency, gastrointestinal symptoms worsened in 54 (31.8%) and 63 patients (42.6%), respectively. The rate of disease exacerbation did not significantly differ between all participants and those with UC ($P = 0.671$) or CD ($P = 0.519$) who did not undergo additional treatment due to disease exacerbation within 1 year before the state of emergency. Regarding smoking, alcohol intake, and drug adherence, an increase in smoking was seen in 1 (0.4%) and 14 (6.7%), alcohol intake in 29 (12.0%) and 23 (11.0%), and a deterioration of drug-adherence in 3 (1.2%) and 2 (1.0%), UC and CD patients,

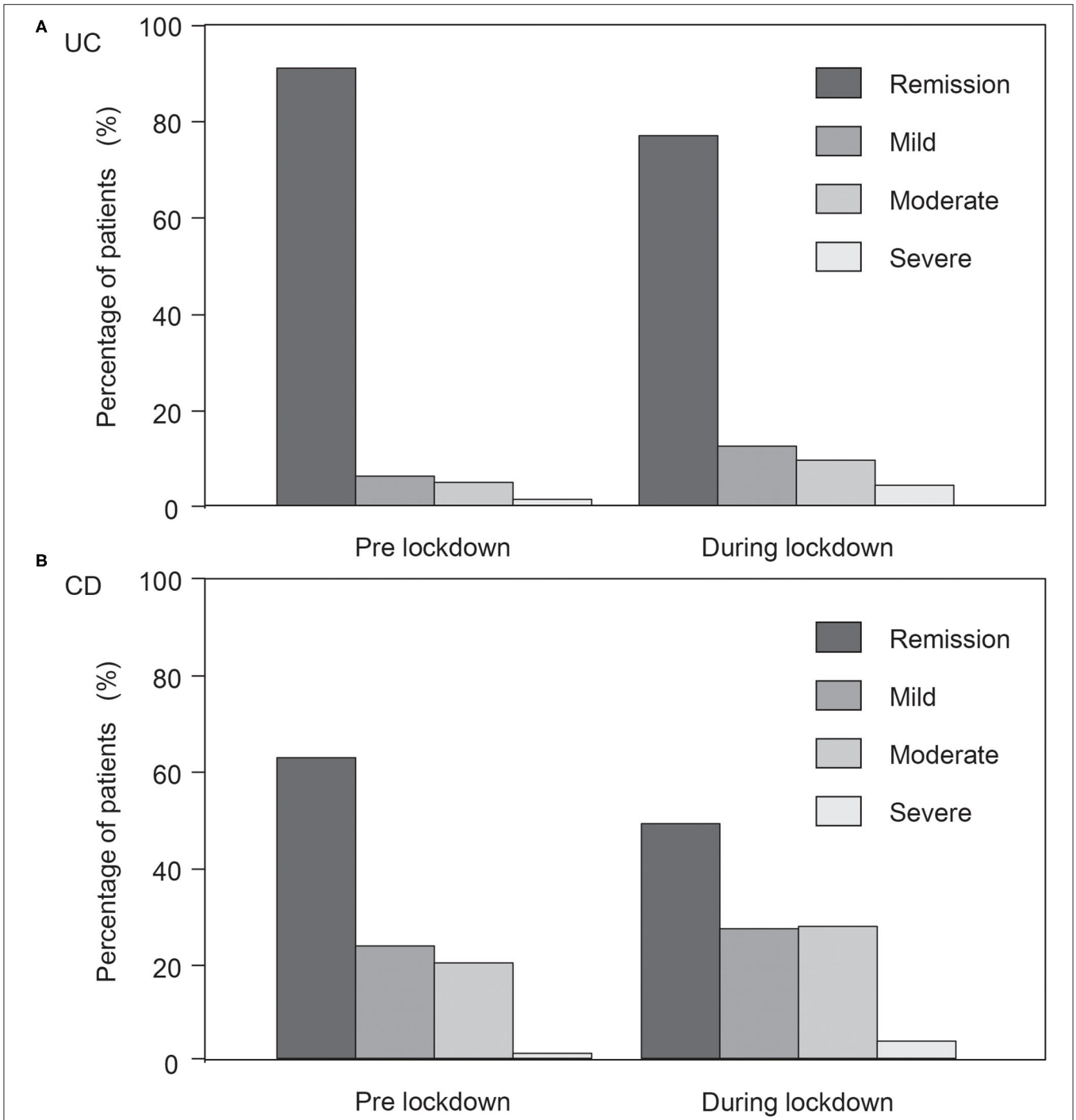

FIGURE 1 | Comparison of disease activity before and during lockdown. **(A)** Ulcerative colitis (UC). **(B)** Crohn's disease (CD). Disease activity in UC changed as follows: remission, 213 (88.4%); mild activity, 14 (5.8%); moderate activity, 11 (4.6%); severe activity, 3 (1.2%) to remission, 180 (74.7%); mild activity, 29 (12.0%); moderate activity, 22 (9.1%); severe activity, 10 (4.1%). Disease activity in CD changed as follows: remission, 123 (58.6%); mild activity, 46 (21.9%); moderate activity, 39 (18.6%); severe activity, 2 (1.0%) to remission, 96 (45.7%); mild activity, 53 (25.2%); moderate activity, 54 (25.7%); severe activity, 2 (1.0%).

respectively. Regarding psychological stress, a high percentage of people felt stress due to being forced to stay indoors or the inability to exercise, whereas the proportion of people with stress due to IBD or nutrition was not significantly high (**Table 1**).

Risk Factors for Exacerbation

Table 2 shows a comparison of patient data. Patients with exacerbated UC (within 2 months after the declaration of the state of emergency) tended to be younger and had less

sleep and more stress due to COVID-19 than patients with non-exacerbated UC. Multivariate logistic regression analysis identified age (odds ratio, OR 0.98, 95% CI 0.96–0.99; $P < 0.05$), sleep hours (OR, 0.74; 95% CI, 0.57–0.97; $P < 0.05$), and increased stress due to the COVID-19 pandemic (OR, 6.06; 95% CI, 1.79–20.50; $P < 0.01$) as independent risk factors associated with UC exacerbation (**Table 3**). Regarding patients with CD, those with exacerbations were lower age at enrollment, lower age at CD onset, and had active disease before the state of emergency than patients with non-exacerbated CD. However, multivariate analysis identified age (OR, 0.97; 95% CI, 0.95–0.99; $P < 0.01$) and active disease before the state of emergency (OR, 2.20; 95% CI, 1.23–3.95; $P < 0.01$) as independent factors associated with CD exacerbation (**Table 3**). Alcohol increase, smoking increase, and drug adherence change were not identified as independent risk factors for exacerbation.

DISCUSSION

Our results suggest that changes in daily life and stress status due to the pandemic and lockdown measures were associated with worsening IBD symptoms, especially in young patients. Possible explanations for these findings could be as follows. First, according to a recent report, the negative impact of lockdown measures on daily life may be more prevalent in younger people than in older people (4). The impact of age at IBD onset on the natural history, severity, and surgical rates have been reported to be higher in patients with elderly-onset UC than in patients with non-elderly-onset UC (17–20), whereas the rates of disease progression have been shown to be lower in patients with elderly-onset CD than in patients with non-elderly-onset CD (21, 22). In the current study, although both patients with UC and CD with worsening IBD symptoms were younger than those without worsening symptoms at enrollment, only patients with CD with worsening symptoms were younger at CD onset than those without worsening symptoms. These results indicate that patients with UC might experience episodes of exacerbations due to the impact of the lockdown, but not natural history, in contrast to patients with CD.

Second, sleep disturbances are commonly seen in patients with active IBD (23, 24) and are associated with the onset of UC (24). Ananthakrishnan et al. reported that sleep disturbance was associated with an increased risk of CD but not UC exacerbation (25). In the current study, multivariate logistic regression analysis identified fewer sleep hours as an independent risk factor associated with UC but not with CD exacerbation. This discrepancy may occur owing to the quality of sleep. Only sleep time could be evaluated as a sleep factor, as the questionnaire used in this study did not include questions associated with sleep disturbance or use of medications that could estimate the quality of sleep. Therefore, further studies will be required to explain this discrepancy.

Finally, stress resulting from the fear of contracting a potentially lethal disease that affects mostly immunosuppressed individuals might aggravate IBD symptoms, though an inverse relation cannot be excluded (IBD exacerbation could cause psychological stress). Several studies have reported the psychological impact of the pandemic on the general population and demonstrated an increase in the level of anxiety during the pandemic, and patients with IBD are more likely to develop anxiety disorders (26–28).

In this study, no case of COVID-19 was registered; however, this does not mean patients with IBD were less likely to contract COVID-19. This is probably owing to the small sample size and low infection rate of COVID-19 during this period in Japan. Indeed, IBD *per se* does not increase the risk of developing COVID-19 (29), and patients with IBD receiving immunomodulators, biological agents, or JAK inhibitors do not have an increased risk of contracting SARS-CoV-2 infection or developing a more severe course of infection (30). Only corticosteroid use was reported to be associated with severe COVID-19 among patients with IBD (31). However, elderly patients or those with comorbidities have a poorer clinical outcome after contracting COVID-19 (30, 32, 33). Based on this evidence and the results of our study, older patients or those with current use of corticosteroid treatments need thorough observation and early intervention to prevent the potential development of severe COVID-19. In addition, younger patients should be careful to prevent exacerbations of IBD during lockdown because they are likely to worsen. Additionally, the results of our study suggested that patients in remission or those who did not require additional treatment within 1 year before the state of emergency had a similar risk of disease exacerbation during the state of emergency. The multivariate logistic regression analysis that included scores for gastrointestinal symptoms also supported this finding.

This study has some limitations, including its single-center nature and relatively small cohort, which could be prone to bias in data selection and analysis. Moreover, the results of our study should have been compared with the rate and factors for gastrointestinal disease exacerbation before the COVID-19 pandemic occurred; however, this comparison could not be performed because of the lack of relevant pre-COVID-19 pandemic data for these diseases. Additionally, we were unable to evaluate objective factors (e.g., laboratory examinations, endoscopic activities, disease locations) because anonymity needed to be maintained in the questionnaire. Patients who experienced disease exacerbation may have had a functional disorder, but it was difficult to assess the influence of such based only on subjective factors. Only 14.6 and 12.4% of patients with exacerbated UC and CD, respectively, required additional treatment, some of which may have had a functional disorder or experienced mild exacerbation of the disease. Disease exacerbation in this study was defined as an increase of 1 point or more in the 6-point Mayo or PRO2 scores. However, since disease exacerbation was not evaluated objectively, it could not be quantified. Further, change in the line of treatments for IBD flare-up during the COVID-19 pandemic should have been evaluated, as alterations could have been made throughout this period that may have affected our results. For example, the European Crohn's and Colitis Organization position statement recommended the use of subcutaneous drugs for IBD flare-ups to minimize hospital visits (34). However, we could not evaluate

TABLE 2 | Descriptive comparison of participants with and without exacerbation.

		Patients with ulcerative colitis			Patients with Crohn's disease		
		Non-exacerbated	Exacerbated	*P*-value	Non-exacerbated	Exacerbated	*P*-value
Demographics	Number of patients	159	82		113	97	
	Sex (male/female)	82/77	47/35	0.416	85/28	73/24	1
	Age at enrolment (years), median (IQR)	51 (39–66)	46.5 (39–56.5)	0.051	46 (35–54)	42 (31–48)	0.014
	Age at diagnosis (years), median (IQR)	32 (24.5–43)	30 (22–41.75)	0.169	26 (20–38)	24 (18–28)	0.013
	Disease duration (years), median (IQR)	13 (7–23)	13.5 (5–22.25)	0.533	13 (5–25)	15 (7–22)	0.788
	6-point Mayo score before the declaration of the state of emergency	0 (0–1)	0 (0–1)	0.221			
	PRO2 score before the declaration of the state of emergency				5 (0–10)	8 (2–13)	0.025
Lifestyle during the state of emergency	Sleeping time (hours/day), mean (IQR)	6 (6–7)	6 (6–7)	0.073	6 (6–7)	7 (6–7)	0.763
	Working time (hours/week), median (IQR)	10 (0–40)	16 (0–40)	0.171	8 (0–9)	8 (0–8)	0.901
	Walking time (hours/day), median (IQR)	1 (0–1)	1 (0–1)	0.295	1 (1–1)	1 (0–1)	0.491
	Exercise time (minutes/week), median (IQR)	0 (0–120)	0 (0–120)	0.917	10 (0–60)	15 (0–30)	0.819
	Number of meals per day, median (IQR)	3 (3–3)	3 (3–3)	0.493	3 (2–3)	3 (2–3)	0.593
	Increased smoking	1 (0.6%)	0 (0.0%)	1	8 (7.1%)	6 (6.2%)	1
	Increased alcohol intake	17 (10.7%)	12 (14.6%)	0.406	12 (10.6%)	11 (11.3%)	1
	Deterioration of drug-adherence	1 (0.6%)	2 (2.4%)	0.268	1 (1.0%)	1 (1.1%)	1
Stress related to the state of emergency†	Stress due to childcare burden	1 (0.6%)	1 (1.2%)	1	0 (0%)	0 (0%)	NA
	Stress due to COVID-19	4 (2.5%)	10 (12.2%)	0.006	2 (1.8%)	5 (5.2%)	0.253
	Stress due to family budget	7 (4.4%)	3 (3.7%)	1	1 (0.9%)	2 (2.1%)	0.597
	Stress due to inability to exercise	12(7.5%)	9 (11%)	0.47	4 (3.5%)	6 (6.2%)	0.519
	Stress due to staying indoors	15(9.4%)	10 (12.2%)	1	11 (9.7%)	7 (7.2%)	0.624
	Stress due to inflammatory bowel disease	3 (1.9%)	4 (4.9%)	0.51	2 (1.8%)	1 (1.0%)	1
	Stress due to worsening of diet and nutritional status	3 (1.9%)	2 (2.4%)	0.55	0 (0%)	2 (2.1%)	0.212
Medication	Mesalamine	142 (89.3%)	72 (87.8%)	0.83	67 (59.3%)	56 (57.7%)	0.888
	Enteral nutrition	0 (0%)	0 (0%)	NA	36 (31.9%)	30 (30.9%)	1
	Corticosteroids	5 (3.1%)	3 (3.7%)	1	3 (2.7%)	5 (5.2%)	0.475
	Immunomodulators (azathioprine or 6-mercaptopurine)	47 (29.6%)	17 (20.7%)	0.167	35 (31%)	35 (36.1%)	0.465
	Anti-TNF therapy	17 (10.7%)	14 (17.1%)	0.222	55 (48.7%)	54 (55.7%)	0.335
	Ustekinumab	0 (0%)	0 (0%)	NA	13 (11.5%)	13 (13.4%)	0.681
	Vedolizumab	8 (5%)	3 (3.7%)	0.754	2 (1.8%)	5 (5.2%)	0.253
	Tofacitinib	3 (1.9%)	3 (3.7%)	0.404	Not approved in Japan		
	Molecularly targeted therapies**	28 (17.6%)	20 (24.4%)	0.235	70 (61.9%)	71 (73.2%)	0.105

† *"Stress related to the state of emergency" was defined as newly emerging stress during the state of emergency. *Tofacitinib is not approved for the treatment of Crohn's disease in Japan. **"Molecularly targeted therapies" include anti-TNF therapy, ustekinumab, vedolizumab, and tofacitinib. COVID-19, coronavirus disease; IQR, interquartile range; PRO2, patient-reported outcome 2; TNF, tumor necrosis factor.*

the details of the additional treatment for the exacerbation because the questionnaire did not include these items. Further, we used simple questions rather than validated ones for psychological factors to reduce the burden on respondents and increase the response rate in consideration of the large number of questions.

Another possible limitation of this study was possible selection bias. Since patients visiting the clinic are likely to have more symptoms (or less), the results may be biased and not generalizable to all patients with IBD. In addition, this study was conducted in a single-tertiary center, which may suggest the patients have more complicated disease. However, in Japan,

TABLE 3 | Logistic regression analyses of factors associated with exacerbation.

Patients with UC	Univariate OR (95% CI)	*P*-value	Multivariate OR (95% CI)†	*P*-value
Age at enrollment	0.98 (0.96–0.99)	0.036	0.98 (0.96–0.99)	<0.05
Age at onset	0.98 (0.98–1.00)	0.106		
Sleep hours	0.81 (0.63–1.03)	0.086	0.74 (0.57–0.97)	<0.05
Stress due to the COVID-19 pandemic				
No	Ref		Ref	
Yes	5.38 (1.63–17.70)	< 0.01	6.06 (1.79–20.50)	<0.01
Disease activity				
Remission	Ref		Ref	
Active	1.29 (0.58–2.91)	0.53	1.27 (0.53–3.04)	0.59
Smoking habit				
Decrease / No change	Ref		Ref	
Increase	9.10e-7 (0–inf)	0.99	7.28e-7 (0–inf)	0.98
Alcohol intake				
Decrease / No change	Ref		Ref	
Increase	1.43 (0.65–3.16)	0.38	1.69 (0.74–3.85)	0.22
Drug adherence				
Improvement / No change	Ref		Ref	
Deterioration	3.95 (0.35–44.20)	0.27	3.02 (0.25–36.5)	0.39
Patients with CD	**Univariate OR (95% CI)**	***P*-value**	**Multivariate OR (95% CI)**	***P*-value**
Age at enrollment	0.97 (0.95–0.99)	<0.05	0.97 (0.94–0.99)	<0.01
Age at onset	0.97 (0.94–0.99)	<0.01		
Sleep hours	1.00 (0.77–1.29)	0.99	0.97 (0.74–1.27)	0.81
Stress due to the COVID-19 pandemic				
No	Ref		Ref	
Yes	3.02 (0.57–15.90)	0.19	3.69 (0.64–21.40)	0.15
Disease activity				
Remission	Ref		Ref	
Active	2.01 (1.15–3.52)	<0.05	2.20 (1.23–3.95)	<0.01
Smoking habit				
Decrease / No change	Ref		Ref	
Increase	0.86 (0.29–2.59)	0.8	0.88 (0.26–3.03)	0.84
Alcohol intake				
Decrease / No change	Ref		Ref	
Increase	1.08 (0.45–2.56)	0.87	0.89 (0.35–2.30)	0.82
Drug adherence				
Improvement / No change	Ref		Ref	
Deterioration	1.17 (0.07–18.90)	0.91	1.21 (0.07–21.80)	0.90

Age at enrollment, age at onset and sleep hours were considered as continuous variables. † *Adjusted for factors, including age at enrollment, sex, short sleep, stress due to the COVID-19 pandemic, increased smoking, increased alcohol intake, drug adherence deterioration, and active disease. COVID-19, coronavirus disease; PRO2, patient-reported outcome 2; UC, ulcerative colitis; CD, Crohn's disease; CI, confidence interval; OR, odds ratio.*

almost all patients with IBD visit the clinic regularly even if they have no symptoms. In addition, it is difficult to include patients with IBD without clinical visits. Although our hospital is a tertiary medical institution, it also provides regular follow-up for patients with remission or mild IBD. Therefore, this may only moderately limit the generalizability of the findings.

Furthermore, as the questionnaire was completed based on memory recall, a response bias could have influenced the answers of the study participants. This may be owing to fatigue from answering many questions or difficulty in remembering gastrointestinal symptoms or lifestyles before the state of emergency; thus, 60 of 511 participants (11.7%) could not complete the questionnaire. As this was a retrospective study, a possibility of reverse causality may have occurred. It is possible that patients were having more symptoms from their disease due to exacerbation, which in turn led to poor sleep and increasing stress. Therefore, further large prospective studies are needed to confirm the impact of a lockdown on patients with IBD.

This study is the first to provide data on the association between IBD activity and lifestyle changes/psychological stress

due to the state of emergency during the COVID-19 pandemic. Our finding suggests that improving the quality of sleep and preventing psychological stress may be significant factors in improving IBD management during a pandemic, especially among young patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Osaka City University Graduate School of Medicine. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SH conceived the study and supervised the overall study. YNi and SH wrote the manuscript. Data collection and analysis were performed by YNi, SH, KF, SI, NK, and SO. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank all the patients that completed the survey.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Medicine

SYSTEMATIC REVIEW
published: 22 December 2021
doi: 10.3389/fmed.2021.676581

Elevated De Ritis Ratio Is Associated With Poor Prognosis in COVID-19: A Systematic Review and Meta-Analysis

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OPEN ACCESS

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Specialty section:
This article was submitted to Gastroenterology, a section of the journal Frontiers in Medicine

Received: *05 March 2021*
Accepted: *22 November 2021*
Published: *22 December 2021*

Citation:
Pranata R, Huang I, Lim MA, Yonas E, Vania R, Lukito AA, Nasution SA, Siswanto BB and Kuswardhani RAT (2021) Elevated De Ritis Ratio Is Associated With Poor Prognosis in COVID-19: A Systematic Review and Meta-Analysis. Front. Med. 8:676581. doi: 10.3389/fmed.2021.676581

Objective: This meta-analysis aims to assess whether elevated De Ritis ratio is associated with poor prognosis in patients with coronavirus 2019 (COVID-19).

Methods: A systematic literature search was performed using PubMed, Embase, and EuropePMC databases up until September 17, 2021. De Ritis ratio is also known as Aspartate aminotransferase/alanine transaminase (AST/ALT) ratio. The main outcome was poor prognosis, a composite of mortality, severity, the need for ICU care, and intubation. The effect measure was odds ratios (ORs) and mean differences. We generated sensitivity and specificity, negative and positive likelihood ratio (NLR and PLR), diagnostic odds ratio (DOR), and area under curve (AUC).

Results: There were eight studies with 4,606 patients. De Ritis ratio was elevated in 44% of the patients. Patients with poor prognosis have higher De Ritis ratio [mean difference 0.41 (0.31, 0.50), $p < 0.001$; I^2: 81.0%] and subgroup analysis showed that non-survivors also have higher De Ritis Ratio [mean difference 0.47 (0.46, 0.48), $p < 0.001$; I^2: 0%]. Elevated De Ritis ratio was associated with poor prognosis [OR 3.28 (2.39, 4.52), $p < 0.001$; I^2: 35.8%]. It has a sensitivity of 55% (36–73), specificity of 71% (52–85), PLR 1.9, NLR.63, DOR of 3 (2–4), and AUC of.67 (0.63–0.71). The posterior probability of poor prognosis was 38% if De Ritis is elevated, while 17% if De Ritis is not elevated.

Conclusion: Elevated De Ritis ratio is associated with poor prognosis in patients with COVID-19.

Systematic Review Registration: PROSPERO ID: CRD42020216634.

Keywords: coronavirus—COVID-19, liver enzyme, transaminase, SARS-CoV-2, De Ritis ratio

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread rapidly and causes a considerable number of deaths worldwide (1). Although most patients with coronavirus 2019 disease (COVID-19) have mild-to-moderate symptoms, they may develop severe COVID-19 with multi-organ dysfunction, cardiorespiratory collapse, coagulopathy and thrombosis, sepsis, and even death (2, 3). Common symptoms include fever, cough and dyspnea, and minor symptoms are dysgeusia, anosmia, gastrointestinal symptoms, cutaneous manifestation, and headache (4–6). Although the virus primarily affects the lungs, it may invade and damage other organs, such as the heart and vasculature, coagulation system, liver, kidneys, intestine, and central nervous system (7–12).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported to cause a varying degree of liver injury (13). Liver injury is more frequently found in patients with severe COVID-19 and is associated with an increased risk of poor outcomes (14). The ratio between the two most routinely requested liver function panel, the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio or more commonly known as the De Ritis ratio, was recently reported as a possible biomarker for prognostication in patients with COVID-19 (15). Therefore, we conducted a systematic review and meta-analysis to evaluate the association between De Ritis ratio and composite poor outcomes in COVID-19.

MATERIALS AND METHODS

The study was registered in the PROSPERO database (CRD42020216634) and was conducted per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Eligibility Criteria

Research articles (both prospective and retrospective cohorts) that contain information on De Ritis ratio and mortality, severity, intensive care unit (ICU) care admission or need for intubation were included in the study. We excluded preprints, review articles, editorial, commentaries, conference abstracts, letters, and case reports/series.

Search Strategy and Study Selection

We performed a systematic literature search from PubMed database, Embase database, and EuropePMC database with the search terms "COVID-19" OR "2019-nCoV" OR "SARS-CoV-2" AND "De Ritis Ratio" OR "AST ALT Ratio." The search was finalized on September 17, 2021. The PubMed search strategy was [(COVID-19) OR (2019-nCoV) OR (SARS-CoV-2)] AND [(De Ritis Ratio) OR (AST ALT Ratio)]. Two independent authors performed the initial search and duplicate removal. The inclusion and exclusion criteria served as the basis for article exclusion during the title or abstract screening and evaluation of full-text articles.

Abbreviations: ACE2, Angiotensin receptor enzyme 2; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; AUC, Area under curve; COVID-19, Coronavirus disease 2019; DOR, Diagnostic odds ratio; OR, Odds ratio; PLR, Positive likelihood ratio; NLR, Negative likelihood ratio; SARS-CoV-2, Severe cute respiratory syndrome coronavirus 2.

Data Collection

Data extraction from the eligible studies was conducted by two authors who are independently using pre-built forms containing the author, study design, origin, AST, ALT, cut-off for elevated De Ritis ratio, sample size, age, gender, obesity, diabetes, elevated liver enzymes, and outcome of interests.

The main outcome was poor prognosis, a composite of mortality, severity, need for ICU care, and need for intubation. Mortality was defined as non-survivor or death.

Severity was defined according to the studies inclusion parameters, need for ICU care, and intubation. The effect measure was the odds ratios (ORs) and mean differences. Diagnostic meta-analysis was performed to generate diagnostic values, which consisted of sensitivity, specificity, negative and positive likelihood ratio (NLR and PLR), diagnostic odds ratio (DOR), and area under curve (AUC).

Risk of Bias Assessment

The risk of bias assessment was performed independently by two authors with the help of Newcastle-Ottawa Scale (NOS). Discrepancies were resolved by discussion. The Egger's test and Deek's funnel plot asymmetry test was used to assess the presence of small-study effects and publication bias, respectively.

Statistical Analysis

STATA 16 (College Station, TX) was used to perform statistical analysis. Meta-analysis of proportions was performed to pool the incidence of elevated De Ritis Ratio. DerSimonian and Laird method random-effects models were used to pool ORs and mean differences, notwithstanding heterogeneity. $p < 0.05$ were considered statistically significant. Inter-study heterogeneity was evaluated using the I-squared (I^2) and Cochrane Q test, an $I^2 > 50\%$ or $p < 0.10$ indicates substantial heterogeneity. We performed pooling of sensitivity and specificity and generated a summary receiver operating characteristic (SROC) curve. Relationship between prior probability and posterior probability was evaluated using Fagan's nomogram. Subgroup analysis was performed for mortality outcome.

RESULTS

Baseline Characteristics

There were eight studies with 4,606 patients in this meta-analysis (**Figure 1**) (7, 16–19). The mean age of patients in this study was 64.3 years, whereas 46.3% of the patients were male. The characteristics of the studies are presented in **Table 1**. Patients with poor prognosis have higher AST levels [mean difference 8.82 (5.47, 12.17), $p < 0.001$; I^2: 71.7%, $p = 0.007$] (**Figure 2A**), but not ALT levels [mean difference 0.43 (−5.03, 5.88), $p = 0.878$; I^2: 88.3%, $p < 0.001$] (**Figure 2B**). De Ritis ratio was elevated in 24% of the patients. Poor prognosis occurs in 26% of the patients.

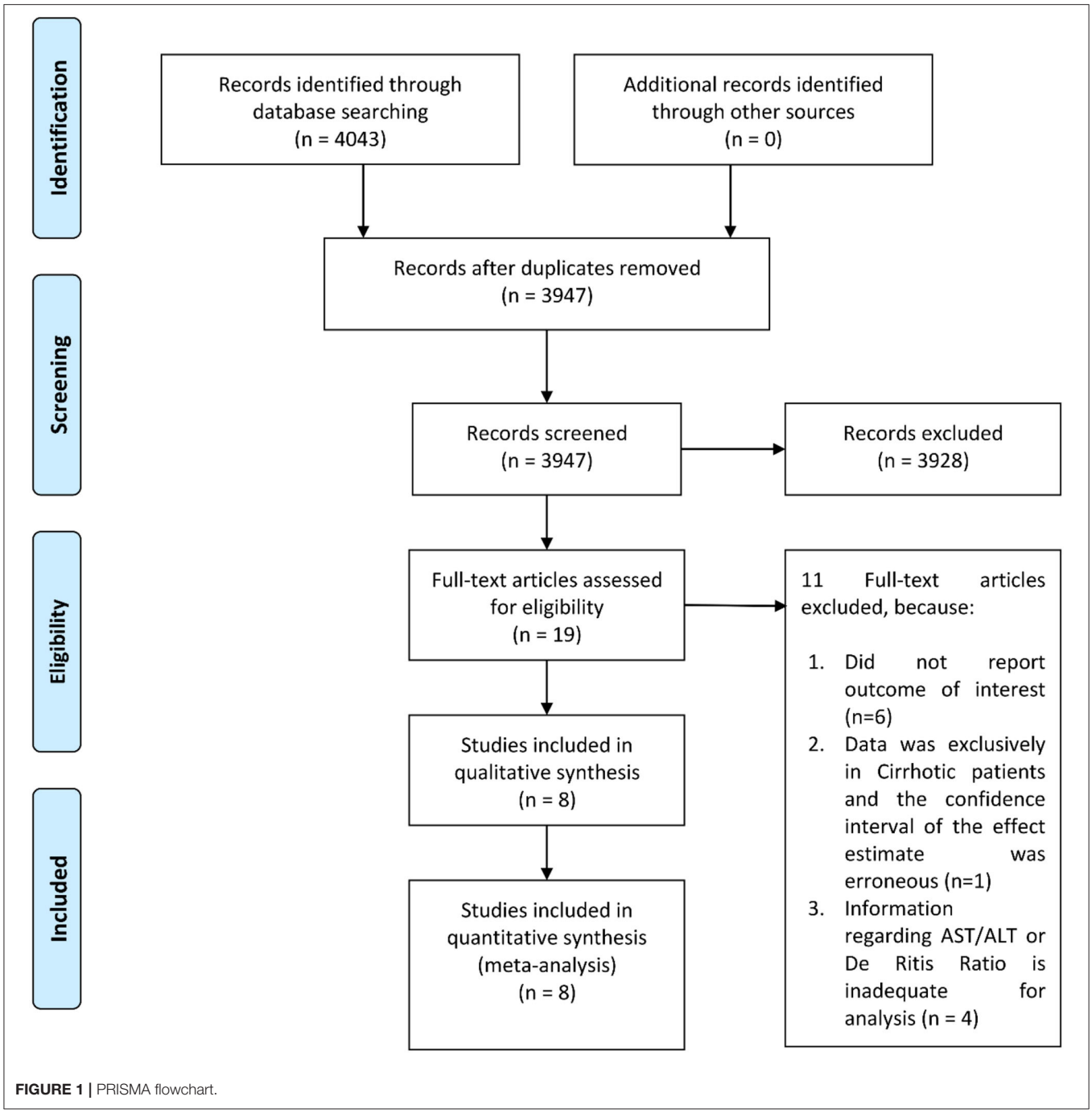

FIGURE 1 | PRISMA flowchart.

Elevated De Ritis Ratio and Poor Prognosis

Patients with poor prognosis have higher De Ritis ratio [mean difference 0.41 (0.31, 0.50), $p < 0.001$; I^2: 81.0%, $p < 0.001$] (**Figure 3**) and subgroup analysis showed that non-survivors also have higher De Ritis Ratio [mean difference 0.47 (0.46, 0.48), $p < 0.001$; I^2: 0%, $p = 0.463$]. Elevated De Ritis ratio was associated with poor prognosis [OR 3.28 (2.39, 4.52), $p < 0.001$; I^2: 35.8%, $p = 0.182$] (**Figure 4**) and subgroup analysis also showed that elevated De Ritis ratio was associated with mortality [OR 3.36 (1.93, 5.85), $p < 0.001$; I^2: 51.7%, $p = 0.102$]. It has a sensitivity of 55% (36–73), specificity of 71% (52–85), PLR 1.9, NLR 0.63, DOR of 3 (2–4), and AUC of 0.67 (0.63–0.71) (**Figure 5**). The posterior probability of poor prognosis was 38% if De Ritis was elevated, while 17% if De Ritis was not elevated (**Figure 6**).

Risk of Bias Assessment

Newcastle-Ottawa Scale (NOS) indicates a low-moderate risk of bias (**Table 1**). There is no indication of small-study effects in the relationship between elevated De Ritis ratio and poor

TABLE 1 | Characteristics of the included studies.

Authors	Study design	Study origin	Cut-off for elevated De Ritis	Samples	Age (mean)	Male (%)	Obesity	Diabetes (%)	Elevated liver enzymes (%)	Outcome	NOS
Benedé-Ubieto (20)	RC	Spain	NA	799	Stratified	54.7	NA	NA	NA	Mortality (17.5%)	6
Chen (18)	RC	China	>1	227	51	45.5	NA	9	4.5	Mortality (11.8%)	5
Medetalibeyoglu (17)	RC	Turkey	>1	554	66.2	58.7	BMI (29.39)	22.7	153/554 (27.6)	Severity (13.9%), Mortality (7.2%)	7
Paliogiannis (19)	RC	Italy	NA	60	71.5	60	NA	25	NA	Mortality (30.0%)	6
Qin (16)	RC	China	>1.38	567	55	43.6	NA	15	103/567 (18.2)	Mortality (11.5%)	8
Ramos-Lopez (21)	RC	Spain	1.29	2,094	66.9	39.4	NA	NA	NA	Mortality + ICU (21.4%)	8
Yadlapati (22)	RC	United States	1.2	200	66.5	45.5	50	NA	110/200 (55)	Mortality (26%)	6
Zinellu (7)	RC	Italy	>1.63	105	72	66.7	21.9	21	51/105 (48.6)	Mortality (26.7%)	9

BMI, body mass index; NOS, newcastle-ottawa scale; RC, retrospective cohort.

prognosis ($p = 0.488$). Deek's funnel plot asymmetry test was non-significant ($p = 0.81$).

DISCUSSION

Early identification of patients at risk for developing severe COVID-19 is crucial during the pandemic. Previous studies highlighted that individuals with advanced age, high body mass index, and physical inactivity had greater morbidity and mortality from COVID-19, along with the presence of various comorbidities, such as cardiovascular disease, diabetes, chronic obstructive pulmonary disease, hypertension, and chronic kidney disease (23–31). Several inflammatory parameters, comprising C-reactive protein, D-dimer, procalcitonin, interleukin-6, and ferritin, are often higher in patients with severe and critically ill with COVID-19 (8). An increase in liver-related biomarkers, particularly AST, ALT, total bilirubin concentrations, and gamma-glutamyl transferase in patients with COVID-19 have been reported (32, 33).

Although hepatic damage is not commonly seen as a major characteristic of COVID-19, liver injury is an emerging concern because it may indicate a severe disease course (2). The mechanism for liver involvement in COVID-19 remains obscure. Previous liver pathology reports showed the presence of moderate microvesicular steatosis along with mild inflammation in several areas (34). These patterns are also observed in drug-induced liver injury and sepsis, although these findings are not unique, they might provide insight into the mechanism involved in liver injury induced by COVID-19 (35). The SARS-CoV-2 may invade the liver directly through the angiotensin receptor enzyme 2 (ACE2) receptor, which serves as the novel coronavirus' entry point. It has been found that bile duct epithelial cells (cholangiocytes) express a high amount of ACE2 receptors (36). Liver dysfunction may also be caused by drug-induced liver injury or an overactive inflammatory response, including cytokine storm and pneumonia-associated hypoxia (2, 7). Antivirals used in the treatment of COVID-19 are postulated to cause drug-induced liver injury (37).

Serum concentrations of ALT and AST, without exception, are the most frequently ordered liver panel for evaluating liver injury in all laboratories. ALT is present in the cytosol of hepatocytes, while AST is present in the cytoplasm and mitochondria of the hepatocyte (38). ALT activity in the liver is ∼10-fold higher than that of the heart and skeletal muscles, which emphasizes its function to indicate parenchymal liver disease or injury. Meanwhile, AST has the greatest activity in the liver, cardiac, and skeletal muscle, but also exhibits in other tissues including kidneys, pulmonary, brain, pancreas, red blood cells, and white blood cells. Therefore, ALT is a more specific biomarker for liver damage compared to AST, indicating liver-biliary disease, myocardial injury, and rhabdomyolysis (7, 15). AST and ALT are found in the liver with a 2.5:1 ratio but with different turnaround time, resulting in a relatively similar level of serum of AST and ALT in healthy populations (38).

The De Ritis ratio or the AST/ALT ratio is a promising biochemical parameter for prognostication in COVID-19. In the

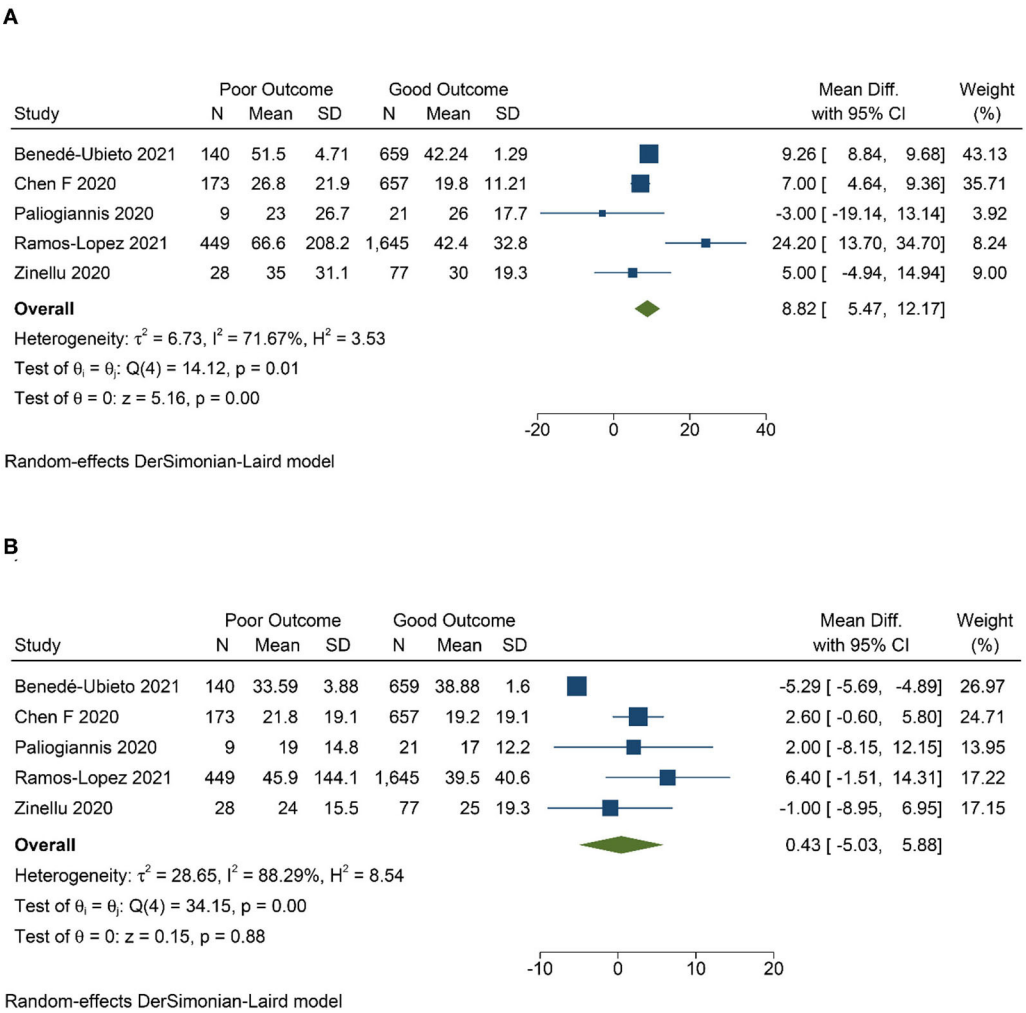

FIGURE 2 | Mean difference in aspartate aminotransferase (AST) **(A)** and alanine transaminase (ALT) **(B)** level between poor and good prognosis.

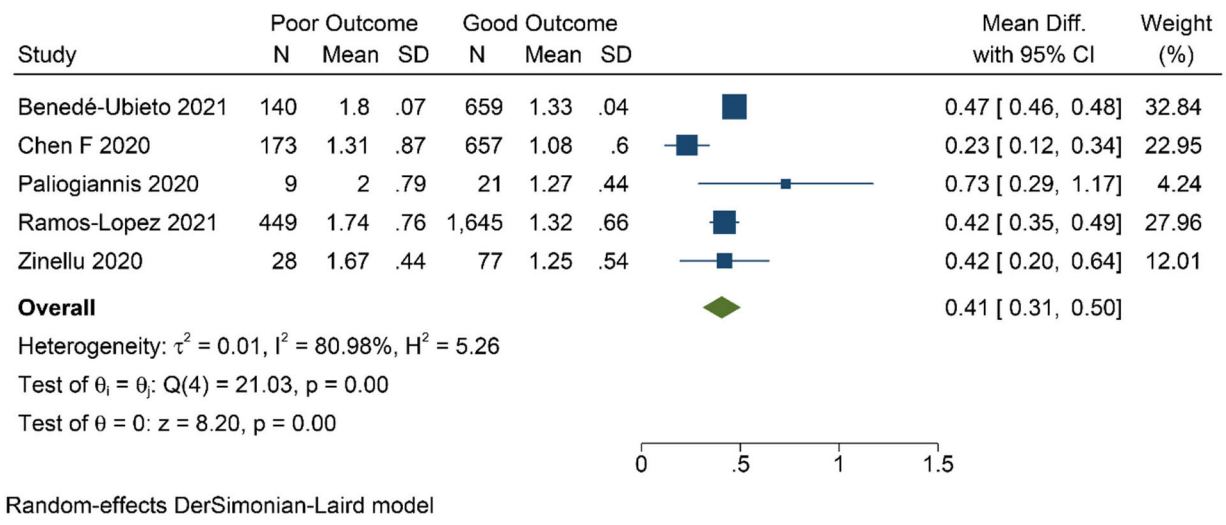

FIGURE 3 | Mean difference in De Ritis ratio between poor and good prognosis.

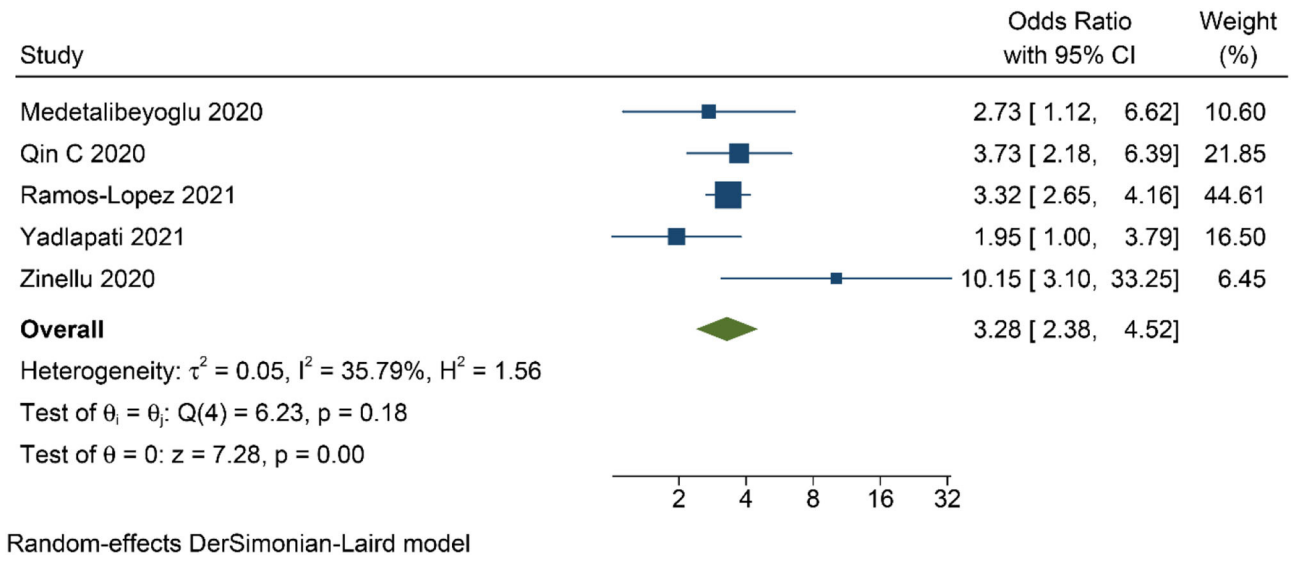

FIGURE 4 | Odds ratio for elevated De Ritis ratio and poor prognosis.

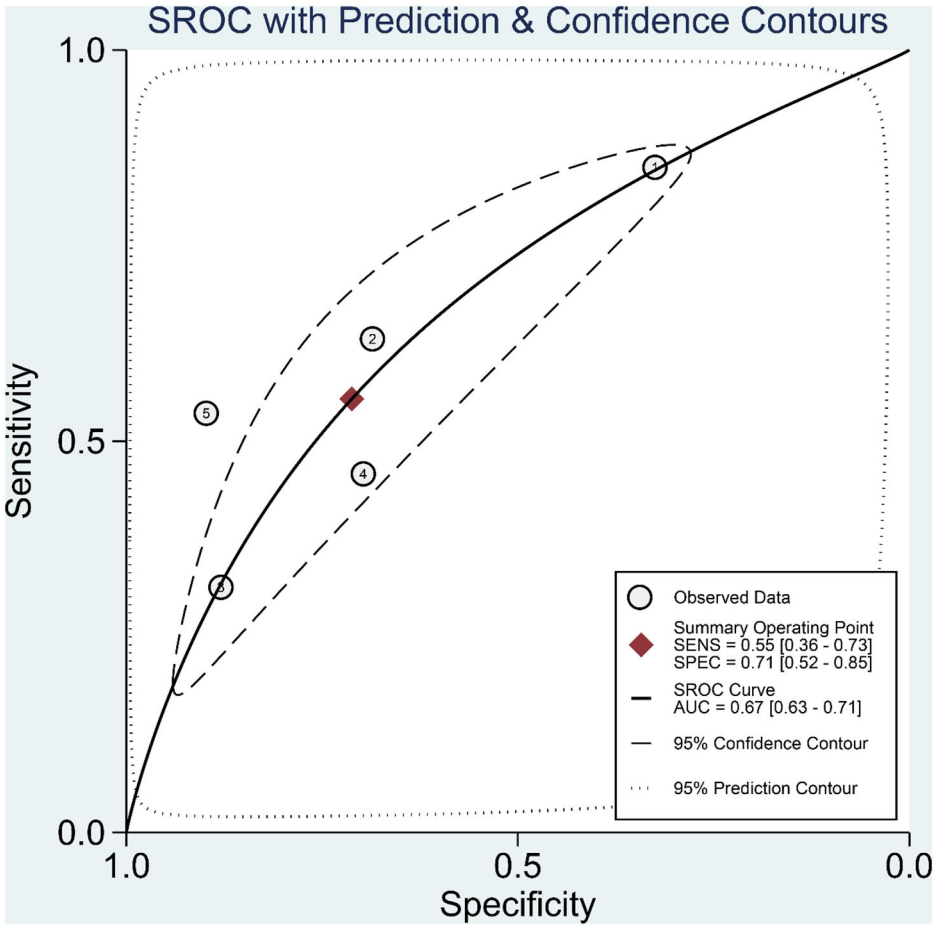

FIGURE 5 | Summary receiver operating characteristic (SROC) curve.

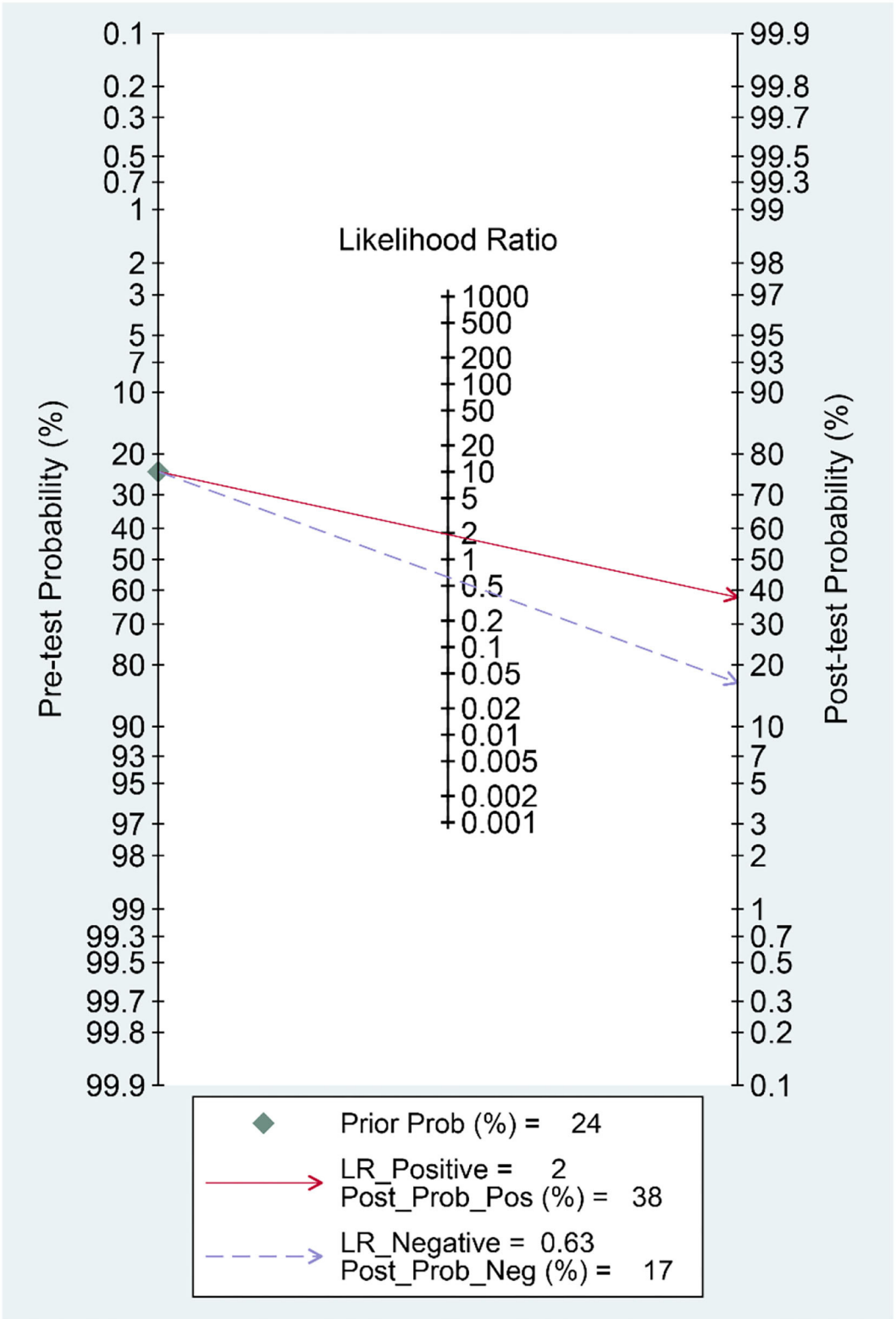

FIGURE 6 | Fagan's nomogram.

present study, elevated De Ritis ratio was associated with 3-fold increased risk for poor prognosis in patients with COVID-19. Although the cut-off values for elevated De Ritis ratio are different from these five studies (**Table 1**), the result of this meta-analysis has low heterogeneity (I^2: 35.8%). Nonetheless, the difference in the cut-off value used between those studies caused a highly varied diagnostic value (**Figure 3**) with an overall sensitivity of 55%, specificity of 71%, and AUC of 0.67. These variations further translate into the uncertainty of the optimal cut-off value for De Ritis Ratio as a prognostic factor in COVID-19 and merit further investigations.

Interestingly, Qin et al. indicated that De Ritis ratio of ≥1.38 was independently associated with poor prognosis irrespective of AST elevation (≤40 and >40 U/L) (16). They showed that AST/ALT ratio elevation was associated with a more severely computed tomography scan findings, higher severity, and positive linear association with other prognostic markers (e.g., c-reactive protein, procalcitonin, interleukin-6, D-Dimer, lactate, LDH, and creatine Kinase-MB). Additionally, Chen et al. showed the association of AST/ALT ratio with liver injury and severity of COVID-19. However, the number of outcomes or risk estimates (e.g., OR) of this study interest was not available (18).

There were two studies on the association of De Ritis ratio with other specific biochemical parameters (e.g., creatinine kinase and serum ALT), but were excluded from the analysis due to its irrelevance with our outcome of interest (15, 39).

The limitations of the current study were primarily caused by the small quantity of the included studies. Moreover, the retrospective-observational nature and the small sample size of the included studies should be taken into account in extrapolating the results of this meta-analysis, where selection bias and confounding factors may be evident. We also could not dismiss the possibility of publication bias due to the small number of studies. Despite our limitations, this meta-analysis has brought early evidence of using the De Ritis ratio for prognostication in COVID-19.

Implication for Clinical Practice

Although this "traditional" ratio was initially found in 1957 as a diagnostic test for viral hepatitis (40), it is still commonly used and proves to be a valuable indicator of liver disease (38). It is a promising, straightforward, and readily available parameter for poor prognosis in COVID-19. This meta-analysis showed that AST, but not ALT, was significantly associated with poor prognosis in COVID-19. This supports the use of De Ritis ratio in addition to AST and ALT levels. However, we suggest, including this parameter and other accessible hematological markers, to improve the prognostic performance of the model for COVID-19. De Ritis ratio would be better for this marker to be a part of a prognostic model rather than a stand-alone examination. A predictive model comprising of readily available tools may be of value, especially in rural areas where sophisticated prognostic biomarkers are often not available.

In conclusion, elevated De Ritis ratio is associated with poor prognosis in patients with COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RP: conceptualization, methodology, formal analysis, investigation, and writing—original draft. IH: data curation, investigation, writing—original draft, and project administration. ML: data curation, investigation, and writing—original draft. EY and RV: investigation and writing—original draft. AL, SN, BS, and RK: investigation and writing—review and editing. All authors contributed to the article and approved the submitted version.

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