Priority Research Paper

Serum IgM against SARS-CoV-2 correlates with in-hospital mortality in severe/critical patients with COVID-19 in Wuhan, China

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ABSTRACT

Severe/critical patients with coronavirus disease 2019 (COVID-19) have become the central issue in the current global pandemic due to their high mortality rate. However, the relationship between antibody response and clinical outcomes has not been well described in this group. We conducted a single-center, retrospective, cohort study to investigate the relationship between serum immunoglobulin G (IgG) and IgM and clinical outcomes in severe/critical patients with COVID-19. Seventy-nine severe/critical patients with COVID-19 admitted in Wuhan Asia General Hospital in Wuhan, China during January 22, 2020 to March 6, 2020 were included. Serum antibodies were measured at day 25 (SD, 7) post illness onset. The median IgG titer was 113 (IQR 81-167) AU/ml, and IgM titer was 50 (IQR, 23-105) AU/ml. Patients whose IgM titer ≥ 50 AU/ml had higher in-hospital mortality (p=0.026). IgM titer ≥ 50 AU/ml was also correlated with higher incidences of Acute Respiratory Distress Syndrome (ARDS) and sepsis shock. Antibody remeasurements were performed in 42 patients, where IgM titer declined significantly in survivors (p=0.031). Serum IgM titer changes according to the COVID-19 progression. The severe/critical patients with COVID-19 have a higher risk of clinical adverse events when IgM titer ≥ 50 AU/ml. Further decreasing of IgM could imply a better outcome in severe/critical cases.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China in December 2019. The highly contagious pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) soon spread all over the country, and has become a global pandemic [1–4]. Patients infected by SARS-CoV-2 might present from asymptomatic to critical illness with respiratory failure and multi-organ dysfunction, therefore, the disease was categorized into 4 types based on the disease state: mild, moderate, severe, and critical [5, 6]. Severe/critical patients with COVID-19 contributed only 4~15% to overall infected population in different countries [7, 8], however, attentions have been paid to them not only because of their rapid progression in disease, but also due to the greater difficulties in treatment and higher mortality rate [7, 9, 10].

Antibody response in human might be activated at early stage of infectious disease, then be kept stable for a long time. Specific serum immunoglobulin G (IgG) and IgM against SARS-CoV or Middle East Respiratory Syndrome-coronavirus (MERS-CoV) became detectable in patients as early as 11-15 days post illness onset [11, 12]. Similar changes were observed in patients with COVID-19 as IgM and IgG could be detected on 5-14 days after symptom onset [13].

Additionally, the titers of IgM and IgG were significantly correlated with viral load in patients infected by SARS-CoV-2 in a recent finding [14], which promoted the hypothesis that specific antibody against virus might be associated with disease progression in COVID-19. However, reports on clinical profiles of antibody response in severe/critical patients with COVID-19 are scarce.

Hereby, we investigated the serum titers of specific antibodies, IgG and IgM, in severe/critical patients with COVID-19 to explore the association between serum antibody titers and the clinical adverse events in those patients.

RESULTS

Characteristics of the patients

A total of 105 severe/critical patients with COVID-19 admitted to Wuhan Asia General Hospital from 2020.01.22 to 2020.03.06 were enrolled, Of which, 23 were excluded due to the incomplete data, 3 due to negative in antibody measurements. Therefore, 79 patients were reviewed in final analysis, whose mean age was 63 (SD 13) years. Seven (9%) patients were smokers, and comorbidities included 5 (6%) chronic obstructive pulmonary disease (COPD), 31 (39%) hypertension, 13 (16%) diabetes, 6 (8%) coronary artery disease (CAD), and 2 (3%) chronic kidney disease (CKD). The most common symptoms were fever in 64 (81%) patients, cough in 57 (72%), dyspnea in 49 (62%), and fatigue in 44 (56%). The average time from illness onset to admission was 12 days (SD, 6). All patients had significantly change on lung computerized tomography (CT).

Antibody response and in-hospital mortality

Eleven (14%) patients died during hospitalization, who were older than survivors (73 [SD 9] vs 61 [SD 2], *P*=0.002). There were 16 (20%) Acute Respiratory Disease Syndrome (ARDS) and 11 (14%) septic shock happening during hospitalization. Patients had their measurements of serum antibody against SRAS-CoV-2 on day 13 (SD, 7) post admission when tests were available, which was 25 (SD, 7) days after illness onset. The median IgG titer was 113 (IQR, 81-167) AU/ml, and that of IgM was 50 (IQR, 23-105) AU/ml. The difference of IgG titer between survivors and non-survivors was trivial (113 [IQR, 81-167] vs 135 [IQR, 82-158] AU/ml, *P*=0.887), however, IgM titer was significantly increased in non-survivor when comparing with survivors (106 [IQR, 50-128] vs 48 [IQR, 22-84] AU/ml, *P*=0.049) (Figure 1). Forty-two patients had antibody remeasurements 5 (SD, 3) days later. The median IgG titer was 150 (IQR 88-179) AU/ml at 2nd time, and that of IgM was 66 (IQR 32-133) AU/ml. IgG titer remained stable during two measurements in both survivors and non-survivors. Change of IgM titer in survivors showed a significantly decreasing (-4 [IQR -14-0], *P*=0.031), but that in non-survivors didn't show statistical difference (3 [IQR -19-29], *P*= 0.779) (Figure 2).

Serum IgM and clinical outcomes

We further divided patients into two groups using median serum IgM titer as cutoff. Clinical characteristics, such as age, gender, comorbidity, symptoms, time intervals, and vital signs at admission, were similar between the two groups (Table 1). Disease severity was quite different, as a higher incidence of critical cases was seen in the high IgM group (p=0.006) (Table 1). Laboratory measurements presented differently between groups (Table 2). All patients received basic therapy as well as specific treatment based on their disease progression in hospital. More Intensive medical supports were applied in patients whose IgM titer ≥ 50 AU/ml (Table 3).

DISCUSSION

In this retrospective cohort study, IgG and IgM against SARS-CoV-2 in severe/critical patients with COVID-19 were profiled, and relationship between antibody titers

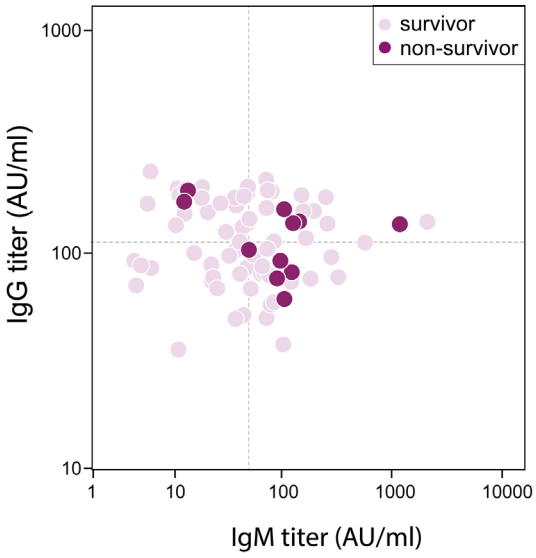

Figure 1 Correlation between Antibody titer and in-hospital mortality in severe/critical patients with COVID-19. Dash lines represent median value as cutoff in IgG (113 AU/ml) and IgM (50 AU/ml) respectively.

and outcomes was also assessed. Specifically, compared with survivors, IgM titer increased in non-survivors while IgG remained unchanged when measurements were performed on 25 (SD, 7) days after illness onset. IgM further decreased in survivors when taking remeasurement 5 (SD, 3) days later. Accompanied by significantly changes in laboratory measurements, more critical cases were seen in patients with IgM titer ≥ 50 AU/ml. Higher frequencies of applying corticosteroids and mechanical ventilation were also observed in patients with IgM titer ≥ 50 AU/ml.

Pneumonia caused by SARS-CoV-2, which was later known as COVID-19, occurred in Wuhan, China in December 2019 [1, 15]. The estimated reproductive number rose from 2.2 to 3.28 [14], and overall mortality rate was around 2-4% [16–18], which might be still increasing as more than one million patients have been confirmed infection, and new deaths are reported globally. Nearly 80% of patients with COVID-19 might present only mild or moderate symptoms, such as fever, and cough [8, 19], however, more than 50% death could be seen in severe/critical cases [7, 20]. Similar to previous studies, non-survivors in our study were older than survivors. There were no differences in comorbidities between survivors and non-survivors in our study, probably due to the variation in the spectrum of underlying diseases. In-hospital mortality (14%) in our study was lower than that in other reports, nonetheless, at least 5 folds higher mortality in severe/critical patients, again, strengthened that great efforts should be paid on this group.

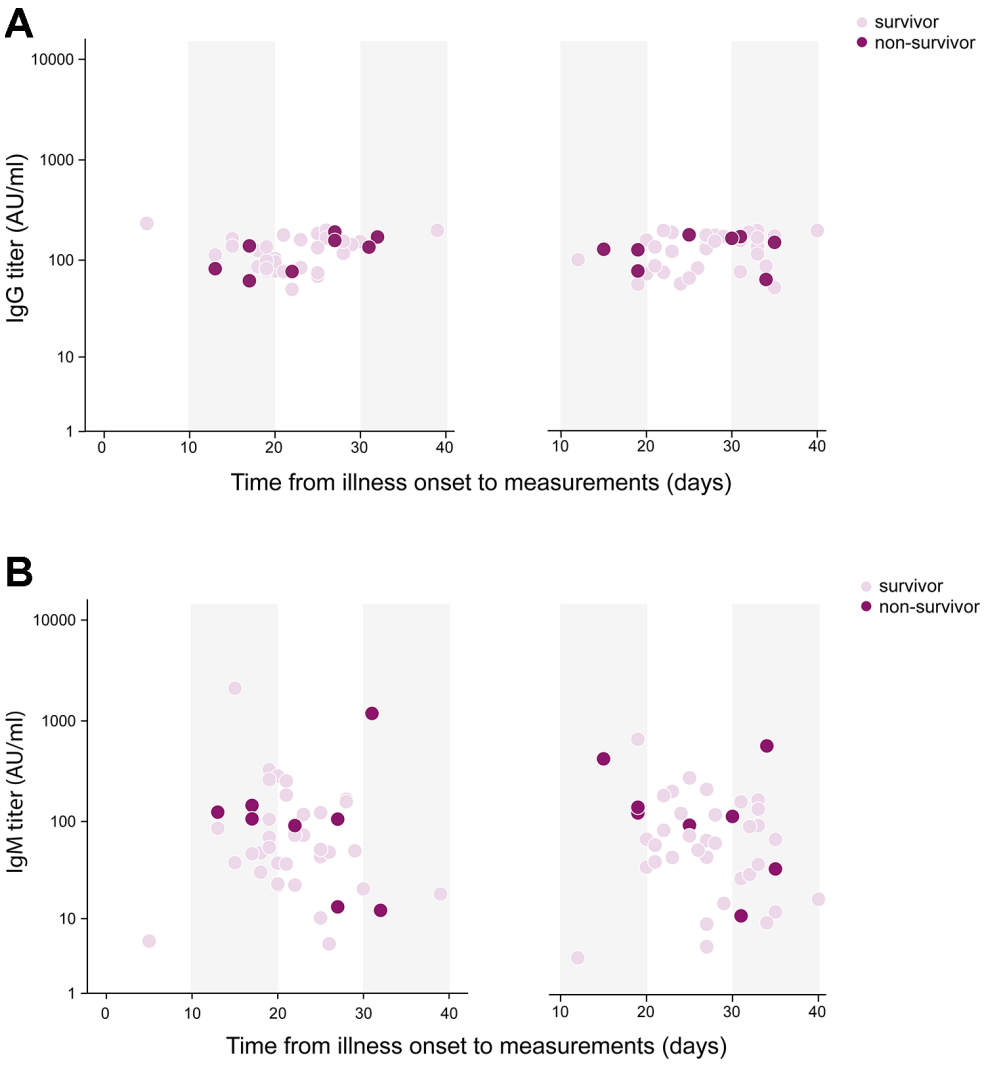

Figure 2. Temporal profile of serum antibodies in severe/critical patients with COVID-19. 42 patients had two antibody measurements on day 25 (SD, 7) and on day 27 (SD, 6) post illness onset respectively. (**A**) IgG titer remained stable during two measurements in both survivors and non-survivors. (**B**) Change of IgM titer in survivors showed a significantly decreasing (-4 [IQR -14-0], P=0.031), but that in non-survivors didn't show statistical difference (3 [IQR -19-29], P=0.779).

Table 1. Clinical characteristics of patients with different IgM titers.

	IgM < 50 AU/ml (n=39)	IgM ≥ 50 AU/ml (n=40)	*P*
Age, years	64±11	61±14	0.315
Men	25(64)	25(63)	0.883
Current smoker	5(13)	2(5)	0.221
Comorbidity			
Chronic obstructive lung disease	3(8)	2(5)	0.623
Hypertension	17(44)	14(35)	0.434
Diabetes	7(18)	6(15)	0.724
Coronary heart disease	4(10)	2(5)	0.378
Chronic kidney disease	0(0)	2(5)	0.494
Symptoms			
Fever	30(77)	34(85)	0.360
Cough	28(72)	29(73)	0.944
Sputum	15(38)	11(28)	0.300
Myalgia	1(3)	5(13)	0.201
Fatigue	22(56)	22(55)	0.900
Diarrhoea	6(15)	6(15)	0.962
Dyspnea	25(64)	24(60)	0.707
Time from illness onset to hospital admission, days	10(7-14)	12(10-14)	0.172
Time from illness onset to first antibody detection, days	26(21-31)	23(19-29)	0.183
Time from hospital admission to first antibody detection, days	13(9-21)	11(7-15)	0.153
Vital signs on admission			
Temperature, °C	36.9±0.6	36.9±0.9	0.774
Systolic pressure, mmHg	129±18	128±18	0.857
Diastolic pressure, mmHg	78±12	76±9	0.461
Heart rate, beats/min	91±18	87±14	0.275
Disease severity state			0.003
Severe	36(92)	26(65)	
Critical	3(8)	14(35)	

Data are mean ± SD, median (IQR) or n (%). IgM = Immunoglobulin M.

Table 2. Laboratory measurements of patients with different IgM titers.

	IgM < 50 AU/ml (n=39)	IgM ≥ 50 AU/ml (n=40)	*P*
Arterial blood gas analysis			
PH	7.38±0.06	7.40±0.05	0.136
PaCO2, mmHg	44±7	42±8	0.277
PaO2, mmHg	59±6	56±7	0.044
SaO2, %	91±4	89±4	0.039
White blood cell count, $\times 10^9$/L	6.9±3.0	7.1±2.8	0.777
Neutrophil count, $\times 10^9$/L	5.5±2.9	5.8±2.9	0.608
Lymphocyte count, $\times 10^9$/L	0.9±0.4	0.9±1.0	0.800
Haemoglobin, g/L	126±15	126±19	0.812
Platelet count, $\times 10^9$/L	249±118	228±87	0.369
ALT, U/L	24(18-44)	39(16-63)	0.161
Albumin, g/L	34±4	32±5	0.010
Creatinine, μmol/L	86±26	83±38	0.730
Prothrombin time, s	12.0±0.8	12.4±1.2	0.085

Fibrinogen, g/L	5.0±1.9	5.2±1.7	0.623
D-dimer, mg/L	0.95(0.44-2.59)	1.81(0.77-9.06)	0.020
Cardiac troponin T, pg/ml	10(6-18)	12(8-20)	0.666
NT-proBNP, pg/ml	80(59-252)	264(73-590)	0.031
C-reactive protein, mg/L	40(12-107)	69(27-126)	0.119
IL-6, pg/mL	17(6-70)	42(12-119)	0.141
TNF-α, pg/mL	11(8-17)	9(5-12)	0.111

Data are mean ± SD or median (IQR). IgM = Immunoglobulin M. PH = Pondus Hydrogenii. PaCO2 = partial pressure of carbon dioxide. PaO2 = partial pressure of oxygen. SaO2 = arterial oxygen saturation. ALT = alanine aminotransferase. NT-proBNP = N-terminal pro-brain natriuretic peptide. IL-6=interleukin-6. TNF-α = tumor necrosis factor-α.

Table 3. Treatments and outcomes of patients with different IgM titers

	IgM < 50 AU/ml (n=39)	**IgM ≥ 50 AU/ml (n=40)**	***P***
Drugs			
Antiviral treatment	36(92)	38(95)	0.675
Antibiotics	36(92)	39(98)	0.359
Corticosteroids	16(41)	32(80)	<0.001
Chinese traditional medicine	39(100)	39(98)	1.000
Oxygen inhalation	38(97)	38(95)	0.571
Mechanical ventilation	3(8)	14(35)	0.003
Non-invasive	3(8)	13(33)	0.006
Invasive	0(0)	9(23)	0.002
Other advanced supportive therapy	1(3)	4(10)	0.175
IABP	0(0)	1(3)	0.320
CRRT	1(3)	4(10)	0.175
ECMO	0(0)	2(5)	0.157
Outcomes			
ARDS	2(5)	14(35)	0.001
Septic shock	2(5)	9(23)	0.026
In-hospital mortality	2(5)	9(23)	0.026
Hospital length of stay, days	29(21-30)	29(19-31)	0.941

Data are median (IQR) or n (%). IgM = Immunoglobulin M. IABP = intra-aortic balloon pump. CRRT = continuous renal replacement therapy. ECMO = extracorporeal membrane oxygenation. ARDS = Acute Respiratory Distress Syndrome.

Serum IgM is the first protein producing in human in response to the exposure to an antigen, such as bacterial, virus, and others. IgM titer could increase in hours to respond antigen attack followed by degradation in weeks. Being a secondly important antibody, IgG would be activated in a moderate but long-lasting way. It might slowly rise in weeks after recognizing antigen, and reach a plateau for years. Guo et al. examined 208 samples from confirmed and suspected patients with COVID-19. Specific antibodies could be positive as early as day 1 after illness onset. For most patients, IgM appeared at day 5 and became stable at days 15-21 after increasing at day 8. IgG showed same change as IgM at acute phase but continued its rising until plateau at day 21 [13]. Our patients had their antibody measurement on day 25 (SD7), and repeated on day 27 (SD6). Despite of the stable levels in IgG and IgM, our measurements were performed later than other studies. We believed the results were still robust because the measurements were performed at the time when both IgG and IgM were in plateau according to previous studies [21], and the IgG and IgM titers remained high and detectable in our study. Moreover, we observed IgM might decrease on day 27 (SD 6) if patients recovered. As Mo et al mentioned in their study, IgM against SARS-CoV declined much earlier than IgG [22]. The decreasing of IgM against SARS-CoV-2 in survivors from our study might be a natural change of IgM in COVID-19. On the other hand, To et al. investigated the correlation between serum antibody response and viral load. They found IgG and IgM titers were highly correlated with viral load in patients with COVID-19, which might explain why our patients had a recover in their illness in consistent with IgM decreasing [14]. One thing might be noticed, there were 10 patients having negative molecular tests in our study, even though they presented critical illness. Similar findings were seen in the

study by Zhang et al. They observed positive rate in molecular tests might be reducing as time from illness onset prolonged, while IgG and IgM titers were stable in all patients [23]. The reasons for this were discussed before: viral RNA might vary from oral swabs to anal swabs; mismatch in the detection probes; fluctuation in viral load unparalleled with illness progression [24, 25].

Efforts have been made to distinguish patients at high risk of mortality. Studies proposed age, comorbidities, CT imaging, and other parameters, which showed differences in survivors and non-survivors [26, 27], to predict risks in patients with COVID-19. Nonetheless, we didn't find many differences between survivors and non-survivors in our study. Severe and critical illness in our patients might eliminate the influence by other factors. On the contrary, our study supported the clinical application of serum IgM in severe/critical patients with COVID-19 for risk stratification. Significantly higher mortality rate was seen in patients when their serum IgM was higher than 50 AU/ml. Additionally, serial changes in IgM titer also helped to follow the disease progression in patients with poor prognosis.

Our study showed that advanced supportive treatment together with combination therapy were more applied in patients with high mortality. The high levels of IgM in our patients might indicate a disease worsening despite of the treatment. Treatment strategy was proposed based on the disease stage, however, no evidence had been shown to be most specific to COVID-19 [28]. Patients might show different response to corticosteroids [29, 30]. Although patients admitted into ICU required more medical treatments, the effect of advanced support seemed to be controversial in critical patients [31, 32]. The ideally strategy to treat viral pneumonia has always been remove the virus as soon as possible. The antivirus effect by Remdesivir in patient and cells might bring hope in further treatment [33, 34].

There were some limitations in our studies. Firstly, there were only 79 patients included in our study. The small size of study population might bring bias to data distribution. Further study should involve more patients to investigate the clinical profile of antibody response. Secondly, our antibody measurements started on 25 days post illness onset. The late measurements missed early change of antibody in patients. New studies might consider a broader interval to cover more changes. Thirdly, we focused on in-hospital mortality for severe/critical patients. However, there were reports that patients might have disease progression after discharge [24]. We might follow-up our patients for a longer time to see the relationship between antibody titer and their prognosis.

CONCLUSIONS

Our study demonstrated the dynamic change of antibody titer in consistent with disease progression. A higher risk of in-hospital mortality was seen in severe/critical patients of COVID-19 when their IgM titer ≥ 50 AU/ml. Further decreasing of IgM could imply a better prognosis in severe/critical patients. Serial measurements of serum antibody provide comprehensive evaluation to the process of COVID-19.

MATERIALS AND METHODS

Study design and patients

A retrospective cohort study was conducted in Wuhan Asia General Hospital, Wuhan, China to investigate the clinical profile of serum antibodies against SARS-CoV-2 in severe/critical patients with COVID-19. The study protocol was reviewed and approved by the Ethics Committee of Wuhan Asia General hospital with a waiver of informed consent (WAGHMEC-KY-2020007). Personal information of patients was re-identified before analysis.

A total of 105 severe/critical patients with COVID-19 admitted in Wuhan Asia General hospital between 2020.01.22 and 2020.03.06 were reviewed. COVID-19 was diagnosed according to the Chinese management guideline for COVID-19 (version 7.0) [6]. New laboratory criteria of COVID-19-specific IgM and IgG positive, and 4 folds increasing of COVID-19-specific IgG titer in recovery period were added in guideline 7.0 [6]. Severe patients with COVID-19 met any of the followings: (1) Shortness of breath, respiratory rate ≥ 30 times per minute; (2) Oxygen saturation ≤ 93% at rest; (3) Alveolar oxygen partial pressure/fraction of inspiration O2 (PaO2/FiO2) ≤ 300 mmHg (1mmHg=0.133kPa). Critical patients had any of the conditions: (1) Respiratory failure requiring mechanical ventilation; (2) Shock; (3) Patients combined with other organ failure needed intensive care unit (ICU) monitoring and treatment [6]. Fever was defined as axillary temperature greater than 37·3°C.

Data collection

Clinical data including age, gender, vital signs, comorbidity were collected from medical records at admission. Laboratory biomarkers such as IgG titer, IgM titer, blood gas analysis, white blood cell count (WBC), alanine aminotransferase (ALT), D-dimer, and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were also collected. Specifically, serum IgG and IgM that against SARS-CoV-2 nucleocapsid protein and envelop protein were measured by

chemiluminescence immunoassay (CLIA) in automatic system when it was available on February 18, 2020. Antibody titer > 10 AU/ml was taken as positive. All blood tests were analyzed in fresh blood and determined by standard quantitative assay techniques in our Department of Clinical Laboratory according to the manufacturer's protocol.

Outcomes

The primary outcome was in-hospital mortality. The secondary outcomes included ARDS related to SARS-CoV-2 infection and sepsis shock secondary to COVID-19. ARDS was diagnosed according to the Berlin Definition [35]. Sepsis shock was defined according to the 2016 Third International Consensus Definition [36].

Statistical analysis

Data are shown as number for categorical data, and mean ± standard deviation or median with interquartile range (IQR) as appropriate for continuous data. Data were compared with student t test or Wilcoxon signed-rank test for continuous variables depending on the normality of their distributions and with the $\chi 2$ test for categorical variables. Comparison between the first and second antibody titer is performed by paired samples Wilcoxon test. A two-side $P < 0.05$ was considered as statistic significant. All statistical analyses were performed with SPSS 23.0 (IBM Corp, Armonk, NY).

Abbreviations

ALT: alanine aminotransferase; ARDS: acute respiratory distress syndrome; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; COVID-19: coronavirus disease 2019; CLIA: chemiluminescence immunoassay; ICU: intensive care unit; IgG: immunoglobulin G; IQR: interquartile range; MERS-CoV: Middle East Respiratory Syndrome-coronavirus; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; PaO2/FiO2: Alveolar oxygen partial pressure/fraction of inspiration O2; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SD: standard deviation; WBC: white blood cell count.

AUTHOR CONTRIBUTIONS

Xintian Liu and Xuan Zheng designed the study. Bo Liu and Mingxiang Wu collected the epidemiological and clinical data. Zhenlu Zhang provided laboratory measurements and collected the data. Xintian Liu, Xuan Zheng and Xi Su summarized all data. Gangcheng Zhang, Xi Su, Xintian Liu and Xuan Zheng drafted the manuscript. All the authors proved the final version of this manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Research Paper

Questionnaire assessment helps the self-management of patients with inflammatory bowel disease during the outbreak of Coronavirus Disease 2019

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ABSTRACT

Objective: This study aimed to assess the disease conditions of patients with inflammatory bowel disease (IBD) in Hubei Province during the outbreak of Coronavirus Disease 2019 (COVID-19) by questionnaire online and guide their self-management during this epidemic.

Results: A total of 102 eligible questionnaires were included. No patient we surveyed reported a diagnosis of COVID-19. Our result showed that 67.86% of patients with ulcerative colitis (UC) and 80.43% of patients with Crohn's disease (CD) were in remission, 85.29%of patients had a good quality of life. Part of the patients (21.57%) reported their disease conditions worsening. The reduction in physical exercise was a risk factor for worsening conditions (OR=17.593, p=0.009). Some patients reported an alteration of medication regimens during the epidemic.

Conclusions: The epidemic of COVID-19 might have a certain impact on many aspects of Hubei IBD patients within four weeks after the traffic control. Doctors could utilize the results from our questionnaire to guide IBD patients' self-management.

Methods: A questionnaire was designed containing the Harvey-Bradshaw Index (HBI), the 6-point Mayo Score, the short inflammatory bowel disease questionnaire (SIBDQ) and distributed to Hubei IBD patients online within four weeks of traffic control after the outbreak, it also included questions about patients' self-reported disease conditions and their epidemiological features of COVID-19.

INTRODUCTION

In December 2019, an outbreak of COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, the capital of Hubei Province, China [1, 2]. On January 23, 2020, the Chinese government implemented traffic controls to prevent the spread of COVID-19 [3]. We distributed questionnaires to IBD patients from February 18th to 20th, which is approximately four weeks after traffic control. As of 20 February 2020, there had been 75,465 cases of COVID-19 confirmed in mainland China, including 62,662 cases in Hubei Province. The number of confirmed diagnoses in Hubei Province is 83.03% of that in China, accounting for the majority. At the same time, medical resources had also shifted more towards

the diagnosis and treatment of COVID-19 in Hubei Province. These factors made routine medical treatment and follow-up of patients with chronic diseases inconvenient. Under these influences, it is essential for patients with chronic diseases to self-manage under the guidance of the doctor in this particular period. Self-management is the process by which patients participate in decision-making and self-care under the guidance of the doctors [4]. Patient's effective self-management can relieve symptoms and control disease activity to a certain extent [5].

Inflammatory bowel disease (IBD) is a type of chronic idiopathic bowel disease, includes Crohn's disease (CD) and ulcerative colitis (UC). IBD patients have varying degrees of immune disorders [6] so that they may be considered as virus-susceptible. It is particularly crucial for IBD patients to know how to manage by themselves during the epidemic. Self-management requires monitoring of diseases by doctors first [7]. We monitored the patient's disease status through a questionnaire in our study. The content of the survey and the concept of the questionnaire design came from the patient-reported outcome (PRO). PRO is a visual report of the patient's treatment and disease management results, emphasizing the patient's self-evaluation and subjective perception [8]. PRO contains objective and subjective evaluation contents, which may include disease status, changes in functional status before and after the intervention, HRQoL and the patient's personal impressions [9–12].

To assess patients' disease activity, HRQoL, and self-reported disease conditions, we design a verified 60-item questionnaire based on the concept of PRO. First of all, our questionnaire included the 6-point Mayo, HBI and SIBDQ. These indexes were objective quantitative indicators designed to obtain detailed knowledge of the disease activity and HRQoL of these IBD patients. Secondly, there were questions to understand patients' subjective perceptions of disease conditions. Finally, this questionnaire also included questions about COVID-19 epidemiological features of these IBD patients. We gave feedback to these IBD patients and guided them to develop targeted self-management programs after obtaining the information through the questionnaire.

RESULTS

Demographic characteristics of study participants

A proximately 350 electronic questionnaires were distributed and a total of 111 were returned. There were 102 valid questionnaires, with an effective rate of 91.89%. The nine questionnaires excluded were due to some missing items. The median age of participants was 34 years (IQR, 27.25-42.25; range, 14-66), and 66.67% of participants were men. There were 56 (54.90%) patients with ulcerative colitis and 46 (45.10%) patients with Crohn's disease. Among all the participants in our survey, no one has reported infection with SARS-CoV-2; no one had symptoms related to COVID-19 or had a history of exposure. Table 1 shows the demographic data and disease-related variables for all participants who agreed and completed the survey.

Disease activity

We used the 6-point Mayo and HBI to score and grade disease activity in UC and CD patients respectively. The results were shown in Table 2. The median 6-point Mayo score of UC patients was 1 (IQR, 0-3; range, 0-6). Of the 56 UC patients, 38 (67.86%) UC patients were in remission, 4 (7.14%) patients had mild activity, 13 (23.21%) patients had moderate activity, and 1 (1.79%) patients had severe activity. The median HBI of CD patients was 2 (IQR, 1-4; range, 0-12). Of the 46 CD patients, 37 (80.43%) CD patients were in remission, 4 (8.70%) patients had mild activity, 5 (40.87%) patients had moderate activity, and no patients had severe activity. There was not a statistically significant difference in the proportion of the disease activity stage between UC and CD patients ($p = 0.301$).

Quality of life

The median SIBDQ of all participants was 59 (IQR, 52.25-63; range, 34-70). The median SIBDQ of UC patients was 60 (IQR, 54.75-64; range, 35-70), and the median SIBDQ of CD patients was 58 (IQR, 52-62.75; range, 34-69). Among all participants, 87 (85.29%) patients had the good health-related quality of life (HRQoL) (SIBDQ $\geq$ 50). There were 49 (87.50%) UC patients who had good HRQoL, compared with 38 (82.61%) CD patients who had good HRQoL ($p=0.338$) (Table 3), suggesting HRQoL is not significantly different between UC and CD patients.

Self-reported disease conditions

In this questionnaire, we investigated the change of the patient's self-reported disease condition through the patient's subjective report. Approximately half of the patients ($n = 55$, 53.92%) thought that their disease condition did not change during the epidemic, 25 (24.51%) considered their disease condition improved, and 22 (21.57%) considered their disease condition worsening. We attempted to study the risk factors of change in patients' self-reported disease conditions. The

Table 1. Baseline characteristics of the study population. (n=102).

Characteristics	Value
Age, Median (min-max) (IQR), y	34 (14-66) (27.25-42.25)
Gender	
Female	34 (33.33%)
Male	68 (66.67%)
Diagnosis	
Ulcerative Colitis	56 (54.90%)
Crohn's Disease	46 (45.10%)
Diagnosed with COVID-19	0
Huanan seafood market exposure	0
Signs and symptoms of COVID-19	0
Habitation*	
Wuhan	26 (25.49%)
Xiaogan	22(21.57%)
Jingzhou	12 (11.76%)
Suizhou	8 (7.83%)
Xiangyang	6 (5.88%)
Huangshi	6 (5.88%)
Huanggang	5 (4.90%)
Yichang	5 (4.90%)
Jingmen	4 (3.92%)
Xianning	2 (1.96%)
Xiantao	2 (1.96%)
Enshi Tujia and Miao Autonomous Prefecture	2(1.96%)
Tianmen	1 (0.98%)
Qianjiang	1 (0.98%)

*All participants are located in Hubei Province.

Table 2. Evaluation of participants' IBD disease activity.

	Participants with Ulcerative Colitis(n=56)	Participants with Crohn's Disease(n=46)	P-value*
Index, Median (IQR) (range)	6-point Mayo,1(0-3)(0-6)	HBI, 2 (1-4) (0-12)	
Disease activity stage			0.301
Remission phase, n (%)	38 (67.86%)	37 (80.43%)	
Mild active phase, n (%)	4 (7.14%)	4 (8.70%)	
Moderate active phase, n (%)	13 (23.21%)	5 (10.87%)	
Severe active phase, n (%)	1 (1.79%)	0 (0%)	

HBI: Harvey-Bradshaw Index.
*Chi-square test was used to test whether there was a statistically significant difference in the proportion of the disease activity between UC and CD patients.

Table 3. Evaluation of participants' SIBDQ.

	Total participants (n=102)	Participants with UC and CD respectively		
		Participants with UC (n=56)	Participants with CD (n=46)	P-value*
SIBDQ, Median (IQR)	59 (52.25-63)	60 (54.75-64)	58 (52-62.75)	
HRQoL				0.338
Good (≥ 50[1]), n (%)	87 (85.29%)	49 (87.50%)	38 (82.61%)	
Poor (<50), n (%)	15 (14.71%)	7 (15.50%)	8 (17.39%)	

HRQoL: health-related quality of life
1: SIBDQ score of more than 50 are considered to have a good HRQoL
*Chi-square test was used to test whether there was a statistically significant difference in the proportion of HRQoL between UC and CD patients.

result showed that reduced physical exercise was a risk factor for worse in the disease condition (OR=17.593, 95%CI 2.035 to 152.097, p=0.009). The other factors did not have a significant risk for change in the patient's disease condition. These data are shown in Table 4. The status of these factors during the epidemic came from the patients' personal reports.

The change in medication regimen

We studied participants' medication regimens before and after the outbreak of COVID-19. Among UC patients, there was an increase of 3 patients who took no medication due to the discontinuation of mesalazine. The reason for the withdrawal of mesalazine was that the medicine was not available. Among CD patients, the number of patients who used adalimumab or took no medication increased, and the number of patients who used Remicade or methotrexate decreased. The details were shown in Table 5. The reasons for changing the medication regimens of these CD patients were "inability to purchase medication" and "inability to go to the hospital for routine treatment". Among the reasons for these patients who changed their medication regimens, no one chose the options of "forgetting to take medicine" and "reducing medicine on your own". This result represented that IBD patients' medical compliance during the epidemic was excellent in our survey.

Emotional state

Negative emotions are significantly correlated with clinical recurrence and are also considered to be independent risk factors for more frequent relapse of disease [13, 14]. Therefore, we also investigated the emotional states of IBD patients in this survey. Changes in emotional states came from the patients' self-judgments. More than half of the participants (57.85%) thought they had the ordinary moods during this epidemic, 35.29% had positive moods, and 6.86% had negative moods.

DISCUSSION

The COVID-19 epidemic outbreak emerged in Wuhan, Hubei Province of China in December 2019 [15]. The population was generally susceptible to SARS-CoV-2, especially for the elderly and the people with underlying diseases, who are prone to serious consequences [16, 17]. In our survey, there was no patient reported infection with SARS-CoV-2, but this did not mean that IBD patients were not susceptible to SARS-CoV-2 since our small sample size and the participants of this study were not obtained by randomized sampling. Until now, there is no evidence to prove the susceptibility of IBD patients to COVID-19 from other studies [18]. The epidemic led to the difficulty of maintaining previous disease management for IBD patients due to the contraction in routine medical resources. Our research focused on guiding the patient's self-management through the results of the questionnaire.

The traffic control in China from late January might largely change the lifestyle, psychological, and physical condition of the Chinese population [19–21], especially for residents in Hubei Province. We used the web questionnaire to evaluate the disease activity, HRQoL and the self-reported disease condition of IBD patients. Then we provided feedback on the patients' conditions and advised on their self-management. This manner helped IBD patients to adjust their treatment plans and develop a home-based self-management medical intervention model. Active doctor-patient communication can improve patients' confidence with treatment, shared decision-making capacity and then provide a good impact on disease activity [4, 22]. We will continue to distribute questionnaires to this group of IBD patients in Hubei Province every month to guide patients' home-based self-management during the epidemic of COVID-19.

Our results showed that 67.86% of UC patients and 80.43% of CD patients were in remission assessed by the 6-point Mayo score and HBI index. 85.29% of patients had a good HRQoL during the epidemic through the SIBDQ test. This suggested that more than half of the patients were in remission and had a good HRQoL in the early period of traffic control after the outbreak of COVID-19. With regard to the patients' self-reported results, although 78.43% of the patients thought that their disease conditions had not changed or even improved, there were still 21.57% of the patients considered that their disease conditions worsening. This showed that the early epidemic also had a certain impact on the patient's disease condition.

We studied the influencing factors of the self-reported disease condition of IBD patients during this epidemic. The factors included "reduction of exercise", "emotional state", "change of medication regimen", "daily rest" and "subsequent visit". The results showed that the reduction of exercise was a risk factor for worsening disease (OR=17.593, p=0.009). The other factors that could affect the disease conditions of IBD patients in previous studies [23–27] didn't show a statistical correlation in our survey. This might be because of our small sample size. Therefore, we still recommend that IBD patients maintain a positive emotional state, retain the medication regimen, have adequate rest and make timely doctor-patient communication during the self-management process.

Table 4. Multi-variable logistic regression of the causes of change in participants' self-evaluation disease condition.

Disease condition	Characters	n (%)	OR(95%CI)	P-value
Condition worsening (n=22)		Subsequent visit		
	No	18 (81.82%)	3.785(0.871, 16.458)	0.076
	Yes	4 (18.18%)		
		Medication regimen		
	Not changed	14 (63.64%)	0.264 (0.060, 1.167)	0.079
	Changed	8 (36.36%)		
		Emotional status		
	Negative	4 (18.18%)	3.306(0.413, 26.454)	0.260
	Positive	3 (13.64%)	0.830 (0.178, 3.880)	0.813
	Normal	15 (68.18%)		
		Physical exercise		
	Reduced	5 (22.73%)	17.593 (2.035, 152.097)	0.009
	Not reduced	17 (77.27%)		
		Rest		
	Inadequate	21 (95.45%)	1.071 (0.262, 4.378)	0.924
	Adequate	1 (4.55%)		
		Smoking		
	No	19 (86.36%)	0.665 (0.123, 3.591)	0.636
	Yes	3 (13.64%)		
Condition improved (n=25)		Subsequent visit		
	No	21 (84.00%)	2.730 (0.731, 10.199)	0.135
	Yes	4 (16.00%)		
		Medication regimen		
	Not changed	19 (76.00%)	0.374 (0.094, 1.496)	0.165
	Changed	6 (24.00%)		
		Emotional status		
	Negative	1 (4.00%)	2.094 (0.138, 31.710)	0.594
	Positive	14 (56.00%)	2.259 (0.751, 6.792)	0.147
	Normal	10 (40.00%)		
		Physical exercise		
	Reduced	1 (4.00%)	0.694 (0.222, 2.167)	0.529
	Not reduced	24 (96.00%)		
		Rest		
	Inadequate	9 (36.00%)	0.136 (0.015, 1.249)	0.078
	Adequate	16 (64.00%)		
		Smoking		
	Yes	4 (15.38%)	0.481 (0.104, 2.228)	0.350
	No	22 (84.62%)		

*The category with no change in the condition (n=55) was used as the reference category for the two groups of condition worsening (n=22) and condition improved (n=25).

Pharmacological intervention is a key part of managing symptoms and maintaining remission in patients with IBD [28]. Our results showed that the number of people who took no medication increased among UC and CD patients after the outbreak of COVID-19. The number of UC patients taking mesalazine decreased due to the inconvenience of obtaining medications. For CD patients, the use of Remicade or methotrexate decreased because Remicade infusion in the hospital was not accessible and methotrexate could not be purchased.

Table 5. The comparison of the medication regimens before and after the outbreak of COVID-19 and the changes in medication regimens.

	Medication	Before the outbreak, n (%)	After the outbreak, n (%)	The number of changes*, n
UC patients, (n=56)	No medication	1 (1.75%)	4 (5.26%)	+3
	Mesalazine	50 (89.47%)	47 (85.96%)	-3
	Enteral nutrition	1 (1.75%)	1 (1.75%)	0
	Glucocorticoid	2 (3.51%)	2 (3.51%)	0
	Probiotics	4 (5.26%)	4 (5.26%)	0
	Biological therapy			
	Remicade	3 (5.26%)	3 (5.26%)	0
	Adalimumab	1 (1.75%)	1 (1.75%)	0
CD patients, (n=46)	No medication	1 (1.92%)	3 (5.77%)	+2
	Mesalazine	8 (17.31%)	8 (17.31%)	0
	Enteral nutrition	10 (19.23%)	10 (21.15%)	0
	Glucocorticoid	2 (3.85%)	2 (3.85%)	0
	Probiotics	1 (1.92%)	1 (1.92%)	0
	Biological therapy			
	Remicade	21 (46.15%)	16 (36.54%)	-5
	Adalimumab	1 (1.92%)	2 (3.85%)	+1
	Immunomodulators			
	Methotrexate	2 (3.85%)	1 (1.92%)	-1
	Azathioprine	20 (40.38%)	20 (40.38%)	0
	Thalidomide	1 (1.92%)	1 (1.92%)	0

*Plus sign indicates an increase in quantity and minus sign indicates a decrease in quantity.

The use of adalimumab increased because we recommended patients to replace inaccessible Remicade with other available biologics. We tried to develop personalized treatment plans for specific patients on time to minimize the impact of changes in medication regimens on the patient's disease condition during the epidemic. It was an important step to increase the self-management of IBD patients.

The result showed that 93.14% of patients had normal or even positive emotional states, which implied that the epidemic did not have a very negative impact on the mood of these IBD patients in such a relatively early month. However, since previous studies have shown that emotional stress is significantly associated with decreased quality of life [29, 30], it was very important to intervene in the emotional state in the process of IBD patient self-management guidance.

Limitations

The major limitation was that the sample size was small and our participants were not obtained by randomized sampling. In addition, there was no IBD patient diagnosed with COVID-19 in our study. Further researches need to be done to get more evidence about the susceptibility to SARS-CoV-2 in IBD patients and the clinical manifestations of IBD patients complicated with COVID-19.

CONCLUSION

In the middle of February, although more than half of IBD patients we studied in Hubei Province were in remission and possessed a good HRQoL, the outbreak of COVID-19 still had a certain impact on IBD patients in Hubei Province, such as worsening of their self-reported disease conditions and changes in their treatment options. These changes deserved attention to the impact of epidemics on IBD patients. In our survey, doctors used the questionnaire to assess patients' disease conditions, give timely feedback and suggestions to IBD patients. This method facilitated the effective self-management of patients under the circumstance of the COVID-19 outbreak.

MATERIALS AND METHODS

Study design and participants

This study was an online questionnaire survey among IBD patients from the region of Hubei Province. From February 18, 2020 to February 20, 2020, the questionnaire was administered to a sample of IBD patients with regular

follow-up in our IBD center. There were two items of the inclusion criteria for this study. One was that the patient (aging from 14 to 80) was established diagnosis of IBD for at least three months, another was that the patient is available to finish the online questionnaire (by Wechat, QQ, website, email) by himself or with the help of others. The exclusion criterion was that the patient is not able to finish the questionnaire. During this study, patients with IBD had been informed of the study's aim. An IBD specialist nurse is explicitly trained in this questionnaire contacted them to explain the study objectives. Participants completed the questionnaire with an online survey portal. They completed questionnaires voluntarily and independently, under uncompensated conditions. This study was approved by the National Health Commission of China and Ethics Commission of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Questionnaire design

The questionnaire was a validated 60-item questionnaire that assesses IBD disease activity across multiple domains, including the 6-point Mayo, HBI and SIBDQ (Supplementary File 1). HBI score ranges from 0 to 16 or more, and the highest score depends on the number of liquid stools per day. HBI scores of 0 and 4 are assigned to the remission phase; 5 to 7 are assigned to mildly active disease phase; 8 to 16 are assigned to moderately active disease phase; ≥17 are assigned to severely active disease phase [31, 32]. We used 6-point Mayo, which composed of the stool frequency, bleeding components. 6-point Mayo score of 0 and 1.5 are assigned to the remission phase; 1.5 to 2.5 are assigned to mildly active disease phase; 2.5 to 4.5 are assigned to moderately active disease phase; 8 to 4.5 are assigned to severely active disease phase [33, 34]. Total SIBDQ score ranges from 10 to 70, to find out how the patients have been feeling during the last two weeks. They will be asked about symptoms related to IBD diagnosis, as well as the general emotional status during the period and their attitude to the plague. Patients with a SIBDQ score of more than 50 are considered to have a good HRQoL [35–37].

In addition to the question about HBI, 6-point Mayo score and SIBDQ, the questionnaire included questions regarding the subjective feeling about their change in disease conditions and epidemiological history questions about COVID-19.

Statistical analysis

Data collection and its statistical analysis were carried out using the SPSS software system (SPSS for Windows, Version 23.0, SPSS Inc., Chicago). Data analysis excluded incomplete items. Categorical data obtained were presented as frequency counts and percentages. Median, range and frequency were used to describe the demographic, SIBDQ scores and clinical characteristics. Chi-square tests were used to test the significant difference of categorical variables between two groups, such as the participants' distribution in different disease active phases and HRQoL between UC and CD patients, with fisher exact test as appropriate. The logistic regression analysis was used to identify variables significantly associated with disease activity, and a multivariate model was built to assess the effect of each potential confounding factor and determined independent and significant factors associated with the disease index. The odds ratio (OR) was calculated to quantify the corresponding risk. For all analyses, $p<0.05$ was considered statistically significant.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Please browse Full Text version to see the data of Supplementary File 1.

Supplementary File 1. Questionnaire for follow-up of patients with inflammatory bowel disease during the outbreak of Corona Virus Disease 2019

Research Paper

A qualitative study of the vocational and psychological perceptions and issues of transdisciplinary nurses during the COVID-19 outbreak

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ABSTRACT

Background: Due to its high infectivity and concealment, the coronavirus disease 2019 (COVID-19) outbreak that occurred in Wuhan attracted global attention. A special nursing group of transdisciplinary nurses (TNs) who had not worked in respiratory medicine, infection departments, or emergency and intensive medicine but who accounted for a large proportion of all nurses also drew our attention. Few studies have examined this special group of TNs. Therefore, this study collected the experiences and views of TNs at the forefront of the COVID-19 outbreak to investigate their potential problems.

Results: Twenty-five TNs and 19 nurses with experience in infectious diseases (non-TNs) were enrolled in the study. Compared with non-TNs, TNs showed higher levels of perceived stress and relatively less perceived social support. For TNs, the ambiguous roles, transition of operating mode, unfamiliar work content, and reversal of their daily schedule were the most common vocational problems. Additionally, most TNs had psychological problems such as anxiety, pain and insomnia. The incomprehension of parents, concern for family members and long-term isolation were the most common causes of psychological stress.

Conclusion: This survey is the first to focus on the group of TNs at the forefront of the COVID-19 outbreak and to investigate their experiences, vocational issues and psychological stresses qualitatively and quantificationally. We found that TNs had more perceived stress and less perceived social support than non-TNs. The vocational and psychological issues of TNs should be highlighted. These findings identify important issues and offer insights into the underlying issues to help TNs ultimately win the battle against novel coronavirus epidemics.

Methods: Semi-structured and face-to-face individual interviews and quantitative assessments were conducted. The Braun Clarke Thematic Analysis method and the strategy outlined by Miles and Huberman were used in the data analysis process of the qualitative study. The perceived stress scale and perceived social support scale were utilized to quantificationally evaluate the perceived stress level and the amount of perceived social support. Both qualitative and quantitative methods were adopted to assess the vocational and psychological perceptions and issues.

INTRODUCTION

In recent decades, there have been a variety of global coronavirus outbreaks, including severe acute respiratory syndrome coronavirus (SARS-COV) and Middle East respiratory syndrome coronavirus (MERS-COV), which have brought serious losses to human society [1, 2]. The coronavirus disease 2019 (COVID-19) outbreak, which occurred in Wuhan, HuBei Province, spread rapidly throughout the country and quickly attracted global attention [3, 4]. Given the high infectivity and concealment surrounding this outbreak, the government of China quickly generated containment strategies and performed a series of measures in the

early stage of the outbreak. Throughout the defensive and therapeutic system, crucial roles were played by medical workers, a large proportion of whom were nurses. In this study, we employ semi-structured interviews in combination with a scale assessment to focus on a special group of nurses and investigate their experiences, issues and challenges during their frontline work against COVID-19.

As the "gatekeepers" of the health care system, nurses at the forefront of the COVID-19 outbreak played key roles in identifying suspected and confirmed COVID-19 patients by carefully evaluating disease manifestations and exposure history [5]. In addition, as "interrupters", they implemented and maintained high-quality infection control measures to control the spread of COVID-19 [3, 5]. Because of the large-scale outbreak, multi-disciplinary nurses from all over the country participated in epidemic prevention and control. A special nursing group of transdisciplinary nurses (TNs) who had not worked in respiratory medicine, infection departments, or emergency and intensive medicine but who accounted for a large proportion of all nurses attracted our attention [6].

A series of studies have highlighted the important roles of appropriate emotions and stress management, the satisfaction of basic needs, sufficient social support, clear task distribution and flexible working schedules on nurses' work and psychological stress [7–9]. High-frequency and high-intensity work, including close contact with patients, produces occupational hazards and psychological stress for nurses. However, most researchers have placed more emphasis on nurses with experience in infectious diseases (non-TNs), especially those working in emergency and intensive medicine [10–12]. As a result, existing studies on TNs' experiences during the COVID-19 pandemic are limited, and quantitative and qualitative studies are lacking. Given TNs' limited experience in nursing during infectious pandemics, we suspected that these nurses likely endured even greater vocational and psychological stress [13, 14]. Therefore, we designed the study to collect the experiences and views of TNs at the forefront of the COVID-19 outbreak and to evaluate their psychological stresses. The results will emphasize an important issue and offer insights into the underlying issues to help TNs ultimately win the battle against novel coronavirus epidemics. In the long run, these findings may help health care institutions prepare for future pandemics.

RESULTS

In the present research, we primarily focused on the group of TNs at the front line against the COVID-19 outbreak and investigated their experiences, vocational issues and psychological stresses. In this part, the interrater reliability showed at least substantial agreement for every theme (K = 0.63-0.85).

Awareness of nurses' responsibilities and roles

When we asked the participants about the responsibilities and obligations of nurses in the face of sudden acute infectious diseases threatening public health, we heard many similar and unmistakable voices promoting "the Nightingale spirit". One of the participants said,

"From the first day I became a nurse, I was deeply conscious of my responsibility to heal the wounded and rescue the dying. In the face of sudden novel coronaviruses, the lives and health of the world's population are under serious threat. As an angel in white, I have to summon "the Nightingale spirit" and go to the front lines where I am most needed to treat patients using professional knowledge and skills."

In addition, all participating nurses described their roles in the COVID-19 outbreak. Most of them thought of themselves as nurses, friends or even family. They not only focused on physical fitness but also maintained the mental health of patients:

"First, I should assist doctors in treating patients and do my job well. Furthermore, most patients in isolation for a long time are bored, alone and scared. I should also be their friend and family to establish a harmonious and friendly relationship with them to help them maintain their mental health."

When the participants mentioned changes in perceptions of nurses' job responsibilities and obligations, some of them noted that they had obtained a more divine sense of purpose and would continue their future work with this sense of professional mission:

"In the past, I used to do my job well, stick to my post, or try to be a "five-star" nurse. But this outbreak has made me see that everyone has a responsibility in the face of the epidemic, and the numerous serious cases have made my sense of responsibility and mission stronger. Our essential work is to help patients alleviate pain. Facing great disaster, I should have great love!"

Other nurses thought that their increased experience and excellent professional skills; particularly techniques for dealing with acute respiratory infections, would be beneficial for further work:

"Although most nursing work in daily life is closely related to my primary major, mastering more comprehensive nursing skills is a better guarantee for

the safety of patients. I can more skillfully and unhurriedly face the outbreak of other acute diseases endangering public health."

Recognition of responsibilities of transdisciplinary nursing work

We asked the participants, "With regard to respiratory infections, as a transdisciplinary nurse, how do you think the responsibilities differ from your usual work?" Differences in working contents and working patterns were the most common answers. One of the nurses in the surgical system said,

"Surgical work is based on 'panic-mode', and the faster work pace and turnover of patients is also significant. In addition to the routine work, I have to leave time to deal with some emergencies. However, the work mode here is mainly 'process-based', and the patient's condition is more complex; the disease is relatively continuous, and the work pace is also slower."

Another nurse in another medical system said the work patterns were not exactly the same, with an obvious difference in the process of observing patients' condition:

"For patients infected with COVID-19, I prefer to monitor the basic vital signs, such as temperature, blood pressure, respiration and oxygen saturation. I need to be constantly vigilant and accurately judge the changes in patients' conditions, especially the transition from mild to severe."

We further investigated the challenges and problems produced by the transdisciplinary field in this epidemic prevention and control work, and we mainly heard three types of answers: acquiring new knowledge, enforcing new regulations and improving physical and psychological quality.

"Although I had learned nursing knowledge in different specialties before, with the update of knowledge and technology and in order to accurately treat patients, I need to relearn nursing knowledge about the COVID-19 outbreak. In addition, when facing large-scale respiratory infectious diseases, the work regulations are completely different from daily work, which also requires an adaptation process. Moreover, we went almost eight hours without eating and drinking in the isolation ward, so it is also a great test of physical quality and mental state. These [issues] were not encountered in my previous work."

There is no doubt that there are many risks in nursing work, and the risks for transdisciplinary nurses are even greater in the fight against the COVID-19 outbreak.

"What I shouldn't ignore is the risk of occupational exposure. Improper protection or careless operation will greatly increase the risk of infection. The unfamiliar operation of a protective suit can significantly increase the risk of infection. In addition, contradictions between patients and nurses remain. The increased workload and the adaptation to the new environment will lead to mental stress and physical fatigue; in this state, the quality of nursing work will also decline."

When we mentioned the new understanding of nursing risks and risk prevention during frontline work against the epidemic, one of the TNs highlighted the importance of protection awareness and standardized operations:

"Acute infectious diseases are highly contagious and carry a high risk of infection, especially for health care workers who are in direct contact with patients. We must achieve accurate and standard operations, such as environment disinfection, detail control and protective suit operation. In addition, we should have a scientific understanding of the disease, enhance the awareness of protection, and successfully popularize knowledge. Ideological vigilance, attention to work, ensure mental health and physical health."

Psychological problems caused by transdisciplinary work

TNs play key roles in fighting at the forefront against the new coronavirus. However, when faced with acute respiratory infections, they have a relative lack of experience, and their psychological burdens increase remarkably under intense working pressure. Close social attention to their mental health is needed. When we asked about psychological problems when they were confronted with tough issues, worked in a strange and specific environment, faced high morbidity and mortality and worked under enormous pressure, most TNs answered that they experienced anxiety, grief, pain and insomnia:

"The professional preparation of disinfection, the intervention in patients' psychological problems and the document records were almost daily tasks, but I was not familiar and hadn't been specifically trained. With the heavy workload every day, I sometimes felt anxious and had insomnia at night."

"The high work intensity in the isolation ward, the disordered internal clock, and the restrictions and challenges of protective clothing all led me to be distressed."

"Although facing life and death is common for nurses, I have never experienced so many deaths in the past.

There was a growing body of critical patients dying while other new patients were constantly transferred to the intensive care unit every day. I often felt exhausted or even powerless. Not only was I sad that I could not treat my patients, but I also was sorrowful that I was not clear how long this situation would last."

Given that the nursing areas are transdisciplinary, most of the participants' families like did not understand why they had to be sent to the front. Therefore, the concerns of their families increased their psychological stress to some extent. Some nurses said they mainly worried about their children and parents:

"When I told my parents that I was going to the front, they didn't object, but it was clear they were worried and kept asking me why the TNs have to charge up. Although I explained patiently, I still worried about them."

In some families, both members of a couple are medical workers and sign up to go to the front to treat patients. In addition to worrying about each other, they are concerned about their children's lives and safety:

"My husband is a doctor majoring in respiratory medicine, and facing the epidemic, he resolutely went to the front. Although I am a surgical nurse, I believe I can also make contributions to epidemic prevention and control. However, I still feel sorry and deeply miss my child."

The levels of perceived stress and perceived social supports

In addition, we conducted a quantitative comparison of the perceived stress levels and the amount of perceived social supports between TNs and non-TNs. The result of PSS showed TNs had the higher level of perceived stress, with the significantly higher perceived stress scores than non-TNs (9.88±2.12 vs. 2.58±3.65) (Supplementary Figure 2A and Table 1). Furthermore, in terms of the perceived social supports, TNs got the remarkably less scores of PSSS (71.72±3.29 vs. 78.68±2.45), which represented the lower level of the perceived social supports of TNs than non-TNs (Supplementary Figure 2B and Table 1).

DISCUSSION

This interview survey is the first to pay attention to TNs, who constitute a large proportion of all nurses at the front line against the COVID-19 outbreak, and to provide insight into their vocational and psychological issues caused by the transdisciplinary work. Based on a survey of 25 TNs and 19 non-TNs, higher perceived stress levels and less perceived social support were detected in the TN group. Following further interviews with TNs, we found that ambiguous roles, the transition of operating modes, unfamiliar work contents, the environment and intensity and the reversal of daily schedules were the most common vocational problems for TNs. Additionally, almost all of the TNs had psychological problems such as anxiety, grief, pain and insomnia. Unacceptable mortality and the resulting powerlessness, incomprehension of parents, concern for family members and long-term isolation were the most common causes of psychological stress. These findings are consistent with other studies investigating nurses in emergency departments [15]. However, TNs seem to suffer from more psychological stress.

From conventional nursing to risk-averse infection control, the transformation of responsibility is the first challenge for TNs [16]. Most TNs have never received training for acute respiratory infections, nor have they been exposed to similar tasks, such as environmental disinfection or special care paperwork. For example, the related high risk of infection among TNs is partly because they are trained to temporarily wear and remove protective equipment and are unfamiliar with their operation. Therefore, these nurses believe that improving the ability and experience of TNs and nursing students in epidemic prevention and control is necessary to face epidemic outbreaks. In addition, efficient and reasonable pre-job training is an effective way for TNs to more quickly adapt to epidemic prevention and control-related nursing work.

Many studies have shown that clear role recognition is an important prerequisite for better nursing work [17–19]. For example, Lam K. emphasized that detailed role classification was beneficial for improving work efficiency [20]. The present study showed that more than half of TNs play ambiguous roles, and most of them play the role of psychologists many times to assist patients with psychological disorders. To some extent, the ambiguous roles of TNs at the forefront of the epidemic resulted in vocational issues. The TNs in this study believed that although medical resources were scarce during the specific period of the outbreak of the new coronavirus, more detailed role classification, clearer role definitions and job descriptions, and appropriate suggestions for expanded responsibilities would be effective methods to alleviate role ambiguity and improve work efficiency.

An important but overlooked problem is the psychological issues of nurses on the front line of epidemics. Arnaud Duhoux [21] and Sarah K. Schäfer [22] summarized general mental health problems and posttraumatic stress symptoms as the two most common types of psychopathological issues among

Table 1. Results of PSS and PSSS.

Group	N	PSS		PSSS	
		Means±SD	95%CI	Means±SD	95%CI
Non-TNs	19	2.58±3.65	0.77-4.38	78.68±2.45	77.47-79.90
TNs	25	9.88±2.12	8.93-11.07	71.72±3.29	70.37-73.73
t	-	-7.585		7.556	
P	-	<0.001		<0.001	

All data were normally distributed.

CI: confidence interval, N: numbers, Non-TNs: nurses experienced in infectious diseases, PSS: perceived stress scale, PSSS: perceived social support scale, TNs: transdisciplinary nurses.

nurses. Unfavorable working hours, including long work weeks, night shifts, weekend work, and quick returns, severely affect biological rhythms and work-life balance [23]. Intensive job attributes, including long-term emergency situations and a fast working pace, lead to a constantly high-pressure state [15]. These job characteristics considerably increase the risk of general mental health problems, such as depression, anxiety, insomnia, pain, and grief [24]. Furthermore, the pandemic and the high number of sudden patient deaths can result in posttraumatic stress symptoms reflected in the four aspects of intrusion, avoidance, negative alterations in cognition and mood and alterations in arousal and reactivity [22]. Because of their long-term working experiences that involve intensive and specific work content on the front line of the epidemic, most non-TNs are more familiar with the situation and can readily accommodate it. In contrast, TNs who lack experience with this type of working schedule, environment and intensity on the front line have more difficulty adapting and consequently are more likely to develop psychological disorders.

Although the psychological stress of nurses has been demonstrated to be higher than that of other professions and although nursing is also a high-risk occupation for psychological disease, in the context of a large-scale epidemic of infectious diseases, more attention should be paid to the special group of TNs [25–27]. The present survey about the psychological stresses of TNs found that anxiety, pain, grief and insomnia were the most common psychological problems, which is similar to other nurse-related studies, but with different causes: (1) their colleagues may be infected, and the number of infected TNs is significantly higher than non-TNs, which leads to anxiety among the TNs; (2) TNs have not been in a closed working environment and worn protective suits for a long time, which greatly challenges their psychological and physical limits; (3) unfamiliar working modes and a lack of skill in the content of their work increase their psychological burden; (4) most TNs have difficulty accepting high mortality and helplessness in the face of the large number of severe patients; and (5) compared with non-TNs, TNs suffer from more pressure from their family, and combined with concerns about their family members, their psychological burdens are significantly increased.

The results of the study suggest that in addition to patients' mental disorders, more attention should be paid to the psychological health of nurses, especially TNs. We can establish a psychological consultation platform for medical workers and increase the rear security of front-line medical workers to reduce psychological pressure and maintain their mental health. Furthermore, entertainment and sports facilities, such as running and dancing, could be established, which would be helpful to adjust emotions and relieve pressure.

In the present study, we highlighted the existing issues of TNs at the front line of the COVID-19 outbreak and provided some insights to further address vocational problems and alleviate psychological stress. In subsequent work against pandemics, a more appropriate work schedule, effective pre-job training and more detailed role classification will ameliorate the related vocational issues. In addition, measurements such as psychological consultation platforms and entertainment and sports facilities should be provided to protect the psychological health of TNs.

The present results offer a new perspective on the group of TNs, evaluate the transdisciplinary deficiencies and address existing issues in the treatment of pandemic infectious diseases. However, some limitations remain to be further discussed. (1) Most enrolled TNs worked in the same hospital, which likely resulted in directivity caused by locality and reduced credibility and objectivity. (2) The sample size was relatively low, and a larger-scale survey might further enhance the practical value. In the future, more participants, including TNs and non-TNs from various hospitals, will be recruited to expand the study. (3) The quantifiable measurements are limited, and quantitative follow-up and assessments should be combined to more accurately identify the existing vocational and psychological issues caused by

the transdisciplinary work and further improve the validity and quality of the research.

CONCLUSION

This study aimed to investigate the existing vocational and psychological issues of TNs against the novel coronavirus and attempted to offer possibilities for this special nursing group. This is the first survey to focus on the group of TNs and to investigate their experiences and vocational and psychological problems during the COVID-19 outbreak. We found that TNs had higher perceived stress levels and less perceived social support. Ambiguous roles, unfamiliar work patterns, a lack of skill in the work content leading to higher infection rates among colleagues, and family factors are prominent problems. These findings provide important information and insights into the underlying issues to help TNs ultimately win the battle against novel coronavirus epidemics.

MATERIALS AND METHODS

Design

We conducted a qualitative study utilizing semi-structured and face-to-face interviews to investigate the experiences, vocational issues and psychological stresses of front-line nurses in the process of fighting against the COVID-19 outbreak. The qualitative descriptive method is usually employed to explore individual experiences, cognitions, and inclinations regarding a specific phenomenon [28]. The utilization of a qualitative descriptive method can promote understanding of the phenomenon by soliciting rich viewpoints and opinions from the perspective of participants [29]. Besides, the perceived stress scale (PSS) (Supplementary File 1) and perceived social support scale (PSSS) (Supplementary File 2) were employed to assess their perceived stress levels and the amount of perceived social support. PSS is a psychological instrument to measure nonspecific perceived stress, and PSSS contains seven-point Likert scale ranging from 'strongly disagree' to 'strongly agree'. The flowchart of the entire study, from the screening of eligible nurses to data collection and analysis, is illustrated in Figure 1.

Selection of participants

A purposeful sampling method was used in this study to recruit eligible participants. This sampling method is beneficial in helping researchers collect relevant and valuable information by identifying different participants [30]. In the selection of TNs, nurses were invited to participate in the study if they met the following criteria: (1) registered nurses who had not worked in respiratory medicine, infection departments, or emergency and intensive medicine; (2) nurses in the frontline hospital for COVID-19 in Hubei; (3) actively and directly provided care for patients; and (4) were willing to share their opinions and ideas. In contrast, eligible non-TNs were required to be registered nurses who had experience in respiratory medicine, infection departments, or emergency or intensive medicine. In addition, the non-TNs completed only some of the

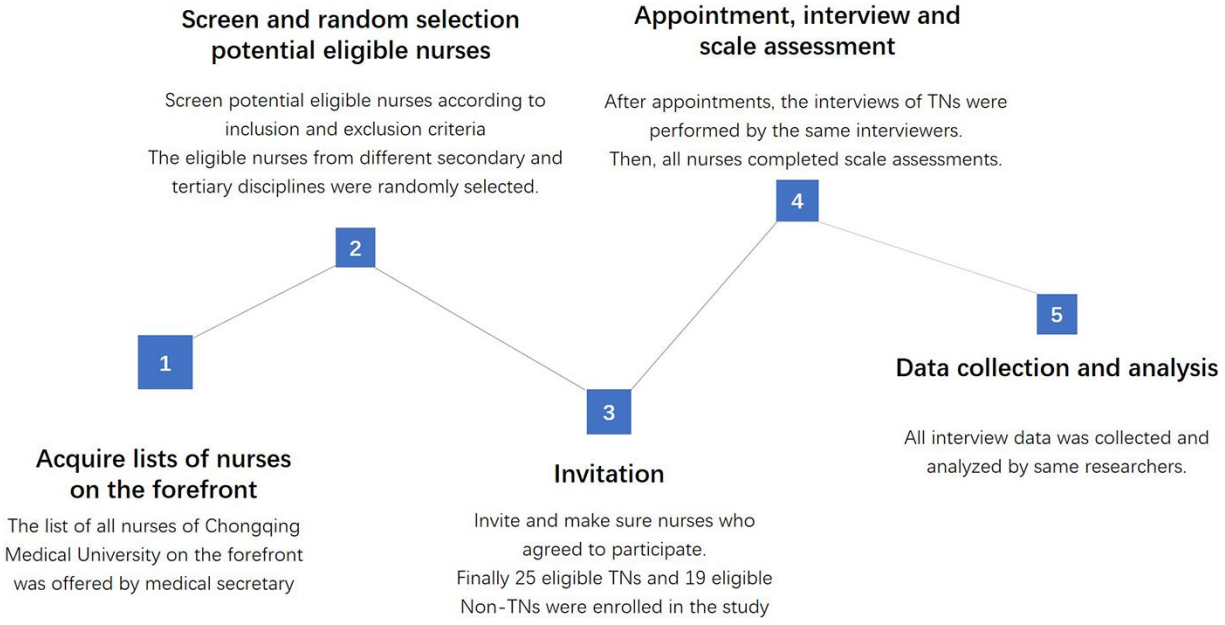

Figure 1. The flowchart of the interview study.

Table 2. Basic information.

Items	TNs [n(%), n=25]	Non-TNs [n(%), n=19]	P values
Gender			0.47
Female	21 (84)	17 (89.5)	
Male	4 (16)	2 (10.5)	
Age (years)			0.51
20-25	8 (32)	6 (31.6)	
26-30	9 (36)	8 (42.1)	
31-35	5 (20)	3 (15.8)	
36-40	2 (8)	2 (10.5)	
>40	1 (4)	0 (0)	
Job title			0.67
Nurse practitioner	20 (80)	15 (78.9)	
Supervisor nurse	5 (20)	4 (21.1)	
Work experience (years)			0.45
1-5	7 (28)	7 (36.8)	
6-10	11 (44)	8 (42.1)	
11-15	4 (16)	2 (10.5)	
>15	3 (12)	2 (10.5)	
Marital status			0.60
Single	6 (24)	4 (21.1)	
In love	2 (8)	2 (10.5)	
Ever-married	17 (68)	13 (68.4)	
Childbearing history			0.43
No children	9 (36)	8 (42.1)	
Be pregnant	0 (0)	0 (0)	
With children	16 (64)	11 (57.9)	

assessment scales. Because this study focused on the experience of front-line nurses in Hubei, nurses in management positions were excluded. Eligible individuals who were interested in participating in the study were contacted through email and were provided with detailed information on the research and the nature of their participation. Participants who were willing to participate in the study were asked to sign an informed consent form. Finally, 25 front-line TNs and 19 front-line non-TNs were enrolled in this study. Table 2 summarizes the demographic data of the participants. The demographic characteristics did not differ significantly between TNs and non-TNs. The various departments of the TNs and non-TNs are listed in Tables 3 and 4, respectively.

Ethical considerations

The research protocol was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. The study conformed to the ethical principles of medical research involving human subjects in the Helsinki Declaration [31]. The informed consent rights, privacy and anonymity of participants were protected.

Data collection

Semi-structured face-to-face interviews with the participants were conducted by the first author to solicit their experiences, vocational issues and psychological states in the forefront of the COVID-19 outbreak. The interviews were arranged in a convenient place for the participants, such as the lounge. To facilitate the follow-up data analysis, all interviews were recorded and backed up with the permission of the participants. The participants were encouraged to express their views and opinions freely. An interview guide comprising open-ended questions was utilized to lead the conversations to the study areas [32] (Figure 2 and Supplementary Figure 1). The average time for an interview was 45 minutes, ranging from 30 to 60 minutes.

In the scale assessments, all participants completed the PSS and the PSSS. The PSS was the version reorganized by Mota-Cardoso et al. [33], consisting of

Table 3. Different departments of TNs.

Departments	Results [n(%), n=25]
Surgical Department	
General Surgery	5 (20)
Urology	2 (8)
Orthopedics	2 (8)
Neurosurgery	1 (4)
Cardiothoracic Surgery	1 (4)
Internal Medicine	
Vasculocardiology	2 (8)
Gastroenterology	2 (8)
Endocrine Medicine	2 (8)
Nephrology	1 (4)
Hematopathology	1 (4)
Obstetrics and Gynaecology	1 (4)
Gerontology	1 (4)
Neurology	1 (4)
Dermatology and Venereology	**1 (4)**
Oncology	1 (4)
Otolaryngology	1 (4)

Table 4. Different departments of Non-TNs.

Departments	Results [n(%), n=19]
Respiratory medicine	5 (26.3)
Infections department	4 (21.1)
Emergency and intensive medicine	10 (52.6)

14 items with 5 alternatives per item, ranging from 0 to 4 points. The points indicated how often they felt or thought about certain events in the past month, from *never* (1 point) to *very often* (4 points). The internal consistency of PSS has been verified, with Cronbach's alpha = 0.90 in the study. The PSSS was composed of 12 items including the aspects of family, friend and others. Participants responded to the items on a 7-point scale representing the degree of agreement, form v*ery strongly disagree* (1 point) to *very strongly agree* (7 points). The internal consistency reliability coefficient of the PSSS was 0.91 in the present study. All

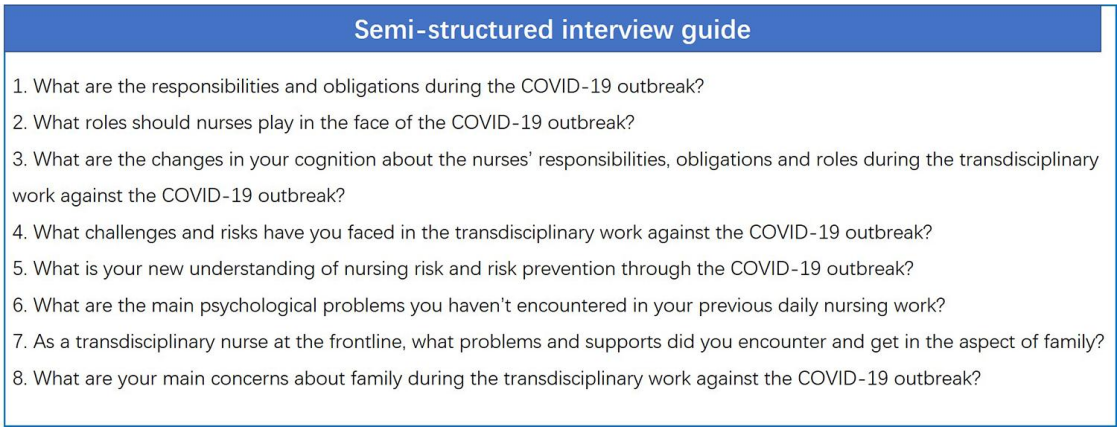

Figure 2. The semi-structured interview guide.

participants completed the scales in a lounge and the whole process of filling in the two scales took around 40 minutes. Subsequently, three researchers simultaneously converted the results of the paper scales to the online version of the scales.

Data analysis

Before the final data analysis of the interviews, the contents of each interview were recorded verbatim at the end of the day. All records were checked to ensure the accuracy of the transcription. Each record was analyzed within three days after the end of the corresponding interview. The Braun Clarke Thematic Analysis method and the strategy outlined by Miles and Huberman were used in the data analysis process [34, 35].

At the beginning of the data analysis process, all seven reliable researchers repeatedly read the interview records line by line and paragraph by paragraph to become familiar with the contents of the data. To develop preliminary codes, the narratives that were considered to be related to the phenomena in the study were emphasized. The records were scrutinized, and the relevant codes were further classified to form themes. Themes were also generated by codes that organized all of the data. We then reviewed these themes to refine the framework within and between themes to establish a network of themes and subthemes (Supplementary Table 1)**.**

The data dependability was established by checking codes among all researchers according to the strategy outlined by Miles and Huberman. All records were double coded by the research team. In all cases, we reached at least 80% agreement in assigning codes between two researchers. Disagreements were resolved by further discussion among the researchers. The investigator triangulation method enhanced the trustworthiness of the results.

Before analyzing the data of the PSS and PSSS, all paper scales were manually entered into the online scale version by three researchers simultaneously. When identical and credible results were obtained from the three researchers, the relevant data were further analyzed by SPSS (version 24). All quantitative data were normally distributed. MANOVAs, chi-square tests and t-tests for independent samples were employed to assess differences between non-TNs and TNs.

Trustworthiness

Trustworthiness is the standard that constitutes the rigor of qualitative research [36]. The trustworthiness of this study was maintained by establishing four main standards, including credibility, confirmability, transferability and dependability. In terms of credibility, the content of the study was discussed through continuous communications between the researchers and the supervisor. The supervisor conducted a critical assessment to identify defects in the investigation and corrected them with the researchers. In terms of confirmability, member-checking with all participants was completed to validate the interpreted findings [37]. Participants were asked to verify the survey results to ensure that their opinions were accurately reflected in the data and to check the consistency between the results of the researchers and the actual intentions of the participants. In terms of transferability, we used a vivid description method to ensure sufficient and accurate contextual information. The findings and conclusions can be transferred to other studies with similar situations [38].

Dependability is achieved through the accurate records and the in-depth description of the methods used in the research. Besides, Cohen's weighted kappa was employed to evaluate the interrater reliability. The poor agreement was considered if $\kappa < 0.00$, slight agreement if between 0.00 and 0.20, fair agreement if between 0.21 and 0.40, moderate agreement of between 0.41 and 0.60, substantial agreement if between 0.61 and 0.80, and almost perfect agreement of between 0.81 and 1.00 [39].

CONFLICTS OF INTEREST

These authors declare no conflicts of interest.

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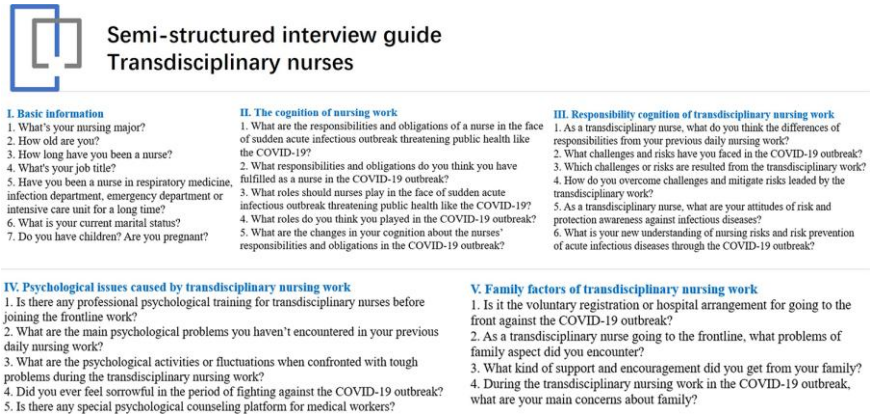
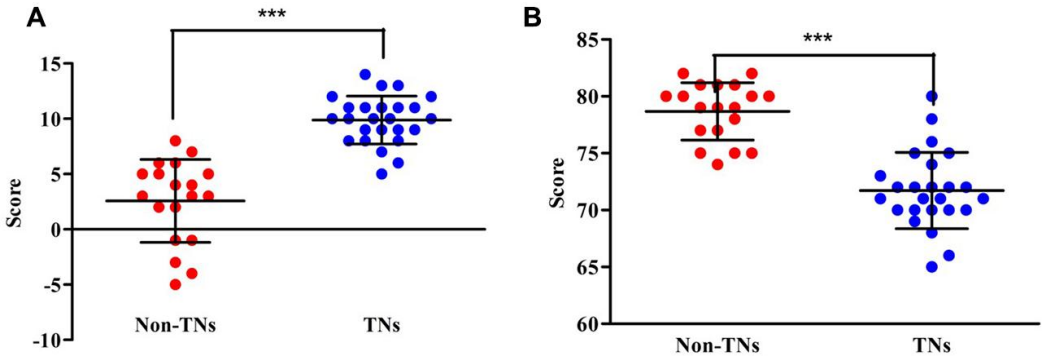
SUPPLEMENTARY MATERIALS

Supplementary Figures



Supplementary Figure 1. The semi-structural interview guide including five parts.



Supplementary Figure 2. The perceived stress levels and the amount of perceived social supports of TNs and Non-TNs. (**A**) the perceived stress scores, the higher score represents the higher level of perceived stress. (**B**) the perceived social support scores, the higher score represents the higher perceived social support level. The data are normally distributed, and are expressed as the means ± SD. ***P<0.001 TNs *vs.* Non-TNs.

Supplementary Table

Supplementary Table 1. The data record table.

Themes and Sub-themes	Results [n (%), n=25]
Responsibility cognition	
healing the wounded and rescuing the dying	25 (100)
the Nightingale spirit	19 (76)
win the battle against the COVID-19	18 (72)
relieve mental and psychological pressure of patients	15 (60)
take care of the daily life of patients	7 (28)
Role cognition	
nurse	25 (100)
friend	22 (88)
family	17 (68)
psychotherapist	13 (52)
patient care	5 (20)
New cognition of nursing work	
With new cognition	25 (100)
stronger sense of responsibility and mission	21 (84)
more comprehensive nursing skills	20 (80)
playing a variety of different roles	16 (64)
full of love to patients and the job	13 (52)
Without new cognition	0 (0)
Challenges of transdisciplinary nursing work	
unfamiliar working patterns	25 (100)
unfamiliar working contents	23 (92)
standardized professional operations	20 (80)
occupational exposure and self-protection	20 (80)
physical and psychological quality	19 (76)
Psychological issues	
grief	22 (88)
insomnia	18 (72)
anxiety	16 (64)
pain	13 (52)
depressed	7 (28)
dysphoria	4 (16)
Family factors of transdisciplinary nursing work	
miss and worry about families	25 (100)
parents don't understand	12 (48)
spouse doesn't understand	8 (32)
guilt towards families	5 (20)

Supplementary Files

Please browse Full Text version to see the data of Supplementary Files 1 and 2.

Supplementary File 1. Perceived Stress Scale.

Supplementary File 2. Multidimensional Scale of Perceived Social Support.

Research Paper

Epidemiological, comorbidity factors with severity and prognosis of COVID-19: a systematic review and meta-analysis

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ABSTRACT

A systematic review and meta-analysis was conducted in an attempt to systematically collect and evaluate the associations of epidemiological, comorbidity factors with the severity and prognosis of coronavirus disease 2019 (COVID-19). The systematic review and meta-analysis was conducted according to the guidelines proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Sixty nine publications met our study criteria, and 61 studies with more than 10,000 COVID-19 cases were eligible for the quantitative synthesis. We found that the males had significantly higher disease severity (RR: 1.20, 95% CI: 1.13-1.27, P <0.001) and more prognostic endpoints. Older age was found to be significantly associated with the disease severity and six prognostic endpoints. Chronic kidney disease contributed mostly for death (RR: 7.10, 95% CI: 3.14-16.02), chronic obstructive pulmonary disease (COPD) for disease severity (RR: 4.20, 95% CI: 2.82-6.25), admission to intensive care unit (ICU) (RR: 5.61, 95% CI: 2.68-11.76), the composite endpoint (RR: 8.52, 95% CI: 4.36-16.65,), invasive ventilation (RR: 6.53, 95% CI: 2.70-15.84), and disease progression (RR: 7.48, 95% CI: 1.60-35.05), cerebrovascular disease for acute respiratory distress syndrome (ARDS) (RR: 3.15, 95% CI: 1.23-8.04), coronary heart disease for cardiac abnormality (RR: 5.37, 95% CI: 1.74-16.54). Our study highlighted that the male gender, older age and comorbidities owned strong epidemiological evidence of associations with the severity and prognosis of COVID-19.

INTRODUCTION

Since publicly characterized as a pandemic by the World Health Organization on March 11th, 2020, the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2, has raised public concerns globally [1]. As of April 30th, 2020, it has caused 3,023,788 confirmed cases and 208,112 deaths [2]. Further, this number is expected to continue to grow rapidly for some time to come, and will threaten the lives, physical and mental health of more people worldwide [3]. To date, there are still no proven specific therapies

available for COVID-19, other than supportive cares [4, 5]. It's a matter of urgency that identifying potential factors affecting the severity and prognosis of COVID-19, and implementing individualized treatment, focused prevention and nursing.

Case-series or retrospective cohort studies have initially explored the associations of epidemiological, comorbidity factors with severity and prognosis of COVID-19 [the multi-stage endpoints including disease severity, acute respiratory distress syndrome (ARDS), an intensive care unit (ICU), the use of mechanical ventilation, or death, etc.] [6–13]. Huang et al. first explored the contribution of demographic and comorbidity factors for ICU admission in 41 COVID-19 cases, and got null results [6]. Further, they conducted a retrospective cohort study with 137 discharged and 54 patients who died, and concluded older age and comorbidities were associated with prognosis of COVID-19 [7]. Meanwhile, in another study with 201 patients, Wu et al. found that older age was associated with higher risk of ARDS and death [8]. A study with 1,590 patients revealed that comorbidities were associated with poorer clinical outcomes [9]. Of note, some studies reported inconsistent, even contradictory conclusions, which might be caused by limited sample size or low endpoint rate [10–13]. Besides, reporting of the same patients in different articles was another concern [14]. Against this context, a thorough understanding of the epidemiological and comorbidity factors upon COVID-19 is urgently warranted. Herein, a systematic review and meta-analysis was conducted to sought to collect and comprehensively evaluate the associations of epidemiological, comorbidity factors with the severity and prognosis of COVID-19.

RESULTS

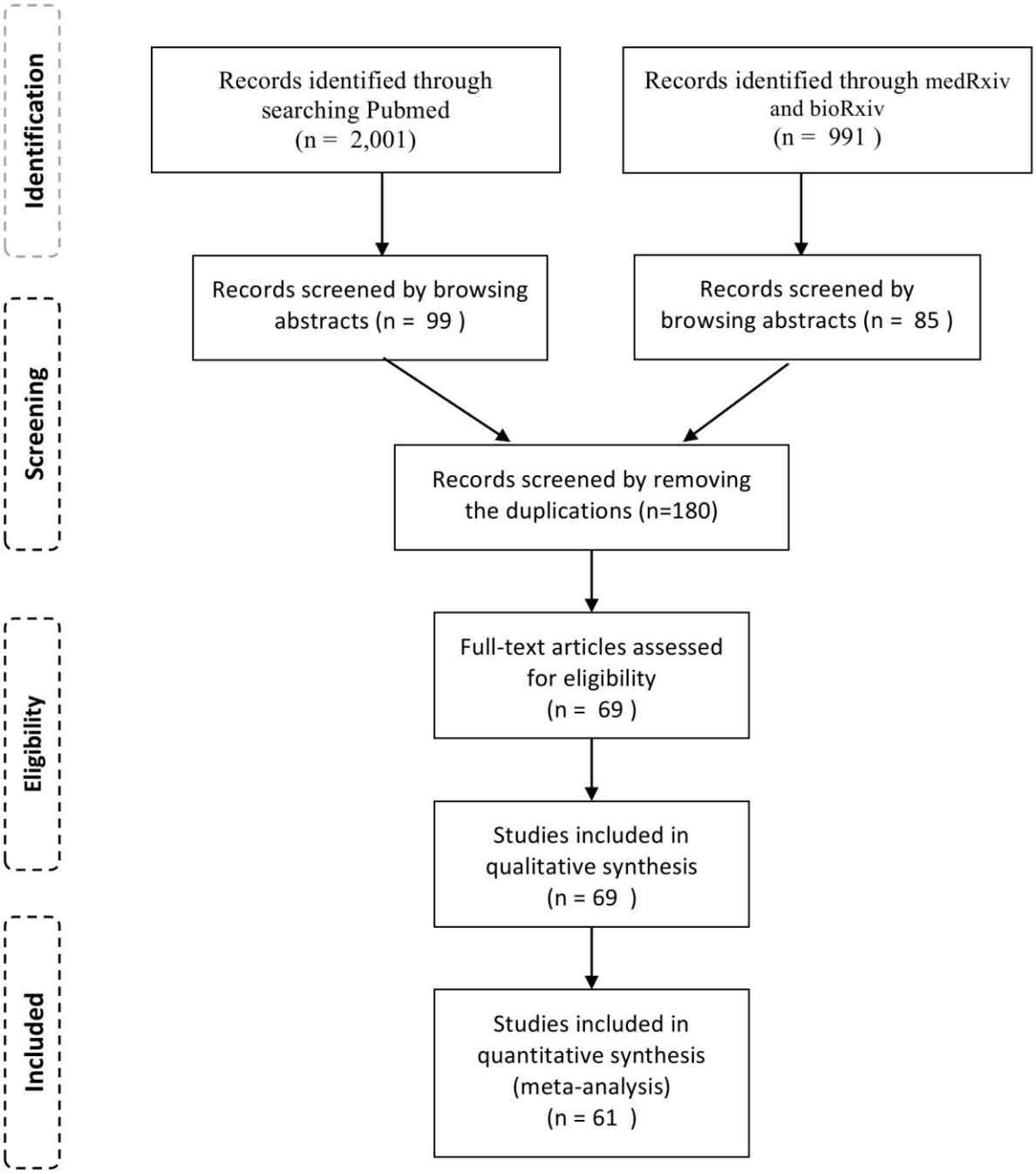
Study characteristics

Figure 1 presents the PRISMA flow diagram of this study. First, an initial search generated 2,992 potentially relevant papers, of which 2001 identified form Pubmed, and 991 from medRxiv or bioRxiv. After a number of screenings, 69 studies were identified (Supplementary Table 1). Of them, 67 (97.1%) reported Chinese COVID-19 patients, and 2 from either Japan or Singapore. The case number of each study ranged from 21 to 1780, with a mean of 218. The NOS score ranged from 5 to 7, which means a moderate methodological quality. Of the 69 publications, 2 duplicated studies (endpoints, exposure indicators and populations are completely covered by other studies), 4 studies with unique endpoints (survival ≤3d, refractory, liver injury, and time since symptom onset > 10 days), and 2 studies with different grouping methods for disease severity were excluded, which resulted that 61 studies were eventually eligible for the quantitative synthesis (Supplementary Table 1).

Quantitative data synthesis

The forest plots for all quantitative data synthesis of the epidemiological, comorbidity factors with severity and prognosis of COVID-19 were shown in supplementary materials (Supplementary Figures 1–120). Table 1 presents the quantitative results for the associations of the dichotomous epidemiological, comorbidity factors with severity of COVID-19. First, we found that the males had significant higher disease severity (RR: 1.20, 95% CI: 1.13-1.27, P <0.001, No. of cases: 8916). Besides, comorbidities, including any comorbidities, hypertension, diabetes, malignancy, cardiovascular disease, coronary heart disease, cerebrovascular disease, cardiovascular/cerebrovascular disease, chronic obstructive pulmonary disease (COPD), respiratory system disease, chronic kidney disease, hepatitis B infection, and digestive disease were significantly associated with the disease severity (all P <0.05). Of them, the top 3 effect sizes for the severity of COVID-19 were detected for COPD (RR: 4.20, 95% CI: 2.82-6.25, P<0.001), respiratory system disease (RR: 3.25, 95% CI: 2.48-4.27, P<0.001), and cerebrovascular disease (RR: 2.77, 95% CI: 1.70-4.52, P<0.001).

We also explored the associations of the dichotomous epidemiological, comorbidity factors with prognosis of COVID-19 (Supplementary Table 2, and Table 2). The males had higher risk of developing the endpoints including death, ARDS, admission to ICU, invasive ventilation, and cardiac abnormality. Hypertension was found to be associated with all seven endpoints, cardiovascular disease and cerebrovascular disease with 6 (except for disease progression), respiratory system disease with 6 (except for Cardiac abnormality), COPD with 5 (except for ARDS and cardiac abnormality), diabetes with 5 (except for the composite endpoint, cardiac abnormality), malignancy with 2 (death, and admission to ICU), etc. Among them, chronic kidney disease contributed mostly for death (RR: 7.10, 95% CI: 3.14-16.02, P<0.001), COPD for admission to ICU (RR: 5.61, 95% CI: 2.68-11.76, P<0.001), the composite endpoint (RR: 8.52, 95% CI: 4.36-16.65, P<0.001), invasive ventilation (RR: 6.53, 95% CI: 2.70-15.84, P<0.001), and disease progression (RR: 7.48, 95% CI: 1.60-35.05, P =0.011), cerebrovascular disease for ARDS (RR: 3.15, 95% CI: 1.23-8.04, P =0.016), coronary heart disease for cardiac abnormality (RR: 5.37, 95% CI: 1.74-16.54, P =0.003). Besides, the associations of continuous age with severity and prognosis of COVID-19 were presented in Table 3. Older age was found to be significantly associated with the disease severity and six endpoints (all P value <0.001, except a marginal



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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Figure 1. PRISMA flow diagram.

Table 1. Quantitative data synthesis for the associations of the epidemiological, comorbidity factors with severity of COVID-19.

Variables	No of studies	Total cases	P heterogeneity	I^2 (%)	RR (95% CIs)	P value	P Egger
Sex, male	33	8916	0.078	27.2	1.20 (1.13-1.27)	<0.001	0.040
Smoking	11	5237	<0.001	80.8	1.56 (0.95-2.57)	0.082	0.956
Current smoking	2	2879	0.133	55.6	1.17 (0.92-1.50)	0.198	-
Ex-smoking	2	2879	0.019	81.7	2.17 (0.61-7.70)	0.232	-
Drinking	4	2274	0.067	58.0	0.83 (0.48-1.44)	0.516	0.722
Local residents of Wuhan	4	1931	<0.001	90.9	0.66 (0.32-1.36)	0.256	0.441
Exposure to Hubei Province	10	3127	<0.001	90.9	1.21 (0.88-1.65)	0.240	0.115
Contact with confirmed or suspect cases	13	5007	0.041	45.8	0.98 (0.86-1.13)	0.801	0.072
Family cluster	5	2578	0.857	0.0	0.94 (0.86-1.04)	0.224	0.856
Huanan seafood market exposure	5	2342	0.001	79.9	1.79 (0.38-8.35)	0.459	0.212
Comorbidities	16	6219	<0.001	83.4	1.72 (1.44-2.06)	<0.001	0.710
Hypertension	23	7739	<0.001	75.0	2.09 (1.74-2.52)	<0.001	0.154
Diabetes	23	7739	0.017	42.6	1.95 (1.60-2.36)	<0.001	0.272
Malignancy	14	5905	0.137	30.0	1.56 (1.11-2.21)	0.011	0.644
Cardiovascular disease	18	6841	0.019	45.5	2.74 (2.03-3.70)	<0.001	<0.001
Coronary heart disease	8	3899	0.087	43.7	2.03 (1.39-2.15)	<0.001	0.040
Cerebrovascular disease	12	5756	0.074	40.0	2.77 (1.70-4.52)	<0.001	0.595
Cardiovascular/cerebrovascular disease	6	3057	<0.001	84.0	2.31 (1.31-4.08)	0.004	0.502
COPD	14	6609	0.492	0.0	4.20 (2.82-6.25)	<0.001	0.580
Respiratory system disease	18	7522	0.661	0.0	3.25 (2.48-4.27)	<0.001	0.577
Chronic kidney disease	15	4861	0.173	25.5	2.27 (1.55-3.32)	<0.001	0.179
Chronic liver disease	11	3248	0.201	25.5	1.35 (0.89-2.05)	0.165	0.782
Hepatitis B infection	3	1710	0.448	0.0	2.69 (1.32-5.51)	0.007	0.735
Lithiasis	2	308	0.873	0.0	3.03 (0.73-12.58)	0.127	-
Autoimmune disease	5	2202	0.727	0.0	2.52 (0.80-7.90)	0.113	0.997
Abnormal lipid metabolism	4	2246	0.648	0.0	0.57 (0.26-1.25)	0.162	0.080
Digestive disease	5	1013	0.492	0.0	1.80 (1.13-2.87)	0.014	0.717
Thyroid disease	3	348	0.350	0.0	2.37 (0.66-8.50)	0.186	0.387
Tuberculosis	2	592	0.473	0.0	2.74 (0.72-10.4)	0.141	-
Nervous system disease	3	796	0.368	0.0%	1.64 (0.68-3.93)	0.270	0.160
Endocrine system disease	3	796	<0.001	89.6	3.09 (0.70-13.64)	0.136	0.622

Table 2. Quantitative data synthesis for the associations of the epidemiological, comorbidity factors with prognosis of COVID-19 (P value<0.05).

Variables	No of studies	Total cases	P heterogeneity	I^2 (%)	RR (95% CIs)	P value	P Egger
Death							
Sex, male	10	4214	0.443	0.0	1.23 (1.14-1.33)	<0.001	0.276
Comorbidities	8	4499	<0.001	88.7	1.68 (1.32-2.13)	<0.001	0.248
Hypertension	11	4860	<0.001	84.4	1.74 (1.31-2.30)	<0.001	0.418
Diabetes	10	4748	0.001	67.1	1.75 (1.27-2.41)	0.001	0.057
Malignancy	6	3978	0.262	22.8	3.09 (1.59-6.00)	0.001	0.006
Cardiovascular disease	11	4860	<0.001	75.9	2.67 (1.60-4.43)	<0.001	0.654
Coronary heart disease	5	2452	<0.001	87.7	3.16 (1.45-6.91)	0.004	0.435
Cerebrovascular disease	6	3771	0.457	0.0	4.61 (2.51-8.47)	<0.001	0.766
COPD	4	3677	0.279	22.0	5.31 (2.63-10.71)	<0.001	0.107
Respiratory system disease	7	4472	0.185	31.8	3.22 (2.12-4.90)	<0.001	0.761
Chronic kidney disease	5	2219	0.477	0.0	7.10 (3.14-16.02)	<0.001	0.772
Admission to ICU							
Sex, male	5	2224	0.011	69.6	1.29 (1.13-1.47)	<0.001	0.651

Comorbidities	5	3747	0.038	60.5	1.82 (1.45-2.29)	<0.001	0.646
Hypertension	5	3747	0.601	0.0	2.31 (1.97-2.70)	<0.001	0.312
Diabetes	5	3747	0.084	51.4	1.88 (1.10-3.23)	0.021	0.457
Malignancy	5	3747	0.427	0.0	2.52 (1.38-5.59)	0.003	0.158
Cardiovascular disease	5	3747	0.511	0.0	2.74 (1.92-3.92)	<0.001	0.692
Cerebrovascular disease	3	3508	0.349	4.9	5.12 (2.86-9.17)	<0.001	0.273
COPD	4	3549	0.800	0.0	5.61 (2.68-11.76)	<0.001	0.740
Respiratory system disease	4	3549	0.613	0.0	4.66 (2.59-8.40)	<0.001	0.637
Composite endpoint							
Smoking	2	2879	0.604	0.0	2.67 (1.91-3.73)	<0.001	-
Comorbidities	2	3370	<0.001	95.3	1.96 (1.06-3.60)	0.031	-
Hypertension	2	3370	0.011	84.5	2.20 (1.44-3.36)	<0.001	-
Cardiovascular disease	2	3370	0.927	0.0	3.09 (2.09-4.57)	<0.001	-
Coronary heart disease	2	3370	0.473	0.0	3.36 (2.15-5.25)	<0.001	-
Cerebrovascular disease	2	3370	0.225	32.0	4.10 (2.34-7.18)	<0.001	-
COPD	2	3370	0.185	43.0	8.52 (4.36-16.65)	<0.001	-
Respiratory system disease	2	3370	0.185	43.0	8.52 (4.36-16.65)	<0.001	-
ARDS							
Sex, male	3	2090	0.464	0.0	1.15 (1.01-1.30)	0.033	0.353
Hypertension	3	2090	0.377	0.0	1.90 (1.57-2.30)	<0.001	0.520
Diabetes	3	2090	0.068	62.9	3.07 (1.28-7.36)	0.012	0.066
Cardiovascular disease	3	2090	0.244	29.2	2.26 (1.43-3.58)	<0.001	0.422
Cerebrovascular disease	2	1889	0.152	51.2	3.15 (1.23-8.04)	0.016	-
Respiratory system disease	2	1889	0.303	5.6	2.44 (1.20-4.97)	0.014	-
Invasive ventilation							
Sex, male	2	1825	0.403	0.0	1.35 (1.11-1.64)	0.002	-
Family cluster	2	1825	0.646	0.0	1.58 (1.13-2.14)	0.006	-
Comorbidities	3	3415	0.005	81.2	1.83 (1.19-2.79)	0.006	0.569
Hypertension	3	3415	0.131	50.9	2.35 (1.92-2.89)	<0.001	0.366
Diabetes	3	3415	0.131	50.8	1.85 (1.24-2.76)	0.003	0.021
Cardiovascular disease	3	3415	0.844	0.0	2.90 (1.63-5.15)	<0.001	0.618
Cerebrovascular disease	2	3370	0.602	0.0	3.98 (1.77-8.93)	0.001	-
COPD	2	3370	0.383	0.0	6.53 (2.70-15.84)	<0.001	-
Respiratory system disease	3	3415	0.260	25.7	4.34 (2.04-9.26)	<0.001	0.567
Cardiac abnormality							
Sex, male	4	439	0.211	33.6	1.33 (1.02-1.72)	0.036	0.624
Hypertension	4	439	0.947	0.0	2.97 (1.65-5.34)	<0.001	0.610
Cardiovascular disease	4	439	0.915	0.0	4.90 (1.82-13.21)	0.002	0.177
Coronary heart disease	3	386	0.819	0.0	5.37 (1.74-16.54)	0.003	0.408
Disease progression							
Hypertension	2	219	0.547	0.0	2.90 (1.45-5.81)	0.003	-
Diabetes	2	219	0.746	0.0	3.30 (1.08-10.07)	0.036	-
COPD	2	219	0.848	0.0	7.48 (1.60-35.05)	0.011	-
Respiratory system disease	2	219	0.848	0.0	7.48 (1.60-35.05)	0.011	-

association for disease progression). The biggest standard mean difference (SMD) was detected for death (SMD: 1.06, 95% CI: 0.85-1.26, P<0.001). However, we didn't find any statistically significant associations for epidemiological factors, including drinking, local residents of Wuhan, exposure to Hubei Province, contact with confirmed or suspect cases, family cluster, and Huanan seafood market exposure. Sensitivity analyses by changing the pooling model and statistical variables, or using one-at-a-time method, were performed to assess the stability of the results. However, we found the results were not materially changed (data not shown). Further, we applied the Egger test to evaluated the potential publication bias, and very litter evidence (among all

Table 3. Quantitative data synthesis for the associations of age with severity and prognosis of COVID-19.

Variables	No of studies	Total cases	$P_{heterogeneity}$	I^2 (%)	SMD (95% CIs)	P value	P_{Egger}
Severity	32	8140	<0.001	92.4	0.73 (0.53-0.94)	<0.001	0.331
Death	9	3725	0.005	63.9	1.06 (0.85-1.26)	<0.001	0.610
Admission to ICU	5	2224	0.189	34.9	0.78 (0.60-0.96)	<0.001	0.538
Composite endpoint	2	2879	0.055	72.9	0.88 (0.56-1.21)	<0.001	-
ARDS	3	2090	0.939	0	0.83 (0.67-0.99)	<0.001	0.882
Invasive ventilation	2	1825	0.493	0.0	0.84 (0.54-1.14)	<0.001	-
Cardiac abnormality	4	439	0.041	63.6	0.92 (0.44-1.41)	<0.001	0.885
Disease progression	2	219	<0.001	95.4	2.37 (0.00-4.74)	0.050	-

120 associations, only 6 presented the existence of possible publication bias) was detected (Tables 1–3 and Supplementary Table 2).

DISCUSSION

To our knowledge, this should be the most comprehensive assessment of epidemiological, comorbidity factors with the severity and prognosis of COVID-19 conducted to date. We systematically evaluated data for more than ten thousand COVID-19 cases from 69 publications in the past several months, and identified that the males had higher risk of reaching severe disease and adverse prognostic endpoints. Older age was found to be significantly associated with the disease severity and six prognostic endpoints. Comorbidities, including hypertension, diabetes, cardiovascular disease, cerebrovascular disease, COPD, chronic kidney disease, and malignancy, contributed significantly to the disease severity and prognostic endpoints of COVID-19. Results from the current study would be helpful for implementing individualized treatment, focused prevention and nursing of COVID-19.

The "Gender and COVID-19 Working Group" first raised the concern of the gendered impacts of the COVID-19 outbreak [15], then echoed by another two publications [16, 17]. In a large epidemiological investigation in China with 72,314 cases, 51.0% of the patients were the males [18]. In a recent report of 1,590 hospitalized COVID-19 patients in China [9], the male rate was 57.3%, and it was 51.3% in Huoshenshan study with 1780 hospitalized cases. All these evidence indicated the almost equal sex distribution and disease susceptibility. Despite this, we identified that the males had higher rate of severity and prognostic endpoints in our meta-analysis. This finding was indirectly proved by a Italian study with 1591 ICU patients, the male rate of which was 82.0%, and higher than that previously reported [19]. However, the smoking status which has significant gender predisposition, showed no statistical associations with disease severity and prognosis of COVID-19, except for composite endpoint. The relationship between smoking and COVID-19 has become a very controversial topic, and should be interpreted with caution, as many factors could affect the results, such as the statistical power, definition of smoking status, the presence of confounding factors, and the potential role of angiotensin-converting enzyme-2 (ACE-2) [20–22]. Older age was another strong determinant of disease referral and outcomes in our results, which has been proved by a model-based analysis [23], and supported by studies of severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) [24, 25].

In addition to epidemiological factors, comorbidities are also potentially important aspects which could affect the disease severity and prognosis of COVID-19. As a key regulator of blood pressure, angiotensin-converting enzyme (ACE) was also the binding site of SARS-CoV, making hypertension the most focused comorbidity [26, 27]. In our meta-analysis, we found hypertension was associated with higher rate of the disease severity and all prognostic endpoints. Of note, using of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type 1 receptor blockers (ARBs) could contribute to the improvement of outcomes of COVID-19 patients with hypertension [28]. COPD was a major predominant indicator for the disease severity and prognosis of COVID-19. In our study, COPD contributed most to the admission to ICU, the composite endpoint, and invasive ventilation of COVID-19. Among the comorbidities, the contribution of malignancy to the prognosis of COVID-19 was a controversial topic. Liang et al. [29] first reported that patients with cancer had a higher risk of COVID-19 and with a poorer prognosis than those without cancer, then challenged by two other publications because of the sample size, and confounding factors [30, 31]. Our meta-analysis temporarily supported Liang's conclusion that malignancy contributed to death, and admission to ICU with a moderate sample size, although we can't adjusted for the potential confounding bias. An interesting finding

was that chronic kidney disease contributed mostly to the death. It is likely an immunologic explanation, given our current understanding of weakened immune system in patients with chronic kidney disease [32]. A more targeted and intensive health protection strategy for the patients with comorbidities above may be warranted.

The strengths of our study included an extensive systematic search strategy, a thorough examination of duplicate data, and a comprehensive quality assessment of the primary studies. The findings in the current study are also affected by several limitations. First, although we have systematically searched the literature to identify eligible studies, it is possible that some studies might have been missed. Despite the wide ranging search strategy, non-English language studies might not have been indexed in the databases we searched. Second, all studies except for two studies from Japan or Singapore, were from China in the early stage of the COVID-19 outbreak. This limited the findings' applicability across different populations and geographic regions during the COVID-19 pandemic. Third, although we have screened the hospital name, date of recruitment aiming to find duplicated usage of cases, it is inevitable as some studies used samples from multiple hospitals and have not reported detailed patient composition. This might affect the accurate estimates of disease prevalence or outcomes of COVID-19. Fourth, significant study heterogeneity or publication bias which may lead to questionable interpretation of result were detected for some associations (especially for comorbidities). Thus, these results should be interpreted with caution. Finally, moderate NOS score means the flawed methodological quality. However, as these studies were dealing with urgent public health concerns and carried out in a state of emergency, the quality was within acceptable limits. Taken together, despite some limitations, our study provides an important basis for a comprehensive understanding of disease severity and prognosis-related factors.

CONCLUSIONS

In our systematic review and meta-analysis, we highlighted that the male gender, older age and comorbidities showed strong epidemiological evidence of associations with the severity and prognosis COVID-19. Taken together, this large-scale meta-analysis not only summarizes the current literatures upon associations of epidemiological, comorbidity factors with the severity and prognosis of COVID-19, but also provides helpful clues for implementing individualized treatment, focused prevention and nursing of COVID-19. Further well-designed prospective cohort studies and randomized controlled trials are warranted to explore the severity and prognosis related factors of COVID-19.

MATERIALS AND METHODS

Search strategy and selection criteria

The systematic review and meta-analyses were conducted and reported according to the guidelines proposed by the PRISMA [33]. Studies were eligible for inclusion in this meta-analysis if they met the following criteria: (1) data published in a peer-reviewed journal in English or Chinese; (2) the study is a case-control, cohort, or a cross-sectional design in human beings; (3) the studies provide sufficient information for epidemiological, comorbidity factors with severity or prognosis of COVID-19; (4) When multiple publications reported on the same hospital, date of recruitment, exposures and endpoints, we defined it as duplicated studies. Some studies, although duplicate in terms of hospital and date of recruitment, were not judged to be duplicate because they evaluated different exposure indicators or endpoints.

Literature retrieval was conducted through a two-step strategy with a cut-off date of April 5th, 2020 (Figure 1). In step 1, we searched the PubMed database using the following key terms in combination: "2019-nCoV OR COVID-19 OR covid-2019 OR novel Coronavirus–Infected Pneumonia OR novel coronavirus OR SARS-CoV-2 OR Wuhan Coronavirus OR Wuhan pneumonia". In step 2, the COVID-19 or SARS-CoV-2 preprints were also retrieved from the medRxiv (https://www.medrxiv.org/) and bioRxiv (https://www.biorxiv.org/) databases using the above terms sequentially. We also searched the references and related articles of all gathered papers, and checked previously published meta-analyses and reviews. In the current study, we also incorporated the data of 1780 COVID-19 cases from Wuhan Huoshenshan hospital, the first and largest emergency specialty field hospital in epicenter Wuhan, China. Finally, 69 publications met our study criteria were included in the systematic review, and 61 studies were eligible for the quantitative synthesis.

Data extraction and quality control

All data were extracted by at least two authors (FX, LS, YH, and WP) according to the pre-specified selection criteria. Disagreement was resolved by discussion with a third party personnel. The details for each study including the first author, country, city or province, year of publication, source hospitals, date duration of the patient recruitment, PubMed identifier number (PMID) or the digital object identifier (DOI) number, total sample size, disease severity, clinical endpoints, the distribution of epidemiological and comorbidity factors, were extracted using a structured data sheet. Frequency numbers of dichotomous variables, and median (IQR,

interquartile range) or mean (SD, standard deviation) for continuous variables were recorded. Median (IQR) were transferred to the form of mean (SD) using the method recommended by the Cochrane handbook version 6, 2019. The endpoints consisted of disease severity, ARDS, admission to ICU, death, a composite endpoint, invasive ventilation, cardiac abnormality, and disease progression (the detailed definitions of the endpoints were presented in supplementary methods). The degree of severity of COVID-19 was determined using the American Thoracic Society guidelines for community-acquired pneumonia or the New Coronavirus Pneumonia Prevention and Control Guidelines of China [34, 35]. Two authors independently evaluated the methodological quality of included studies using the Newcastle-Ottawa Scale (NOS) for cohort study [36]. Any disagreement in the quality assessment was resolved by discussion with a third author. The NOS includes 8 items (up to 9 stars), each one of these items was scored from 0 to 1, except that a maximum of two stars can be given for comparability.

Data synthesis

All statistical analyses for this study were performed by STATA, version 12.0 (Stata Corporation, College Station, Texas). All tests were two-sided, and a *P*-value of less than 0.05 for any test or model was considered statistically significant unless otherwise stated. Meta-analysis was performed for all associations with data available from 2 or more independent samples. Summary relative ratios (RRs), or standard mean difference (SMD) with their 95% confidence intervals (CIs) were used to assess the strength of associations between epidemiological, comorbidity factors with the severity and prognosis of COVID-19 by either the fixed-effect model (Mantel–Haenszel method) or, in case of heterogeneity, the random-effect model (DerSimonian–Laird method). To assess inter-study heterogeneity, we calculated the chi-square-based Cochran's *Q* statistic test and I^2 statistic. Because of the low power of Cochran's *Q* statistic, heterogeneity was considered significant if $P < 0.10$. For I^2, values around 25% indicated low heterogeneity, around 50% moderate heterogeneity, and around 75% high heterogeneity. Publication bias was assessed visually by funnel plots and quantitatively with Egger' s regression test for asymmetry.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Please browse Full Text version to see the data of Supplementary Methods, Figures and Tables related to this manuscript.

Research Paper

Risk factors influencing the prognosis of elderly patients infected with COVID-19: a clinical retrospective study in Wuhan, China

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ABSTRACT

The mortality rate of elderly patients with Coronavirus Disease 2019 (COVID-19) was significantly higher than the overall mortality rate. However, besides age, leading death risk factors for the high mortality in elderly patients remain unidentified. This retrospective study included 210 elderly COVID-19 patients (aged ≥ 65 years), of whom 175 patients were discharged and 35 died. All deceased patients had at least one comorbidity. A significantly higher proportion of patients in the deceased group had cardiovascular diseases (49% vs. 20%), respiratory diseases (51% vs. 11%), chronic kidney disease (29% vs. 5%) and cerebrovascular disease (20% vs. 3%) than that in the discharged group. The median levels of C-reactive protein (125.8mg/L vs. 9.3mg/L) and blood urea nitrogen (7.2mmol/L vs. 4.4mmol/L) were significantly higher and median lymphocyte counts (0.7×10^9/L vs. 1.1×10^9/L) significantly lower in the deceased group than those in the discharged group. The survival curve analysis showed that higher C-reactive protein (≥5mg/L) plus any other abnormalities of lymphocyte, blood urea nitrogen or lactate dehydrogenase significantly predicted poor prognosis of COVID-19 infected elderly patients. This study revealed that the risk factors for the death in these elderly patients included comorbidities, increased levels of C-reactive protein and blood urea nitrogen, and lymphopenia during hospitalization.

INTRODUCTION

Since the outbreak in December 2019, COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly grown into a pandemic worldwide [1–3]. While the overall mortality rate during the early phase of the pandemic in both China and Italy was around 2.3% [1, 4, 5], the mortality rate was significantly higher in the elderly especially in those aged 65 years or older. A report from the United States Centers for Disease Control and Prevention COVID-19 response team showed that 80% of deaths associated with COVID-19 were among adults aged ≥65 years [6], which is similar to that initially reported from China regarding the high mortality rate in elderly patients with COVID-19 [7, 8]. Available evidence

suggest that old age *per se*, especially aged ≥65 years [9], is an independent risk factor for COVID-19 related mortality irrespective of whether there may exist underlying comorbidities. In addition, the majority of the persons aged ≥65 years may have one or more pre-existing complications which would further increase the rate of mortality. At present, clinical research on COVID-19 has been mainly focused on the epidemiological characteristics, clinical manifestations, prognosis of the general population or comparisons between aged or young populations [9]. However, studies that specifically aimed to identify fatal risk factors for elderly COVID-19 patients (aged ≥65 years) are rare. Of note, a recent study showed that despite both age≥ 65 years and pre-existing comorbidities, including hypertension or diabetes, were independently associated with the development of acute respiratory distress syndrome (ARDS), age ≥ 65 years was a major risk factor associated with the progression from ARDS to death [9]. However, the main risk factors that are responsible for the death of elderly infected with SARS-CoV-2 have yet to be determined. Here, we report on the characteristics of the largest cohort of elderly COVID-19 patients in Wuhan city, China, the epicenter of the SARS-CoV-2 outbreak, and describe the fatal risk factors for the most fragile elderly patients with COVID-19 infection in the hope that this will help to guide the clinicians to identify the elderly people who are at higher danger to progress to severe illness from COVID-19 infection at an early stage and adjust treatment plans to reduce mortality.

RESULTS

Demographics and characteristics

A total of 302 patients aged ≥65 years old admitted between January 23, 2020 to February 29, 2020 were screened, and excluded 69 suspected cases admitted without laboratory confirmation tests for SARS-CoV-2, as were 9 cases transferred to Huoshenshan Hospital or Leishenshan Hospital, and 14 patients with only one laboratory test during hospitalization. Overall, 210 patients were finally included in this study (Table 1). The median age of the 210 patients was 71 (interquartile range [IQR] 67-77) years, and the ratio of male and female was approximate equal. 175 patients were in the discharged group, with a median age was 70 (IQR 67-74) years and 79 (45%) were male, while 35 patients were in the deceased group, with a median age was 74 (IQR 70-82) years, 22 (63%) were male. Patients in the deceased group were significantly older (74 years, IQR 70-82) than that in the discharged group (70 years, IQR 67-74). In the deceased group, the median time from onset of symptoms to admission and death were, respectively, 8 (IQR 6-14) days and 14 (IQR 12-24) days. In the discharged group, the median time from onset of symptoms to admission and discharge were 10 (IQR7-15) days and 26 (IQR21-29) days, respectively. A total of 18 cases required intensive care admission, with 16 cases in the deceased group.

Among the elderly patients, 159 (76%) had comorbidities, with hypertension (115 [55%]) and cardiovascular disease (52 [25%]) being the most common comorbidities, followed by diabetes (38 [18%]), respiratory disease (38 [18%]), and digestive disease (21 [10%]). There were 124 (71%) cases having comorbidities in the discharged group compared with 35 (100%) cases in the deceased group. Additionally, significantly higher percentage of patients in the deceased group had cardiovascular disease (17 [49%] vs. 35 [20%]), respiratory disease (18 [51%] vs. 20 [11%]), cerebrovascular disease (7 [20%] vs. 6 [3%]), chronic liver disease (6 [17%] vs. 12 [7%]), chronic kidney disease (10 [29%] vs. 8 [5%]) or malignancy (3 [9%] vs. 3 [2%]) than in the discharged group (Table 1).

Of the hospitalized elderly patients, the most common symptoms were fever (72%) and cough (71%), followed by chest stuffiness (36%), fatigue (35%), anorexia (11%), diarrhea (11%), pharyngalgia (10%), dyspnea (8%), headache (6%), myalgia (6%), and nausea or vomiting (5%). Among all the patients, half of them had fever (51%) as the first symptom, nearly one third had cough (31%), and a small proportion had pharyngalgia (8%), fatigue (4%), chest tightness (3%), diarrhea (2%), anorexia (0.5%), dyspnea (0.5%) as the first symptom.

Treatment and complications

Most of the elderly patients received antiviral therapy (90%), antibiotic therapy (82%), oxygen inhalation (67%), and one third of patients were treated with glucocorticoid (33%), part of the patients received gamma globulin therapy (19%), albumin therapy (11%), mechanical ventilation (11%) or continuous renal replacement therapy (CRRT) treatment (2%). Of all the patients, ARDS (13%, 27 of 210) was the most frequently complication, followed by acute renal failure (2%) and large cerebral infarction (1%) (Table 1). 71% (25/35) of patients in the deceased group developed ARDS as compared to 2 (1%) in the discharged group. And typical pulmonary Computed Tomographic (CT) changes from a deceased and a discharged patient were shown in Supplementary Figure 1A–1F.

Laboratory findings

Comparison of laboratory findings within 24 hours at admission were shown in Table 2. The median leucocyte counts (6.4×10^9/L) in patients in the deceased

Table 1. Baseline characteristics, treatments, complications of patients infected with COVID-1.

	Total (n=210)	Discharged group (n=175)	Deceased group (n=35)	P Value[a]
Age, median (IQR), y	71 (67-77)	70 (67-74)	74 (70-82)	<0.001
Sex, n (%)				
Male	101 (48)	79 (45)	22 (63)	0.056
Female	109 (52)	96 (55)	13 (37)	
Duration from onset of symptoms to admission, median (IQR), d	10 (7-15)	10 (7-15)	8 (6-14)	0.889
Duration from admission to outcome, median (IQR), d	14 (10-17)	14 (11-17)	9 (5-15)	<0.001
Duration from onset of symptoms to outcome, median (IQR), d	23 (17-28)	26 (21-29)	14 (12-24)	<0.001
ICU cases, n (%)	18/198 (9)	2/167 (1)	16/31 (52)	<0.001
Comorbidities, n (%)	159 (76)	124 (71)	35 (100)	<0.001
Hypertension	115 (55)	97 (55)	18 (51)	0.664
Diabetes	38 (18)	29 (17)	9 (26)	0.200
Cardiovascular disease	52 (25)	35 (20)	17 (49)	<0.001
COPD	3 (1)	2 (1)	1 (3)	0.435
Respiratory disease	38 (18)	20 (11)	18 (51)	<0.001
Cerebrovascular disease	13 (6)	6 (3)	7 (20)	<0.001
Chronic liver disease	18 (9)	12 (7)	6 (17)	0.047
Digestive diseases	21 (10)	15 (9)	6 (17)	0.123
Chronic kidney disease	18 (9)	8 (5)	10 (29)	<0.001
Malignancy	6 (3)	3 (2)	3 (9)	0.026
Number of Comorbidities, n (%)				
None	51 (24)	51 (29)	0 (0)	<0.001
One	56 (27)	48 (27)	8 (23)	
Two	60 (29)	54 (31)	6 (17)	
Three	22 (10)	16 (9)	6 (17)	
Four	13 (6)	4 (2)	9 (26)	
Five	4 (2)	1 (1)	3 (9)	
Six or more	4 (2)	1 (1)	3 (9)	
Signs and symptoms, n (%)				
Fever	151 (72)	122 (70)	29 (83)	0.115
Cough	148 (71)	118 (67)	30 (87)	0.031
Headache	13 (6)	10 (6)	3 (9)	0.523
Pharyngalgia	20 (10)	18 (10)	2 (6)	0.400
Fatigue	73 (35)	64 (37)	9 (26)	0.219
Anorexia	23 (11)	19 (11)	4 (11)	0.921
Nausea or vomiting	11 (5)	9 (5)	2 (6)	0.890
Myalgia	12 (6)	11 (6)	1 (3)	0.426
Chest stuffiness	76(36)	65(37)	11 (31)	0.522
Dyspnea	17 (8)	12 (7)	5 (14)	0.142
Diarrhea	24 (11)	21 (12)	3 (9)	0.562
First symptom, n (%)				
Fever	107 (51)	88 (50)	19 (54)	<0.001
Cough	65 (31)	55 (31)	10 (28)	
Pharyngalgia	16 (8)	14 (8)	2 (6)	
Fatigue	9 (4)	9 (5)	0 (0)	
Anorexia	1 (0.5)	1 (0.5)	0 (0)	
Chest tightness	6 (3)	4 (3)	2 (6)	
Dyspnea	1 (0.5)	1 (0.5)	0 (0)	
Diarrhea	5 (2)	3 (2)	2 (6)	
Temperature, °C	36.8 (36.5-37.0)	36.7 (36.5-36.9)	37.0 (36.5-37.8)	0.069
Heart rate, median (IQR), beat per minute	80 (78-88)	80 (78-86)	85 (80-104)	0.016

Respiratory rate, median (IQR), beat per minute	20 (20-22)	20 (20-22)	22 (20-26)	0.008
Mean arterial pressure, median (IQR), mm Hg	97 (92-105)	97 (93-104)	100 (92-109)	0.585
Treatment, n (%)				
Antiviral therapy	179/198 (90)	148/167 *(89)	31/31* (100)	0.048
Antibiotic therapy	163/198 (82)	133/167 (80)	30/31 (97)	0.022
Glucocorticoid therapy	65/198 (33)	42/167 (25)	23/31 (74)	<0.001
Gamma globulin therapy	37/198 (19)	23/167 (14)	14/31 (45)	<0.001
Albumin therapy	22/198 (11)	10/167 (6)	12/31 (39)	<0.001
Oxygen inhalation	133/198 (67)	103/167 (62)	30/31 (97)	<0.001
Mechanical ventilation	21/198 (11)	1/167 (1)	20/31 (66)	<0.001
CRRT	3/198 (2)	1/167 (1)	2/31 (7)	0.014
Complications, n (%)				
ARDS	27 (13)	2 (1)	25 (71)	<0.001
Acute renal failure	4 (2)	0 (0)	4 (11)	<0.001
Cerebral infarction	3 (1)	0 (0)	3 (9)	<0.001

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; ICU, intensive care unit; COPD, chronic obstructive pulmonary diseases; CRRT, continuous renal replacement therapy; ARDS, acute respiratory distress syndrome. [a] P values indicate differences between the discharged group and the deceased group. P < 0.05 was considered statistically significant. *Record/data were missing in 8 patients in the discharged group, and in 4 patients in the deceased group regarding whether or not the patients received a treatment.

group were higher than those in the discharged group (5.1×10^9/L). Among them, 9 (26%) patients had leucocytes counts above the normal range in the deceased group, compared with 3 (2%) in the discharged group. The concentrations of C-reactive protein in the deceased group were significantly higher than that in the discharged group, and the levels of C-reactive protein in deceased patients were all elevated beyond the normal range. The lymphocyte counts of the deceased group were progressively decreased compared with that of the discharged group, and 60% patients in the deceased group had lymphopenia while 18% patients in the discharged group had lymphopenia. The neutrophil counts in the deceased group were higher than that in the discharged group, and 43% cases in the deceased group had neutrophil counts above the normal range as compared to 6% in the discharged group. Compared with discharged group, the platelet counts were significantly lower in the deceased group.

Biochemical test results were shown in Table 2. The concentrations of aspartate aminotransferase (AST), serum creatinine (Cr), and blood urea nitrogen (BUN) of the deceased group were all significantly higher than that of the discharged group. And, nearly half (49%) of the deceased patients had BUN concentrations elevated beyond the normal range as compared to 11% of the patients in the discharged group. The levels of creatine kinase (CK) and creatine kinase isoenzyme (CK-MB) in the deceased group were significantly higher than those in the discharged group. Significantly more patients in the deceased group (86%) had procalcitonin concentrations above the normal range than in discharged group (21%, 35 of 170).

The concentrations of lactate dehydrogenase (LDH) were significantly higher in the deceased group than those in the discharged group, with 79% (27 of 34) deceased patients had LDH concentrations above the normal range as compared to 29% (50 of 171) in the discharged group. The median concentrations of fasting blood glucose did not differ significantly between the two groups, however relatively more patients in the deceased group (68%, 23 of 34) had acute hyperglycemia (glucose ≥6.1 mmol/L) as compared to 33% (57 of 171) in the discharged group. Albumin concentrations were significantly lower in the deceased group than in the discharged group, with 38% (13 of 34) deceased patients and 10% (17 of 172) discharged patients developed hypoalbuminemia. In addition, D-dimer level in the deceased patients was significantly higher than that in the discharged patients. The median activated partial thromboplastin time and prothrombin time as well as the total bilirubin concentrations in the deceased group were all significantly higher than those in the discharged group. Monocytes count, concentrations of alanine aminotransferase (ALT), triglyceride and thrombin time did not significantly differ between the two groups.

Receiver operating characteristic curve, survival curve and dynamic profile

The relationships between routine blood test results, including blood biochemistry, inflammatory markers

Table 2. Laboratory findings of patients infected with COVID-19.

Laboratory finding within 24 hours on admission	normal range	Median (IQR)			P value[a]
		Total (n=210)	Discharged group (n=175)	Deceased group (n=35)	
Leucocytes, $\times10^9$/L, n (%)	(3.5-9.5) $\times10^9$/L	5.2 (3.9-6.4)	5.1 (3.9-6.1)	6.4 (4.1-10.7)	0.037
<3.5 $\times10^9$/L		30 (14)	24 (14)	6 (17)	
3.5-9.5 $\times10^9$/L		168 (80)	148 (84)	20 (57)	<0.001
≥9.5		12 (6)	3 (2)	9 (26)	
C-reactive protein, mg/L, n (%)	(0-5) mg/L	15.5 (3.2-63.6)	9.3 (2.6-37.2)	125.8 (49.1-200.0)	<0.001
0-5 mg/L		68 (32)	68 (39)	0 (0)	<0.001
≥5 mg/L		142 (68)	107 (61)	35 (100)	
Lymphocyte, $\times10^9$/L, n (%)	(1.1-3.2) $\times10^9$/L	1.1 (0.8-1.5)	1.1 (0.9-1.6)	0.7 (0.4-0.9)	<0.001
<0.8 ×109/L		52 (25)	31 (18)	21 (60)	
0.8-1.1 ×109/L		42 (20)	35 (20)	7 (20)	<0.001
1.1-3.2×109/L		114 (54)	107 (61)	7 (20)	
≥3.2×109/L		2 (1)	2 (1)	0 (0)	
Neutrophils, $\times10^9$/L, n (%)	(1.8-6.3) $\times10^9$/L	3.3 (2.4-4.5)	3.0 (2.4-4.3)	5.2 (2.7-10.0)	0.003
<1.8 ×109/L		20 (10)	16 (9)	4 (11)	
1.8-6.3 ×109/L		165 (78)	149 (85)	16 (46)	<0.001
≥6.3 ×109/L		25 (12)	10 (6)	15 (43)	
NLR		2.9 (1.9-5.1)	2.8 (1.8-3.8)	8.4 (3.1-13.1)	<0.001
Monocytes, $\times10^9$/L	(0.1-0.6) $\times10^9$/L	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.4 (0.2-0.5)	0.225
Procalcitonin, ng/mL, n (%)	(0.02-0.05) ng/mL				
<0.02 ng/mL		0	0	0	
0.02-0.05 ng/mL		140/205 (68)	135/170 (79)	5 (14)	<0.001
≥0.05 ng/mL		65/205 (32)	35/170 (21)	30 (86)	
Platelet count, $\times10^9$/L, n (%)	(125-350) $\times10^9$/L	206.0 (159.8-267.0)	216.0 (169.0-285.0)	168.0 (124.0-216.0)	<0.001
<125 ×109/L		20/208 (10)	19/174 (11)	1/34 (3)	
125-350 ×109/L		164/208 (79)	139/174 (80)	25/34 (74)	0.010
≥350 ×109/L		24/208 (11)	16/174 (9)	8/34 (23)	
ALT, IU/L, n (%)	(7-40) IU/L	29.5 (17.0-45.5)	27.0 (17.0-43.0)	35.0 (18.0-77.0)	0.427
<7 IU/L		1/208 (0.5)	0/173 (0)	1 (3)	
7-40 IU/L		140/208 (67)	121/173 (70)	19 (54)	0.261
≥40 IU/L		67/208 (32.5)	52/173 (30)	15 (43)	
AST, IU/L, n (%)	(0-45) IU/L	26.0 (21.0-38.3)	25.0 (20.0-35.5)	45.0 (26.0-72.0)	0.008
<45 IU/L		165/208 (79)	149/173 (86)	16 (46)	<0.001
≥45 IU/L		43/208 (21)	24/173 (14)	19 (54)	
Cr, μmol/L, n (%)	(40-105) μmol/L	67.1 (56.5-84.1)	65.2 (55.2-80.2)	76.0 (64.0-107.4)	0.012
<40 μmol/L		2/208 (1)	1/173 (0.5)	1 (3)	
40-105μmol/L		197/208 (95)	167/173 (96.5)	30 (86)	0.139
≥105μmol/L		9/208 (4)	5/173 (3)	4 (11)	
BUN, mmol/L, n (%)	(3.1-7.2) mmol/L	4.7 (3.4-6.1)	4.4 (3.3-5.6)	7.2 (5.0-11.1)	<0.001
<3.1 mmol/L		28 (13)	26 (15)	2 (5)	
3.1-7.2 mmol/L		146 (70)	130 (74)	16 (46)	<0.001
≥7.2 mmol/L		36 (17)	19 (11)	17 (49)	
CK, IU/L, n (%)	(30-180) IU/L	65.0 (43.0-116.0)	60.0 (41.0-91.5)	137.0 (54.0-363.0)	0.008
<30 IU/L		19/208 (9)	16/173 (9)	3 (9)	
30-180 IU/L		162/208 (78)	144/173 (83)	18 (51)	<0.001
≥180 IU/L		27/208 (13)	13/173 (8)	14 (40)	
CK-MB, IU/L, n (%)	(0-25) IU/L	10.0 (7.0-13.0)	9.0 (7.0-12.0)	13.0 (8.0-19.0)	0.002
<25 IU/L		204/208 (98)	171/173 (99)	33 (94)	0.074
≥25 IU/L		4/208 (2)	2/173 (1)	2 (6)	
APTT, s	(21-35) s	27.6 (23.7-31.4)	27.3 (23.5-30.6)	31.3 (26.0-35.4)	0.012
Prothrombin time, s	(10-13) s	11.6 (11.0-12.4)	11.6 (10.9-12.3)	12.1 (11.5-12.8)	0.035
Thrombin time, s	(13-21) s	19.5 (16.4-22.2)	19.6 (14.9-22.3)	18.7 (17.4-21.4)	0.180
D-dimer, μg/mL, n (%)	(<0.5) μg/mL	0.6 (0.2-1.9)	0.6 (0.2-1.1)	3.6 (0.6-5.7)	<0.001

0-0.5 μg/mL		42/168 (25)	40/139 (29)	2/29 (7)	0.014
≥0.5 μg/mL		126/168 (75)	99/139 (71)	27/29 (93)	
Albumin, g/L, n (%)	(40-55) g/L	35.7 (31.9-39.3)	36.2 (32.8-39.6)	31.2 (26.7-34.6)	<0.001
<30 g/L		30/206 (15)	17/172 (10)	13/34 (38)	<0.001
Glucose, mmol/L, n (%)	(3.9-6.1) mmol/L	5.7 (4.7-7.4)	5.5 (4.7-6.9)	6.9 (5.0-7.9)	0.105
<3.9 mmol/L		2/205 (1)	2/171 (1)	0/34 (0)	<0.001
3.9-6.1 mmol/L		123/205 (60)	112/171 (66)	11/34 (32)	
≥6.1 mmol/L		80/205 (39)	57/171 (33)	23/34 (68)	
Total bilirubin, μmol/L	(2-21) μmol/L	8.9 (6.6-11.9)	8.4 (6.5-11.6)	9.6 (6.7-16.7)	0.043
Triglyceride, mmol/L	(0.5-1.72) mmol/L	1.2 (0.9-1.7)	1.2 (0.9-1.6)	1.3 (0.9-1.9)	0.635
Total cholesterol, mmol/L	(3.1-5.7) mmol/L	3.9 (3.3-4.6)	4.0 (3.5-4.6)	3.4 (2.8-4.2)	0.051
Lactate dehydrogenase, IU/L, n (%)	(114-240) IU/L	208.0 (165.8-270.8)	199.0 (164.0-244.0)	367.0 (251.0-547.0)	<0.001
<240 IU/L		128/205 (62)	121/171 (71)	7/34 (21)	<0.001
≥240 IU/L		77/205 (38)	50/171 (29)	27/34 (79)	

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; NLR, neutrophil-to-lymphocyte ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, serum creatinine; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, creatine kinase isoenzyme; APTT, activated partial thromboplastin time. [a] P values indicate differences between the discharged group and the deceased group. $P < 0.05$ was considered statistically significant.

and the prognosis were analyzed. As shown in Figure 1A, the values of area under curve (AUC) of C-reactive protein, lymphocytes, BUN, glucose, LDH, and neutrophil to lymphocyte ratio (NLR) were respectively 0.857, 0.214, 0.769, 0.660, 0.766, and 0.774. The optimal cut-off values of C-reactive protein, BUN, glucose, LDH and NLR were 63 mg/L, 6.1 mmol/L, 6.5 mmol/L, 265 IU/L, 6.48 respectively (Table 3). It showed that higher C-reactive protein, BUN, LDH and NLR on admission could significantly predict poor prognosis of COVID-19 infected elderly patients.

Survival curves derived from C-reactive protein, lymphocyte, BUN, glucose, LDH and NLR individually and from the frequency of abnormal findings in relation to C-reactive protein, lymphocyte, BUN, and LDH were shown in Figure 1B–1H. The survival rate was much higher in patients with normal values of C-reactive protein, LDH and NLR. Abnormally high levels of BUN and glucose were associated with lower survival rate. Patients suffered severe lymphopenia had decreased survival rate, and the lower the lymphocyte count the lower the survival rate. All the deceased patients had abnormally high C-reactive protein level plus at least one abnormal value of either lymphocyte, BUN or LDH at admission. And, all elderly patients that concomitantly had abnormally high C-reactive protein plus two abnormalities of lymphocyte, BUN or LDH were in the deceased group.

Dynamic profile of the three major findings/predictors (i.e. C-reactive protein, lymphopenia and BUN), were tracked from 24 hours at admission, during hospitalization and from the last laboratory findings before discharge or death, respectively. As shown in Figure 2, at admission, all deceased patients had markedly high level of C-reactive protein than that in the discharged patients. The level of C-reactive protein slightly reduced during the time impending death, however, it was still higher than that in the discharged patients. Most deceased patients had lymphopenia at admission, and lymphopenia became more serious when approaching death. By contrast, few discharged patients had lymphopenia, and it returned to normal during hospitalization. At admission, most deceased patients had BUN above normal range as compared to that in the discharged patients, and the BUN levels increased when impending death (Figure 2).

DISCUSSION

This study, to our knowledge, is the largest cohort study to date of elderly COVID-19 patients with definitive outcomes of the disease and describes the fatal risk factors for the most fragile elderly patients. In keeping with the findings that aging is a risk factor for patients with COVID-19 in the overall population, the median age of patients in the deceased group was significantly older than the discharged group, suggesting that the older the patients, the higher the mortality. Consistently, epidemiological studies conducted among 72,314 patients across the China showed that the mortality of patients aged 70-79 years was 8.0%, and the mortality of patients aged over 80 years was 14.8% [7]. The relatively high mortality of elderly patients in our study (16.7%, 35/210) was possibly related to the lack of medical resources caused by the outbreak of COVID-19 initially in Hubei Province, China, and similarly in Europe and north America later on. But, most likely, the high mortality in the elderly may be attributable to the

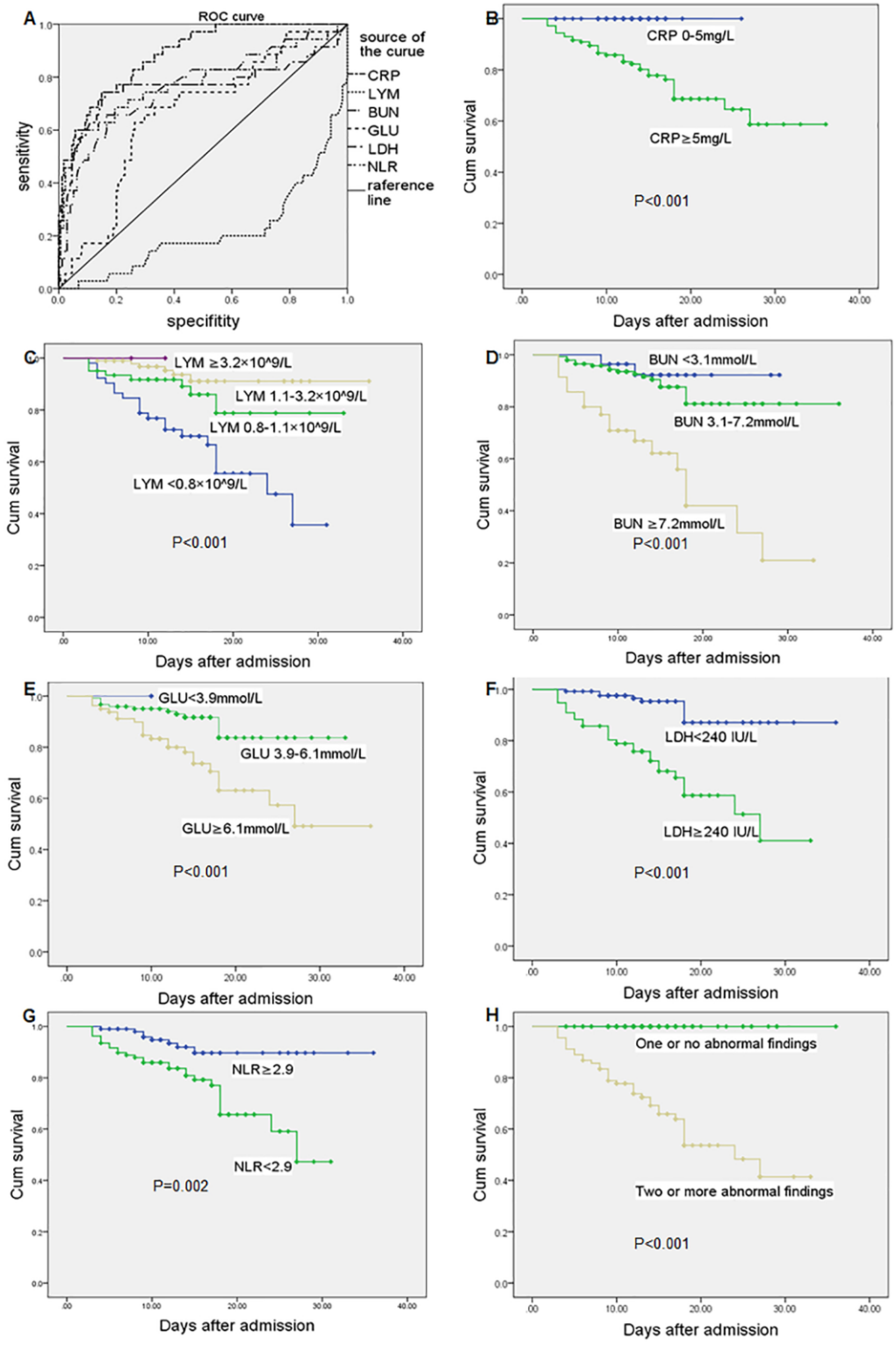

Figure 1. Receiver operating characteristic curve and survival curve. (**A**) ROC in CRP, LYM, BUN, GLU, LDH, NLR at admission. Survival curves in elderly COVID-19 patients with different levels of CRP (**B**), LYM(**C**), BUN (**D**), GLU (**E**), LDH (**F**), NLR (**G**, NLR value take median value in total patients) at admission. (**H**) Two or more abnormal values of CRP, LYM, BUN, LDH in the patients at admission can significantly predict poor prognosis of COVID-19 infected elderly patients. Abbreviations: COVID-19, coronavirus disease 2019; ROC, receiver operating curve; CRP, C-reactive protein; LYM, lymphocytes; BUN, blood urea nitrogen; GLU, glucose; LDH, lactate dehydrogenase; NLR, neutrophil-to-lymphocyte ratio. P-value reported in each subplot indicates the difference between survival curves by Kaplan-Meier method with log-rank test. $P < 0.05$ was considered statistically significant.

Table 3. Areas under the curve (AUC) of CRP, LYM, BUN, GLU, LDH, and NLR.

Test result variable (s)	AUC	highest specificity	highest sensitivity	optimal cut-off values
CRP	0.857	0.85	0.74	63 mg/L
LYM	0.214			
BUN	0.769	0.82	0.66	6.1 mmol/L
GLU	0.660	0.76	0.63	6.5 mmol/L
LDH	0.766	0.79	0.74	265 IU/L
NLR	0.774	0.69	0.60	6.48

Abbreviations: AUC, areas under the curve; BUN, blood urea nitrogen; CRP, C-reactive protein; GLU, glucose; LYM, lymphocytes; NLR, neutrophil-to-lymphocyte ratio.

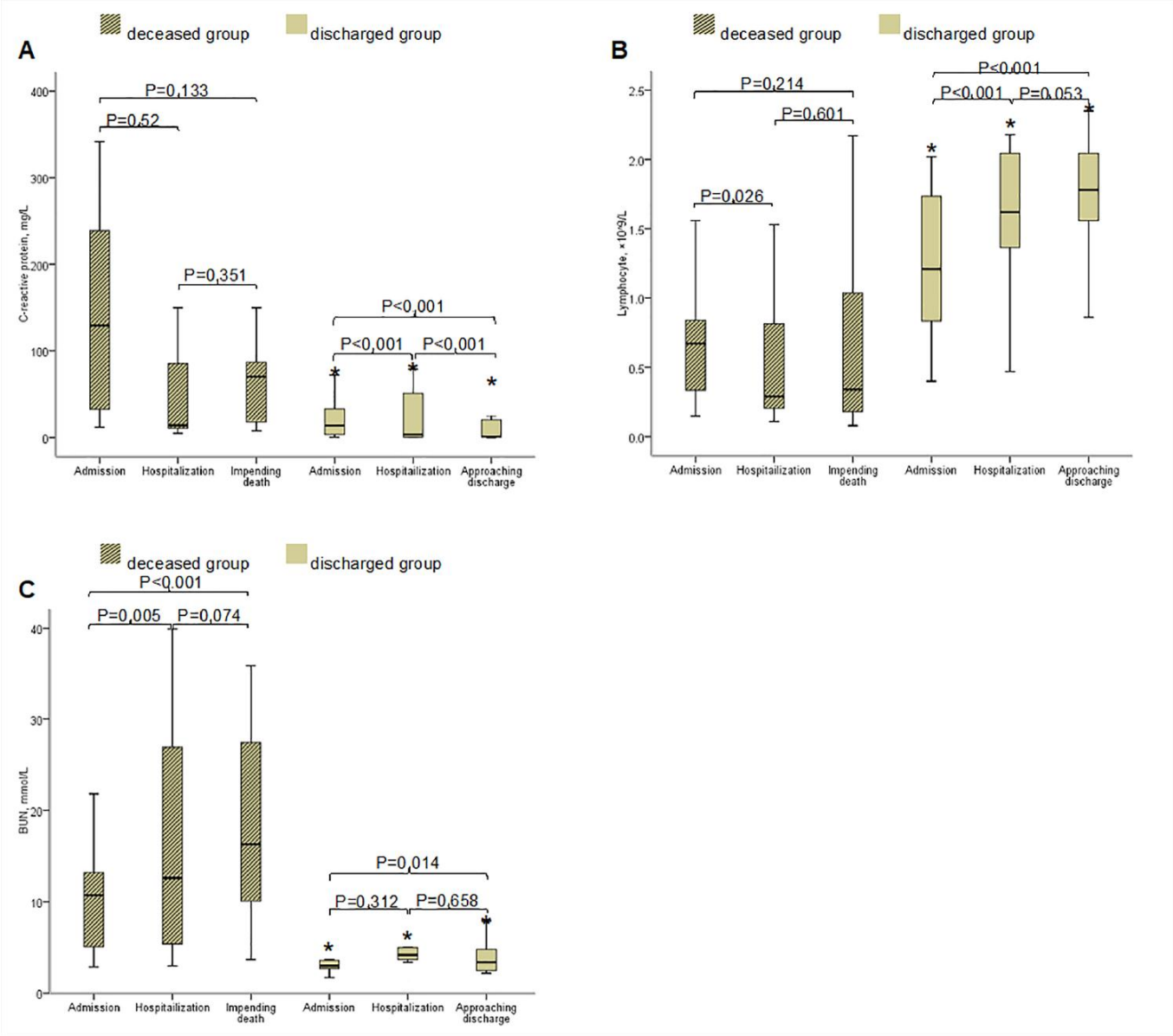

Figure 2. Dynamic Changes of C-reactive protein (**A**), lymphocyte (**B**) and BUN (**C**) within 24 hours at admission, during hospitalization and before discharge or death. Abbreviations: BUN, blood urea nitrogen. The horizontal lines represent the median value in each group. P values indicate differences among admission, hospitalization, impending death between the discharged group and the deceased group. *P<0.05 vs. deceased group. P<0.05 was considered statistically significant.

lack of adequate information or experience regarding what are the most fatal risk factors for the elderly patients, in addition to the generally known risk factors such as comorbidities. The higher mortality in the elderly patients could be in part due to the hypoimmunity, as less robust immune responses in elderly patients may render them more susceptible to ARDS after SARS-CoV-2 infection and die from respiratory failure [10]. Indeed, in the present study, significantly more patients in the deceased group suffered from lymphopenia and failed to survive ICU and wean from mechanical ventilation, while those who survived usually had relatively normal lymphocyte level or lymphocyte levels could gradually recover.

A study showed that male patients accounted for 67% of critically ill patients in the general population [11]. However, our study did not identify significant gender difference between the deceased and discharged elderly. This is possibly because that female patients in our study are at postmenopausal age. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a functional receptor [12, 13] and infects type 2 alveolar epithelial cells, which subsequently generates strong immune response and even induces cytokine storm [14]. In our study, the lymphocytes in the deceased group decreased progressively while neutrophils increased, leading to most significantly increased NLR, which is predictive of mortality. Another manifestation of cytokine storm is the elevation of C-reactive protein. In our study, the lever of C-reactive protein in the deceased group was significantly higher when compared with the discharged group. And, the receiver operating characteristic curve analysis indicates that high level of C-reactive protein is a risk factor of mortality in elderly patients. The fact that the levels of C-reactive protein significantly decreased after treatment in the discharged group but not in the deceased group (Figure 2A) provides support that the dynamic changes of C-reactive protein may serve as good indicator of prognosis of the elderly patients with COVID-19. Also, recent study showed that SARS-CoV-2 may directly affect kidney cells [15] and the myocardium [16], these may explain why high BUN and LDH are also highly predictive of mortality in the elderly, despite that LDH is a non-specific myocardial injury marker.

Limitations

This study has several limitations. Firstly, it is a single-center, retrospective study, and included participants were elderly patients who aged over 65 years, therefore, it is limited in sample size. Secondly, elderly patients are special, especially patients with older age, may cause recall bias when conducting epidemiological investigations, especially if there are comorbidities that are used as an analysis of prognosis-related factors.

CONCLUSIONS

This study shows that elderly patients with comorbidities had a greater risk of death, and, the enhanced level of C-reactive protein, blood urea nitrogen or lactate dehydrogenase at admission, progressively lowered lymphocyte counts during hospitalization, alone and especially in combination predict the poor prognosis in elderly patients with COVID-19.

MATERIALS AND METHODS

Study population

This study was a single-center, retrospective, observational study. We included elderly patients aged ≥65 years who were admitted to Wuhan Third Hospital, Wuhan, China, one of the designated hospitals for the treatment of COVID-19 assigned by the government, during the period from January 23, 2020 to February 29, 2020. For all patients, the ethics committee of Wuhan Third Hospital approved this study (Wu San Yi Lun KY2020-019) and granted a waiver of informed consent from study participants.

We included patients who were confirmed with COVID-19 according to World Health Organization interim guidance [17], and laboratory confirmation of SAR-CoV-2 was done by quantitative RT-PCR on samples from the respiratory tract, which was performed by the local health authority. Discharge criteria for patients include: body temperature returned to normal for more than 3 days; the respiratory symptoms had improved significantly, the pulmonary imaging showed a significant improvement of acute exudative lesions, and the nucleic acid test result of respiratory specimens of sputum and/or nasopharyngeal swabs became negative for two successive times (sampling interval more than 24 hours). Patients who were transferred to Huoshenshan Hospital and Leishenshan Hospital during the disease progress and thus the records were not complete at the Wuhan Third Hospital, and patients who were only subjected to one laboratory test during their admission were excluded. The included patients were divided into the discharged group and the deceased group according to the prognosis of patients.

Data collection

A trained team of physicians and medical staffs reviewed and collected epidemiological, demographic, clinical, and prognosis data from electronic medical records, and the records were double checked and confirmed by two researchers (SG and WJ) respectively. The recorded comorbidities included hypertension, diabetes, cardiovascular diseases, chronic

obstructive pulmonary diseases (COPD), respiratory diseases, cerebrovascular diseases, chronic liver disease, digestive diseases, chronic kidney disease and malignancy. The signs and symptoms including fever, cough, headache, fatigue, nausea or vomiting, anorexia, myalgia, chest stuffiness, dyspnea, and diarrhea were recorded. The patients' life vital signs including heart rate, respiratory rate, and mean arterial pressure (MAP) were also collected.

The laboratory findings were collected within 24 hours on admission, which included leucocytes, C-reactive protein, lymphocytes, neutrophils, NLT, ALT, AST, Cr, BUN, CK, CK-MB, coagulation function, fasting blood glucose, albumin, total bilirubin, triglycerides, total cholesterol, and LDH. Lymphopenia was diagnosed as the counts of lymphocytes below 0.8×10^9/L according to the Common Terminology Criteria for Adverse Events version 5.0 [18]. Hyperglycemia was defined as concentrations of fasting blood glucose above 6.1 mmol/L. Hypoalbuminemia was diagnosed as concentrations of albumin below 30 g/L according to American Society of Chest Physicians/Society of Critical Care Medicine criteria [19].

The treatments included antiviral treatment, antibiotic therapy, glucocorticoid therapy, gamma globulin therapy, albumin therapy, oxygen inhalation, mechanical ventilation, and CRRT. The duration of antiviral therapy was 7-10 days, which included the applications of oseltamivir, ganciclovir, and arbidol. While the antibiotic therapy lasted for 14 days, which included the use of cefoperazone sulbactam and moxifloxacin. No patients received treatments for specific interleukin 6 (IL-6) inhibition or anti-cytokine-storm medications. The complications included ARDS, acute renal failure, and cerebral infarction.

Definitions

The COVID-19 onset time was defined as the date when the first sign or symptom was noticed. Acute cardiac injury was identified if the cardiac biomarkers (e.g. hypersensitive troponin I, Creatine kinase–MB) were above the 99% upper reference limit or new abnormalities were shown in electrocardiography and echocardiography [20]. Respiratory failure was identified according to the guidance of World Health Organization for COVID-19 [17]. Acute kidney injury was defined according to the KDIGO clinical practice guidelines [21]. Cerebral infarction was diagnosed according to the 2018 Stroke Guidelines [22].

Outcomes

The primary outcomes were death and successful discharge of the patients. The second outcomes were laboratory results, radiological data, treatments, and complications of the groups and the analysis of their prognostic values.

Statistical analysis

The categorical variables were compared by chi-square test or Fisher's test, and expressed as frequency and percentage; the continuous variables were compared by rank sum test, and presented as median (IQR) between the discharged group and deceased group. AUC of receiver operating characteristic (ROC) was calculated to predict the prognosis of elderly patients. Survival curve was developed using the Kaplan-Meier method with log-rank test to predict death or discharge in the elderly. A two-sided P value less than 0.05 was considered statistically significant. The SPSS 21.0 software was used for all the analyses.

AUTHOR CONTRIBUTIONS

SG had the idea for the study. JP and ZX designed the study and have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. WJ, SG, YS, LY, YX, LJ and BW collected the data. SG, FJ and WJ performed data analysis. SG, YC, HL and ZX participated in discussion/data interpretation. SG, FJ and YC drafted the manuscript. ZX and JP revised the final manuscript.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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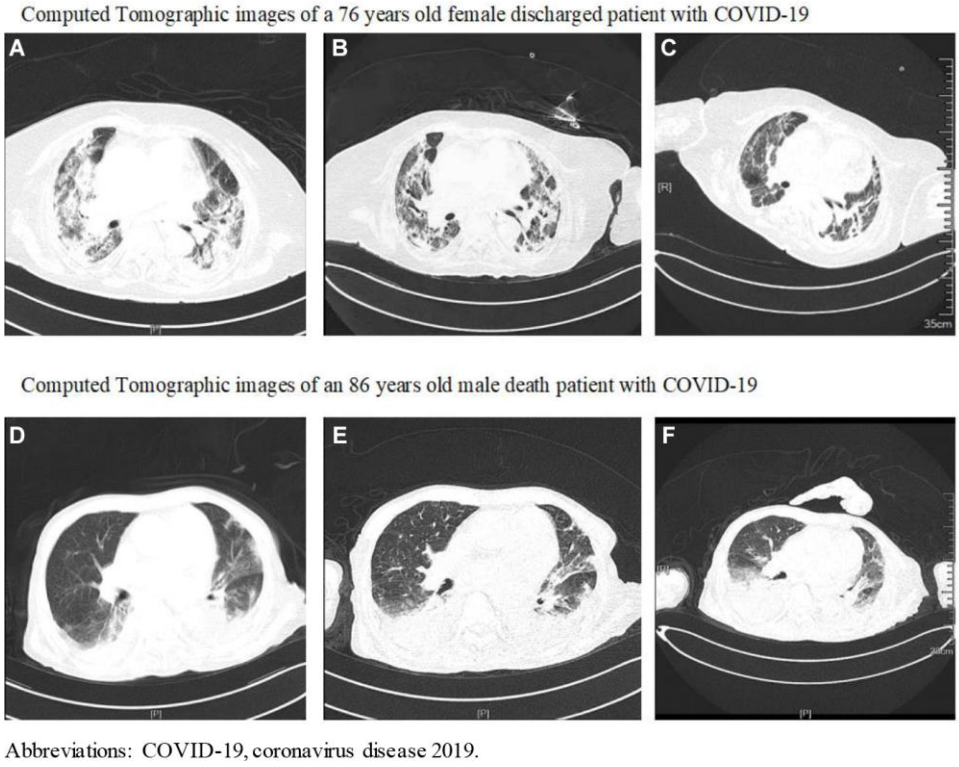
SUPPLEMENTARY MATERIAL

Supplementary Figure



Supplementary Figure 1. Computed Tomographic (CT) findings of two patients. As shown in (**A**–**C**) a 76 years old female discharged patient, she had fever and headache for 5 days before admission on January 28, 2020. (**A**) image obtained on day 23 after symptom onset shows progressive multiple ground glass opacities, massive high-density shadows in bilateral lungs. (**B**) image obtained on day 28 after symptom onset shows multiple ground glass opacities and high-density shadows in bilateral lungs. (**C**) image obtained on day 34 after symptom onset showed that the consolidation was obviously resolved and pulmonary interstitial fibrosis attenuated. The patient was discharged on March 17, 2020, the duration from admission to discharge was 49 days, and the duration from onset of symptoms to discharge was 54 days. (**D**–**F**) an 86 years old male death patient, he had cough and chest tightness for 7 days before admission on February 25, 2020. (**D**) image obtained on day 8 after symptom onset showed multiple ground glass opacities, high-density shadows in bilateral lungs. (**E**) image obtained on day 14 after symptom onset showed progressive multiple ground glass opacities and mass shadows of high-density shadows in bilateral lungs. (**F**) image obtained on day 21 after symptom showed progressive multiple ground glass opacities and mass shadows of high-density shadows in bilateral lungs. The patient died on March 10, 2020, and the duration from admission to death was 14 days, while the duration from onset of symptoms to death was 21 days.

Review

Pathophysiology of SARS-CoV-2 infection in patients with intracerebral hemorrhage

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ABSTRACT

Intracerebral hemorrhage (ICH) is associated with old age and underlying conditions such as hypertension and diabetes. ICH patients are vulnerable to SARS-CoV-2 infection and develop serious complications as a result of infection. The pathophysiology of ICH patients with SARS-CoV-2 infection includes viral invasion, dysfunction of the ACE2–Ang (1–7)–MasR and ACE–Ang II–AT_1R axes, overactive immune response, cytokine storm, and excessive oxidative stress. These patients have high morbidity and mortality due to hyaline membrane formation, respiratory failure, neurologic deficits, and multiple organ failure.

INTRODUCTION

Novel coronavirus (2019-nCoV)–associated pneumonia cases first appeared in Wuhan, Hubei Province, China, in December 2019 [1]. Whole-genome sequencing identified a novel coronavirus—severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2, 3]. In the following months, SARS-CoV-2 rapidly spread throughout China and the world. By May 26, 2020, SARS-CoV-2 had resulted in 84,543 infections and 4,645 deaths in China, as reported by National Health Commission of the People's Republic of China. In addition, other countries reported 5,468,627 confirmed cases and 345,544 deaths. The World Health Organization declared SARS-CoV-2 a public health emergency and named the virus Corona Virus Disease 2019 (COVID-19).

Although the source of SARS-CoV-2 and its pathogenesis are still being studied, COVID-19 is a systemic disease that can lead to pneumonia, respiratory failure, and acute respiratory distress syndrome (ARDS) and has high morbidity and mortality. COVID-19 also affects the cardiovascular, renal, cerebrovascular, and blood coagulation systems. Genome sequencing of patients' cerebrospinal fluid has identified the presence of SARS-CoV-2 in the brain, which is also seen in SARS and Middle East respiratory syndrome (MERS) infection [4]. Here, we review the pathophysiology of SARS-CoV-2 infection in patients with intracerebral hemorrhage (ICH).

Characteristics of SARS-CoV-2

Coronaviruses (CoVs), part of the subfamily *Orthocoronavirinae* in the family *Coronaviridae* of the order Nidovirales, are enveloped, nonsegmented, positive-sense, single-stranded RNA viruses [5]. Some CoVs are transmitted from animals to people and have gradually developed as pathogens of the respiratory, gastrointestinal, and central nervous systems in human. Examples include SARS, which caused an outbreak in 2002, and MERS, which caused an outbreak in 2012, both of which affect the lower respiratory tract [6, 7]. Genome sequencing has identified 2019-nCoV as a *Betacoronavirus*. SARS-CoV-2 and two bat-derived SARS-like strains, ZC45 and ZXC21, form an independent clade within lineage B of the subgenus *Sarbecovirus* [8, 9]. The two bat SARS-related

coronaviruses closest to SARS-CoV-2, ZXC21 and ZC45, can infect suckling rats and cause brain tissue inflammation and pathological changes in the lung and intestine [10].

SARS-CoV-2 contains a single positive-sense RNA genome and is around 60 to 140 nm in diameter [5]. The genome sequence of SARS-CoV-2 has 89% nucleotide identity with the bat SARS-like CoV ZXC21, 86.9% with the bat SARS-like CoV ZC45, and 82% with the human SARS-CoV [10–12]. The phylogenetic trees of SARS-CoV-2's orf1a/b, spike, envelope, membrane, and nucleoprotein also cluster closely with those of the bat, civet, and human SARS coronaviruses [10, 11, 13]. However, the external subdomain of spike's receptor binding domain in SARS-CoV-2 shares only 40% amino acid identity with other SARS-related coronaviruses [8, 10].

SARS-CoV-2, like SARS-CoV, manipulates angiotensin-converting enzyme 2 (ACE2) as the viral receptor and invades type 2 alveolar epithelial cells in the lower respiratory tract [11]. ACE2 inhibitors prevent SARS coronavirus from constant viral replication in Vero E6 cells [14]. The receptor binding domain on the S1 subunit of the SARS-CoV-2 spike protein (S glycoprotein) and the transmembrane domain of ACE2 are implicated in SARS-CoV-2 infection [2, 15].

A majority of the earliest confirmed patients infected with SARS-CoV-2 were exposed to wild animals sold in the Huanan Seafood Wholesale Market. Although it is difficult to pinpoint the exact source or the intermediate host of the novel coronavirus, the first cluster of pneumonia cases suggests that person-to-person transmission via the respiratory route occurred [16]. The digestive system is also hypothesized to be a route of SARS-CoV-2 transmission.

ICH patients are vulnerable to SARS-CoV-2 infection and develop serious complications as a result of infection

The general population is susceptible to SARS-CoV-2 infection. As of February 11, 2020, the Chinese Center for Disease Control and Prevention had identified 72,314 cases of COVID-19, including 55,239 confirmed patients, 16,186 suspected infections, and 889 infections without any symptoms [17]. 87% of the patients are between 30 and 79 years old. Clinical symptoms at the beginning of COVID-19 infection include chills, fever, cough, fatigue, myalgia, dyspnea, and diarrhea. Chest computed tomography (CT) images show ground-glass opacity in both lungs and, in severe cases, progressive consolidation of multiple lobular and subsegmental tracts. However, many infected patients are asymptomatic and have normal chest CT scans. Asymptomatic patients with SARS-CoV-2 infection, as well as those with atypical neurologic manifestations such as headache, dizziness, nausea, and vomiting, contribute to misdiagnosis and delayed treatment. According to the Chinese Center for Disease Control and Prevention, 81% of the 72,341 patients diagnosed with COVID-19 had mild disease, and the mortality rate was approximately 2.3%. However, the fatality rate increased to 8.0% in people age 70 to 79 years old and 14.8% in those age 80 or older. Infected patients with underlying diseases also had higher fatality rates: 10.5% in patients with cardiovascular disease, 7.3% for diabetes, 6.0% for hypertension, and 5.6% for cancer. A review of the clinical features of 138 confirmed patients in Zhongnan Hospital of Wuhan University confirmed that ICU patients were obviously elder and were more likely to have underlying diseases, as well as having higher risk for poor outcome [18]. Therefore, the worst complications and outcomes occur in older patients and those with chronic diseases, such as pulmonary disease, diabetes, hypertension, heart failure, atherosclerosis, cerebrovascular disease, and cancer. Patients with severe SARS-CoV-2 infection develop pneumonia and extrapulmonary pathological changes. Complications in patients with severe infection include hypoxemia, pulmonary edema, ARDS, postviral bacterial superinfection, septic shock, metabolic acidosis, blood coagulation dysfunction, and multiple organ damage. A retrospective, single-center study of 99 cases of COVID-19 in Wuhan Jinyintan Hospital revealed that severe patients had high levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), myocardial zymogram, blood urea nitrogen and serum creatinine, all of which were implicated with multiple organ damage. Biopsy samples of tissues from patients with SARS-CoV-2 indicate impairment of alveolar epithelial cells and pneumocytes in both lungs, exudation of extracellular fluid in alveolus, infiltration of lymphocytes and macrophages, and formation of hyaline membrane, indicating ARDS, which also occurs in SARS and MERS coronavirus infection [19, 20].

ICH accounts for 20% to 30% of strokes in China and is associated with high mortality and morbidity, with most survivors experiencing neurologic and cognitive impairment. The physiological status go to the bad with age and elder persons have higher possibility to develop underlying diseases, consisting of hypertension, diabetes, and dysfunction of blood coagulation, all of which are interact with the occurrence and development of ICH [21]. Hypertension is the mainly risk factor of ICH, as well as amyloid angiopathy, hemangioma, arteriovenous malformations, coagulopathy, and cerebroma [22]. Therefore, ICH is associated with old age and underlying conditions such as hypertension and

diabetes. ICH patients, susceptible to SARS-CoV-2 infection, are prone to develop serious complications and need ICU admission.

ICH exerts mass effect and causes primary physical damage that is dependent on the location, volume, and expansion of the hematoma. Secondary injury is caused by brain edema, the inflammatory cascade, and hematoma decomposition products. After the interaction between SARS-CoV-2 and the ACE2 receptor, some infected patients rapidly develop elevated blood pressure, which brings about severe cerebral changes, including activated microglia, accumulated ferritin, damaged neurons, and impaired neurologic function [23]. One report describing 41 cases of COVID-19 indicated that prolonged prothrombin time, elevated D-dimer, and severe platelet reduction occur in ICU patients with SARS-CoV-2 infection [24]. Then ICH patients may develop blood coagulation dysfunction as a result of infection. The high levels of thrombin is a trigger of early perihematomal brain edema; thrombin affects a variety of cells, including microglia, neurons, and brain endothelial cells, and destroys the blood–brain barrier (BBB) [25]. Low platelet activity is a marker of severe ICH, and platelet transfusion in the acute phase can limit hemorrhage volume and attenuate poor outcomes [26]. The BBB inhibits cerebrum invasion, regulates substantial exchange, and maintains homeostasis in the center nervous system. The viral invasion and breakdown of the BBB results in immunocyte recruitment in the central nervous system. Overactivation of the immune response and pro-inflammatory factors can lead to cellular apoptosis and necrosis, endothelial impairment, brain edema, and neuronal loss. In detail, the pathophysiology of ICH patients with SARS-CoV-2 infection includes viral invasion, dysfunction of the ACE2–Ang (1–7)–MasR and ACE–Ang II–AT_1R axes, overactive immune response, cytokine storm, and excessive oxidative stress.

SARS-CoV-2 brain invasion and ACE2

The renin-angiotensin system (RAS) consists of the protease renin, angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensin II. The local brain RAS includes angiotensinogen, peptidases, angiotensins, and specific receptor proteins that play specific roles in development of cerebrovascular disease [27, 28]. ACE2, a homologous enzyme of ACE, is secreted by endothelia and smooth muscle cells. A study pointed that SARS-CoV-2 can manipulate all but mouse ACE2 as the entry receptor in the ACE2-expressing cells, which might permit the viral invasion and replication in multiple organs. ACE2 is found in arterial and venous endothelial cells and arterial smooth muscle cells in most organs, including oral and nasal mucosa, naso-pharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain [14, 29].

Pathologists obtained human brain tissue from autopsies and research on the staining for ACE2; endothelial and smooth muscle cells of cerebrum were stained [14]. The barrier between plasma and brain cells is formed by brain capillary walls and glial cells and the barrier between plasma and cerebrospinal fluid is formed by choroid plexus. The expression of ACE2 in endothelial and smooth muscle cells allow viral invasion and replication in the blood-brain barrier. The BBB breakdown includes swelling of endothelial cells, necrosis, apoptosis, inflammatory injury and systemic vasculitis. Genome sequencing of patients' cerebro-spinal fluid confirmed SARS-CoV-2 infection in the brain [17]. The infected patients with atypical neurologic manifestations such as headache, dizziness, nausea, and vomiting are important signs for SARS-CoV-2 brain invasion. In addition, autopsies from patients with SARS infection have detected SARS-CoV particles and genomic sequence in cerebral neurons, as well as in T lymphocytes and monocytes in the circulating blood of multiple organs [30]. After intranasal inoculation of MERS-CoV in transgenic mice, study of brain tissues indicated viral invasion. Mice infected with the JHM and A59 strains of murine hepatitis virus (MHV) manifest an acute encephalo-myelitis and gradually develop demyelinating disease as a result of persistent viral stimulation. In addition to pulmonary disease, coronaviruses also cause patho-logical changes in the cerebrum due to their neuroinvasive and neurotropic properties [31].

SARS-CoV-2 and the dysfunction of the ACE2–Ang (1–7)–MasR and ACE–Ang II–AT_1R axes

Angiotensin 1-7, which is transferred by endopeptidases, ACE2, and ACE from angiotensin I, binds to the Mas receptor and is an effective and protective vasodilator [32]. Mas receptors are distributed throughout the brain, including the medulla and forebrain, which are associated with cardiovascular regulation, and the hippocampus, amygdala, anterodorsal thalamic nucleus, cortex, and hypoglossal nucleus [33]. In contrast to the effects of Ang II in the brain, Ang-(1-7) regulates the cardiovascular reflex and mediates blood pressure by releasing nitric oxide (NO) and activating the PI3K-Akt-PKB pathway [34]. The interaction between Ang-(1-7) and the Mas receptor decreases reactive oxygen species (ROS) production by cleaving Ang II or inhibiting AT_1 receptors [35]. Ang-(1-7) and the G-protein-coupled receptor Mas, which initiate the release of cytokines and activate and recruit leukomonocytes, reduce inflammation by the restraining Des-Arg9 bradykinin (DABK)-mediated pathway [36, 37]. The ACE2–Ang-(1-7)–Mas axis is a

protective regulator in the center nervous system; it regulates blood pressure and inhibits inflammatory injury, oxidative stress, fibrosis, and cellular apoptosis [38, 39]. Injection of Ang-(1-7) in the ventricle of rats reduces ICH-induced injury, resulting in limited hematoma expansion, decreased microglia, and neuronal recovery [40]. In addition, administration of Ang-(1-7) in mice with aneurysmal rupture inhibits the production of TNF-α and IL-1β and attenuates pathological damage [41]. The ACE2–Ang (1–7)–MasR axis and ACE–Ang II–AT_1R axis counterbalance each other to maintain cerebral homeostasis [42]. Thus, pathological disruption of ACE2 and Ang II can result in neurologic damage [43].

Infection and endocytosis of SARS-CoV-2 particles downregulate active ACE2 and Ang-(1-7) and increase Ang II. The subsequent inhibition of the ACE2–Ang (1–7)–MasR axis and overactivation of the ACE–Ang II–AT_1R axis underlie the progressive pathological deterioration in the cerebrum seen in patients with SARS-CoV-2. Disruption of the ACE–Ang II–AT_1R axis contributes to rapidly elevated blood pressure [44]. Stimulation and production of Ang II in local brain, which binds to AT_1 receptors, activates the inflammatory NF-κB pathway and superoxide production by activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [45]. Increased ROS production damages brain tissue, which is full of polyunsaturated fatty acid. In addition, overactivation of ACE–Ang II–AT_1R is partly responsible for brain inflammation and cellular apoptosis and necrosis, leading to endothelial impairment, brain edema, and neuronal injury. Administration of brainc Ang II receptor inhibitor attenuated acute inflammatory responses in an animal model with bacterial infection [46]. The brain inflammation with positive feedback seen in ICH patients with SARS-CoV-2 infection, which is postulated to be a result of dysfunction of the ACE2–Ang (1–7)–MasR and ACE–Ang II–AT_1R axes, results in excessive oxidative stress and elevated cytokines, chemokines, and toxic substances, which lead to neuronal injury, cell death, brain edema, and neurologic deficits. Hematoma expansion and brain edema contribute to physical pressure on neighboring structures, such as arterial vessels, the aqueduct of Sylvius, and the brainstem, leading to cerebral ischemia, obstructive hydrocephalus, cardiorespiratory dysfunction, intracranial hypertension, and even cerebral hernia.

SARS-CoV-2, immune evasion and over-activated immune responses

Among hospitalized patients with SARS-CoV-2 infection, general laboratory abnormalities include leukopenia and lymphopenia. These abnormalities indicate that both the viral burden and the reaction of immune system play a critical role in SARS-CoV-2 invasion and replication. The immune system can inhibit coronavirus, clean up apoptotic cells, and promote tissue recovery in the cerebrum. Chemotactic factors help leukocytes migrate to the correct position to fight infection, and abnormal secretion can aggravate the cerebral immunopathology. Conversely, weak immune systems and insufficient immune responses are associated with viral survivors and rapid coronavirus invasion. Therefore, the relationship between SARS-CoV-2 infection and the immune response needs to be investigated, with potential measures provided to interfere with viral dissemination, clear the virus, and reduce tissue impairment.

After the internalization of coronavirus particles, host cells recognize the coronavirus and initiate an innate and adaptive immune response against the viral infection; the complement system is also activated. Interaction between cell-surface pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs), activation of proinflammatory signaling proteins and pathways, production and release of several inflammatory factors, and migration of immunocytes occur in the immune and inflammatory settings [36]. In addition, complementary autocrine and paracrine signaling ensures that the infected cells and surrounding uninfected cells express a series of interferon-stimulated genes (ISGs), which establish an antiviral microenvironment [47]. The PRRs in host cells that detect pathogens contain toll-like receptor (TLR), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin-like receptor (CLR), cytoplasmic DNA receptor (CDR), type I interferons (IFNs), and dendritic cells (DCs) and restrict viral pervasion with the help of macrophages, natural killer cells, T/B cells, and immune molecules [48]. The main function of macrophages is to phagocytose and digest cell debris and pathogens and activate lymphocytes or other immune cells in response to pathogens. Macrophages and DCs infected with feline infectious peritonitis virus (FIPV) inhibited the protective Th1 cell response by promoting the signaling pathway of IL-10 expression [49]. Natural killer cells are active in the response to numerous infectious diseases and regulate immune response by activating a series of cytokines including IL-12, IL-1β, IL-18, IL-23, and IFN-β, sometimes resulting in hypersensitivity reactions and autoimmune diseases [50]. B cell, $CD4^+$ T cells, and $CD8^+$ T cells, with migration and secretion features, exert important protective functions during adaptive immune responses in organisms. $CD4^+$ helper T cells fight pathogens by activating T-cell-dependent B cells and supporting humoral and cellular immunity. Cytotoxic $CD8^+$ T cells kill infected cells using a specific antigen response that corresponds with tissue damage [51]. Due to the antigenic stimulation and activation of antigen-

presenting cells and Th cells, activated B cells differentiate into plasma cells and secrete pathogen-specific antibodies to inhibit the effects of pathogens.

Although SARS-CoV-2 is sensitive to cell-surface PRRs, immune evasion is achieved by defending intermediate products of viral replication from immune recognition, resulting in spread of SARS-CoV-2 and restricted immune responses, which are associated with lymphopenia [47, 52]. However, although immune evasion of SARS-CoV-2 temporarily restricts the innate immune response, subsequent overactivation or eruptive initiation of the immune system can occur, leading to multiple organ damage [53]. A hyperactivated immune response contributes to immunopathogenesis, tissue damage, and severe complications. The presence of lymphopenia in 2019-nCoV infection indicates that SARS-CoV-2 affects lymphocytes. Although the $CD4^+$ and $CD8^+$ T cell levels in peripheral blood are largely decreased, the function of lymphocytes is overactivated. Flow cytometric analysis has indicated high levels of proinflammatory $CCR4^+CCR6^+$ Th17 in $CD4^+$ T cells and cytotoxic granules in $CD8^+$ T cells, which are associated with systemic inflammatory responses and toxic reactions [54, 55]. In addition, the depressed immune response also indicates the mechanism of immune evasion in SARS-CoV infection [56]. $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes were shown to be decreased in the acute phase of SARS-CoV infection, indicating lymphocyte deterioration and a suppressed immune system. Nine hours after the cellular infection of SARS-CoV in vitro, the incomplete viral replication of SARS-CoV led to low production of antiviral cytokines (IFN-α, IFN-β, IFN-γ, and IL-12p40), mild generation of proinflammatory cytokines (TNF-α and IL-6), and significantly elevated inflammatory chemokines (MIP-1a, IP-10, and MCP-1) [57].

Activation of the immune system in response SARS-CoV-2 and subsequent signaling cascades lead to innate and adaptive immunity and proliferation of proinflammatory cytokines, neutralizing antibodies, and recruited lymphocytes, such as neutrophils and macrophages. However, surviving virus excessively stimulates immune cells with positive feedback and causes an inflammatory factor storm. A recent report of 138 patients with SARS-CoV-2 at Zhongnan Hospital of Wuhan University indicated that adverse reactions in severe cases included neutrophilia, coagulation activation, and acute multiple organ injury and that these reactions were associated with higher concentrations of white blood cells and neutrophils, D-dimer, creatine, aspartate aminotransferase, and high-sensitivity troponin I [18]. Another report from Wuhan demonstrated that, compared with healthy people, 44 patients with SARS-CoV-2 had higher immune cytokine counts, including IL-1b, IL-6, IL-12, IFN-γ, IP10, and MCP-1, resulting in systematic toxic organ changes and severe tissue damage [24]. Moreover, patients admitted to the ICU presented with higher levels of GCSF, IP10, MCP-1, MIP1A, and TNF-α [58].

It is believed that the immune response that aims to kill SARS-CoV-2 also disrupts tissue homeostasis and induces immunopathological changes, which is similar to MERS-CoV and SARS-CoV infection [49]. In an analysis of 128 serum samples of SARS patients, T cell responses, especially $CD8^+$ T cell responses, and antibody production were found to be major components of the immune response to SARS-CoV infection. The serological manifestation of memory phenotype ($CD27^+/CD45RO^+$) $CD4^+$ T cells producing IFN-γ, TNF-α, and IL-2 and $CD8^+$ T cells producing IFN-γ, TNF-α, and CD107a was correlated with severe disease. High concentrations of plasma IFN-γ, IL-1β, IL-6, IL-8, IL-12, IP-10, MCP-1, CXCL8, CXCL10, and CCL2 granules are a result of hyperactivated inflammatory signaling cascades and cytokine storm and are associated with the immunopathological changes and severity of SARS-CoV infection [59]. In SARS-CoV infection, neutrophils and chemokines such as IL-8 infiltrate the respiratory tract and generate myeloperoxidase and elastase, which causes deterioration of pulmonary tissue and function and leads to ARDS, respiratory failure, and admission to the ICU. A research investigated 27 serum samples of MERS-CoV from patients from South Korea in 2015 [60]. They found that the $CD8^+$ T cell response and proinflammatory factors are associated with severe disease, whereas $CD4^+$ T cell response is associated with less severe disease. $CD8^+$ T cells act on viral S protein in the early phase of MERS-CoV infection, whereas $CD4^+$ T cells interact with E/M/N proteins in the later phase. The invasion of MERS-CoV in host cells triggers the Th1 and Th17 proinflammatory response and stimulates monocytes and lymphocytes, resulting in high levels of IFN-γ, TNF-α, IL-15, and IL-17 and promoting activation of the MAPK, STAT3, and NF-κB signaling pathways. The downstream signaling protein and secreted inflammatory factors fight against the virus, even leading to tissue damage, via the production of IL-6, IL-1β, TGF-β, TNF-α, IL-8, and MCP-1. In addition, an elevated IL-10 level correlates with activated JAK-STAT pathway and indicates an anti-inflammatory effect [61].

Therefore, decreased lymphocytes and the induction of cytokine storm are potent indicators of severe COVID-19 infection. In a study of 228 patients with SARS, patients with severe disease had high levels of IL-6 and reduced concentrations of IL-8 and TGF-β in the acute phase, which correlated with disease severity [62]. The

cytokine profiles caused by excessive immune response lead to ARDS and multiple organ failure, contributing to the mortality of patients with COVID-19 [63]. Plasma exchange can clear inflammatory factors, block cytokine storms, and reduce the damage caused by the inflammatory response.

SARS-CoV-2 and cytokine storms in ICH patients

After mechanical injury by ICH, activated microglia migrate to the position of damage. Although M1 microglia help clear necrotic substances, they also generate inflammatory cytokines and contribute to BBB breakdown and brain edema. Triggered inflammatory cascades, including production of IL-1β, TNF-α, ROS, chemokines, and prostaglandins, damage the BBB [64]. Due to increased BBB permeability, mobilized neutrophils in the perihematomal region generate ROS and release a series of granules, such as collagenase, myeloperoxidase, and elastase. Neutrophils can stimulate nearby microglia, regulate immune response, and exaggerate adverse effects on brain tissue via production of IL-8, IL-6, TNF-α, and IL-1β, resulting in neuronal loss and brain edema. Persistently high neutrophil levels in peripheral blood predict poor prognosis in ICH patients. In addition, neutrophils are important mediators in the recruitment of monocytes [65]. The reactive astrocytes gather around the hematoma and induce MMP-9 [66]. Elevated MMP-9 activity is associated with perihematomal edema, BBB disruption, and neural loss [67]. $CD8^+$ cytotoxic T cells and $CD4^+$ Th cells increase in the perihematomal region and contribute to neuronal apoptosis and endothelial injury. Due to physical damage and BBB impairment, inflammatory cells infiltrate the hematoma, stimulate the production of cytokines and chemotactic factor with active feedback, and initiate cellular apoptosis via NF-κB inflammatory signaling pathways and downstream molecules [68]. Intercellular adhesion molecule-1, IL-1β, TNF-α, chemokines, MMP-9, inducible nitric oxide synthase, free radicals, COX-2, and PLA_2 participate in NF-κB activation. Inflammatory cells contain recruited neutrophils and monocytes and resident microglia and astrocytes. Active cytokines can stimulate the complement system to form the membrane attack complex and generate C3a and C5a, resulting in direct tissue injury and augmented immune response. Infiltration of blood substances affects microcirculation, contributing to hypoxia and producing ROS. Hemoglobin and iron are cytotoxic and cause oxidative and proinflammatory changes that further brain injury, probably in conjunction with oxygen free radicals [69]. Oxidative stress, excitotoxicity, and cellular necrosis and apoptosis result in neuronal injury, brain edema, and cell death [70].

However, there is limited information about the innate immune responses in the center nervous system after SARS-CoV-2 brain invasion in ICH patients. In a lab study, the serum samples of SARS-CoV-2 patients were IgM positive in the early stage of infection and subsequently became IgG positive, indicating a humoral response [11]. Because SARS-CoV-2 invades the brain via ACE2 receptor in ICH patients, viral pathogenicity and replication destroy the blood–brain barrier and induce dynamic immune responses. SARS-CoV-2 infection may disturb the activation and inhibition of related signaling cascades, leading to stimulation of the innate immune system, recruitment of lymphocytes, secretion of toxic substances, and cytokine storm with positive feedback circulation.

Neurologic biopsies of patients with SARS-CoV-2 infection demonstrate congestion, brain edema, and partial neuronal degeneration, similar to the effects seen with SARS and MERS infection. ACE2 receptors are distributed throughout the synaptic membrane of the brain, and center nervous system autopsies of SARS patients demonstrated infiltration of monocytes and lymphocytes in blood vessels, hydrocephalus, demyelination of the nerve myelin sheath, and neuronal degeneration, which is associated with aggravation of the pathological changes seen in ICH patients [19]. After SARS-CoV infection in K18-hACE2 mice, viral particles and antigens were found in the neurons of the brain. Upregulation of cytokines and chemokines contributes to BBB impairment, gliocyte hyperplasia, neuronal damage, and brain edema as a result of cellular oxidative damage, necrosis, and apoptosis [71]. Some patients with severe MERS-CoV infection manifested neurological symptoms, including epilepsy, dystaxia, paralysis, and conscious disturbance. Magnetic resonance imaging performed in hospitalized patients with MERS indicated acute alterations in the white matter and the subcortical areas of the frontal, temporal, and parietal lobes [72]. In MERS, excessive production of proinflammatory cytokines and chemokines leads to rapid increases of RIG-I, MDA5, PKR, MYD88, TNF-α, IL-1β, CCL2, CCL5, and CXCL10 in the brain [73]. MHV affects oligodendrocytes and impairs the myelin sheath via immunologic injury. Although MHV-JHM infection in brain tissue initiates an immune response and activates inflammatory signaling cascades to clear the virus, MMP secretion, immunocyte migration, and increased chemokines and cytokines are associated with BBB breakdown and demyelination [49]. Impairment of brain microvascular endothelial cells (BMECs) in vitro by MHV3 infection is a result of downregulation of zona occludens protein 1 (ZO-1), VE-cadherin, and occludin, which leads to elevated BBB permeability [74]. In addition, stimulation and recruitment of

macrophages and/or microglia in the white matter contributes to demyelination in MHV-JHM-infected mice. Although immune responses help clear pathogens, excessively inflammatory signaling cascades, influx of cytokines and chemokines, a large volume of recruited immune cells, and toxic substances in the center nervous system indicate a poor prognosis.

Whereas SARS-CoV-2 invasion and replication in brain cause direct damage, indirect deterioration is associated with the immune response. Immune mediator dysfunction and autoimmune reactions prolong the immune response and exacerbate tissue damage. Neutrophils, natural killer cells, macrophages, and lymphocytes proliferate and produce IL-1α, IL-1β, IL-6, IL-12, TNF-α, IFN-γ, and CXCR2. Migrated neutrophils swallow viral particles; generate a series of antibacterial peptides, proteases, and ROS to kill the virus; and introduce tissue damage. ROS, superoxide anion, and NADPH oxidase cause excessive oxidative stress. Elevated neutrophil-to-lymphocyte ratio (NLR) has been shown to be a marker of severe SARS-CoV-2 infection in the early phase.

An overactivated immune system affects both virus and host cells. As SARS-CoV-2 combines with ACE2 receptors, the immunopathological injury in center nerve system is the result of the explosive cytokine storm [75]. ACE2 is highly expressed in arterial and venous endothelial cells and arterial smooth muscle cells in brain. The impairment and contraction of vascular endothelial cells, due to the out of control inflammatory response, lead to increased permeability of the capillary wall and diffusion of substances from vessels into the interstitial space. Brain tissues with ACE2 receptors are attacked the extreme immune response, eventually leading to neurologic deficits and bad outcomes.

CONCLUSIONS

Patients with COVID-19 who present with neurologic symptoms need early diagnosis, isolation, and treatment. When new neurologic symptoms occur in hospitalized patients, such as ataxia, focal motor deficits, and conscious disturbance, cerebrospinal fluid examination and SARS-CoV-2 nucleic acid and gene sequencing should be performed. ICH patients with SARS-CoV-2 infection are prone to develop neurological complications and have poor outcomes. Because there is no specific treatment for the virus, airborne precautions and isolation of identified and suspected infected patients is crucial.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

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Research Paper

High neutrophil-to-lymphocyte ratio associated with progression to critical illness in older patients with COVID-19: a multicenter retrospective study

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ABSTRACT

This retrospective cohort study aimed to investigate the correlation of the neutrophil-to-lymphocyte ratio (NLR) with critical illness in older patients with COVID-19, and evaluate the prognostic power of the NLR at admission. We enrolled 232 patients with COVID-19, aged ≥60 y, in Zhejiang province from January 17 to March 3, 2020. Primary outcomes were evaluated until April 13. Cox regression was performed for prognostic factors. Twenty-nine (12.5%) patients progressed to critical illness. Age, shortness of breath, comorbidities including hypertension, heart disease, and chronic obstructive pulmonary disease, higher NLR, lower albumin levels, and multiple mottling and ground-glass opacity were associated with progression. In the multivariate analysis, older age (hazard ratio [HR] 1.121, confidence interval [CI] 1.070-1.174, P<0.001), heart disease (HR 2.587, CI 1.156-5.787, P=0.021), higher NLR (HR 1.136, CI 1.094-1.180, P < 0.001), and multiple mottling and ground-glass opacity (HR 4.518, CI 1.906-10.712, P<0.001) remained critical illness predictors. The NLR was independently associated with progression to critical illness; the relationship was significant and graded (HR: 1.16 per unit; 95% CI: 1.10-1.22; P for trend < 0.001). Therefore, NLR can be adopted as a prognostic tool to assist healthcare providers predict the clinical outcomes of older patients suffering from COVID-19.

INTRODUCTION

In December 2019, a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China [1–3]. Infection with the virus leads to coronavirus disease (COVID-19), which is characterized by rapid human-to-human transmission and varied degrees of fatality, due to acute respiratory distress syndrome, multi-organ failure, and other serious complications [4, 5]. The global spread of this pandemic has been rapid since March 2020. As of mid-April 2020, more than 2 million individuals had been diagnosed with the disease, leading to over 150,000 deaths.

In our previous study, we found that older patients with COVID-19 had significantly greater disease severities, as well as higher rates of critical-type disease and intensive care unit (ICU) admission than their younger counterparts outside Wuhan [6]. Wang et al. [7] found that patients treated in the ICU were older than those without ICU treatment in Wuhan. In the United States, Garg et al. [8] demonstrated that older adults had elevated rates of COVID-19-associated hospitalization, and the majority of people hospitalized with COVID-19 had underlying medical conditions. In Italy, a majority of critically ill patients with laboratory-confirmed COVID-19 who were admitted to ICUs were older men, and a large proportion of them required mechanical ventilation and high levels of positive end-expiratory pressure; the associated ICU-related mortality was 26% [9].

Many studies have shown that older age is an independent risk factor for fatal outcomes in patients with COVID-19 [10–12]. Wang et al. investigated the characteristics of elderly patients with COVID-19 and the associated prognostic factors, and found that the presence of acute respiratory distress syndrome was a strong predictor of death. In addition, high lymphocyte levels were predictive of better outcomes [13]. Lymphopenia is a risk factor for severe illness and death among patients with COVID-19 [14].

The neutrophil-to-lymphocyte ratio (NLR) can be easily determined from the full blood count, and has been reported to be closely related to patients' overall inflammatory status.

Increasing NLR values are risk factors of mortality in not only infectious disease settings but also cancer [15, 16]. A study showed that the NLR is an independent risk factor of mortality in hospitalized patients with COVID-19 [17]. The identification of a good indicator of disease progression can aid clinicians in improving the effect of therapy and reducing the mortality related to COVID-19 without excessive medical resource use. Whether the NLR can predict progression to critical illness in older patients with COVID-19 requires further elucidated.

In this study, we investigated the correlation of the NLR with critical illness in older patients with COVID-19, to evaluate the prognostic power of the NLR at admission in the prediction of progression to critical illness.

RESULTS

Demographic and epidemiologic characteristics

In this study, 232 older (≥60 years) patients with confirmed COVID-19 were enrolled from January 17, 2020 to March 3, 2020 in Zhejiang province. Patients' clinical outcomes were followed-up until April 13, 2020. As shown in Table 1, the median ages in the mild, severe, and critical disease groups were 66 years (interquartile range [IQR]: 63-70), 66 years (IQR: 62-71) and 72 years (IQR: 68-81). The critical group showed a significantly higher age than the mild and severe groups (P<0.001). The proportions of hypertension and heart disease in the critical group were 72.41% and 55.17%, respectively, which were significantly higher than those noted in the mild and severe groups (P<0.001). One case (0.71%) with mild disease, two (3.14%) with severe disease, and six (20.69%) with critical disease had chronic obstructive pulmonary disease (COPD) (P<0.001). There were no significant differences in the other coexisting medical conditions across the three groups, including the rates of diabetes, asthma, cancer, chronic liver disease, chronic renal disease, and immunosuppression.

Clinical features and laboratory abnormalities

On admission, the majority of cases showed decreased or normal leucocyte levels in all subtypes, as shown in Table 2. The median neutrophil levels in the mild, severe, and critical groups were 3.22×10^9 /L [IQR: (2.59-4.20) $\times10^9$], 3.50×10^9/L [IQR: (2.70-4.80) $\times10^9$], and 6.65×10^9/L [IQR: (3.51-9.70) $\times10^9$], respectively; the critical group showed significantly higher values than the mild and severe groups (P<0.001). The median lymphocyte levels in the mild, severe, and critical groups were 1.26×10^9 /L [IQR: (0.90-1.60) $\times10^9$], 0.98×10^9/L [IQR: (0.70-1.26) $\times10^9$], and 0.54×10^9/L [IQR: (0.45-0.80) $\times10^9$], respectively. The critical group showed significantly lower values than the mild and severe groups (P<0.001). The platelet levels were lower in the critically group than the mild and severe groups, but were still within the normal range. The levels of lactate dehydrogenase, creatinine, C-reactive protein, and procalcitonin increased with increasing illness severity (P<0.05). There were no significant differences in the blood test results across the three groups, including the values of albumin, alanine aminotransferase, aspartate aminotransferase, total bilirubin, potassium, sodium, and blood urea nitrogen. Multiple mottling and ground-glass opacity were typical imaging manifestations noted in patients with COVID-19, and their prevalence rates in the mild, severe, and critical groups were 24.29%, 42.86%, and 68.97%, respectively (P<0.001).

Treatment and outcomes

All patients were isolated in designated hospitals and received supportive care as well as the currently recommended medications. As shown in Table 3, 135 cases (84.77%), 60 cases (95.24%), and 29 cases (100%) received antiviral treatment, including interferon-α sprays, arbidol hydrochloride capsules, and

Table 1. Demographic, epidemiologic, and clinical characteristics of the different subtypes in older patients with COVID-19.

Characteristic	Mild type (n=140)	Severe type (n=63)	Critical type (n=29)	*P* value
Age (years)	66(63-70)	66(62-71)	72(68-81)	<0.001
Distribution				
60-70 y	102(72.86)	45(71.435)	7(24.14)	<0.001
70-80 y	30(21.43)	14(22.22)	13(44.83)	0.025
≥80 y	8(5.71)	4(6.35)	9(31.03)	<0.001
Sex (male)	62(44.29)	28(44.44)	19(65.52)	0.102
Body mass index (kg/m^2)	23.52(21.23-25.39)	24.34(22.25-25.16)	24.51(22.89-26.62)	0.227
Current smoker	17(12.14)	4(6.35)	4(13.79)	0.418
Exposure history in Wuhan	25(17.86)	18(28.57)	5(17.24)	0.194
Contact with patients	82(57.14)	25(39.68)	12(41.37)	0.023
Family cluster	50(35.71)	20(31.75)	10(34.48)	0.859
Time from illness onset to first hospital admission (days)	3(1-6)	5(2-7)	3(1-5)	0.048
Coexisting disorder				
Any	76(54.29)	25(38.68)	13(44.83)	0.132
Hypertension	57(40.71)	22(34.92)	21(72.41)	0.004
Heart disease	8(5.71)	7(11.11)	16(55.17)	<0.001
Diabetes	29(20.71)	9(14.29)	4(13.79)	0.431
asthma	1(0.71)	1(1.59)	2(6.90)	0.076
Chronic obstructive pulmonary disease	1(0.71)	2(3.14)	6(20.69)	<0.001
Cancer	2(14.29)	1(1.59)	1(3.45)	0.766
Chronic liver disease	4(2.86)	4(6.35)	2(6.90)	0.397
Chronic renal disease	3(2.14)	1(1.59)	2(6.90)	0.313
Immunosuppression	0(0)	2(3.17)	0(0)	0.064
Symptoms on admission				
Fever	110(78.57)	55(87.30)	25(86.21)	0.105
Cough	94(67.14)	38(60.2)	22(75.87)	0.461
Sputum production	46(32.86)	26(41.27)	15(51.72)	0.148
Hemoptysis	2(1.43)	1(1.59)	1(3.45)	0.766
Sore throat	13(9.29)	8(12.70)	2(6.90)	0.598
Nasal obstruction	2(1.43)	0(0%)	1(3.45)	0.404
Myalgia	12(8.57)	8(12.70)	4(13.79)	0.552
Fatigue	19(13.57)	11(17.46)	8(27.59)	0.202
Gastrointestinal symptoms	12(8.57)	6(9.52)	7(24.14)	0.06
Headache	5(3.57)	6(9.52)	0(0%)	0.073
Shortness of breath	1(0.71)	7(11.11)	12(41.38)	<0.001

Data are presented as medians (interquartile ranges), n (%) and n/N (%).

lopinavir and ritonavir tablets in the mild, severe, and critical groups, respectively (*P*=0.504). The durations from illness onset to antiviral therapy initiation were 4 days (IQR: 2.0-7.0), 5 days (IQR: 1.5-8.5), and 4 days (IQR: 2.0-8.0) in the mild, severe, and critical groups, respectively (*P*=0.390). With increases in the illness severity, the proportion of the use of glucocorticoids and intravenous immunoglobins rose (*P*<0.001). Ten

Table 2. Laboratory and radiograph findings of the different subtypes in older patients with COVID-19.

Characteristic	Mild type (n=140)	Severe type (n=63)	Critical type (n=29)	*P* value
Blood routine				
Leucocyte count ($\times 10^9$/L)	5.20(4.38-6.48)	5.0(4.1-6.88)	8.08(4.4-10.8)	0.02
Neutrophil count ($\times 10^9$/L)	3.22(2.59-4.20)	3.50(2.70-4.80)	6.65(3.51-9.70)	<0.001
Lymphocyte count ($\times 10^9$/L)	1.26(0.90-1.60)	0.98(0.70-1.26)	0.54(0.45-0.80)	<0.001
Neutrophil count/lymphocyte count	2.45(1.82-3.65)	4.08(2.39-6.20)	9.67(6.86-21.10)	<0.001
Hemoglobin (g/L)	125.0(113.0-138.0)	122.0(113.5-133.5)	121.0(110.5-137.5)	0.535
Platelet count ($\times 10^9$/L)	204(170-279)	175(139-236)	156(123-191)	<0.001
Coagulation function				
International normalized ratio	1.02(0.96-1.06)	1.01(0.96-1.10)	1.0(0.97-1.06)	0.895
Blood biochemistry				
Albumin (g/L)	38.40(35.43-41.25)	36.30(33.30-39.50)	34.60(30.65-38.45)	0.001
Alanine aminotransferase (U/L)	25(16-36)	24(16-31)	21(14-31)	0.664
Aspartate aminotransferase (U/L)	25(20-33)	25(19-34)	29(18-38)	0.891
Total bilirubin (umol//L)	9.70(7.0-12.55)	10.10(7.90-13.15)	9.10(5.70-14.30)	0.671
Potassium (mmol/L)	3.99(3.70-4.37)	3.89(3.45-4.25)	3.81(3.50-4.14)	0.072
Sodium (mmol/L)	138.0(135.72-140.15)	137.50(134.95-140.0)	136.0(130.60-139.0)	0.027
Blood urea nitrogen (mmol/L)	4.51(3.83-5.47)	4.59(3.60-7.10)	6.16(4.48-8.72)	0.032
Creatinine (umol/L)	64.0(54.0-76.5)	68.0(57.0-84.0)	76.0(63.0-96.5)	0.003
Creatinine kinase (U/L)	56.50(41.25-88.75)	62.0(26.25-113.75)	80.0(52.0-173.50)	0.038
Lactate dehydrogenase (U/L)	218.0(175.0-256.50)	233.0(190.0-313.0)	273.0(243.0-354.0)	<0.001
Infection-related biomarkers				
C-reactive protein (mg/L)	16.02(4.41-39.26)	19.10(5.89-44.70)	41.86(6.33-70.10)	0.039
Procalcitonin (ng/mL)	0.09(0.04-0.14)	0.05(0.04-0.08)	0.19(0.04-0.25)	0.046
Chest radiography/Computed tomography findings				
Multiple mottling and ground-glass opacity	34(24.29)	27(42.86)	20(68.97)	<0.001

Data are presented as medians (interquartile ranges), n (%) and n/N (%).

patients received extracorporeal membrane oxygenation (ECMO) therapy, and six underwent continuous renal-replacement therapy (CRRT) in the critical group; none of the patients received ECMO therapy and only one underwent CRRT in the severe group. Three patients had shock in the critical group, while there were no cases with shock in the mild and severe groups (*P*<0.001). The viral RNA shedding durations were 16 days (IQR: 12-22), 17 days (IQR: 14-21), and 25 days (IQR: 17-30) in the mild, severe, and critical groups, respectively (P<0.001).

By April 13, one patient had died, two had received lung transplantation, and eight remained hospitalized in the critical group. By May 27, among the eight patients who were still hospitalized, two withdrew from the ECMO treatment and were transferred to the general ward, while the other six patients were still receiving the ECMO therapy. In the other two groups, all patients had survived and were discharged. The number of days of hospitalization were 18 days [IQR: 14-23], 22 days [IQR: 19-26], and 32 days [IQR: 21-68] in the mild, severe, and critical groups, respectively (P<0.001).

Risk factors associated with progression to critical illness

Univariate Cox regression was used to analyze the risk factors for critical illness in the older patients with COVID-19, as shown in Table 4. Older age was shown to increase the likelihood of critical illness even in older patients (≥60 years) (hazard ratio [HR] 1.107, confidence interval [CI] 1.065-1.151, *P*<0.001). Shortness of breath as a symptom (HR 11.328,

Table 3. Treatments and clinical outcomes of the different subtypes in older patients with COVID-19.

Characteristic	Mild type (n=140)	Severe type (n=63)	Critical type (n=29)	*P* value
Shock	0(0)	0(0)	3(10.34)	<0.001
Time from illness onset to antiviral treatment initiation (days)	4.0(2.0-7.0)	5.0(1.5-8.5)	4.0(2.0-8.0)	0.390
Antiviral treatment	135(96.43)	60(95.24)	29(100)	0.504
Viral RNA shedding time	16(12-22)	17(14-21)	25(17-30)	<0.001
Glucocorticoids	22(15.71)	29(46.03)	26(89.66)	<0.001
Use of intravenous immunoglobulin	17()	21()	23(79.31)	<0.001
Use of extracorporeal membrane oxygenation	0(0)	0(0)	10(34.48)	<0.001
Use of continuous renal-replacement therapy	0(0)	1(1.59)	6(20.69)	<0.001
Clinical outcomes at data cutoff				
Discharge from hospital	140(100)	63(100)	20(68.97)	0.098
Hospitalization	0(0)	0(0)	8(27.59)	0.098
Number of days in hospital	18(14-23)	22(19-26)	32(21-68)	<0.001
Lung transplantation	0(0)	0(0)	2(6.90)	0.001
Death	0(0)	0(0)	1(3.45)	0.030

Data are presented as medians (interquartile ranges), n (%) and n/N (%).

Table 4. Risk factors for critical illness.

Variables	Mild/Severe type (n=203)	Critical type (n=29)	Univariate analysis		Multivariate analysis	
			HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years)	66(63-70)	72(68-81)	1.107(1.065-1.151)	<0.001	1.121(1.070-1.174)	<0.001
Time from illness onset to first hospital admission (days)	3(1-7)	3(1-5)	0.937(0.836-1.049)	0.258		
Hypertension	79(38.92)	21(72.41)	3.563(1.578-8.047)	0.002		
Heart disease	15(7.39)	16(55.17)	9.638(4.626-20.081)	<0.001	2.587(1.156-5.787)	0.021
COPD	3(1.48)	6(20.69)	7.108(2.891-17.481)	<0.001		
Shortness of breath	8(3.94)	12(41.38)	11.328(5.370-23.894)	<0.001		
NLR	2.68(1.96-4.42)	9.67(6.86-21.10)	1.157(1.117-1.199)	<0.001	1.136(1.094-1.180)	<0.001
Albumin (g/L)	38.0(35.20-41.0)	34.60(30.65-38.45)	0.875(0.807-0.950)	0.001		
C-reactive protein (mg/L)	16.95(4.75-40.62)	41.86(6.33-70.10)	1.012(1.005-1.020)	0.002		
Multiple mottling and ground-glass opacity	61(30.05)	20(68.97)	4.573(2.082-10.045)	<0.001	4.518 (1.906-10.712)	0.001

HR, hazard ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease; NLR, neutrophil-to-lymphocyte ratio.

CI 5.370-23.894, P<0.001), and comorbidities including hypertension (HR 3.563, CI 1.578-8.047, *P*=0.002), heart disease (HR 9.638, CI 4.626-20.081, *P*<0.001), and COPD (HR 7.108, CI 2.891-17.481, *P*<0.001) were predictive of critical illness. The increasing odds of critical illness development in patients with COVID-19 were associated with higher NLR values (HR 1.157, CI 1.117-1.199, *P*<0.001), lower albumin levels (HR 0.875, CI 0.807-0.950, *P*<0.001), higher C-reactive protein levels (HR 1.012, CI 1.005-1.020, *P*=0.002), and multiple mottling and ground-glass opacity (HR 4.573, CI 2.082-10.045, P<0.001). In the multivariate analysis, only older age (HR 1.121, CI 1.070-1.174, *P*<0.001), heart disease (HR 2.587, CI 1.156-5.787, *P*=0.021), higher NLRs (HR 1.136, CI 1.094-1.180, *P* < 0.001), and multiple mottling and ground-glass opacity (HR 4.518, CI 1.906-10.712, P<0.001) remained predictors of critical illness when the other factors in the model were kept constant.

Association of the NLR with progression to critical illness

Figure 1A shows the association between the NLR and progression to critical illness, as identified using a Cox proportional hazards model adjusted for the baseline covariates. For the sensitivity analysis, we converted the NLR from a continuous variable to a categorical

variable (the quartile of NLR), and the P for trend of the NLR with categorical variables in the fully adjusted model (model II) was consistent with that obtained when the NLR was a continuous variable. The relationship between the NLR and progression was significant and graded (HR: 1.16 per unit; 95% CI: 1.10-1.22; P<0.001). When adjusted for sex and age, the ratio of the highest quartile of the NLR compared to the lowest quartile was 33.017 (95% CI 4.436-245.732, P <0.001), and in the fully adjusted model, the odds of the NLR as a clinical risk factor was 21.755 (95% CI 2.854-165.860, P<0.001) (Table 5). Figure 1B shows the Kaplan-Meier analyses graphs for progression to critical illness based on the quartiles of the NLR.

DISCUSSION

In the present study, we described the clinical characteristics and outcomes of older patients who had COVID-19 with the highest risk of critical illness after SARS-CoV-2 infection. Of the 232 older patients with COVID-19, 29 (12.5%) had critical disease; one patient died and two received lung transplantation in the critical group. Eight patients remained in the hospital, and received ECMO therapy for more than two weeks. The median duration of hospitalization was 32 days in the critical group, which was significantly longer than that in the mild and severe groups.

Disease typing and prognostic indicators are of great significance in the guidance of classified treatment and prevention of medical runs, and saving patients with a critical status. In our study, some independent risk factors for progression to critical illness were found using multivariate Cox regression analysis, such as older age, multiple mottling and ground-glass opacity, heart disease, and a high NLR.

Previously, older age was reported as an important independent predictor of fatal outcomes in patients with COVID-19 [18–21]. Older age was shown to increase the likelihood of critical illness even in older patients (HR 1.107, CI 1.065-1.151, P<0.001). Our results are consistent with those of previous reports [13]. Elderly patients experience a marked cell-mediated immune function decline and a reduced degree of humoral immune function. The cytokine and chemokine signaling networks are altered in elderly patients, and tend to favor a type 2 cytokine response over type 1 cytokine responses, potentially leading to poor outcomes [22].

Advanced imaging in patients with COVID-19 is capable of demonstrating disease progression. Generally, imaging manifestations are in line with the severity of COVID-19 [23]. Zhong et al. found that the computed tomography (CT) images in patients with different clinical types of COVID-19 had characteristic manifestations, and that the presence of solid shadows may be predictive of severe and critical illness [24]. Our study found that the presence of multiple mottling and ground-glass opacity on CT was an independent predictor of progression to critical illness (HR 4.518, CI

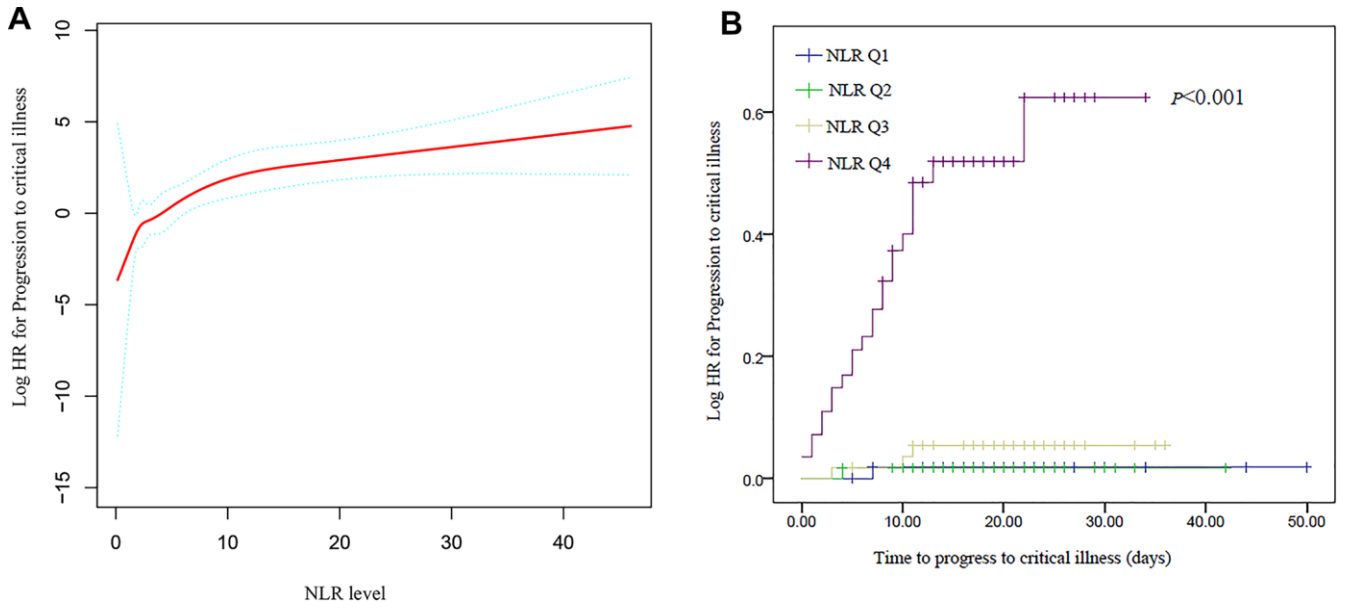

Figure 1. Association between the neutrophil-to-lymphocyte ratio (NLR) and progression to critical illness. (**A**) Adjusted hazard ratio (HR) for progression to critical illness according to the NLR. (**B**) Cumulative probability of progression to critical illness with increasing NLR values.

Table 5. Relationships between the neutrophil-to-lymphocyte ratio and critical disease development using different models.

Neutrophil-to-lymphocyte ratio (quartile)	Total, n	Event (%)	HR (95% CI)		
			Crude Model	Model I	Model II
Q1	58	1(1.72)	Reference	Reference	Reference
Q2	60	1(1.61)	0.980(0.061-15.662)	1.186(0.074-18.984)	1.324(0.081-21.591)
Q3	57	3(5.26)	2.914(0.303-28.014)	2.966(0.308-28.533)	3.867(0.399-37.461)
Q4	57	24(42.11)	29.769(4.024-220.233)	33.017(4.436-245.732)	21.755(2.854-165.860)
P for trend	—	—	<0.001	<0.001	<0.001
Increase per unit	—	—	1.16(1.12-1.20)	1.15(1.11-1.19)	1.16(1.10-1.22)

Note: Model I adjusted for age, sex.
Model II adjusted for age, sex, hypertension, heart disease, COPD, shortness of breath, albumin, C-reactive protein and multiple mottling and ground-glass opacity.
HR, hazard ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

1.906-10.712, *P*=0.001). We also found that older patients with COVID-19 who had heart disease were likelier to progress to critical illness. Several studies have shown that coexisting heart disease was an independent risk factor associated with fatal outcomes in patients with COVID-19 [12, 25]. Cardiac complications, including new or worsening heart failure, new or worsening arrhythmia, and myocardial infarction are commonly observed in patients with severe pneumonia. Cardiac arrest occurs in about 3% of inpatients with severe pneumonia [26].

Chen et al. showed that, compared to cases with moderate disease severity, those with a severe disease status more frequently had lymphopenia [27]. Mo et al. found that patients with refractory disease had higher neutrophil levels than general COVID-19 patients [28]. The prognostic role of the NLR has been documented in multiple settings, including malignancies, infectious diseases, liver cirrhosis, and cerebrovascular disease [29–32]. In this study, we investigated the correlation of the NLR with critical illness in older patients with COVID-19 to evaluate the prognostic power of the NLR at admission in the prediction of progression to critical illness. In the sensitivity analysis, we converted the NLR from a continuous variable to a categorical variable, and found that the higher the NLR the greater the likelihood of progression to critical illness. Liu et al. also found that the NLR is an independent risk factor of in-hospital mortality in COVID-19 patients, especially male patients [17]. Our previous study suggested that a change in the NLR on admission among older patients with COVID-19 might be a biomarker specific to the prediction of progression to critical illness. A future study, conducted to elucidate this specificity, will further our understanding of the prognostic value of the NLR.

Our study has several limitations. First, its retrospective nature may decrease the accuracy of the findings; there is a need for a validation cohort to assess the predictive accuracy and confirm our findings. Second, owing to the retrospective design, data on some relevant factors such as interleukin-6 and D-dimer were incomplete and could not be included in the risk factor analysis. Third, data on the outcomes of older patients with COVID-19 in the critical group require further investigation, as, at the time of this study, there were still eight patients who were undergoing treatment at the hospital.

MATERIALS AND METHODS

Patients

This retrospective study, focusing on the epidemiological and clinical characteristics of older (age≥60 years) patients with confirmed COVID-19, was conducted from January 17 to March 3, 2020. All the enrolled cases showed real-time reverse transcriptase polymerase chain reaction (RT-PCR) positivity for SARS-CoV-2, and were retested several times during their hospitalization. Data were collected uniformly by the Health Commission of Zhejiang Province, wherein all patients were assigned to specific hospitals for unified treatment according to Zhejiang Province's emergency rule. The diagnosis of COVID-19 infection was based on the interim guidance of the World Health Organization (WHO) [33], and all data were shared with the WHO, with the primary analytic results reported to the authority of Zhejiang Province. Since the collection and analysis of all cases were determined by the Health Commission of Zhejiang Province under national authorization and considered as part of the continuing public health outbreak investigation, our retrospective study was exempt from institutional review board approval.

The subtype definition of COVID-19 patients was based on the diagnosis and treatment scheme for COVID-19 in China, based on a minor modification of WHO standards [34]. The degree of COVID-19 was categorized as mild, severe, or critical: the mild type included non-pneumonia and mild pneumonia cases, and the severe type was characterized by dyspnea, respiratory frequency ≥30 min, blood oxygen saturation ≤93%, PaO2/FiO2 ratio <300, and/or rate of lung infiltration >50% within 24–48 h. Critical cases were those that exhibited respiratory failure, septic shock, and/or multiple organ dysfunction/failure.

Procedures

We obtained epidemiological, demographic, laboratory, clinical, management, and outcome data from patients' medical records. Data were retrieved and reviewed by two independent observers. Clinical outcomes were followed-up until April 13, 2020. Missing or unclear data were confirmed by direct communication with healthcare providers. Throat swab specimens obtained from the upper respiratory tract and sputum of all patients were collected at admission. Laboratory confirmation of COVID-19 was performed at the First Affiliated Hospital at Zhejiang University, under the authorization of the Centers for Disease Control and Prevention at the Zhejiang Province/city level, by previously reported RT-PCR methods. All patients underwent chest CT at admission. Patients with other common respiratory viruses, including respiratory syncytial virus, parainfluenza virus, influenza A and B virus, and adenovirus were excluded from this study.

Data collection

In this study, we collected data on epidemiology, anthropometrics, demographics, as well as symptoms and signs at the time of admission to the hospital. We analyzed the blood collected within 48 hours of admission. Additional data collected included those on the results of laboratory tests and chest CT, comorbidities, co-infection with other respiratory pathogens, treatment (including drugs, intensive care and mechanical ventilation), and other clinical outcomes.

Statistical analysis

Continuous variables are expressed as medians (range), and were compared using t tests or Mann-Whitney U tests, and categorical variables were compared using chi-squared tests or Fisher's exact tests. Follow-up was initiated on the day of admission, and ended at the patient's death or until the last follow-up. The Kaplan-Meier method was used to evaluate the cumulative rate of progression to critical illness, and a log-rank test was used to assess differences between groups. HRs were calculated using the Cox regression model. Variables with $P < 0.05$ in the univariate analysis were included in a stepwise Cox proportional hazards regression model. We performed tests for linear trend by entering the median value of each quartile of the NLR as a continuous variable in the models. The Cox proportional hazards model was used to estimate the HRs associated with the NLR for the risk of progression to critical illness with adjustment for pertinent variables. The HRs and 95% CIs of the progression to critical illness in each subgroup were estimated, and their interactions tested. Statistical analyses were conducted using SPSS version 26.0 (IBM Corporation, Armonk) and R version 3.4 (R Foundation). A two-sided P value < 0.05 was considered to indicate statistical significance.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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Research Paper

Clinical imaging characteristics of inpatients with coronavirus disease-2019 in Heilongjiang Province, China: a retrospective study

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ABSTRACT

Objective: To investigate the clinical, laboratory, and radiological characteristics of patients with coronavirus disease-2019 (COVID-19) in Heilongjiang Province.

Results: Patients in the ICU group were older and their incidence of cardiovascular disease was higher than those in the non-ICU group. Lymphocyte levels were lower and neutrophil and D-dimer levels were higher in the ICU than that in the non-ICU group. Compared to the non-ICU group, the incidence of pulmonary consolidation and ground-glass opacity with consolidation was significantly higher in the ICU group, all lung lobes were more likely to be involved, with higher number of lung lobes and areas surrounding the bronchi. Of the 59 patients with COVID-19 in this group, 15 received mechanical ventilation. All intubated patients involved lung lobes, and a large number of lesions were observed in the area around the bronchial vessels.

Conclusion: Significant differences were observed in clinical symptoms, laboratory tests, and computed tomography features between the ICU and non-ICU groups.

Methods: A total of 59 patients with COVID-19, comprising 44 patients in the intensive care unit (ICU) and 15 in the non-ICU, were retrospectively analyzed. Characteristics of the two groups of patients were compared.

INTRODUCTION

An outbreak of new pneumonia caused by the 2019 novel coronavirus (2019-nCoV) started in Wuhan, China, in December 2019 [1]. In January 2020, Chinese scientists isolated this 2019-nCoV from patients with viral pneumonia, officially naming it as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. Since then, the disease has rapidly spread from Wuhan to other regions. In February 2020, the World Health Organization (WHO) named the disease caused by this virus as coronavirus disease 2019 (COVID-19). At the time of this article's submission, some cases have been reported internationally across the six continents.

The COVID-19 pandemic has caused severe illness in infected patients, such as pneumonia and acute respiratory distress syndrome, which even resulted in death. According to the COVID-19 joint study report released by the National Health Commission of the People's Republic of China, about 80% of patients have light and common infection, whereas 13.8% have

severe/critical infections, making them highly at risk for mortality [3]. In addition, prevention and control of severe and critically ill patients are yet to be implemented [3]. Thus, clinicians and radiologists should identify the characteristic imaging manifestations in chest CT findings of critically ill individuals, so that they can perform specific symptomatic treatment at the earliest, prevent complications, and provide organ functional support. Compared to other methods, computed tomography (CT) is the best technique for the early detection of pneumonia. Only a few reports demonstrated the clinical imaging features of severe and critically ill patients during the epidemic in Heilongjiang Province. This study describes the clinical and radiological characteristics and laboratory examination data of 59 patients with COVID-19 and compares between those admitted in the intensive care unit (ICU) and non-ICU departments. Thus, we hope that these current results could be used by clinicians in Heilongjiang Province and worldwide for the treatment plan of COVID-19.

RESULTS

A total of 59 patients confirmed with COVID-19 in Heilongjiang Province were included in this study. The general clinical data of patients are shown in Table 1. The median age was 64.0 (IQR, 56–72) years. The most common complication in the patient group was cardiovascular disease (44%), followed hypertension (42%) and diabetes (15%), and the rarest complication was chronic obstructive disease (3%), followed by malignancy (2%) and chronic liver disease (2%). Compared to non-ICU patients, ICU patients were older (median age: 67 *vs*. 56); $P = 0.037$) and more likely at risk for cardiovascular diseases (52% *vs*. 20%; $P = 0.030$). The most common clinical symptoms in this study were fever (41/59, 69%), cough (30/59, 51%), and muscle soreness (15/59, 25%), whereas the less common were dyspnea (14/59, 24%), headache (8/59, 13%), abdominal pain, diarrhea (5/59, 8%), and nausea (3/59, 5%). However, compared to non-ICU patients, the incidence of muscle soreness in the ICU patients was reduced (18% *vs*. 47%; $P = 0.042$).

Laboratory examination results of 59 patients are summarized in Table 2. White blood cell count ($<4 \times 10^9$/L; 11/59, 19%) and lymphocyte count ($<1.0 \times 10^9$/L; 26/59, 44%) were low in some patients. Compared to non-ICU patients, ICU patients are more likely to have lymphopenia (52% *vs*. 20%; $P = 0.003$), with higher neutrophil and D-dimer levels (median: 3.5 [IQR, 2.6–5.2] *vs*. median 1.7 [IQR, 0.8–3.1], $P = 0.003$; median 364.6 [IQR, 3.5–1475.0] *vs*. median 0.5 [IQR, 0.4–6.5], $P = 0.000$, respectively) and lower hemoglobin levels (median, 100.5 [IQR, 86.0–115.0] *vs*. median, 128.0 [IQR, 122.0–136.0], $P < 0.001$).

All patients (59/59; 100%) showed abnormal CT findings (Table 3). The main features of the imaging examination were ground-glass opacity (58/59; 98%; Figure 1A), consolidation (37/59; 63%), and ground-glass opacity combined with consolidation (36/59; 61%; Figure 1B). Compared to non-ICU patients, the incidence of consolidation and ground-glass opacity combined with consolidation in ICU patients was higher (73% *vs*. 33%, $P = 0.006$; 70% *vs*. 33%, $P = 0.011$, respectively). Furthermore, 40/59 (68%) patients showed involvement of all lung lobes in the ICU group (Figure 1C) as compared to the non-ICU patients, whereas the incidence of all lung lobes (75% *vs*. 47%, $P = 0.043$) and the number of lung lobes were higher in patients with ICU (median, 5 [IQR, 5–5] *vs*. median, 4 [IQR, 2–5], $P = 0.012$). Among 59 patients with COVID-19, 43 (73%) were multifocal, 15 (25%) were diffuse, and only 1 (2%) was focal. A significant difference was detected in the degree of lung involvement between ICU and non-ICU patients ($P = 0.032$). Furthermore, 23 (39%) patients had abnormal density shadows around the bronchi: 21/44 (48%) ICU patients and 2/15 (13%) non-ICU patients. The incidence of bronchovascular involvement in ICU patients was significantly higher than that in non-ICU patients (48% *vs*. 13%, $P = 0.040$), which might be observed by breathing difficulty and need for mechanical ventilation (Figure 1D). Unilateral or bilateral pleural effusion occurred in 7/59 (12%) patients: 6 in the ICU group (6/44, 14%) and 1 in the non-ICU group (1/15, 7%). In addition, mediastinal lymphadenopathy (short axis, >1 cm) was observed in 13 of 59 patients (22%), fibrous cord shadow in 22 (37%), and arterial plaque in 32 (54%).

A total of 15 (25%) patients were intubated with respiratory failure. All of them (100%) had ground-glass opacity, showed bilateral lung involvement, and involved more than three lung lobes. Compared to the non-mechanically ventilated patients, these patients requiring mechanical ventilation were more likely to have abnormal lung changes in the area around the bronchi (53% *vs*. 34%) and showed diffuse distribution (47% *vs*. 18%).

DISCUSSION

COVID-19 is a new viral outbreak that may have a profound impact on public health. With the increased number of confirmed cases, the number of severe and critical cases in Heilongjiang Province is also continuously increasing. This might be caused by lung tissue inflammation, which in turn, causes organ dysfunction and is even life-threatening. In addition, patients who are severely/critically ill have poor prognosis and higher mortality than non-critically ill

Table 1. Demographics and baseline characteristics of two groups of patients infected with 2019-nCoV.

	All patients (n=59)	ICU care (n=44)	No ICU care (n=15)	*P* value
Characteristics				
Age (y)	64.0(56.0-72.0)	66.5(57.3-75.8)	56.0(50.0-68.0)	0.037
Gender				0.552
Male	29(49%)	23(52%)	6(40%)	
Female	30(51%)	21(48%)	9(60%)	
Exposure history				0.516
Contact with infected patients	42(71%)	30(68%)	12(80%)	
Unknown history	17 (29%)	14(32%)	3(20%)	
Any comorbidity				
Diabetes	9(15%)	6(14%)	3(20%)	0.680
Hypertension	25(42%)	20(45%)	5(33%)	0.412
Cardiovascular disease	26(44%)	23(52%)	3(20%)	0.030
COPD	2(3%)	1(2%)	1(7%)	0.447
Malignancy	1(2%)	1(2%)	0(0%)	--
Chronic liver disease	1(2%)	0(0%)	1(7%)	--
Signs and symptoms				
Fever	41(69%)	31(70%)	10(67%)	0.785
Highest temperature, °C				0.412
<37.3	18(31%)	14(32%)	4(27%)	
37.3–38.0	25(42%)	16(36%)	9(60%)	
38.1–39.0	15(25%)	13(30%)	2(13%)	
>39.0	1(2%)	1(2%)	0(0%)	
Cough	30(51%)	20(45%)	10(67%)	0.205
Myalgia or fatigue	15(25%)	8(18%)	7(47%)	0.042
Headache	8 14%)	4(9%)	4(27%)	0.184
Diarrhoea, bellyache	5(8%)	4(9%)	1(7%)	0.624
Dyspnoea	14(24%)	9(20%)	5(33%)	0.316
Nausea	3(5%)	1(2%)	2(13%)	0.156

Data are median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. *P* values comparing Group1 and Group2 are from χ^2 test, Fisher's exact test, or Mann-Whitney U test. 2019-nCoV=2019 novel coronavirus. COPD=Chronic obstructive pulmonary disease.

Table 2. Laboratory findings of two groups of patients infected with 2019-nCoV.

Laboratory Findings	All patients (n=59)	ICU care (n=44)	No ICU care (n=15)	*P* value
White blood cell count($\times 10^9$/L)	5.5(4.3-7.1)	5.2(4.1-7.0)	5.8(4.6-7.0)	0.334
<4	11(19%)	9(20%)	2(13%)	0.894
4-10	42(71%)	30(68%)	12(80%)	
>10	6(10%)	5(11%)	1(7%)	
Neutrophil count($\times 10^9$/L)	3.2(1.9-4.8)	3.5(2.6-5.2)	1.7(0.8-3.1)	0.003
Lymphocyte count($\times 10^9$/L)	1.1(0.6-1.5)	0.9(0.6-1.3)	1.6(0.9-2.3)	0.004
<1.0	26(44%)	23(52%)	3(20%)	0.030
≥1.0	33(56%)	21(48%)	12(80%)	
Haemoglobin, g/L	104.0(92.0-122.0)	100.5(86.0-115.0)	128.0(122.0-136.0)	0.000
Platelet count($\times 10^9$/L)	189.0(145.0-260.0)	194.5(142.0-264.5)	189.0(152.0-255.0)	0.734
<100	11(19%)	9(20%)	2(13%)	0.712
≥100	48(81%)	35(80%)	13(87%)	
Prothrombin time, s	12.4(12.0-13.3)	12.6(12.0-13.4)	12.0(11.9-13.0)	0.458
Activated partial thromboplastin time, s	30.9(28.0-33.3)	31.0(27.0-33.9)	30.5(29.0-31.8)	0.651

D-dimer, mg/L	6.1(1.5-1090.0)	364.6(3.5-1475.0)	0.5(0.4-6.5)	0.000
C-reactive protein, mg/L	8.4(2.0-30.9)	9.9(0.3-180.7)	8.0(0.2-77.9)	0.807
Alanine aminotransferase, U/L	37.6(30.2-45.0)	37.8(25.9-46.7)	36.7(34.4-40.7)	0.862
Aspartate aminotransferase, U/L	26.5(21.2-33.3)	26.5(19.3-35.0)	26.1(23.8-33.3)	0.708
≤40	51(86%)	36(82%)	15(100%)	0.100
>40	8(14%)	8(18%)	0(0%)	
Creatinine, μmol/L	57.1(44.7-89.9)	55.7(42.0-83.0)	89.9(57.0-133.0)	0.008
≤133	53(90%)	41(93%)	12(80%)	0.165
>133	6(10%)	3(7%)	3(20%)	
Creatine kinase, U/L	116.0(34.6-175.3)	130.1(34.8-200.0)	113.9(31.5-167.7)	0.676
≤185	45(76%)	32(73%)	13(87%)	0.483
>185	14(24%)	12(27%)	2(13%)	

Data are median (IQR) or n/N (%), where N is the total number of patients with available data. p values comparing Group1 and Group2 are from χ^2, Fisher's exact test, or Mann-Whitney U test. 2019-nCoV=2019 novel coronavirus.

Table 3. CT diagnosis characteristics of two groups of patients infected with 2019-nCoV.

Imaging Findings	All patients (n=59)	ICU care (n=44)	No ICU care (n=15)	*P* value
Parenchymal opacities				
Consolidation	37(63%)	32(73%)	5(33%)	0.006
GGO	58(98%)	43(98%)	15(100%)	0.746
GGO and consolidation	36(61%)	31(70%)	5(33%)	0.011
Reticular opacities	13(22%)	7(16%)	6(40%)	0.073
Nodular opacities	11(19%)	8(18%)	3(20%)	0.574
Laterality				0.265
Bilateral	4(7%)	2(5%)	2(13%)	
Unilateral	55(93%)	42(95%)	13(87%)	
Involvement range of lung lobes				
All lung lobe	40(68%)	33(75%)	7(47%)	0.043
Right upper lobe	51(86%)	38(86%)	7(47%)	0.673
Right middle lobe	49(83%)	39(89%)	10(67%)	0.104
Right lower lobe	54(92%)	42(95%)	12(80%)	0.099
Left upper lobe	51(86%)	39(89%)	12(80%)	0.407
Left lower lobe	52(88%)	41(93%)	11(73%)	0.062
Number of lung lobes, mean	5(4-5)	5(5-5)	4(2-5)	0.012
Distribution				
Central and peripheral	9(15%)	8(18%)	1(7%)	0.424
Central	12(20%)	11(25%)	1(7%)	0.160
Peripheral	53(90%)	39(89%)	14(93%)	0.518
Peribronchovascular	23(39%)	21(48%)	2(13%)	0.040
Extent				0.032
Single shot	1(2%)	0(0%)	1(7%)	
Multiple	43(73%)	30(68%)	13(87%)	
Diffuse	15(25%)	14(32%)	1(7%)	
Pleural effusion	6(10%)	3(7%)	3(20%)	0.165
Arterial plaque	22(37%)	15(34%)	7(47%)	0.384
Fiber rope	32(54%)	27(61%)	5(33%)	0.060
Mediastinal lymphadenopathy	13(22%)	10(23%)	3(20%)	0.569

Data is n/N (%), where N is the total number of patients with available data. Abbreviations: CT, computed tomography; GGO, ground-glass opacity.

ones [6, 7]. A recent assessment showed that the fatality rate of severe pneumonia is 30–50%, leading to severe complications and increasing the medical burden [8]. Thus, early identification of such cases based on changes in chest radiography and clinical features is crucial. In the present study, clinical and imaging characteristics of patients with COVID-19 in the ICU group were determined by comparing the ICU and non-ICU patients.

The most common clinical symptoms in this group of patients were fever and cough. We found that the ICU group was older and more likely to have cardiovascular disease than the non-ICU group. Moreover, older people or people with poor health conditions were found to have a worsening pneumonia, which might be due to the weakened immune system [9]. According to a study report on patients with COVID 19 in Wuhan [10], the probability of all patients with hypertension and cardiovascular disease is 15% and 15%, whereas the corresponding incidence in patients with COVID 19 in Heilongjiang Province is 42% and 44%, which may be attributed to the specific geographical environment of Heilongjiang Province, resulting in a high incidence of cardiovascular diseases. Studies on SARS-CoV and Middle East Respiratory Syndrome (MERS)-CoV infections demonstrated that the risk of exacerbation markedly increases with age and presence of underlying diseases [11–13], which was consistent with the conclusions of this study. The difference in the male-to-female ratio was not significant between the two groups, indicating that gender is not a high-risk cause of disease severity, which is consistent with that of a recent report [14]. Compared to the ICU group, the incidence of muscle soreness was significantly higher in the non-ICU group. This clinical symptom is rarely observed in other related studies and may be related to regional environmental characteristics. Taken together, these clinical manifestations can help clinicians determine the disease severity in clinical practice. Other

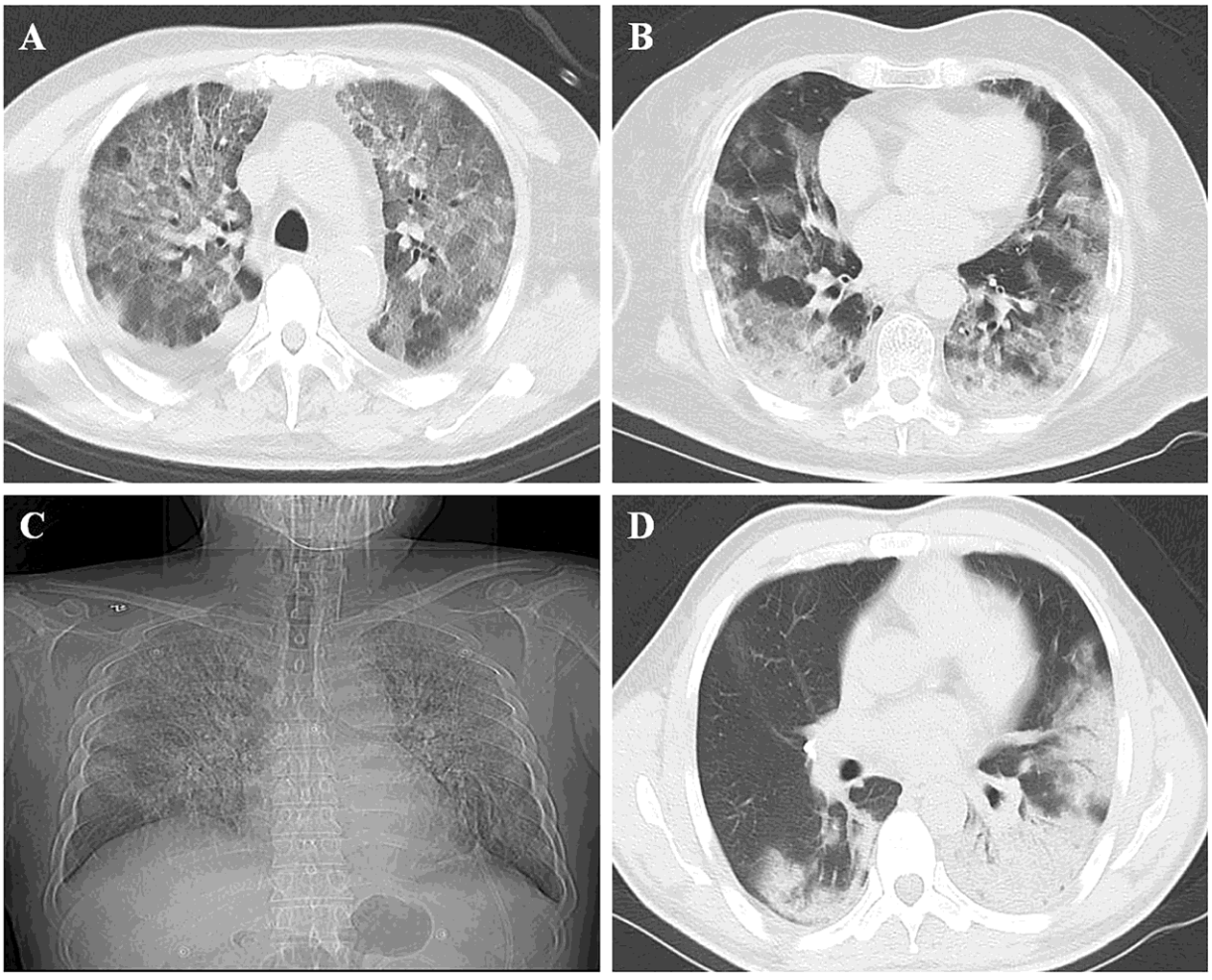

Figure 1. Chest imaging of patients with COVID-19. (**A**) Ground-glass opacity; (**B**) Lesion with ground-glass opacity and consolidation; (**C**) Lesion involving all lung lobes of both lungs; (**D**) Lesion involving the surrounding area of the bronchial blood vessel.

symptoms in our patients with COVID-19 were similar to that of other coronavirus infections, including dyspnea, headache, abdominal pain, diarrhea, and nausea. For example, SARS and MERS may belong to the same attributed infection and also indicate that the SARS-CoV-2 target cells are located in the lower respiratory tract [15–17].

The present study identified multiple laboratory index differences between non-ICU groups and ICU groups, including lymphocyte, neutrophil, and D-dimer levels. Compared to the non-ICU group, the ICU group is prone to lymphopenia, which is consistent with the results of the latest research report of patients with COVID-19 in Wuhan and China [10, 18]. Lymphopenia in the ICU group indicates that a large number of immune cells are consumed and the immune function is suppressed, demonstrating that lymphocyte damage may be the key to the deterioration of the patient's condition; therefore, decreased lymphocyte count could be a critical indicator of disease severity [19]. Increased neutrophil and D-dimer levels in patients in the ICU group may be related to cytokine storms caused by the viral invasion, which is supported by recent studies [9, 20]. Notably, patients with high D-dimer levels for the first time are predictive of poor prognosis [20], which is consistent with the opinion of this study.

From a broad perspective, CT manifestations of COVID-19 pneumonia are similar to that of other viral pneumonia. Imaging findings of viral pneumonia include reticular pattern and patchy or diffuse ground-glass opacity, with or without consolidation [21]. In influenza pneumonia, lobular septal thickening and grid-like density shadows are frequently observed, whereas pleural effusion is rare [21]. Despite similarities, some of our patients' imaging findings are different from those of the traditional seasonal flu.

In this study, all patients with COVID-19 had abnormal chest CT findings. Additionally, ground-glass opacity (98%) and consolidation (63%) are the most common imaging findings in the current study, which is consistent with the results of the recent COVID-19 studies [22]. This phenomenon may be related to exudative inflammation caused by alveolar and interstitial edema of the lung due to viral invasion, and CT is mainly manifested as ground-glass opacity [23]. An autopsy report of patients with COVID-19 pneumonia deaths shows that the ground-glass opacity corresponds to the gray-white alveolar lesions observed by the naked eye, suggesting that the virus mainly causes inflammatory reactions characterized by deep airway and alveolar damage [24]. Herein, we found that compared to the non-ICU group, the incidence of consolidation and ground-glass opacity combined with consolidation in patients in the ICU group was higher (P = 0.006; P = 0.011), indicating that the alveoli of critically ill patients were filled with inflammatory exudates. This means that the virus has spread to the respiratory tract, leading to necrotic bronchitis and diffuse alveolar damage [25, 26], which is consistent with the results of recently published studies [27–29]. Among the 59 (68%) patients, 40 displayed imaging abnormalities involving all lung lobes (5) as compared to 7/15 (47%) of non-ICU patients, whereas 33/44 (75%) of all ICU patients were involved; the difference between the two groups was statistically significant (P = 0.043). In addition, we found that the degree of involvement of lung lesions was statistically significant between the two groups (P = 0.032). Chest imaging features may help the early prediction of the patients' clinical development early.

In this group of patients, 15 needed mechanical ventilation. Compared to non-mechanical ventilation patients, CT abnormalities in the lungs of patients requiring mechanical ventilation were primarily distributed around the bronchial blood vessels, and diffuse distribution was likely to occur, making patients prone to dyspnea. Some other studies demonstrated that the distribution of abnormal lesions during CT examination may be the decisive factor for the clinical course of patients with COVID-19 [22, 30]. Other imaging features in this study included bilateral lung involvement in 93% of patients, and majority of them (90%) had lung lesions in the peripheral area without emphysema or pulmonary nodules; these imaging abnormalities and distribution patterns are consistent the previously published results [31, 32]. Among the patients in this study, only 7 (12%) had pleural effusion, including 6 (14%) in the ICU group and 1 (7%) in the non-ICU group. Furthermore, pleural effusion is a rare imaging manifestation in patients with COVID-19, and the incidence rate in the ICU group is higher than that in the non-ICU group, which is consistent with the results of Junhua et al.'s study [33].

Nevertheless, this study has some limitations. (1) None of the patients underwent lung biopsy or autopsy, which might have established a correlation between imaging and histopathology. (2) The sample size of the non-ICU group is relatively small. Collecting standardized data for larger populations will help explore clinical manifestations and high-risk factors. (3) As most patients are still in the hospital at the time of submission of this manuscript, risk factors for poor prognosis were not assessed.

CONCLUSIONS

In summary, existing cardiovascular disease, fever, and cough in elderly patients with COVID-19 may worsen

the condition. Lymphopenia and elevated neutrophil and D-dimer levels are also indicators of COVID-19 disease progression. In addition, imaging findings of patients with severe COVID-19 mainly include consolidation and ground-glass opacity combined with consolidation, which putatively involves all lung lobes and the area around the bronchi. Since several patients are currently in the critical stage, we hope that the results of this study would be beneficial for the disease control, diagnosis, treatment, and prognosis in Heilongjiang Province and worldwide and even reduce the mortality rate.

MATERIALS AND METHODS

Study population

The study has been approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University and is in accordance with the Helsinki Declaration. According to the COVID-19 pneumonia diagnostic criteria for the diagnosis and treatment of new coronavirus-caused pneumonia (trial version 6) issued by the National Health Commission of the People's Republic of China [4], the inclusion criteria were as follows: (1) real-time fluorescent reverse transcription-polymerase chain reaction (RT-PCR) for detection of positive cDNA of SARS-CoV-2; (2) untreated newly diagnosed patients; (3) patients with complete clinical data; and (4) all patients who underwent at least one CT scan. Exclusion criteria were as follows: (1) treated non-newly diagnosed patients and (2) missing clinical data. This study included a total of 76 patients confirmed with COVID-19 between February and March 2020, and 59 of them met the above criteria. The cohort was divided into the ICU (n = 44) and non-ICU groups (n = 15). Clinical data of all patients were evaluated: background information such as gender and age and clinical symptoms such as fever, cough, and underlying diseases (hypertension, diabetes, cardiovascular disease, and chronic obstructive pulmonary disease). Laboratory examination results upon admission, including white blood cells, lymphocytes, neutrophils, D-dimer, and C-reactive protein levels, as well as imaging data, were collected.

Image analysis

All CT images were analyzed and diagnosed by two radiologists trained for novel coronavirus. Both radiologists have >5 years of diagnostic experience. Two doctors independently diagnosed all patient images and reached a consensus. In case of disagreement between the two radiologists, a third trained radiologist with >10 years of diagnostic experience was consulted to reach a consensus. Imaging features (ground-glass opacity, consolidation, reticular pattern, and nodular opacity), lesion distribution (unilateral/bilateral, upper/middle/lower lobe, and central/peripheral/bronchial blood vessel surrounding), and degree of involvement (focal/multifocal/diffuse and number of lung lobes) were all abnormal. Radiographic images and CT scans using descriptors were defined using the Fleischner Society Naming Committee [5]. Ground-glass opacity is defined as a hazy area showing increased lung opacity with indistinct pulmonary vessel margins on a radiograph but with preserved bronchial and vascular margins on CT. Consolidation is defined as a homogeneous increase in parenchymal attenuation that obscures vessel margins and airway walls. The reticular pattern is defined as small linear opacities forming a net pattern. Nodular opacity is defined as a well- or poorly defined rounded opacity, measuring up to 3 cm in diameter. Lesion distribution features include unilateral/bilateral and upper/middle/lower lobes. The extent of lesion involvement was divided into focality, multifocality, and diffuse. Focality is defined as an abnormal single lesion, whereas multifocality is defined as the presence of more than one lesions; if it is diffusely distributed, it involves one or both lungs. Moreover, whether the lesion occurs centrally (<4 cm from the hilum) or peripherally or involves the bronchi should be determined. The presence of pleural effusion, laterality, and any other lung findings such as mediastinal lymphadenopathy was also noted.

Statistical analysis

The SPSS 19.0 statistical software was used for analysis. Continuous variables were expressed as median (interquartile ratio [IQR]) and compared using the Mann–Whitney U test. Categorical variables were expressed as number of cases (n) and percentage/rate (%); χ^2 test or Fisher's exact test was used to compare ICU and non-ICU groups. $P < 0.05$ was considered statistically significant.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Research Paper

Risk of death by age and gender from CoVID-19 in Peru, March-May, 2020

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ABSTRACT

Peru implemented strict social distancing measures during the early phase of the epidemic and is now experiencing one of the largest CoVID-19 epidemics in Latin America. Estimates of disease severity are an essential indicator to inform policy decisions about the intensity and duration of interventions needed to mitigate the outbreak. Here we derive delay-adjusted case fatality risks (aCFR) of CoVID-19 in a middle-income country in South America.

We utilize government-reported time series of CoVID-19 cases and deaths in Peru stratified by age group and gender.

As of May 25, 2020, we estimate the aCFR for men and women at 10.8% (95%CrI: 10.5-11.1%) and 6.5% (95%CrI: 6.2-6.8%), respectively, whereas the overall aCFR was estimated at 9.1% (95%CrI: 8.9-9.3%). Our results show that senior individuals have been the most severely affected by CoVID-19, particularly men, with an aCFR of nearly 60% for those aged 80- years. We also found that men have a significantly higher cumulative morbidity ratio across most age groups (proportion test, p-value< 0.001), with the exception of those aged 0-9 years.

The ongoing COVID-19 pandemic is generating a substantial mortality burden in Peru. Senior individuals, especially those older than 70 years, are being disproportionately affected by the COVID-19 pandemic.

INTRODUCTION

As of May 25, 2020, more than 5.5 million CoVID-19 cases and about340,000 deaths have been reported from almost every country and territory around the globe [1, 2]. The ongoing CoVID-19 pandemic has imposed a substantial burden on health systems, economies, and societies globally, and there are strong indicators pointing to a disproportionate impact on low- and middle-income countries [3–5]. Since its initial outbreak in China, the world has tracked the CoVID-19 pandemic proliferating across Europe and Asia, and later seeding hotspots in

North America, the Middle East, and more recently in Latin America [6]. Brazil reported its first case on February 26, 2020 [7]. Neighboring countries started to report CoVID-19 cases in subsequent days; South America has registered more than 600,000 cases and 30,600 deaths as of May 24, 2020 [1]. Although many South American countries imposed strict control measures, including travel bans, school closures, and lockdowns early in the epidemic, the magnitude of their epidemics now rival those observed in European hotspots, with CoVID-19 cases and death counts increasing rapidly in the region [1, 5]. Other factors, including high poverty rates, informal economies, frail healthcare systems, insufficient medical supplies as well as inadequate water, sanitation, and hygiene infrastructure further exacerbate the health and socioeconomic impacts of the CoVID-19 pandemic [5, 8–10]. Governments in South America are now facing the social and economic consequences from SARS-COV-2 containment measures, while struggling to contain the rapidly expanding outbreaks of the deadly virus [9].

Peru, a country of about 30 million people, is experiencing one of the largest CoVID-19 epidemics in Latin America. With a rapidly rising case tally, Peru has reported almost 129,148 cases and 7660 deaths as of May 25, 2020 [11]. The majority (63%) of CoVID-19 cases have been confirmed in Lima, the capital of Peru [11]. The government of Peru initiated social distancing measures soon after the confirmation of the first imported case in Peru on March 6, 2020 [12]. The initial epidemic control measures included school closures on March 11, 2020 followed by the suspension of large gatherings and flights from Europe and Asia the next day. Subsequently the government declared a national emergency and closed its borders on March 16, 2020 [13]. Despite these forthcoming and swift control measures, untraced community transmission was reported by March 17, 2020, forcing the implementation of a night time curfew as of March 18, 2020 [13].

Estimates of the reproduction number from the early stage of the epidemic in Peru (March 2020) showed sustained transmission in Lima with a reproduction number R estimated at 2.3 (95% CI: 2.0, 2.5) [14]. Moreover, the 20-days ahead forecast for Lima suggested that the prompt social distancing measures had significantly slowed down the initial spread of the virus in the region [14]. Despite the implementation of non-pharmaceutical interventions in Peru, case and death counts have continued to rise rapidly. The crude case fatality risk (CFR), defined as the number of cumulative deaths and cases as of May 25, 2020, in Peru is estimated at 5.9%, which is in good agreement with the global crude CFR average of 6.3% [15]. Statistical analyses and mathematical models using data from Peru suggest that under current epidemic growth trends, the number of CoVID-19 infected individuals could surpass the country's healthcare system capacity [16].

The clinical spectrum of CoVID-19 ranges from asymptomatic cases to clinical conditions characterized by respiratory failure, to multiorgan and systemic manifestations which can cause death [17–19]. The SARS-CoV-2 virus is more likely to generate severe disease among individuals ≥60 years of age, especially those with preexisting medical conditions that include heart disease, lung disease, diabetes or cancer [20]. Further, CoVID-19 associated deaths occur more frequently (about 80% of total deaths) in persons aged ≥65 years based on data from the USA, and consistent with data from China indicating that >80% CoVID-19 deaths occur among persons aged ≥60 years [21]. Moreover, a higher crude fatality risk has been reported among men (2.8% for men versus 1.7% for women) in China [22]. Age adjusted CFR estimates from Peru can be useful to gauge the mortality impact of the pandemic and assess whether the severity patterns are consistent in the South America, a region with fragmented health systems, vast inequality, and high poverty rates.

CFR is a key epidemiological metric that quantifies the severity of an epidemic [23], aiding public health officials assess the type and intensity of interventions that need to be implemented to mitigate its impact [24]. However, it becomes challenging to estimate CFR during an epidemic as CFR estimates are sensitive to right censoring of the data that occurs because of the time lag between the symptoms onset and death [25–27]. Moreover, under-reporting of cases because mild or asymptomatic cases can go undetected by disease surveillance systems also overestimates CFR [25, 28], while CFR estimates by subgroup are less prone to sampling bias and help identify the most vulnerable subpopulations. For comparison, the infection fatality risk (IFR) is calculated by the ratio of cumulative deaths over the cumulative number of infected individuals.

Given the importance of timely CFR estimates for public health decision making, we provide real-time estimates of adjusted age-specific CFR during the CoVID-19 epidemic in Peru, through May 25, 2020 to assess the pandemic's severity variation in this southern hemisphere setting, which helps pinpoint the most vulnerable segments of the population and tailor public health interventions.

RESULTS

As of May 25, a total of 129,148 cases and 7,660 deaths due to CoVID-19 have been reported by the Ministry of

Table 1. Distribution of the cases by sex and age groups, as of May 25, 2020.

Age group	Men				Women			
	Cases (%)	Deaths (%)	cCFR (%)	Mortality per 100,000 population	Cases (%)	Deaths (%)	cCFR(%)	Mortality per 100,000 population
All	78264 (100)	5508 (100)	7.0%	34.0	50884 (100)	2152 (100)	4.2	13.1
0-9	1416 (1.8)	10 (0.2)	0.7	0.4	1362 (2.7)	11 (0.5)	0.8	0.4
10-19	2475 (3.2)	8 (0.1)	0.3	0.3	2128 (4.2)	6 (0.3)	0.3	0.2
20-29	14306 (18.3)	32 (0.6)	0.2	1.2	8707 (17.1)	19 (0.9)	0.2	0.7
30-39	18052 (23.1)	169 (3.1)	0.9	6.6	11487 (22.6)	49 (2.3)	0.4	2.0
40-49	16258 (20.8)	499 (9.1)	3.1	23.7	10005 (19.7)	140 (6.5)	1.4	6.7
50-59	13274 (17.0)	1107 (20.1)	8.3	67.7	8124 (16.0)	323 (15.0)	4.0	19.7
60-69	7034 (9.0)	1615 (29.3)	23.0	150.4	5023 (9.9)	649 (30.2)	12.9	56.6
70-79	3620 (4.6)	1279 (23.2)	35.3	207.6	2488 (4.9)	558 (25.9)	22.4	85.1
80 -	1769 (2.3)	789 (14.3)	44.6	279.2	1536 (3.0)	397 (18.4)	25.8	108.8

Health, Peru. Among men, reported cases were mostly observed among individuals aged 30-39 years (23.1%), followed by those aged 40-49 years (20.8%), and those aged 20-29 years (18.3%). In contrast, most deaths were reported among those aged 50 years and above, especially among men aged 60-69 (29.3%) followed by those aged 70-79 (23.2%), aged 50-59 years (20.1%), and aged 80 years and above (14.3%). (Table 1, Figure 1A, 1B). Data show a similar pattern for women. The majority of reported cases occur in females aged 20-69 years, and the majority of reported deaths occur among women aged 50 years or more. More specifically, most reported cases occur among women aged 30-39 (22.6%), followed by women aged 40-49 (19.7%), and 50-59 year olds (16.0%). In contrast, most deaths are reported among those aged 60-69 (30.2%), followed by women aged 70-79 (25.9%), and lastly, women aged 80 years and above (18.4%). Regarding CoVID-19 mortality per 100,000 population, seniors (individuals >70 years of age) were the most affected age group; mortality burden per 100,000 is 279.2 among men aged 80 years and above, and 207.6 among men aged 70-79 years. For women of 80 years of age or more mortality is 108.8 and 85.1 for women aged 70-79 years (Table 1, Figure 1F).

The gender proportions of reported cases by age groups are presented in Figure 1C and Figure 1D. The proportion of cases among men is higher than 50% across all age groups (χ^2 test, p-value<0.001). Similarly, the proportion of male deaths is also higher than 50% except for those aged 10-19 years (χ^2 test, p-value<0.001). Cumulative morbidity ratio by gender and age group is presented in Figure 1E, indicating that cumulative morbidity ratio among men is higher than women across all age groups (proportion test, p-value < 0.001) except for individuals aged 0-9 years (proportion test, p-value =0.85). Figure 1F illustrates the mortality per 100,000 population directly caused by CoVID-19 by gender and age group. Mortality is higher than among females aged 20 years and above (proportion test, p-value <0.05), and it is not significantly different among those aged 0-19 years.

Figure 2 shows the cumulative cases and deaths of CoVID-19 by age group for males and females (A through J) over time. The figure suggests cumulative deaths increases after an increase in cumulative cases. The growth curve for overall cumulative cases (all age groups) for men and women appears to increase exponentially until around day 60 (April 29th, 2020), while exponential growth in cumulative deaths overall (all age groups) for men and women appears to occur until around day 70 (May 9th, 2020).

Figure 3 illustrates observed and model based posterior estimates of the crude CFR by age group (A-J) and time-delay adjusted CFR by age group (K-T) for men

and women. Black dots show crude case fatality risks, and light and dark indicate 95% and 50% credible intervals (CrI) for posterior estimates, respectively.

Overall, our model based crude CFR fitted the observed data well, except for individuals aged 80 years and above, probably influenced by low reporting rate/ ascertainment bias of cases at an early stage. Crude CFR for most of age groups increased at the early stage of the epidemic, peaked amidst the outbreak day 34 (April 3rd, 2020) and followed a decreasing trend turning into an almost flat curve.

Overall, our model-based posterior estimates for the time-delay adjusted CFR are substantially higher than the crude observed CFR. These estimates fluctuated at the early stage of the epidemic and then followed a decreasing trend.

The most recent estimates, as of May 25, 2020, of the time-delay adjusted CFR for men and women are 10.8% (95%CrI: 10.5-11.1%) and 6.5% (95%CrI: 6.2-6.8%), respectively, while overall national estimate is 9.1% (95%CrI: 8.9-9.3%) (Figure 4 and Table 2). Among men, senior citizens appear to be severely affected; the

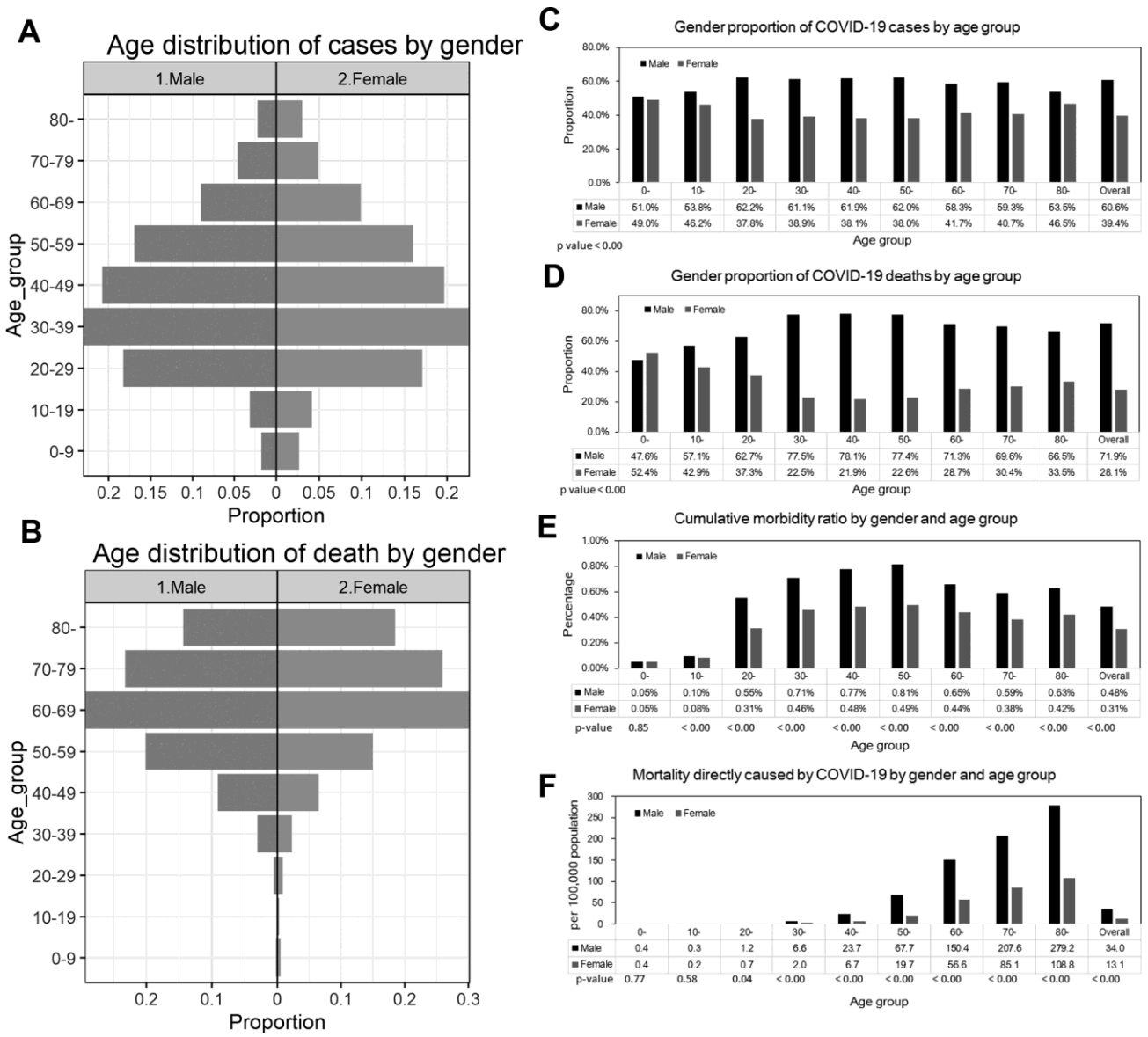

Figure 1. Epidemiological characterization of CoVID-19 in Peru, as of May 25, 2020. (**A**) Age distribution of reported cases by gender, (**B**) Age distribution of reported deaths by gender. (**C**) Gender proportion of CoVID-19 cases by age group, (**D**) Gender proportion of CoVID-19 deaths by age group, (**E**) Cumulative morbidity risk by gender and age group, (**F**) Mortality directly caused by CoVID-19 by gender and age group.

adjusted CFR is 33.1% (95%CrI: 31.7-34.6%) for men aged 60-69 years, 49.4% (95%CrI: 47.3-51.6%) for those aged 70-79 years, and 64.3% (95%CrI: 60.9-67.8%) for those 80 years old and above. We observe a similar pattern for women. The adjusted CFR is 19.2% (95%CrI: 17.9-20.6%) for women aged 60-69 years, 32.2% (95%CrI: 29.9-34.7%) for those aged 70-79 years, and 35.1% (95%CrI: 32.1-38.1%) for women aged 80 years old or more.

DISCUSSION

This study estimates the time-delay adjusted CFR by age group for the ongoing CoVID-19 epidemic in Peru. The crude CFR varies across countries due to differences in testing and timing of tests [29]. The results from our analysis show that the CoVID-19 epidemic in Peru disproportionately impacts senior individuals, especially those who are 70 years of age or older, consistent with CFR estimates obtained from recent studies conducted in China [30, 31], Chile [32], and Italy [33, 34]. This pattern suggests that an aging population could aggravate the fatality impact of CoVID-19, influenza and respiratory syncytial virus [32], as was probably an important factor for its high impact in Italy [33, 34]. While the population in Lain America, including Peru, is aging at a rapid rate, still a relatively small percentage of the population in the region are older than 65 years of age [35]. Hence, the age structure in the region could favor a lower overall CFR than would be expected otherwise with a relatively older population, as in other regions.

Our estimate of adjusted CFR among men (10.8% (95%CrI: 10.5-11.1%)) is 1.7-fold higher than the estimated adjusted CFR for women (6.5% (95%CrI: 6.2-6.8%)), consistent with the estimates given in ref [37]. Men aged 80 years or older have an estimated adjusted CFR as high as 64.3% (95%CrI: 60.9-67.8%), 58-fold higher than our estimates for men aged 0-9, and 1.3-fold higher than our estimates for men aged 70-79. Similarly, the adjusted CFR estimates for women of aged 80 years or older are as high as 35.1% (95%CrI: 32.1-38.1%), 29-fold

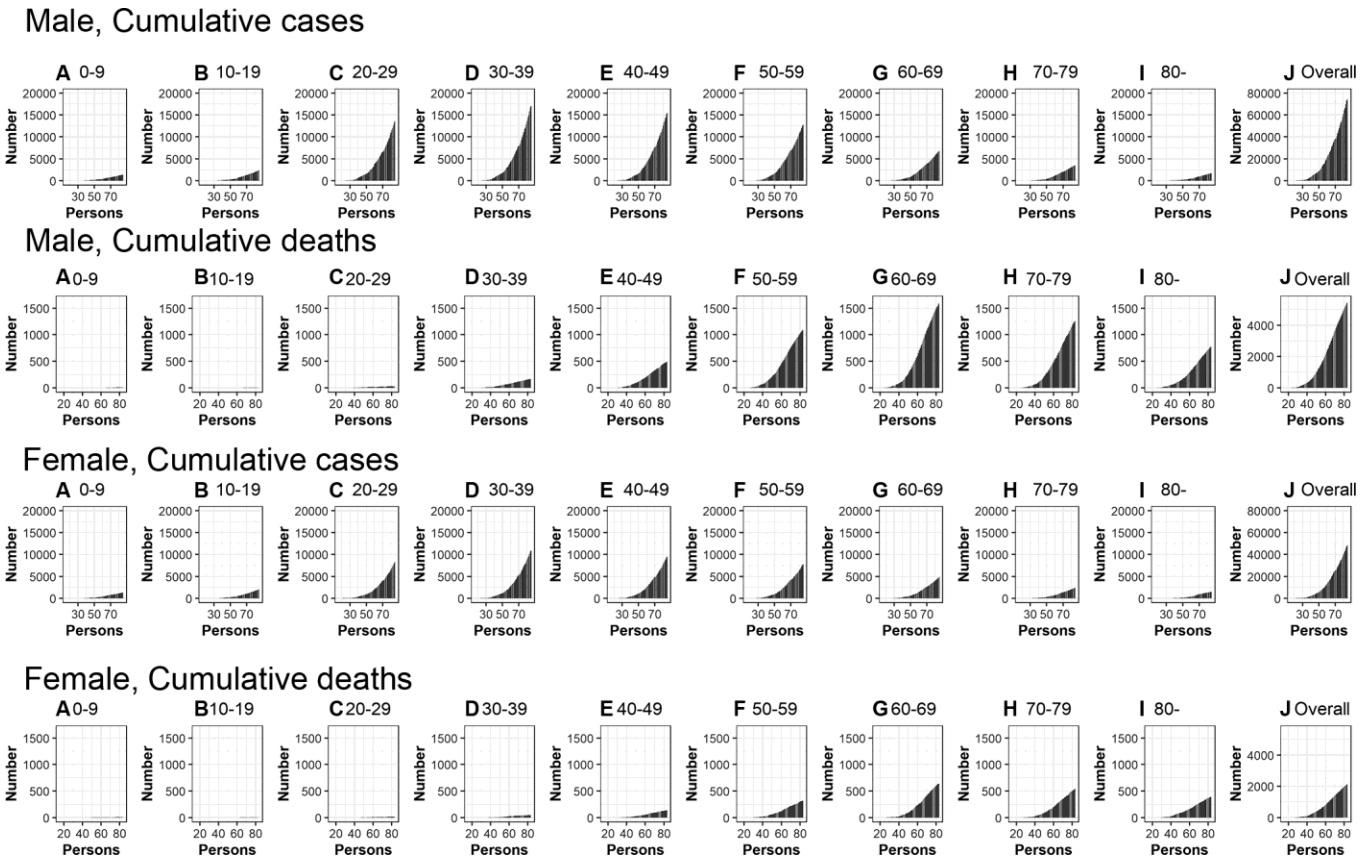

Figure 2. Temporal distribution of cases and deaths by age group due to CoVID-19, March-May 2020, Peru. Top: Male, cumulative cases, Second top: Male, cumulative cases, Second bottom: Female, cumulative cases, Bottom: Female cumulative deaths (**A**) aged 0-9, (**B**) aged 10-19, (**C**) aged 20-29, (**D**) aged 30-39, (**E**) aged 40-49, (**F**) aged 50-59, (**G**) aged 60-69, (**H**) aged 70-79, (**I**) aged 80- and (**J**) Overall (all age groups). Day 1 corresponds to March 1st in 2020.

higher than the estimates obtained for female aged 0-9 and 1.1-fold higher than the estimates obtained for female aged 70-79, consistent with recent findings in Chile [32]. In comparison, a study conducted in China, reported much lower estimates of CFR for individuals >80 years of age (13.4%) [31].

An upward trend in the crude CFR for overall population suggests the disease transmission may be spreading to more vulnerable populations. The majority of social distancing measures in Peru were implemented between March 11-March 18, 2020. However, since 72.4% of the economically active population works in informal jobs, which are concentrated in the poorest areas of the country, compliance with government mitigation strategies can be challenging despite the government's efforts to support the population [37]. Another factor possibly contributing to the upward trend in crude CFR may be an increase in unreported cases due to saturated testing capacity [29]. However, since Peru's testing capacity has substantially increased since the beginning of the outbreak, going from >0.01 test per 1000 population to 0.09 per 1000 in May 22 [15], and the positivity rate estimated at 8.6% for March, 2020, this seems an unlikely cause. In Peru, about 85% of ICU beds with ventilators are currently occupied by patients [37], therefore our present estimates are not affected by excess deaths due to health

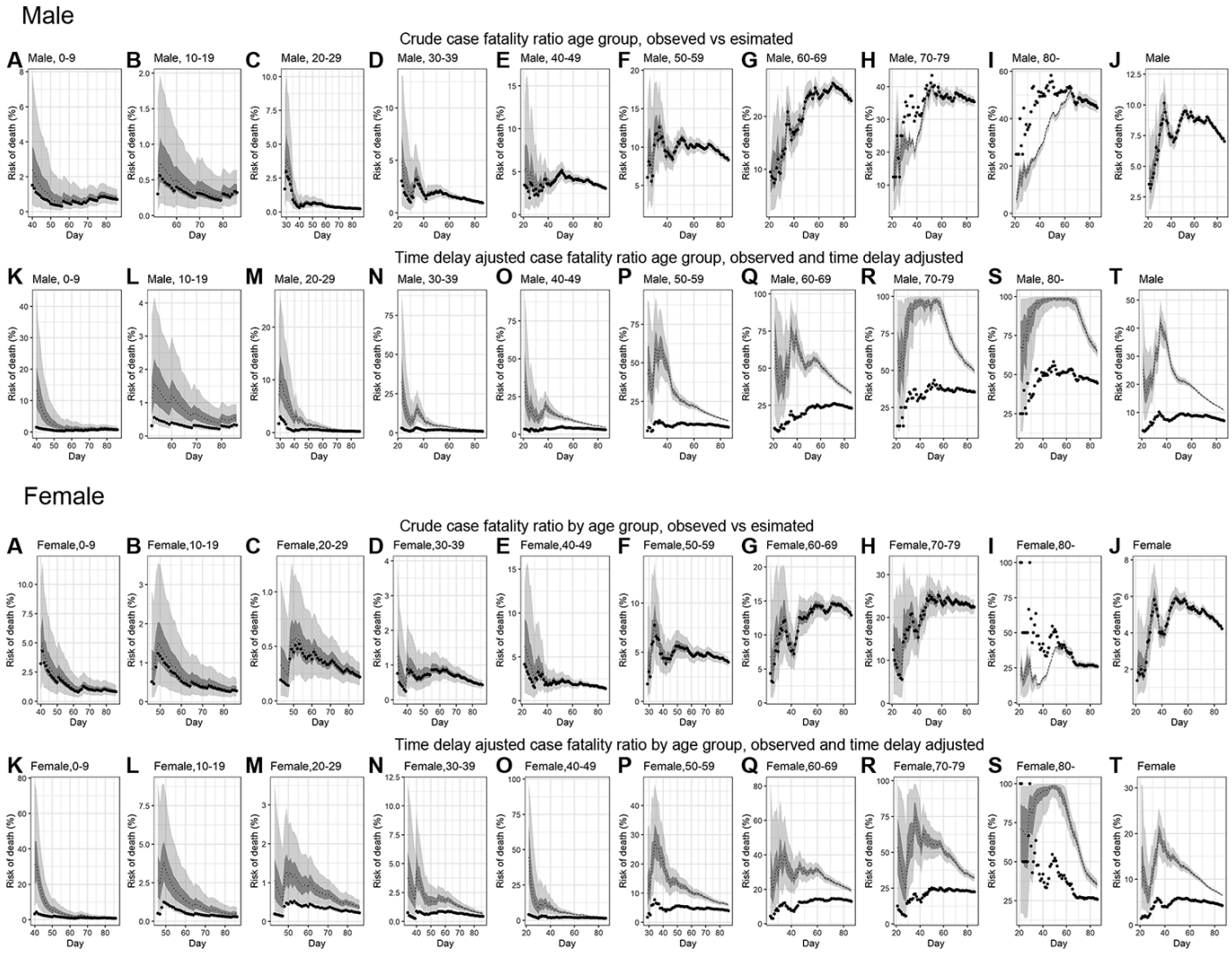

Figure 3. Temporal variation of male and female risk of death by age group caused by CoVID-19, March-May 2020, Peru. Upper two rows; Male risk of deaths, Lower two rows; Female risk of deaths. Observed and posterior estimated of crude case fatality risk of (**A**) aged 0-9, (**B**) aged 10-19, (**C**) aged 20-29, (**D**) aged 30-39, (**E**) aged 40-49, (**F**) aged 50-59, (**G**) aged 60-69, (**H**) aged 70-79, (**I**) aged 80-, (**J**) all age groups and time-delay adjusted case fatality risk of (**K**) aged 0-9, (**L**) aged 10-19, (**M**) aged 20-29, (**N**) aged 30-39, (**O**) aged 40-49, (**P**) aged 50-59, (**Q**) aged 60-69, (**R**) aged 70-79, (**S**) aged 80-, (**T**) all age groups. Day 1 corresponds to March 1st in 2020. Black dots show crude case fatality risk, and light and dark indicates 95% and 50% credible intervals for posterior estimates, respectively.

care demand exceeding health care capacity. However, as the epidemic continues to expand, healthcare capacity may be reached in the short term [37]. Furthermore, the results show an increasing trend in crude CFR around day 45 (May 14th, 2020), probably reflecting the exponential increase of cumulative cases around day 40 (May 9th, 2020).

The downward trend in the adjusted CFR at the early stage may indicate the existence of a reporting delay and the shift of the outbreak to a less vulnerable segment of the population. In particular, the observed differences in estimates between the crude CFR and adjusted CFR can be attributed to the time-delay that is assumed fixed during the course of the epidemic.

The relatively small proportion of males (53.5%) among CoVID-19 cases in the individuals aged 80 years and above can be attributed to the relatively small male population size for that age group; with men comprising only 1.7% of the population >80 years of age in Peru, consistent with estimates for Chile [32]. As higher mortality among male has been reported in China and the U.S. [38], additional data on deaths stratified by gender provides the opportunity to examine the CFR by gender and age.

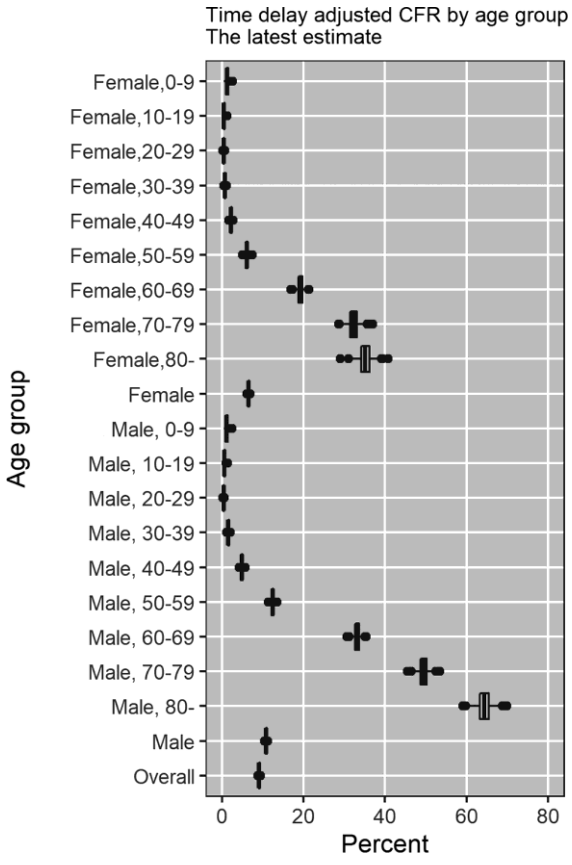

Figure 4. Most recent estimates of time-delay adjusted risk of death caused by CoVID-19 by age group and gender, March-May 2020, Peru. Distribution of time-delay adjusted risk of death from the latest estimates (May 25, 2020) is presented. Top to bottom: female aged 0-9, female aged 10-19, female aged 20-29, female aged 30-39, female aged 40-49, female aged 50-59, female aged 60-69, female aged 70-79, female aged 80 and over, female overall.

Several studies documenting the IFR of CoVID-19 have been reported based on an observational study [39], modeling studies [31, 40] and serological studies [41, 42]. While IFR estimates may be more realistic indicators compared to estimates derived from observed cases alone [43, 44], the external validity of these serological studies, e.g., whether the results can be applied to the generalized population in the region where they are performed, needs to be closely examined, as pointed out elsewhere [40, 45, 46]. In particular, to derive IFR estimates, prevalence, the cumulative number of infected people, is estimated based on the result of serological studies. Then, the cumulative number of deaths in the region is divided by the estimated cumulative number of infected individuals.

Indeed, serological studies based on blood donors and outpatients/hospitalized patients will easily lead to overestimation and underestimation, respectively, because the number of infected individuals is expected to be lower among the blood donors and higher among the outpatients/hospitalized patients. In contrast, the death risk derived from the CFR is less affected by the sampling bias and a convenient indicator to identify the vulnerable subpopulations, especially focusing on a single country with relatively uniform testing capacity across the population.

Our study has at least two limitations. First, our estimates are probably overestimated, due to the effect of under reporting rates and ascertainment rates, as has been underscored in other studies [25, 27, 47]. But a recently enhanced testing capacity in Peru is expected to mitigate these effects, and an ongoing mass serological study will provide data to generate more accurate estimates of the death risk. Second, adjusted CFR, especially among seniors, has displayed fluctuations, highlighting the importance of focusing on sub-group analyses. Additional information such as line lists that include related risks including information on underlying diseases may help to identify subgroups with elevated risks.

CONCLUSIONS

The CoVID-19 pandemic is imposing a large death toll in Peru. Senior individuals, especially those who are

Table 2. Summary results of time-delay adjusted case fatality risk of CoVID-19 in each age group in Peru, 2020 as of May 25, 2020.

Age group	Gender	Latest estimate	Range of median estimates	Crude case fatality rate
Overall		9.1% (95%CrI[a]: 8.9-9.3%)	9.1-32.0%	5.9% (95%CI[b]: 5.8-6.1%) 7660/129148 [c]
Male		10.8% (95%CrI: 10.5-11.1%)	10.8-42.3%	7.0% (95%CI: 6.9-7.2%) 5508/78264
Female		6.5% (95%CrI: 6.2-6.8%)	6.4-20.0%	4.2% (95%CI: 4.1-4.4%) 2152/50884
0-9	Male	1.1% (95%CrI: 0.5-1.8%)	1.0-13.3%	0.7% (95%CI:0.3-1.3%) 10/1416
	Female	1.2% (95%CrI: 0.7-2.0%)	1.2-31.4%	0.8% (95%CI: 0.4-1.4%) 11/1362
10-19	Male	0.5% (95%CrI: 0.3-1.0%)	0.4-1.6%	0.3% (95%CI: 0.1-0.6%) 8/2475
	Female	0.5% (95%CrI: 0.2-0.9%)	0.4-3.8%	0.3% (95%CI: 0.1-0.6%) 6/2128
20-29	Male	0.4% (95%CrI: 0.2-0.5%)	0.4-10.0%	0.2% (95%CI: 0.2-0.3%) 32/14306
	Female	0.4% (95%CrI: 0.2-0.6%)	0.4-1.3%	0.2% (95%CI: 0.1-0.3%) 19/8707
30-39	Male	1.5% (95%CrI: 1.3-1.7%)	1.5-32.3%	0.9% (95%CI: 0.8-1.1%) 169/18052
	Female	0.7% (95%CrI: 0.5-0.9%)	0.9-4.4%	0.4% (95%CI: 0.3-0.6%) 49/11487
40-49	Male	4.8% (95%CrI: 4.4-5.2%)	4.8-34.7%	3.1% (95%CI: 2.8-3.3%) 499/16258
	Female	2.2% (95%CrI: 1.8-2.5%)	2.2-44.6%	1.4% (95%CI: 1.2-1.6%) 140/10005
50-59	Male	12.4% (95%CrI: 11.7-13.1%)	12.4-60.0%	8.3% (95%CI: 7.9-8.8%) 1107/13274
	Female	6.0% (95%CrI: 5.4-6.7%)	7.5-27.7%	4.0% (95%CI: 3.6-4.4%) 323/8124
60-69	Male	33.1% (95%CrI: 31.7-34.6%)	33.1-77.8%	23.0% (95%CI: 22.0-24.0%) 1615/7034
	Female	19.2% (95%CrI: 17.9-20.6%)	19.2-40.9%	12.9% (95%CI: 12.0-13.9%) 649/5023
70-79	Male	49.4% (95%CrI: 47.3-51.6%)	48.7-97.8%	35.3% (95%CI: 33.8-36.9%) 1279/3620
	Female	32.2% (95%CrI: 29.9-34.7%)	24.1-74.9%	22.4% (95%CI: 20.8-24.1%) 558/2488
80-	Male	64.3% (95%CrI: 60.9-67.8%)	64.3-98.9%	44.6% (95%CI: 42.3-47.0%) 789/1769
	Female	35.1% (95%CrI: 32.1-38.1%)	35.1-98.3%	25.8% (95%CI: 23.7-28.1%) 397/1536

[a] 95%CrI: 95% credibility intervals (CrI), [b] 95%CI: 95% confidence interval, [c] Cumulative cases over cumulative deaths

older than 70 years of age, are being disproportionately affected by the CoVID-19 pandemic, particularly elderly men. CFR was as high as 64.3% (95%CrI: 60.9-67.8%) for men aged 80 older, 58-fold higher than our estimates for men aged 0-9. The overall adjusted CFR in Peru is estimated to be higher than in other countries, which is worrying, particularly because healthcare demand has not yet exceeded capacity, but probably will do in the coming weeks. The relatively younger age structure in Latin America may help ameliorate the overall CFR than would otherwise be expected with an older age structure in the population.

MATERIALS AND METHODS

Data

We obtained daily cumulative numbers of reported laboratory confirmed CoVID-19 cases and deaths stratified by age group and gender through May 25, 2020. Different age groups had different starting times, which correspond to the day when death was reported. Confirmed CoVID-19 cases were retrieved from three surveillance systems: a) national surveillance system (confirmed and suspected cases based on a case definition), b) Netlab system (molecular test) and c) SICOVID system (rapid serological test). CoVID-19 deaths were obtained from two surveillance systems: a) national surveillance system (confirmed and suspected deaths based on a case definition) and b) Vital statistics system (National System of mortality -SINADEF- which is an online system that keeps track of death certificates) [48]. A suspected case presents with acute respiratory infection and with two or more of the following symptoms (cough, sore throat, respiratory distress, nasal congestion or fever), close contact with a CoVID-19 case within 14 days of symptoms onset, or people who live or traveled to cities with community transmission of SARS-CoV-2 within 14 days of symptoms onset. On the other hand, the definition of confirmed cases is a suspected case with a positive lab test. [49].

Population size by age, group, and gender in 2020 were retrieved from the Ministry of Health in Peru [50].

Statistical analysis

The crude CFR is defined as the number of cumulative deaths over the number of cumulative cases. For the estimation of CFR in real time, we employed the delay from hospitalization to death, h_s, which is assumed to be given by $h_s = H(s) - H(s-1)$ for $s>0$ where $H(s)$ is a cumulative density function of the delay from hospitalization to death and follows a gamma distribution with mean 10.1 days and SD 5.4 days, as given in ref, Mizumoto and Chowell [24]. Let $\pi_{a,ti}$ be the time-delay adjusted case fatality risk on reported day t_i in area a, the likelihood function of the estimate π_{a,t_i} is given by equation:

$$L(\pi_{a,t_i}; c_{a,t}, D_{a,t}) = \prod_{t_i} \left(\sum_{t=1}^{t_i} c_{a,t} \atop D_{a,t_i} \right) \left(\pi_{a,t_i} \frac{\sum_{t=2}^{t_i} \sum_{s=1}^{t-1} c_{a,t-s} h_s}{\sum_{t=1}^{t_i} c_{a,t}} \right)^{D_{a,t_i}} \left(1 - \pi_{a,t_i} \frac{\sum_{t=2}^{t_i} \sum_{s=1}^{t-1} c_{a,t-s} h_s}{\sum_{t=1}^{t_i} c_{a,t}} \right)^{\sum_{t=1}^{ti} c_{a,t} - D_{a,t_i}}$$

where $c_{a,t}$ represents the number of new cases with reported day t in area a, and D_{a,t_i} is the cumulative number of deaths until reported day t_i in area a [51, 52]. Among the cumulative cases with reported day t in area a, D_{a,t_i} have died and the remainder have survived the infection. The contribution of those who have died with biased death risk is shown in the middle parenthetical term and the contribution of survivors is presented in the right parenthetical term. We assume that D_{a,t_i} is the result of the binomial sampling process with probability π_{a,t_i}.

We used a Monte Carlo Markov Chain (MCMC) method in a Bayesian framework to estimate model parameters. We evaluated the convergence of MCMC chains using the potential scale reduction statistic [53, 54]. Estimates and 95% credibility intervals for these estimates are based on the posterior probability distribution of each parameter and samples drawn from the posterior distributions. All statistical analyses were conducted in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) using the 'rstan' package.

AUTHOR CONTRIBUTIONS

GC and KM conceived the early study idea. KM implemented statistical analysis. AT and KM wrote the first full draft. CM performed data acquisition. All authors contributed to the revision of the manuscript. AU advised the study, and revised the manuscript. GC advised on and helped shape the research. All authors contributed to the interpretation of the results and edited and commented on several earlier versions of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

All authors report no conflicts of interest.

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Research Paper

Development and validation of a risk stratification model for screening suspected cases of COVID-19 in China

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ABSTRACT

How to quickly identify high-risk populations is critical to epidemic control. We developed and validated a risk prediction model for screening SARS-CoV-2 infection in suspected cases with an epidemiological history. A total of 1019 patients, ≥13 years of age, who had an epidemiological history were enrolled from fever clinics between January 2020 and February 2020. Among 103 (10.11%) cases of COVID-19 were confirmed. Multivariable analysis summarized four features associated with increased risk of SARS-CoV-2 infection, summarized in the mnemonic COVID-19-REAL: radiological evidence of pneumonia (1 point), eosinophils < 0.005 × 10^9/L (1 point), age ≥ 32 years (2 points), and leukocytes < 6.05 × 10^9 /L (1 point). The area under the ROC curve for the training group was 0.863 (95% CI, 0.813 - 0.912). A cut-off value of less than 3 points for COVID-19-REAL was assigned to define the low-risk population. Only 10 (2.70%) of 371 patients were proved to be SARS-CoV-2 positive, with a negative predictive value of 0.973. External validation was similar. This study provides a simple, practical, and robust screening model, COVID-19-REAL, able to identify populations at high risk for SARS-CoV-2 infection.

INTRODUCTION

At the end of December 2019, an outbreak of pneumonia caused by a novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) was reported in Wuhan, China [1]. Transmission takes place through respiratory droplets and other routes such as ocular surfaces [2–4]. This highly contagious virus spread rapidly to other cities of China, and gave rise to a global outbreak. As of Mar 23, 2020, over 300,000 cases of COVID-19 have been confirmed worldwide, and more than 10,000 have died. The number of confirmed cases is still increasing. One study estimates the basic reproductive number (R0) to be 2.68, and the epidemic doubling time to be 6.4 days [5]. The control of COVID-19 must include detection and isolation of latent infection. A considerable proportion of COVID-19 cases are infected by those who only had mild

symptoms [6, 7]. COVID-19 patients have the highest viral load near symptom presentation [8]. Moreover, the rapid spread of COVID-19 has meant that large numbers of patients with suspicious symptoms are often crowded into fever clinics for diagnosis.

At present, cases are confirmed by a positive result with high-throughput sequencing or real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay of samples from nasal or pharyngeal swabs [9]. However, nucleic acid tests are not available to all suspected patients in pandemic areas due to the shortage of equipment and reagents [10, 11]. Testing for all cases with mild symptoms and/or an epidemiological history can lead to competition for resources. In addition, undiagnosed mild-type COVID-19 patients who were not properly isolated could become sources of infection as their viral load peaks near symptom presentation, which could explain the rapid spread of this epidemic [12]. A large proportion of infected cases continue to test negative for viral RNA, even after they develop clinical manifestations, and positive chest CT (computed tomography) results [13, 14]. This dilemma demands a fast and accurate model for early screening for SARS-CoV-2 infections to prioritize high-risk patients for clinical care, isolation, and contact tracking. Previous studies reported that a number of COVID-19 patients exhibit lymphopenia and thrombocytopenia [15–17]. Blood counts and high-sensitivity C-reactive protein (hsCRP) are commonly used for early identification of fever [18], and CT is used to assess pneumonia. These tests are simple and fast, and nearly all patients with fever or respiratory symptoms can be tested. We first compared alterations of hematological parameters between cases with and without SARS-CoV-2 infection, then developed and validated a novel score-based prognostic model (COVID-19-REAL) for SARS-CoV-2 infection.

RESULTS

Patient characteristics

A total of 1019 patients were enrolled in this study out of the 1076 patients who presented to fever clinics until 5 February 2020. Fifty-seven patients were excluded, including one with stroke, two with organ transplantation, one with HIV, 12 with cancer, one with active tuberculosis, 18 with age < 12 years, and 22 unconfirmed cases until 10 February 2020 (Figure 1). Of the 1019 patients, 485 (48%) were female, and the median age was 34 years (range 13 to 91 years). The characteristics of the patients are shown in Table 1. All received sequencing or nucleic acid testing using RT-PCR; 103 (10.11%) tested positive for SAR-CoV-2 (Supplementary Table 1).

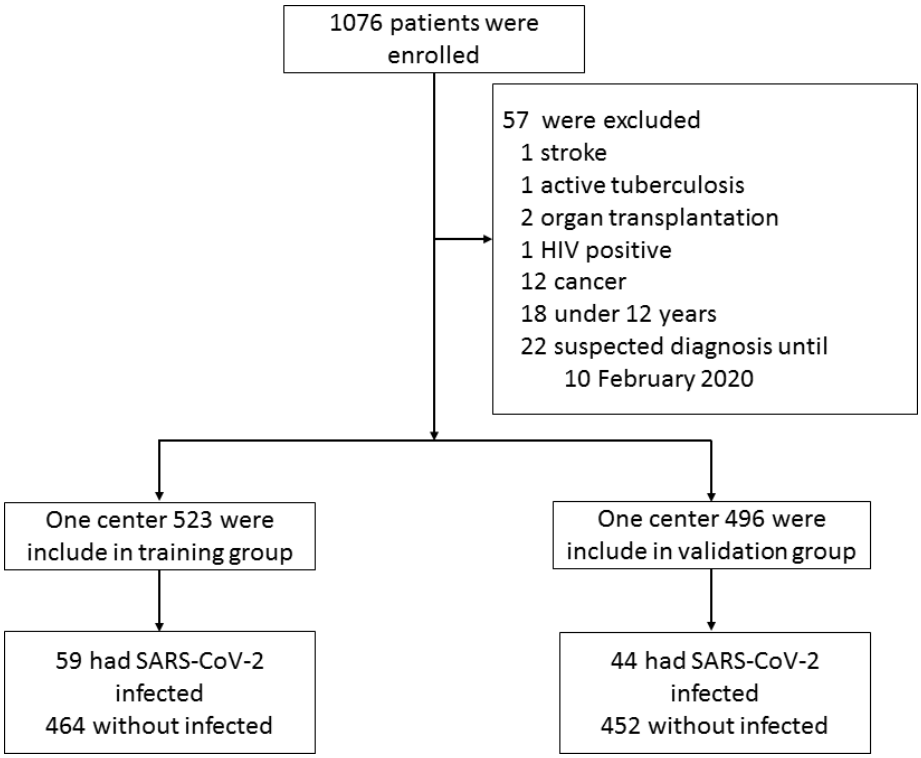

Figure 1. Flowchart of patient selection.

Table 1. Characteristics of patients in this study.

Characteristic	Development group	Validation group	P-value
Number	523	496	
Female	253 (48.38%)	232 (46.77%)	0.609
Age (years)	33 (24-45)	32 (26-40)	0.895
Symptom			
Fever	412 (78.78%)	367 (73.99%)	0.072
Dry cough	209 (39.96%)	171 (34.48%)	0.070
Fatigue	45 (8.60%)	43 (8.669%)	0.970
Pharyngalgia	84 (16.06%)	89 (17.94%)	0.424
Diarrhea	12 (2.29%)	13 (2.62%)	0.736
Coexisting comorbidity			
Hypertension	29 (5.54%)	34 (6.85%)	0.386
Cardiovascular diseases	6 (1.15%)	5 (1.01%)	0.83
Diabetes	11 (2.10%)	7 (1.41%)	0.48
Chronic lung disease	0 (0.00%)	3 (0.60%)	0.115
Chronic liver disease	11 (2.10%)	19 (3.83%)	0.103
Chronic renal disease	1 (0.19%)	2 (0.40%)	0.615
Blood parameters			
Leucocyte (109/L)	6.9 (5.30-8.80)	7.0 (5.20-9.03)	0.74
hsCRP (mg/L)	5.07 (0.90-15.95)	9.10 (2.75-22.56)	<0.001
Monocyte (109/L)	0.50 (0.40-0.70)	0.55 (0.41-0.76)	0.477
RBC (1012/L)	4.78 (4.44-5.22)	4.74 (4.37-5.14)	0.031
Hematocrit (%)	0.42 (0.40-0.46)	0.42 (0.39-0.46)	0.538
Lymphocyte (109/L)	1.30 (0.90-1.80)	1.25 (0.86-1.69)	0.592
MCH (pg)	30.30 (29.30-31.00)	30.30 (29.48-31.20)	0.074
MCHC (g/L)	339.00 (333.00-345.00)	339.00 (332.00-345.00)	0.251
MPV	10.00 (9.60-10.60)	10.00 (9.40-10.60)	0.04
Basophilic granulocyte (109/L)	0.02 (0.01-0.02)	0.02 (0.01-0.03)	<0.001
Eosinophil (109/L)	0.04 (0.01-0.08)	0.03 (0.01-0.09)	0.612
Hemoglobin (g/L)	143 (133-157)	144.00 (132-156)	0.318
PDW (%)	11.70 (10.80-12.85)	11.20 (10.10-12.60)	0.003
Platelet (109/L)	216 (181-256)	212 (173-256)	0.874
Platelet hematocrit (%)	0.22 (0.18-0.25)	0.21 (0.18-0.25)	0.37
Neutrophil (109/L)	4.70 (3.40-6.60)	4.75 (3.30-7.10)	0.7
Radiological evidence of pneumonia	92 (17.59%)	63 (12.70%)	0.03
Confirmed with COVID-19	59 (11.28%)	44 (8.87%)	0.202

Abbreviations: SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; HsCRP: high-sensitivity C-reactive proteins; RBC: red blood cell; MCH: mean corpuscular hemoglobin; MPV: mean platelet volume; MCHC: mean corpuscular hemoglobin concentration; PDW: platelet distribution width; CT: chest computed tomography scan.

Association factors for SARS-CoV-2 infection

The association between age and infection rate is presented in Figure 2A. The rate of SARS-CoV-2 infection increased with age. After stratifying patients by age quartile, the positive rate of SARS-CoV-2 infection from first to fourth quartile was 2.90%, 3.06%, 12.14%, and 23.81% in the training group, and 2.97%, 3.45%, 6.72%, and 23.28% in the validation group (Figure 2B, C). The risk of infection in last two quartiles was relatively higher than the first two quartiles. The infection rate was lower (less than 5%) for patients with age < 32 years. Subgroup analyses were performed for patients with age ≥ 32 years to stratify those as high-risk population.

The factors associated with a positive result of SARS-CoV-2 infection in univariate analysis are shown in

Table 2. Compared to non-COVID-19 patients, COVID-19 patients had a lower count of leukocytes (5.10×10^9/L vs 7.15×10^9/L, $p < 0.001$), monocytes (0.40×10^9/L vs 0.55×10^9/L, $p < 0.001$), lymphocytes (1.10×10^9/L vs 1.30×10^9/L, $p = 0.02$), eosinophils (0.01×10^9/L vs 0.04×10^9/L, $p < 0.001$), neutrophils (3.40×10^9/L vs 5.00×10^9/L, $p < 0.001$), and platelets (192×10^9/L vs 220×10^9/L, $p < 0.001$). They had a higher age (47 years vs 32 years, $p < 0.001$) in the training group, and similar characteristics were found in validation group (Supplementary Tables 2). After multivariate analysis, age, leukocytes, and eosinophils remained as significant factors; lymphocytes, leukocytes, monocytes, platelets, and neutrophils were not significant indicators (Table 2).

A COVID-19 prediction model based on age, leukocyte, and eosinophil and radiological evidence of pneumonia

The AUROC value for the prediction of leukocytes and eosinophils in the training group for COVID-19 diagnosis were 0.747 and 0.729, respectively. This was comparable to the validation group, where the AUROC value for leukocytes and eosinophils were 0.763 and 0.772 (Supplementary Figure 1). Using Youden's index, the optimal cut-off value for leukocytes and eosinophils were 6.05×10^9/L and 0.005×10^9/L.

Significantly higher infection rate was observed in those with leukocytes $< 6.05\times10^9$/L (23.66% vs 4.45% in leukocytes $\geq 6.05\times10^9$/L), and eosinophils $< 0.005\times10^9$/L (33.72% vs 6.68% in eosinophils $\geq 0.005\times10^9$/L) in the training group. The trend was consistent in the validation group, where the infection rate was 18.13% vs 3.5% for leukocyte subgroups, and 28.13% vs 4.25% for eosinophil subgroups (Figure 3).

Based on multivariate logistic regression analysis, the major criterion was age ≥ 32 years (2 point). Minor criteria included leukocytes $< 6.05\times10^9$/L (1 point), eosinophils $< 0.005\times10^9$/L (1 point), and radiological evidence of pneumonia (1 point) (Table 3). The model showed good discrimination (AUROC = 0.863, 95% CI, 0.81 - 0.91) and calibration. Internal verification shows AUROC = 0.863 (95% CI, 0.81 - 0.91) and external verification showed good discrimination (AUROC = 0.871, 95% CI, 0.82-0.93) (Table 4, Supplementary Figure 2)

The following four risk groups were developed: very low risk (0 point), with a risk of infection of 0.84%; low risk (1 - 2 points), with a risk of 3.57%; moderate risk (3 points), with a risk of 19.05%; and high risk (4 - 5 points), with a risk of 61.70%. For the validation group, the infection risk was 0% (0 point); 3.49% (1 - 2 points); 10.87% (3 points); and 55.32% (4 - 5 points) (Figure 4). A cut-off value of less than 3 points for COVID-19-REAL was used to stratify 371 out of 523 (70.94%) cases as low risk, of whom only 10 (2.70%) were infected with SARS-CoV-2 in the training group. The remaining 152 patients were classified as higher risk of infection; about 49 (32.24%) were infected with SARS-CoV-2. According to the cut-off value of 3 points, the sensitivity, specificity, positive predictive value, and negative predictive value was 0.778, 0.831, 0.322, and 0.973 respectively (Table 4).

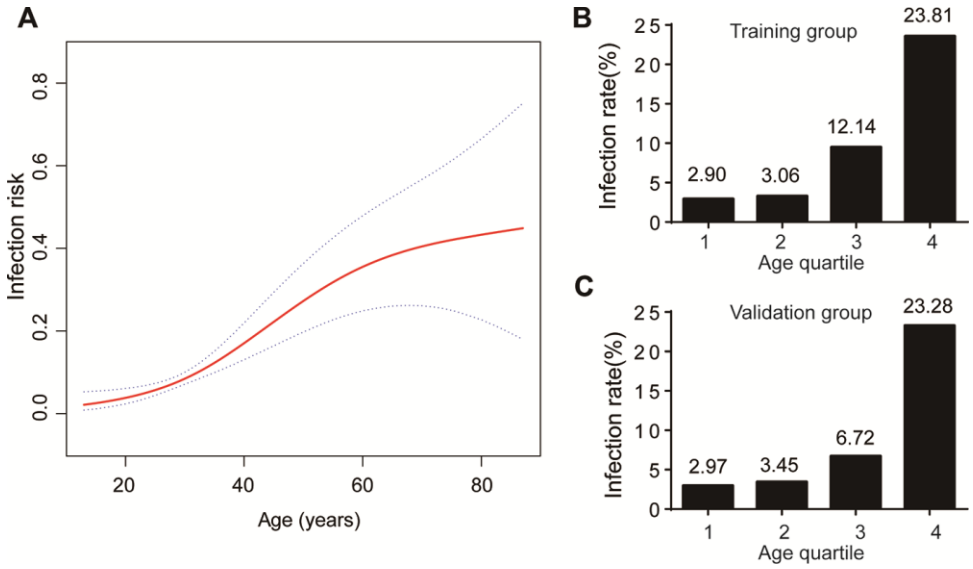

Figure 2. Age and COVID-19 infection. (**A**) The infection risk increased with increasing age; (**B**) Infection rate at age quartile in training group; (**C**) Infection rate at age quartile in validation group.

Table 2. Univariate and multivariate analyses of indicators for SARS-CoV-2 infection in training group.

Variable	non-COVID-19	COVID-19	Univariate		Multivariate	
	N = 464	N = 59	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (years)	32 (23-42)	47 (38-56)	1.05 (1.04- 1.07)	<0.001	1.06 (1.04- 1.08)	<0.001
Leucocyte (109/L)	7.15 (5.70-9.03)	5.10 (4.05-6.05)	0.72 (0.63- 0.83)	<0.001	0.74 (0.64- 0.85)	<0.001
Monocyte (109/L)	0.55 (0.40-0.70)	0.40 (0.30-0.50)	0.06 (0.01- 0.24)	<0.001		
RBC (1012/L)	4.80 (4.45-5.24)	4.70 (4.25-5.01)	0.46 (0.27- 0.78)	0.004		
Lymphocyte (109/L)	1.30 (0.90-1.90)	1.10 (0.85-1.50)	0.57 (0.35- 0.91)	0.019		
Basophilic granulocyte (109/L)	0.02 (0.01-0.03)	0.01 (0.01-0.02)	0.00 (0.00- 45.46)	0.098		
Eosinophil (107/L)	4.00 (1.00-9.00)	1.00 (0.00-3.00)	0.88 (0.82- 0.95)	0.001	0.91 (0.85- 0.98)	0.009
Platelet (109/L)	220.00 (184.00-259.00)	192.00 (144.50-234.00)	0.99 (0.99- 1.00)	<0.001		
Neutrophil (109/L)	5.00 (3.60-6.80)	3.40 (0.80-22.20)	0.75 (0.65- 0.87)	<0.001		
Radiological evidence of pneumonia	68 (14.66%)	24 (40.68%)	3.99 (2.24- 7.13)	<0.001	4.00 (2.04- 7.86)	<0.001

Abbreviations: SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; RBC: red blood cell; HsCRP: high-sensitivity C-reactive proteins; CT: chest computed tomography scan; CI: confidence interval; OR: odds ratio.

DISCUSSION

Beginning in mid-January 2020, a large number of people living in Wuhan left the area via public transportation due to Chinese New Year, leading to a dramatic increase in confirmed or suspected cases nationwide. The management of these suspected cases is of major concern. Nucleic acid testing is currently the main diagnostic method, but the sensitivity and specificity of nucleic acid tests are yet to be verified, and the overall detection rate is constrained by virus concentration and sampling method. Another problem is that some patients with positive chest CT images test negative for COVID-19 by RT-PCR [14]. With such issues in mind, we proposed a robust, high-throughput screening model to help prioritize high-risk patients. We used the data of routine blood tests and CT images to develop a score system (COVID-19-REAL) that can stratify patients into risk groups. Suspected cases with 0 - < 3 points had a predicted probability of 99.16% in training and 97.3% in validation groups for not being infected by SARS-CoV-2. This risk classification can be employed by clinicians and medical institutions, especially those with inadequate detection reagents or equipment, to make rational allocation of resources.

Previous investigations have revealed valuable information about demographics for COVID-19. Most patients with COVID-19 are older [16]. We first stratified patients according to age. Two earlier studies stated the median age of the patients was 56 and 59 years [15, 19]. In our study, the median age was 47 years. We found the risk of infection significantly increased with age, from less than 3% to over 23% from the first to last quartile.

The level of leukocytes, monocytes, lymphocytes, eosinophils, neutrophils, and platelets was dramatically lower in COVID-19 patients. Our results are consistent with previous research that patients exhibited leukopenia, lymphopenia, and thrombocytopenia after SARS-CoV-2 infection [15, 20]. Some researchers suggested a decreased level of white blood cells could serve as an auxiliary diagnosis [20]. Similar patterns emerged in SARS-CoV, with cases of lymphopenia and neutropenia [21, 22], and decreased levels of leukocytes and platelets [23]. A SARS-CoV model showed that neutrophils, lymphocytes, and leukocytes were significantly reduced the day after infection [24]. In a SARS-CoV MA15 infection model, the decrease of peripheral blood cells was explained by inflammatory cell infiltration to the lungs [25]. The N protein of

SARS-CoV enhances eosinophilic infiltration into the lungs and aggravates lung inflammation [26]. Lung lesions were the most important feature of SARS-CoV-2 infections [20], and eosinophilopenia may indicate a poor prognosis of COVID-19 [27]. These results shed light on the neglected role that eosinophils might play in the progression of respiratory disease.

To better stratify SARS-CoV-2 infection risk for the suspected cases, four criteria including leukocytes < 6.05×10^9 /L (1 point), eosinophils < 0.005×10^9/L (1 point), radiological evidence of pneumonia (1 point), and age ≥ 32 years (2 point) were used to determine the likelihood of SARS-CoV-2 infection. We defined four risk groups: very low risk (0 point), low risk (1 - 2 points), moderate risk (3 points), and high risk (4 - 5 points). According to the cut-off value that was assigned as less than 3 points of COVID-19-REAL score, the number of suspected cases who required priority examination and hospitalization decreased by 70.94% and 71.98%, while maintaining a false negative rate of 2.70% and 2.24% in training and validation group, respectively.

Clinical decision models have been explored to predict infection of SARS-CoV-2. Sun et al. [28] studied 788 cases in Singapore to identify populations at high risk for COVID-19. From their large population-based study, a model that combined laboratory blood tests, clinical findings, and radiology was proposed, and the AUROC was 0.88 (95% CI: 0.83- 0.93). Similar to our cohort, those authors found that eosinophils and CT imaged pneumonia were strong predictors. However, their conclusions were limited by a lack of external verification, clinical inapplicability caused by redundant parameters, and missing data in laboratory blood tests.

The advantage of present study is that a simple and applicable prediction model, COVID-19-REAL, which combines age, radiological image, and two functionally related hematological indicators (i.e., leukocytes and eosinophils) has been developed to stratify and distinguish between high- and low-risk populations suspected of SARS-CoV-2 infection. This evaluation of suspected cases based on age, radiological image, and two dichotomous criteria could be easily implemented in routine clinical practice. In clinical settings where resources and testing kits are limited, patients with advanced respiratory symptoms are usually tested first. However, those undiagnosed mild-type COVID-19 patients who were not properly isolated would become sources of infection as the viral load peaked near symptom presentation. This score system will be of great help for early infection screening and offer more information for physicians to help prioritize high-risk patients.

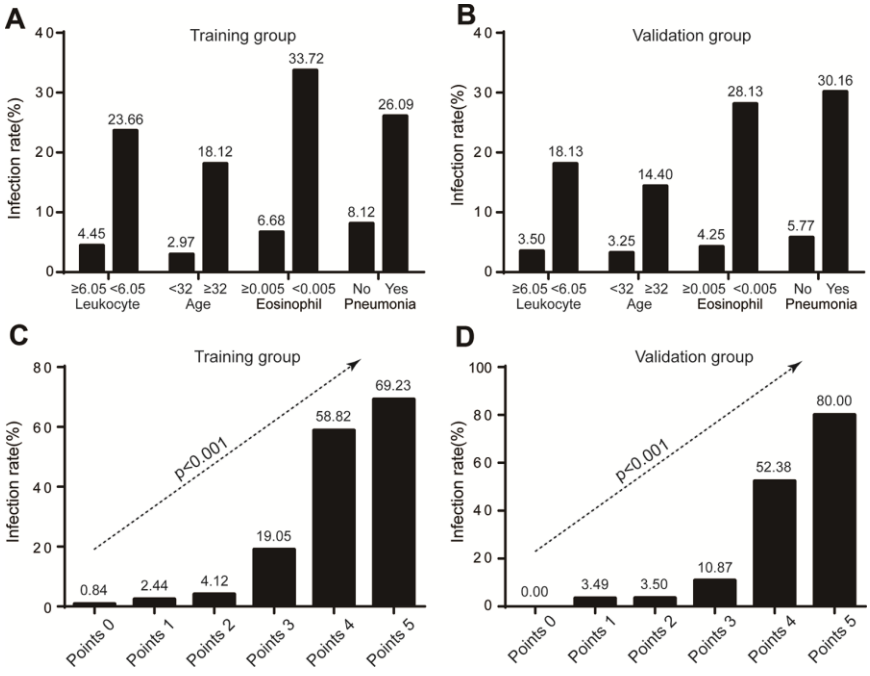

Figure 3. Infection rate in risk stratification. (**A**) Infection rate stratified by leukocyte, age, eosinophil, and radiological evidence of pneumonia in training group; (**B**) Infection rate stratified by leukocyte, age, eosinophil, and radiological evidence of pneumonia in validation group; (**C**) Infection rate according to COVID-19-REAL score in training group; (**D**) Infection rate according to COVID-19-REAL score in validation group.

Table 3. Multivariate analyses of indicators for SARS-CoV-2 infection in training group.

Variable	OR (95% CI)	β Coefficient (95% CI)	P-value	Point score
Age (years)				
<32 (n = 236)	1	1		
≥32(n = 287)	8.63 (3.60 - 20.64)	2.16 (1.28- 3.03)	<0.001	2
Eosinophil (109/L)				
>0.005 (n = 437)	1	1		
≤0.005 (n = 86)	4.92 (2.50 - 9.69)	1.59 (0.94 - 2.27)	<0.001	1
Leucocyte (109/L)				
>6.05 (n =337)	1	1		
≤6.05 (n =186)	6.23 (3.14 - 12.35)	1.83 (1.14 - 2.51)	<0.001	1
Radiological evidence				
No Pneumonia(n = 431)	1	1		
Pneumonia(n = 92)	3.73 (1.83 - 7.62)	1.32 (0.60 – 2.03)	<0.001	1

Abbreviations: SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CT: chest computed tomography scan; CI: confidence interval; OR: odds ratio.

Table 4. Performances of the risk stratification algorithm in the diagnosis of SARS-CoV-2 infection in training and validation groups.

Group	AUROC (95% CI)	Specificity	Sensitivity	Positive PV	Negative PV
Training group	0.863 (0.813-0.912)	0.778	0.831	0.322	0.973
Validation group	0.871 (0.816-0.925)	0.772	0.818	0.259	0.978

Abbreviations: SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CI: confidence interval; Positive PV: positive predictive value; Negative PV: negative predictive value.

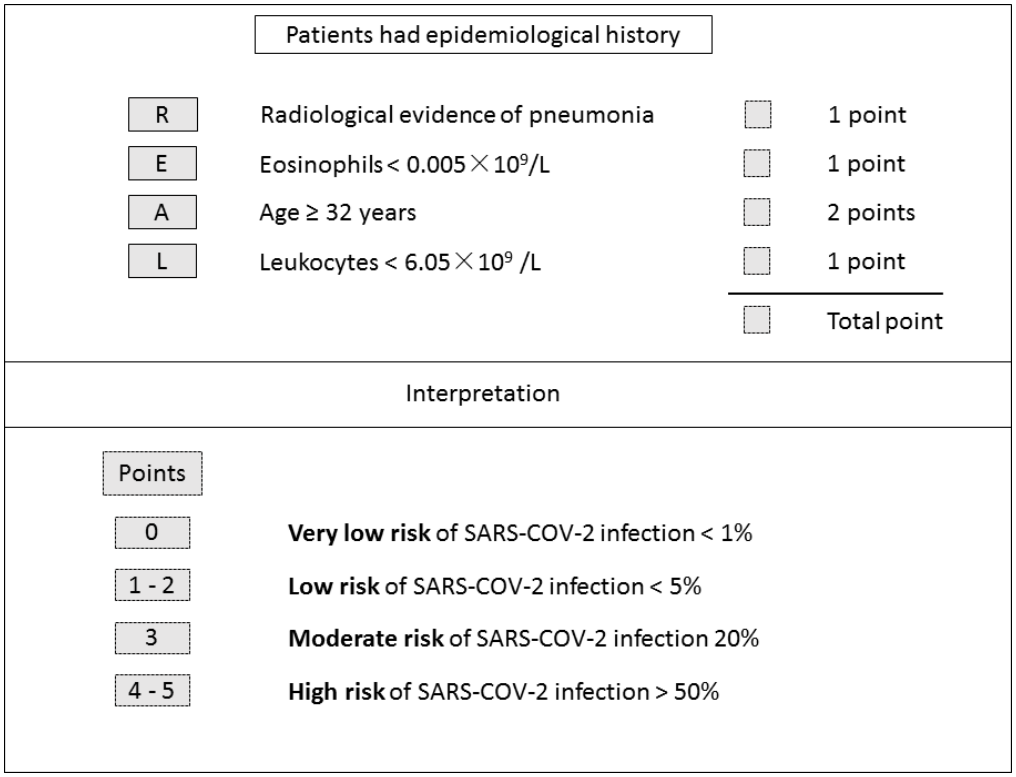

Figure 4. COVID-19-REAL model for risk stratification of SARS-CoV-2 infection.

There are limitations in current study. Our training and validation data comes from China; their applicability to Western populations must be separately evaluated. The results were obtained from people over 12 years of age, and may not be applicable to younger people. Only routine tests including hsCRP, radiological image, and blood cell count were performed, and other hematological indicators including liver and kidney function are lacking.

In conclusion, this study provides a simple, practical, and robust screening model (COVID-19-REAL) to identify high risk populations for SARS-CoV-2 infection. This prediction model will help reduce the burden on hospitals in pandemic areas and help them allocate resources more rationally.

MATERIALS AND METHODS

Patients

Suspect cases of COVID-19 with age ≥13 years with an epidemiological history were included from fever clinics of the First Affiliated Hospital, College of Medicine, Zhejiang University and Taizhou Enze Medical Center (Group), Enze Hospital, between 23 January 2020 and 5 February 2020. All suspected cases received sequencing or RT-PCR assay for SARS-CoV-2. According to National Health Commission, an epidemiological history of COVID-19 is defined as follows: within 14 days before the onset of the disease (1) there were tourism or residence histories of Wuhan or its surrounding areas, or other communities with confirmed cases; (2) there were contacts with confirmed cases of COVID-19; (3) there were contacts with suspected cases (having fever or respiratory symptoms) from Wuhan or its surrounding areas, or other communities with confirmed cases; (4) one confirmed case was found in an enclosed environment (such as a family house, a construction site, an office, etc.), with one or more cases of fever/respiratory tract infection re found at the same time

The patient-selection process is shown in Figure 1. The COVID-19 cases were all confirmed by sequencing or RT-PCR assay [9]. The RT-PCR was mainly performed using a commercial kit for SARS-CoV-2 detection (BoJie, Shanghai, China) which was approved by China Food and Drug Administration. We excluded patients with HIV infection, cancer, organ transplantation, stoke, active tuberculosis, severe and critical COVID-19 patients according to the National Health Commission [17], and suspected cases without confirmed laboratory evidence until 10 February 2020. The study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, and complied with the ethical guidelines of the Declaration of Helsinki. The researchers only analyzed anonymous data, so informed consent was waived. Age, gender, laboratory assessments consisting of hsCRP, complete blood count, and radiological images were obtained from electronic medical records. Radiological evidence of pneumonia was defined as lung consolidation and/or ground-glass opacity [20]. The images were reviewed independently by two radiologists, and if there were disagreements, a third radiologist would perform further examination.

Statistical analysis

Continuous variables were expressed as medians and interquartile range (IQR), and were compared by t-test or Mann–Whitney U-test. Chi-squared test or Fisher's exact test was used to compare categorical variables and expressed as percentages. Generalized linear models with a logit link were used to test the association between age and the risk of COVID-19 infection. Univariate and multivariate analyses were performed to identify indicators of COVID-19 patients. Variables with $P < 0.1$ in a univariate analysis were then included in a forward stepwise regression model. A score for the final model was developed by rounding the coefficients of the logit model. Predicted and observed risk was calculated for each score. The area under receiver operating characteristic (AUROC) curve was used to assess the accuracy of different scores in diagnosis power. Internal validation was performed using a bootstrap procedure with 500 bootstrapped samples. The Youden's index was used to determine the optimal cut-off level for predicting clinical outcomes. All statistical analysis was performed by Statistical Package for the Social Sciences version 19.0 (International Business Machines Corporation, Armonk, NY) and R version 3.4 (R Foundation, Vienna, Austria). All tests were two tailed and $P < 0.05$ was considered to indicate statistical significance.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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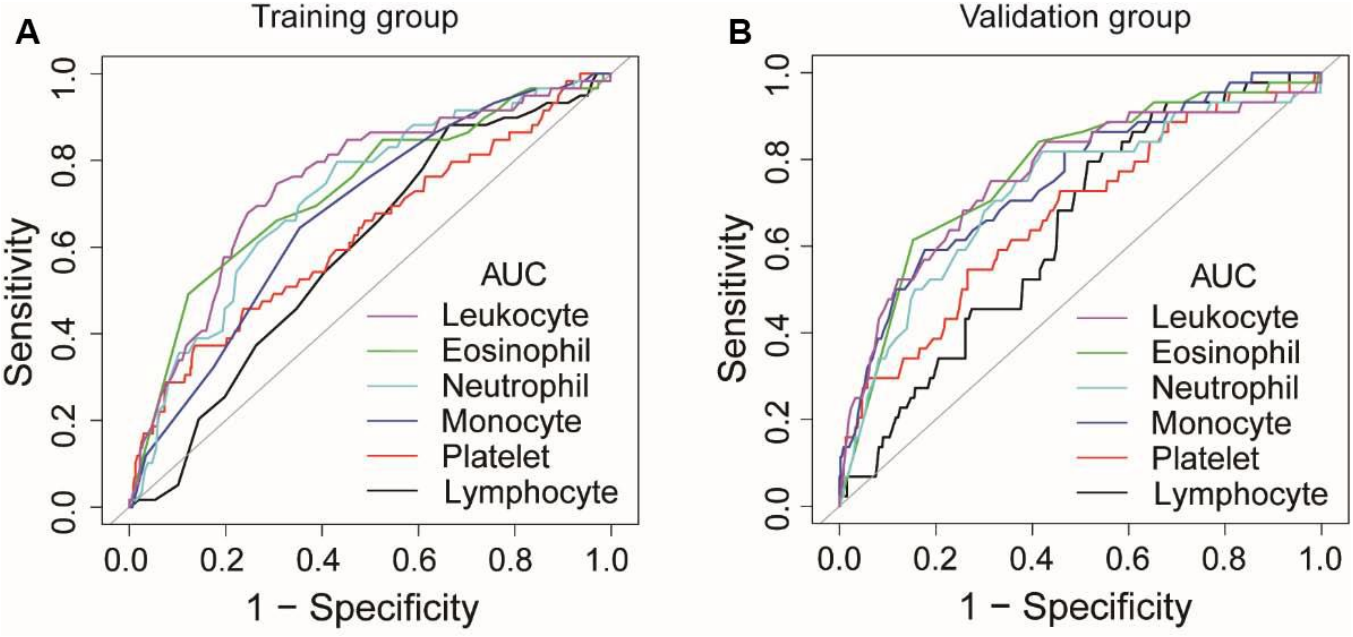
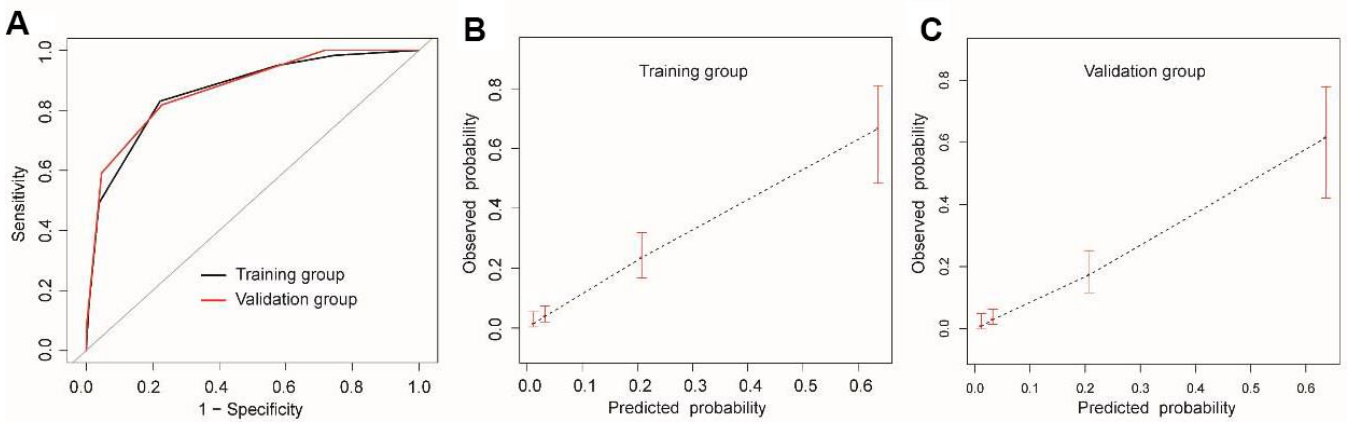
SUPPLEMENTARY MATERIALS

Supplementary Figures



Supplementary Figure 1. AUROC of leukocyte, monocyte, lymphocyte, eosinophil, neutrophil and platelets in COVID-19 diagnosis. (**A**) training group; (**B**) validation group.



Supplementary Figure 2. AUROC of model in COVID-19 diagnosis (**A**), and Calibration chart for predicted versus observed probability (**B**, **C**) in training and validation group.

Supplementary Tables

Supplementary Table 1. Characteristics of patients with SARS-CoV-2 infected.

Characteristic	Development group	Validation group	P-value
Number	59	44	
Female	24 (40.68%)	15 (34.09%)	0.495
Age (years)	47 (38-56)	48 (35-57)	0.812
Symptom			
Fever	37 (62.71%)	32 (72.73%)	0.285
Dry cough	24 (40.68%)	18 (40.91%)	0.981
Fatigue	6 (10.17%)	4 (9.09%)	0.855
Pharyngalgia	14 (23.73%)	8 (18.18%)	0.497
Blood parameters			
Leucocyte (109/L)	5.10 (4.05-6.05)	4.50 (3.63-6.03)	0.738
hsCRP (mg/L)	10.17 (2.62-21.88)	14.80 (5.35-30.10)	0.043
Monocyte (109/L)	0.40 (0.30-0.50)	0.35 (0.27-0.52)	0.224
RBC (1012/L)	4.70 (4.25-5.01)	4.69 (4.22-5.01)	0.88
Hematocrit (%)	0.42 (0.38-0.45)	0.42 (0.38-0.44)	0.668
Lymphocyte (109/L)	1.10 (0.85-1.50)	1.10 (0.73-1.30)	0.134
MCH (pg)	30.60 (29.65-31.30)	30.15 (29.30-31.33)	0.439
MCHC (g/L)	341.00 (334.00-346.50)	341.00 (334.50-348.00)	0.939
MPV	10.40 (9.90-10.85)	10.35 (9.95-11.00)	0.707
Basophilicgranulocyte (109/L)	0.01 (0.01-0.02)	0.01 (0.00-0.01)	0.066
Eosinophil (109/L)	0.01 (0.00-0.03)	0.00 (0.00-0.02)	0.757
Hemoglobin (g/L)	144.00 (129.00-153.00)	143.00 (129.00-152.00)	0.641
PDW (%)	12.00 (11.20-13.10)	11.90 (10.73-13.03)	0.403
Platelet (109/L)	192.00 (144.50-234.00)	177.00 (140.00-226.00)	0.4
Platelet hematocrit (%)	0.20 (0.15-0.23)	0.18 (0.15-0.24)	0.453
Neutrophil (109/L)	3.40 (2.60-4.45)	3.00 (2.18-4.15)	0.981
Radiological evidence of pneumonia	24 (40.68%)	19 (43.18%)	0.799

Abbreviations: HsCRP: high-sensitivity C-reactive proteins; RBC: Red Blood Cell; MCH: mean corpuscular hemoglobin; MPV: mean platelet volume; MCHC: mean corpuscular hemoglobin concentration; PDW: Platelet distribution width; CT: chest computed tomography scan.

Supplementary Table 2. Characteristics of patients visited fever clinics.

Characteristic	Non-COVID-19 infected	COVID-19 infected	*P*-value
Number	452	44	
Female	217 (48.01%)	15 (34.09%)	0.077
Age (years)	31 (25-38)	48 (35-57)	<0.001
Symptom			
Fever	335 (74.12%)	32 (72.73%)	0.841
Dry cough	153 (33.85%)	18 (40.91%)	0.347
Fatigue	39 (8.63%)	4 (9.09%)	0.917
Pharyngalgia	81 (17.92%)	8 (18.18%)	0.966
Blood parameters			
Leucocyte (10^9/L)	7.20 (5.50-9.50)	4.50 (3.63-6.03)	<0.001
hsCRP (mg/L)	8.80 (2.40-22.22)	14.80 (5.35-30.10)	0.547
Monocyte (10^9/L)	0.57 (0.43-0.78)	0.35 (0.27-0.52)	<0.001
RBC (10^{12}/L)	4.74 (4.38-5.14)	4.69 (4.22-5.01)	0.068
Hematocrit (%)	0.42 (0.40-0.46)	0.42 (0.38-0.44)	0.042
Lymphocyte (10^9/L)	1.29 (0.87-1.76)	1.10 (0.73-1.30)	0.004
MCH (pg)	30.30 (29.50-31.20)	30.15 (29.30-31.33)	0.442
MCHC (g/L)	339.00 (332.00-344.00)	341.00 (334.50-348.00)	0.046
MPV	9.95 (9.30-10.60)	10.35 (9.95-11.00)	<0.001
Basophilicgranulocyte (109/L)	0.02 (0.01-0.03)	0.01 (0.00-0.01)	<0.001
Eosinophil (109/L)	0.03 (0.01-0.09)	0.00 (0.00-0.02)	0.002
Hemoglobin (g/L)	144.00 (132.00-156.00)	143.00 (129.00-152.00)	0.177
PDW (%)	11.20 (10.10-12.45)	11.90 (10.73-13.03)	0.15
Platelet (109/L)	214.00 (176.00-260.00)	177.00 (139.75-226.00)	<0.001
Platelet hematocrit (%)	0.22 (0.18-0.25)	0.18 (0.15-0.24)	0.002
Neutrophil (109/L)	4.90 (3.50-7.22)	3.00 (2.18-4.15)	<0.001
Radiological evidence of pneumonia	44 (9.73%)	19 (43.18%)	<0.001

Abbreviations: HsCRP: high-sensitivity C-reactive proteins; RBC: Red Blood Cell; MCH: mean corpuscular hemoglobin; MPV: Mean platelet volume; MCHC: mean corpuscular hemoglobin concentration; PDW: Platelet distribution width; CT: chest computed tomography scan.

Research Paper

COVID-19 induced liver function abnormality associates with age

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) is a novel infectious disease that may cause fever, dry cough, fatigue and shortness of breath. The impact of COVID-19 on liver function is not well described.
Results: We found that the overall frequency of LFT abnormality was 17.6%. Frequency of LFT abnormality was significantly greater in patients with severe/critical (SC) COVID-19 compared to those with mild/moderate (MM) COVID-19 (32.4% vs 11.6%, p=0.011). Among patients with LFT abnormality, the median age was significantly higher in the SC group compared to the MM group (52 vs 39 years, p=0.021).
Conclusion: COVID-19 is frequently associated with mild liver function abnormality, particularly in individuals with severe/critical COVID-19 who were older. Liver function should be monitored carefully during infection, with judicious use of hepatotoxic agents where possible and avoidance of prolonged hypotension to minimize liver injury in older patients.
Methods: The No. 2 People's Hospital of Fuyang City in China has admitted a total of 159 patients with confirmed COVID-19 since the outbreak from January 2020 to March 2020. We analyzed the incidence of liver function test (LFT) abnormality in these patients with confirmed COVID-19 infection.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a novel infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS coronavirus 2 or SARS-CoV-2) [1–5]. The COVID-19 outbreak was first described in November/December 2019 in China, and has since spread to over 180 countries around the world [6–10]. Due to this rapid spread and severity of the illness, the World Health Organization characterized COVID-19 as a pandemic [11–13]. COVID-19 continues to be a serious threat to public health worldwide, with a global morality rate of 5.15% as of June 24th 2020 [10]. However, the mortality rate has varied significantly across regions, ranging from low rates in Qatar (0.11%), Russia (1.40%) and South Africa (1.98%), to intermediate rates in India (3.17%), Germany (4.63%), Iran (4.70%), China (5.48%) and the United States (5.16%), to very high rates in Spain (11.48%), the United Kingdom (13.98%), Italy

(14.52%), France (15.03%) and Belgium (15.97%) (see the Coronavirus Resource Centre or the latest worldwide data [10, 14]). COVID-19 most commonly causes fever, cough, shortness of breath, myalgia, fatigue, and sore throat [1], ranging from mild in severity to severe, with around a quarter requiring intensive care admission in the largest case series to date [1, 15]. Asymptomatic infection with confirmed transmission and atypical presentations with abdominal pain, nausea, vomiting and diarrhea have also been reported [15]. However, the frequency of liver dysfunction in COVID-19 infection has not been well described, and in particular have been difficult to interpret due to co-administration of hepatotoxic agents and varied timing of liver function abnormality in the course of the illness and across age groups [16–18]. In this brief report, we sought to analyze the association between COVID-19 infection, liver function test (LFT) abnormality and age in the 159 patients hospitalized for confirmed COVID-19 at the No. 2 People's Hospital of Fuyang City, Fuyang, Anhui Province, China.

RESULTS

Patients

A total of 159 patients were admitted with confirmed COVID-19 and enrolled in this study. Baseline demographics and patient characteristics according to severity of COVID-19 are presented in Table 1. Overall, the median age was 43 years, and 56.6% (90/159) were male (Table 1). Thirty-four patients (21.4%) were classified to have severe or critical illness (SC) and the remaining 125 patients (78.6%) were classified to have mild/moderate illness (MM) (Tables 1, 2). In brief, patients in the MM group were significantly younger, had a lower body mass index (BMI), were less likely to have fever, and had a lower heart rate, lower respiratory rate and higher oxygen saturations at admission compared to the SC group (Table 1), reflecting the severity of their COVID-19. There was a significantly higher proportion of patients with underlying chronic hepatitis B virus (HBV) infection in the SC group compared to the MM group (Table 1). There was a significantly higher proportion of patients with hypertension in the SC group compared to the MM group (Table 1). Other comorbidities were similar between both groups. Patients with chronic HBV were treated with entecavir (ETV) if they met the APASL guidelines for HBV treatment [23].

Liver function test abnormality frequency

Twenty-eight of the 159 (17.6%) hospitalized patients had LFT abnormality at the time of hospital admission (n=19), and a further 9 patients (5.7%) developed LFT abnormality during the first week of admission. The proportion of patients with LFT abnormality was significantly higher in the SC group compared to the MM group (32.4% vs 11.6%, p=0.011, Table 1).

Among patients with LFT abnormality, the median age was significantly higher in the SC group compared to the MM group (52 vs 39 years, p=0.021). Three patients had a history of chronic HBV infection, 2 of whom were receiving antiviral therapy with ETV (Tables 2, 3). The distribution of comorbidities was similar among the subset of patients with liver function test abnormality in the MM and SC groups, and in particular there was a similar number of patients with chronic HBV and patients receiving ETV in each group (Tables 2, 3 and Figure 1).

Pattern and degree of liver function test abnormality

We analyzed the components of the LFT panel, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of hepatocellular damage, and gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP), which are markers of cholestasis, in COVID-19 patients at the time admission (week 0), at 1, 2 and 6 weeks following date of admission as an inpatient or outpatient depending on length of admission. In addition, we analyzed markers of liver synthetic function including total bilirubin (TBIL), and coagulation profiles using the international normalized ratio (INR). We found that there was only a mild to moderate derangement in LFTs in both MM and SC patient groups (Tables 3, 4 and Figures 1, 2), with a mixed pattern of both hepatocellular injury and cholestasis (GGT elevations observed but no changes in ALP were observed), without significant liver synthetic dysfunction.

In more detail, the majority of patients had ALT, AST and GGT levels below 5 times the ULN (Table 4 and Figure 2). In the SC group, 4 (36.4%) patients had an ALT 1-2x ULN, 4 (36.4%) patients had an ALT 2-5x ULN, and 3 (27.3%) patients had an elevated ALT >5x ULN. In the MM group, 9 (52.9%) patients had an ALT 1-2x ULN, 7 (41.2%) patients had an ALT 2-5x ULN, and 1 (5.9%) patient had an ALT >5x ULN. AST was abnormal in 8 SC patients and 7 MM patients with LFT abnormalities. In the SC group, 4 (50%) patients had an AST of 1-2x ULN, 2 (25%) patients had AST 2-5x ULN, and 2 (25%) patients had an AST greater than 5 ULN (Table 4 and Figure 2). A total of 7 (100%) patients had an AST 1-2x ULN in the MM group, and no elevations >2x ULN were noted in this group (Table 4 and Figure 2). Median ALT and AST values failed to reach statistical significance between the SC and MM groups (Table 4). GGT levels were abnormal in 10 SC

Table 1. Comparison of baseline demographics and clinical characteristics between SC and MM groups. Values are expressed as median (interquartile range (IQR), 25-75%). P value is the comparison between severe/critical (SC) and mild/moderate (MM) patients. *P<0.05, **P<0.01, ***P<0.001.

Characteristic	SC	MM	P value
Total number (n, %)	34 (21.4%)	125 (78.6%)	
with liver function test abnormality (n, %)	11 (32.4%)	17 (11.6%)	0.011
Age (years) (median, IQR)	49.5 (42.5-65.3)	41.0 (29.0-50.0)	<0.0001
Male gender (n, %)	23 (67.6%)	67 (53.6%)	0.143
Fever (n, %)	34(100%)	84(67.2%)	<0.0001
Temperature (°C) (Median, IQR)	37.1 (36.8-37.9)	36.8 (36.5-37.5)	0.014
Heart rate (beats / minute) (Median, IQR)	96 (78-102)	84 (80-91)	0.039
Blood Pressure (mmHg) (Median, IQR)	130 (116-142)/ 84 (73-93)	128 (119.5-140)/ 85 (75.5-92)	0.671/ 0.711
Respiratory rate (breaths / minute) (Median, IQR)	20 (19-23)	20 (19-21.5)	0.031
Oxygen saturation (%) (Median, IQR)	91.5(89.5-94.3)	98(97-98)	<0.0001
Treatment with lopinavir/ritonavir (n, %)	30(88.2%)	109(87.2%)	0.798
Treatment with lopinavir/ritonavir and hydroxychloroquine (n, %)	1(2.9%)	15(12%)	0.07
Body mass index (kg/m^2) (Median, IQR)	25.8 (23.4-27.6)	24.2 (22.1-26.1)	0.022
Comorbidities	**SC**	**MM**	**P value**
Chronic hepatitis B virus (HBV)	9	3	<0.0001
Chronic HBV receiving entecavir	1	2	0.517
HBV-related cirrhosis	0	0	a/n
HBV cirrhosis	0	0	a/n
Hypertension	13	11	<0.0001
Diabetes	4	10	0.492
Coronary heart disease	3	0	0.009
Fatty liver	1	1	0.321
Other	9	1	<0.0001

Table 2. Comparison of baseline demographics and clinical characteristics between SC and MM groups in the subset with liver function test abnormality.

Characteristic	SC	MM	P value
Number (n, %)	11 (32.4%)	17 (11.6%)	0.011
Age (years) (Median, IQR)	52 (40-63)	39 (30-47.0)	0.021
Male gender (n, %)	9 (81.8%)	11 (64.7%)	0.328
Body mass index (kg/m^2) (Median, IQR)	26.2 (25.7-27.0)	24.5 (22.9-26.1)	0.120
Comorbidities	**SC**	**MM**	**P value**
Chronic hepatitis B virus	2 (1 on ETV)	1 (1 on ETV)	0.543
Hepatitis B virus related cirrhosis	0	0	a/n
Hypertension	3	1	0.269
Diabetes	1	1	1
Other	2	0	0.146
The number of comorbidities	**SC**	**MM**	**P value**
One	1	3	1
Two	3	0	0.05
Three	1	0	0.393

Values are expressed as median (interquartile range (IQR), 25-75%). P value is the comparison between severe/critical (SC) and mild/moderate (MM) patients. *P<0.05. ETV = entecavir.

Table 3. Comparison of liver function test parameters between MM and SC group patients with abnormal liver function tests (Table 3-1) and normal liver function tests (Table 3-2).

Table 3-1. Patients with abnormal liver function within 1 week of admission.

		Week 0			Week 1			Week 2			Week 6		
	NRR	SC n=11	MM n=17	P value	SC n=11	MM n=17	P value	SC n=11	MM n=17	P value	SC n=11	MM n=17	P value
ALT (U/L)	0-50	69 (27-81)	62 (33.0-90.5)	0.962	70 (49-119)	60 (49-106)	0.64	47 (25-210)	41.0 (22-71.5)	0.378	25.5 (15.5-45.5)	42.5 (20.3-49.5)	0.291
AST (U/L)	0-50	46 (30-65)	40 (28-52.5)	0.423	37.50 (25.25-74.0)	24.0 (18.0-44.25)	0.078	34.50 (24.25-38.5)	25.0 (21.0-31.5)	0.128	21.5 (17.8-25.8)	23.5 (20-29)	0.178
GGT (U/L)	10-60	59 (21-130)	35 (21-108.5)	0.48	96 (55-114)	48 (24-99)	0.082	66 (41.5-166.5)	40 (22.5-79)	0.217	49.5 (23-87.5)	34 (22.3-73.5)	0.752
ALP (U/L)	45-125	62 (48-75)	64 (55-72)	0.495	56 (48-63)	61 (51-79)	0.232	58 (49-64)	67 (47-92.4)	0.045	66.5 (57.8-78.3)	71 (58.8-87)	0.598
TBIL (µmmol/L)	0-26	13.40 (8.6-33.1)	11.5 (7.2-15.5)	0.279	13.4 (8.6-33.1)	11.8 (8.5-15.3)	0.264	10.9 (7.8-18.4)	7.6 (6.3-12.6)	0.115	9.4 (5.6-16.9)	12 (9.5-13.5)	0.562
INR	0.94-1.30	1 (0.94-1.11)	0.98 (0.91-1.12)	0.925	0.93 (0.85-0.98)	0.92 (0.85-0.95)	0.744	0.92 (0.9-0.99)	0.88 (0.87-0.93)	0.176	0.93 (0.88-0.97)	0.91 (0.88-0.95)	0.735

Normal reference range (NRR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), and international normalized ratio (INR).

Table 3-2. Patients with normal liver function within 1 week of admission.

		Week 0			Week 1			Week 2			Week 6		
	NRR	SC n=23	MM n=108	P	SC n=23	MM n=108	P	SC n=23	MM n=108	P	SC n=23	MM n=108	P
ALT (U/L)	0-50	25 (18-33)	23 (13-36)	0.515	22 (18-32)	19 (13-34.5)	0.632	30 (22-47.5)	28.5 (15-44)	0.206	18 (13.5-33.8)	26 (15-40)	0.25
AST (U/L)	0-50	28 (23-30)	25 (19-31)	0.091	24 (19-26)	21 (18-24)	0.197	21 (18-28.5)	20 (16-26)	0.394	19 (16-26)	23 (18-30)	0.07
GGT (U/L)	10-60	23 (16-33)	26 (15-41)	0.934	23 (18-29)	22 (15-38)	0.55	30 (21-46.5)	26.5 (16.8-53)	0.416	21 (15.5-26.8)	29 (17-46)	0.099
ALP (U/L)	45-125	61 (46-66)	63 (51-73)	0.17	52 (45-58)	59 (48.5-70)	0.006	54 (40-65)	59 (51-72.3)	0.064	61 (51.8-74)	62 (53-77)	0.602
TBIL (µmmol/L)	0-26	11.4 (7.2-15.2)	9.9 (7.0-15.3)	0.495	13.6 (8.1-17.7)	11.9 (9.4-15.6)	0.951	9.8 (6.4-19.3)	7.5 (5.9-10.2)	0.034	11 (6.5-13)	10.5 (8.4-14.1)	0.562
INR	0.94-1.30	1.01 (0.95-1.07)	0.98 (0.94-1.07)	0.702	0.95 (0.93-1.01)	0.93 (0.88-1.0)	0.123	0.95 (0.88-1.08)	0.93 (0.9-0.95)	0.505	0.91 (0.87-0.97)	0.91 (0.89-0.92)	0.91

Values are expressed as median (interquartile range (IQR), 25-75%). P value is the comparison between Severe, Critical (SC) and Mild, Moderate (MM) group patients.

patients and 17 MM patients with LFT abnormalities (Table 4). In the SC group, 6 (60%) patients had elevated GGT levels 1-2x ULN, and 4 (40%) patients had elevated GGT levels 2-5x ULN. In the MM group, 14 (82.4%) patients had elevated GGT levels 1-2x ULN, and 3 (17.6%) patients had elevated GGT levels 2-5x ULN (Table 4 and Figure 2). Only 1 patient had an abnormal ALP in MM group (1-2x ULN), and no patients had abnormal ALP levels in the SC group (Table 4 and Figure 2). Only 3 patients had an elevated TBIL in the SC group; 1 patient had a TBIL 1-2x ULN, and 2 patients had a TBIL 2-5x ULN (all 3 patients had an elevated ALT, but normal ALP and INR) (Table 4 and Figure 2). TBIL elevation was not observed in the MM group. All patients had a normal INR (Tables 3, 4 and Figures 1, 2). Patients who experienced elevations in ALT above 2x ULN received glycyrrhizin therapy, which is routinely used as a hepatoprotective agent in our institution. LFT abnormalities recovered in all patients, and median time to normalization was 10 days.

In our case series, the most significantly elevated ALT was observed in a patient with chronic HBV who was treatment-naïve, where the peak ALT was 414 U/L, with an AST of 309 U/L, GGT of 290 U/L, ALP of 86 U/L, and an elevated TBIL of 70.5 µmol/L, but normal INR (1.08). Further characterization of the HBV revealed the patient was HBsAg positive, HBeAg positive and the HBV DNA was elevated at 22,800 IU/mL. Given the significant elevation in HBV DNA,

the treating clinician felt that the LFT abnormalities were more likely attributable to their chronic HBV rather than COVID-19, and entecavir was commenced, in addition to glycyrrhizin therapy for 13 days. The LFTs normalized over the following 13 days in this patient.

DISCUSSION

COVID-19 can lead to symptoms including fever, cough, fatigue, shortness of breath and myalgias. In more severe disease, COVID-19 may cause significant shortness of breath, hypoxia and respiratory failure, as well as radiographic features of pneumonia and/or other lung infiltrates. Although the lung is the primary target organ of SARS-CoV-2, confirmed at autopsy and characterized by an inflammatory reaction in the deep airway and alveolar injury [24], there are several reports that COVID-19 may also cause liver function test abnormality [1, 16, 17, 25], however these case series are difficult to interpret due to frequent co-administration of hepatotoxic agents such as lopinavir /ritonavir and other conditions that may lead to liver injury such as ischaemic hepatitis from severe and/or prolonged hypotension/shock. In this report, we observed that LFT abnormality is frequent in COVID-19

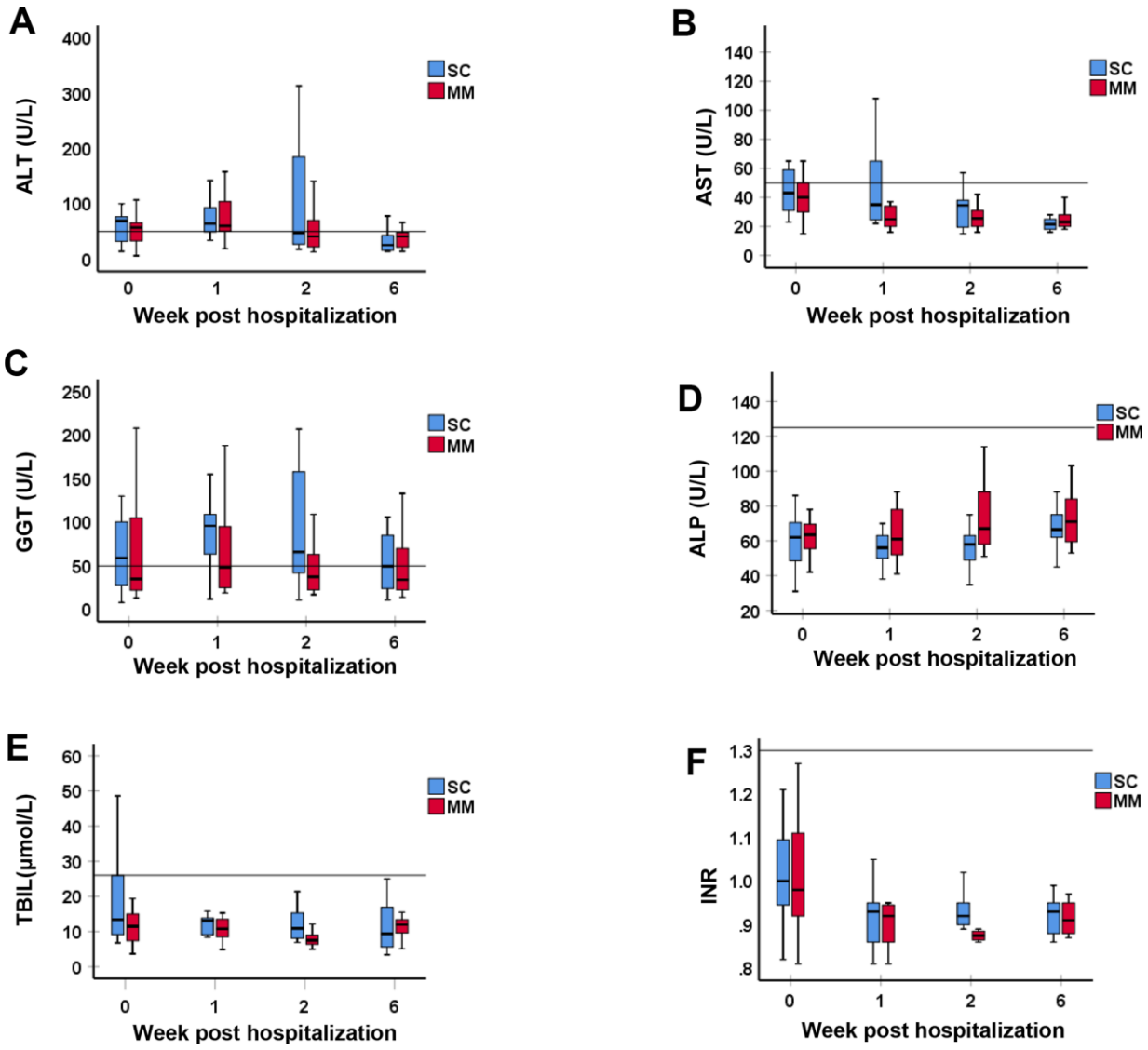

Figure 1. Comparison of liver function test between MM and SC patient groups with liver function test abnormality. The liver function tests including (**A**) ALT, (**B**) AST, (**C**) GGT, (**D**) ALP, (**E**) TBIL, and (**F**) INR, were compared between MM and SC patient groups with liver function test abnormality at Week 0, 1, 2 and 6 post hospitalization for COVID-19. Values are expressed as median (interquartile range (IQR), 25-75%). The horizontal line in each panel is the upper limit of normal (ULN) for each parameter. There was no statistically significant difference in any of the LFT or INR parameters between SC and MM patients.

Table 4. Degree of liver function test abnormality in SC and MM groups in the subset with liver abnormality.

	1-2 ULN		P value	2-5 ULN		P value	> 5ULN		P value
	SC	MM		SC	MM		SC	MM	
ALT (n, %)	4(11,36.4%)	9(17,52.9%)	0.057	4(11,36.4%)	7(17, 41.2%)	0.799	3(11,27.3%)	1(17,5.9%)	0.269
AST (n, %)	4(8,50%)	7(7,100%)	0.799	2(8,25%)	0	0.543	2(8,25%)	0	0.146
GGT (n, %)	6(10,60%)	14(17,82.4%)	0.112	4(10,40%)	3(17,17.6%)	0.264	0	0	a/n
ALP (n, %)	0	1(100%)	0.206	0	0	a/n	**0**	**0**	a/n
TBIL (n, %)	1(3,33.3%)	0	0.206	2(3,66.7%)	0	0.068	0	0	a/n
INR	0	0	a/n	0	0	a/n	0	0	a/n

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), and international normalized ratio (INR).
Values are expressed as median (interquartile range (IQR), 25-75%). P value is the comparison between severe/critical (SC) and mild/moderate (MM) patients.

patients hospitalized at the No. 2 People's Hospital of Fuyang City, with an overall frequency of LFT abnormality of 17.6% in the 159 patients with confirmed COVID-19. In addition, our study demonstrates that older patients are more likely to develop more severe COVID-19, which has been observed throughout the world, and are also more likely to develop LFT abnormality [18]. Furthermore, we observed a significantly higher proportion of patients with liver function test abnormality in the SC group compared to the MM group, suggesting a greater frequency of liver dysfunction in the SC group. These findings are consistent with other reports of COVID-19 in China [15, 25], and importantly demonstrates frequent LFT abnormality prior to the administration of potentially hepatotoxic agents. Our study therefore offers some interesting findings of liver involvement during COVID-19 infection.

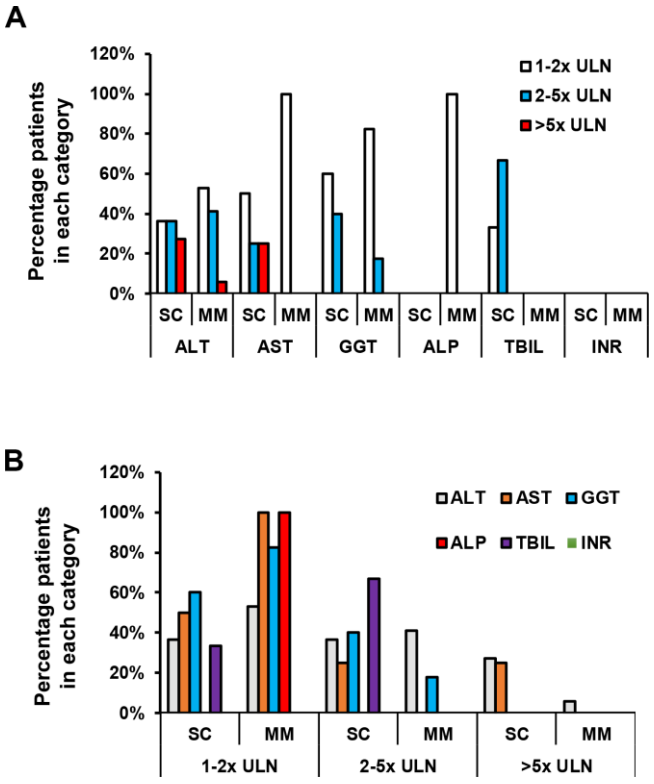

Figure 2. Degree of liver function test abnormality in SC and MM groups in the subset with liver function test abnormality. (**A**) Comparison of liver function abnormality between SC and MM groups with liver abnormality. (**B**) Comparison of liver function subset between SC and MM groups with liver abnormality. There was no statistically significant difference in the degree of LFT abnormality between SC and MM patients.

Liver dysfunction has been seen during other respiratory virus pandemics, although the incidence of LFT dysfunction was more severe with pandemic A/H1N1 influenza in 2009 than during this current COVID-19 outbreak [26], whereby serum levels of AST, ALT, and GGT were significantly higher in the A/H1N1 influenza than observed in COVID-19. Interestingly, abnormalities in serum liver enzymes were strongly correlated with hypoxemia in the A/H1N1 influenza pandemic, suggesting that influenza itself may in some way mediate the hepatotoxicity [26]. When comparing liver function test parameters between our SC and MM COVID-19 patients, we found that the SC patients had a higher incidence of liver injury to MM patients, however the pattern and degree of LFT abnormality was not significantly different between the two groups with regards to the proportion of patients with LFT abnormalities 1-2x ULN, 2-5x ULN and >5x ULN. We did observe that median ALT and AST values were numerically higher in SC patients compared to MM patients, with median ALT values above the ULN in the SC group, however this failed to reach statistical significance. These findings indicate that COVID-19 is associated with mild to moderate liver function test abnormalities with a mixed picture of liver injury,

particularly in SC patients. However, accompanying significant liver synthetic function compromise or liver failure were not observed in this cohort. In addition, our findings indicate that older patients are not only more likely to develop more severe COVID-19 but are also at greater risk of liver function abnormality.

It should be noted that the vast majority of the patients enrolled in this study received lopinavir/ritonavir with or without hydroxychloroquine as potential antiviral agents. Lopinavir/ritonavir is a well described to cause drug-induced liver injury (DILI). Therefore, we designed our study to restrict the definition of LFT abnormality to the first week following admission in order to limit potential confounding from hepatotoxicity from lopinavir/ritonavir. However, liver function parameters did not significantly change from baseline or week 1 to week 2, indicating that lopinavir/ritonavir-induced DILI does not explain our findings.

There are increasing reports of COVID-19 induced liver dysfunction in China, where mild elevations in liver functions tests have also been described [1, 15, 27, 28]. However, the mechanism by which COVID-19 induces liver function abnormality is not well characterized. There is much speculation regarding potential mechanisms, which include direct liver injury from SARS-CoV-2 infection of hepatocytes, cytokine storm syndrome, DILI and ischaemic hepatitis. We speculate that hepatocytes could be infected given SARS caused by SARS-CoV-1, another coronavirus similar to SARS-CoV-2, as SARS-CoV-1 RNA was detected in liver tissue from patients with SARS, although viral inclusions were not seen on electron microscopy [29].

Interestingly, non-specific histological features of microvascular steatosis and mild lobular and portal activity has been observed in the liver at autopsy in a patient who died of severe COVID-19 [27]. However, viral inclusions were not identified in liver tissue at autopsy and therefore it is unclear if these changes were related to direct viral infection of the liver by SARS-CoV-2, DILI, or even due to pre-existing fatty liver disease, although it should be noted that viral inclusions were also not seen in lung tissue (the primary target organ of COVID-19) in this patient. Another potential mechanism that has been considered is the effect of COVID-19 induced cytokine storm syndrome (CSS) on liver injury, but without strong evidence supporting this hypothesis. Liver damage may also be influenced by underlying liver diseases, such as chronic HBV and fatty liver disease, or as a result of pneumonia-associated hypoxia or ischaemic hepatitis from prolonged hypotension. These data highlight that further studies are required to elucidate the mechanism(s) of liver impairment in COVID-19.

In summary, we found that COVID-19 associated liver function test abnormality is more common in patients with severe or critical presentations of COVID-19, as well as older patients. Although the degree of COVID-19 induced liver function abnormality is relatively mild to moderate in our cohort without evidence of significant liver synthetic dysfunction or liver failure, it highlights the frequent incidence of LFT abnormalities in patients with COVID-19, which has implications for the management of these patients in order to preserve liver function with consideration of co-administration of hepatoprotective agents and to minimize exposure to hepatotoxic events, particularly in patients with underlying liver disease and older age. Our study adds to the growing body of evidence that SARS-CoV-2 is associated with liver function test abnormality, and particularly in older patients [18, 30, 31]. A more detailed understanding of the underlying mechanisms of liver injury from SAR-CoV-2, as well as viral pathogenesis and antiviral responses to COVID-19 are therefore required in order to best optimize older age patient outcomes.

MATERIALS AND METHODS

Patients and study design

As of March 4th, 2020, the No. 2 People's Hospital of Fuyang City has admitted 159 patients (including 4 patients transferred from Bozhou City, Anhui Province) with confirmed COVID-19 since the outbreak of the disease in Anhui Province in January 2020. No COVID-19 related deaths have been recorded in this hospital. The majority of the patients enrolled in this study received lopinavir/ritonavir with or without hydroxychloroquine for antiviral therapy. All COVID-19 patients were diagnosed, classified and treated according to the guidelines of the Pneumonia Treatment Plan for the Novel Coronavirus Infection, National Health and Health Commission of the people's Republic of China (Version 1-6) [19–22]. Patients with confirmed COVID-19 were included, and classification of severity criteria was as follows: 1) mild: mild clinical symptoms without pneumonia on imaging; 2) moderate: fever, respiratory tract infection symptoms with pneumonia on imaging; 3) severe: confirmed COVID-19 with one or more of the following 3 features: (a) breathing distress, respiratory rate ≥ 30 breaths/minute, (b) oxygen saturation ≤ 93% on room air, or (c) oxygenation index ≤ 300mmHg; 4) critical: confirmed COVID-19 with one or more of the following 3 features: (a) respiratory failure requiring mechanical ventilation, (b) coma, (c) combined organ failure

requiring ICU monitoring (for example, dysfunction/ failure of more than 2 organ systems that requires ICU support). Exclusion criteria included patients with respiratory symptoms that repeatedly tested negative for COVID-19 and did not have pneumonia on imaging, and those with new onset of liver dysfunction one week after hospitalization. This time point was chosen in order to exclude patients that may have developed abnormal LFTs from another cause such as ischaemic hepatitis from prolonged hypotension/shock or drug-induced liver injury. In this report, we sought to analyze the frequency of LFT abnormality and liver dysfunction in COVID-19 patients, and specifically to compare the incidence of LFT abnormality and liver dysfunction between COVID-19 patients with mild or moderate illness (MM group) and those with severe or critical illness (SC group). LFT abnormality is defined as any parameter of the liver enzyme panel greater than the upper limit of normal (ULN). We also evaluated liver synthetic function abnormality with International Normalized Ratio (INR) and elevated total bilirubin (TBIL).

Ethics approval and consent to participate

This study was approved by the Ethics Review Committee of the No. 2 People's Hospital of Fuyang City (reference number: 2020006) and was conducted in accordance with the ethical standards of the institutional and national research committees, and with the 1964 declaration of Helsinki. This study was registered at the Chinese Clinical Trial Registry (registration number ChiCTR2000031620).

COVID-19 detection and laboratory parameter testing

Nasopharyngeal aspirates and sputum from patients with suspected COVID-19 were used for COVID-19 testing. COVID-19 RNA was detected by using real-time quantitative PCR (qPCR) (Shanghai BioGerm Medical Biotechnology Co., Shanghai, China). Liver function was measured by using the Hitachi 7600 fully automatic biochemical analyzer. The complete blood count was measured by using the SYSMEX CA5100 automatic clotting analyzer (Siemens Healthcare, Erlangen, Germany). Internationalized Normalized Ratio (INR) was calculated based on the prothrombin time (PT) test result.

Statistical analyses

Data were analyzed using SPSS Statistics v25.0 (Armonk, New York, USA). Continuous data were expressed as medians with interquartile range, and categorical data as frequencies. Groups were compared using the Mann-Whitney U test, and the correlations between clinical, laboratory parameter were evaluated using the two-tailed chi-squared test.

AUTHOR CONTRIBUTIONS

Shasha Li, Jinsong Li, Tuo Shao, Xiuyong Li, Jacinta A. Holmes, Wenyu Lin and Mingfeng Han designed the research study, analyzed and interpreted the data, and wrote the manuscript. Shasha Li, Jinsong Li, Zhenhua Zhang, Lin Tan, Ming Li, Xiuyong Li and Mingfeng Han were involved in diagnosis and treatment of patients, recruiting patients and collection of clinical data. All authors were involved in critical appraisal of the manuscript and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors have declared that no conflicts of interest exist.

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Research Paper

COVID-19 mortality in Lombardy: the vulnerability of the oldest old and the resilience of male centenarians

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ABSTRACT

Italy was the first European nation to be affected by COVID-19. The biggest cluster of cases occurred in Lombardy, the most populous Italian region, and elderly men were the population hit in the hardest way. Besides its high infectivity, COVID-19 causes a severe cytokine storm and old people, especially those with comorbidities, appear to be the most vulnerable, presumably in connection to inflammaging. In centenarians inflammaging is much lower than predicted by their chronological age and females, presenting survival advantage in almost all centenarian populations, outnumber males, a phenomenon particularly evident in Northern Italy. Within this scenario, we wondered if: a) the COVID-19 mortality in centenarians was lower than that in people aged between 50 and 80 and b) the mortality from COVID-19 in nonagenarians and centenarians highlighted gender differences.

We checked COVID-19-related vulnerability/mortality at the peak of infection (March 2020), using data on total deaths (i.e. not only confirmed COVID-19 cases). Our conclusion is that excess mortality increases steadily up to very old ages and at the same time men older than 90 years become relatively more resilient than age-matched females.

INTRODUCTION

Italy was the first European country to suffer from the COVID-19 pandemic. A main characteristic of this pandemic, besides high infectivity of its causative agent, is a cytokine storm characterized by an IL-6 centered response [1, 2] and the uneven distribution of severity and mortality among different age classes. Indeed, old people, and particularly those with one or more comorbidity, appear to be the most vulnerable [3, 4]. The reasons of such a high vulnerability to COVID-19 is poorly understood but it has been suggested that a major role is played by inflammaging [5–7], i.e. the low-grade chronic inflammation that is a major driver of aging [8] and whose basic underlying mechanisms are shared with those responsible for frailty and age-related diseases (ARDs), including cardiovascular disease, type 2 diabetes, chronic obstructive pulmonary disease, chronic kidney disease and dementia, among others [9–11]. However, a major

characteristic of old people is their heterogeneity regarding not only their health status (presence/absence of comorbidities, frailty, cognitive status) but also, in particular, their different capability to mount an immune response to pathogens and vaccines [12, 13]. At present it is possible to quantify such heterogeneity using a variety of proteomic [15] and epigenetic biomarkers [16] capable of distinguishing between chronological and biological age, and to predict the risk of developing major ARDs. In particular, we showed that centenarians, i.e. subjects who avoided or largely postponed all major ARDs, and their offspring, are characterized by being healthier than age-matched controls born from non-long-living parents, i.e. a slower aging and are biologically younger than their chronological age of about 9 and 5 years, respectively. Particularly important within the scenario of COVID-19 pandemic is that centenarians have a peculiar state/degree of inflammaging, which is much lower than that predicted by their chronological age and is biased toward anti-inflammaging, i.e. the production of anti-inflammatory molecules and cells that the body produces lifelong as an adaptive, compensatory mechanisms to continuously down-regulate the inflammatory process and avoid its chronic detrimental effects [18–20]. Accordingly, the oldest old, including centenarians, are high-selected, exceptionally robust subjects that can be taken as a model of successful/healthy aging [21].

A major characteristic of human longevity is the ubiquitous female survival advantage. In particular, centenarian females outnumber males [22], and this demographic phenomenon is particularly evident in Northern Italy, including Lombardy [23]. However, although women live longer, they suffer greater morbidity, particularly late in life. In Trieste, a city situated in the North-East of Italy with 204,000 inhabitants, the prevalence of centenarians is high: in mid-June 2020 there were 148 centenarians, number obtained from the list of the public health service considering only subjects who have reached 100 years of age. In 2014 we started the Centenari a Trieste (CaT) Study, to examine the centenarians living in Trieste. From 2014 to January 2020 we enrolled, visited and collected data of 130 centenarians, using the annual lists provided by the public health service mentioned above. 90% of our centenarian population are women, but the few males are all in excellent health [24]. The complex reasons of such a female longevity advantage/paradox is still unclear [25, 26] but it is likely the result of a mixture of biological (e.g. genetics) and non-biological (e.g. cultural, anthropological) factors [27, 28].

Within this scenario, and considering that the population age 100 years and older is part of the fastest growing segment of the population worldwide, we thought worthwhile to check COVID-19-related vulnerability/mortality in old people across the above-mentioned large and heterogeneous age spectrum, focusing on nonagenarians and centenarians and gender, in Lombardy, the largest (10 million inhabitants) and most populous Italian Region, heavily affected by COVID-19 pandemic. To this regard, our study refers to the peak of infection (March 2020) when the number of (reported) infected people was 76,586 and the number of deaths was 11,399 (data from the Italian Civil Protection Department available at: http://opendatadpc.maps.arcgis.com/apps/opsdashboard/index.html-/b0c68bce2cce478eaac82fe38d4138b1).

The following questions/ hypotheses were addressed/ tested: i) is the COVID-19-related mortality of exceptionally long-living subjects, lower than that of people in the age-range between 50 and 80 years of age? ii) do the COVID-19-related mortality data show any gender difference in nonagenarians and centenarians?

RESULTS

Lombardy municipalities

During March 2020 a large increase in mortality was seen in Lombardy relative to previous years, both in absolute and in relative terms: against a background of 8492 deaths (mean of March deaths between 2015 and 2019), in 2020 there were 24,330 deaths, constituting an increase of 15,838 in absolute numbers and of 286% in percentage. Men contributed more to this increase with 9021 (57.0%) extra deaths. Increase in mortality is apparent in older age groups. In fact, while excess mortality under 40 years of age totalized less than 50 persons, its maximum was reached in the 80-84 and 85-89 age categories, with about 3300 more deaths each (Supplementary Figure 1).

When percent excess death by age class was plotted, a continuous increase in mortality by age was apparent (Figure 1, panel A). This phenomenon was clearly visible in both men (Figure 1, panel B) and in women (Figure 1, panel C), where March 2020 mortality is compared with mean March mortality of the previous years. However, the two patterns had also some differences: in women the increase resulted in approximately a doubling in mortality risk in each age class, whereas in men the greatest relative increase was in "younger" ages, were 2020 mortality was more than three times that of previous years, while in later ages the increase is about 80%. These different changes result in two different patterns of increase by age, i.e. women increase in excess mortality was lower in "younger"

ages, but reached that of men in later ages (Figure 1, panel D): while excess mortality under 90 was much higher in men, it was similar in the nonagenarians and in centenarians women even had a higher mortality.

We tested if the increase in mortality by age was different between men and women entering an interaction (age*sex) term in a logistic model: the effect was statistically significant ($p<0.0001$). We further refined the model entering age also as a quadratic term, together with its interaction with sex (age squared*sex): the interaction term resulted statistically significant ($p<0.0001$). This latter model was statistically better than the simpler one ($p<0.0001$). Both models indicated that the probability of dying was much higher in "young" men, but that at older ages the difference was less pronounced (simpler model) or even reversed (model with quadratic age).

Mortality in Trieste

Owing to the above-mentioned large heterogeneity of the health status of elderly subjects including nonagenarians and centenarians [12–14], and considering that morbidity, mortality and longevity outcomes are largely context-dependent [27, 28], it is interesting to look at what can be observed with a higher "granularity".

In March 2020 in Trieste there were 138 centenarians, 90% of them were women (Figure 2). 71 centenarians were tested with swab for COVID-19: three of them resulted positive but subsequently became negative at test and were therefore considered cured of COVID-19 infection. The remaining 68 centenarians tested negative for COVID-19: four of them died of old age from March to mid-June 2020.

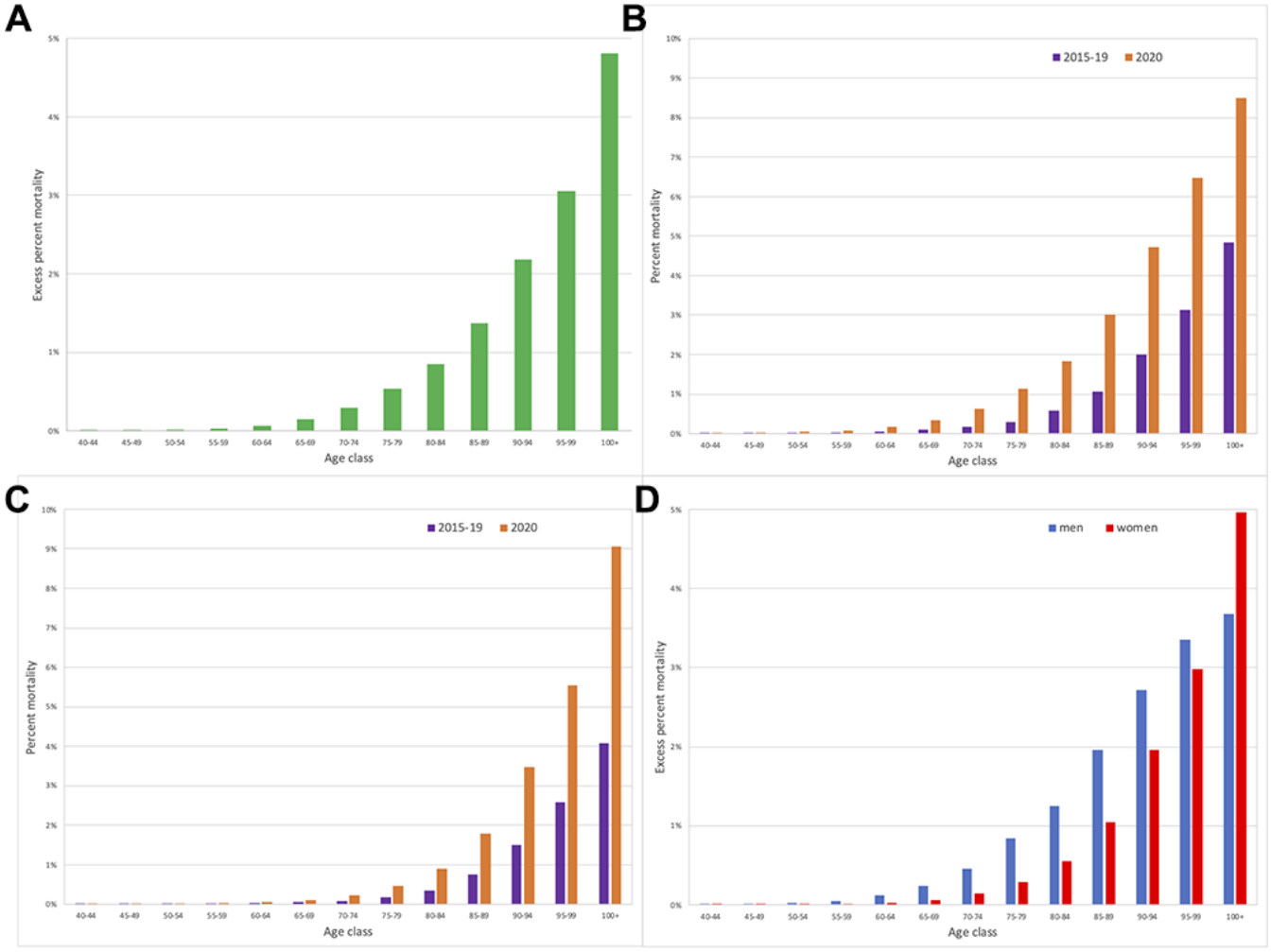

Figure 1. Mortality in March 2020 in Lombardy compared with mean mortality in March in 2015-2019. (**A**) Percent March 2020 excess mortality, by age class. (**B**) Men percent mortality by age class and year. (**C**) Women percent mortality by age class and year. (**D**) Percent March 2020 excess mortality by age class and sex.

As part of the CaT Study, from October 2019 to January 2020, immediately before medical emergency for COVID-19, we enrolled 42 centenarians using the list of the public health service, 39 women and 3 men (Figure 2). Six centenarians died before April 2020: five women for senectus and without symptoms related to COVID-19 infection. A man was admitted in a ward COVID-19 infected and was the only one dying with COVID-19 pathology. A woman of the 42 centenarians enrolled in the Study tested for COVID-19 was negative despite living in a nursing home with a COVID-19 outbreak, where most of the other elderly guests became positive. She was one of the centenarians belonging to the group of 68 tested negative and mentioned above.

DISCUSSION

The answer to the first question (is the COVID-19-related mortality of exceptionally long-living subjects lower than that of people in the age-range between 50 and 80 years of age?) is negative. In a region such as Lombardy which experienced a high SARS-CoV-2 infection rate, we found a continuous increase in mortality by age when percent excess death by age class was plotted. On the whole, nonagenarians and centenarians, despite their capability to survive until an extreme age and to avoid/postpone most of the ARDs, could be highly vulnerable during personal and societal stressful events like as seen during the SARS-CoV-2 pandemic. These data are in accord with the hypothesis that a major reason of such increasing vulnerability of the elderly, including nonagenarians and centenarians, to COVID-19 infection and related stressful conditions is inflammaging, the age-related increase of the inflammatory status which is particularly deleterious in those old subjects affected by one or more comorbidities. Inflammaging is a complex phenomenon at present only partially understood which can be highly different and personalized in different individuals [29]. Accordingly, the conceptual framework of inflammaging could help in understanding both the higher vulnerability of the elderly to COVID-19 but also the different responsiveness to COVID-19 infection and related contextual stressors in different subsets of elderly people.

The take home message is that nonagenarians and centenarians need particular attention, protection and special care in situations challenging the capability of hospitals, nursing homes and Health Service to cope with exceptional events like the COVID-19 pandemic.

However, when mortality is disentangled according to gender a peculiar gender-specific crossing emerged. The excess of mortality presumably due, directly or indirectly, to COVID-19 explosively grew in males from 50 years of age up to 80 years but thereafter the

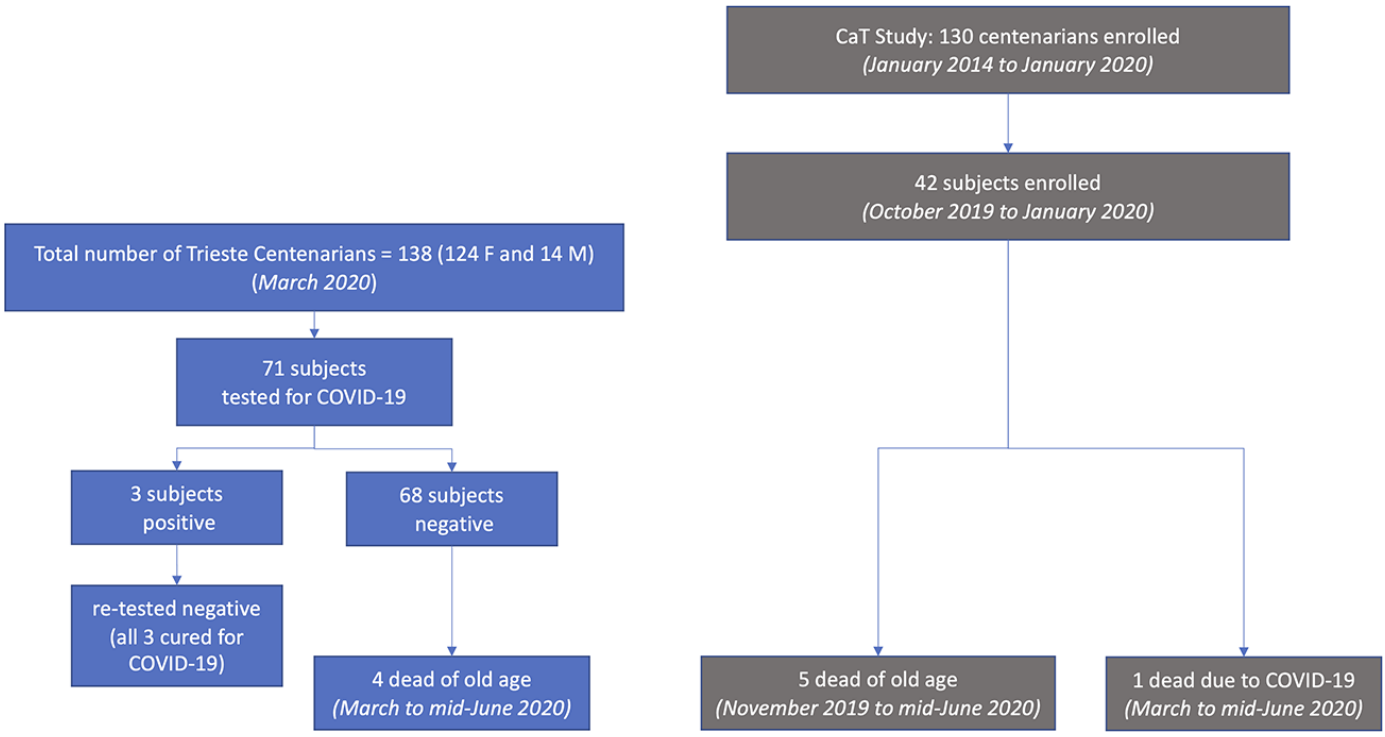

Figure 2. COVID-19 testing and deaths in Trieste (left) and in the CaT (Centenari a Trieste) Study (right).

rise tended to slow down. Females had a similar age trend, but their risk was lower in lower ages than in males and the decrease in higher age groups was less marked. Thus, very old people such as nonagenarian and centenarian males appears to be more resilient than age-matched females.

Accordingly, the answer to the second question (do the COVID-19-related mortality data show any gender difference in nonagenarians and centenarians?) is positive.

The reasons of such gender-specific trajectories of resilience are unclear. We reported that in men a genetic predisposition to produce high levels of IL-6 is detrimental for longevity [30]. In subjects with ages ranging from 22 to 93 years the age-related decline in adaptive immunity (particularly T cells) and especially the activation of innate immunity despite being present in both women and men were significantly greater in magnitude in men, suggesting that they experience a stronger inflammaging than women even when the subjects were otherwise healthy and clinically comparable in terms of age, BMI, and ethnicity [31]. Men have also a stronger inflammatory state in circulating monocytes compared to women [31]. Thus, men-specific immune characteristics interacting with/related to inflammaging, such as a blunted acquired immune system and type I interferon response, coupled with the downregulation of ACE2 (SARS-CoV-2 receptor) (particularly in patients with age-related comorbid diseases such as type II diabetes) and an accelerated biological aging (measured by epigenetic markers and telomere shortening), could help in explaining the higher vulnerability of men to COVID-19 infection [32].

To understand why men older than about 90 years become relatively more resilient than age-matched females it is important to consider the above-mentioned female-male health-survival paradox [25, 26, 33]. Indeed, despite women live longer than men and appear to be stronger even during severe famines and epidemics [34] when they became nonagenarians and centenarians show a much worse health status than that of nonagenarian and centenarian men who have a much better physical and cognitive health. The more years of life expectancy of women are mostly years of disease and disability [27, 28]. In any case, nonagenarians and centenarians are a mix of those aging well and those aging poorly, and in this heterogeneous scenario men capable of reaching age 90 and especially 100 are likely the more robust. Centenarian men are fewer but more selected and healthier and likely more resilient than centenarian women in highly stressful conditions like COVID-19 pandemic.

Finally, it is important to note that the two general conclusions of our study, i.e. the high COVID-19-related mortality of nonagenarians and centenarians and the relative resilience of male centenarians, resulting from epidemiological investigations can be at variance with anecdotal observations that centenarians and sometime supercentenarians (people over 110 years old) survived and recovered after COVID-19 infection. As an example, our data on a low number of very well characterized subjects of the CaT Study, suggest that both centenarian women and men looked strong during the peak of COVID-19 pandemic which profoundly challenged the entire health system and care of the elderly. What can be observed and reported at a higher magnification and higher granularity in single cities, institutions and settings is the consequence of the basic heterogeneity of the aging phenotype which is particularly evident at the extreme ages and suggests that outcomes may differ by robustness or other characteristics of the individual and are always highly diverse and context-dependent. To this regard, it can be predicted that the use of proteomic [15], epigenetic [16, 17] and glycomic biomarkers [35], among others, capable of distinguishing between chronological and biological age, will help in disentangling the heterogeneity of the aging phenotypes and in identifying the elderly characterized by an accelerated aging and lower robustness and thus at higher risk of morbidity and mortality in normal as well in exceptional circumstances such the COVID-19 pandemic.

Strength and limitations

We had access to open data provided by Istat, which despite being a non-representative subset of Italian municipalities covers the Lombardy population almost completely (about 97%). The data on the entire Italian population would have diluted the results here presented, owing to the much lower mortality in the other Italian regions.

We analyzed total mortality and not COVID-19-related deaths. This is both a limitation and a strength. Due to the great strain imposed on the Italian National Health Service, particularly in the hardest hit provinces, we cannot exclude an increase in general mortality due to a missing response to needs that would have been otherwise met. Even if this may not be excluded, we find difficult to think of logistical reasons that would differentially impact men and women and spare oldest men. Analyzing only confirmed COVID-19 deaths is more specific but, due to the impressive surge in mortality, only a part of those who died due to the infection were reported as being infected, and only a part of them was subject to a verification. Also, in absence of clear typical manifestations, a part of

COVID-19 mortality could be incorrectly attributed to other causes, even after a closer reanalysis, since swabs were only partially available, and their sensitivity is far from perfect.

In conclusion, we reported data that clearly show that old people, including nonagenarians and centenarians, suffered a high COVID-19-related mortality in the Lombardy region and suggested that the conceptual framework of inflammaging could help in understanding such age-related vulnerability. The remarkable difference between women and men in life expectancy, disability, mortality and longevity which emerged also in circumstances such as the COVID-19 pandemic is complex but still poorly understood and deserves attention and a closer scrutiny. Preventive strategies focused on the elderly preparing us better for the next pandemic are urgently needed [6].

MATERIALS AND METHODS

We used publicly available online data from the Istat (Italian Institute of Statistics) site: https://www.istat.it/it/archivio/240401 (accessed on June 15, 2020). Mortality raw data in a large dataset of Italian municipalities were collected by ANPR (National Registry of Resident Population) operated by the Ministry of the Interior. These data were successively merged with the dataset of the Registry Tax operated by the Ministry of Economy and Finance, validated and made available on-line by Istat.

Mortality data were made available for each day starting from January 1 to Apr 30, 2020 by municipality, 5-year age classes, and sex. Reference mortality data are available for the years 2015 to 2019, with same granularity.

Since we wanted to study the effect of the virus on mortality by age we concentrated on the Lombardy region, which presently (June 15, 2020) accounts for almost half of the confirmed COVID-19 deaths in Italy, and on the peak of infection (March 2020). Notably the dataset covers 97.1% of the Lombardy population.

Population in Italy (and as a consequence also in Lombardy) is gradually ageing, rendering impossible a direct comparison of 2020 deaths to 2015-2019 deaths. In order to correct for this imbalance we calculated the 2020 death percentage comparing the number of deaths within age classes with the respective age class populations, i.e. [(March 2020 number of deaths)/(March 2020 Lombardy population)]*100, for each age class, by sex. Reference 2015-2019 death percentage was calculated similarly as [(mean March 2015-2019 number of deaths)/(mean March 2015-2019 Lombardy population)] *100, for each age class, by sex. Percent excess mortality was calculated as a difference between 2020 mortality percentage and previous years mean mortality percentage. Lombardy population data was retrieved from the demo.istat.it site. Population data for 2020 is not available yet, so we used the data from the Istat population projections for 2020 available from same site.

We used logistic regression models in which age and sex were used as predictors for March 2020 probability of excess mortality, i.e. we disregarded "usual" (mean 2015-2019) number of March deaths. Age was modelled as a continuous factor, and age classes were given an intermediate value: for example 80-84 class was given an 82.5 value. Last class (100+) was given a 102.5 value. Models tried were hierarchically related: first only age and sex, then an interaction age*sex was entered into the model to test if the rate of increase was different between sexes. A quadratic effect, and its interaction was tried in a subsequent model.

The protocol of the CaT study to obtain, after informed consent, demographic, anamnestic, clinical and lifestyle data from the subjects enrolled in the study was already published [36].

JMP Pro v 15.0 (SAS Institute Inc.) was used to manage data and perform statistics.

Abbreviations

ACE: Angiotensin-converting-enzyme; ARD: age-related diseases; BMI: body mass index; CaT: Centenari a Trieste; COVID-19: coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus; IL: interleukin.

AUTHOR CONTRIBUTIONS

GM, GiuF, and MS collected data; MT performed data analysis; GM, CF and MT prepared draft manuscript; AN, CF, GiaF and GC critically reviewed manuscript; all the authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

No financial or conflicts of interest related to the manuscript.

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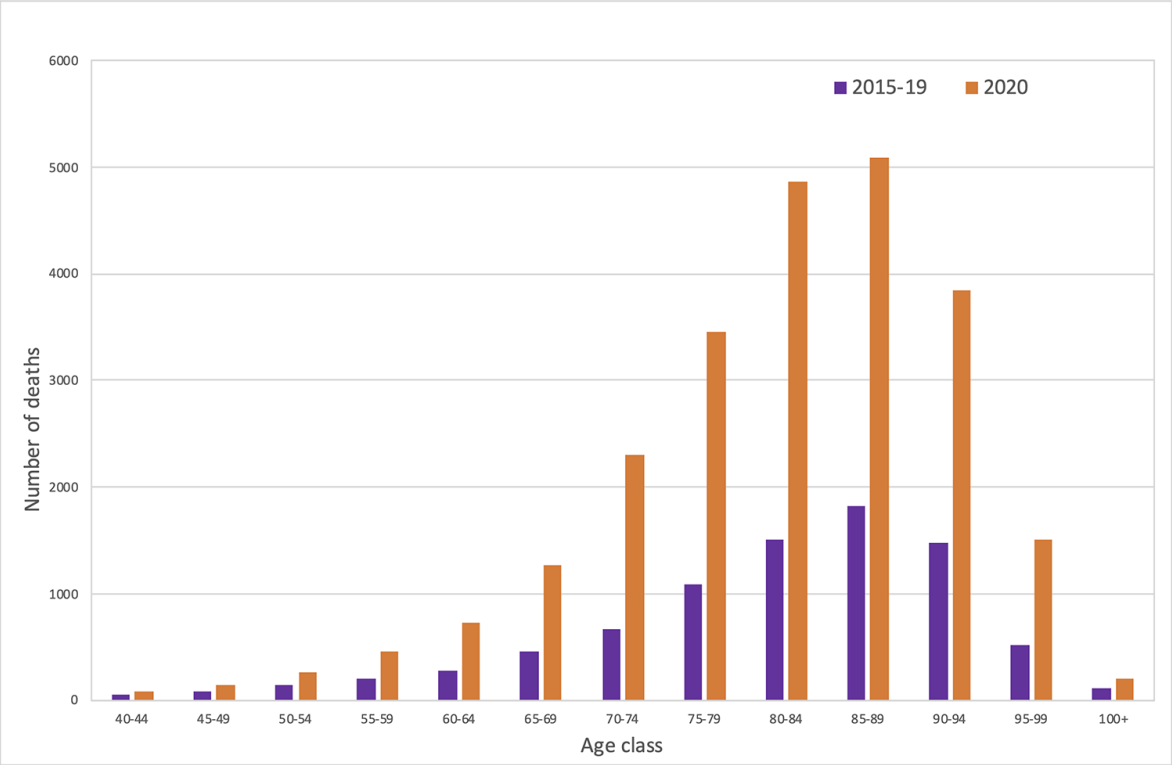
SUPPLEMENTARY MATERIAL

Supplementary Figure



Supplementary Figure 1. Total number of deaths in March in Lombardy, by age class and year.

Research Paper

Elevated Lactate Dehydrogenase (LDH) level as an independent risk factor for the severity and mortality of COVID-19

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ABSTRACT

Early identification of severe patients with coronavirus disease 2019 (COVID-19) is very important for individual treatment. We included 203 patients with COVID-19 by propensity score matching in this retrospective, case-control study. The effects of serum lactate dehydrogenase (LDH) at admission on patients with COVID-19 were evaluated. We found that serum LDH levels had a 58.7% sensitivity and 82.0% specificity, based on a best cut-off of 277.00 U/L, for predicting severe COVID-19. And a cut-off of 359.50 U/L of the serum LDH levels resulted in a 93.8% sensitivity, 88.2% specificity for predicting death of COVID-19. Additionally, logistic regression analysis and Cox proportional hazards model respectively indicated that elevated LDH level was an independent risk factor for the severity (HR: 2.73, 95% CI: 1.25-5.97; P=0.012) and mortality (HR: 40.50, 95% CI: 3.65-449.28; P=0.003) of COVID-19. Therefore, elevated LDH level at admission is an independent risk factor for the severity and mortality of COVID-19. LDH can assist in the early evaluating of COVID-19. Clinicians should pay attention to the serum LDH level at admission for patients with COVID-19.

INTRODUCTION

Since the end of 2019, Wuhan, China, has experienced an outbreak of coronavirus disease 2019 (COVID-19) caused by a novel coronavirus later named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. SARS-CoV-2 is an RNA virus that can be transmitted from person to person, and all people are susceptible to

this infection. At present, COVID-19 has progressed into a pandemic and become a major global health concern. It is reported that most cases are nonsevere type with a good prognosis; however, severe cases may deteriorate rapidly to multiple organ damage, impaired immune function and even death [2]. Therefore, early identification of severe COVID-19 is very important for individual or precise management, including antiviral, organ support and intensive care unit (ICU) care, to improve the prognosis.

Lactate dehydrogenase (LDH) is an intracellular enzyme involved in anaerobic glycolysis that catalyzes the oxidation of pyruvate to lactate [3]. Serum LDH is routinely tested in various diseases clinically. It has been reported that elevated serum LDH levels are associated with poor prognosis in various diseases, especially in tumors and inflammation [4–6]. To date, studies have shown that patients with severe COVID-19 have elevated serum LDH levels [7, 8], but no study has specifically evaluated its effect on the severity and mortality of COVID-19. Therefore, this multicenter retrospective, case-control study aimed to explore whether the serum LDH levels at admission can assist in evaluating the severity and mortality of COVID-19.

RESULTS

Results of propensity score matching and baseline of patients

Sex, age, hypoproteinemia or anemia, tumor history, chronic kidney disease, stroke history, hyperlipidemia, hypertension, diabetes, coronary heart disease, viral hepatitis, smoking and drinking were included as covariates in the logistic regression model of the propensity score matching. We matched 203 patients (128 nonsevere and 75 severe cases) from among 523 patients (424 nonsevere and 99 severe cases) with laboratory confirmed SARS-Cov-2 infection by propensity score matching. The quality assessment of the propensity score matching is shown in Supplementary Figure 1, and the comparison before and after propensity score matching is shown in Table 1. Overall, the results of propensity score matching were satisfactory. After propensity score matching, the difference in covariables between the nonsevere group and the severe group were controlled within no statistical differences (Table 1).

In the current study, 26 (5.0%) out of 523 patients before propensity score matching and 16 (7.9%) out of 203 patients after propensity score matching died of COVID-19. Considering that the patients were not continuously enrolled, we cannot calculate the case fatality rate.

Comparison of laboratory indicators between the nonsevere group and the severe group

We analyzed the levels of laboratory indicators at admission between nonsevere group and severe group. There were significant differences ($P<0.05$) in the levels of white blood cells (WBCs), neutrophils, lymphocytes, C-reactive protein (CRP), fibrinogen, D-dimer, creatine kinase and LDH between two groups (Figure 1 and Supplementary Table 1). Considering the relationship among laboratory indicators, we conducted Pearson correlation analysis on these laboratory indicators with significant differences. As a result, CRP and LDH exhibited powerful correlations with other indexes (Supplementary Table 2), which suggested that CRP and LDH were significant factors associated with the severity of COVID-19.

Role of the serum LDH in severity and death among COVID-19 cases

We performed ROC curves on the above laboratory indicators with significant differences to assess their value in patients with COVID-19. Lymphocyte counts were the most specific predictor (specificity 94.7%) for severe COVID-19, but with a low sensitivity of 20.3% (Table 2). In contrast, D-dimer had a high sensitivity (86.7%) but a very poor specificity (37.5%) in predicting severe COVID-19. Overall, serum LDH levels had an AUC of 0.76 (95% CI: 0.70 - 0.83) for predicting severe COVID-19, with a 58.7% sensitivity and 82.0% specificity, based on a best cut-off of 277.00 (U/L) (Table 2). However, there seems to be no significant difference between CRP and LDH in predicting severe COVID-19 (Figure 2).

The AUC values of the above indicators, even the CRP and LDH, were not very satisfactory. Therefore, we further analyzed the role of these indicators in predicting the mortality due to COVID-19. Unexpectedly, a cut-off of 91.39 mg/L for serum CRP levels had a sensitivity of 81.3% and a specificity of 88.2% for predicting death in patients with COVID-19 (Table 2). In addition, when the best cut-off of was 359.50 U/L, serum LDH levels had an AUC of 0.92 (95% CI: 0.84 - 0.99) for predicting death due to COVID-19, with a sensitivity of 93.8% and specificity of 88.2% (Table 2). Similarly, there was no significant difference in the ROC curve between CRP and LDH (Figure 3).

Elevated serum LDH as an independent risk factor for the severity of COVID-19

We detected the risk factors for the severity of COVID-19 by univariate and multivariate logistic regression

Table 1. Baseline of included patients.

	Before matching			After matching		
	Nonsevere (n=424)	Severe (n=99)	P values	Nonsevere (n=128)	Severe (n=75)	P values
Female	209(49.3%)	39(39.4%)	0.096	52(40.6%)	31(41.3%)	1.000
Age	51.45±15.08	61.54±13.36	<0.001	57.13±14.55	58.49±13.35	0.508
Hypoproteinemia or anemia	24(5.7%)	25(25.3%)	<0.001	13(10.2%)	13(17.3%)	0.208
Tumor history	8(1.9%)	1(1.0%)	0.861	2(1.6%)	1(1.3%)	1.000
Chronic kidney disease	10(2.4%)	7(7.1%)	0.039	6(4.7%)	3(4.0%)	1.000
Stroke history	8(1.9%)	11(11.1%)	<0.001	3(2.3%)	1(1.3%)	1.000
Hyperlipidemia	48(11.3%)	8(8.1%)	0.448	11(8.6%)	7(9.3%)	1.000
Hypertension	82(19.3%)	43(43.4%)	<0.001	44(34.4%)	25(33.3%)	1.000
Diabetes	61(14.4%)	23(23.2%)	0.045	32(25.0%)	18(24.0%)	1.000
Coronary heart disease	17(4.0%)	12(12.1%)	0.003	10(7.8%)	6(8.0%)	1.000
Viral hepatitis	7(1.7%)	1(1.0%)	0.99	3(2.3%)	1(1.3%)	1.000
Smoking	27(6.4%)	13(13.1%)	0.008	10(7.8%)	9(12.0%)	0.566
Drinking	28(6.6%)	16(16.2%)	0.002	12(9.4%)	10(13.3%)	0.628
Death	0	26(26.3%)	<0.001	0	16(21.1%)	<0.001

analysis. Neutrophils were excluded from logical regression analysis because neutrophils and leukocytes were collinear. In univariate analysis, high levels of WBC (HR: 2.32, 95% CI: 1.29, 4.16; P=0.005), CRP (HR: 4.91, 95% CI: 2.61-9.24; P<0.001), neutrophil-to-lymphocyte ratio (HR: 3.51, 95% CI: 1.93-6.39; P<0.001), fibrinogen, D-dimer (HR: 3.26, 95% CI: 1.60-6.64; P=0.001), creatine kinase and LDH (HR: 6.48, 95% CI: 3.40-12.34; P<0.001), and low levels of lymphocytes (HR: 4.53, 95% CI: 1.51-13.53; P=0.007) were risk factors for the severity of COVID-19 (Table 3). Furthermore, we took indicators that were P<0.1 in univariate logistic regression into multivariate logistic regression analysis. Of the 8 indicators, the P value of

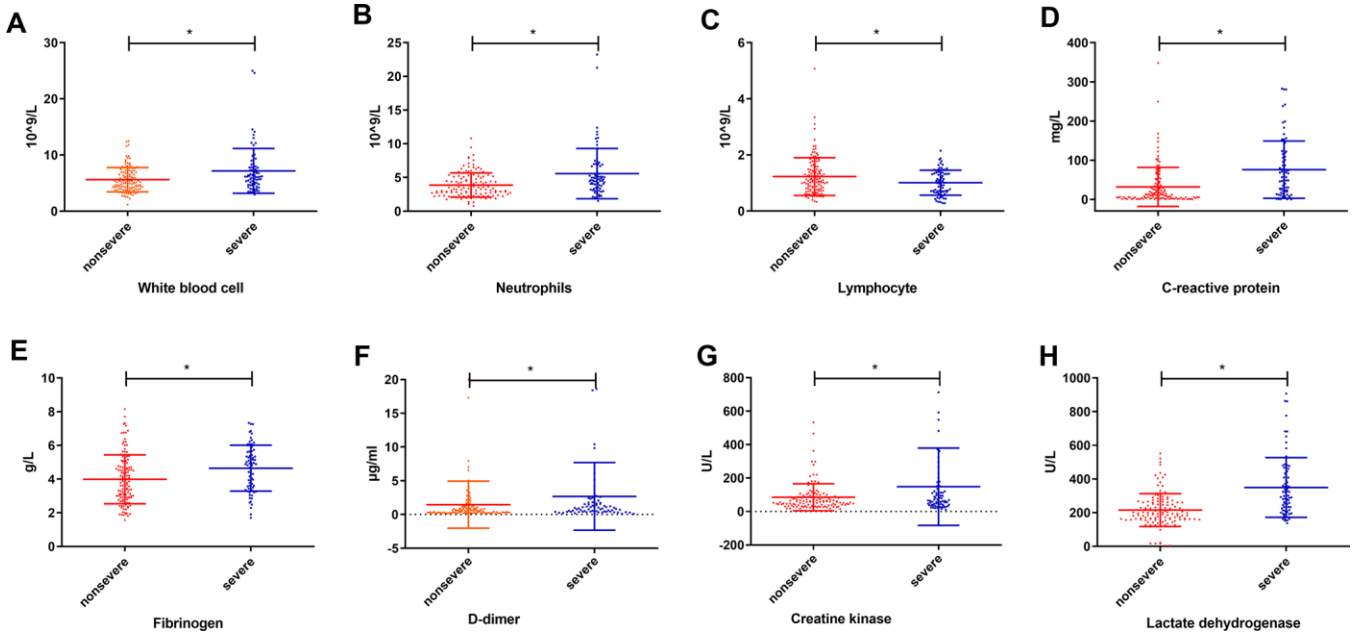

Figure 1. Levels (mean ± SD) of laboratory indicators at admission between the nonsevere group and severe group. (**A**) white blood cell; (**B**) neutrophils; (**C**) lymphocyte; (**D**) c-reactive protein; (**E**) fibrinogen; (**F**) d-dimer; (**G**) creatine kinase; (**H**) lactate dehydrogenase. * P<0.05.

Table 2. Role of laboratory indicators in predicting the severity and death of COVID-19.

	Predicting severity of COVID-19				Predicting death of COVID-19			
	AUC	Best cut-off *	Sensitivity	Specificity	AUC	Best cut-off *	Sensitivity	Specificity
WBC	0.63±0.04	5.65 (×10^9/L)	0.627	0.594	0.78±0.07	7.45(×10^9/L)	0.688	0.797
Neutrophils	0.66±0.04	3.85 (×10^9/L)	0.707	0.586	0.82±0.05	4.87(×10^9/L)	0.813	0.711
Lymphocyte	0.58±0.04	1.72 (×10^9/L)	0.203	0.947	0.76±0.06	0.73(×10^9/L)	0.759	0.750
NLR	0.68±0.04	3.83	0.640	0.660	0.87±0.06	7.42	0.750	0.900
CRP	0.73±0.04	20.14 (mg/L)	0.747	0.625	0.89±0.05	91.39 (mg/L)	0.813	0.882
Fibrinogen	0.64±0.04	4.79 (g/L)	0.533	0.758	0.69±0.06	3.96 (g/L)	0.875	0.497
D-dimer	0.65±0.04	0.33 (μg/ml)	0.867	0.375	0.80±0.06	1.09 (μg/ml)	0.813	0.706
CK	0.55±0.04	109.50 (U/L)	0.347	0.812	0.62±0.08	120.50 (U/L)	0.438	0.818
LDH	0.76±0.04	277.00 (U/L)	0.587	0.820	0.92±0.05	359.50 (U/L)	0.938	0.882

* Chosen by maximizing the Youden index. Abbreviations: AUC, area under the curve; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; CRP, c-reactive protein; CK, creatine kinase; LDH, lactic dehydrogenase.

the serum LDH levels was still less than 0.05, which suggested that elevated serum LDH (HR: 2.73, 95% CI: 1.25-5.97; P=0.012) is an independent risk factor for the severity of COVID-19 (Table 3).

Elevated serum LDH as an independent risk factor for mortality of COVID-19

We applied the Cox proportional hazards model to evaluate the effect of LDH on the survival time of patients. In univariable Cox regression analysis, male sex (HR: 3.04, 95%: CI 0.87-10.65; P=0.083) and age older than 60 years (HR: 5.88, 95% CI: 1.33-25.90, P=0.019) had a significant effect on the survival time of patients. In addition, elevated serum WBC count (HR: 8.06, 95% CI: 2.8-23.23; P<0.001), neutrophil-to-lymphocyte ratio (HR: 21.11, 95% CI: 6.80-65.51; P<0.001), CRP (HR: 24.06, 95% CI: 6.85-84.50; P<0.001), fibrinogen, D-dimer, CK, LDH (HR: 77.20, 95% CI: 10.20-584.61; P<0.001) and reduced lymphocyte counts were risk factors of mortality (Table 4). We take indicators that were P<0.1 in univariate logistic regression into multivariate logistic regression analysis. We found that the elevated serum LDH (HR: 40.50, 95% CI: 3.35-449.28; P=0.003) remained an independent risk factor for the mortality of COVID-19 (Table 4).

DISCUSSION

In this study, we identified that elevated serum LDH level was an independent indicator for predicting severity and mortality in patients with COVID-19 for the first time. Based on ROC analysis, serum LDH

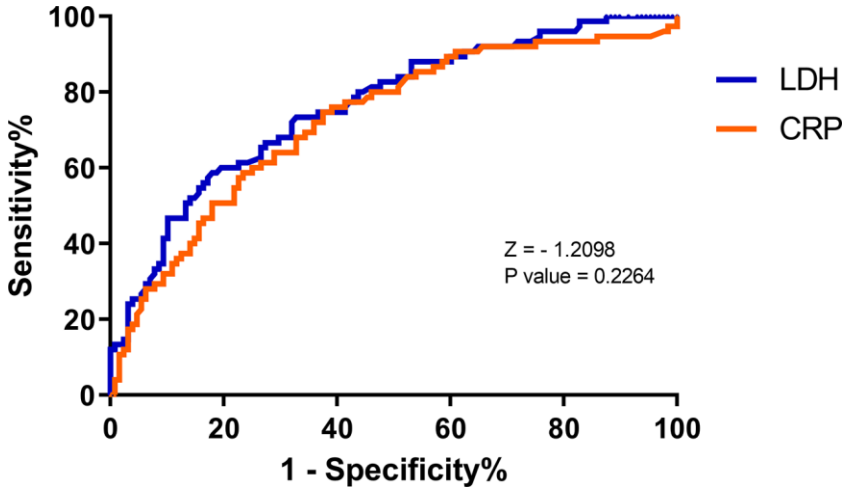

Figure 2. Receiver operating characteristic (ROC) curve for predicting severity of COVID by C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels at admission. LDH: AUC 0.76 ± 0.04, cut-off 277.00 U/L, sensitivity 58.7%, specificity 82.0%. CRP: AUC 0.73 ± 0.04, cut-off 20.14 mg/L, sensitivity 74.7%, specificity 62.5%.

levels at admission had high specificity for predicting the severity of COVID-19 and a satisfactory sensitivity and specificity for predicting death due to COVID-19. Furthermore, logistic regression analysis and Cox proportional hazards model revealed that elevated serum LDH at admission to be an independent risk factor for the severity and mortality of COVID-19.

We regarded sex, age, hypoproteinemia or anemia, tumor history, chronic kidney disease, stroke history, hyperlipidemia, hypertension, diabetes, coronary heart disease, viral hepatitis, smoking and drinking as covariates in the logistic regression model of the propensity score matching, because these covariates may have an impact on the severity and mortality of COVID-19 [9–11]. Autoimmune and inflammatory diseases do have an impact on the severity and mortality of COVID-19. We did not include autoimmune and inflammatory diseases in the logistic regression model of the propensity score matching because there were no patients diagnosed with autoimmune and inflammatory diseases in the enrolled patients. After propensity score matching, the differences in covariables between the nonsevere group and the severe group were controlled at almost the same levels. Controls for confounding factors were the premise of this study, ensuring the reliability of the conclusions.

As suggested by comparison of laboratory indicators, there were significant differences in the levels of WBC, neutrophils, lymphocytes, CRP, fibrinogen, D-dimer, creatine kinase and LDH between nonsevere and severe groups. The differences in these indicators were very similar to those reported by Huang et al. [12]. Notably, LDH showed a powerful correlation with the other indexes by Pearson correlation analysis, which suggested that LDH was a significant factor associated with the severity of patients with COVID-19. When the body experiences acute hypoxia or inflammation, the level of LDH in serum will rise significantly. COVID-19, caused by SARS-Cov-2 infection, mainly involves in the lungs, as well as other tissues and organs [13, 14], leading to hypoxia, thrombogenesis, inflammation and organ injury. Theoretically, elevated serum LDH is an important laboratory indicator for evaluating COVID-19 [15].

In this study, male sex and age older than 60 years old had obvious effects on death due to COVID-19. We found that patients who were aged over 60 years (HR: 5.88, 95% CI: 1.33-25.90, P=0.019) and male (HR: 3.04, 95%: CI 0.87-10.65; P=0.083) were more likely to expire, as suggested by the univariate Cox proportional hazards model. This obtained similar general conclusions as previous studies [16, 17]. However, the effect of age and sex on death due to COVID-19 was reduced in multivariate Cox regression because the risk of age and sex was adjusted for other factors.

Elevated serum LDH as an independent risk factor for COVID-19 is the main conclusion of this study. In univariate analysis, high WBC, NLR, CRP, fibrinogen, D-dimer, creatine kinase and LDH, and low lymphocyte were not only risk factors for severity but also risk indicators for death among patients with COVID-19 (Table 3 and Table 4). Additionally, in multivariate analysis, elevated serum LDH remained an independent risk factor for COVID-19 severity and mortality. A previous study [17], which did not mention the influence of LDH on COVID-19, proved that NLR is an

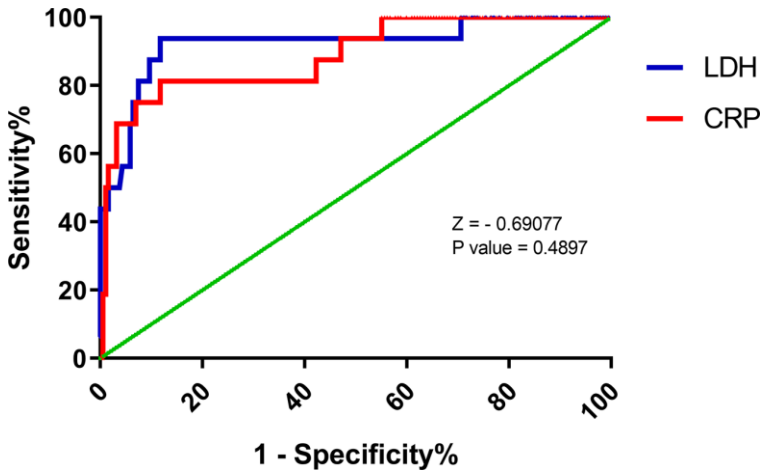

Figure 3. Receiver operating characteristic (ROC) curve for predicting death (B) of COVID by C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels at admission. LDH: AUC 0.92 ± 0.05, cut-off 359.50 U/L, sensitivity 93.8%, specificity 88.2%. CRP: AUC 0.89 ± 0.05, cut-off 91.39 mg/L, sensitivity 81.3%, specificity 88.2%.

Table 3. Uni- and multivariate logistic regression analyses of risk factors for the severity of COVID-19.

Variables	Univariate logistic regression		Multivariate logistic regression	
	P value	Hazard ratio (95%CI)	P value	Hazard ratio (95%CI)
WBC* (> 5.65×10^9/L)	0.005	2.32 (1.29, 4.16)	0.056	2.01 (0.98, 4.09)
Lymphocyte* (< 1.72×10^9/L)	0.007	4.53(1.51, 13.53)	0.240	2.09 (0.61, 7.15)
NLR* (>3.83)	< 0.001	3.51 (1.93, 6.39)	0.633	1.21 (0.55, 2.64)
CRP* (> 20.14 mg/L)	< 0.001	4.91(2.61, 9.24)	0.109	1.93 (0.86, 4.31)
Fibrinogen* (> 4.79 g/L)	< 0.001	3.58(1.95, 6.57)	0.257	1.54 (0.73, 3.22)
D-dimer* (> 0.33 μg/ml)	0.001	3.26(1.60, 6.64)	0.398	1.43 (0.62, 3.29)
CK* (> 109.50 U/L)	0.012	2.30(1.20, 4.41)	0.364	1.43 (0.66, 3.08)
LDH* (> 277.00 U/L)	< 0.001	6.48(3.40, 12.34)	0.012	2.73(1.25, 5.97)

*Take the best cut-off for predicting the severity of COVID-19 as the boundary value of binary variable. Abbreviations: WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; CK, creatine kinase; LDH, lactic dehydrogenase.

Table 4. Uni- and multivariate Cox regression analyses of risk factors for the death due to COVID-19.

Variables	Univariate Cox regression		Multivariate Cox regression	
	P value	Hazard ratio (95%CI)	P value	Hazard ratio (95%CI)
Sex (male)	0.083	3.04 (0.87, 10.65)	0.876	1.13 (0.25, 5.14)
Age (> 60)	0.019	5.88 (1.33, 25.90)	0.914	1.12 (0.15, 8.13)
WBC* (> 7.45×10^9/L)	< 0.001	8.06 (2.80, 23.23)	0.245	2.46 (0.54, 11.19)
Lymphocyte* (< 0.73×10^9/L)	< 0.001	7.47 (2.41, 23.18)	0.843	1.17 (0.24, 5.71)
NLR* (>7.42)	< 0.001	21.11 (6.80, 65.51)	0.131	4.33 (0.65, 28.95)
CRP* (> 91.39 mg/L)	< 0.001	24.06 (6.85, 84.50)	0.558	1.82 (0.25, 13.52)
Fibrinogen* (> 3.96 g/L)	0.016	6.19 (1.41, 27.21)	0.846	1.23 (0.15, 9.76)
D-dimer* (> 1.09 μg/ml)	0.001	8.67 (2.47, 30.45)	0.476	0.51 (0.08, 3.22)
CK* (> 120.50 U/L)	0.023	3.14 (1.17, 8.42)	0.827	1.13 (0.37, 3.41)
LDH* (> 359.50 U/L)	< 0.001	77.20 (10.20, 584.61)	0.003	40.50(3.65, 449.28)

*Take the best cut-off for predicting death due to COVID-19 as the boundary value of binary variable. Abbreviations: WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; CK, creatine kinase; LDH, lactic dehydrogenase.

independent risk factor for in-hospital mortality in COVID-19. Therefore, we included the NLR in Cox proportional hazards model. However, in our study, we proved that LDH was a more independent risk factor compared with NLR as suggested by multivariate Cox regression (Table 4).

There are some limitations in this study that should be noted. Firstly, the number of subjects included is to some extent small which limits the statistical power of this study. Nonetheless, the sample size of this study was sufficient to draw our conclusion. Secondly, on a whole, 16 out of 203 patients died of COVID-19 in this study. Considering the small number of deaths, we performed Cox regression instead of logistic regression to analyze the effect of LDH on COVID-19 mortality. Although the 95% confidence interval of HR is slightly lager, it is enough to ensure that elevated serum LDH is an independent risk indicator for death due to COVID-19. Thirdly, although we have controlled the bias by propensity score matching, multiple potential confounders might not have been fully considered. A small number of patients have taken antiviral drugs, antihypertensive drugs, and antidiabetic drugs prior to admission, the effect of past medical history on the results were not studied.

In conclusion, this study revealed that serum LDH at admission was useful in evaluating the disease severity and in-hospital mortality among patients with COVID-19. Further studies are needed to confirm our findings.

MATERIALS AND METHODS

Study design and participants

We collected data for 523 adult patients admitted to the hospital with laboratory confirmed SARS-Cov-2 infection in 4 designated tertiary hospitals in Hubei

Province, including 2 in Wuhan city and 2 in cities outside Wuhan, Hubei Province, from January 22, 2020 to March 14, 2020. We divided all these 523 patients into two groups: a severe group (severe type and critical severe type of COVID-19) and a nonsevere group (mild type and moderate type of COVID-19).

Considering that this study is a retrospective study, we used propensity score matching [18] to reduce biases and confounders. Ultimately, 203 patients with COVID-19 (75 patients in the severe group and 128 patients in the nonsevere group) were included.

Inclusion and exclusion criteria

Patients who met all the following criteria were included: (1) ≥18 years old, male or female; (2) laboratory confirmed SARS-Cov-2 infection; (3) complete clinical, laboratory, imaging and outcome data. Patients younger than 18 years old, with uncomplete clinical information because of transferring to other designated hospitals were excluded.

Ethical considerations

This study was approved by the Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University (ZDWY2020-K173-1). Written informed consent was waived by the Ethics Committee in consideration of the designated hospital for emerging infectious disease.

Data collection

The data included basic clinical information, diagnosis, comorbidity, and laboratory data at admission including routine blood examination, liver and renal function, myocardial enzyme, blood coagulation, procalcitonin (PCT), CRP and LDH. Additionally, neutrophil-to-lymphocyte ratio (NLR) was calculate. All these data were obtained with a standardized data collection form created by EpiData software (version 3.1). All data were checked by two physicians (Lingling Wang and Jianfang Ye) and a third researcher (Yameng Fan) adjudicated any difference in interpretation between the two primary reviewers.

Diagnosis and classification of COVID-19

COVID-19 was diagnosed and classified according to the newest "Guidelines for the Diagnosis and Treatment of COVID-19 (Trial Version 7)" [19] by the National Health Commission in China (http://www.nhc.gov.cn/). Clinical condition classification criteria are as follows: (1) mild type - clinical symptoms were mild, and no radiological changes; (2) moderate type - fever, respiratory tract or other symptoms, and pneumonia can be seen on imaging; (3) severe type - respiratory rate ≥ 30 times per minute, or the oxygen saturation is lower than 93% at rest state, or the ratio of arterial partial pressure of oxygen (PaO_2) and fraction of inspired oxygen (FiO_2) is lower than 300 mmHg (altitude below 1000 meters), or pulmonary imaging indicate that lung damage deteriorates rapidly within 24 to 48 hours; (4) critical severe type - respiratory failure requiring mechanical ventilation, or signs indicating shock, or multiple organ failure requiring admission to the intensive care unit.

Statistical analysis

Propensity score matching was performed using open source R software (version 3.6.3, Vienna, Austria) based on the "MatchIt" package [20]. The calipers value was set to 0.03, the matching ratio was 1:2, and the matching method was "nearest". Statistical analysis was performed using IBM SPSS software (version 25.0, Chicago, USA). Statistical charts were generated using GraphPad Prism software (version 8.0, San Diego, USA). The statistical results are presented as mean ± standard deviation. Continuous data were analyzed by the Student's t-tests, and the Levene test was used to decide homogeneity of variance. The receiver operating characteristic (ROC) curve, sensitivity, specificity and area under the curve (AUC) were measured to evaluate the levels of laboratory indicators in predicting the severity and mortality of COVID-19. Differences between AUCs were detected by the Z-test. All indicators were further tested by univariate and multivariate logistic regression or Cox regression analysis. The hazard ratio (HR) and 95% confidence intervals (95% CI) are shown. P value less than 0.05 was considered statistically significant.

AUTHOR CONTRIBUTIONS

Jianfang Ye designed the idea and drafted the manuscript. Chang Li, Weihua Hu, Zaishu Chen, Wei Xiao, and Qijian Chen provided the clinical data. Jianfang Ye, Lingling Wang, Yameng Fan, Zhanjin Lu, Shiyan Chen, Wei Xiao, Zaishu Chen and Junlu Tong collected the data for this study. Jianfang Ye, Lingling Wang and Yameng Fan checked the data for this study. Jie Chen performed the statistical analysis. Jin Mei and Hongyun Lu guided the entire process of this study and checked the final manuscript.

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We acknowledge all health-care workers involved in the diagnosis and treatment of patients in Hubei Province.

CONFLICTS OF INTEREST

All these authors declare no conflicts of interest.

FUNDING

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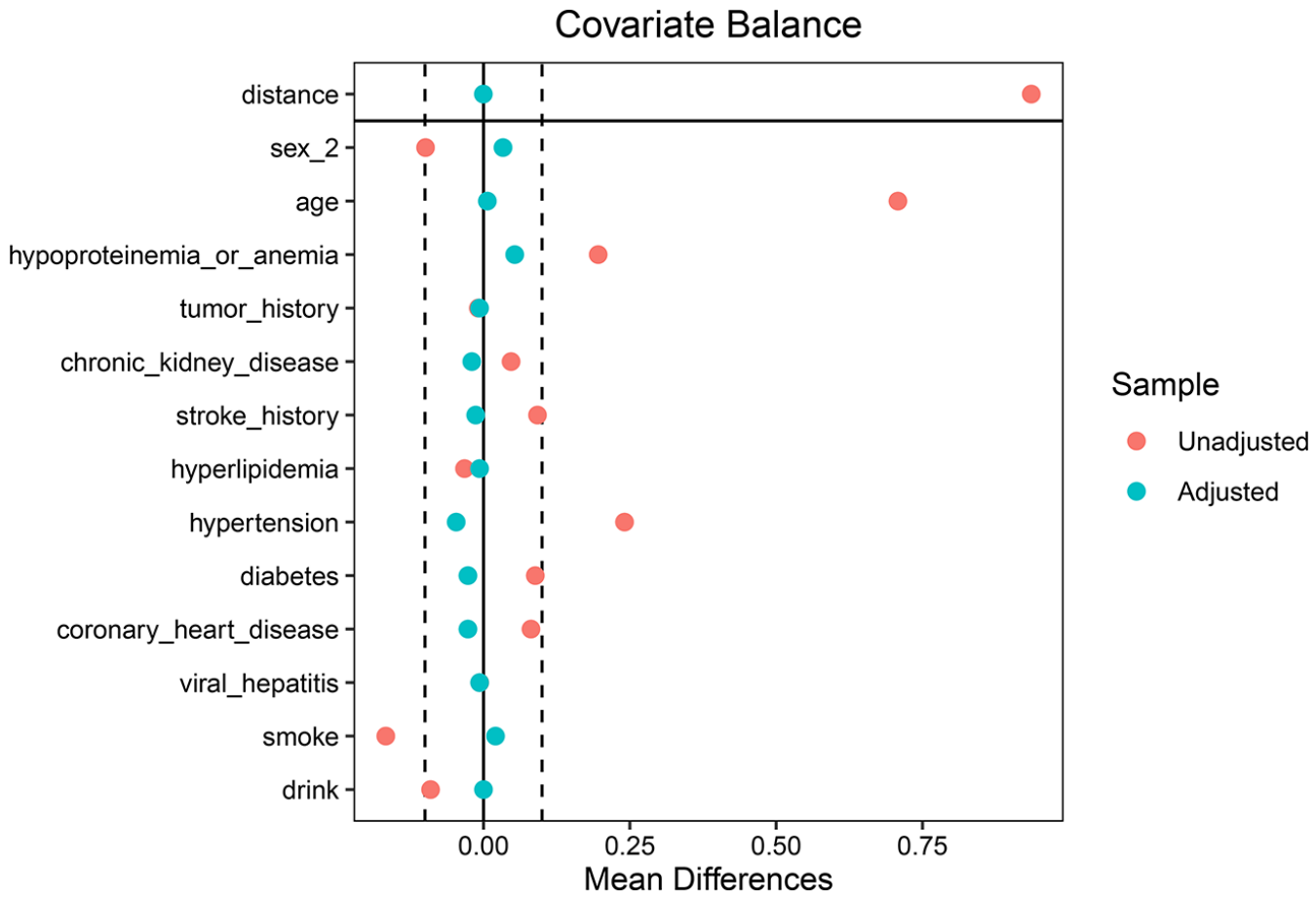
SUPPLEMENTARY MATERIALS

Supplementary Figure



Supplementary Figure 1. Mean differences in covariate balance before and after being adjusted.

Supplementary Tables

Supplementary Table 1. Laboratory indicators at admission between the nonsevere group and severe group.

	Nonsevere (n=128)	Severe (n=75)	P value*
WBC ($\times 10^9$/L)	5.61±2.16	7.17±3.99	0.002
Neutrophils ($\times 10^9$/L)	3.87±1.81	5.57±3.73	<0.001
Lymphocyte ($\times 10^9$/L)	1.23±0.67	1.01±0.45	0.014
NLR	3.93±3.17	7.2±6.41	<0.001
RBC ($\times 10^{12}$/L)	4.28±0.57	4.41±0.56	0.113
Platelet($\times 10^9$/L)	224.34±103.38	214.08±83.01	0.465
Albumin (g/L)	37.47±5.77	36.12±6.04	0.115
TBIL (μmol/L)	12.38±7.58	13.49±6.89	0.250
DBIL (μmol/L)	4.42±5.63	5.02±3.21	0.401
ALT (U/L)	35.49±32.48	35.61±29.96	0.980
AST (U/L)	33.54±22.04	37.60±22.39	0.209
Creatinine(μmol/L)	83.53±127.53	100.44±150.76	0.395
TG (mmol/L)	1.47±1.11	1.43±0.69	0.814
TC (mmol/L)	4.00±0.99	3.83±0.99	0.261
UA (μmol/L)	272.97±104.19	280.56±113.09	0.628
PCT (ng/mL)	0.20±0.70	0.32±0.90	0.296
CRP (mg/L)	31.84±49.83	75.52±73.09	<0.001
Fibrinogen (g/L)	3.99±1.45	4.65±1.36	0.002
D-dimer (μg/ml)	1.45±3.50	2.69±5.01	0.041
CK (U/L)	85.37±80.53	148.48±231.03	0.025
LDH (U/L)	215.23±97.36	349.28±177.60	<0.001

*Data were analyzed by Student's t-tests and Levene test was used to evaluate homogeneity of variance. Abbreviations: WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; RBC, red blood cell; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; TC, total cholesterol; UA, uric acid; PCT, procalcitonin; CRP, c-reactive protein; CK, creatine kinase; LDH, lactic dehydrogenase.

Supplementary Table 2. Pearson correlation coefficient among levels of laboratory indicators.

	WBC	Neutrophils	Lymphocyte	CRP	fibrinogen	D-dimer	CK	LDH
WBC	1.00							
Neutrophils	0.96**	1.00						
Lymphocyte	0.22**	- 0.01	1.00					
CRP	0.31**	0.37**	- 0.37**	1.00				
Fibrinogen	0.13	0.21**	- 0.40**	0.54**	1.00			
D-dimer	0.17*	0.23**	- 0.20**	0.29**	0.04	1.00		
CK	0.02	0.00	- 0.08	0.20**	0.05	- 0.07	1.00	
LDH	0.34**	0.41**	- 0.36**	0.63**	0.34**	0.33**	0.40**	1

*There was a statistical difference at the level of $P < 0.05$. ** There was a statistical difference at the level of $P < 0.01$.
Abbreviations: WBC, white blood cell; CRP, C-reactive protein; CK, creatine kinase; LDH, lactic dehydrogenase.

Research Paper

Risk factors for severe cases of COVID-19: a retrospective cohort study

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ABSTRACT

Background: SARS-CoV-2 has raged around the world since March, 2020. We aim to describe the clinical characteristics and risk factors of severe patients with COVID-19 in Guangzhou.

Results: The severity and mortality of COVID-19 was 10.4% and 0.3% respectively. And each 1-year increase in age (OR, 1.057; 95% CI, 1.018-1.098; P=0.004), Wuhan exposure history greater than 2 weeks (OR, 2.765; 95% CI, 1.040-7.355; P=0.042), diarrhea (OR, 24.349; 95% CI, 3.580-165.609; P=0.001), chronic kidney disease (OR, 6.966; 95% CI, 1.310-37.058; P = 0.023), myoglobin higher than 106 μg/L (OR, 8.910; 95% CI, 1.225-64.816; P=0.031), white blood cell higher than 10×10^9/L (OR, 5.776; 95% CI, 1.052-31.722; P=0.044), and C-reactive protein higher than 10 mg/L (OR, 5.362; 95% CI, 1.631-17.626; P=0.006) were risk factors for severe cases.

Conclusion: Older age, Wuhan exposure history, diarrhea, chronic kidney disease, elevated myoglobin, elevated white blood cell and C-reactive protein were independent risk factors for severe patients with COVID-19 in Guangzhou.

Methods: We included 288 adult patients with COVID-19 and compared the data between severe and non-severe group. We used univariate and multivariate logistic regression methods to explore risk factors of severe cases.

INTRODUCTION

In December 2019, a large-scale infectious pneumonia of unknown origin broke out in Wuhan, China. Chinese scientists isolated a new coronavirus, SARS-CoV-2, causing the pneumonia on Jan 7, 2020 [1, 2]. And WHO named it Coronavirus Disease 2019 (COVID-19) in February 2020 [3]. Since March, justifying the previous data model [4], COVID-19 has raged across world. Up to Jun 3, 2020, there have been more than 6.4 million diagnosed cases in more than 200 countries, with a mortality rate of about 6% [5].

The clinical manifestations of COVID-19 range from mild to critical [6]. A lot of observational studies have described the clinical characteristics of patients with COVID-19 in Wuhan [7–10], but studies outside Wuhan have rarely been reported. Because of the virus variation, the clinical characteristics of the patients in Wuhan and outside Wuhan maybe different. In this study, we aimed to investigate patients with COVID-19 in Guangzhou to find their clinical characteristics and the risk factors for severe cases. Monitoring these factors can help clinicians identify severe patients early and take subsequent interventions to reduce their illness.

RESULTS

Baseline characteristics

Among the 288 patients, 30 cases were in severe group and only 1 case died by the end of the study. Thus, the severity and mortality were 10.4% and 0.3% respectively. The median age of all patients was 48.5 years (IQR 34.3-62), of which women accounted for 54.5% (Table 1). 134 (46.5%) patients had comorbidities, of which cardiovascular disease (CVD) (85, 29.5%) was the most common one, followed by hypertension (84, 29.2%), diabetes (24, 8.3%) (Table 1). 132 patients (45.8%) had a history of exposure to Wuhan 2 weeks before onset (Table 1). The most common symptoms on admission were fever (201, 69.8%) and cough (163, 56.6%), followed by sputum (58, 20.1%), fatigue (43, 14.9%), and myalgia (35, 12.2%) (Table 1).

Laboratory and radiological findings

216 (75%) patients had white blood cells (WBC) in normal range and lymphopenia occurred in 91 (31.6%) patients (Table 2). Compared with non-severe patients, severe patients had significantly reduced serum hemoglobin, platelet and myoglobin, as well as significantly increased WBC, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, creatine kinase, C-reactive protein (CRP), procalcitonin (PCT), brain natriuretic peptide (BNP), and troponin I (Table 2). 31 (10.8%) patients had unilateral pneumonia, and all of them were non-severe patients; 241 (83.7%) patients had bilateral pneumonia, of which 29 (96.7%) were severe patients (Table 2). Their chest CTs showed varying degrees of patchy ground-glass opacity, with lung lesion area of severe patients usually larger than that of non-severe patients (Figure 1).

Treatments and outcomes

244 (84.7%) patients received antibiotics, and 233 (80.9%) patients received antiviral drugs (oseltamivir / ribavirin; Table 3). There was a significant difference in the use of glucocorticoids and vasoactive drugs between non-severe and severe patients (Table 3). Five patients were treated with continuous renal replacement therapy (CRRT) and four patients were treated with extracorporeal membrane oxygenation (ECMO), and they were all severe patients (Table 3). 98.9% of the non-severe patients did not take oxygen or took normal-flux oxygen, while 43.3% of the severe patients took high-flux oxygen (Table 3). Eight patients were tracheal intubated and they were all severe patients (Table 3). Severe patients had a significant increase in use of non-invasive mechanical ventilation than non-severe patients (Table 3). Compared with non-severe patients, severe patients were more likely to be transferred to the intensive care unit (ICU), and suffer from ARDS, acute kidney injury and acute cardiac injury (Table 3).

Univariate and multivariate analysis of risk factors of severe cases

In univariate logistic regression analysis, we found that older patients with hypertension, chronic kidney disease (CKD), and a history of exposure in Wuhan were more likely to develop severe disease (Table 4). In addition, fever, shortness of breath, diarrhea, WBC, CRP, lymphocytes, COPD, CVD, hemoglobin, ALT, AST, myoglobin, creatinine, creatine kinase, PCT, BNP and TNI were also related with severe cases (Table 4).

The multivariable logistic regression model was constructed using all variables of significant statistical differences in univariate logistic regression analysis. We found that each 1-year increase in age (OR, 1.057; 95% CI, 1.018-1.098; P=0.004), Wuhan exposure history greater than 2 weeks (OR, 2.765; 95% CI, 1.040-7.355; P=0.042), CKD (OR, 6.966; 95% CI, 1.310-37.058; P = 0.023), diarrhea (OR, 24.349; 95% CI, 3.580-165.609; P=0.001), Myoglobin higher than 106 μg/L (OR, 8.910; 95% CI, 1.225-64.816; P=0.031), WBC higher than 10×10^9/L (OR, 5.776; 95% CI, 1.052-31.722; P=0.044), and CRP higher than 10 mg/L (OR, 5.362; 95% CI, 1.631-17.626; P=0.006) were independent risk factors for severe cases (Table 4).

DISCUSSION

Of the 288 patients in our database, only one case (0.3%) died, while the early mortality rate in Wuhan was as high as 28.3% [3]. And the severity of COVID-19 in Guangzhou is 10.4%, which was far less than that in early Wuhan of 31.7% [11].

It was interesting to note Guangzhou patients with Wuhan exposure history had a higher risk of becoming severe cases (Table 4). Earlier reports reported that some patients had SARS-CoV-2 gene fragments missing, suggesting that their virulence gradually weakened [12]. And an article reported that COVID-19 patients in Zhejiang Province had relatively mild symptoms compared with Wuhan [13]. Later, it was reported that SARS-CoV-2 has genomic diversity. It mutated through replication and may evolve under the pressure of immune surveillance in human body, with its virulence, infectivity and transmission being affected [14]. Therefore, the virulence of SARS-CoV-2 may increase or decrease during transmission, and certain populations in different regions may also have a screening effect on it, resulting in different disease

Table 1. Baseline characteristics of non-severe or severe patients of COVID-19 in Guangzhou.

Demographics and clinical characteristics	No. (%)			P value
	Total (288)	Non-severe (258)	Severe (30)	
Age, median (IQR), years	48.5 (34.3-62)	47 (33-61)	61.5(51-71.3)	<0.0001
Age groups (years):	..	..	..	<0.0001
≤30	44(15.3)	44(17.1)	0(0)	..
31-45	87(30.2)	83(32.2)	4(13.3)	..
46-65	116(40.3)	101(39.1)	15(50)	..
≥66	41(14.2)	30(11.6)	11(36.7)	..
Sex:	..	..	..	0.194
Male	131(45.5)	114(44.2)	17(56.7)	..
Female	157(54.5)	114(55.8)	13(43.3)	..
Comorbidity:	..	..	..	..
Hypertension	84(29.2)	69(26.7)	15(50)	0.008
SBP (mm Hg), median (IQR)	125(117-136)	125(117-136)	124.5(117-138.3)	0.186
DBP (mm Hg), median (IQR)	80(74-87)	80(75-87)	80.5(67.3-85)	0.028
MAP (mm Hg), median (IQR)	94.7(87.8-103)	94.7(88-103)	94.8(85.1-102.6)	0.415
Diabetes	24(8.3)	20(7.8)	4(13.3)	0.295
COPD	5(1.7)	3(1.2)	2(6.9)	0.025
CVD	85(29.5)	70(27.1)	15(50)	0.009
Carcinoma	6(2.1)	6(2.3)	0(0)	0.399
CKD	8(2.8)	4(1.6)	4(13.3)	<0.0001
CLD	10(3.5)	8(3.1)	2(6.7)	0.313
Exposure history in Wuhan >2 weeks:	..	..	..	0.016
Yes	132(45.8)	112(43.4)	20(66.7)	..
No	156(54.2)	146(56.6)	10(33.3)	..
Respiratory rate >24 breaths per min	19(6.6)	12(4.7)	7(23.3)	<0.0001
Oxygenation index, median (IQR)	98(97-98.8)	98(97-98.8)	98(97-99)	0.986
Fever (tempetature≥37·3°C)	201(69.8)	174(67.4)	27(90)	0.011
Cough	163(56.6)	142(55)	21(70)	0.118
Sputum	58(20.1)	54(20.9)	4(13.3)	0.326
Myalgia	35(12.2)	30(11.6)	5(16.7)	0.424
Fatigue	43(14.9)	37(14.3)	6(20)	0.410
Nausea or Anorexia	28(9.7)	22(8.5)	6(20)	0.045
Vomiting	6(2.1)	5(1.9)	1(3.3)	0.613
Diarrhea	11(3.8)	6(2.3)	5(16.7)	<0.0001
Headache	26(9)	22(8.5)	4(13.3)	0.385

SBP, Systolic blood pressure; DBP, Diastolic blood pressure; MAP, Mean arterial pressure; COPD, Chronic obstructive pulmonary disease; CVD, Cardiovascular disease; CKD, Chronic kidney disease; CLD, Chronic liver disease.
***P* values** indicate differences between Severe and Non-severe patients. P<0.05 was considered statistically significant.

Table 2. Laboratory and radiological findings of non-severe or severe patients of COVID-19 in Guangzhou.

	Median (IQR)			P value	Normal range
	Total (288)	Non-severe (258)	Severe (30)		
Laboratory findings					
WBC (×10^9/L)	5.20(4.14-6.44)	5.14(4.10-6.38)	5.33(4.42-7.18)	0.934	4-10
WBC (×10^9/L) (No (%)):	..	..	..	<0.0001	..
<4	62(21.5)	57(22.1)	5(16.7)	..	..

4-10	216(75)	197(76.4)	19(63.3)	..	..
>10	10(3.5)	4(1.6)	6(20)	..	..
Lymphocyte count (×10^9/L)	1.42(1.04-1.96)	1.46(1.09-1.97)	1.03(0.84-1.38)	0.511	1.1-3.2
Lymphocyte count (×10^9/L) (No (%)):	..	..	..	..	..
<1.1	91(31.6)	73(28.3)	18(60)	<0.0001	
Hemoglobin (g/L)	135.5(123-147)	136(125-147.3)	123(114-143.3)	0.001	130-175
Platelet count (×10^9/L)	194.5(158-247)	199(160.1-249.3)	167(140.3-188.5)	0.043	125-350
D-dimer (mg/L)	1110(700-1700)	1090(680-1600)	1855(865-3442.5)	0.052	<1000
D-dimer (mg/L) (No (%)):	..	..	..	0.106	..
≤1000	125(43.9)	116(45.5)	9(30)	..	..
>1000	160(56.1)	139(54.5)	21(70)	..	..
ALT (U/L)	22.5(14.3-34.5)	22.1(14.2-33.9)	25(16.1-49.1)	0.912	9-50
ALT (U/L) (No (%)):	..	..	..	0.039	..
≤50	254(88.2)	231(89.5)	23(76.7)	..	..
>50	34(11.8)	27(10.5)	7(23.3)	..	..
AST(U/L)	18.4(14.9-25.6)	18.1(14.5-24.5)	21.9(16.8-41.1)	0.161	15-40
AST(U/L) (No (%)):	..	..	..	0.004	..
≤40	256(88.9)	234(90.7)	22(73.3)	..	..
>40	32(11.1)	24(9.3)	8(26.7)	..	..
Myoglobin (μg/L)	15(8.85-22.4)	14.4(8.6-21.2)	27.3(13.1-86.6)	0.212	17.4-105.7
Myoglobin (μg/L) (No (%)):	..	..	..	<0.0001	..
≤106	269(97.1)	247(99.2)	22(78.6)	..	..
>106	8(2.9)	2(0.8)	6(21.4)	..	..
Creatinine (μmol/L)	61.8(50.25-76.56)	62.0(50.4-76.4)	59.6(45.9-78.1)	0.428	54-106
Creatinine (μmol/L) (No (%)):	..	..	..	0.022	..
≤106	279(96.9)	252(97.7)	27(90)	..	..
>106	9(3.1)	6(2.3)	3(10)	..	..
Creatinine kinase (U/L)	52(36-80)	52(37-80)	44.5(27.5-128)	0.238	50-310
Creatinine kinase (U/L) (No (%)):	..	..	..	0.009	..
≤310	283(98.6)	255(99.2)	28(93.3)	..	..
>310	4(1.4)	2(0.8)	2(6.7)	..	..
CRP (mg/L)	9(8-22.72)	9(8-18.9)	24(11.7-51.2)	0.005	<10
CRP (mg/L) (No (%)):	..	..	..	<0.0001	..
≤10	175(60.8)	169(65.5)	6(20)	..	..
>10	113(39.2)	89(34.5)	24(80)	..	..
PCT (ng/mL)	0.13(0.04-32.6)	0.106(0.035-32.58)	0.2(0.09-51)	0.241	<0.05
PCT (ng/mL) (No (%)):	..	..	..	<0.0001	..
<0.05	99(35.5)	96(38.4)	3(10.3)	..	..
0.05-1.0	73(26.2)	56(22.4)	17(58.6)	..	..
1..0-10	6(2.2)	6(2.4)	0(0)	..	..
>10	101(36.2)	92(36.8)	9(31)	..	..
BNP (ng/L)	35(13-117.5)	18.5(9.75-40.25)	213(45-399)	0.014	<100
TNI (μg/L)	0.004(0.001-0.009)	0.003(0.001-0.007)	0.027(0.010-0.099)	0.033	<0.03
TNI (μg/L) (No (%)):	..	..	..	<0.0001	..
≤0.03	168(88.4)	159(92.4)	9(50)	..	..
>0.03	22(11.6)	13(7.6)	9(50)	..	..
Chest radiography findings					
Unilateral pneumonia	31(10.8)	31(12.1)	0(0)	0.044	..
Bilateral pneumonia	241(83.7)	212(82.2)	29(96.7)	0.042	..

WBC, White blood cell; ALT, Alanine transaminase; AST, Aspartate aminotransferase; CRP, C-reactive protein; PCT, Procalcitonin; BNP, Brain natriuretic peptide; TNI, Troponin I.

***P* values** indicate differences between Severe and Non-severe patients. P<0.05 was considered statistically significant.

degrees and influencing factors of COVID-19 in different regions.

According to previous reports, older age was an important independent predictor of SARS and MERS mortality [15, 16]. Previous studies have confirmed increased severity and mortality of COVID-19 in old patients [3, 7, 17]. A recent study comparing the clinical characteristics and results of COVID-19 patients of different ages showed that the symptoms of elderly patients were more atypical, with more comorbidities, secondary infection, organ injuries, immunodeficiency and a higher risk of critical illness [18]. Many comorbidities in the elderly such as hypertension, diabetes and CKD were treated with ACE inhibitors and angiotensin II receptor blockers, which would upregulate the ACE2 receptor, thereby increasing the risk of SARS-CoV-2 infection and the risk of disease [19]. In our study cohort, age was also one of the risk factors for severe patients (Table 4). Therefore, it's very important for old patients to have early diagnosis and treat systemic comorbidities carefully.

SARS-CoV-2 was reported to be detected in stool samples from patients [20], and a study of a family cluster have reported two COVID-19 patients who had only diarrhea symptom [9]. Besides diarrhea, some patients also had other gastrointestinal symptoms such as vomiting and abdominal pain [21]. Our analysis showed that diarrhea was a risk factor for severe cases (Table 4), which suggested that beside of damaging the respiratory system, the virus may also have a certain function on the digestive system. This finding may be related to the expression of SARS-CoV-2 receptor ACE2 in both the epithelial cells of lungs and digestive tract [21, 22]. Given the small number of diarrhea cases

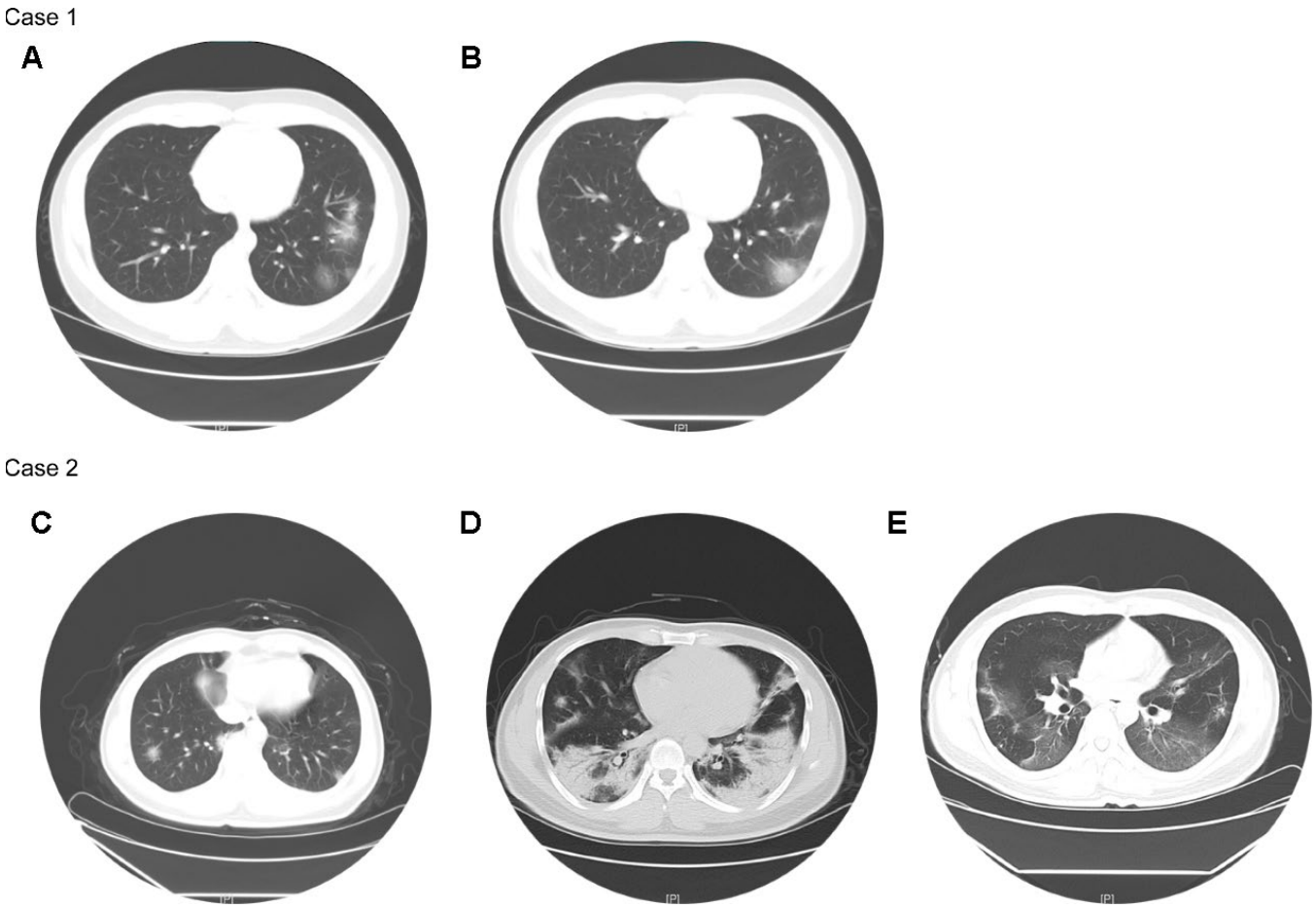

Figure 1. Chest CTs of two representative cases. Case 1 (non-severe): Chest CT on Feb 24 (**A**) showed multiple patchy ground-glass opacity in both lungs, with unclear borders and uneven density. Chest CT on Feb 28 (**B**) showed better status, and some lesions were slightly absorbed than before. Case 2 (severe): Chest CT on Jan 29 (**C**) showed the texture of both lungs was slightly increased, and both lungs were scattered in patchy shadows, whose edges were blurred. Chest CT on Feb 11 (**D**) showed the scope of the bilateral lung lesions was enlarged, the density was increased, and the local consolidation and bronchial signs were seen. Chest CT on Mar 4 (**E**) showed improved status, and both lung lesions were significantly less than before.

Table 3. Treatments and outcomes of non-severe or severe patients of COVID-19 in Guangzhou.

	No. (%)			P value
	Total (288)	Non-severe (258)	Severe (30)	
Treatments				
Antiviral	233(80.9)	204(79.1)	29(96.7)	0.020
Antibiotics	244(84.7)	214(82.9)	30(100)	0.014
Vasoactive drugs	5(1.7)	1(0.4)	4(13.3)	<0.0001
Glucocorticoid	21(7.3)	12(4.7)	9(30)	<0.0001
CRRT	5(1.7)	0(0)	5(16.7)	<0.0001
ECMO	4(1.4)	0(0)	4(13.3)	<0.0001
Oxygen uptake:	..	..	..	..
None	88(30.6)	84(32.6)	4(13.3)	0.030
Normal-flux	184(63.9)	171(66.3)	13(43.3)	0.013
High-flux	16(5.6)	3(1.2)	13(43.3)	<0.0001
Tracheal intubation	8(2.8)	0(0)	8(26.7)	<0.0001
Tracheotomy	0(0)	0(0)	0(0)	..
Non-invasive mechanical ventilation	32(11.1)	13(5)	19(63.3)	<0.0001
Outcomes				
ICU Admission	27(9.4)	12(4.7)	15(50)	<0.0001
ARDS	3(1)	0(0)	3(10)	<0.0001
Acute kidney injury	5(1.7)	0(0)	5(16.7)	<0.0001
Acute cardiac injury	22(11.6)	13(7.6)	9(50)	<0.0001

CRRT, continuous renal-replacement therapy; ECMO, Extracorporeal membrane oxygenation; ICU, intensive care unit; ARDS, Acute respiratory distress syndrome.

***P* values** indicate differences between Severe and Non-severe patients. P<0.05 was considered statistically significant.

Table 4. Univariate and multivariate analysis of risk factors of severe cases in Guangzhou.

	Univariable OR (95% CI)	P value	Multivariable OR (95%) CI)	P value
Demographics and clinical characteristics				
Age, years	1.063(1.033-1.095)	<0.0001	1.057 (1.018-1.098)	0.004
Female sex (vs male)	0.605(0.282-1.298)	0.197	..	..
Comorbidity present (vs not present)				
Hypertension	2.739(1.272-5.898)	0.010	..	..
COPD	6.296(1.007-39.354)	0.049	..	..
CVD	2.686(1.248-5.780)	0.012	0.986(0.052-18.588)	0.992
CKD	9.769(2.307-41.376)	0.002	6.966(1.310-37.058)	0.023
Respiratory rate >24 breaths per min	6.239(2.238-17.397)	<0.0001	..	..
Exposure history in Wuhan >2 weeks	2.607(1.174-5.791)	0.019	2.765(1.040-7.355)	0.042
Fever (tempetature≥37·3°C)	4.345(1.282-14.730)	0.018	..	..
Nausea or Anorexia	2.682(0.991-7.258)	0.052	..	..
Diarrhea	8.400(2.392-29.494)	0.001	24.349(3.580-165.609)	0.001
Laboratory and radiography findings				
White blood cell count (10^9/L) (No (%)):	..	..	..	..
≤4	0.910(0.325-2.543)	0.857	0.968(0.289-3.245)	0.958
4-10	1(ref)	..	..	..
≥10	15.553(4.032-59.988)	<0.0001	5.776(1.052-31.722)	0.044
Lymphocyte count (×10^9/L)	..	..	..	..
<1.1	0.263(0.121-0.573)	0.001	0.697(0.246-1.975)	0.497

Hemoglobin (g/L)	0.966(0.946-0.986)	0.001	..	..
Platelet count ($\times 10^9$/L)	0.993(0.987-1.000)	0.042	..	..
D-dimer (mg/L)	..	..	..	..
≤1000	1(ref)	..	..	..
>1000	1.947(0.859-4.416)	0.111	..	..
ALT (U/L)	..	..	..	..
≤50	1(ref)	..	..	..
>50	2.604(1.022-6.634)	0.045	..	..
AST(U/L)	..	..	..	..
≤40	1(ref)	..	..	..
>40	3.545(1.425-8.823)	0.007	..	..
Myoglobin (μg/L)	..	..	..	..
≤106	1(ref)	..	..	..
>106	33.682(6.413-176.905)	<0.0001	8.910(1.225-64.816)	0.031
Creatinine (μmol/L)	..	..	..	..
≤106	1(ref)	..	..	..
>106	4.667(1.104-19.728)	0.036	..	..
Creatinine kinase (U/L)	..	..	..	..
≤310	1(ref)	..	..	..
>310	9.107(1.234-67.188)	0.030	..	..
CRP (mg/L)	..	..	..	..
≤10	1(ref)	..	..	..
>10	7.596(2.995-19.264)	<0.0001	5.362(1.631-17.626)	0.006
PCT (ng/mL)	..	..	..	..
≤0.05	1(ref)	..	..	..
>0.05	5.333(1.572-18.098)	0.007	..	..
BNP (ng/L)	1.022(1.005-1.040)	0.014	..	..
TNI (μg/L)	..	..	..	..
≤0.03	1(ref)	..	..	..
>0.03	12.231(4.14-36.131)	<0.0001	..	..
Bilateral pneumonia	6.292(0.836-47.378)	0.074	..	..

OR=odds ratio.

***P* values** indicate differences between Severe and Non-severe patients. P<0.05 was considered statistically significant.

(11, 3.8%) (Table 1), SARS-CoV-2-induced digestive system damage may also be related to other physical factors of these patients, which deserves further study.

Previous studies have reported that COVID-19 non-survivors had more neutrophil counts than survivors, which may be related to cytokine storms caused by virus invasion [7, 11]. Our analysis found that elevated WBC and CRP were risk factors for severe cases (Table 4). Like neutrophils, WBC and CRP are also indicators of inflammatory status in the body. When they elevated, there may be a cytokine storm caused by virus invasion in the body, which may cause severe inflammation in lungs and other organs, and aggravate the disease. Therefore, paying close attention to changes in WBC, CRP and making timely correction can effectively reduce the number of severe cases and deaths.

In our study, myoglobin, creatine kinase, BNP, and TNI were increased in severe patients compared to non-severe patients (Table 2), and myoglobin was a risk factor for severe patients, which indicated that COVID-19 may be related to acute cardiac injury. ACE2 is also expressed in heart [23], and SARS-CoV has been shown in animal models to directly mediate myocardial inflammation and damage by down-regulating myocardial ACE2 and lead to poor cardiac prognosis [24]. A meta-analysis involving 4189 patients showed that more severe COVID-19 was associated with increased troponin, creatine kinase, myoglobin, and NT-proBNP [25]. Myoglobin was also included in the COVID-19 severity score table as one of the biomarkers [26]. The severity of COVID-19 may be related to acute cardiac injury, which prompts us to effectively monitor heart condition to prevent

COVID-19 patients from myocarditis and avoid poor cardiac prognosis.

Many studies have reported that comorbidities were major risk factors for increasing COVID mortality and poor prognosis [7, 8], and CKD was one of them. Due to older age, previous comorbidities, impaired immune system, and regular visits to crowded outpatient dialysis centers, CKD patients have increase susceptibility to SARS-COV-2 [27]. On one hand, the above factors have greatly reduced the ability of CKD patients to overcome the virus and may lead to severe disease or even death. On the other hand, SARS-COV-2 can directly damage kidney by combine with ACE2 [28], and cause kidney inflammation and acute kidney injury [13, 29], which was consistent with the increase creatinine level of severe patients in our study. AKI could further aggravate CKD as well as worsening the patients' whole conditions, leading patients to develop severe illness.

The study has several limitations. First, the sample size of our study cohort was relatively small including only 288 patients from a single center. Due to the exploratory nature of the study, which was not driven by formal hypotheses, we did not estimate the sample size, but included as many cases as possible. Second, this study lacked laboratory data such as serum cytokines and chemokines, so that we cannot evaluate the inflammation levels and cytokine storms of these patients. Third, this was a retrospective study. The data in this study was only a preliminary assessment of clinical characteristics and risk factors of COVID-19 severe patients. Further researches are still needed.

In conclusion, our research showed that the severity and mortality of COVID-19 in Guangzhou were much lower than those in early Wuhan. The risk factors for severe cases of COVID-19 in Guangzhou included older age, Wuhan exposure history greater than 2 weeks, diarrhea, elevated Myoglobin, elevated WBC and CRP, and CKD. Investigating and monitoring these factors can help clinicians identify patients with poor prognosis at an early stage, and take proactive interventions to benefit patients and reduce severity and mortality. It also provided significant experience and reference for countries around the world to fight against COVID-19.

MATERIALS AND METHODS

Study design and participants

This single-center, retrospective cohort study was conducted at Guangzhou Eighth People's Hospital (Guangzhou, China), which was the designated hospital to treat patients with COVID-19 in Guangzhou. From Jan 15, 2020 to Mar 10, 2020, we recruited 288 adult patients with COVID-19 (the total number was 292, including 4 underage patients).

This study was approved by the Ethics Committee of Guangzhou Eighth People's Hospital, and informed consent was obtained from all patients enrolled.

Definitions

According to the Chinese diagnosis and treatment guideline for COVID-19 (trial version 7.0) [6], 288 patients were divided into non-severe group (258 cases), including light and general patients, and severe group (30 cases), including severe and critical patients. A case was defined as severe if it met any of the following: (1) shortness of breath, respiratory rate ≥ 30 times / minute; (2) blood oxygen saturation ≤ 93% at rest; (3) oxygenation index (PaO2 / FiO2) ≤ 300 mmHg; (4) pulmonary infiltrates > 50% of the lung lesions within 24-48 hours; (5) respiratory failure, requiring mechanical ventilation; (6) shock; (7) combine with multiple organ dysfunction, needing ICU monitoring treatment. Acute Respiratory Distress Syndrome (ARDS) was defined according to WHO's guidance for COVID-19 [30]. Acute renal injury (ARI) was determined from serum creatinine [31]. Acute cardiac injury (ACI) was determined based on the serum concentration of troponin I (TNI) [11]. The reference ranges of all laboratory inspection indicators were measured in the laboratory of Guangzhou Eighth People's Hospital.

Data collection

This study reviewed the clinical electronic medical records, nursing records, laboratory tests and radiological findings of 288 adult patients with COVID-19, who were confirmed by nucleic acid testing. And we extracted epidemiology, demographics, clinical manifestations, laboratory data, chest radiography findings, treatment and outcome data for statistical analysis and research.

Statistical analysis

The purpose of this study was to analyze the risk factors of severe patients by comparing severe group and non-severe group in terms of their clinical data. Therefore, no formal assumptions were used to facilitate the calculation of the sample size, and we included the largest number of patients who met the inclusion criteria.

We represented continuous variables as median and interquartile range (IQR), and categorical variables as

frequency (N) and percentage (%). We assessed differences between severe group and non-severe group using two-sample *t* test or the Mann-Whitney U test depending on parametric or nonparametric data for continuous variables, and χ 2 test or Fisher's exact test for categorical variables. Univariate and multivariate logistic regression models were used to explore the risk factors for severe cases.

A P value of less than 0.05 was considered statistically significant. All data were statistically analyzed using SPSS software (version 25).

AUTHOR CONTRIBUTIONS

Dr. Feng He, Dr. Qingqing Luo and Dr. Ming Lei contributed equally, they all developed conceptualization and wrote the manuscript under the supervision of Dr. Na Yu, Dr. Liuping Yang and Dr. Jie Cao. Lixin Fan, Xinning Shao, Guanglie Huang, Jun Zeng, Ziwen Zhao, Shuguang Qin, Zhi Yang has participated substantially in the conceptualization and design of this work as well as the writing of the manuscript. All authors have reviewed the final version of the manuscript and have approved it for publication.

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CONFLICTS OF INTEREST

We declare no conflicts of interest.

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Editorial note

&This corresponding author has a verified history of publications using the personal email addresses for correspondence.

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Research Paper

The effect of emergency surgery on acute abdomen patients with COVID-19 pneumonia: a retrospective observational study

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ABSTRACT

During the COVID-19 outbreak, some patients with COVID-19 pneumonia also suffered from acute abdomen requiring surgical treatment; however, there is no consensus for the treatment of such patients. In this study, we retrospectively reviewed 34 patients with acute abdomen who underwent emergency surgery during the COVID-19 outbreak. Among the 34 patients with acute abdomen, a total of six cases were found with COVID-19 pneumonia (clinical classification for COVID-19 pneumonia: all were the common type). On the premise of similar demographics between both groups, patients with COVID-19 pneumonia had worse indicators of liver and coagulation function. Compared with acute abdomen patients without COVID-19, patients with COVID-19 pneumonia had a longer hospital stay, but there were no significant differences in postsurgical complications (P = 0.58) or clinical outcomes (P = 0.56). In addition, an obvious resolution of lung inflammation after surgery was observed in five COVID-19 patients (83.3%). No new COVID-19 cases occurred during the patients' hospital stays. Therefore, for the common type of COVID-19 pneumonia, emergency surgery could not only improve the outcomes of COVID-19 pneumonia patients with acute abdomen, but also benefit the resolution of pulmonary inflammation.

INTRODUCTION

In the last two decades there have been two large-scale pandemics caused by coronaviruses, severe acute respiratory syndrome (SARS) [1] and Middle East respiratory syndrome (MERS) [2]. At the end of 2019, another novel coronavirus, designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan and subsequently spread rapidly throughout the world [3, 4]. Due to accumulating evidence of continuous person-to-person transmission and a general susceptibility of humans to the virus [5–7], the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a public health emergency of international concern on January 30, 2020. As of May 16, 2020, COVID-19 caused 309,713 deaths among over 4.5 million patients across more than 200 countries, with a case-fatality rate of 6.8%. Although SARS-CoV-2 was found to predominantly infect the

lower airways and cause life-threatening pneumonia [8, 9], evidence has revealed that the digestive system might be another potential viral target [7, 10, 11].

Acute abdomen is defined as acute onset of abdominal pain which requires accurate diagnosis and treatment within a particular time limit to prevent mortality and morbidity [12]. During COVID-19 outbreaks, some patients with COVID-19 pneumonia also suffered from acute abdomen requiring immediate interventions [13]. However, previous studies have demonstrated that preoperative pneumonia is a significant risk factor for poor postsurgical outcomes [14, 15]. In addition, surgical treatment might increase medical staff exposure to SARS-CoV-2 [16, 17] and trigger excessive inflammation in the patient, resulting in worsening of COVID-19 pneumonia [18]. Therefore, an investigation of the impact of emergency surgery on patients with both acute abdomen and COVID-19 pneumonia is urgently needed.

RESULTS

Clinical characteristics of patients

Among the 34 patients with acute abdomen who underwent emergency surgery, six patients had COVID-19, and the remaining 28 patients did not. The baseline characteristics of all patients are summarized in Table 1. No new infections were found in medical staff or patients throughout the hospitalization period.

Of the 28 patients who did not have COVID-19 pneumonia (9 female and 19 male; mean age 55 years (range 17–87)), 12 (43%) patients were diagnosed with acute appendicitis, 10 (36%) with gastrointestinal perforation, 5 (18%) with intestinal obstruction and 1 (4%) with bladder rupture. The typical abdominal CT appearance is shown in Figure 1. Comorbidities were found in 17 (61%) patients and included diabetes mellitus in 7 (25%), coronary heart disease in 7 (25%), hypertension in 6 (21.4%), chronic obstructive pulmonary disease in 2 (7.1%), chronic renal failure in 1 (3.6%), chronic liver failure in 1 (3.6%), acute myeloid leukemia in 1 (3.6%), and rheumatoid arthritis in 1 (3.6%). Five (17.9%) patients were reported to have postoperative complications: one had intra-abdominal infection, one had a wound infection, and three had multiple organ dysfunction syndrome (MODS). All three patients with postoperative MODS had preoperative comorbidities (case 1: coronary heart disease and chronic renal failure; case 2: chronic liver failure; case 3: hypertension and coronary heart disease). In total, 25 (89.3%) patients were cured, and the remaining 3 patients died due to severe septic shock and MODS.

The detailed clinical characteristics of the six patients with both acute abdomen and COVID-19 pneumonia are shown in Table 2. Age of the six patients (4 women and 2 men) ranged from 66 to 78 years. Three patients were diagnosed with intestinal obstruction, two with acute appendicitis, and one with gangrenous cholecystitis. The clinical classification of COVID-19 pneumonia in all patients was the common type. Three patients had hypertension, and one had coronary heart disease. The most common clinical manifestations were abdominal pain and fever. Two (33.3%) patients tested positive for SARS-CoV-2 by RT-PCR, and one patient tested positive for IgM-IgG antibodies; however, typical CT imaging manifestations of COVID-19 pneumonia were found in all six patients. Postoperative complications occurred in two patients: one had aspiration pneumonia and the other had MODS. All patients received antiviral therapy (ribavirin, 500 mg each time, twice times a day, 5-7 days; arbidol, 200 mg each time, three times a day, 5-7 days; Interferon α-2b, 5.0×10^5 IU, nebulized inhalation, twice times a day) and antibacterial therapy, and four patients received immunoglobulins (human immunoglobulin, 10g/d). Two patients with postoperative complications received mechanical ventilation and systematic corticosteroid treatment (methylprednisolone, 1–2 mg/kg.d, 3–5 days). In total, five patients were cured, and one patient died of postoperative MODS.

Emergency surgery could not only improve the outcomes of acute abdomen patients with COVID-19 pneumonia, but also benefit the resolution of pulmonary inflammation

The baseline characteristics in patients with and without COVID-19 pneumonia are shown in Table 1. Differences in demographics, including age ($P = 0.12$), sex ($P = 0.17$), diagnosis ($P = 0.06$) and comorbidities ($P = 0.67$), between both groups were not significant. However, patients with COVID-19 pneumonia had higher ALT (70.7 ± 108.3 U/L vs. 18.7 ± 7 U/L, $P = 0.012$), AST (72.7 ± 93.7 U/L vs. 20.6 ± 13.7 U/L, $P = 0.006$), APTT (50.7 ± 10 s vs. 36.1 ± 3.6 s, $P < 0.001$), and PT (16.9 ± 4.5 s vs. 14.1 ± 1.2 s, $P = 0.006$), and lower albumin (30 ± 10.8 g/L vs. 41.6 ± 6.5 g/L, $P = 0.012$) and hemoglobin (107.2 ± 26.8 g/L vs. 143.9 ± 17.4 g/L, $P < 0.001$) than patients who did not have COVID-19 pneumonia. In addition, although there were no significant differences, patients with COVID-19 pneumonia had lower infection-related biomarkers, including WBC ($(10.4 \pm 6.5)\times10^9$/L vs. $(11.8 \pm 3.8)\times10^9$/L, $P = 0.49$), lymphocyte ($(0.7 \pm 0.3)\times10^9$/L vs. $(1.1 \pm 0.7)\times10^9$/L, $P = 0.26$), neutrophil ($(8.9 \pm 5.9)\times10^9$/L vs. $(10.1 \pm 3.5)\times10^9$/L, $P = 0.51$), CRP (82.6 ± 72.9 mg/L vs.

Table 1. The baseline characteristics of all patients with acute abdomen.

Characteristics	Patients with acute abdomen		P-value
	With COVID-19 (n = 6)	Without COVID-19 (n = 28)	
Age (years)	70 ± 4.2	55 ± 22	0.120
Gender			0.170
Female	4 (67%)	9 (32%)	
Male	2 (33%)	19 (68%)	
Diagnosis			0.060
Acute appendicitis	2 (33%)	12 (43%)	
Gastrointestinal perforation	0 (0%)	10 (36%)	
Intestinal obstruction	3 (50%)	5 (18%)	
Gangrenous cholecystitis	1 (17%)	0 (0%)	
Bladder rupture	0 (0%)	1 (4%)	
Comorbidities			0.670
No	3 (50%)	11 (39%)	
Yes	3 (50%)	17 (61%)	
Laboratory findings			
WBC ($\times 10^9$/L)	10.4 ± 6.5	11.8 ± 3.8	0.490
Neutrophil($\times 10^9$/L)	8.9 ± 5.9	10.1 ± 3.5	0.510
Lymphocyte ($\times 10^9$/L)	0.7 ± 0.3	1.1 ± 0.7	0.260
HGB (g/L)	107.2 ± 26.8	143.9 ± 17.4	<0.001
CRP (mg/L)	82.6 ± 72.9	139.2 ± 67.1	0.074
PCT (μg/L)	3.4 ± 5.3	8.8 ± 8.7	0.160
Albumin (g/L)	30 ± 10.8	41.6 ± 6.5	0.001
ALT (U/L)	70.7 ± 108.3	18.7 ± 7	0.012
AST (U/L)	72.7 ± 93.7	20.6 ± 13.7	0.006
D-Dimer (mg/L)	2.6 ± 3.3	1.4 ± 1.2	0.140
APTT (s)	50.7 ± 10	36.1 ± 3.6	<0.001
PT (s)	16.9 ± 4.5	14.1 ± 1.2	0.006

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; APTT, activated partial thromboplastin time; PT, prothrombin time; HGB, hemoglobin; WBC, white blood cell; CRP, C-reaction protein; PCT, procalcitonin.

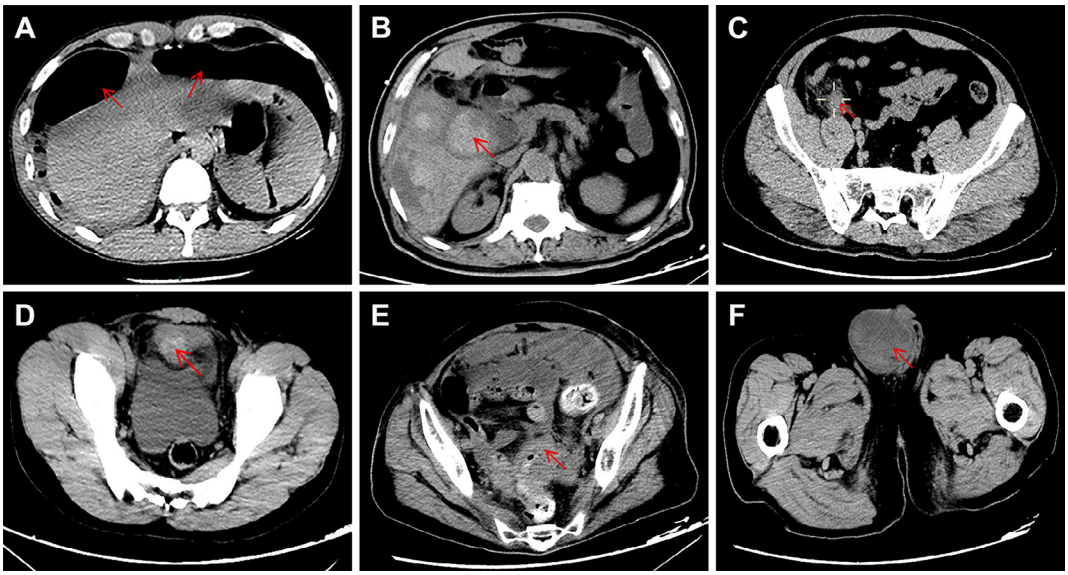

Figure 1. Typical appearance of abdominal CT showing the causes of acute abdomen in the present study. (**A**) duodenal perforation accompanied by free intraperitoneal gas; (**B**) gangrenous cholecystitis; (**C**) acute appendicitis; (**D**) bladder rupture; (**E**) intestinal obstruction caused by carcinomas in the rectosigmoid junction; (**F**) intestinal obstruction caused by inguinal incarcerated hernia.

Table 2. The clinical characteristics of the six patients with both acute abdomen and COVID-19 pneumonia.

Characteristics	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age, years	69	78	68	68	66	69
Gender	Female	Male	Female	Male	Female	Female
Evidence of COVID-19						
RT-PCR	Negative	Positive	Negative	Negative	Negative	Positive
IgM-IgG antibodies	NA	NA	NA	NA	Negative	Positive
Typical CT manifestation	Unilateral	Bilateral	Bilateral	Unilateral	Bilateral	Bilateral
Diagnosis	Intestinal volvulus Pneumonia (mild)	Gangrenous cholecystitis Pneumonia (mild)	Acute appendicitis Pneumonia (mild)	Malignant intestinal obstruction Pneumonia (mild)	Acute appendicitis Pneumonia (mild)	Malignant intestinal obstruction Pneumonia (mild)
Symptoms and signs						
Fever	No	Yes	Yes	Yes	Yes	No
Cough	Yes	No	No	Yes	No	No
Expectoration	No	Yes	No	Yes	No	Yes
Abdominal pain	Yes	Yes	Yes	Yes	Yes	Yes
Diarrhea	No	No	No	No	Yes	No
Nausea and vomiting	Yes	Yes	No	Yes	No	Yes
Comorbidities	No	Hypertension	No	Hypertension, CHD	Hypertension	No
Postoperative complications	No	MODS	No	Aspiration pneumonia	No	No
Treatment						
Mechanical ventilation	No	Yes	No	Yes	No	No
Antibiotics	Yes	Yes	Yes	Yes	Yes	Yes
Antivirals	Yes	Yes	Yes	Yes	Yes	Yes
Immune globulins	Yes	Yes	No	Yes	Yes	No
Hormones	No	Yes	No	Yes	No	No
Clinical outcome	Discharged	Death	Discharged	Discharged	Discharged	Discharged

Abbreviations: NA, not available; CHD, coronary heart disease; MODS, multiple organ dysfunction syndrome.

139.2 ± 67.1 mg/L, P = 0.074) and PCT (3.4 ± 5.3 μg/L vs. 8.8 ± 8.7 μg/L, P = 0.16), than patients who did not have COVID-19 pneumonia.

The comparative data of postsurgical outcomes between the two groups are shown in Figure 2. Compared with patients who did not have COVID-19 pneumonia, patients with COVID-19 pneumonia had a longer hospital stay (19.3 ± 10 days vs. 10.4 ± 6.6 days, P = 0.009), but no significant differences in postsurgical complications (P = 0.58) and clinical outcomes (P = 0.56) were found between groups. Furthermore, the majority of worsening preoperative laboratory indicators, including ALT (P = 0.43), AST (P = 0.93), APTT (P = 0.1), PT (P = 0.14), albumin (P = 0.44) and hemoglobin (P = 0.06), had improved by the third postoperative day. As outlined in Figure 3, when compared with preoperative indicators, postoperative infection-related biomarkers also decreased, including WBCs ((10.4 ± 6.5)×10^9/L vs. (5.4 ± 3.2)×10^9/L, P = 0.19), neutrophils ((8.9 ± 5.9)×10^9/L vs. (3.9 ± 3.4)×10^9/L, P = 0.16), CRP (82.6 ± 72.9 mg/L vs. 56.1 ± 49.8 mg/L, P = 0.55) and PCT (3.4 ± 5.3 μg/L vs. 0.3 ± 0.2 μg/L, P = 0.29).

To remove the potential impact of age on the above results, we further compared the pre- and postsurgical differences between patients with COVID-19 and those without COVID-19 pneumonia (between 60 and 80 years old). After age-matching between both groups, the majority of preoperative and postoperative results were consistent with the previous results. As

shown in Table 3, patients with COVID-19 pneumonia still had poor preoperative liver and coagulation function. However, the bulk of abnormal preoperative laboratory findings were significantly and rapidly corrected after surgical treatment (Figure 4). In addition, an obvious resolution of lung inflammation was observed after surgery in five patients (83.3%) (Figure 5). These results indicated that COVID-19 pneumonia is associated with poor liver function and coagulation function in acute abdomen patients with COVID-19 pneumonia. Nevertheless, emergency surgery could not only improve the outcomes of

A

Characteristics	Patients with acute abdomen		P value
	With COVID-19 (n = 6)	Without COVID-19 (n = 28)	
Length of hospitalization (days)	19.3 ± 10	10.4 ± 6.6	0.009
Postoperative complications			0.580
No	4 (67%)	23 (82%)	
Yes	2 (33%)	5 (18%)	
Clinical outcome			0.560
Discharged	5 (83%)	25 (89%)	
Death	1 (17%)	3 (11%)	

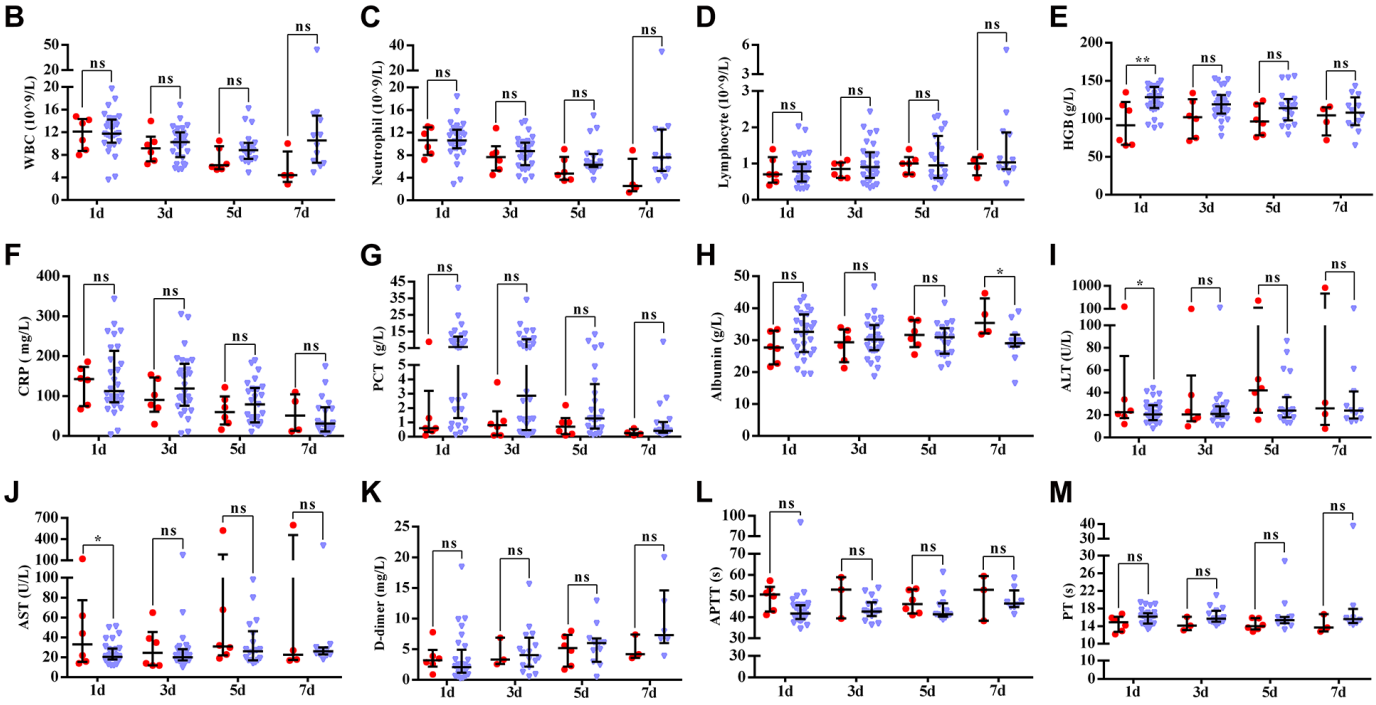

Figure 2. Postoperative outcomes of all patients with acute abdomen. Data are presented as numbers and percentages for categorical variables, and continuous data are expressed as the mean ± standard deviation (SD). *P< 0.05, **P< 0.01, ***P <0.001, based on Student's t-test. (**A**) the difference between both groups in clinical outcomes; (**B**–**M**) shows the differences between patients with and without COVID-19 pneumonia in postoperative laboratory findings, including (**B**) WBCs (white blood cells); (**C**) neutrophils; (**D**) lymphocytes; (**E**) HGB (hemoglobin); (**F**) CRP (C-reactive protein); (**G**) PCT (procalcitonin); (**H**) Albumin; (**I**) ALT (alanine aminotransferase); (**J**) AST (aspartate aminotransferase); (**K**) D-dimer; (**L**) APTT (activated partial thromboplastin time); (**M**) PT (prothrombin time). Red and blue marks represent patients with and without COVID-19 pneumonia, respectively.

COVID-19 pneumonia patients with acute abdomen, but also benefit the resolution of pulmonary inflammation.

DISCUSSION

Accurate diagnosis and appropriate intervention within a particular time limit is crucial to prevent deterioration and mortality in patients with acute abdomen [12]. Although previous studies revealed that preoperative pneumonia is significantly associated with worse postoperative outcomes [14, 15], there is still no direct evidence suggesting that surgical treatment leads to adverse effects in acute abdomen patients with COVID-19 pneumonia. Using the data from 34 patients with acute abdomen who underwent emergency surgery at our institute, the results of our study show that COVID-19 pneumonia is associated with poor liver function and coagulation function in acute abdomen patients with COVID-19 pneumonia. However, emergency surgery could not only improve the outcomes of COVID-19 pneumonia patients with acute abdomen, but also benefit the resolution of pulmonary inflammation.

COVID-19 might complicate the perioperative course of acute abdomen [13, 19]. The bulk of evidence revealed that SARS-CoV-2 RNA was identified in stool specimens [7, 20] and that the viral receptor angiotensin-converting enzyme 2 (ACE2) was highly expressed in gastrointestinal epithelial cells [21, 22], this evidence supported the conclusion that the digestive system is a potential target of SARS-CoV-2. In addition, infection-related biomarkers (including peripheral blood lymphocytes and WBCs) tend to decrease in patients with COVID-19 pneumonia [3, 4], while these indicators frequently increase in patients who only have acute abdomen. Blanco-Colino et al. also reported a case of suspected acute abdomen as an extrapulmonary manifestation of COVID-19 [19]. All of these results demonstrated that COVID-19 likely interferes with the accurate diagnosis and clinical assessment of acute abdomen.

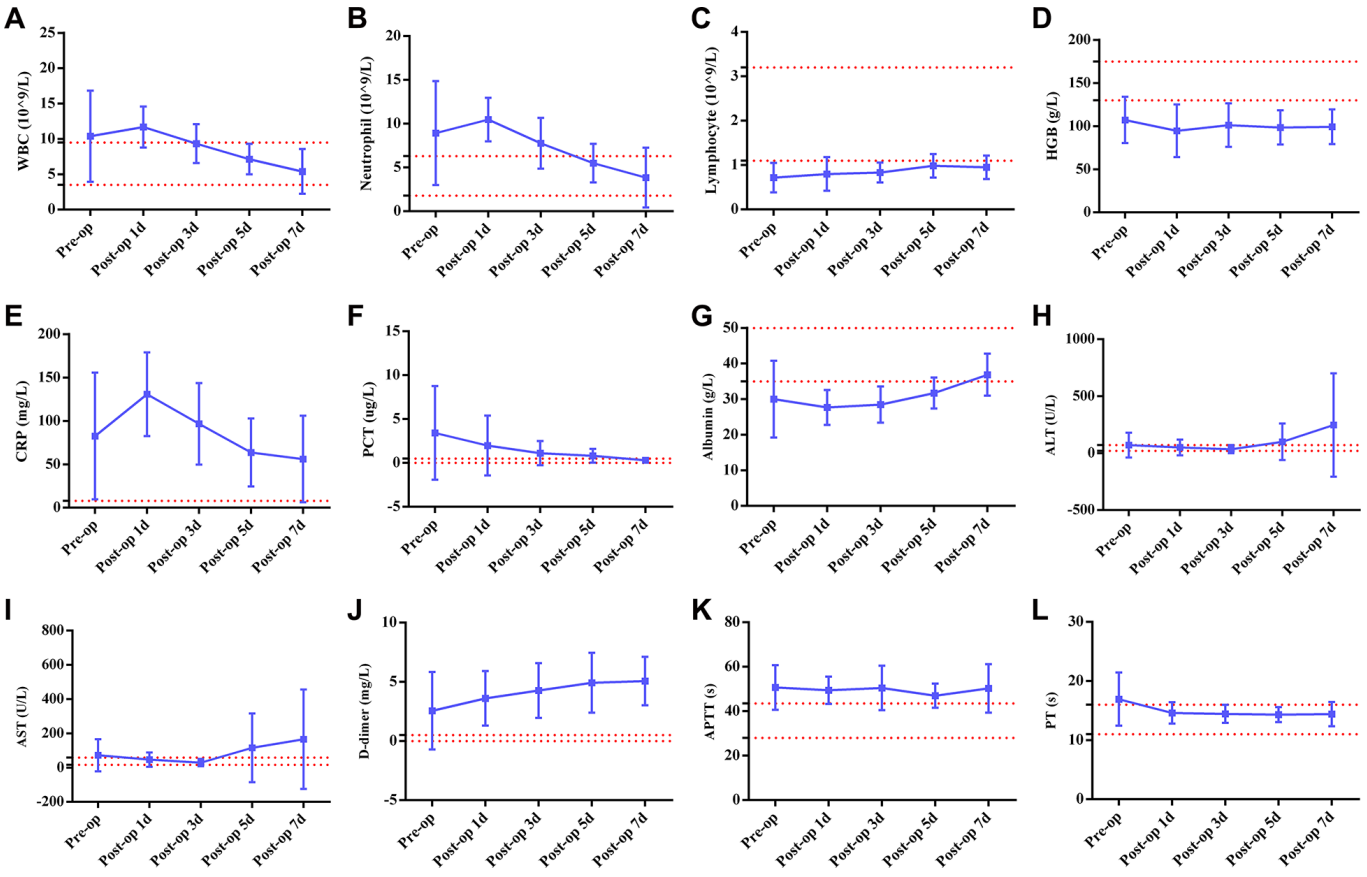

Figure 3. Line graphs illustrating detailed changes in laboratory findings in six patients with both acute abdomen and COVID-19 pneumonia. The red line represents the normal range of laboratory findings. (**A**) WBCs (white blood cells); (**B**) neutrophils; (**C**) lymphocytes; (**D**) HGB (hemoglobin); (**E**) CRP (C-reactive protein); (**F**) PCT (procalcitonin); (**G**) Albumin; (**H**) ALT (alanine aminotransferase); (**I**) AST (aspartate aminotransferase); (**J**) D-dimer; (**K**) APTT (activated partial thromboplastin time); (**L**) PT (prothrombin time).

Table 3. The preoperative differences between patients with COVID-19 pneumonia and those without COVID-19 pneumonia after age-matching.

Characteristics	Patients with acute abdomen		P-value
	With COVID-19 (n = 6)	Without COVID-19 (n = 12)	
Age (years)	70 ± 4.2	71.2 ± 5.9	0.590
Gender			0.620
Female	4 (67%)	5 (42%)	
Male	2 (33%)	7 (58%)	
Diagnosis			0.110
Acute appendicitis	2 (33%)	5 (42%)	
Gastrointestinal perforation	0 (0%)	5 (42%)	
Intestinal obstruction	3 (50%)	2 (17%)	
Gangrenous cholecystitis	1 (17%)	0 (0%)	
Comorbidities			0.340
No	3 (50%)	3 (25%)	
Yes	3 (50%)	9 (75%)	
Laboratory findings			
WBC ($\times 10^9$/L)	10.4 ± 6.5	9.6 ± 2.6	0.730
Neutrophil($\times 10^9$/L)	8.9 ± 5.9	8.3 ± 2.4	0.750
Lymphocyte ($\times 10^9$/L)	0.7 ± 0.3	1.0 ± 0.7	0.300
HGB (g/L)	107.2 ± 26.8	143.6 ± 13.2	0.001
CRP (mg/L)	82.6 ± 72.9	148.9 ± 79.7	0.110
PCT (μg/L)	3.4 ± 5.3	11.9 ± 11.5	0.110
Albumin (g/L)	30 ± 10.8	38.3 ± 5.2	0.040
ALT (U/L)	70.7 ± 108.3	15.3 ± 4.8	0.086
AST (U/L)	72.7 ± 93.7	15.8 ± 6.3	0.046
D-Dimer (mg/L)	2.6 ± 3.3	1.4 ± 1.1	0.290
APTT (s)	50.7 ± 10	37.2 ± 4.4	<0.001
PT (s)	16.9 ± 4.5	14.1 ± 0.8	0.042

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; APTT, activated partial thromboplastin time; PT, prothrombin time; HGB, hemoglobin; WBC, white blood cell; CRP, C-reaction protein; PCT, procalcitonin.

To better carry out emergency surgery during the outbreak, our hospital has developed a detailed management strategy for acute abdomen patients. For patients with stable vital signs and local involvement (such as acute appendicitis alone, acute cholecystitis alone, and incomplete ileus) not requiring emergency surgery, conservative treatment in the outpatient department can be considered. If conservative treatment fails, emergency surgery should be performed immediately. The goal of emergency surgery is to remove the patient's lesions rapidly and effectively while minimizing the operation time and limiting the medical staff's exposure.

The indications for emergency surgery should be strictly managed during the COVID-19 outbreak. The possible reasons for opposing surgical interventions for acute abdomen accompanied with COVID-19 pneumonia are as follows: 1) Surgical interventions on patients with COVID-19 may lead to contamination of the operating room and surgical equipment and risk transmission of the infection to healthcare providers and other patients in the hospital [17, 23]; 2) surgical treatment may trigger oxidative stress [24] and immunosuppression [25], which might hinder the clearance of SARS-CoV-2 and accelerate the progression of COVID-19 pneumonia. However, the scientific foundation of this theory is very weak. Jamali et al. reported that preoperative pneumonia only moderately increased the risk of mortality (OR= 1.2) in patients undergoing emergency surgery [14]. Moreover, an improvement of acute abdomen and pneumonia after surgery was observed in our study. A possible explanation for such results is that surgical

treatment alleviated excessive inflammation and persistent immunosuppression caused by acute abdomen, which in turn contributed to clearance of the virus and resolution of lung inflammation. In addition, medical staff could effectively prevent SARS-CoV-2 infection through adherence to strict infection prevention and control protocols [16, 26]. No new infections were found in medical staff or patients throughout the hospitalization of patients with or without COVID-19 pneumonia in our study.

A

Characteristics	Patients with acute abdomen		P value
	With COVID-19 (n = 6)	Without COVID-19 (n = 12)	
Length of hospitalization (days)	19.3 ± 10	13.3 ± 5.2	0.032
Postoperative complications			0.792
No	4 (67%)	9 (75%)	
Yes	2 (33%)	3 (25%)	
Clinical outcome			1.000
Discharged	5 (83%)	10 (83%)	
Death	1 (17%)	2 (17%)	

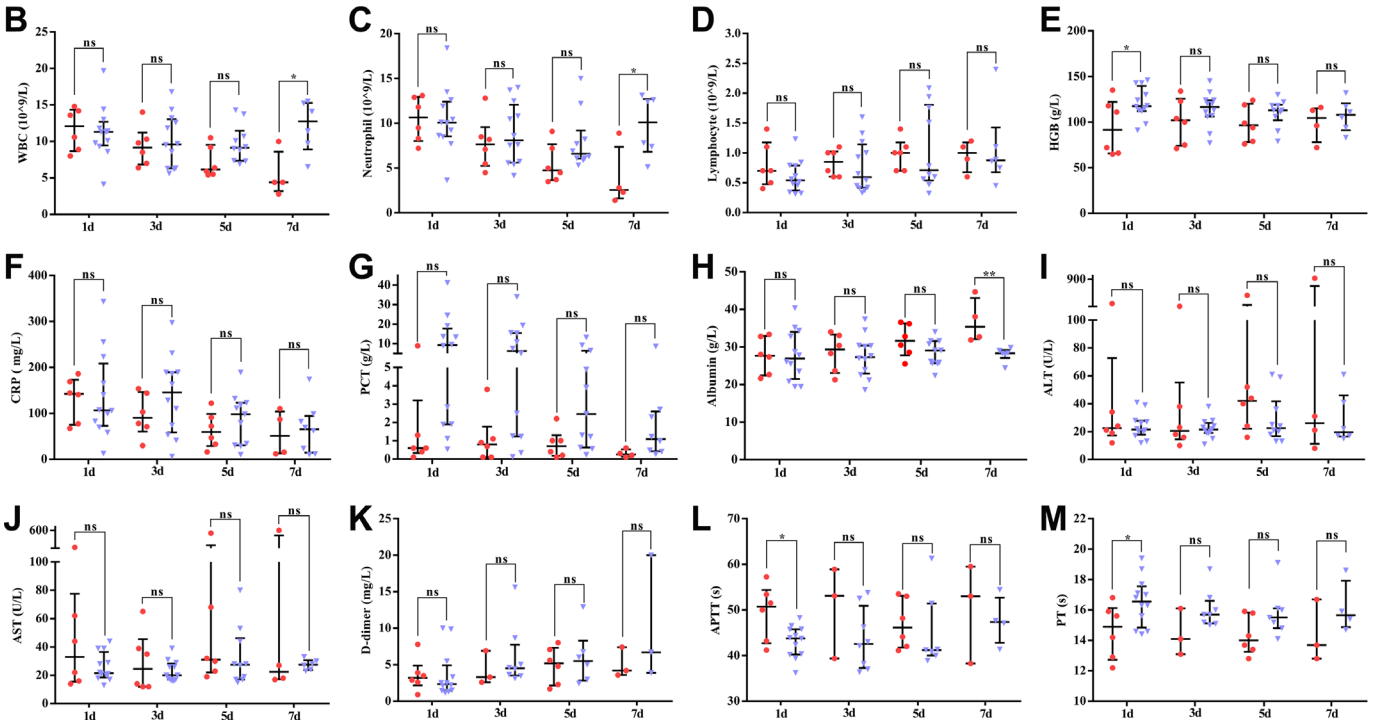

Figure 4. The difference between patients with COVID-19 pneumonia and those without COVID-19 pneumonia (aged between 60 and 80) in postoperative outcomes. Data are presented as numbers and percentages for categorical variables, and continuous data are expressed as the mean ± standard deviation (SD). *P< 0.05, **P< 0.01, ***P <0.001, based on Student's t-test. (**A**) The difference between both groups in clinical outcomes; (**B**–**M**) shows the differences in postoperative laboratory findings between patients with and without COVID-19 pneumonia, including (**B**) WBCs (white blood cells); (**C**) neutrophils; (**D**) lymphocytes; (**E**) HGB (hemoglobin); (**F**) CRP (C-reactive protein); (**G**) PCT (procalcitonin); (**H**) Albumin; (**I**) ALT (alanine aminotransferase); (**J**) AST (aspartate aminotransferase); (**K**) D-dimer; (**L**) APTT (activated partial thromboplastin time); (**M**) PT (prothrombin time). Red and blue marks represent patients with and without COVID-19 pneumonia, respectively.

Current clinical observations have found that most COVID-19 patients have fever and acute abdomen patients often have fever. In our study, 5 patients (17.9%) presented with fever before emergency surgery. Some postoperative patients may present with fever, which may result from postoperative traumatic stress or residual abdominal infection. This makes it extremely difficult to identify the cause of fever and to identify COVID-19 in a timely manner. Elderly patients, especially those with pulmonary infections, are more susceptible to COVID-19 during the postoperative hospitalization period. Therefore, we monitored the patient's body temperature closely, and routine blood parameters, including PCT and CRP, were regularly retested. If necessary, a chest CT scan was performed again to monitor COVID-19 pneumonia progression. To ensure therapeutic efficacy, we streamlined treatments to reduce doctor-patient contact and avoid cross-infection.

This study had certain limitations that should be discussed. First, due to the lack of definite practical guidance for patients with both acute abdomen and COVID-19 pneumonia, the indication and timing of the surgical treatment was decided empirically instead of being based on evidence. Second, this was a small-sample nonrandomized retrospective study without strict inclusion and exclusion criteria, and as such, there were potential biases that could affect the comparison analysis. Third, the availability of clinical care and the diversity of COVID-19 management may limit the applicability of our results. However, to our knowledge, the results of our study provide the first evidence that emergency surgery could not only improve the outcomes of acute abdomen patients with COVID-19 pneumonia, but also benefit the resolution of pulmonary inflammation. These results hopefully lead to a consensus on the treatment and management of acute abdomen patients with or without COVID-19 during the COVID-19 outbreak.

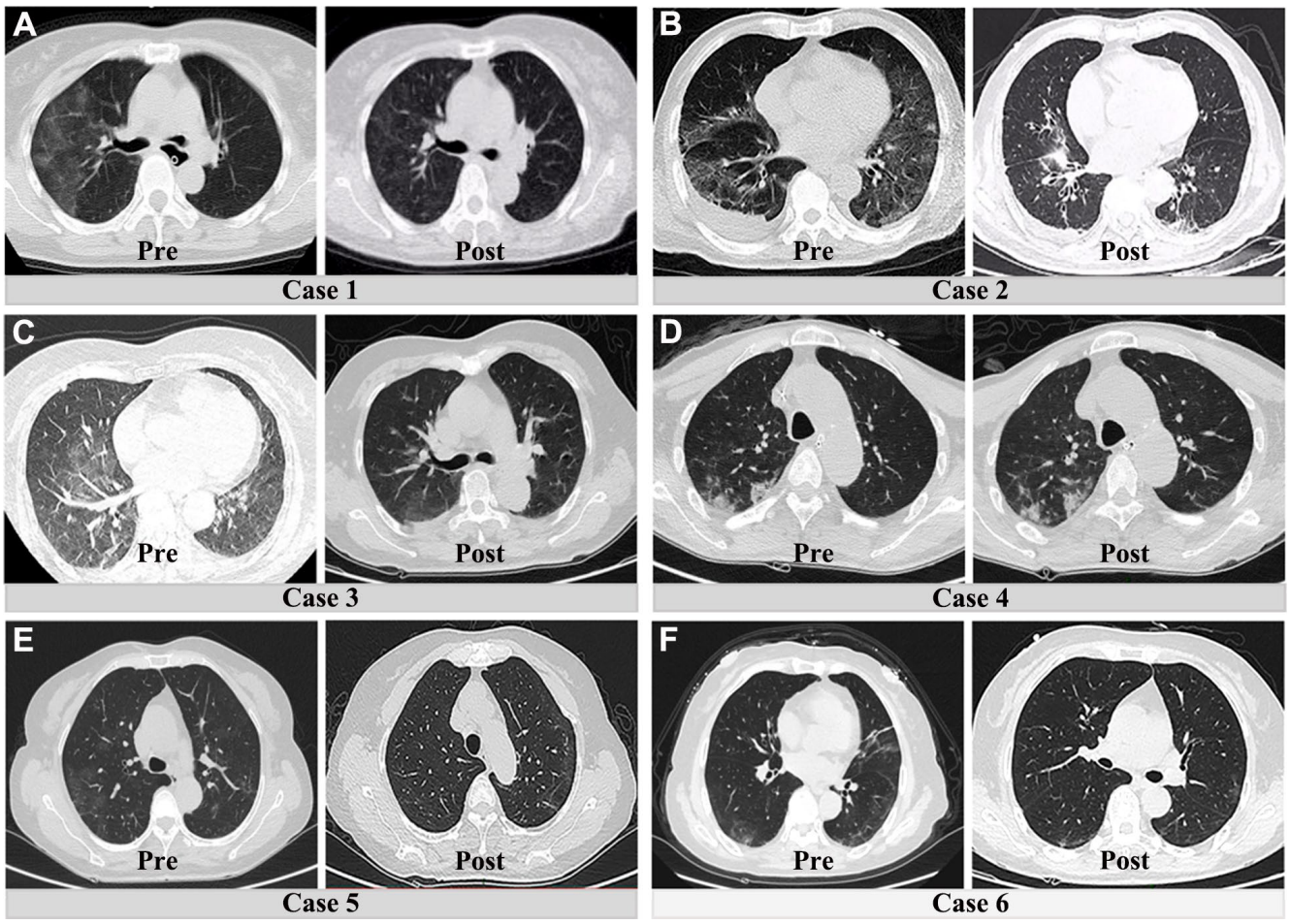

Figure 5. Preoperative and postoperative CT lung manifestations in six patients with both acute abdomen and COVID-19 pneumonia. (**A**–**C**) and (**E**, **F**) show the obvious resolution of pulmonary inflammation. The fourth patient had no significant change of pulmonary inflammation after surgical treatment (**D**).

MATERIALS AND METHODS

Study design and patient cohort

We retrospectively reviewed 34 patients with acute abdomen who underwent emergency surgery from February 2, 2020, to March 18, 2020, at the Union Hospital affiliated with Tongji Medical College, Huazhong University of Science and Technology. This study protocol was approved by the ethics committee of our college. All patients signed an informed consent document indicating their understanding of the procedure and its potential complications as well as their approval to participate in the research study. A flow diagram of the emergency surgery protocol for patients with acute abdomen during COVID-19 outbreaks is presented in Figure 6.

Preoperative work-up

After a detailed history and a complete physical examination, all patients with acute abdomen underwent routine laboratory testing (such as complete blood counts, serum biochemistry and tumor-marker screening) and imaging examination (such as chest X-ray, abdominal ultrasound, contrast-enhanced computed tomography (CT)). Prior to admission, all patients also completed a detailed risk assessment for COVID-19, including typical clinical manifestation and contact history with suspicious or confirmed COVID-19 patients within 14 days. CT lung imaging, quantitative reverse transcription-polymerase chain reaction (RT-PCR) and IgM-IgG antibodies against SARS-CoV-2 were also required for all patients to screen for potential infections. All suspected COVID-19 cases were treated as positive until confirmed. If emergency surgery was required, patients with positive indicators for infection must be taken directly to a designated COVID operation room through a predefined path. Due to the possible false negatives of test kits, all surgical procedures were carried out using a high level of protection, including masks, eye protection, gloves, caps and protective clothing, for the entire duration of the procedure.

Postoperative work-up

All patients who were not excluded from possibly having COVID-19 were transferred to the isolation ward and transitional ward after surgery according to the status of the preoperative screening results. Medical staff were required to adhere to strict prevention and infection control protocols in addition to routine universal precautions, and all patients were advised to wear a mask throughout hospitalization. Patients in the transitional ward underwent another round of RT-PCR for SARS-CoV-2. If the screening yielded negative results, the patient was transferred and treated in a single room of the general ward for three to five days prior to transfer to a shared room. If patients presented with pyrexia of unknown origin, typical respiratory symptoms or CT imaging manifestations indicating viral pneumonia, they were transferred to the

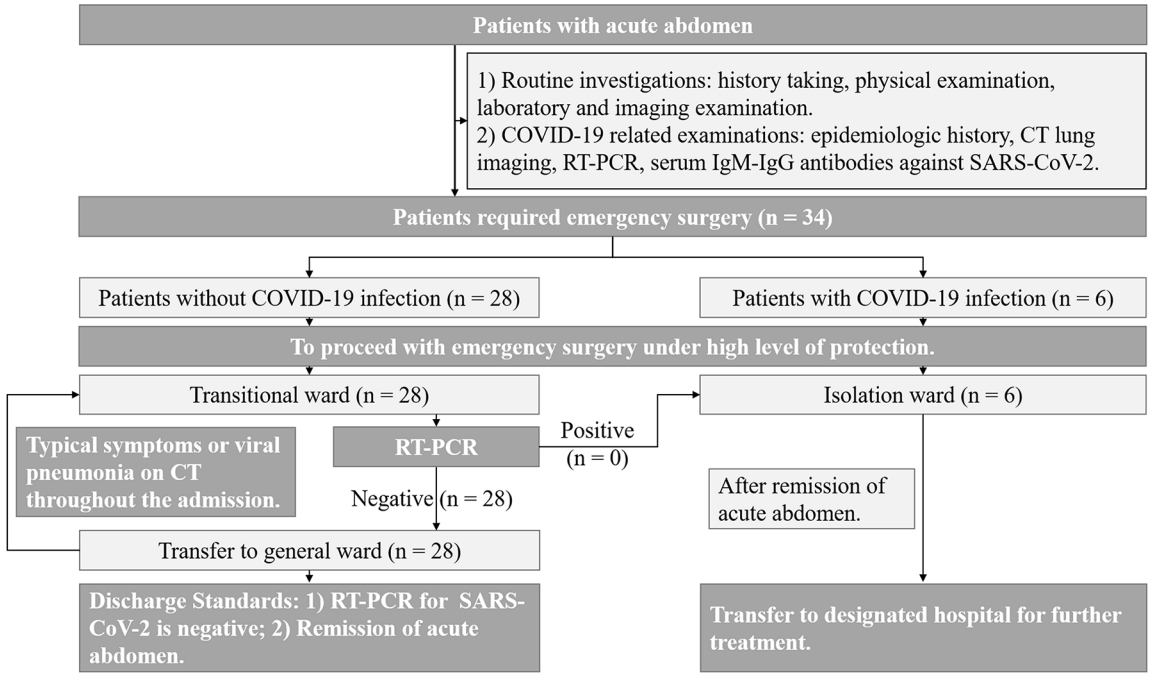

Figure 6. Flow diagram for performing emergency surgery for acute abdomen patients during COVID-19 outbreak.

transitional ward to retest for SARS-CoV-2 infection by RT-PCR. If the screening yielded positive results, patients were transferred to the isolation ward for further treatment. After the remission of acute abdomen, patients in the isolation ward were advised to transfer to designated hospitals for the treatment of COVID-19.

Data collection

Primary data, including clinical characteristics, the laboratory and imaging examination results, evidence of COVID-19, treatments, and clinical outcomes, were identified from medical reports. Blood samples from all participants were obtained through peripheral venipuncture. The following thresholds were considered the normal range of indicators: creatinine, 58-110 μmol/L; total bilirubin, 3-22 μmol/L; albumin, 35-50 g/L; alanine aminotransferase (ALT), 21-72 U/L; aspartate aminotransferase (AST), 17-59 U/L; hemoglobin (HGB), 130-175 g/L; white blood cell (WBC) count, $(3.5\text{-}9.5)\times10^9$/L; neutrophil count, $(1.8\text{-}6.3)\times10^9$/L; lymphocyte count, $(1.1\text{-}3.2)\times10^9$/L; C-reaction protein (CRP), < 8 mg/L; procalcitonin (PCT), < 0.5 μg/L; D-dimer, < 0.5 mg/L; activated partial thromboplastin time (APTT), 28-43.5 s; and prothrombin time (PT), 11-16 s. We adopted the classification system of the New Coronavirus Pneumonia Prevention and Control Program (7th edition). According to this system, COVID-19 pneumonia cases were divided into four groups: mild, moderate, severe and critically ill. The discharge requirements for patients who only had acute abdomen include 1) remission of acute abdomen and 2) negative SARS-CoV-2 results by RT-PCR. However, for acute abdomen patients with COVID-19 pneumonia, obvious resolution of pulmonary inflammation and negative results by RT-PCR for two consecutive evaluation times were necessary for discharge.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA), and diagrams of curves were drawn using Prism (version 6.0; GraphPad). Data are presented as numbers and percentages for categorical variables, and continuous data are expressed as the mean ± standard deviation (SD). Differences were considered significant at *p<0.05, **p <0.01 and ***p <0.001. ns: no significance.

AUTHOR CONTRIBUTIONS

All authors conceived the study and study design, iteratively drafted the article, and approved the final article. ZG and CJH obtained institutional review board approval. CYF, ZH, HP, HCJ, CD, XP, CQY and CP coordinated the data collection. ZN, WL, and CYF coordinated discussions with statisticians and performed the analyses. ZG and CJH take responsibility for the paper as a whole.

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CONFLICTS OF INTEREST

The authors have declared that no conflicts of interest exist.

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Research Paper

Early coagulation tests predict risk stratification and prognosis of COVID-19

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ABSTRACT

The ongoing outbreak of Coronavirus Disease 2019 (COVID-19) is hitting the world hard, but the relationship between coagulation disorders and COVID-19 is still not clear. This study aimed to explore whether early coagulation tests can predict risk stratification and prognosis. PubMed, Web of Science, Cochrane Library, and Scopus were searched electronically for relevant research studies published up to March 24, 2020, producing 24 articles for the final inclusion. The pooled standard mean difference (SMD) of coagulation parameters at admission were calculated to determine severe and composite endpoint conditions (ICU or death) in COVID-19 patients. Meta-analyses revealed that platelet count was not statistically related to disease severity and composite endpoint; elevated D-dimer correlated positively with disease severity (SMD 0.787 (0.277-1.298), P= 0.003, I^2= 96.7%) but had no significant statistical relationship with composite endpoints. Similarly, patients with prolonged prothrombin time (PT) had an increased risk of ICU and increased risk of death (SMD 1.338 (0.551-2.125), P = 0.001, I^2 = 92.7%). Besides, increased fibrin degradation products (FDP) and decreased antithrombin might also mean the disease is worsening. Therefore, early coagulation tests followed by dynamic monitoring is useful for recognizing coagulation disorders accompanied by COVID-19 and guiding timely therapy to improve prognosis.

INTRODUCTION

In December 2019, a group of patients with unexplained pneumonia in Wuhan, China was found to be infected with a previously unknown coronavirus, officially named later as Coronavirus Disease 2019 (COVID-19). The coronavirus was initially called 2019-nCoV but was subsequently renamed severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) because it has 75-80% genomic similarity to SARS-CoV and 50% resemblance to the Middle East Respiratory Syndrome coronavirus (MERS-CoV) [1]. SARS-CoV2 is the third known kind of coronavirus that causes severe acute respiratory distress syndrome (ARDS) in humans, the others being SARS-CoV and MERS-CoV. As of April 7, 2020, 1,342,184 cases have been confirmed worldwide. Although the fatality rate will continue to change until all infected persons have recovered, it appears that SARS-CoV2 is less deadly (approximately 3.7%) than SARS-CoV (~10%) and much less than MERS-CoV (~40%) [2, 3]. Regrettably, the outbreak of COVID-19 is spreading wide and amplifying mainly because of the long incubation period and high infection rates, raising great public health concerns globally.

Unfortunately, some studies have revealed that mortality rates in critical COVID-19 patients are high (~41.7%), possibly because of the association of the disease with severe complications, including organ failure, sepsis/septic shock, and sepsis-associated coagulopathy [4–11]. Generally, the three conditions mentioned above are complexly linked in critical patients. Sepsis is consistently common in severe patients with SARS-CoV2 infection as a secondary disease [5]. Septic shock and sepsis-associated coagulopathy are severe conditions of sepsis, both of which can result in organ failure. The early reported incidence of at least one organ dysfunction is about 30%~60% in critically ill patients and non-survivors [5, 6, 12, 13], while the reported incidence of shock varies from 23% to 70% [5, 6, 13]. However, coagulopathy in COVID-19 has been reported rarely; only three articles have mentioned this problem up to now.

In the first report of the occurrence of disseminated intravascular coagulation (DIC), the worst form of coagulopathy, in a large epidemiological study on COVID-19, only 0.6% of the patients with severe cases had DIC; the standard used for diagnosis was not mentioned, and no one had DIC among non-severe patients [8]. Tang's analysis focusing on abnormal coagulation parameters revealed that 71.4% (15/21) of non-survivors with COVID-19 met the criteria for overt-DIC [11]. Zhou and his colleagues later found that 50% of non-survivors with COVID-19 had coagulopathy, and only 7% of survivors had coagulopathy [5]. However, DIC encompasses a broad spectrum of clinical manifestations, ranging from a prothrombotic state to bleeding or both [14], and there is a lack of a golden approach to diagnosing DIC, easily leading to misdiagnosis and missed diagnoses. To optimize patient care and resource allocation during this pandemic, coagulation parameters reflecting coagulopathy and DIC are urgently needed for risk stratification and for actively monitoring illness severity.

Abnormal coagulation parameters reflecting coagulopathy, including platelet count, D-dimer level, prothrombin time (PT), and activated partial thromboplastin time (APTT), are common in many COVID-19 patients at admission. However, these indicators, as presented in different articles, are providing contradictory messages to guiding risk stratification and predicting outcomes. Although two independent teams have shown that severe COVID-19 patients have significantly lower platelet counts than non-severe patients [10, 15], other teams have demonstrated that there is no significant difference between the two groups [6, 7, 13, 16–18]. Almost all related articles have reported that critical or non-survivor patients had statistically significantly higher levels of D-dimer than non-severe or survivor patients [4, 6, 10, 19–22], except for one [15]. PT is more prolonged in severe patients in some articles [6, 10, 11], but not so in other reports [4, 13, 19, 23]. APTT in severe COVID-19 patients appears more complicated, longer than in non-severe patients [10] or shorter than in non-severe patients [4, 21] or similar to the one in non-severe patients [6, 11, 13, 23, 24]. Some reports have shown that there is no significant difference in fibrinogen levels between severe COVID-19 patients and non-severe patients [11, 17, 19], but one article found higher levels in severe patients [23]. Therefore, we did a meta-analysis and a systematic review to comprehensively analyze the significance of early coagulation tests and understand coagulopathy during COVID-19 progression for disease stratification and prediction of the composite endpoint (ICU admission or death).

RESULTS

The outcome of the electronic search

Overall, 3370 documents were initially identified based on our search criteria and a reference list (Figure 1). Subsequently, 1669 files were excluded because of duplication, and 1627 were excluded after reading the title and abstract and finding that the materials were not related to medicine (n = 488) or failed to report clinical characteristics or laboratory tests (n = 657) or that they were reviews (n = 271), or expert consensus (n = 96), meta-analyses (n = 9), or case reports (n = 74). Additionally, 32 documents relating to children were excluded. As a result, 74 articles were selected for full-text assessment. Of the 74 studies, 50 were disqualified for lacking information on coagulation test data (n = 34), or having no definition of disease severity (n = 12), or lacking descriptive summary analyses (n = 3), or being a review (n = 1). In the end, 24 articles were included for the meta-analysis. To eliminate bias, the detailed endpoint was split into severity and composite endpoint instead of a rough poor outcome. Also, we analyzed several biomarkers individually rather than treat them as one entity.

Characteristics of the 24 selected studies

Of the articles included, 23 were full-length articles published in peer-reviewed journals, and one article was provided by the corresponding author after we reached out to them. Most of the studies were from China (n = 22), except for two from Singapore. All the investigations were case-control trials assessing 3544 adult COVID-19 patients; the sample size of each study varied from 21 to 1099 participants. The vast majority of patients were diagnosed using laboratory nucleic acid tests, except for three patients who were diagnosed based on clinical characteristics and imaging data. The details of the selected studies are provided in Table 1.

Ottawa quality assessment scale (NOS) was used to evaluate the quality of the chosen literature, and all literature scored ≥ 8 points (Supplementary Table 1), indicating that the quality of each of the 24 studies was high.

The relationship between platelets and disease severity or composite endpoint

The relationship between disease prognosis and platelets was analyzed in 16 articles with 2980 COVID-19 patients (Table 2). Of the 16 articles, 12 studies with 2152 patients were used to analyze the relationship between platelets and disease severity, [7, 8, 10, 12, 15, 17, 18, 22, 25–27] and 1778 patients in 6 articles were used to analyze the relationship between platelets and composite endpoint [6, 8, 12, 13, 16]. Pooled analyses revealed that platelet count was not statistically linked to disease severity (standard mean difference (SMD) -0.271 (-0.547-0.005), P = 0.054, I^2 = 84.6%) and composite endpoint (SMD -0.541 (-1.109-0.028), P = 0.062, I^2 = 92.5%) on admission (Table 2, Figures 2 and 3). Because the heterogeneity value was over 50%, the random effect model was used for the meta-analysis of these articles.

The relationship between D-dimer and disease severity or composite endpoint

In this meta-analysis, we explored the relationship between D-dimer and prognosis in 1762 patients with COVID-19 from 13 investigations (Table 2). Based on the data from 1438 participants in 11 trials, [4, 10, 15, 17, 19, 20, 22, 23, 25, 28].

We found that D-dimer correlated positively with disease severity in patients with COVID-19 (SMD 0.787 (0.277-1.298), P = 0.003, I^2 = 96.7%), suggesting that D-dimer levels were significantly elevated in critically ill patients. Also, 410 patients in three articles were assessed for the relationship between D-dimer and composite endpoint [5, 13], but we found no statistical relationship between the two parameters (SMD 1.523 (-0.221-3.267), P = 0.0087, I^2 = 97.5%), see Table 2, Figures 2 and 3.

The relationship between PT and disease severity or composite endpoint

Eleven articles with 1641 patients were analyzed for PT; 7 articles with 940 cases were evaluated for the relationship between PT and disease severity [4, 10, 19, 21, 23], and 5 articles with 645 cases were examined for the relationship between PT and composite endpoint [5, 11–13]. The analyses showed that prolonged PT during admission indicated a more serious disease, with the two correlating positively (SMD 0.803 (0.254-1.352), P = 0.004, I^2 = 91.3%). Similarly, patients with prolonged PT had an increased risk of ICU during admission and increased risk of death (SMD 1.338 (0.551-2.125), P = 0.001, I^2 = 92.7%), see Table 2, Figures 2 and 3.

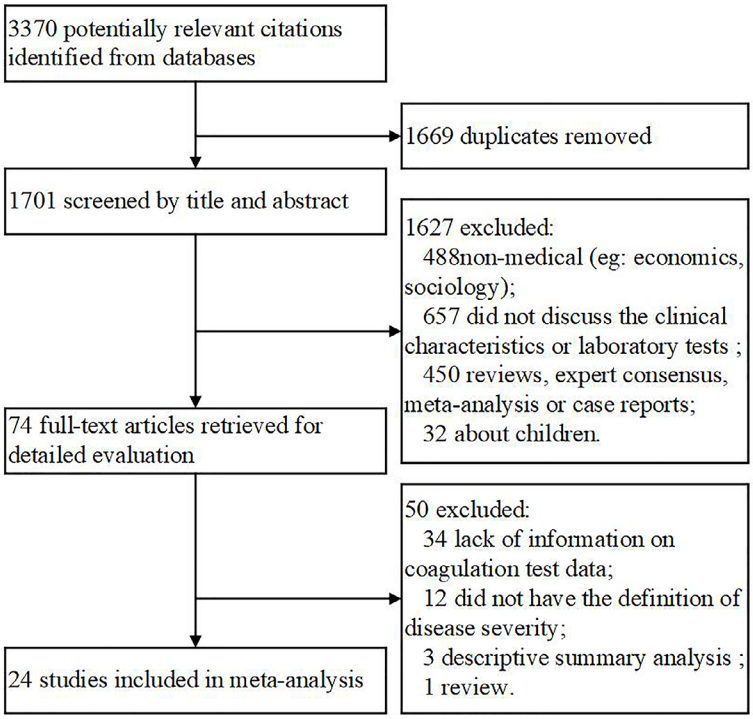

Figure 1. Flow chart of the included studies.

Table 1. Basic information of included studies.

Num	Study cohort	Journal	Institute/region	Period	Follow-up	Study type	No.(M/F)	Diagnose	Age (year)	Compared endpoint	NOS
1	Cao B [6]	Lancet	Jinyintan Hospital & Wuhan Pulmonary Hospital	2019/12/29-2020/1/31	NA	case control	191 (119/72)	laboratory-confirmed	56.0 (46.0–67.0)	composite endpoint	8
2	Sun ZY [11]	J. Thromb. Hemost.	Tongji Hospital	2020/1/1 - 2020/2/3	2020/2/13	case control	183 (98/85)	laboratory-confirmed	54.1 (14-94)	composite endpoint	8
3	Cao B (2) [5]	Lancet	Jinyintan Hospital	2019/12/16 -2020/1/2	2020/1/22	case control	41 (30/11)	laboratory-confirmed	49.0 (41·0-58.0)	composite endpoint	8
4	Ning Q [21]	NA	Tongji Hospital	2019/12/19-2020/1/27	2020/2/2	case control	21 (17/4)	laboratory-confirmed	56.3 (42.0-70.6)	severity status	8
5	Peng ZY [13]	JAMA	Zhongnan Hospital	2020/1/1-2020/1/28	2020/2/3	case control	138 (75/63)	laboratory-confirmed	56 (42-68)	composite endpoint	8
6	Zhong NS [8]	NA	552 hospitals	2020/1/29	2020/1/29	case control	1099 (640/459)	laboratory-confirmed	47.0 (35.0-58.0)	severity status/composite endpoint	8
7	Song YL [2]	JAMA Internal Medicine	Jinyintan Hospital	2019/12/24-2020/1/26	2020/2/13	case control	201 (128/73)	laboratory-confirmed	51(43-60)	severity status/composite endpoint	8
8	Hu B [22]	NA	Union Hospital	2020/1/16-2020/2/19	NA	case control	214 (127/87)	laboratory-confirmed	52.7 (37.2-68.2)	severity status	8
9	Zhang YX [15]	Clin Infect Dis	Zhongnan Hospital	2020/1/1-2020/2/5	NA	case control	155 (86/69)	laboratory-confirmed	54 (42-66)	severity status	8
10	Li LJ [36]	BMJ	Zhejiang Province	2020/1/10-2020/1/26	2020/1/26	case control	62 (36/27)	laboratory-confirmed	41 (32-52)	severity status	8
11	Shang Y [12]	The Lancet Respiratory Medicine	Jinyintan Hospital	2019/12-2020/1/26	2020/2/9	case control	52 (35/17)	laboratory-confirmed	59.7 (46.4-73.0)	composite endpoint	8
12	Ong, K H [16]	Am J Hematol	Singapore	2020/1/23-2020/2/28	2020/2/28	case control	67 (37/30)	laboratory-confirmed	42(35-54)	composite endpoint	8
13	Wang Q [18]	Journal of medical virology	Huizhou municipal central hospital from	2020/1-2020/2	2020/2/21	case control	30 (16/14)	laboratory-confirmed	50.5 (36-65)	severity status	8
14	Hu Y [27]	Chin Med J	Tongji Hospital	2019/12/30-2020/1/15	2019/12/30-2020/1/15	case control	78 (39/39)	laboratory-confirmed	38 (33-57)	severity status	8
15	Chen XM [17]	QJM	Zhejiang province	2020/1/20-2020/2/11	2020/2/16	case control	91 (37/54)	88 laboratory-confirmed & 3 clinical-confirmed	50 (36.5-57)	severity status	8
16	Gao YD [20]	Allergy	No. 7 Hospital of Wuhan	2020/1/16-2020/2/3	NA	case control	140 (71/69)	laboratory-confirmed	57 (25-87)	severity status	8
17	Zhang RG [7]	Clin Infect Dis	Union Hospital	2020/1/16-2020/1/29	2020/2/4	case control	69 (32/37)	laboratory-confirmed	42.0 (35.0-62.0)	severity status	8
18	Zhu CL [19]	Clinical chemistry and laboratory medicine	Renmin Hospital	2020/1/31-2020/2/10	NA	case control	134 (76/68)	laboratory-confirmed	NA	severity status	9
19	Wang LD [23]	Journal of medical virology	Fuyang Second people's hospital	2020/1/23-20202/2	NA	case control	43 (26/17)	laboratory-confirmed	43.74 ± 12.12	severity status	8
20	Zeng QT [24]	Zhonghua xin xue guan bing za zhi	Union Hospital	2020/1/20-2020/2/15	NA	case control	112 (53/59)	NA	62 (55-67)	severity status	8

21	Li CH [28]	Chinese journal of tuberculosis and respiratory diseases	Jianghan university hospital	2020/1/10-2020/1/31	NA	case control	30 (10/20)	laboratory-confirmed	35(27-43)	severity status	8
22	Barnaby EY [26]	JAMA	Singapore	2020/1/23-2020/2/3	2020/2/25	case control	18 (9/9)	laboratory-confirmed	47 (31-73)	severity status	8
23	Yang SR [10]	J Med Virol	Chongqing Three Gorges Central Hospital	2020/1/23-2020/2/8	NA	case control	135 (72/63)	laboratory-confirmed	47 (36-55)	severity status	8
24	Hu Y	NA	3 designated hospitals in Wuhan	2020/1/15-2020/2/15	2020/3/10	case control	380 (207/173)	laboratory-confirmed	64 (53-73)	severity status/composite endpoint	8

The second column is the corresponding author of the article. Composite endpoint means ICU or death. Not applicable (NA); M/F (male/female); Newcastle-Ottawa quality assessment scale (NOS).

Table 2. Summary of the meta-analysis results.

Biomarker	Total no. of studies	Total no. of patients	Endpoint	No. of studies	No. of patients	Statistical method	pooled Standard Mean Difference (SMD)	P	I^2	P (Heterogeneity)	P Begg's Test	P Egger's test
Platelet	16	2980	severity status	12	2152	I-V, Random	-0.271 (-0.547-0.005)	0.054	84.60%	<0.001	0.732	0.951
			composite endpoint	6	1778	I-V, Random	-0.541 (-1.109-0.028)	0.062	92.50%	<0.001	0.462	0.413
PT	11	1641	severity status	7	940	I-V, Random	0.803 (0.254-1.352)	**0.004**	91.30%	<0.001	0.368	0.224
			composite endpoint	5	645	I-V, Random	1.338 (0.551-2.125)	**0.001**	92.70%	<0.001	1.000	0.300
APTT	10	1388	severity status	7	940	I-V, Random	-0.133 (-0.668-0.402)	0.625	91.50%	<0.001	0.368	0.499
			composite endpoint	4	593	I-V, Random	0.327 (-0.630-1.285)	0.503	94.90%	<0.001	0.734	0.591
D-dimer	13	1762	severity status	11	1438	I-V, Random	0.787 (0.277-1.298)	**0.003**	96.70%	<0.001	0.062	0.510
			composite endpoint	3	410	I-V, Random	1.523 (-0.221-3.267)	0.087	97.50%	<0.001	1.000	0.805
Fibrinogen	5	682	-	-	-	I-V, Random	0.559 (-0.599-1.718)	0.344	96.70%	<0.001	0.806	0.317
FDP	3	548	-	-	-	I-V, Random	1.046 (0.371-1.722)	**0.002**	88.90%	<0.001	1.000	0.806
Antithrombin	3	548	-	-	-	I-V, Random	-0.798(-1.217 - -0.379)	**<0.001**	72.20%	0.027	0.296	0.190

prothrombin time (PT); activated partial thromboplastin time (APTT); fibrin/fibrinogen degradation products (FDP).

The relationship between APTT and disease severity or composite endpoint

10 articles with 1388 COVID-19 patients were analyzed for the relationship between disease prognosis and APTT (Table 2); 7 articles with 940 patients were assessed for the relationship between APTT and disease severity [4, 10, 19, 21, 23, 24], and 593 patients in four articles were studied for the relationship between APTT and composite endpoint [5, 11, 13]. Our results revealed that APTT was not statistically associated with disease severity and composite endpoint at admission (Table 2, Figures 2 and 3).

The relationship between fibrinogen, fibrin/ fibrinogen degradation products (FDP), anti-thrombin, and prognosis

Five studies with 682 patients were analyzed for the effect of fibrinogen on prognosis [11, 17, 19, 23]. We found that fibrinogen had no value in predicting disease prognosis in COVID-19 patients (SMD 0.559 (-0.599-1.718), P = 0.344, I^2 = 96.7%) (Supplementary Figure 1). Furthermore, 548 cases in three articles were evaluated for the relationship between FDP, antithrombin, and prognosis [11, 19]. Our results revealed that increased FDP (SMD 1.046 (0.371-1.722, P = 0.002, I^2 = 88.9%) and decreased antithrombin (SMD -0.798 (-1.217-0.379), P<0.001, I^2 = 72.2%) were associated with the worsening of COVID-19 (Table 2, Supplementary Figure 2).

Sensitivity analysis and publication bias

A Funnel plot was drawn to test publication bias, and Egger's test and Begg's test indicated that there was no publication bias (Supplementary Figures 3, 4). Sensitivity analysis revealed that no study greatly interfered with the results of this meta-analysis study greatly interfered with the results of this meta-analysis, suggesting that the study was stable (Supplementary Figures 5, 6).

DISCUSSION

COVID-19 has raised great public health concerns globally over the last three months. Like with SARS, abnormal coagulation disorders are common in severe patients with COVID-19. Our meta-analysis combined the outcomes of 3544 COVID-19 patients from 24 separate studies and established that elevated D-dimer significantly predicted more severe classifications of COVID-19 patients. Prolonged PT at baseline also suggested poor outcomes, both in severity status and composite endpoint. Increased FDPs and decreased antithrombin might also signal severe conditions.

The platelet count at admission had no remarkable relationship with outcome. However, a meta-analysis

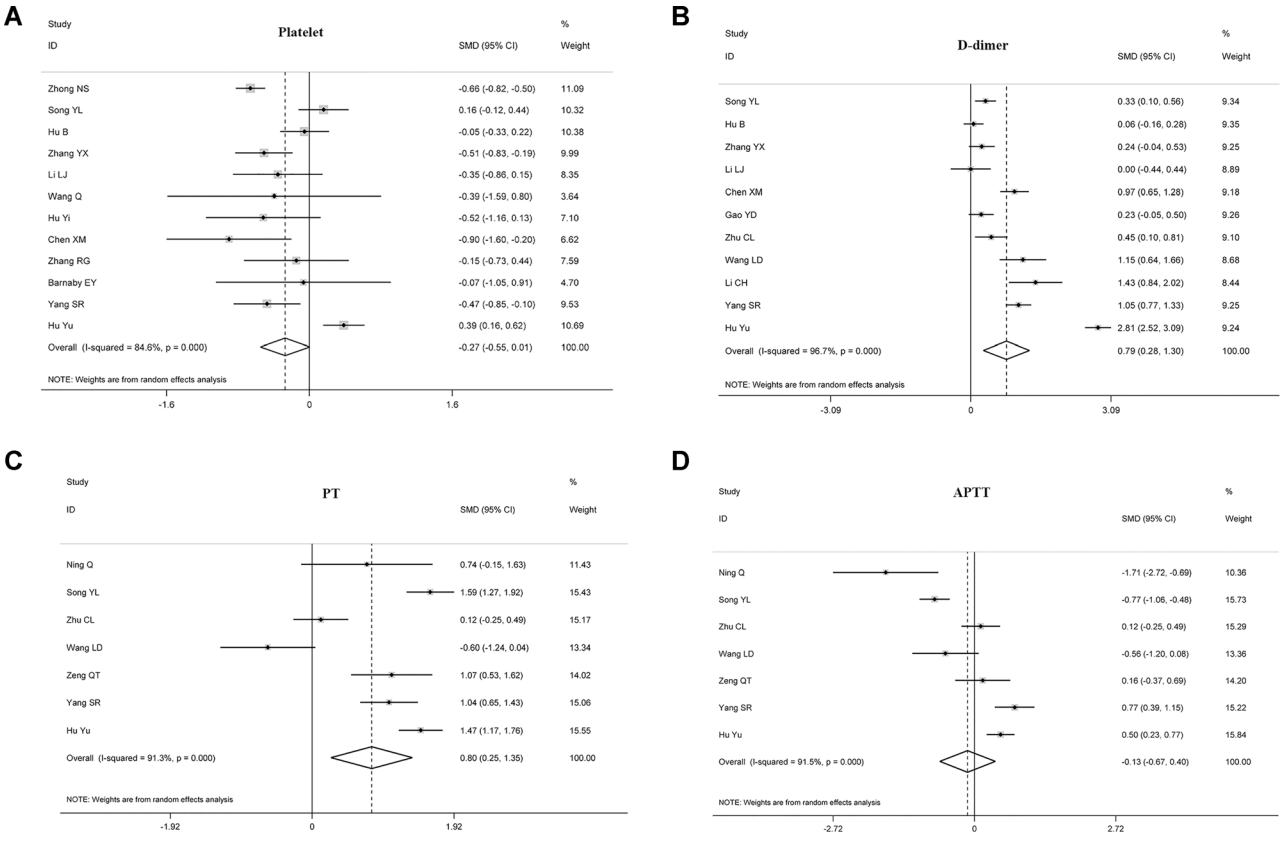

Figure 2. Forest plots assessing the severity status of COVID-19 patients, as determined using coagulation parameters. The sizes of the blocks or diamonds represent the weights, and the lengths of the straight lines represent the widths of the 95% CIs. (**A**) comparing patients by platelet counts; (**B**) comparing patients by D-dimer levels; (**C**) comparing patients by PT; (**D**) comparing patients by APTT. prothrombin time (PT); activated partial thromboplastin time (APTT).

involving 399 subjects showed that platelet counts at admission were significantly lower in more severe and non-survivor COVID-19 patients [29]. This discrepancy in outcome regarding platelet counts may be due to inconsistencies in the selected literature. A national multi-center retrospective study led by Academician Zhong supported the conclusion that platelet count is not statistically linked to a composite endpoint, although the authors also found that severe patients had lower platelets on admission than non-severe patients. One possible reason was the difference in the research objects. In other selected articles, the patients were either in Wuhan or outside Wuhan. The objects in Zhong's article included hospitalized patients both in Wuhan and outside-Wuhan. The early epidemic situation in Wuhan was overwhelming, medical resources were tight, and patients with a milder disease were isolated at home while more severe patients were admitted to the hospital. Patients hospitalized outside-Wuhan got sufficient resources due to they having relatively few cases at the time.

Platelets play a crucial role in hemostasis and thrombosis. While platelet activation and thrombocytosis increase the risk of thrombotic complications, platelet function disorders and thrombocytopenia increase bleeding risk. Thrombocytopenia and reactive thrombocytosis are both common in a variety of viral infections [30–35]. During SARS, most patients' platelet counts were normal at the onset of the disease, but, with time, 55% developed thrombocytopenia (platelet count $< 140\times10^9$/L), and 49% harbored reactive thrombocytosis (platelet count $\geq 400\times10^9$/L) [32]. Similarly, in COVID-19 patients, platelet counts were also within the normal range in most cases at admission [4, 7, 13, 15, 16, 26, 36]; thrombocytopenia (platelet count $< 100\times10^9$/L) was reported primarily in severe patients or non-survivors (20%~66.1%) [5, 8, 11], while thrombocytosis was reported in a few articles, and the proportion was not assessed [21]. The outcome of platelet count changes for the entirety of COVID-19 infection in patients has rarely been reported. Until recently, according to the article with 1476 COVID-19 patients by Yang et al., platelet counts in survivors tended to be stable during hospitalization, but they progressively decreased in non-survivors [37]. Furthermore, the lower the nadir platelet count during hospitalization, the higher the risk of death [37].

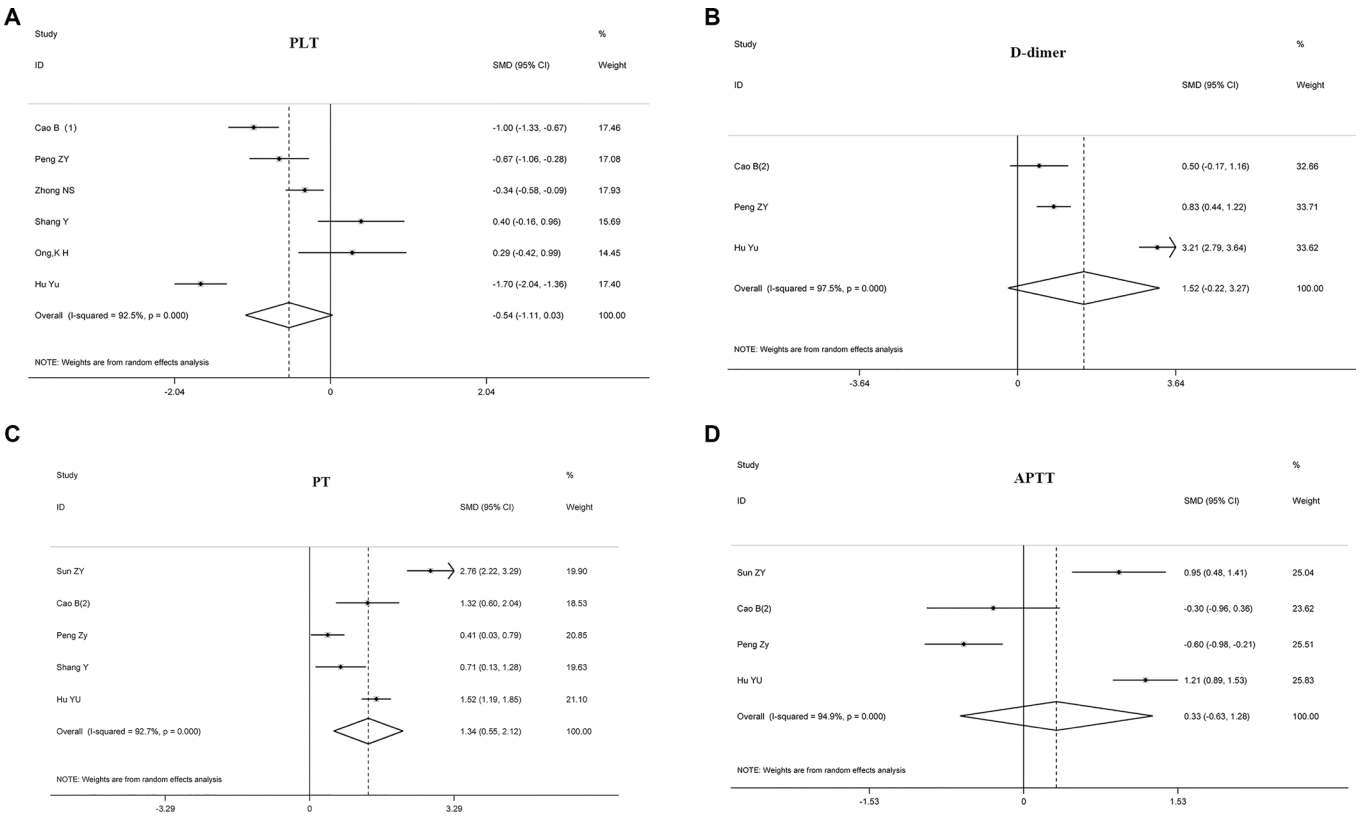

Figure 3. Forest plots assessing the composite endpoint of COVID-19 patients, as determined using coagulation parameters. The sizes of the blocks or diamonds represent the weights, and the lengths of the straight lines represent the widths of the 95% CIs. (**A**) Comparing patients by platelet counts; (**B**) comparing patients by D-dimer levels; (**C**) comparing patients by PT; (**D**) comparing patients by APTT. prothrombin time (PT); activated partial thromboplastin time (APTT).

Thrombocytopenia is often considered an indicator of bleeding and mortality in critical patients [38]. Decreased platelet counts help recognize the presence and severity of coagulopathy [39]. The mechanisms of thrombocytopenia during COVID-19 might include direct or indirect factors induced by the SARS-Cov2 infection, such as inappropriate platelet activation and consumption, immunological platelet destruction, and impaired megakaryopoiesis [40]. Recently, Levi M et al. proposed that localized pulmonary thrombotic microangiopathy where platelet consumption is a common feature, may partly account for thrombocytopenia [41]. Additionally, two independent teams found that COVID-19 patients in ICU had markedly elevated levels of the von Willebrand factor [42, 43], further supporting Levi M's opinion. Though COVID-19-associated coagulopathy belongs to sepsis-induced coagulopathy, thrombocytopenia is less profound [43], which may be related with that COVID-19-accociated-coagulopathy was a severe hypercoagulability rather than consumptive coagulopathy [44]. Bleeding events are less documented or reported in current articles looking at the clinical features of COVID-19, although autopsies have revealed focal hemorrhage in the lungs and spleen and decreased myelopoiesis in the bone marrow [45]. Mao's team found that one of 88 severe patients had a cerebral hemorrhage [21]. Yang et al. showed that 6% of 32 non-survivors had a gastrointestinal hemorrhage [12]. In addition to low platelets, bleeding events in critical COVID-19 patients may also be linked to corticosteroid therapy in more critically ill patients. In the interim guidance of coagulopathy in COVID-19, the ISTH recommends that platelet counts be kept above 50×10^9/L in bleeding patients and above 20×10^9/L in non-bleeding patients.

D-dimer, a more specific marker than FDP reflecting the dissolution of microthrombi, is amplified in septic patients [46], consistent with what is reported in COVID-19 patients [6, 10, 11, 20, 22, 23]. In non-COVID-19 septic patients, D-dimer concentrations do not reach the high values seen in patients with COVID-19 [41, 43]. Generally, FDP correlates positively well with D-dimer, except in some situations, like primary hyperfibrinolysis, and simultaneous measurements of FDPs and D-dimer are useful for more accurate estimations of fibrinolytic states [47]. However, of the articles that met our inclusion criteria, only three provided FDP information, whereas, many articles recorded D-dimer changes. Strikingly, 43.2%~68% of COVID-19 patients had elevated levels of D-dimer [5, 8, 20], and this proportion was as high as 92% in dead patients [5]. Increased D-dimer levels generally indicate a high risk of thrombotic diseases [48]. By the time we started this meta-analysis, the incidence of thrombosis had rarely been reported in COVID-19 patients, although thrombosis and microthrombosis in multiple organs had been observed during autopsies [45]. In a study specifically looking at neurological manifestations, Mao and colleagues revealed that 4.5% of severe COVID-19 patients had an acute ischemic stroke [22]. Another study found that 3.4% of severe COVID-19 patients had a stroke [20]. Recently, several teams in different countries emphasized the high incidence of thrombotic events in severe COVID-19 patients. In a study of 81 ICU patients without routine thromboprophylaxis in China, the incidence of deep vein thrombosis was 25% [49]. In Netherlands, two independent researches where routine low molecular weight heparin prophylaxis was applied, reported similar (even higher) incidence of venous thromboembolism (VTE) among ICU patients with COVID-19 [50, 51]. Most recently, Helms et al. showed that COVID-19-ARDS patients developed significantly more thrombotic complications than non-COVID-19-ARDS patients based on a multicenter prospective cohort study [43].

In COVID-19 patients, especially severe patients, the mechanisms of elevated D-dimer or thrombosis may include older age, chronic diseases, hypoxemia, hypercytokinemia, coagulopathy, and inevitable prolonged bed rest. It is already well-established that older individuals and those who have co-morbidities and hypercytokinemia are more likely to die from COVID-19 infection [4, 5, 7, 8, 11, 20, 21, 23, 24]. Aging and chronic diseases are recognized risk factors for sepsis, which is characterized by excessive inflammation, including hypercytokinemia and endothelial dysfunction, resulting in a hypercoagulability state [42, 52]. Refractory hypoxemia may lead to vasoconstriction reducing blood flow and promoting vascular occlusion [53]. SARS- and COVID-19-associated coagulopathy is sepsis-induced, generally characterized by markedly increased levels of plasminogen activator inhibitor-1 (PAI-1) [46, 54]. Consistently, the PAI-1 level in SARS patients is significantly higher, not only compared to healthy controls but also patients with other cases of pneumonia [55], and whether this is so in COVID-19 patients is a matter still to be verified.

Generally, coagulation tests are prolonged when the level of coagulation factors is below 50%, and an abnormality may occur up to the decompensation period of DIC because of the consumption of clotting factors during DIC progression [46, 56]. However, at the early stage of septic DIC, coagulation tests may be shortened because of hypercoagulability. This meta-analysis showed that PT, but not APTT, had an increased risk of ICU and death on admission, perhaps because coagulopathy in COVID-19 is sepsis-induced, where mostly the exogenous, but not the endogenous,

coagulation pathway is activated. Given that PT and APTT are within the reference ranges on admission in most COVID-19 patients, baseline PT and APTT have limited values for risk stratification and prognosis in COVID-19 patients [5, 13, 19]. However, PT can progressively extend in nonsurvivors [11], due to the continuous activation and consumption of the exogenous coagulation pathway. As an acute reactive protein, hyperfibrinogenemia is common in the early phase of COVID-19 in both survivors and non-survivors [11]. Yet, the level of fibrinogen can progressively decrease in non-survivors, and hypo-fibrinogenemia may be observed at the late stage of consumption coagulopathy [11]. Antithrombin may be readily exhausted during continuous thrombin generation, with low levels of antithrombin found in approximately 50% of critically ill patients and 90% of DIC patients [56]. Therefore, the dynamic monitoring of these coagulation tests is highly recommended.

The combination of thrombocytopenia, increased D-dimer, prolonged PT, and decreased antithrombin is suggestive of DIC, though the majority of COVID-19 patients would not meet the Overt-DIC criteria established by the International Society on Thrombosis and Hemostasis (ISTH) [41, 43]. The ISTH positively recommends anticoagulants when septic patients meet the diagnostic criteria of sepsis-induced coagulopathy (SIC) [54], which could result in a significant reduction in mortality [57, 58]. However, patients with advanced coagulopathy may have a disease progression that is no longer amenable to anticoagulant therapy [59]. For that reason, the ISTH recommends a two-step diagnosis for sepsis-associated coagulopathy and emphasizes that therapeutic doses of heparin should be considered in coagulopathic patients to avoid progression from coagulopathy to DIC [54]. Increasing evidence demonstrates that there is a high risk of thrombotic complications in severe COVID-19 patients, and early anticoagulation therapy seems to improve the outcome of severe COVID-19 patients [43, 49–51, 60–62]. Tang's team specifically looking at anticoagulant treatments showed that the 28-day mortality rate of COVID-19 patients using heparin was lower than that of nonusers in cases of severe COVID-19 patients meeting SIC criteria or with D-dimer > 3.0 ug/mL [60]. Llitjos et al. revealed that, among the twenty-six COVID-19 patients with mechanical ventilation, the incidence of VTE in patients treated with prophylactic anticoagulation was significantly higher than that in the group receiving therapeutic anticoagulation [63]. In a prospective observational study with sixteen ICU COVID-19 patients, Ranucci et al. showed that the pro-coagulant situation of patients gradually improved after thromboprophylaxis was increased [64]. Zhang et al. revealed that the thromboprophylaxis halved the incidence of DVT in COVID-19 patients with a Padua prediction score≥4 [65]. Given that COVID-19-ARDS patients had higher risk of thrombotic complications than non-COVID-19-ARDS patients, Helms et al. suggested the presence of higher anticoagulation targets in critically ill patients than usual [43]. However, the efficacy of anticoagulant therapy needs to be verified in high-quality RCT experiments. Clinicians should closely monitor indicators during the laboratory examination of patients to stay alert for side effects after anticoagulant treatment [66].

Our study has several limitations. First, all the studies included in this meta-analysis are retrospective studies with large heterogeneity. Second, the data came mainly from China; factors such as virus strain types, medical levels, countries, races, etc., may affect the results. However, at the moment, more detailed subgroup analyses cannot be conducted to comprehensively understand COVID-19 because the material for this is limited. Third, for some parameters, the number of studies included in the meta-analysis was less than 10. In this case, the publication bias may, therefore, not have been detected by Egger's and Begg's tests because of the relatively lower power. Fourth, the pooled sample sizes were not large enough. Precise estimates of these parameters should be assessed further.

CONCLUSIONS

Elevated D-dimer and FDP, prolonged PT, and decreased antithrombin predict higher risk stratification and poorer prognosis in COVID-19, which is perhaps not fully in line with the facts because the studies selected for the meta-analysis were limited. There is, however, no doubt that early coagulation tests and dynamically monitoring coagulation indicators during hospitalization are helpful in the early identification of coagulation disorders, and the rational use of these parameters and the scoring systems help guide treatment and improve the prognosis of COVID-19.

MATERIALS AND METHODS

Search strategy

We conducted this systematic review and meta-analysis using a predefined protocol under PRISMA guidelines [67]. We searched PubMed, Web of Science, Cochrane Library, and Scopus electronically. Medical subject headings and random words (e.g. COVID-19) were combined to search the databases without language or ethnic origin restriction and dated up to March 24, 2020 (for detailed search methods, see Supplementary Table 2). The titles, abstracts, and full texts of all documents were identified independently by two investigators, and disagreements were adjudicated by a third investigator.

The reference list of all identified documents was scrutinized to identify additional potentially eligible studies.

Inclusion and exclusion

The criteria for including a study in the meta-analysis were as follows: (I) the COVID-19 patient cohort was confirmed primarily by laboratory detection; (II) the endpoint was severity status and/or composite endpoint (including ICU monitoring and death); (III) groups were established for comparison; (IV) the correlation of coagulation biomarkers with endpoints was recorded. Exclusion criteria were as follows: (I) review articles, case reports, and laboratory studies; (II) studies with insufficient data for estimating pooled standard mean differences (SMD).

Data extraction

We collected the following items from each study, if available: the corresponding author's name, the study type, the institute or region, the period of case collection and follow-up, the number of reported cases, disease severity, complications (e.g., coagulopathy and DIC), outcome, and laboratory findings (e.g., platelet, D-dimer, PT, APTT, or fibrinogen) were entered in a well-designed form independently by two investigators. If different articles published by the same institution overlapped during case inclusion, the research with the largest number of cases was selected, and the others were excluded. A third investigator checked the article list and data extraction to ensure that there were no duplicate articles or duplicate information and made a judgment on controversial articles.

Quality assessment

Two reviewers independently evaluated the methodological quality of each selected study. The quality of case-control studies was evaluated using the Newcastle-Ottawa quality assessment scale (NOS) [68], which comprises 9 points; 4 points for selection, 2 points for comparability, and 3 points for the outcome. Six or more points in case-control studies were regarded as high quality. Disagreements were resolved by discussion.

Statistical analysis

Version 12.0 of the STATA statistical software (STATA, College Station, TX) was used to calculate the combined survival impact of indicators of coagulation. The impact of biomarkers on endpoints was determined by calculating pooled mean values and their 95% CIs. Results suggested statistical significance if the 95% CI was no more than 0. Also, increased indicator levels contributed to an adverse survival effect, compared to control-patients, when the pooled mean value was more than 0. The heterogeneity of the selected studies was evaluated using the chi-squared test, with significance set at a p-value of less than 0.10. The statistic I^2 was used to quantify heterogeneity; an I^2 value less than 25% was regarded as low heterogeneity, a value between 25 and 50% indicated moderate heterogeneity, and a value over 50% signaled high heterogeneity [69]. The random-effect model was used if high heterogeneity was observed; otherwise, a fixed-effect model was used for the meta-analysis. Sensitivity analysis was applied to explore the origin of heterogeneity. Funnel plots, Begg's test, and Egger's test were used to screen for potential publication bias of the total population. Poor stability resulting from the inclusion and exclusion of studies was reappraised.

Abbreviations

COVID-19: Coronavirus Disease 2019; SARS-Cov-2: severe acute respiratory syndrome coronavirus 2; ARDS: acute respiratory distress syndrome; MERS-CoV: Middle East Respiratory Syndrome coronavirus; DIC: disseminated intravascular coagulation; PT: prothrombin time; APTT: activated partial thromboplastin time; FDP: fibrin/fibrinogen degradation products; SMD: standard mean differences; VTE: venous thromboembolism. NOS:Newcastle-Ottawa quality assessment scale.

AUTHOR CONTRIBUTIONS

MH and HY conceived and designed the study. LLL, XM, DMY, KHM, LDY and CZP contributed to the literature searches, study selection, data extraction, quality assessment, data analysis and interpretation. LLL, XM and DMY drafted the initial manuscript, and MH and HY made critical revisions to the intellectual content. All authors approved the final version of the study.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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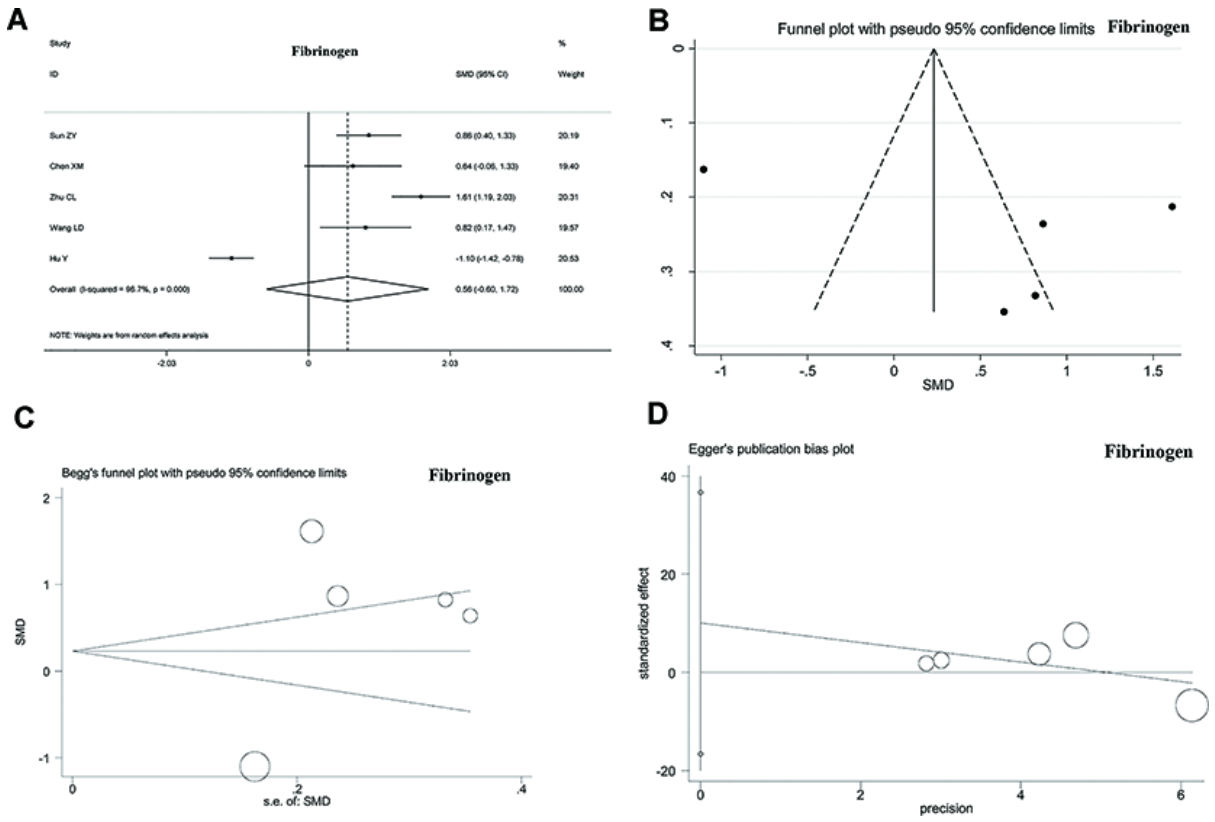
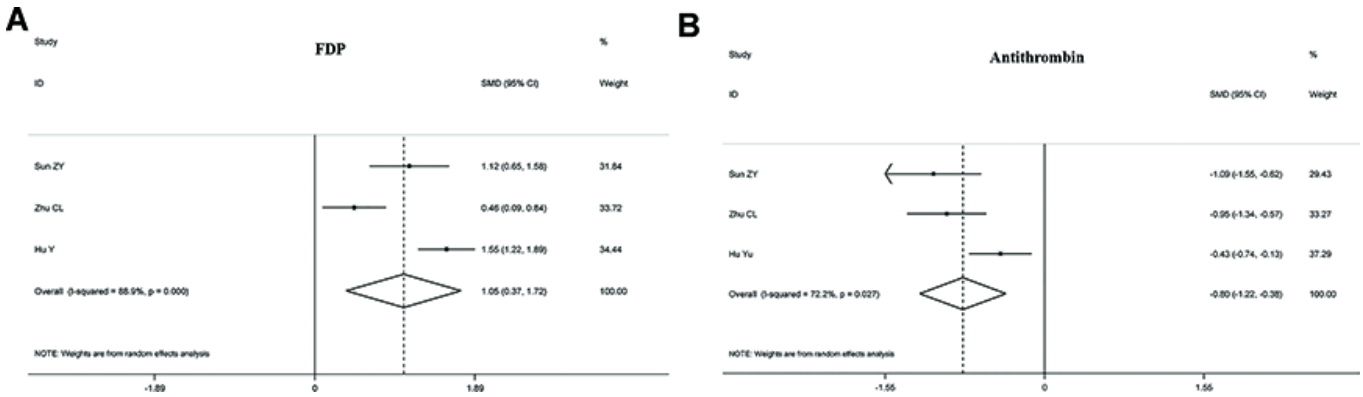
SUPPLEMENTARY MATERIALS

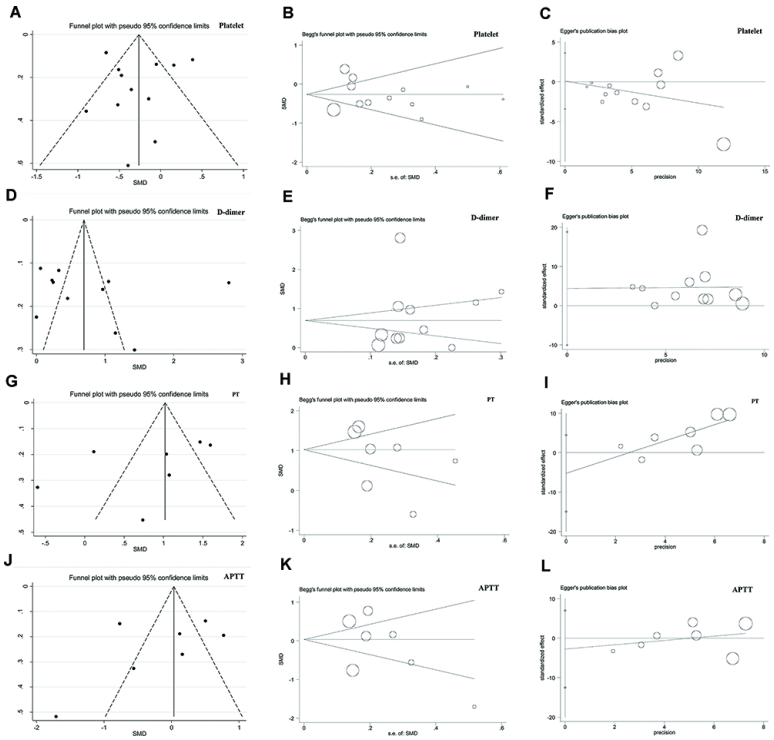
Supplementary Figures



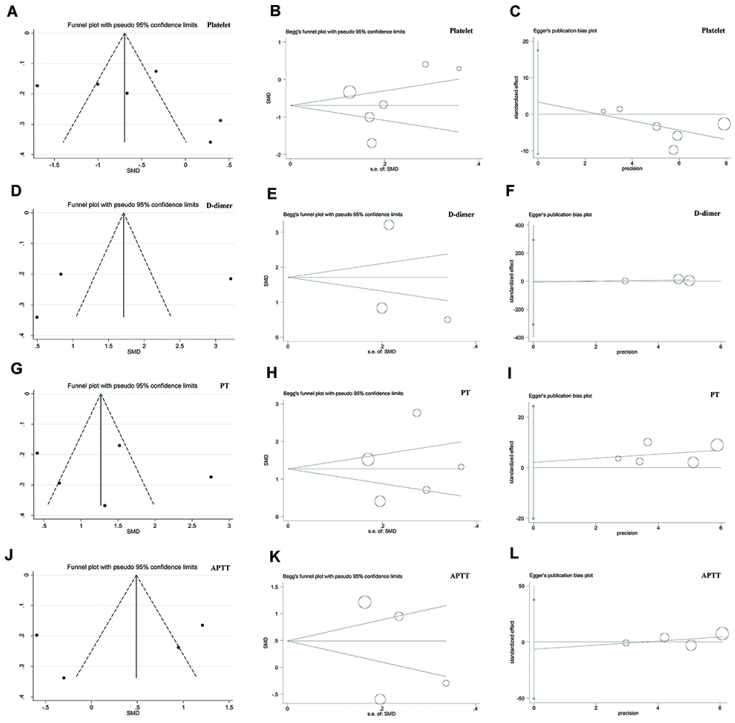
Supplementary Figure 1. Forest plots and publication bias of fibrinogen. Forest plots of pooled standard mean difference and 95% CIs assessing the severity status of COVID-19 patients by fibrinogen. The sizes of the blocks or diamonds represent the weights, and the lengths of the straight lines represent the widths of the 95% CI (**A**) Funnel plot (**B**) Egger's test (**C**) and Begg's (**D**) test assessing the publication bias of fibrinogen.



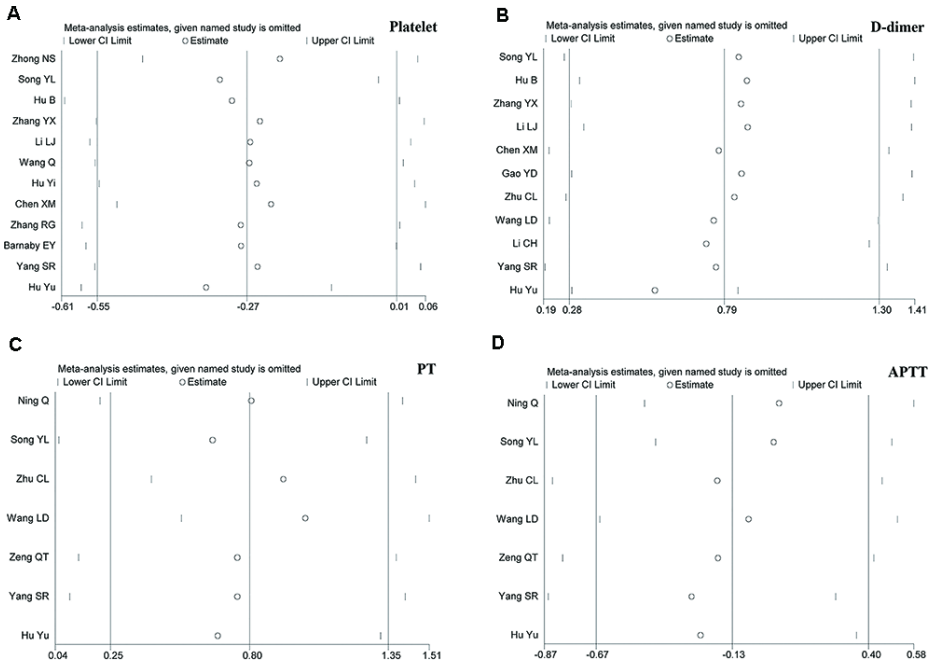
Supplementary Figure 2. Forest plots of pooled standard mean difference and 95% CIs assessing the severity status of COVID-19 patients by fibrin/fibrinogen degradation products (FDP) (**A**) and antithrombin (**B**). The sizes of the blocks or diamonds represent the weights, and the lengths of the straight lines represent the widths of the 95% CIs.



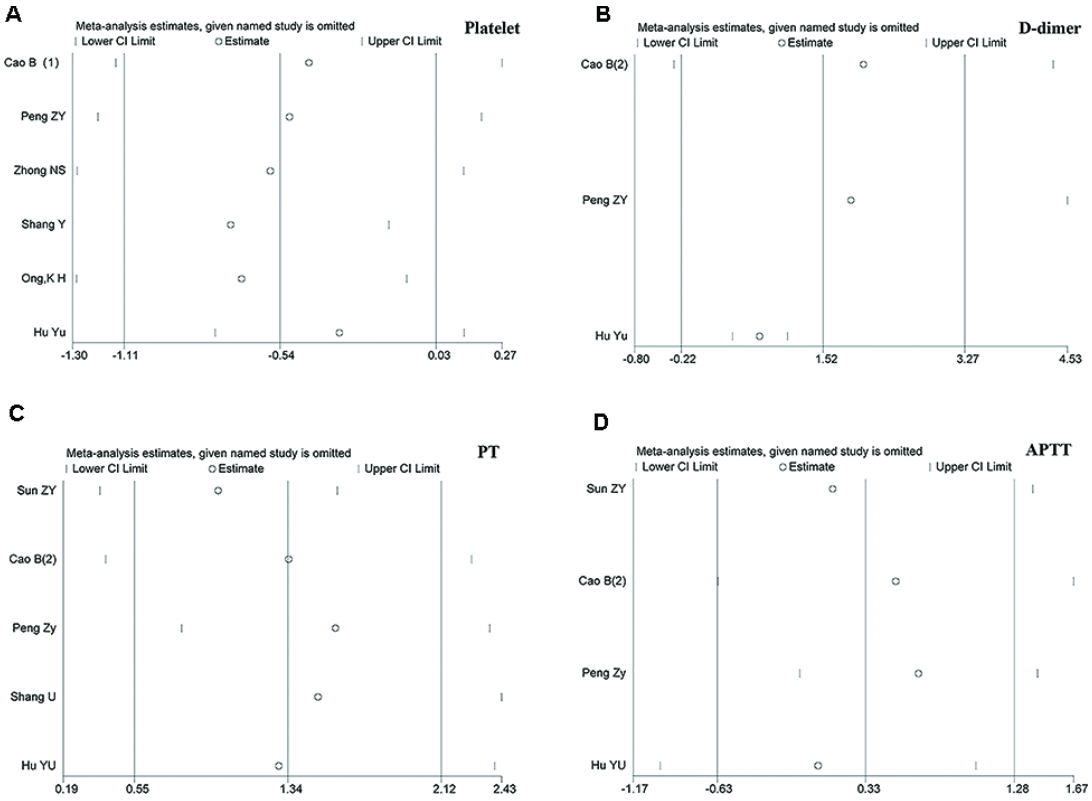
Supplementary Figure 3. Funnel plot, Egger's test and Begg's test assessing the publication bias of platelet (**A**–**C**) D-dimer (**D**–**F**) prothrombin time (PT) (**G**–**I**) and activated partial thromboplastin time (APTT) (**J**–**L**) associated with the severity status, respectively.



Supplementary Figure 4. Funnel plot, Egger's test and Begg's test assessing the publication bias of platelet (**A**–**C**) D-dimer (**D**–**F**) prothrombin time (PT) (**G**–**I**) and activated partial thromboplastin time (APTT) (**J**–**L**) associated with the composite endpoint, respectively.



Supplementary Figure 5. Sensitivity analysis of studies involving platelet (**A**) D-dimer (**B**) prothrombin time (PT) (**C**) and activated partial thromboplastin time (APTT) (**D**) associated with the severity status. None of the articles removed would have a significant effect on the results.



Supplementary Figure 6. Sensitivity analysis of studies involving platelet (**A**) D-dimer (**B**) prothrombin time (PT) (**C**) and activated partial thromboplastin time (APTT) (**D**) associated with the composite endpoint. None of the articles removed would have a significant effect on the results.

Supplementary Tables

Supplementary Table 1. Newcastle-Ottawa quality assessment scale (NOS).

NUM	Study	Selection (****)				Comparability (**)	Exposure (***)			Score
		Case definition adequate	Representativeness of the cases	Selection of controls	Definition of controls		Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	
1	Cao B	*	*		*	**	*	*	*	8
2	Sun ZY	*	*		*	**	*	*	*	8
3	Cao B (2)	*	*		*	**	*	*	*	8
4	Ning Q	*	*		*	**	*	*	*	8
5	Peng ZY	*	*		*	**	*	*	*	8
6	Zhong NS	*	*		*	**	*	*	*	8
7	Song YL	*	*		*	**	*	*	*	8
8	Hu B	*	*		*	**	*	*	*	8
9	Zhang YX	*	*		*	**	*	*	*	8
10	Li LJ	*	*		*	**	*	*	*	8
11	Shang Y	*	*		*	**	*	*	*	8
12	Ong, K H	*	*		*	**	*	*	*	8
13	Wang Q	*	*		*	**	*	*	*	8
14	Hu Y	*	*		*	**	*	*	*	8
15	Chen XM	*	*		*	**	*	*	*	8
16	Gao YD	*	*		*	**	*	*	*	8
17	Zhang RG	*	*		*	**	*	*	*	8
18	Zhu CL	*	*	*	*	**	*	*	*	9
19	Wang LD	*	*		*	**	*	*	*	8
20	Zeng QT	*	*		*	**	*	*	*	8
21	Li CH	*	*		*	**	*	*	*	8
22	Barnaby EY	*	*		*	**	*	*	*	8
23	Yang SR	*	*		*	**	*	*	*	8
24	Hu Y	*	*		*	**	*	*	*	8

Supplementary Table 2. Research strategy.

PubMed	**Mesh**: severe acute respiratory syndrome coronavirus 2 COVID-19; spike glycoprotein; COVID-19 virus **Entry Terms**: Wuhan coronavirus; Wuhan seafood market pneumonia virus; COVID19 virus; coronavirus disease 2019 virus; SARS-CoV-2; SARS2; 2019-nCoV; 2019 novel coronavirus; 2019 novel coronavirus infection; COVID19; coronavirus disease 2019; coronavirus disease-19; 2019-nCoV disease; 2019 novel coronavirus disease; 2019-nCoV infection; COVID-19 virus spike glycoprotein; 2019-nCoV spike glycoprotein **Search** ((((((((((((((((((severe acute respiratory syndrome coronavirus 2) OR COVID-19) OR spike glycoprotein, COVID-19 virus) OR Wuhan coronavirus) OR Wuhan seafood market pneumonia virus) OR COVID19 virus) OR coronavirus disease 2019 virus) OR SARS-CoV-2) OR SARS2) OR 2019-nCoV) OR 2019 novel coronavirus) OR 2019 novel coronavirus infection) OR COVID19) OR coronavirus disease 2019) OR coronavirus disease-19) OR 2019-nCoV disease) OR 2019 novel coronavirus disease) OR 2019-nCoV infection) OR COVID-19 virus spike glycoprotein) OR 2019-nCoV spike glycoprotein
Web of Science	**TS=**(severe acute respiratory syndrome coronavirus2 OR COVID-19 OR spike glycoprotein, COVID-19 virus OR Wuhan coronavirus OR Wuhan seafood market pneumonia virus OR COVID19 virus OR coronavirus disease 2019 virus OR SARS-CoV-2 OR 2019-nCoV OR 2019 novel coronavirus OR 2019 novel coronavirus infection OR COVID19 OR coronavirus disease 2019 OR coronavirus disease-19 OR 2019-nCoV disease OR 2019 novel coronavirus disease OR 2019-nCoV infection OR COVID-19 virus spike glycoprotein OR 2019-nCoV spike glycoprotein)
Cochrane Library Scopus	We put “COVID-19” into the Mesh box, but no Mesh terms and Tree were available **Search** ("severe acute respiratory syndrome coronavirus2") OR (COVID-19) OR ("spike glycoprotein, COVID-19 virus") OR ("Wuhan coronavirus")OR("Wuhan seafood market pneumonia virus ")OR(" COVID19 virus") OR ("coronavirus disease 2019 virus ")OR ("SARS-CoV-2") OR ("2019-nCoV ")OR ("2019 novel coronavirus") OR ("2019 novel coronavirus infection") OR (COVID19) OR ("coronavirus disease 2019") OR ("coronavirus disease-19") OR ("2019-nCoV disease ")OR ("2019 novel coronavirus disease") OR ("2019-nCoV infection") OR ("COVID-19 virus spike glycoprotein") OR ("2019-nCoV spike glycoprotein")

Research Paper

Clinical characteristics of chronic liver disease with coronavirus disease 2019 (COVID-19): a cohort study in Wuhan, China

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ABSTRACT

Background: Previous work has described acute liver injury (ALI) in coronavirus disease 2019 (COVID-19) pneumonia patients, However, there is limited analyses available investigating chronic liver disease (CLD) in COVID-19 patients. This study aimed to investigate clinical characteristics and outcomes of CLD confirmed in COVID-19 patients.

Results: A total of 104 cases (each group containing 52 patients) were analyzed in this study. The CLD group showed an average of 14 (10.0~21.2) length of stay (LOS) days, compared to the group without CLD that only showed an average of 12.5 (10~16) LOS days (Relative Risk [RR] = 1.34, 95% CI (1.22~1.48), P<0.001; Adjusted Relative Risk was 1.24 (95% CI: 1.12~1.39)). The CLD group contained a higher mortality rate and slight liver injury. Furthermore, COX regression model analyses suggested that the neutrophil-to-lymphocyte ratio (NLR) was an independent predictor of mortality risk (P < 0.001) in the CLD group. Additionally, a high NLR significantly correlated with a shorter overall survival (P <0.001).

Conclusions: COVID-19 patients also diagnosed with CLD suffered longer LOS, slight liver injuries and a higher mortality when compared to COVID-19 patients without CLD. The NLR was an independent risk factor for in-hospital deaths. Increased expression of NLR was an indicator of poor prognosis in COVID-19 patients with CLD. Thus, COVID-19 patients diagnosed with CLD and who show a higher NLR need additional care.

Methods: A retrospective cohort study was performed at the Wuhan Jin Yin-tan Hospital from February 2, 2020 to April 2, 2020. COVID-19 patients diagnosed with CLD or not diagnosed with CLD were enrolled in this study. The clinical characteristics and outcomes of these patients were compared.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) pneumonia, emerged in Wuhan (Hubei, China), has rapidly spread worldwide, infecting over 3.48 million patients. Previous work has mainly described acute liver injury (ALI) in general COVID-19 pneumonia patients [1–4]. There is little research available that focuses on patients with liver disease, especially chronic liver disease (CLD). Although some studies and reviews had reported that CLD is not associated with severity or mortality of COVID-19, all these used small sample sizes and most likely failed to analyze the characteristics and mortality rates of these patients. To our knowledge, the study presented here is the first to investigate clinical features and outcomes of CLD patients who have been diagnosed with COVID-19 pneumonia in a largest cohort study.

RESULTS

A total 104 patients were analyzed in this study, with both groups containing 52 patients (Table 1). In addition to CLD, a total of 39 (37.5%) patients showed other comorbidities. The median age of the patients analyzed was 59 (SD 12.9) years of age and a total of 39 (37.5%) patients were female. The most common symptoms experienced by the patients included cough (85[81.7%]), expectoration (38[36.5%]), dyspnea (19[18.3%]), and fatigue or myalgia (13 [12.5%]). All patients showed bilateral infiltrates on chest CT, while 92 (88.5%) patients had bilateral infiltrates. A total of 34.6%,35.6% and 5.77% of patients showed elevated ALT, AST and TBil levels, respectively. There was a total of 9 death patients in the CLD group, including 6 patients died of respiratory and circulatory failure, 3 patients died of multiple organ dysfunction syndrome (MODS). There were no significant differences observed in demographics, initial common symptoms, laboratory findings without lymphocyte count, PLT, INR, Glu IL-6 or PCT levels, liver function and treatment when comparing the two groups ($P > 0.05$; Table 1). The CLD group had showed a LOS of 14 (10.0~21.2) days compared to12.5 (10~16) for the non- CLD group (Relative Risk [RR] = 1.34, 95% CI (1.22~1.48), $P<0.001$; Adjusted RR was 1.24(95% CI: 1.12–1.39)) (Table 2). However, no differences in severity outcome were observed between the two groups ((Hazard Ratio [HR] = 1.78, 95% CI (0.80~4.04), P=0.16; Adjusted HR was 1.19, 95% CI(0 .45~3.19), P=0.73) (Table 2). There was no difference in severity ratio between the two groups (39 [37.5%] vs 16 [30.8%], p=0.22). The CLD group showed a higher mortality rate (9 [8.7%] vs 0[0.0%]) and slight liver injuries compared to the non-CLD group. Furthermore, univariate and multivariate COX regression analyses were performed to explore risk factors for death in the CLD group. Univariate survival analyses revealed that age, NLR, GLU and PCT were risk factors for death. However, only the NLR (OR, 1.04; 95% CI, 1.01–1.06) was found to be an independent predictor of death based on the multivariate analysis. (Table 3). To further assess prognostic significance of NLR in CLD patients with COVID-19, Kaplan-Meier survival analysis was performed to analyze overall survival (OS) (cutoff = 4.00). CLD patients with high NLR showed a significantly shorter OS (Figure 1, $P <0.001$). Thus, CLD was not associated with severity, but was associated with LOS, liver injury and mortality in patients diagnosed with COVID-19. Furthermore, NLR was shown to be an independent risk factor and prognostic factor for mortality in CLD patients confirmed to have COVID-19.

DISCUSSION

COVID-19 patients diagnosed with CLD showed a prolong LOS, slightly liver injuries and higher mortality rates compared to general COVID-19 patients. Furthermore, the NLR was found to be an independent risk factor for mortality in COVID-19 patients with CLD. Moreover, increased expression of NLR is an independent indicator of poor prognosis in COVID-19 patients diagnosed with CLD.

Previous work conducted by our team and others has shown that COVID-19 patients were more prone to liver injury whether general patients or critically ill patients [1, 5–11]. In the other hand, a severe outcome of COVID-19 disease was associated with liver dysfunction [12]. Although there are some reports that liver injury is uncommon in these cases [13], CLD should also be analyzed related to COVID-19 risk due to poor immune function. However, for CLD patients, some studies [5] showed that no patient should be receiving ICU care. Similarly, there were no significant differences for AST and ALT between the ICU and non-ICU groups, where both groups showed a normal distribution [5]. There were also no differences in liver function between survivors and non-survivors [14]. At the same time, other studies revealed that COVID-19 patients diagnosed with CLD did not show associations with severity or mortality [6]. This research only included rare CLD patients, where in some studies only a single CLD patient was included. Thus, these studies failed to explore the risk of severity or mortality associated with CLD. Here, we focused on all CLD confirmed COVID-19 cases presented in the Jin Yin-tan Hospital. CLD patients showed a prolonged LOS, slight liver injures and an increased risk for death, but not severity.

Table 1. Demographics and clinical features of patients with COVID-19.

	All patients (N= 104)	Non-CLD (n = 52)	CLD (n = 52)	*P*
Demographics				
Age	59 ± 12.9	59.7 ± 14	58.2 ± 11.7	0.58
sex, n (%)				
Female	39 (37.5)	19 (36.5)	20 (38.5)	1
Male	65 (62.5)	33 (63.5)	32 (61.5)	
Comorbidities, n (%)				
No	65 (62.5)	33 (63.5)	32 (61.5)	1
Yes	39 (37.5)	19 (36.5)	20 (38.5)	
Smoking, n (%)				
No	40 (38.5)	20 (38.5)	20 (38.5)	1
Yes	64 (61.5)	32 (61.5)	32 (61.5)	
Initial common symptoms				
Dyspnoea, n (%)				0.13
No	85 (81.7)	46 (88.5)	39 (75)	
Yes	19 (18.3)	6 (11.5)	13 (25)	
Cough, n (%)				
No	19 (18.3)	9 (17.3)	10 (19.2)	1
Yes	85 (81.7)	43 (82.7)	42 (80.8)	
Expectoration, n (%)				
No	66 (63.5)	28 (53.8)	38 (73.1)	0.07
Yes	38 (36.5)	24 (46.2)	14 (26.9)	
Myalgia or fatigue, n (%)				
No	91 (87.5)	46 (88.5)	45 (86.5)	1
Yes	13 (12.5)	6 (11.5)	7 (13.5)	
Sore throat, n (%)				
No	104 (100)	52 (100)	52 (100)	1
Thoracodynia, n (%)				
No	104 (100)	52 (100)	52 (100)	1
Systolic Pressure	125(117.8,133.3)	125 (118.0, 130.5)	125.5(116.3, 135.5)	0.39
Respiratory Rate	22 (20, 24)	22 (20, 23)	22 (20, 25)	0.51
Laboratory findings				
White blood cell count ($\times 10^9$ cells per L)	5.5 (4.4, 7.36)	5.5 (4.36, 6.86)	5.48 (4.58, 8.02)	0.22
Neutrophil count ($\times 10^9$ cells per L)	3.85(2.96, 5.39)	3.79 (2.96, 5.06)	3.92(2.91, 5.85)	0.69
Lymphocyte count ($\times 10^9$ cells per L)	0.88(0.69, 1.18)	1.02 (0.85, 1.24)	0.79(0.55, 1.04)	< 0.001
MNM ($\times 10^9$ cells per L)	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.39
Hb (g/L)	124(114.0,134.3)	124(114.3, 132.0)	124(114.0,136.3)	0.49
PLT ($\times 10^9$ per L)	211.5(164, 268)	233.5(183.75, 296)	186(155, 230)	<0.001
INR	0.96(0.9, 1.04)	0.94 (0.9, 0.99)	1 (0.92, 1.12)	0.03
Potassium (mmol/L)	4.1 ± 0.6	4 ± 0.6	4.2 ± 0.6	0.18
Sodium (mmol/L)	140.5 (139, 142)	141 (139, 143)	140 (138, 142)	0.13
Cl (mmol/L)	106 (104, 108)	107 (105, 108)	106 (103, 108)	0.08
BUN	4 (3.4, 5.23)	4 (3.3, 5.05)	4.1 (3.5, 5.7)	0.28
Cr (μmol/L)	70 (59.45, 79.98)	70.65(59.45, 82.05)	69.85(59.52, 78.15)	0.69
Glu (mmol/L)	5.8 (5.07, 7.05)	5.65 (5, 6.2)	6.3 (5.27, 7.7)	0.02
CK (U/L)	65(42.75, 185.25)	63 (41.00, 143.25)	73 (47.50, 208.25)	0.23
IL-6 (mmol/L)	8.52(6.52,11.52)	7.77 (6.5, 10.52)	9.58 (7.15, 15.45)	0.04
Infection, n (%)				
No	29 (27.9)	15 (28.8)	14 (26.9)	1
Yes	75 (72.1)	37 (71.2)	38 (73.1)	

PCT, Median (IQR)	0 (0, 0.07)	0 (0, 0.05)	0.05 (0, 0.23)	< 0.001
Chest CT				
Lobi Pulmonis, n (%)				
Unilateral	12 (11.5)	5 (9.6)	7 (13.5)	0.76
Bilateral	92 (88.5)	47 (90.4)	45 (86.5)	
Ground-glass opacity, n (%)				
No	45 (43.3)	23 (44.2)	22 (42.3)	1
Yes	59 (56.7)	29 (55.8)	30 (57.7)	
ALT (IU/L, baseline)	36.5(22.75,63.25)	42.5 (22.75, 68)	36 (24.25, 57.5)	0.62
AST (IU/L, baseline)	33 (25, 50)	32 (25, 46.5)	36.5 (25.75, 51.5)	0.4
Total bilirubin(μmol/L)	12.85 (10.38, 16.22)	11.95 (10.2, 14.12)	13.3 (10.95, 17.5)	0.1
ALB(g/L)	32 ± 4.2	31.3 ± 3.5	32.7 ± 4.7	0.08
PT(s)	11.25 (10.5, 12)	11 (10.5, 11.7)	11.65 (10.57, 12)	0.05
PTA	108.35 (89, 127.55)	111 (92.78, 130.6)	105.55 (88.4,124.35)	0.52
Treatment				
Antibiotic therapy, n (%)				
No	18 (17.3)	7 (13.5)	11 (21.2)	0.44
Yes	86 (82.7)	45 (86.5)	41 (78.8)	
Use of corticosteroid, n (%)				
No	79 (76)	43 (82.7)	36 (69.2)	0.17
Yes	25 (24)	9 (17.3)	16 (30.8)	
Oxygen support, n (%)				
No	19 (18.3)	7 (13.5)	12 (23.1)	0.31
Yes	85 (81.7)	45 (86.5)	40 (76.9)	
Ventilation, n (%)				
Non-invasive ventilation	7 (6.7)	2 (3.8)	5 (9.6)	
Invasive mechanical ventilation	6 (5.8)	1 (1.9)	5 (9.6)	0.1
NO ventilation	91 (87.5)	49 (94.2)	42 (80.8)	
Prone position ventilation, n (%)				
No	103 (99)	52 (100)	51 (98.1)	1
Yes	1 (1)	0 (0)	1 (1.9)	
ECMO, n (%)				
No	104 (100)	52 (100)	52 (100)	1
Nebulization inhalation, n (%)				
No	99 (95.2)	50 (96.2)	49 (94.2)	1
Yes	5 (4.8)	2 (3.8)	3 (5.8)	
Vasoconstrictor, n (%)				
No	103 (99)	52 (100)	51 (98.1)	1
Yes	1 (1)	0 (0)	1 (1.9)	
Immunoglobulin therapy, n (%)				
No	86 (82.7)	47 (90.4)	39 (75)	0.07
Yes	18 (17.3)	5 (9.6)	13 (25)	

Abbreviations: CLD, Chronic liver disease; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate; INR, International Normalized Ratio; PT, Prothrombin time; *PLT*, *blood platelet; CK, creatine kinase;* BUN, blood urea nitrogen; *RR*: Respiratory Rate; MNM: monocyte counts; IL-6, interleukin-6; ECMO, extracorporeal membrane oxygenation.

To the best of our knowledge, this is the first study focusing on COVID-19 patients diagnosed with CLD. The COVID-19 with CLD group showed an increased lymphocyte count as well as increased IL-6 and PCT levels, suggesting pathogenic effects from excessive inflammation in acute lung injury caused by COVID-19 infection. Inflammation may reflect disease severity and defects in innate immune regulation, especially in CLD patients who have poor immune function [13]. At the same time, blood sugar levels were elevated to support

Table 2. Unadjusted and adjusted risk ratios of LOS, severity, and mortality in COVID-19.

Outcome	β	Unadjusted risk ratio	95% CI	*P*	β	Adjusted risk ratio	95% CI	*P*
LOS Outcome								
	0.30	1.34	(1.22~1.48)	<0.01	0.22	1.24	1.12~1.39	<0.001
Severity Outcome								
	0.58	1.78	0.80~4.04	0.16	0.17	1.19	0.45~3.19	0.73
Mortality Rate Outcome								
			CLD (n = 52)			Non-CLD (n = 52)		*P*
	Survivors		43 (82.7)			52 (100)		<0.01
	Death		9 (17.3)			0 (0)		
Liver Injury Outcome								
		Total (N = 104)	CLD (n = 52)			Non-CLD (n = 52)		*P*
ALT		37 (24.75, 57.25)	39.5 (28.75, 55.5)			33.5 (23, 62)		0.47
AST		28.5 (19, 38.75)	30 (23, 49.5)			24 (17.75, 34.25)		< 0.001
TBil		10.05 (6.7, 15.12)	13.9 (7.18, 20.8)			8.6 (6.7, 11.93)		< 0.001

(CLD group verse Non-CLD group).

Table 3. Unadjusted and adjusted risk factors of mortality for CLD group.

	β	Unadjusted hazard ratio	95%CI	*P*	β	Adjusted hazard ratio	95%CI	*P*
Age	2.13493	8.46	1.73~41.37	0.0084				
NLR	0.046	1.05	1.02~1.07	<0.001	0.03624	1.04	1.01~1.06	<0.001
GLU	0.24	1.27	1.03~1.57	0.028				
IL-6	0.005009	1.01	0.97 ~1.05	0.799				
PCT	2.28	9.74	1.85~51.20	<0.001				
INR	0.04832	0.95	0.45~2.02	0.9				

NLR, neutrophil-to-lymphocyte ratio.

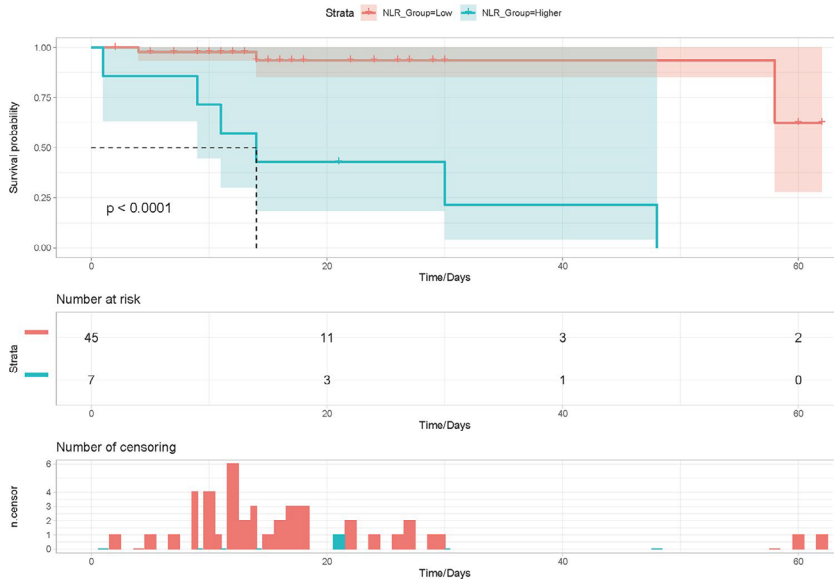

Figure 1. Kaplan-Meier analysis of overall survival (OS) according to NLR expression in CLD with COVID-19 infection patients.

the specific energetic demands needed by inflammatory responses [15, 16]. This work also revealed that the CLD group had relatively low PLT and increased total bilirubin and INR levels, supporting hepatic dysfunction in the activation of coagulative and fibrinolytic, which consistent with previous studies [13, 17] In this study, risk of CLD was found to be related to LOS in COVID-19. Compared to patients without CLD, the patient group diagnosed with COVID-19 and CLD showed a prolonged LOS. With a prolonged LOS, CLD may improve the risk of nosocomial infections (NIs), which may result in a worse prognosis. Similarly, the CLD group showed an increased mortality rate and higher incidence of liver injury. Interestingly, although the risk of CLD was positively associated with LOS, liver function risk and mortality, it was negatively associated to severity which was consistent with other studies [6, 13]. We performed additional work to explore risk factors related to mortality in the CLD group. The most important finding was that the NLR was associated with mortality and severity, suggesting it as a potential indicator for poor prognosis in CLD patients diagnosed with COVID-19 infection. The high NLR accounts for increased neutrophil and decreased lymphocyte counts, reflecting systemic inflammation. Systemic inflammation is believed to play an important role in the severity of virus-induced disease, such as COVID-19. Meanwhile, patients diagnosed with CLD have poor immune function. Thus, CLD may aggravate the dysregulation of the immune response associated with COVID-19 [18]. An easily accessible and less costly biological marker for systemic inflammatory diseases, NLR has been reported as an independent risk factor for the severity and mortality in COVID-19 [3, 18, 19], especially in elder or male patients [19]. Since NLR could be quickly calculated based on a blood routine test on admission, clinicians may identify high risk COVID-19 patients at an early stage. Thus, treatments can be modified accordingly to reduce the in-hospital death. In particular, this study confirmed the risk of NLR associated with mortality in CLD patients.

This study also exhibits some limitations. (a) The sample size was limited to a single-center hospital, clinical characteristics COVID-19 patients, diagnosed with CLD, should be verified with a randomized controlled trial enrolling more patients. (b) The diagnosis of CLD needs systematic testing such as a biopsy of the liver, blood tests and imaging including ultrasound. This study was unable to widely diagnose all CLD cases through systematic testing when the COVID-19 outbreak occurred in Wu Han since there were limited medical resources. However, CLD was diagnosed based on written medical and *oral health records with the goal to minimize bias* of diagnosis. (c) The study population included older COVID-19 patients diagnosed with CLD, suggesting that these conclusions may not be applicable to younger patients. In the future, a larger multicenter study analyzed CLD patients diagnosed with COVID-19 is needed to further understand the pathological mechanisms behind CLD associated with COVID-19. This would contribute to further knowledge defining the clinical characteristics and outcome for these patients.

CONCLUSIONS

This retrospective study revealed that COVID-19 patients diagnosed with CLD showed a longer LOS, slight liver injuries and higher mortality compared to general COVID-19 patients. The NLR was found to be an independent risk factor for in-hospital deaths. Increased expression of NLR was found to be a potential indicator for poor prognosis in COVID-19 patients diagnosed with CLD. Thus, CLD patients with COVID-19 who have a higher NLR should be critically cared for.

MATERIALS AND METHODS

Study design, participants and data collection

The retrospective cohort study presented here included all CLD and random non-CLD patients at Wuhan Jin Yin-tan Hospital. Wuhan Jin Yin-tan Hospital (Wuhan Isolation Hospital) is known to have treated the largest number of COVID-19 patients. All enrolled patients were treated from February 2, 2020 to April 2, 2020. The diagnostic and treatment criteria of COVID-19 and its severity were based on guidelines provided by the WHO and China Trial Seventh Edition. Patients diagnosed with acute liver injury or who showed incomplete medical records were excluded from this study. Clinical data such as demographics, initial symptoms, laboratory findings, chest CT pneumonia compromise and treatment was reviewed using digital medical records by the Fujian Medical Team to aid Wuhan Jin Yin-tan Hospital. Patients were divided into two groups including the COVID-19 with CLD group and non-CLD group. CLD was defined as a progressive deterioration of liver functions, leading to fibrosis and cirrhosis of liver parenchyma. It refers to liver disease at least 6 months. CLD consists of diverse liver pathologies including hepatocellular carcinoma, liver cirrhosis, and inflammation (chronic hepatitis). Our team diagnosed CLD based on clinical features. The COVID-19 with CLD group included all CLD patients that were diagnosed with chronic viral hepatitis B and C, autoimmune liver disease, cryptogenic liver cirrhosis, NAFLD, methotrexate related liver fibrosis and alcoholic liver disease. At the same time, we used computer-generated random same size to enroll

non-CLD group during the same period. We analyzed the clinical characteristics of all patients, then compared the baseline information and the outcome of LOS, severity, mortality rate and liver function. This retrospective cohort study approved by Ethics Commission of Jin Yin-tan hospital, Wuhan (KY-2020–55.01).

Statistical analysis

The mean ± SD or median (IQR) value and number (%) were used to descriptive data of continuous and categorical variables. For continuous variables, independent group t tests were used to compare the two group or Mann–Whitney test was performed when data were normally distributed. For categorical variables, the χ^2 or Fisher exact tests were performed to compared the two groups. Furthermore, Poisson regression was used to verify independent risk of CLD for LOS. Stepwise Logistic Regression models were used to test independent risks of CLD for severity. Cox models were used to calculate the hazard ratio of mortality in the CLD group. Kaplan-Meier survival analysis was used to analyze overall survival (OS) of the CLD group based on neutrophil-to-lymphocyte ratio (NLR) levels. R project (version 3.6.0) was used to perform all statistical analyses. Statistical significance was recognized at a P value of 0.05 or less.

AUTHOR CONTRIBUTIONS

ZR Yang designed study, analyzed and revised the manuscript; Chaowei Li and Qingshi Chen collected data and wrote the manuscript; Jianwen Wang, Huasong Lin, Yalan Lin, Jinhuang Lin, Fangzhan Peng and Jiangmu Chen searched the literature.

CONFLICTS OF INTEREST

The authors disclose no conflicts of interest.

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Research Paper

Clinical course and characteristics of patients with coronavirus disease 2019 in Wuhan, China: a single-centered, retrospective, observational study

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible for the coronavirus disease 2019(COVID-19) pandemic. Despite the extensive studies aiming to understand the pathology of COVID-19, the clinicopathological characteristics and risk factors associated with COVID-19 remain mostly unclear. In this study, we assessed the clinical course and features of COVID-19 patients.

Findings: There were 59 patients (54.1%) that had no fever. One-hundred (91.7%) patients required oxygen therapy, which improved percutaneous oxygen saturation (SpO_2). Seventy-two (66.1%) patients aged over 60; these patients were more likely to develop respiratory symptoms. Only 13 (11.9%) patients were positive for anti-SARS-CoV-2 antibodies, SARS-CoV-2 nucleic acid, and computed tomography (CT) findings. We found significant differences in age, respiratory symptoms, and heart rates between patients with and without underlying conditions.

Conclusions: Our findings suggest that oxygen plays an important role in the treatment of COVID-19 patients and that age and underlying diseases are significant risk factors for COVID-19. Most COVID-19 patients have no fever, and CT provides higher detection rates than antibody- and nucleic acid-based detection methods.

Methods: We analyzed data from 109 confirmed COVID-19 cases. We compared the clinicopathological characteristic of patients stratified according to age and underlying diseases, as well as assessed the detection rates of different diagnostic methods.

INTRODUCTION

In December 2019, an outbreak of severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) in Wuhan, China, marked the beginning of the coronavirus disease 2019(COVID-19) pandemic [1–3]. As of April 8, 2020, the number of confirmed cases has risen to 1.35 million worldwide.[4] COVID-19 often present with persistent fever, cough, chest distress, dyspnea, and sore throat. Some patients also develop gastrointestinal symptoms, including diarrhea, while other patients have no obvious symptoms, making the virus spread containment extremely challenging [5, 6]. In COVID-19 patients, lung computed tomography (CT) findings include bilateral scattered patchy ground-glass density shadows and consolidation stripe shadows in both lungs [7, 8].

Despite the extensive efforts of the last months to understand the pathology of COVID-19 and identify

therapeutic targets, the clinicopathological characteristics and risk factors associated with COVID-19 remain largely unclear. In this study, we investigated the clinicopathological characteristics and treatment outcomes in 109 patients diagnosed with COVID-19. The findings reported herein provide a better understanding of the clinical course, treatment efficacy, and risk factors in COVID-19 patients, providing a step forward toward the development of novel strategies to contain the pandemic.

RESULTS

Patient demographics and characteristics

In this study, we included 109 patients diagnosed with COVID-19 from February 13, 2020, to February 29, 2020, at Wuhan Union Hospital. The demographics and characteristics of these patients are summarized in Supplementary Table 1. The median age of all patients was 63 (range, 29-97), and 72 (66.1%) patients were aged over 60. There were 51 (46.8%) female patients and 58 (53.2%) male patients. Fourth-seven (43.1%) patients had chronic diseases. Among all patients, 100 (91.7%) required oxygen therapy, after which percutaneous oxygen saturation (SpO_2) values returned to physiological levels ($SpO_2 \geq 94\%$).

Clinical features

The clinical features of COVID-19 patients are summarized in Supplementary Table 2. Common symptoms included increased heart rate (n = 57; 52.3%), cough (n = 56; 51.4%), mild fever (37.3°C-38°C), and chest tightness (n = 36; 33.0%); high fever (39°C-40°C) was observed in three patients (2.8%). Only 50 (45.9%) patients presented with fever, while the remaining 59 (54.1%) patients did not develop fever throughout the disease course. Among the patients who developed fever, the median body temperature was 37.8°C (ranges, 37.3°C -40°C), and the median fever duration was 2.5 days (ranges, 1-8 days). 95 (87.1%) patients presented with respiratory symptoms at the time of diagnosis, and in 31 (28.5%) patients, respiratory symptoms continued even after O_2 supplement. Of the 47 (43.1%) patients who had chronic diseases, 41 (87.2%) had respiratory symptoms at the time of diagnosis, and 13 (31.7%) had respiratory symptoms after oxygen therapy. A total of 31 (28.4%) patients were diagnosed with abnormal SpO_2; in these patients, SpO_2 values returned to physiological levels after O_2 supplement.

Among all patients with underlying diseases, 17 (36.2%) were O_2 unsaturated on admission. The median age of patients with respiratory symptoms after oxygen therapy was 65.5 (ranges, 29-97), whereas the median age of patients without respiratory symptoms after O_2 supplement was 62 (range, 29-91). The median age of patients with respiratory symptoms requiring oxygen therapy was 73 (range, 60-83), whereas that of patients not requiring oxygen therapy was 65 (range, 58-65) (Supplementary Table 1).

In this study, we also compared the demographics and clinical characteristics of patients with and without chronic diseases (Table 1). While the median age of patients with chronic diseases was 69 (range, 38-97), that of patients without underlying conditions was 60 (range, 29-91) (Table 1); this difference was statistically significant. Compared with patients with chronic diseases, respiratory symptoms were less frequent, and heart rates were lower in patients without underlying conditions ($P<0.0284$ and $P<0.0001$, respectively; Table 1). No significant differences in gender or other clinical characteristics were observed between patients with chronic diseases and those without chronic conditions (Table 1). Significant factors identified by univariate analyses (age, respiratory symptoms, and heart rate) were included in multivariate analyses; age and heart rate were identified as significant risk factors of chronic diseases (Table 1).

Laboratory parameters and imaging findings

The results of laboratory examination and computed tomography (CT) in COVID-19 patients are shown in Figure 1 and Supplementary Table 3. Although 101 (92.6%) of the patients had positive CT findings, and 91 (83.5%) were positive for anti-SARS-CoV-2 antibodies, only 24 (22.0%) were positive for SARS-CoV-2 nucleic acid. Only 13 (11.9%) of the cases were positive for anti-SARS-CoV-2 antibodies, SARS-CoV-2 nucleic acid, and CT findings. Eighty-three (76.1%) cases were positive for both anti-SARS-CoV-2 antibodies and CT findings, and 18 (16.5%) were positive for SARS-CoV-2 nucleic acid and CT findings.

Eighty-two (75.3%) of the patients with anti-SARS-CoV-2 antibodies presented with respiratory symptoms at the time of diagnosis, whereas 9 (8.2%) had no respiratory symptoms at diagnosis (Table 2). Eighty-eight (80.7%) patients with CT findings had respiratory symptoms at the time of diagnosis, while 13 (11.9%) cases with CT findings had no respiratory symptoms at diagnosis. Importantly, the number of patients who had respiratory symptoms after oxygen therapy was lower among individuals with anti-SARS-CoV-2 antibodies and CT findings. Among the patients with anti-SARS-CoV-2 antibodies and who received O_2 supplement, 24 (29.3%) had respiratory symptoms even after oxygen therapy. Additionally, among the patients with CT findings and who received O_2 supplement, 27 (29.0%) had respiratory

Table 1. Unvariate and multivariate analysis of patients with or without chronic diseases.

	All patients	Chronic diseases	Non-chronic diseases	Univariate P value	Multivariate P value
Sex	109(100)	47(43.1)	62(56.9)		
Female	51(46.8)	20(18.3)	31(28.4)	0.4403	
Male	58(53.2)	27(24.6)	31(28.4)		
Age, median(range)	63(29-97)	69(38-97)	60(29-91)	<0.0001	<0.0001
≤39	6(5.5)	1(0.9)	5(4.6)	0.0061	
40-59	31(28.4)	7(6.4)	24(22.0)		
60-79	56(51.4)	28(25.7)	28(25.7)		
≥80	16(14.6)	11(10.0)	5(4.6)		
Initial respiratory symptoms[a]					
Yes	95(87.2)	41(37.6)	54(49.5)	0.9831	
No	14(12.8)	6(5.5)	8(7.3)		
Respiratory symptoms after O_2 supplement					
Yes	31(28.4)	18(16.5)	23(21.1)	0.2676	
No	69(63.3)	23(21.1)	46(42.2)		
Median temperature,℃	37.2(36.6-40)	37.2(36.6-39.8)	37.2(36.8-40)	0.6853	
Initial median SpO2 value (%)	95(64-98)	95(91-97)	102(86-135)	0.4961	
Median SpO2 value after O_2 supplement (%)	98(96-100)	98(96-100)	98(96-100)	0.9876	
Median heart rate, beats per min	101(84-135)	100(84-126)	95(80-98)	<0.0001	<0.0001
Median respiratory rate, breaths per min	22(20-34)	22(20-34)	21(20-28)	0.0284	

Data are n (%), unless otherwise specified. Abbreviations: COVID-19, coronavirus disease 2019; SpO2, Percutaneous oxygen saturation; O_2, oxygen.

[a]including cough, sore throat, short of breath, chest tightness, expectoration and dyspnea.

symptoms after oxygen therapy. However, among the patients with positive SARS-CoV-2 RT-PCR results, respiratory symptoms continued after oxygen therapy in 12 (52.2%) of them.

DISCUSSION

SARS-CoV-2 is the third coronavirus discovered so far; it is considerably more infectious than SARS-CoV and MERS-CoV [9–12], leading to the rapid spread of COVID-19 across almost every country [13, 14]. In this study, we reported on the clinicopathological characteristics and clinical course of 109 COVID-19 patients. The median age was 63 (ranges 29-97), and 72 (66.1%) patients aged over 60. Forty-seven (43.1%) patients had underlying conditions. Among all patients, 100 (91.7%) received oxygen therapy, after which SpO_2 values returned to physiological levels, suggesting that O_2 supplementation played an important role in improving the condition of patients infected with SARS-CoV-2.

Interestingly, more than half of the patients (54.1%) did not develop fever, in contrast to the study by Goyal P et al., which reported that 77.1% of patients had a fever [15]. Interestingly, Zhiliang Hu et al. reported that only 20.8% of COVID-19 patients developed fever [16], highlighting the high variation in the symptoms of COVID-19 across cohorts. We believe that the fact that SARS-CoV-2 has been circulating for more than half a year has contributed to the attenuation of the symptoms caused by the virus. Additionally, as some COVID-19 patients remain asymptomatic, routine testing using antibody detection tests, nucleic acid testing, and chest CT, should be implemented in every country to contain the pandemic. Among patients who had a fever, the median body temperature was 37.8°C, and the median duration of the fever was 2.5 days; therefore, SARS-CoV-2 infection screening solely by measuring body temperature is insufficient. Additionally, a few patients did not have respiratory symptoms on admission but developed such symptoms later during the disease course. This finding highlights the need for early thorough clinical examination, CT scan, and laboratory testing in suspected cases. Moreover, self-isolation for at least 14 days is crucial for the prevention of community spread of SARS-CoV-2.

In our cohort, more than half of COVID-19 patients were elderly (over 60 years old), consistent with findings from previous studies [1, 17]; hence, we conclude that elderly patients are more likely to be infected with SARS-CoV-2 and that strict measures should be implemented to prevent the spear of the virus in the elderly. Our findings also revealed that people over 60 years old were more likely to develop respiratory symptoms, including abnormal SpO_2, compared with younger individuals. Furthermore, the elderly were less likely to improve after oxygen therapy, further supporting the need for close monitoring of COVID-19 patients aged more than 60. We observed significant differences in age, respiratory symptoms, and heart rates between patients with chronic diseases and those without underlying conditions, suggesting that these factors may indicate the presence of chronic diseases.

It has been reported that patients with underlying diseases were more likely to contract SARS-CoV-2 [17, 18]. In this study, we found that 43.1% of the patients had chronic diseases. After oxygen therapy, the respiratory symptoms continued in one-third of the patients with underlying conditions. However, SpO_2 returned to the physiological levels in all patients after oxygen therapy, pinpointing the importance of O_2 supplement for the treatment of COVID-19 patients with underlying conditions.

The combination of laboratory examination and CT scans plays an important role in the diagnosis of COVID-19. In this cohort, although 92.6% of patients received CT-based diagnosis, and 83.5% were positive for anti-SARS-CoV-2 antibodies, only 22.0% of them were positive for SARS-CoV-2 nucleic acid. Importantly, only 11.9% of the patients were positive for anti-SARS-CoV-2 antibodies, SARS-CoV-2 nucleic acid, and CT diagnosis. Previous studies have shown that some COVID-19 patients were negative for anti-SARS-CoV-2 antibodies and viral nucleic acid at early stages [19]. CT provided a higher detection rate than laboratory examination, highlighting the importance of CT imaging to confirm SARS-CoV-2 infection.

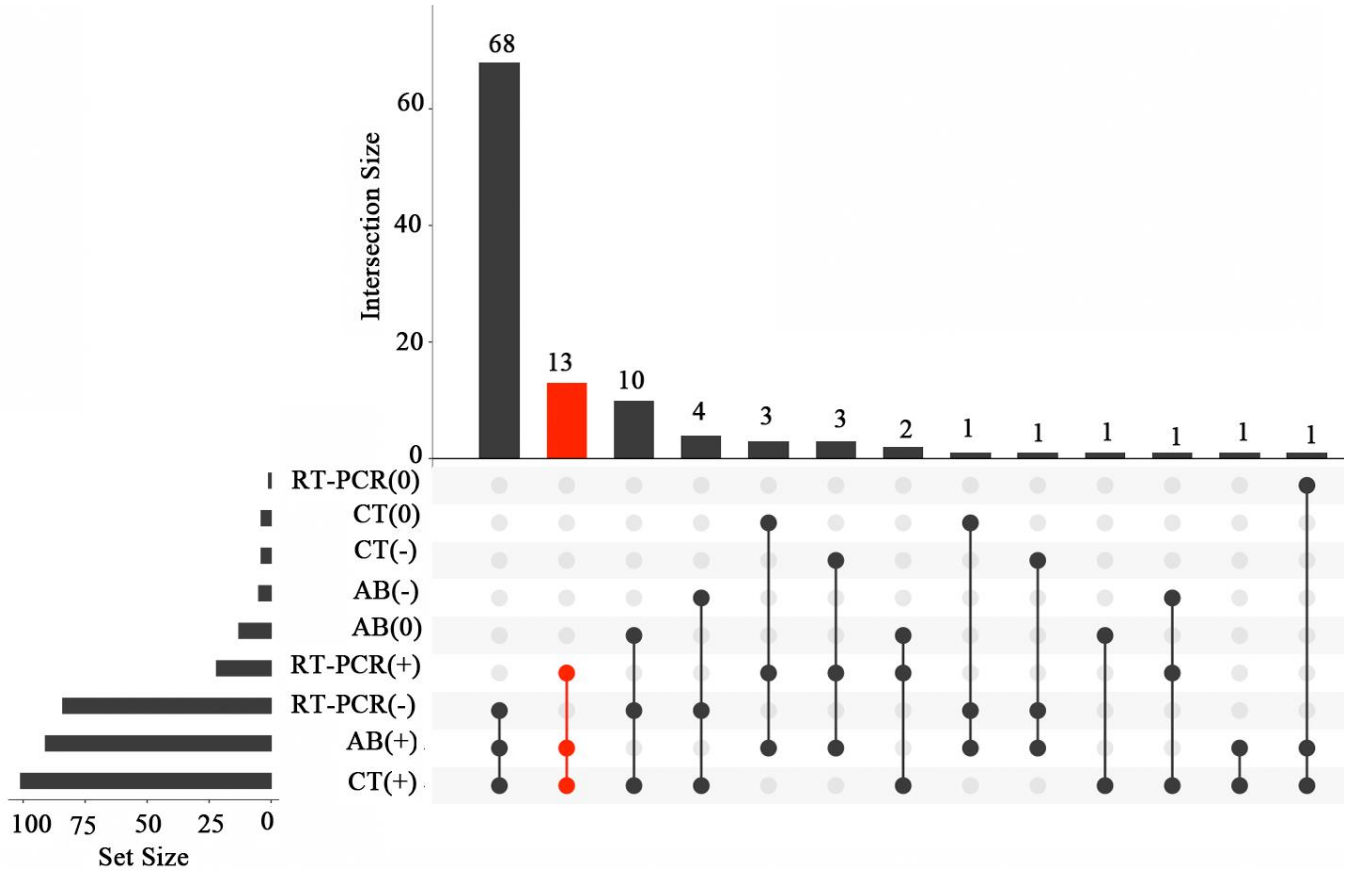

Figure 1. Venn diagram showing the laboratory parameters and CT findings in COVID-19 patients. AB (+), positive antibody assay; AB (-), negative antibody assay; AB (0), antibody assay was not performed; RT-PCR (+), positive RT-PCR assay; RT-PCR (-), negative RT-PCR assay; RT-PCR (0), RT-PCR assay was not performed; CT (+), positive CT diagnosis; CT (-), negative CT diagnosis; CT (0), CT was not performed.

Table 2. Examinations and clinical symptoms of patients with COVID-19.

	All patients	Initial respiratory symptoms[a]		Initial SpO2 value (%)		Supplemental O_2		Respiratory symptoms after O_2 supplement		SpO2 value after O_2 supplement (%)		Respiratory symptoms without O_2 supplement		SpO2 value without O_2 supplement (%)	
		Yes	No	≥94	<94	Yes	No	Yes	No	≥94	<94	Yes	No	≥94	<94
Antibody assay[b]															
Positive	91(83.5)	82(75.3)	9(8.2)	68(62.4)	23(21.1)	82(75.3)	9(8.2)	24(22.0)	58(53.2)	82(75.3)	0(0)	1(0.9)	8(7.3)	9(8.2)	0(0)
Negative	5(4.6)	3(2.8)	2(1.8)	3(2.8)	2(1.8)	5(4.6)	0(0)	1(0.9)	4(3.7)	5(4.6)	0(0)	0(0)	0(0)	0(0)	0(0)
Not performed	13(11.9)	10(9.1)	3(2.8)	7(6.4)	6(5.5)	13(11.9)	0(0)	6(5.5)	7(6.4)	13(11.9)	0(0)	0(0)	0(0)	0(0)	0(0)
RT-PCR assay[c]															
Positive	24(22.0)	22(20.2)	2(1.8)	17(15.5)	7(6.4)	23(21.1)	1(0.9)	12(11.0)	11(10.1)	23(21.1)	0(0)	0(0)	1(0.9)	1(0.9)	0(0)
Negative	84(77.1)	72(66.1)	12(11.0)	60(55.0)	24(22.1)	76(69.7)	8(7.3)	19(17.4)	57(52.3)	76(69.7)	0(0)	1(0.9)	7(6.4)	8(7.3)	0(0)
Not performed	1(0.9)	1(0.9)	0(0)	1(0.9)	0(0)	1(0.9)	0(0)	0(0)	1(0.9)	1(0.9)	0(0)	0(0)	0(0)	0(0)	0(0)
CT diagnosis															
Positive	101(92.6)	88(80.7)	13(11.9)	72(66.1)	29(26.6)	93(85.3)	8(7.3)	27(24.8)	66(60.6)	93(85.3)	0(0)	1(0.9)	7(6.4)	8(7.3)	0(0)
Negative	4(3.7)	3(2.8)	1(0.9)	3(2.8)	1(0.9)	3(2.8)	1(0.9)	2(1.8)	1(0.9)	3(2.8)	0(0)	0(0)	1(0.9)	1(0.9)	0(0)
Not performed	4(3.7)	4(3.7)	0(0)	3(2.8)	1(0.9)	4(3.7)	0(0)	2(1.8)	2(1.8)	4(3.7)	0(0)	0(0)	0(0)	0(0)	0(0)

Data are n (%), unless otherwise specified. Abbreviations: COVID-19, coronavirus disease 2019; SpO2, Percutaneous oxygen saturation; O_2, oxygen.

[a] including cough, sore throat, short of breath, chest tightness, expectoration and dyspnea; [b]Anti-SARS-CoV-2 antibody assay; [c]SARS-CoV-2 RT-PCR assay.

There are several limitations to this study. First, the patient cohort consisted of only 109 cases, but the patients' characteristics were similar to those in previous studies [1, 6, 18]. Second, we did not test for hematological indicators of heart, liver, and kidney function. Additionally, we did not assess for important observation indexes, such as clinical outcomes, medication plans, living conditions, and less common symptoms, which might be vital for clinical decision making and outcome prediction.

In conclusion, our findings suggest that oxygen therapy plays an important role in the treatment of COVID-19 patients. Old age and underlying diseases are the main risk factors of SARS-CoV-2 infection; therefore, the elderly and individuals with chronic diseases should be closely monitored after contracting the virus. Most COVID-19 patients have no fever; hence, thorough clinical examination, CT, and laboratory examination are pivotal for COVID-19 diagnosis. Additionally, the detection rate of CT is superior to anti-SARS-CoV-2 antibody testing and SARS-CoV-2 RT-PCR; thus, CT imaging should be implemented in the clinical practice to confirm SARS-CoV-2 infection.

MATERIALS AND METHODS

Study design and participants

In this study, we analyzed data from 109 COVID-19 patients admitted to the Wuhan Union Hospital in Hubei, China, from February 13, 2020, to February 29, 2020, according to the WHO Interim Guidelines [20]. The study was approved by the Ethics Committee of Wuhan Union Hospital. Informed consent was provided by all patients.

Data collection

We collected the following data from 109 patients diagnosed with SARS-CoV-2 infection: age, gender, respiratory symptoms (fever, cough, dyspnea, chest tightness, sore throat), vital signs at admission (temperature, heart rate, respiratory rate, SpO_2), chronic medical history (chronic heart disease, coronary heart disease, hypertension, hyperlipidemia, diabetes), treatment (O_2 therapy), respiratory symptoms after treatment (fever, cough, dyspnea, chest tightness, sore throat), SpO_2 after treatment, presence of anti-SARS-CoV-2 antibodies, SARS-CoV-2 RT-PCR assay results, and CT findings. Continuous variables were expressed as medians and ranges, whereas categorical variables were expressed as numbers and percentages (%).

Statistical analysis

Differences among groups were analyzed using Fisher's exact test, χ^2 test, univariate analysis, and multivariate analysis according to the type of data. Statistical analyses were conducted using GraphPad Prism 8.0 and TBtools software. *P*-values <0.05 were considered statistically significant.

Abbreviations

COVID-19: coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CT: computed tomography; O_2: oxygen; RT-PCR: real-time quantitative polymerase chain reaction; MERS: Middle East respiratory syndrome; SpO_2: percutaneous oxygen saturation.

AUTHOR CONTRIBUTIONS

Lina Liu and Jing Yao collected the data. Yanfang Liu, Ye Wang, and Xinyang Du summarized and analyzed all data. Yanfang Liu and Lina Liu drafted the manuscript. Jing Yao and Hong Ma revised the final manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Tables

Please browse Full Text version to see the data of Supplementary Tables 1, 2.

Supplementary Table 1. Demographics characteristics of patients with COVID-19.

Supplementary Table 2. Clinical features of patients with COVID-19.

Supplementary Table 3. Laboratory examination and CT diagnosis of patients with COVID-19.

	All patients	Antibody assay positive	Antibody assay negative	Antibody assay not performed	RT-PCR assay positive	RT-PCR assay negative	RT-PCR assay not performed	CT diagnosis positive	CT diagnosis negative	CT diagnosis not performed
Antibody assay[a]										
Positive	91(83.5)	91(83.5)	0(0)	0(0)	20(18.3)	70(64.3)	1(0.9)	83(76.1)	4(3.7)	4(3.7)
Negative	5(4.6)	0(0)	5(4.6)	0(0)	1(0.9)	4(3.7)	0(0)	5(4.6)	0(0)	0(0)
Not performed	13(11.9)	0(0)	0(0)	13(11.9)	3(2.8)	10(9.1)	0(0)	13(11.9)	0(0)	0(0)
RT-PCR assay[b]										
Positive	24(22.0)	20(18.3)	1(0.9)	3(2.8)	24(22.0)	0(0)	0(0)	18(16.5)	3(2.8)	3(2.8)
Negative	84(77.1)	70(64.3)	4(3.7)	10(9.1)	0(0)	84(77.1)	0(0)	82(75.2)	1(0.9)	1(0.9)
Not performed	1(0.9)	1(0.9)	0(0)	0(0)	0(0)	0(0)	1(0.9)	1(0.9)	0(0)	0(0)
CT diagnosis										
Positive	101(92.6)	83(76.1)	5(4.6)	13(11.9)	18(16.5)	82(75.2)	1(0.9)	101(92.6)	0(0)	0(0)
Negative	4(3.7)	4(3.7)	0(0)	0(0)	3(2.8)	1(0.9)	0(0)	0(0)	4(3.7)	0(0)
Not performed	4(3.7)	4(3.7)	0(0)	0(0)	3(2.8)	1(0.9)	0(0)	0(0)	0(0)	4(3.7)

Data are n (%), unless otherwise specified. Abbreviations: COVID-19, coronavirus disease 2019; CT, computed tomography; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, real-time quantitative polymerase chain reaction. [a]Anti-SARS-CoV-2 antibody assay; [b]SARS-CoV-2 RT-PCR assay.

Research Paper

COVID-19: a probable role of the anticoagulant Protein S in managing COVID-19-associated coagulopathy

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ABSTRACT

The COVID-19 pandemic has caused monumental mortality, and there are still no adequate therapies. Most severely ill COVID-19 patients manifest a hyperactivated immune response, instigated by interleukin 6 (IL6) that triggers a so called "cytokine storm" and coagulopathy. Hypoxia is also associated with COVID-19. So far overlooked is the fact that both IL6 and hypoxia depress the abundance of a key anticoagulant, Protein S. We speculate that the IL6-driven cytokine explosion plus hypoxemia causes a severe drop in Protein S level that exacerbates the thrombotic risk in COVID-19 patients. Here we highlight a mechanism by which the IL6-hypoxia curse causes a deadly hypercoagulable state in COVID-19 patients, and we suggest a path to therapy.

INTRODUCTION

The menacing SARS-CoV2 virus has caused a pandemic with over 6.9 million cases and around 400,000 deaths. As of to date (07/11/2020), there are more than 3.2 million confirmed cases and ~134,729 deaths in the U.S. Clinical features of patients admitted to the hospital with the viral disease COVID-19 are bilateral pneumonia, systemic inflammation, endothelial dysfunction, coagulation activation, acute respiratory distress syndrome, and multi-organ failure. Signs of myocardial injury are also observed in at least one quarter of severe cases. Although the lung is the main target organ, the virus can infect other tissues (small intestine, testis, kidneys, heart, thyroid, adipose tissue, colon, liver, bladder, adrenal gland [1]).

The infection risk of SARS-CoV2 has no remarkable correlation with age because the expression of the virus receptor ACE2 does not vary much between young and old; however, mortality is significantly higher in older people compared with the young, (Table 1). SARS-CoV2 infection was found to reduce the expression of ACE2 in lungs, leading to a renin-angiotensin system (RAS) dysfunction. This RAS dysfunction, in turn, would enhance inflammation and vascular permeability in the airways [2].

However, unlike the SARS-CoV pandemic in 2003, COVID-19 is not simply a disease of the upper respiratory tract. COVID-19 patients experience hypercoagulability and increased risk of venous thromboembolism (Table 2). These thrombotic complications have been referred to as *thrombo-inflammation* or *COVID-19-associated coagulopathy* [3–7]. Moreover, several reports indicate that hypercoagulability, as measured by the D-Dimer levels, is present mostly in critically ill and deceased patients (Table 2). In addition to blood clots of all sizes throughout the body, doctors who treat coronavirus patients report a range of other odd and frightening syndromes, such as kidney failure, cardiac inflammation, and immune complications. These syndromes appear to arise from a SARS-CoV2 virus-induced local inflammatory response.

Table 1. Different parameters of COVID-19 patients.

Place	Time and Date	No of patient	Sex		Age	Mortality
			Male	Female		
Netherlands[42]	7th March - 5th April, 2020	184	139	45	Average : 64	23
Lombardy region of Italy[43]	20th February -18th March, 2020	1591	1304	287	Median : 63	405
Italy[44]	Until 15th March, 2020	22512	13462	9050	Median : 64	1625
Zhongnan Hospital of Wuhan University in Wuhan, China[4]	1st January to 13th March, 2020	449	268	181	Average : 65.1	134
Fatal Cases of COVID-19 from Wuhan China[20]	9th January-15th February, 2020	85	62	23	Median: 65.8	All
Wuhan Jin Yin-tan Hospital, Wuhan, China[45]	Late December, 2019-26th January, 2020	52	36	17	Average: 59.7	32

Table 2. Studies which indicate that hypercoagulability (supra-physiological levels of D-dimer), is almost always associated with disease severity and mortality of COVID-19.

Study	Sample size	Mean D-dimer (<0.5 µg/ml)	p-values	Comment
Tang et al, Feb 2020,[34]	Survivors (162) Non-survivors (21)	0.6 2.12	<0.001	Disseminated intravascular coagulation (DIC) was found in most deaths
Han et al, Mar 2020, [33]	Ordinary patient (49) Critical (10)	2.14 ±2.88 20.04 ± 32.39	<0.001 <0.05	Huge increase in D-dimer in critically ill COVID patients
Wang et al, Mar 2020, [46]	ICU (36) Non-ICU (102)	4.14 1.66	<0.001	In the non-survivors, D-dimer increased continuously
Zhang et al, April 2020, [47]	Ordinary (276) Severe (67)	0.41 4.76	<0.001	12 non-survivors had D-dimer values greater than 2.0
Spiezia et al, April 2020, [48]	ICU (22)	5.343 ±2.099	<0.0001	All ICU patients with acute respiratory failure showed severe hypercoagulability, one patient with the most hypercoagulable state died.
Ranucci et al, April 2020, [35]	Total (16)	3.5	0.017	Seven patients died of hypoxia and multi-organ failure
Tang et al, May 2020, [49]	Survivors (315) Non-survivors (134)	1.47 4.7	<0.001	30 of the non survivors died even after treated with low molecular weight heparin

Because of lung involvement, most COVID-19 patients have exceedingly low blood oxygen levels, but, inexplicably, some of these hypoxic patients hardly gasp for breath. Alarmingly, these individuals are subject, without warning, to sudden shortness of breath and massive pulmonary embolism [8]. Note that bleeding is rare in the current onset of the disease.

Our past studies [9–13] with the natural anticoagulant Protein S illuminated our understanding about the significance of Protein S -Factor IXa interaction in hemostasis. Further, we identified a critical role of Protein S in regulating hypoxia and associated thrombotic complications [9].

The overarching goal of this article is to propose a strategy to better control the hypoxemia associated hypercoagulability in severe COVID-19 patients.

Inflammation, coagulation and hypoxia

Inflammation, as a part of the innate immunity response to an infection, triggers activation of coagulation pathways. Activation of coagulation influenced by

inflammation, in turn, can modulate the inflammatory response. The coordinated activation of both coagulation and inflammation during a severe infection is a well-recognized phenomenon known as thrombo-inflammation. Thrombo-inflammation is associated with microvascular thrombosis, hypoxemic respiratory failure, and, in extreme cases, it may lead to death due to development of multiple organ dysfunction syndrome (MODS) [14]. A disproportionate inflammatory response to SARS-CoV2 is associated with exorbitant circulating levels of inflammatory cytokines, which is thought to be a major cause of disease severity and death [15].

The main mediators of inflammation-activated coagulation are the pro-inflammatory cytokines [16]. In severe sepsis, the pro-inflammatory cytokines stimulate mononuclear cells expressing more and more tissue factor that initiate the coagulation pathways. Interleukin 6 (IL-6) is the most important cytokine that influences the expression of tissue factor which activates coagulation.

Thrombo-inflammation – occurs by overproduction of early response proinflammatory cytokines (TNFα, IL-6, and IL-1β) that create a "cytokine storm" [4, 15, 17–22]. This cytokine explosion leads to increased risk of vascular hyperpermeability, multi-organ failure, and eventually death when high cytokine concentrations persist [23, 24]. The inflammatory effects of cytokines also activate vascular endothelial cells and cause endothelial injury with resultant prothrombotic properties [25]. Independent transcriptome datasets from infection models revealed that IL6 is the major cytokine differentially expressed after infection with SARS- CoV2 [26–30].

Autopsies revealed microthrombi in lungs and other organs with associated foci of hemorrhage [31]. Such observations suggest that severe endothelial dysfunction, driven by the cytokine storm and associated hypoxemia, lead to disseminated intravascular coagulation and thromboembolic complications. Importantly, development of local hypoxia will progressively intensify endothelial cell disruption, tissue factor expression, and activation of the coagulation cascade, thereby establishing a deadly positive thrombo-inflammatory feedback loop with thrombosis and hemorrhage occurring in the small vessels of the lungs.

In summary, severe hypoxia is now considered associated with gravely ill COVID-19 patients, and IL6 is upregulated in COVID-19 and promotes *cis* and *trans* signaling to produce a cytokine storm [32]. Further, it is reasonable to conclude that subtle clotting begins early in the lungs, perhaps due to an inflammatory reaction in their fine web of blood vessels, which then sets off a cascade of proteins that prompt blood to clot and prevent proper oxygenation. Blood clots are clearly a major contributor to COVID-19 disease severity and mortality.

Crosstalk between thrombotic complications and inflammation/cytokine storm in SARS-CoV2 infection

Severity of COVID-19 is commonly associated with coagulopathy; disseminated intravascular coagulation (DIC) being the predominant condition along with high venous thromboembolism rates, and pulmonary congestion with microvascular thrombosis [33]. In general, hemostatic system alterations were indicated by prolonged aPTT, elevated platelet count, increased D-dimer level and fibrin degradation product for patients with severe COVID 19 [34]. Fibrin deposition in alveolar and interstitial lung spaces, in addition to microcirculation thrombosis, may exacerbate respiratory symptoms that require prolonged mechanical ventilation, and which are associated with poor prognosis and death. D-dimer levels have been identified as markers of severity of the disease and predictive of mortality [34]. Ranucci et. al. [35] incorporated viscoelastic tests for ICU patients along with the other commonly performed examinations. The test provides information about clot time (CT), clot strength (CS), fibrinogen contribution (FCS), and platelet contribution (PCS) to clot strength. Patient procoagulant profiles were confirmed by increased CS, FCS and PCS. Increased clot strength has been correlated to high fibrinogen level and somewhat to elevated platelet count.

COVID-19 disease severity is also associated with acute lung injury and hypoxemic respiratory failure, the most common cause of death. High levels of cytokines and chemokines associated with T cell depletion, pulmonary inflammation, and extensive lung damage have been documented in individuals who experienced similar viral respiratory diseases such as SARS and MERS. Thus, the wide-spread lung damage associated with this kind of infection may be caused more by an exaggerated immune response than by the virus itself. In addition, supraphysiological levels of IL-6, IL-10 and TNF-α have been found in the sera of severely ill COVID 19 patients [35]. Therefore, all patients with severe COVID-19 should be screened for excessive inflammation by measuring cytokine levels to stratify patients eligible for a specific immunosuppressive treatment [36].

The prevalence of both a cytokine storm and derangement of coagulation in critically ill COVID-19

patients signifies the aforesaid synergy between inflammation and coagulation. A clear association between increased IL-6 and fibrinogen level was reported for a set of COVID 19 ICU patients [35]. Recent guidance from the International Society on Thrombosis and Hemostasis (ISTH) stresses the need for monitoring coagulation parameters for patients who develop sepsis from the infection. The only widely available standard of care in this respect is a prophylactic dose of low molecular weight heparin, which should be considered for all COVID 19 patients (including non-critically ill) with high D-dimer levels, except for patients in whom anticoagulants are not advisable. For patients allergic to heparin, fondaparinux, a synthetic pentasaccharide, is an alternative. Fondaparinux has antithrombotic activity due to anti-thrombin-mediated selective inhibition of FXa. Systematic anticoagulation therapy for hospitalized COVID 19 patients is now routine treatment.

IL6, Hypoxia and Protein S

An overlooked aspect of hypoxia and the IL6-induced cytokine storm is that *both* factors downregulate a key anticoagulant, Protein S [9, 32] (Figure 1). For example, in a population of stroke patients, IL6 was upregulated, and it caused downregulation of Protein S that resulted in venous thrombosis [37]. We demonstrated that hypoxia downregulates Protein S expression in HepG2 cells [9]. Further, we showed that Protein S supplementation in thrombotic mice (mimicking hypoxic niche due to constitutive stabilization of HIF1α) plasma was able to alleviate the thrombotic risk [9]. Notably, addition of Protein S in normal mice plasma reduced thrombin generation as well [9]. These data indicate that Protein S supplementation could be useful in treating thrombotic complications. A substantial number of severe COVID-19 patients manifest both hypoxia and prothrombotic complications [34, 38–40] and we speculate that reduced Protein S level might play a key role in the disease progression of these patients.

Ordinarily, Protein S deficiency is due either to homozygous or heterozygous genetic alteration, and Protein S deficiency can result from various pathological states and diseases. In all cases, Protein S deficiency is associated with a higher risk of venous thrombosis. Because both hypoxia and IL6-induced inflammation depress Protein S abundance, it's reasonable to consider administration of Protein S as an effective therapy in severe Covid19 patients. Indeed, therapeutic heparin has improved the conditions of COVID-19 patients who experienced hypoxia. However, heparin targets FIXa, FXa and thrombin [41] through antithrombin. Therefore, direct administration of Protein S should have a highly specific anticoagulant effect in any thrombotic complications caused by Protein S deficiency. Of course, the possibility of

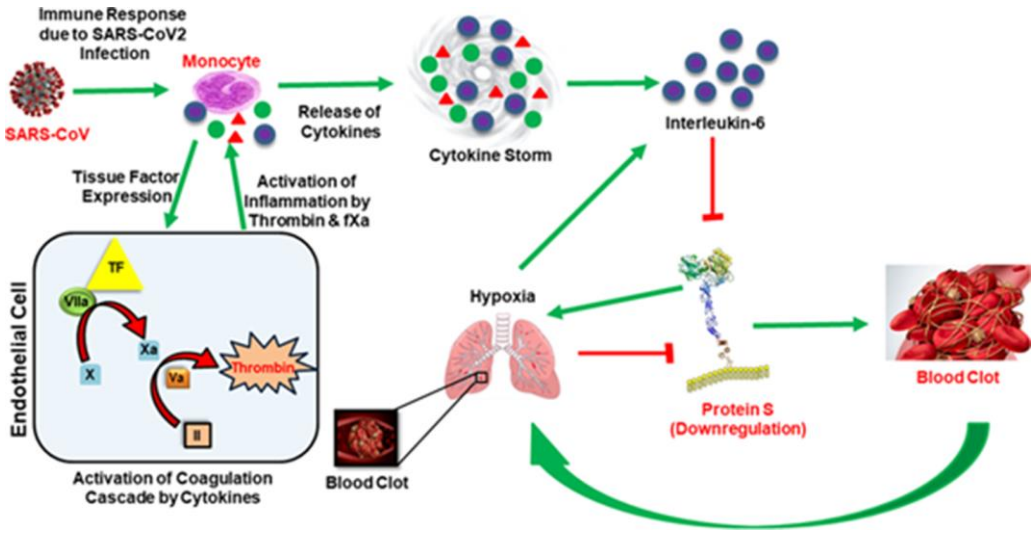

Figure 1. In the presence of the SAR-COV2 virus, early response proinflammatory cytokines (IL-6, TNFα, IL-1β etc.) are induced and activate the coagulation cascade by stimulating tissue factor (TF) expression from monocytes. The presentation of tissue factor leads to the formation of thrombin by the TF-VIIa pathway. Thrombin produces clots, and clots get wedged into arteries in the lungs and cause thrombotic complications and hypoxia. Hypoxia also induces IL-6. Simultaneously, thrombin augments inflammation and accelerates the production of proinflammatory cytokines, termed 'cytokine storm'. Both cytokine storm and hypoxia downregulate Protein S, leading to coagulopathy. Green arrows represent upregulation and red blockage represent downregulation.

bleeding would need attention, but, fortunately, even high doses of heparin have not caused bleeding in COVID-19 patients. Nonetheless, before Protein S administration can be deemed a new therapeutic approach, it is necessary to determine the extent to which Protein S is downregulated in a large cohort of COVID-19 patients. In view of the double curse of hypoxia and IL6, we expect Protein S deficiency to be severe in COVID-patients. However, we acknowledge that testing for safety and efficacy as well as FDA approval would be required before this approach could be implemented.

AUTHOR CONTRIBUTIONS

SC and TS - contributed to writing the first draft, assembling the references, and composing the Figure. SM - provided critical biological input, edited the final Figure and revised the resubmitted manuscript. RM-conceptualized the work and wrote the final draft.

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CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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Letter to the Editors

A newborn with normal IgM and elevated IgG antibodies born to an asymptomatic infection mother with COVID-19

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ABSTRACT

Pregnant women are susceptible population of COVID-19 which are more likely to have complications and even progress to severe illness. Pregnancy with COVID-19 and neonates are rarely reported. We report a newborn with normal IgM and elevated IgG antibodies born to an asymptomatic infection mother with COVID-19. We assessed whether there was intrauterine vertical transmission potential of COVID-19.

DEAR EDITOR

We reported a newborn with normal IgM and elevated IgG antibodies born to an asymptomatic infection mother with coronavirus disease 2019 (COVID-19). In our present case, the mother of the neonate was a 30-year-old pregnant woman. There were no confirmed or suspected cases of COVID-19 in her family. She denied having a history of exposure to COVID-19 patients. She was pregnant for the first time. She claimed that she had never had syphilis, hepatitis b, AIDS and other infectious diseases.

March 4, 2020, the gestation pregnant woman was 39 weeks pregnant. Color ultrasound indicated that the fetus was in the breech position with the umbilical cord around the neck. At 02:05 on March 6, 2020, the pregnant woman went to Wuhan Central Hospital for treatment due to excessive amniotic fluid and umbilical cord around the neck. There were no typical symptoms of COVID-19, such as fever and cough, in this pregnant woman. Thoracic computerized tomography scan revealed no abnormality. The nucleic acid test of pharyngeal swab showed positive, and the results of serum IgM and IgG antibody (colloidal gold method) were weak positive and strong positive, respectively, suggesting that the pregnant woman might be an asymptomatic infection case of COVID-19. Blood tests showed lymphocytes (0.82×10^9/L, normal: $1.1\text{-}3.2\times10^9$/L) reduced. She was hospitalized for suspected viral pneumonia.

On admission, her body temperature was 36.4°C and her blood pressure was 108/65 mmHg, with respiratory rate of 20 breaths per minute, pulse of 76 beats per minute. Cardiopulmonary function was normal, and there was no edema in the lower limbs. No intrauterine distress was presented throughout the pregnancy. Amniotic fluid slant overloaded, without amniotic fluid pollution. Fetal heart monitoring showed no abnormalities, and the fetal heart rate was 140 bpm. Emergency cesarean section was operated for pregnant women. The pregnant woman wore an N95 mask throughout the operation, without cough or produce sputum.

At 14:00 on March 6, 2020 a baby girl was born, weighted 3,460g. Apgar scores at 1 and 5 minutes were 9 and 10, respectively. The baby did not get groan, fever, cough, and vomit. The baby had a ruddy face and a powerful cry. Since there was no isolation ward in the neonatal department of Wuhan Central Hospital, the neonate was transferred to COVID-19 children's designated hospital-Wuhan Children's Hospital immediately after 3 hours of birth. The newborn could eat normal breast milk. The newborn's mental response was good, with blood oxygen saturation maintaining more than 92%. Her body temperature and body length were 36.9°C and 50 cm, with respiratory rate of 36 breaths per minute, pulse of 135 beats per minute.

Laboratory reports of this infant were negative, including toxoplasma, herpes simplex virus 1/2, cytomegalovirus (CMV), and rubella virus. Neutrophils percentage (69.5%, normal: 31% ~ 52%), basophilic cells percentage (0.70%, normal: 0% ~ 0.6%), neutrophils total (13.45%, normal: 3.9% ~ 9.4%) were all increased. Liver dysfunction (AST 92U/L, normal: ≤41 U/L), creatine kinase (189U/L, normal: 30 ~ 170 U/L), creatine kinase isoenzyme MB (60U/L, normal: 0 ~ 24U/L) levels increased. Procalcitonin increased (0.880ng/ml, normal: ≤0.05ng/ml). IL-6 (49.00pg/ml, normal: 0 ~ 20.9pg/ml) and IL-10 (6.28pg/ml, normal: 0 ~ 5.9pg/ml) increased. Renal function and electrolytes were normal. Figure 1A Chest X-ray showed enhanced lung veins, reticular and patchy shadows, and no abnormalities in heart and palate (image). She was closely monitored in isolation, treated with a nourishing cardiac muscle and a spray of interferon. Intravenous injection of penicillin G (15wu q.d, intravenous bolus) and vitamin K1 (1mg q.d, intravenously) were used as antibiotics and to prevent coagulation disorders.

March 7, 2020 the second day after the surgery, the newborn was in good condition and the mother's vital signs were stable. Baby was closely monitored and given 30ml of formula every three hours.

From March 7 to March 12, 2020 the newborn's vital signs were stable, the blood oxygen saturation maintaining above 90%, and there was no apnea or vomit. On March 9, 2020 the nucleic acid test of neonatal COVID-19 pharyngeal swab was negative, however, and serum COVID-19 IgM and IgG antibodies were normal and strong positive, respectively. The blood routine, liver function, calcitonin, and creatine kinase levels all returned to normal. Chest X-ray of neonate showed a few flaky shadow, with no abnormality in heart and palate. Compared with the chest X-ray on March 6, the Chest X-ray Figure 1B of March 12, 2020 revealed that most of the lung lesions were absorbed. She did not receive any special treatment since March 12. On March 17, laboratory tests and chest radiographs were normal, the nucleic acid test of neonatal COVID-19 pharyngeal swab and serum IgM were both negative. The newborn was discharged from hospital on March 18, 2020.

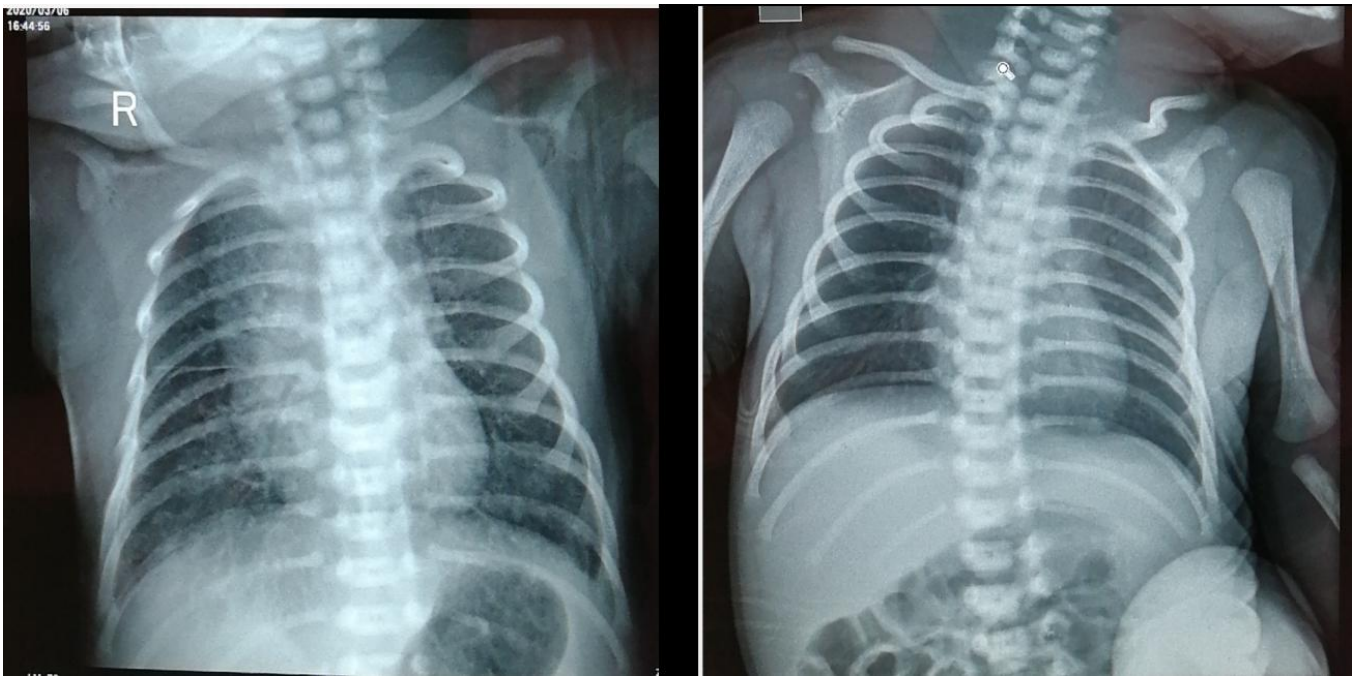

Figure 1. Chest X-ray of neonate on March 6th, 2020 (**A**) and March 12th, 2020 (**B**).

June 17, 2020 about one hundred days after the neonate was born, we detected the serum COVID-19 IgM and IgG antibodies of mother and infant again. The serum COVID-19 IgM and IgG antibodies of mother were still negative and positive, while the IgG antibody of infant decreased rapidly and changed into negative.

Since December 2019, pneumonia caused by SARS-CoV-2 has become a highly contagious disease. As of April 27, 2020, a total of 2,878,196 COVID–19 cases and 198,668 related deaths have been confirmed [1]. Since COVID-19 was a brand new infectious disease and the immunological detection reagent has just been developed, there were few reports of COVID-19 vertical transmission tracing in pregnant women. Here, we reported a newborn with normal IgM and elevated IgG antibodies born to an asymptomatic infection mother with COVID-19. The viral nucleic acid of the pharyngeal swab in newborn was negative. The pathogenic test found that the serum of IgM of the newborn was normal, and IgG was strongly positive. In general, after the body infecting with pathogenic microorganisms, the immune system carries out immune defense against the virus and produces specific antibodies. Specific IgM may indicate a current or recent infection. IgG is the main antibody produced in the immune response, indicating that the disease has entered the recovery period or the presence of previous infection [2–3].

When the infant was born, the level of IgG antibody in her serum was similar to that of her mother, both strong positive. However, the infant IgG antibody decreased rapidly and turned negative after about one hundred days, while the maternal IgG antibody remains at a high level. According to the examination results of mother and neonate, we suspected that, on the one hand, the neonate might acquire the IgG antibody from mother via placenta. The infant did not produce IgG antibody, therefore, the level of IgG antibody decreased remarkably at time passed. On the other hand, the mother might expose to small numbers of virus before, and was an asymptomatic infection case of COVID-19, thus, the neonate might be in the recovery period of COVID-19 at present. Because of the children's immune systems were underdeveloped, the level of IgG antibody in infant reduced rapidly.

There were several limitations in this study. 1) Lack of nucleic acid detection results in breast milk and placenta; 2) Our present report include the single case, more information was need to confirm the observation.

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CONFLICTS OF INTEREST

All the authors declare no conflicts of interest.

CONSENT FOR PUBLICATION

The patient and his parents all agreed to publish this study via written consent. The consent was obtained from a parent of legal guardian of the patient.

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Research Paper

Clinical course and risk factors for recurrence of positive SARS-CoV-2 RNA: a retrospective cohort study from Wuhan, China

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The objective of this study was to determine the clinical course and risk factors for patients showing recurrent SARS-CoV-2 RNA positivity. A total of 1087 COVID-19 patients confirmed by RT-PCR from February 24, 2020 to March 31, 2020 were retrospectively enrolled. Advanced age was significantly associated with mortality. In addition, 81 (7.6%) of the discharged patients tested positive for SARS-CoV-2 RNA during the isolation period. For patients with recurrent RT-PCR positivity, the median duration from illness onset to recurrence was 50 days. Multivariate regression analysis identified elevated serum IL-6, increased lymphocyte counts and CT imaging features of lung consolidation during hospitalization as the independent risk factors of recurrence. We hypothesized that the balance between immune response and virus toxicity may be the underlying mechanism of this phenomenon. For patients with a high risk of recurrence, a prolonged observation and additional preventative measures should be implemented for at least 50 days after illness onset to prevent future outbreaks.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2]. As of April 18, 2020, 2,121,675 confirmed cases of COVID-19 and 142,299 related deaths have been reported from 213 countries according to the World Health Organization (WHO) [3]. Although several studies have summarized the epidemiological and clinical features of SARS-CoV-2 infection [4–6], and research is going on viral pathogenicity and mechanism. However, the exact origin of SARS-CoV-2 is controversial and a potential threat to a new outbreak [7, 8]. Furthermore, little is known regarding the immune response against SARS-CoV-2 infection, which in turn makes it difficult to assess complete recovery with no further risk of infection. The latter is a crucial factor in "flattening the curve" of COVID-19 and preventing additional outbreaks.

In the early stages of the COVID-19 outbreak that was located to Wuhan, China, the severe shortage and limitations in the detection and accuracy of the RT-PCR test restricted identification of infected patients. The diagnostic techniques have improved substantially since [9], and two or more multipoint throat-swabs are taken over 24 hours apart prior to discharge in order to minimize the false negative rate of RT-PCR tests [10]. Lan L et al. [11] reported that four medical professionals with COVID-19 who met the criteria for hospital discharge (including two consecutive negative RT-PCR results) reverted to SARS-CoV-2 positivity, indicating a potential asymptomatic carrier state. It remains to be determined whether patients with recurrent SARS-CoV-2 RNA positivity remain infectious after discharge. Furthermore, the clinical and radiological characteristics of the COVID-19 patients with recurrence is largely unknown.

Herein, we retrospectively analyzed 1087 patients with confirmed COVID-19 and explored the clinical course and risk factors of SARS-CoV-2 RNA recurrence by RT-PCR during post-discharge isolation.

RESULTS

Clinico-demographic characteristics of patients

A total of 1087 consecutive COVID-19 pneumonia patients positive for SARS-CoV-2 RNA were enrolled in this study. The median age of the cohort was 60 years (9 to 100 years; IQR - 49-69 years) and 635 (58.4%) of the patients were women. The majority (83.1%) of the cases were mild, whereas the proportion of severe and critical cases were 13.2% and 3.7% respectively. Most patients (874, 80.4%) had bilateral pulmonary infiltration on the chest CT, while 730 (67.2%) and 525 patients (48.3%) respectively showed ground-glass appearance and consolidation. In addition, 887 out of 1007 (88.1%) patients were positive for serum IgG, while 797 out of 1057 (75.4%) patients were positive for serum IgM against COVID 19.

The median length of hospitalization was 12 days (1-38 days; IQR, 8-17 days), and 20 patients died during hospitalization whereas 1067 were discharged. The total mortality rate was 1.8% and the discharge rate was 98.2%. Among the fatalities, 5 patients were graded as severe with mortality rate of 3.5%, and 15 were critical cases with a high mortality rate of 37.5%. The total mortality rate of the severe and critical cases was 10.6%. The median age of the deceased patients was 83 years (65 to 92 years; IQR, 79.3-87.8 years), which was significantly higher than that of the discharged patients ($P<0.001$). The main causes of deaths were multiple organ failure (MOSF), most commonly affecting the lungs, heart, liver and kidneys. Other clinical features, laboratory examinations and imaging findings are summarized in Supplementary Table 1.

Characteristics of patients with SARS-CoV-2 RNA recurrence

Eighty-one (7.6%) of the discharged patients reverted to SARS-CoV-2 RNA positive after two negative RT-PCR tests during the post-discharge isolation period. The median age of the recurring cases was 62 years (range 16-90 years; IQR, 50.5-68 years), and 51 (63.0%) were female. Twenty (24.7%) patients had accompanying hypertension and 9 (11.1%) had diabetes. Furthermore, 84.0% (68), 14.8% (12) and 1.2% (1) of the cases were mild, severe and critical respectively. Most of these patients had the initial symptoms of COVID-19 infection prior to positive SARS-CoV-2 RNA diagnosis, and only 15 (18.5%) were asymptomatic when first diagnosed. Before hospitalization, pulmonary infection was confirmed in 70 patients via CT scan, and 65 (65.3%) received anti-viral agents.

Laboratory and CT imaging results from the inpatient hospital-stay are summarized in Table 1. Seven (8.6%) patients had lymphocytopenia and only 4 (4.9%) patients had neutrophilia. High-sensitivity CRP was elevated in 8 (9.9%) patients, and the ESR, procalcitonin and IL-6 levels were increased in 27 (33.3%), 14 (17.3%) and 11 (13.6%) patients. Furthermore, 10 (12.3%) patients developed liver injury with elevated ALT, 4 (4.9%) demonstrated myocardial damage with elevated Accu-Tell troponin, and 11 (13.6%) patients had kidney injury with elevated serum BUN and creatinine levels. CT images revealed consolidation, ground-glass opacity and bilateral pulmonary infiltration in 49 (60.5%), 56 (69.1%) and 70 (86.4%) patients, respectively. Finally, 72 of 77 (93.5%) patients were positive for serum IgG, whereas 68 of 79 (86.1%) were positive for serum IgM against COVID-19.

Clinical course of patients with SARS-CoV-2 recurrence

As shown in Figure 1, the median length of hospitalization for patients that reverted to SARS-CoV-2 RNA positive state was 12 days (range, 4-27 days; IQR, 7-17 days). The median duration from discharge to recurrence was 9 days (range, 3-18 days; IQR, 7-10 days), and that from the onset of illness to RT-PCR confirmation was 11 days (range, 0-57 days; IQR, 1.5-21 days) (Figure 2A). In addition, the time from illness onset to complete RNA negative status was 33 days (range, 6-82 days; IQR, 20-41 days), and from illness onset to recurrence was 50 days (range, 21-95 days; IQR, 36.5-59.5 days). As shown in Figure 2B, the

Table 1. Clinico-demographic characteristics of patients with recurrence of SARS-CoV-2 RNA positivity.

Variables	No. (n=81)	Percentage (%)
General features		
Clinical severity of disease		
Mild	68	84.0%
Severe	12	14.8%
Critical	1	1.2%
Age		
Median (IQR)	62.0 (50.5-68.0)	
Gender		
Male	30	37.0%
Female	51	63.0%
Hypertension		
Yes	20	24.7%
No	61	75.3%
Diabetes		
Yes	9	11.1%
No	72	88.8%
Illness onset		
Fever		
Yes	41	50.6%
No	40	49.4%
Cough		
Yes	44	54.3%
No	37	45.7%
Chest congestion		
Yes	18	22.2%
No	63	77.8%
Weak		
Yes	34	42.5%
No	46	57.5%
Muscular soreness		
Yes	15	18.5%
No	66	81.5%
Pulmonary infection (CT)		
Yes	70	86.4%
No	5	6.2%
Unknown	6	7.4%
Anti-virus therapy		
Yes	53	65.4%
No	15	18.5%
Unknown	13	16.0%
In hospital		
Fever		
Yes(>=37.3 °C once or more)	18	22.2%
No	63	77.8%
Internal visceral dysfunctions		
Yes	30	37.0%
No	51	63.0%
Comorbid diseases		
Yes	46	56.8%
No	35	43.2%
White blood cell count, $\times 10^9$ per L		
<4		
4-10	6	7.4%
>10	71	87.7%
Unknown	3	3.7%
	1	1.2%
Neutrophil count, $\times 10^9$ per L		
<1.8	3	3.7%
1.8-6.3	73	90.1%
>6.3	4	4.9%
Unknown	1	1.2%
Lymphocyte count, $\times 10^9$ per L		
<1.1	7	8.6%

1.1-3.2	72	88.9%
>1.1	1	1.2%
Unknown	1	1.2%
Platelet count, ×10⁹ per L		
<125	4	4.9%
125-350	69	85.2%
>350	7	8.6%
Unknown	1	1.2%
ALT		
<40	71	87.7%
>=40	10	12.3%
Albumin		
<35	10	12.3%
>=35	71	87.7%
C-reactive protein		
<10	71	87.7%
>=10	8	9.9%
Unknown	2	2.5%
ESR 30min		
<20	13	16.0%
>=20	27	33.3%
Unknown	41	50.6%
Procalcitonin		
<=0.05	47	58.0%
>0.05	14	17.3%
Unknown	20	24.7%
D-dimer		
<0.5	47	58.0%
>=0.5	18	22.2%
Unknown	16	19.8%
BUN		
<=6.5	68	84.0%
>6.5	11	13.6%
Unknown	2	2.5%
Creatinine		
<90	68	84.0%
>=90	11	13.6%
Unknown	2	2.5%
Accu-Tell Troponin		
<15.6	47	58.0%
>=15.6	4	4.9%
Unknown	30	37.0%
IL-6		
<10	43	53.1%
>=10	11	13.6%
Unknown	27	33.3%
IgG		
Positive	72	88.9%
Negative	5	6.2%
Unknown	4	4.9%
IgM		
Positive	68	84.0%
Negative	11	13.6%
Unknown	2	2.5%
Imaging features		
Consolidation		
Yes	49	60.5%
No	32	39.5%
Ground-glass opacity		
Yes	56	69.1%
No	25	30.9%
Bilateral pulmonary infiltration		
Yes	70	86.4%
No	11	13.6%

median duration from initial RT-PCR diagnosis to recurrence was 36 days (range, 16-64 days; IQR, 26.5-45 days). In addition, the median duration between the initial diagnostic RT-PCR and complete RNA negative status was 17 days (range, 1-45 days; IQR, 8-29 days), while that between complete RNA negative status and recurrence was 12 days (range, 4-27 days; IQR, 7-17 days).

Amongst these 81 patients, 37 (45.7%) received oxygen support. However, no invasive mechanical ventilation (IMV) or IMV with extracorporeal membrane

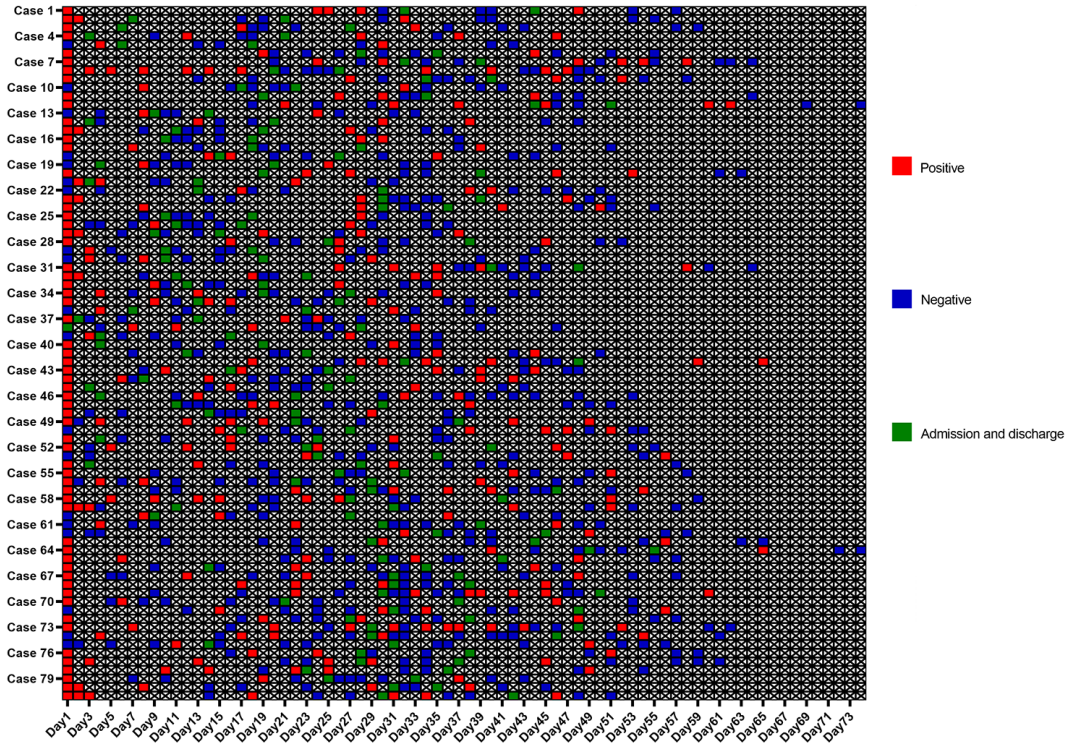

Figure 1. Individual duration of viral shedding and positive SARS-CoV-2 RNA recurrence from illness onset after discharge. The timing and results of RT-PCR examinations for SARS-CoV-2 RNA in details. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. RT-PCR=reverse transcription-polymerase chain reaction.

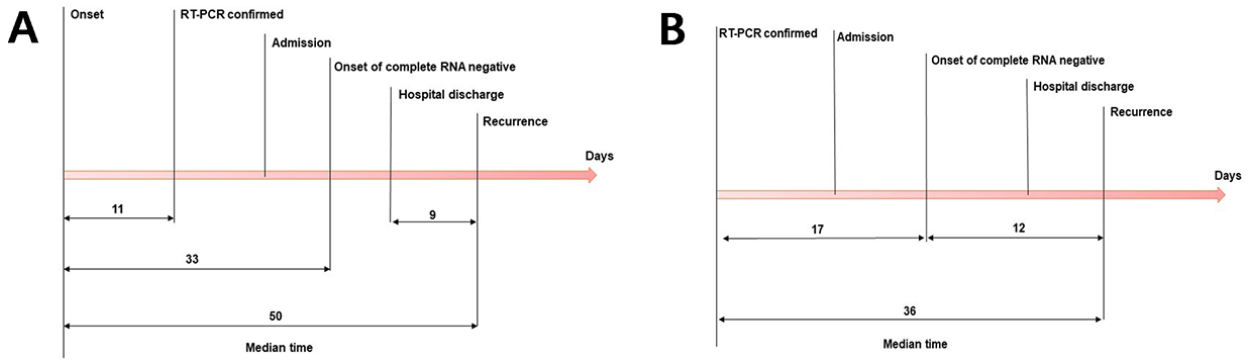

Figure 2. The median duration of different stages in patients with recurrence of positive SARS-CoV-2 RNA after discharge. (**A**) The median duration from illness onset to initial RT-PCR confirmation, onset of complete RNA negative status and recurrent RT-PCR positivity after discharge, and from discharge to recurrence. (**B**) The median duration from initial RT-PCR confirmation to onset of complete RNA negative status and recurrent RT-PCR positivity after discharge, and from onset of complete RNA negative status to recurrence. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. RT-PCR=reverse transcription-polymerase chain reaction.

oxygenation (ECMO) was used. The optimal antiviral therapy was administered in 69 (85.2%) patients, including arbidol hydrochloride (40 patients, 49.4%), interferon alfa (17 patients, 21.0%), entecavir/tenofovir (7 patients, 8.6%) and oseltamivir (5 patients, 6.2%). Fifty-one patients (63%) were treated with Chinese patented drugs, such as Lianhuaqingwen capsule. Vitamin C was given to 41 (50.6%) patients, and immunomodulators like thymopentin and immunoglobulin were administrated to 8 (9.9%) patients.

Associated risk factors with recurrence of positive SARS-CoV-2 RNA

As shown in Table 2, positive SARS-CoV-2 RNA recurrence correlated positively with serum IL-6 level (P=0.010) and CT imaging depicting consolidation (P=0.031). In the univariate analysis, elevated lymphocyte count (P=0.194, OR=1.644; 95% CI, 0.776-3.484), elevated serum IL-6 (P=0.013, OR=2.504; 95% CI, 1.218-5.150), consolidation on CT imaging (P=0.033, OR=1.655; 95% CI, 1.042-2.629) and bilateral pulmonary infiltration (P=0.196, OR=1.540; 95% CI, 0.800-2.966) were identified as potential risk factors for recurrence of SARS-CoV-2 RNA positivity (Table 3). Multivariate analysis concluded that elevated lymphocyte count (P=0.038, OR=2.321; 95% CI, 1.048-5.138), serum IL-6 level (P=0.004, OR=3.050; 95% CI, 1.432-6.499) and consolidation features on CT imaging (P=0.038, OR=1.641; 95% CI, 1.028-2.620) were the independent risk factors of recurrence (Table 4).

DISCUSSION

In this study, we have provided comprehensive data on the demographic and clinical characteristics of 1087 consecutive COVID-19 patients from Wuhan, China. The majority (83.1%) of the cases in our cohort were mild, and the overall mortality rate of the severe and critical cases was 10.6%. The mortality rate of the entire cohort was 1.8%, which is consistent to one previous study [4] but lower than that reported in other studies [5, 12]. This difference can be partly attributed to the higher proportion of severe cases in the other cohorts, as well as the greater medical resources that were allocated in the later stages of this pandemic wherein we enrolled patients for our study. Liang WH et al. [13] reported that the mortality of COVID-19 patients outside of the Hubei Province was limited to 0.3%, as strict public health interventions were initiated in order to prevent further outbreak outside Hubei and adequate medical resources were provided for treatment. In agreement with previous studies that identified older age as a risk factor of mortality in COVID-19 patients [6, 14], the median age of the deceased patients in our cohort was 83 years, distinctly higher than that of the discharged patients (P<0.001), which further suggests that a higher age was significantly associated with mortality.

Among the 1067 patients that were discharged on the basis of negative SARS-CoV-2 RNA results, 81 (7.6%) patients reverted to positive state during their isolation period. Similar findings have been reported previously [11, 15, 16]. However, Yuan J et al. [16] reported a higher repeat positivity rate of 14.5% after discharge, which could be on account the smaller cohort of enrolled patients. These persistent asymptomatic viral carriers may pose a risk for potential future outbreaks despite unprecedented public health interventions [17]. Therefore, we explored the clinical course and risk predictors for recurrent SARS-CoV-2 PCR positivity in order to provide new insights into the disease and help guide the clinical practice against future outbreaks.

In our study, the median duration of viral shedding for patients with positive SARS-CoV-2 RNA recurrence was 33 days from the onset of illness to complete RNA negative status. However, the median duration from illness onset to SARS-CoV-2 RNA reversion was 50 days. Previous studies have reported on duration of viral shedding. Zhou F et al. [6] reported a 20 day median duration of viral shedding in survivors and the longest observed duration was 37 days. Furthermore, Zhou B et al. [18] reported that the median duration of viral shedding was 31 days from illness onset in severe COVID-19 patients. Xu K et al. [19] further showed that 3 out of 4 COVID-19 patients had viral RNA clearance within 21 days of illness onset, and male gender, older age, hypertension, delayed hospital admission, severe illness upon admission, invasive mechanical ventilation and corticosteroid treatment were risk factors for prolonged viral RNA clearance. Our findings underscore the importance of a prolonged treatment or isolation for patients at increased risk of recurrent SARS-CoV-2 RNA positivity.

Nevertheless, we found that age and comorbidities that were previously described to be risk factors of mortality [14] were not identified as significant risk factors when compared to patients without reversion. Instead, high serum IL-6 levels, lymphocyte count greater than $1.1*10^8$ /L and consolidation on CT imaging during hospitalization were associated with a higher likelihood of recurrent SARS-CoV-2 RNA positivity after discharge. This is consistent with a previous study that showed that the lymphocyte count prior to discharge was positively correlated with the time to virus reappearance, which confirms the role of lymphocytes in the potential recurrence of SARS-CoV-2 RNA positivity [16]. Other factors that influence the host defense against viral infections, such as clinical severity

Table 2. Correlations between clinical characteristics and recurrence of SARS-CoV-2 RNA positivity in discharged patients.

Variables	No. (n=1067)	No recurrence n=986	Recurrence n=81	P value
General features				
Clinical severity of disease				
Mild	903	835	68	0.684
Severe	139	127	12	
Critical	25	24	1	
Age				
Median (IQR)	60.0 (49.0-68.0)	60.0 (49.0-68.0)	62.0 (50.5-68.0)	0.700
Gender				
Male	440	410	30	0.424
Female	627	576	51	
Hypertension				
Yes	331	311	20	0.200
No	736	675	61	
Diabetes				
Yes	135	126	9	0.664
No	932	860	72	
In hospital				
Fever				
Yes(>=37.3°C once or more)	246	228	18	0.853
No	821	758	63	
Internal visceral dysfunctions				
Yes	343	313	30	0.327
No	724	673	51	
Comorbid diseases				
Yes	560	514	46	0.419
No	507	472	35	
White blood cell count, $\times 10^9$ per L				
<4	100	94	6	0.579
4-10	927	856	71	
>10	24	21	3	
Unknown	16	15	1	
Neutrophil count, $\times 10^9$ per L				
<=6.3	994	918	76	1.000
>6.3	57	53	4	
Unknown	16	15	1	
Lymphocyte count, $\times 10^9$ per L				
<=1.1	158	150	8	0.190
>1.1	894	821	72	
Unknown	15	15	1	
Platelet count, $\times 10^9$ per L				
<125	44	40	4	
125-350	956	886	69	0.297
>350	52	45	7	
Unknown	15	15	1	
ALT				
<40	852	781	71	0.202
>=40	181	171	10	
Unknown	34	34	0	
Albumin				
<35	154	144	10	0.502
>=35	880	809	71	
Unknown	33	33	0	
C-reactive protein				
<10	914	843	71	0.653
>=10	121	113	8	
Unknown	32	30	2	
ESR 30min				
<20	176	163	13	0.420
>=20	282	255	27	
Unknown	609	568	41	
Procalcitonin				0.571

<=0.05	589	542	47	
>0.05	207	193	14	
Unknown	271	251	20	
D-dimer				
<0.5	507	460	47	0.339
>=0.5	250	232	18	
Unknown	310	294	16	
BUN				
<=6.5	853	785	68	0.822
>6.5	148	137	11	
Unknown	66	64	2	
Creatinine				
<90	907	839	68	0.150
>=90	94	83	11	
Unknown	66	64	2	
Accu-Tell Troponin				
<15.6	590	543	47	1.000
>=15.6	54	50	4	
Unknown	423	393	30	
IL-6				
<10	552	509	43	0.010
>=10	63	52	11	
Unknown	452	425	27	
Imaging features				
Consolidation				
Yes	520	471	49	0.031
No	541	509	32	
Unknown	6	6	0	
Ground-glass opacity				
Yes	720	664	56	0.798
No	341	316	25	
Unknown	6	6	0	
Bilateral pulmonary infiltration				
Yes	859	789	70	0.193
No	202	191	11	
Unknown	6	6	0	

of the disease, CRP, D-dimer level etc., were not significantly different between the recurrent versus non-recurrent groups. IL-6 is one of the major pro-inflammatory cytokines that are instrumental in clearing pathogens. However, the rapid multiplication of SARS-CoV-2 in the lower respiratory tract leads to excessive IL-6 production, which triggers an acute severe systemic inflammatory response known as cytokine release syndrome (CRS) [20]. In fact, the increased serum IL-6 levels in severe and critical COVID-19 patients is associated with poor outcomes [21, 22], which was also observed during severe acute respiratory syndrome (SARS) outbreak [23]. Concurrently, lymphopenia is also common in patients with COVID-19, especially in severe and critical cases [5, 22, 24], suggesting a dysregulated immune response in this sub-cohort. In our study however, only 175 (16.1%) patients showed a decrease in lymphocyte count, which again may be can be attributed to the fewer severe cases. Interestingly, the discharged patients with recurrence of positive SARS-CoV-2 RNA had an elevated serum IL-6 level and lymphocyte count compared to those with no recurrence, indicating that the immune system may still be actively involved in clearing the infection. It is also possible that the immune responses can suppress but not completely eradicate SARS-CoV-2, which may have led to the false-negative results due to lower viral loads. Once the virus started replicating again, the RT-PCR results reverted to positive in the discharged patients.

The chest CT imaging of COVID-19 pneumonia is a useful preliminary diagnostic tool that has lowered the rate of missed diagnoses [25]. Features of consolidation on CT imaging are associated with critical disease [26]. Progression of consolidation might indicate further infiltration of the lung parenchyma and lung interstitium due to virus invasion into the respiratory epithelium, which is characterized by diffuse alveolar damage and necrotizing bronchitis. This eventually leads to complete permeation of the alveoli with the inflammatory exudate [27, 28]. Therefore, SARS-CoV-2 may persist in the respiratory epithelium during lung consolidation in the recovery phase of the infection, which eventually results in the recurrence of positive SARS-CoV-2 RNA after discharge. Interestingly, most patients with recurrence had fluctuating positive and

Table 3. Univariate regression analysis for risk factors of patients with recurrence of SARS-CoV-2 RNA positivity.

Variables	No.	Univariate OR (95% CI)	P value
Age	1067	1.000(0.985-1.015)	0.995
Gender	1067	1.210(0.757-1.933)	0.425
Clinical severity of disease	1067	0.976(0.579-1.645)	0.927
Hypertension	1067	0.712(0.422-1.200)	0.202
Diabetes	1067	0.853(0.416-1.749)	0.665
Fever	1067	0.950(0.551-1.637)	0.853
Internal visceral dysfunctions	1067	1.265(0.790-2.025)	0.328
Comorbid diseases	1067	1.207(0.764-1.906)	0.420
Neutrophil count, $\times 10^9$ per L	1051	0.912(0.321-2.587)	0.862
Lymphocyte count, $\times 10^9$ per L	1051	1.644(0.776-3.484)	**0.194**
Platelet count, $\times 10^9$ per L	1051	1.417(0.676-2.969)	0.356
ALT	1033	0.643(0.325-1.273)	0.205
Albumin	1034	1.264(0.637-2.508)	0.503
C-reactive protein	1035	0.841(0.394-1.792)	0.653
ESR 30min	458	1.328(0.666-2.647)	0.421
Procalcitonin	796	0.837(0.450-1.553)	0.572
D-dimer	757	0.759(0.431-1.337)	0.340
Accu-Tell Troponin	644	0.924(0.320-2.671)	0.884
IL-6	615	2.504(1.218-5.150)	0.013
Consolidation	1061	1.655(1.042-2.629)	0.033
Ground-glass opacity	1061	1.066(0.653-1.740)	0.798
Bilateral pulmonary infiltration	1061	1.540(0.800-2.966)	**0.196**

Table 4. Multivariate regression analysis for risk factors of patients with recurrence of SARS-CoV-2 RNA positivity.

Variables	Multivariate OR (95% CI)	P value
IL-6		
<10	Reference	
>=10	3.050(1.432-6.499)	0.004
Consolidation		
No	Reference	
Yes	1.641(1.028-2.620)	0.038
Lymphocyte count, $\times 10^9$ per L		
<=1.1	Reference	
>1.1	2.321(1.048-5.138)	0.038
Bilateral pulmonary infiltration		
No	Reference	
Yes	1.482(0.764-2.871)	0.244

negative results in the course of the disease, especially in cases 7, 8 and 41 (Figure 1). This is a potential sign of recurrent SARS-CoV-2 positivity after discharge, and also partly ruled out the randomly error probability in RT-PCR detection for one case. Thus, the infected patients may have already been immune to the virus and require a period for complete recovery. However, if the immune response cannot deal with the recurrence, further treatment may be still needed.

Limitations

This study has a few limitations that ought to be noted. First, this study was conducted at a single-center hospital which may have introduced a selection bias that influenced the clinical outcomes. A larger multi-center or even global cohort study of COVID-19 patients would help further define the clinical characteristics and risk factors of recurrence. Second, only multipoint throat-swab

specimens were tested which increases the risk of false negative results. Therefore, multisite samples should be collected for RT-PCR detection, such as the fecal SARS-CoV-2 RNA test for patients with gastrointestinal symptoms [29]. Third, the retrospective design and initial lack of guidelines for drug administration made it difficult to analyze the impact of treatment regimens on the recurrence of positive SARS-CoV-2 RNA.

CONCLUSIONS

Elevated lymphocyte counts and serum IL-6 level, and consolidation on chest CT were associated with a greater risk of recurrent SARS-CoV-2 RNA positivity, possibly due to a balance between immune regulation and virus toxicity. For patients with a higher risk of recurrence, a prolonged treatment or isolation period for at least 50 days after illness onset is recommended in order to identify patients that may pose a risk for future outbreaks.

MATERIALS AND METHODS

Study design and participants

A total of 1087 consecutive COVID-19 patients diagnosed by SARS-CoV-2 RNA detection in accordance with the interim guidelines of World Health Organization at the Guanggu Branch of Hubei Province Maternity and Childcare Hospital (Wuhan, China) were retrospectively enrolled. All patients had been discharged or had died between February 24, 2020 and March 31, 2020. This study was approved by the Research Ethics Committee of Guanggu Branch of Hubei Province Maternity and Childcare Hospital and was granted with a waiver of informed consent from study participants.

Data collection and follow-up

The epidemiological, radiographic, laboratory, treatment and treatment outcome data of these patients were extracted from medical records and through direct communication in order to establish a database. The SARS-CoV-2 RNA RT-PCR records from discharge to April 15, 2020 were obtained from the Health Wuhan App, a database containing all real-time results about SARS-CoV-2 RNA tests conducted in Wuhan. The patients were assigned a number for confidentiality. All data were evaluated by two authors (JC and QC) and thereafter by a third researcher (NP) in case of any differences in interpretation.

Clinical tests

In accordance with the standard procedure, throat-swab specimens were obtained and tested for SARS-CoV-2 infection using RT-PCR by the Academy of Military Medical Sciences and hospital laboratory [14]. The test was repeated during the hospital stay and after clinical remission of symptoms at 24-hour intervals. In addition, serum levels of SARS-CoV-2-specific IgM/IgG measured during hospitalization with the indirect enzyme-linked immunosorbent assay (ELISA) protocol using the N protein of SARS-CoV-2 as the coating antigen. Routine blood tests were performed to determine complete blood counts (including white blood cells, neutrophils, lymphocytes, monocytes and platelets), biochemical indices (liver function, renal function and electrolyte levels), coagulation indices, high-sensitivity C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), procalcitonin, myocardial enzymes, D-dimer and interleukin-6 (IL-6). Computed tomography (CT) scans were routinely performed as recommended by the attending physician.

Clinical definitions

The patients were discharged based on the following criteria: 1) no fever for at least three days, 2) remission of respiratory symptoms, 3) amelioration of pulmonary inflammation on the chest CT scan, 4) two negative SARS-CoV-2 RNA tests at least 24 hours apart, 5) overall good constitution.

The severity of COVID-19 was defined according to the Chinese management guidelines for COVID-19 (version 6.0) [10]. Fever was defined as axillary temperature of at least 37.3°C. Comorbidities during hospitalization included hypertension, diabetes, hypoproteinaemia (< 25 g/L), coagulopathy (3-second increase in prothrombin time or a 5-second increase of activated partial thromboplastin time), hyperuricemia (blood trioxypurine > 420 μmol/L or > 360 μmol/L in males and females respectively), anemia (according to WHO guidelines [30]), acute respiratory distress syndrome (ARDS; diagnosed according to the Berlin Definition [31]), acute liver failure (diagnosed according to EASL Clinical Practical Guidelines [32]), acute kidney injury (diagnosed according to the KDIGO clinical practice guidelines [33]), and acute cardiac injury (diagnosed as previously reported [6])

Statistical analysis

Continuous and categorical variables were respectively presented as median with interquartile range (IQR) and counts with percentages. The differences between the recurrence and non-recurrence groups were compared using the Pearson Chis-squared test, Fisher's exact test or Mann-Whitney U test as appropriate. The risk factors associated with the recurrence of positive SARS-CoV-2 RNA were identified using univariate analysis, and

variables with $P < 0.2$ were selected for multivariate logistic regression model. Missing data was not included in any of the analyses. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS v21.0 software (IBM Corporation, Armonk, NY, USA), and the figures were plotted using the GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA).

AUTHOR CONTRIBUTIONS

JC, XX, NP, and ZC conceived and designed the research. JC, XX, JH, FX, HL, NL, HZ, JL, JX, NP and ZC collected and analyzed data. JC, QC, NP, and ZC participated in evaluation of results. JC, QC, and NP wrote the manuscript, and all authors contributed to manuscript revision, read and approved the submitted version.

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CONFLICTS OF INTEREST

We declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Table

Supplementary Table 1. Clinico-demographic characteristics of all patients with COVID-19 confirmed by RT-PCR.

Variables	No. (n=1087)	Percentage (%)
General features		
Clinical severity of disease		
Mild	903	83.1%
Severe	144	13.2%
Critical	40	3.7%
Age		
Median (IQR)	60.0 (49.0-69.0)	
Gender		
Male	452	41.6%
Female	635	58.4%
Hypertension		
Yes	337	31.0%
No	750	69.0%
Diabetes		
Yes	137	12.6%
No	950	87.4%
In hospital		
Fever		
Yes(>=37.3°C once or more)	254	23.4%
No	833	76.6%
Internal visceral dysfunctions		
Yes	363	33.4%
No	724	66.6%
Comorbid diseases		
Yes	580	53.4%
No	507	46.6%
White blood cell count, $\times 10^9$ per L		
<4	102	9.4%
4-10	938	86.3%
>10	31	2.9%
Unknown	16	1.5%
Neutrophil count, $\times 10^9$per L		
<=6.3	1005	92.5%
>6.3	66	6.1%
Unknown	16	1.5%
Lymphocyte count, $\times 10^9$per L		
<=1.1	175	16.1%
>1.1	896	82.4%
Unknown	16	1.5%
Platelet count, $\times 10^9$per L		
<125	52	4.8%
125-350	967	89.0%
>350	52	4.8%
Unknown	16	1.5%
ALT		
<40	867	79.8%
>=40	185	17.0%
Unknown	35	3.2%
Albumin		
<35	171	15.7%
>=35	883	81.2%
Unknown	33	3.0%
C-reactive protein		
<10	919	84.5%
>=10	136	12.5%
Unknown	32	2.9%
ESR 30min		
<20	178	16.4%

>=20	290	26.7%
Unknown	619	56.9%
Procalcitonin		
<=0.05	594	54.6%
>0.05	221	20.3%
Unknown	272	25.0%
D-Dimer		
<0.5	510	46.9%
>=0.5	265	24.4%
Unknown	312	28.7%
BUN		
<=6.5	859	79.0%
>6.5	162	14.9%
Unknown	66	6.1%
Creatinine		
<90	917	84.4%
>=90	104	9.6%
Unknown	66	6.1%
Accu-Tell Troponin		
<15.6	593	54.6%
>=15.6	66	6.1%
Unknown	428	39.4%
IL-6		
<10	555	51.1%
>=10	76	7.0%
Unknown	456	42.0%
IgG		
Positive	887	81.6%
Negative	120	11.0%
Unknown	80	7.4%
IgM		
Positive	797	73.3%
Negative	260	23.9%
Unknown	30	2.8%
Imaging features		
Consolidation		
Yes	525	48.3%
No	551	50.7%
Unknown	11	1.0%
Ground-glass opacity		
Yes	730	67.2%
No	346	31.8%
Unknown	11	1.0%
Bilateral pulmonary infiltration		
Yes	874	80.4%
No	202	18.6%
Unknown	11	1.0%

Review

Neurological manifestations in COVID-19 and its possible mechanism

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ABSTRACT

In December 2019, the first cases of the acute respiratory illness now known as Corona Virus Disease 2019 (COVID-19) occurred in Wuhan, Hubei Province, China. The main clinical manifestations of COVID-19 are a fever, dry cough and general weakness, although in some patients, a headache, tight chest, diarrhea, etc. are the first clinical manifestations. Neurological practice is involved in all aspects of medicine, from primary care for patients with migraines to consultations with patients in the intensive care unit. Few disorders spare the nervous system, and newly emerging infections are no exception. As neurologists, we are concerned about the effects of SARS-CoV-2 infections on the nervous system. Multiple neuropathy, rhabdomyolysis, cerebrovascular disease, central nervous system infections and other common neurological diseases require attention during this outbreak.

INTRODUCTION

In December 2019, a number of unexplained cases of pneumonia occurred in Wuhan, China, and rapidly spread to other parts of China, then to Europe, North America, Asia and most of the world. This outbreak was confirmed to be caused by a novel coronavirus – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2]. On March 11, 2020, the World Health Organization declared Corona Virus Disease 2019 (COVID-19) as a pandemic [3]. As of May 7, 2020, there were 3,672,238 confirmed cases of COVID-19 and 254,045 deaths due to the disease globally [4]. The most common symptoms in patients diagnosed with SARS-CoV-2 infections are a fever and dry cough [5, 6]. Infections caused by SARS-CoV-2 exhibit many clinical similarities to those caused by SARS-CoV, such as a fever, dry cough and diarrhea during the prodromal phase [7–9].

In addition to the typical respiratory symptoms, some SARS patients have had neurological problems [10, 11]. Similarly, in some patients diagnosed with COVID-19, headaches, muscle aches, confusion and seizures have been the first clinical manifestations [12, 13]. In COVID-19 epidemic areas, people who have had close contact with diagnosed COVID-19 patients should be alert to the possibility of SARS-CoV-2 infection if they develop neurological symptoms such as headaches, slurred speech, hemiplegia and disturbances of consciousness. This article reviews the epidemiology of SARS-CoV-2 infections, the neurological diseases related to SARS-CoV-2, and the possible mechanisms behind these relationships.

SARS-CoV-2

SARS-CoV-2, originally named 2019-nCoV, belongs to the broader family of coronaviruses [1, 2]. Coronaviruses are enveloped, positive-stranded RNA viruses that belong to the family Coronaviridae and the order Nidovirales. Six coronavirus species are known to cause respiratory, enteric, hepatic and neurologic diseases. Four of these

viruses are prevalent – 229E, OC43, NL63 and HKU1 – and typically cause common cold symptoms in immuno-competent individuals [14]. The two other strains – SARS-CoV [15] and Middle East respiratory syndrome coronavirus (MERS-CoV) [16] – are zoonotic in origin, and have been linked to sometimes fatal illness. SARS-CoV-2 is the seventh member of the coronavirus family. Zhou et al. found that SARS-CoV-2 was 96% identical at the whole-genome level to a bat coronavirus, so SARS-CoV-2 may have originated in bats [17].

An epidemic or outbreak can occur when the agent (pathogen), population (hosts) and environment create an ideal situation for spread [18]. The current evidence suggests that SARS-CoV-2 may have spread to humans via wild animals sold illegally in the Huanan Seafood Wholesale Market [19]. The extent of human-to-human transmission of SARS-CoV-2 was unclear at first, but now there is evidence of human-to-human transmission [5, 20, 21]. The main sources of infection are SARS-CoV-2-infected patients, including those who are asymptomatic [22]. The routes of transmission include droplet transmission, contact transmission and aerosol transmission [23, 24]. In a recent study, SARS-CoV-2 was detected in stool samples from patients with abdominal symptoms [20, 25], so some scholars have proposed that SARS-CoV-2 could spread *via* fecal-oral transmission. Further environmental studies will be needed to determine whether the virus remains viable under conditions that would favor fecal-oral transmission [26]. SARS-CoV-2 has not been confirmed to be transmitted vertically from mother to child [27]. Based on the available data, a Chinese team estimated a basic reproduction number (R0) of 3.77 for SARS-CoV-2, basically confirming that the new coronavirus is more contagious than SARS [28].

SARS-CoV-2 employs a densely glycosylated, homotrimeric class I fusion spike (S) protein to enter host cells. The S protein exists in a metastable prefusion conformation that undergoes a dramatic structural re-arrangement to fuse the viral membrane with the host cell membrane [29, 30]. Epidemiological data indicate that the population is generally susceptible to SARS-CoV-2 [31]. Therefore, it is necessary for individuals to wash or disinfect their hands frequently, go outside less, wear a mask, avoid group activities, stay away from patients with COVID-19, maintain good living habits and keep an optimistic attitude [32–34].

Neurological disease

SARS-CoV-2 has been reported to be associated with Guillain-Barré syndrome, rhabdomyolysis, acute cerebrovascular disease, central nervous system infections and other neurological diseases. Four formal reports have described neurological problems in SARS patients, including polyneuropathy [35], myopathy and rhabdomyolysis [36], large artery ischemic stroke [37] and central nervous system infections [38]. Human coronaviruses (HCoVs) can naturally reach the central nervous system, and could potentially cause neurological symptoms. Among the coronavirus-induced animal diseases, feline infectious peritonitis virus, mouse hepatitis virus and hemagglutinating encephalomyelitis virus can all reach the central nervous system and induce different types of neuropathologies [10, 11]. The structure of SARS-CoV-2 is similar to that of the SARS virus, and both viruses invade the human body through the angiotensin converting enzyme II (ACE2) receptor. Thus, in this paper, we mainly describe the neurological diseases associated with SARS-CoV-2, but also briefly introduce the neurological diseases associated with SARS.

Neuromuscular manifestations

Polyneuropathy

Polyneuropathy, also known as peripheral neuropathy, is multiple-nerve damage of the extremities. The clinical manifestations are mostly distal symmetrical motor sensory dysfunction and autonomic nerve dysfunction [39]. The causes of polyneuropathic disorders include metabolic, toxic, infectious, inflammatory, autoimmune and genetic conditions [40]. Zhao et al. reported a case of COVID-19 initially presenting with acute Guillain-Barré syndrome (GBS). The female patient aged 61 years presented with acute weakness in both legs and severe fatigue. She received intravenous immuno-globulin, antiviral drugs of arbidol, lopinavir, and ritonavir, and supportive care. After 30 days of treatment, the muscle strength of the limbs returned to normal and the respiratory symptoms disappeared [41]. In a recently published article, two COVID-19 patients were diagnosed with Miller-Fisher syndrome (MFS) and multiple cranial neuritis, respectively [42]. These cases suggest a possible link between GBS and SARS-CoV-2 infection.

Some patients with severe COVID-19 progress rapidly and need to be transferred to an intensive care unit (ICU) for further treatment [43, 44]. In such patients, the peripheral nerves could be particularly susceptible to peripheral microcirculation disturbances, since the vessels supplying them with blood lack autoregulation [45]. ICU-acquired weakness, which can manifest as critical-illness polyneuropathy, critical-illness myopathy or both, is a frequent and disabling disorder in ICU patients [46]. Critical-illness polyneuropathy, an axonal sensory-motor polyneuropathy, is observed in up to a third of critically ill patients with systemic inflammatory response syndrome. Critical-illness myopathy, an acute

myopathy, develops in a similar setting, often in association with the use of corticosteroids and/or non-depolarizing neuromuscular-blocking agents [47].

Tsai et al. [35] presented data from four patients with probable SARS who developed axonal polyneuropathy, myopathy or both (2004). All of them had received intubation for respiratory distress and a high dose of steroid therapy for multiple organ failure. They developed distal-predominant weakness in all four limbs and a mild decrease in deep-tendon reflexes three to four weeks after the onset of SARS. The most likely diagnoses were critical-illness polyneuropathy and/or critical-illness myopathy. Some viruses, such as cytomegalovirus and varicella zoster virus, may cause peripheral neuropathy by directly attacking the nerves. It is not known whether direct attacks of the peripheral nervous system occur in HCoV-associated neuropathy.

Rhabdomyolysis

Rhabdomyolysis refers to the damage to striated muscle, the destruction of the muscle cell membrane integrity and the release of myoglobin, creatine kinase, other enzymes, small molecules and toxic substances into the systemic circulation due to various traumatic and non-traumatic factors, resulting in a group of clinical syndromes of organ damage [48, 49]. Clinical examination, history evaluation, laboratory studies, muscle biopsies and genetic testing are useful tools for diagnosing rhabdomyolysis and differentiating acquired from inherited cases. Acquired cases may be due to substance abuse, medication or toxic exposures, electrolyte abnormalities, endocrine disturbances and autoimmune myopathies [50].

In several recent studies on COVID-19 [5, 12, 20], a few patients exhibited varying degrees of myalgia, fatigue and elevated creatine and creatine kinase levels. In the study of Guan et al., two patients clearly developed rhabdomyolysis as a complication of COVID-19, while 14.90% (164/1099) exhibited myalgia or arthralgia symptoms and 13.7% (90/657) had creatine kinase levels ≥ 200 U/L [5]. Tong's research group reported that a patient diagnosed with COVID-19 had pain and weakness in both lower limbs and obvious tenderness after the ninth day of admission. Laboratory examination indicated that the patient's myoglobin level was >12,000.0 μg/L (reference 0-140 μg/L), creatine kinase was 11,842 U/L (reference 38-174 U/L) and lactate dehydrogenase was 2,347 U/L (reference 109-245 U/L). The authors added hydration, alkalization, plasma transfusion, gamma globulin and symptomatic support therapy based on the patient's previous treatment with oxygen, antivirals, antibiotics and methylprednisolone [51]. Creatine kinase and myoglobin are important indicators of rhabdomyolysis, but they are not routinely detected in the clinical practice. When patients have local muscle pain and weakness, rhabdomyolysis should be considered.

In previous SARS studies, some patients were clearly diagnosed with critical-illness myopathy [35] and rhabdomyolysis [50, 52]. In such patients, it cannot be ruled out that rhabdomyolysis may have developed due to the use of corticosteroids and/or nondepolarizing neuromuscular-blocking agents; however, the association of rhabdomyolysis with viruses such as influenza viruses A and B, human immunodeficiency virus, Coxsackie virus, cytomegalovirus, West Nile virus and dengue virus has also been well described [53–56]. Nevertheless, there is not yet sufficient evidence that HCoVs can directly invade muscle cells.

Acute cerebrovascular disease

The population is generally susceptible to SARS-CoV-2, but the elderly are more susceptible (the median age of hospitalized patients in one study was 56 years [interquartile range, 42-68 years; range, 22-92 years] [20]), and such patients are already at high risk for cerebrovascular diseases. Viral infections are known to be associated with an increased risk of stroke [57]. In a study by Mao et al., 214 patients diagnosed with COVID-19 were enrolled, and six (2.80%) of them developed acute cerebrovascular disease (five cases of ischemic stroke and one case of cerebral hemorrhage). All but one of these patients (an ischemic stroke patient) died of respiratory failure [13]. In a study of 206 SARS patients in Singapore, large artery stroke was diagnosed in five patients, of whom four were critically ill and three died [58]. Strokes are not uncommon in critically ill patients with multiple comorbidities, so SARS-CoV-2 infections in humans may increase the risk of stroke.

Central nervous system infection

Central nervous system infections are among the most critical problems in public health, as patients frequently exhibit neurologic sequelae. The clinical manifestations include a fever, headache, vomiting, stiff neck, afebrile seizures and status epilepticus. HCoVs cause a certain degree of nerve erosion, but their capacity to infect the central nervous system in humans has not been well characterized [10, 59]. Moriguchi et al. described a patient with SARS-CoV-2-associated meningitis who was brought to the hospital by ambulance due to convulsions and a coma. Interestingly, SARS-CoV-2 RNA was not detected in the patient's nasopharyngeal swab, but was detected in the patient's cerebrospinal fluid [60]. Zhao et al. [61] reported spinal cord involvement in a COVID-19 patient one week after the onset of fever. After admission, his SARS-CoV-2 RNA

nasopharyngeal swab test was positive. Based on the patient's acute flaccid myelitis of the lower limbs, urinary and bowel incontinence, and sensory level at T10, a diagnosis of acute myelitis was more likely. After the patient had been treated with high-flow oxygen, antiviral medication, steroids and human immunoglobulin, his body temperature returned to normal and two subsequent SARS-CoV-2 RNA nasopharyngeal swab tests were negative. The muscle strength of both upper limbs recovered to grade 4/5, while the muscle strength of both lower limbs was grade 1/5. This study indicated that acute myelitis may be a neurological complication of COVID-19. The above cases demonstrate the potential for neurological invasion of SARS-CoV-2.

The presence of HCoV in human central nervous system-related samples was detected as early as 1980 in autopsies of patients with multiple sclerosis [62]. In 2004, genetic material from SARS-CoV was detected in cerebrospinal fluid samples from a 32-year-old woman. The patient had a generalized tonic-clonic convulsions with loss of consciousness and up-rolling eyeballs lasting for one minute [38]. Another patient, a doctor infected with the SARS virus, had symptoms of restlessness, vomiting and confusion on the 33th day of illness. The patient died after treatment failed, and a brain biopsy was performed. A fragment specific for SARS HCoV was amplified from cultures of the brain suspension, and transmission electronic microscopy revealed the presence of an enveloped virus morphologically compatible with a coronavirus in the cultures [63]. Since some COVID-19 patients have complained of headaches, nausea etc, care providers should be alert for central nervous system infections caused by SARS-CoV-2 if such patients also exhibit symptoms such as a fever, epilepsy and disturbances of consciousness.

Mechanisms of nervous system damage due to SARS-CoV-2 infections

In this section, we will explore various mechanisms that may explain the correlation between COVID-19 and neurological disease.

Hypoxemia

In a clinical retrospective study of 138 people, the most common complication of COVID-19 during hospital admission was pneumonia, followed by acute respiratory distress syndrome (19.60%) and shock (8.70%) [20]. The patients in this study had varying degrees of hypoxia, accompanied by hypoxemia. Most patients received oxygen inhalation (ordinary oxygen inhalation, 106 [76.81%]), and many received mechanical ventilation (non-invasive ventilation, 15 [10.09%]; intermittent mandatory ventilation, 17 [12.32%]). More than 20% of the oxygen consumed by humans is used by the brain for ATP production to generate the required membrane potential [64]. As soon as anoxia sets in, ATP synthase begins to pump protons out of the mitochondrial matrix to maintain the mitochondrial membrane potential. Continued lack of oxygen can eventually lead to the loss of high-energy phosphate esters, disturbances of neurotransmitter metabolism, the breakdown of the membrane, the failure of mitochondria and the accumulation of intracellular Ca^{2+}. The immediate consequence is irreversible neurological damage and even neuronal death [64, 65].

Lack of oxygen increases the risk of stroke. For instance, the prolonged hypoxia of obstructive sleep apnea hypopnea syndrome can damage the sleep structure, increase blood pressure, reduce cerebral blood flow and promote microthrombosis and atherosclerosis, thus impacting the prognosis and recurrence of cerebral infarction [66, 67]. Mao et al. reported that six COVID-19 patients had acute cerebrovascular disease: five with severe infections (5/88) and one with a non-severe infection (1/126) (P=0.03) [13]. The symptoms of hypoxia in COVID-19 patients are very obvious, and critical patients need ventilator support. COVID-19 patients admitted to the ICU tend to be older and have a greater number of comorbid conditions (e.g., hypertension, diabetes, cardiovascular and cerebrovascular diseases) than those not admitted to the ICU [20]. This suggests that older age and these comorbidities may be risk factors for poor outcomes [68, 69].

ACE2

The metallopeptidase ACE2 has been confirmed to be the cell receptor for SARS-CoV-2, just as it is for SARS-CoV [70, 71]. However, SARS-CoV-2 cannot enter cells through other coronavirus receptors such as aminopeptidase N and dipeptidyl peptidase [71]. ACE2 is highly expressed not only in the alveolar type II cells of the lungs and the upper and stratified epithelial cells of the esophagus, but also in the absorptive enterocytes of the ileum and colon [72, 73]. The main physiological function of ACE2 is to catalyze the conversion of angiotensin II to angiotensin (1-7), with a vasodilator effect. In brain tissues, angiotensin (1-7) stimulates Mas receptors to promote angiogenesis, and also inhibits oxidative stress, prevents neuroinflammation, improves cerebral blood flow, suppresses apoptosis and protects cerebral blood vessels [74].

Enhancing the expression of ACE2 may be an important strategy for treating cardiovascular and cerebrovascular

diseases [75]. SARS-CoV-2 patients with cerebrovascular disease may be more likely to develop into severe patients with a higher risk of death, so more timely diagnosis is needed for such patients. ACEI and angiotensin II receptor blocker antihypertensive drugs may increase the expression of the ACE2 receptor [76]. In order to avoid aggravating SARS-CoV-2 infection symptoms, it is recommended that hypertensive patients on blood pressure control medications stop using ACEI and angiotensin II receptor blocker antihypertensive drugs, and instead use calcium channel blocker diuretic antihypertensive drugs [77].

Immunization

The responses of the immune system can be divided into innate immunity (also known as non-specific immunity) and adaptive immunity (also known as specific immunity, which can be further divided into humoral immunity and cellular immunity) [76, 78]. The immune mechanisms induced by SARS-CoV-2 are unclear. After SARS-CoV-2 enters the body through ACE2, host factors trigger an immune response against the virus. The virus induces natural immunity, phagocytosis and phagocytic cell death, thus damaging tissues and organs. In four clinical retrospective studies that clearly identified the diagnosis of COVID-19 [12, 19, 20, 79], the absolute value of lymphocytes in most patients was reduced. These findings suggest that SARS-CoV-2 mainly attacks lymphocytes, especially T lymphocytes, similar to SARS-CoV.

CD4+ T cells are well known to regulate or "assist" the functioning of other lymphocytes. CD8+ T cells are cytotoxic and can kill virus-infected cells [80]. Barton et al. [81] reported that in two autopsies of COVID-19 patients, immunohistochemistry revealed a small number of CD3+ T lymphocytes infiltrating the alveolar septum, while CD20+ B-lymphocytes were rare. CD8+ T cells were slightly more prevalent than CD4+ T cells, and CD68 detection revealed a few macrophages. Some studies have suggested that the substantial decrease in the total number of lymphocytes in coronavirus patients may indicate that the virus consumes many immune cells and inhibits cellular immune function [82, 83].

After an antigen enters the body, the corresponding antigen-specific B cells are activated, induced to proliferate and eventually stimulated to differentiate into plasma cells. These plasma cells then produce specific antibodies that can enter the body fluid and exert immune effects. It is widely accepted that immunoglobulin M (IgM) provides the first line of defense during viral infections, prior to the generation of adaptive, high-affinity IgG responses that are important for long-term immunity and immunological memory [84]. Li et al. successfully developed a rapid detection IgG-IgM combined antibody test kit for the diagnosis of COVID-19. The kit has a sensitivity of 88.66% and a specificity of 90.63%, and can detect the infection within 15 minutes [85]. After the rehabilitation of most patients with the novel coronavirus, the body will produce specific antibodies that can kill and eliminate the virus.

On February 8, 2020, with the Pneumonia Diagnosis and Treatment Program for Novel Coronavirus Infection (Trial Version 5) [86] as a guide, The First People's Hospital of Jiangxia District carried out the first phase of a new convalescent plasma treatment on three critically ill patients. After 12 to 24 hours of convalescent plasma therapy, the patients' laboratory examination results, clinical signs and symptoms improved significantly. Plasma therapy not only is safe and potentially effective, but also stimulates humoral immunity [87].

Most COVID-19 patients have a good prognosis, while a few patients have mild symptoms in the early stage and suddenly deteriorate in the later stage of the disease or during the recovery process. A large number of patients have exhibited a 'cytokine storm' (the rapid production of cytokines such as tumor necrosis factor alpha, interleukin-1, interleukin-6 and interferon gamma) due to the viral infection, which sometimes has progressed to acute respiratory distress syndrome and multiple organ failure [12, 19]. It is already known that HCoV can spread from the respiratory tract to the central nervous system through transneuronal and hematogenous routes, resulting in encephalitis and neurological diseases [88]. The invasion of the blood-brain barrier by the coronavirus can destroy vascular endothelial connections, leading to blood-brain barrier dysfunction and enhanced permeability [89]. When the virus invades the human brain, it triggers immune damage, causing brain damage and acute or chronic inflammation, thus creating a vicious cycle.

Inflammation

Several current retrospective clinical studies have described COVID-19 patients with abnormally low lymphocyte counts, Prolonged prothrombin times and significantly increased lactate dehydrogenase levels. Patients transferred to the ICU had significantly higher white blood cell and neutrophil counts than those not transferred to the ICU, as well as higher levels of D-dimer, creatine kinase and creatine [20]. The complications in severe cases have included rhabdomyolysis, shock, acute cardiac injury and acute kidney injury. Several mechanisms are thought to link

infections with acute vascular events, including the release of proinflammatory cytokines, the disruption of atherosclerotic plaques, physiological changes in the heart rate and vasoconstriction [90]. The inflammatory response in severe pneumonia is not limited to lung tissue; rather, the systemic inflammatory response is activated, and its amplification cascade impairs the function of distant organs [57, 91].

Hypercoagulability

Middle-aged and elderly patients account for the majority of COVID-19 patients (especially critically ill patients) with abnormally increased D-dimer levels, and such patients are more prone to embolic vascular events and cerebrovascular disease [20]. Umapathi et al. postulated that a hypercoagulable state predisposed a group of mainly critically ill SARS patients to large cerebral arterial thromboembolism [58]. Providers treating critically ill COVID-19 patients with underlying diseases such as hypertension, diabetes, cancer, etc. should be alert to the potential for hypercoagulability and regularly assess routine blood coagulation.

Ethics statement

Our research does not require an ethics statement.

CONCLUSIONS

SARS-CoV-2 infection may involve the nervous system, and may cause diseases such as poly-neuropathy, myopathy, cerebral infarction and central nervous system infections. Cerebral infarction is the second most common cause of death and the leading cause of adult disability worldwide. Patients with cerebrovascular diseases may face greater risks during infections, so it is necessary to strengthen protection to avoid infection, perform secondary prevention measures and monitor patients' symptoms and vital signs. During the period of high incidence of COVID-19, neurologists need to pay great attention to the treatment of patients, especially those whose first symptoms are neurological symptoms.

AUTHOR CONTRIBUTIONS

Xiaojia Tang, Peipei Liu and Yingzhu Chen conceived and designed the research. Xiaojia Tan wrote the manuscript, and all authors contributed to manuscript revision, read and approved the submitted version.

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CONFLICTS OF INTEREST

No potential conflicts of interest were reported by the authors.

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Review

SARS-CoV-2, immunosenescence and inflammaging: partners in the COVID-19 crime

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ABSTRACT

Pneumonia outbreak in the city of Wuhan, China, prompted the finding of a novel strain of severe acute respiratory syndrome virus (SARS-CoV-2). Here, we discuss potential long-term consequences of SARS-CoV-2 infection, and its possibility to cause permanent damage to the immune system and the central nervous system. Advanced chronological age is one of the main risk factors for the adverse outcomes of COVID-19, presumably due to immunosenescence and chronic low-grade inflammation, both characteristic of the elderly. The combination of viral infection and chronic inflammation in advanced chronological age might cause multiple detrimental unforeseen consequences for the predisposition and severity of neurodegenerative diseases and needs to be considered so that we can be prepared to deal with future outcomes of the ongoing pandemic.

INTRODUCTION

At the end of 2019, the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) emerged in Wuhan, China, as the causative agent of Coronavirus Disease 2019 (COVID-19) [1, 2]. The most prominent clinical symptom of COVID-19 is extensive lung damage, accompanied by respiratory distress of varying severity [3]. Within only 2-3 months, SARS-CoV-2 caused a worldwide health emergency and a pandemic, by infecting over 15 million people and, at the point of writing of this text, taking more than 633,000 lives. Within this short period, the pandemic has also triggered an avalanche of social and economic consequences that promise to continue growing, and that will scar our society [1].

SARS-CoV-2 belongs to the family of coronaviruses (CoV), together with SARS-CoV and Middle East respiratory syndrome CoV – two highly pathogenic viral strains that caused significant medical turmoil in the recent past and were responsible for considerable lethality [4]. The same family also includes several harmless viruses (HKU, 229E) [5]. The coronavirus family shares some overall similarities with the influenza A virus (IAV) H1N1 in the context of immune system activation, which includes allowing interferon-stimulated genes (ISG) effector response, responsible for the first defense against viral infection [6].

SARS-CoV-2 is a large and enveloped virus with positive-sense, single-stranded RNA genome [7]. The

infection is initiated by the binding of the viral spike (S) protein to ACE2 receptor at the host cell surface (Figure 1) [8], followed by the internalization and replication of the virus, culminating in the cell lysis and the exit of newly formed viral particles [9].

Although no treatment or preventive measures against SARS-CoV-2 exist at the present moment, the scientific community is working tirelessly, producing daily results on the molecular properties of the new virus and the plethora of its interaction with the host cells and tissues.

While at the clinical level, the respiratory problems are one of the main hallmarks of the disease, the molecular alterations among the severe cases of COVID-19 include signs of hyperinflammation characteristic of immunopathologies. The most striking example is a systemic inflammatory response known as cytokine release syndrome (or cytokine storm) due to massive T cell stimulation [10].

Here, we address the major clinical features of COVID-19 and discuss its potential effects on the aged population, from the perspective of its incidence and severity, as well as long-term effects in developing age-related diseases of the central nervous system.

On the one hand, aging affects the severity of COVID-19 and, on the other, is the leading risk factor for the development of neurodegenerative diseases [11]. Although the link between the SARS-CoV-2 and neurodegeneration has yet to be established, the cocktail of infection stress, chronic inflammation, and advanced chronological age may cause multiple detrimental unforeseen consequences to the risk and severity of neurodegenerative diseases. Therefore, it needs to be seriously considered so that we can be prepared to deal with future outcomes of the ongoing pandemic.

Clinical aspects of SARS-CoV-2 infection

The clinical spectrum associated with SARS-CoV-2 infection varies among the infected population depending on the time point of the diagnosis. At the moment of seeking medical attention, the most common symptoms are fever (>37.4°C), fatigue, dry cough, myalgia, and dyspnea [12]. The reduced ability to smell, or hyposmia, has been characterized as a major symptom in otherwise mild cases [13]. The other typical symptoms associated with a common viral upper respiratory infection, such as nasal congestion and rhinorrhea, are very uncommon (< 5%) [14, 15].

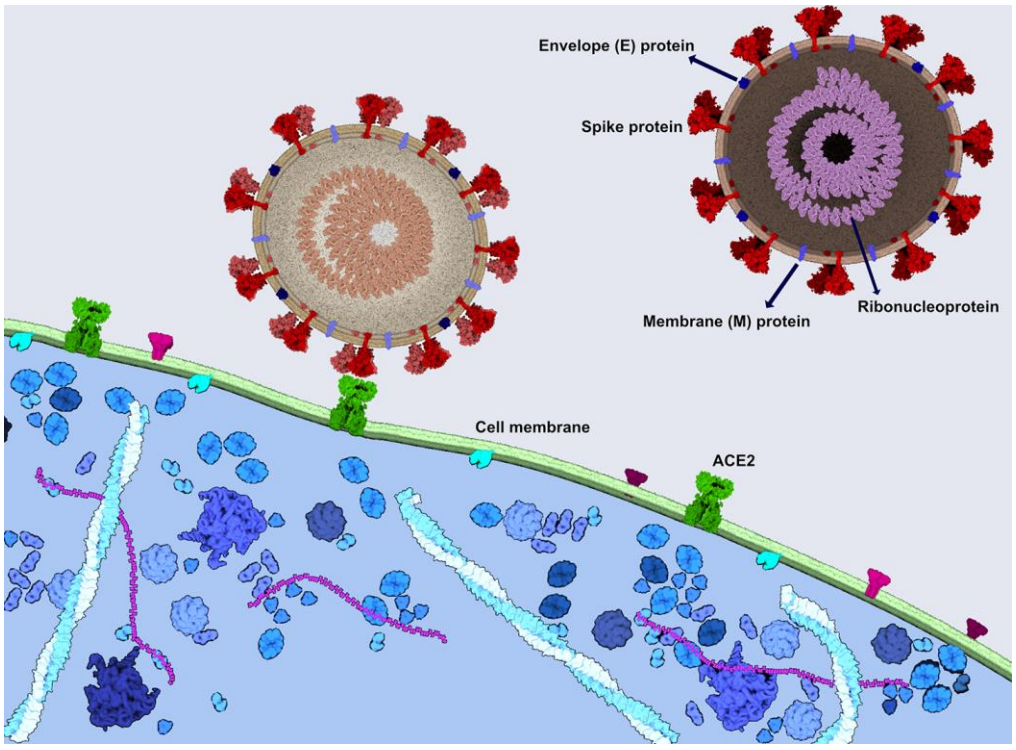

Figure 1. SARS-CoV-2 spike protein binds to the ACE2 receptor to enter the cells. Viral spike protein binds to the ACE2 receptor in the human cell membrane, followed by the internalization of the virus. SARS-CoV-2 consists also of the ribonucleoprotein, envelope protein and a membrane protein. The image was generated using CellPAINT Software [100].

The SARS-CoV-2 infection primarily affects adults, with fewer cases reported in children of 15 years or younger [15, 16]. The virus enters the host through the upper airway, and the viral load peaks at approximately day ten after the onset of symptoms [17]. The highest spread during the initial phase of the epidemic in Wuhan was observed as a human-to-human transmission among otolaryngologists [18]. Subsequent studies conducted on infected patients demonstrated high SARS-CoV-2 titers in the mucosa of the nasal and oral cavity [19], which represents the way SARS-CoV-2 enters the host, most readily transmitted by respiratory droplets and direct contact. The asymptomatic form of transmission may have contributed to the rapid spread of the disease [12], but there is still no scientific consensus regarding this mechanism [20–22].

A significant portion of patients infected with SARS-CoV-2 also shows neurological symptoms such as headache, nausea, and vomiting (<5%). Other described neurologic manifestations associated with SARS-CoV-2 infections are impaired consciousness and cerebro-vascular disease [15, 23]. The first case of meningitis/ encephalitis associated with SARS-CoV-2 infection was also recently reported [24].

SARS-CoV, a closely related virus, enters into human host cells mediated mainly by the angiotensin-converting enzyme 2 (ACE2) receptor, expressed in human airway epithelia and lung parenchyma, but also present in vascular endothelial cells, kidney cells, cells from the small intestine, and the brain (Figure 1) [25, 26]. Usually located on type I and II alveolar cells in the lung, the ACE2 receptor was also found to bind SARS-CoV-2 with an estimated binding affinity 10-20 times greater than the one of SARS-CoV [27]. The mechanism of entry into the host target cells, for both SARS-CoV and SARS-CoV-2, is warranted by the spike (S) protein [28, 29]. When attached to ACE2, the cellular transmembrane serine protease 2 (TMPRSS2) primes the spike protein to trigger the entry of the virus into the cell [19, 29]. Therefore, the spread of SARS-CoV-2 also depends on TMPRSS2 activity [29].

Neurotropism highlights the prerequisite of awareness towards SARS-CoV-2 entering the central nervous system. The neuroinvasive propensity of CoV has been documented for almost all of the β-CoV, including SARS-CoV [30], MERS-CoV [31], HCoV-229E [32] and HCoV-OC43 [23]. Evidence suggests that the virus might first invade peripheral nerve terminals, thus gaining access to the central nervous system via synapse-connected route [33, 34].

SARS-CoV-2: immunosenescence and increased severity among older adults

Epidemiological studies show that older adults are the most affected by this pandemic [35], rendering the chronological age a risk factor in COVID-19. Moreover, studies reveal the variable host resistance between patients from the same age groups.

Casualties in all age groups are also associated with pre-existing conditions such as reduced lung function, cardiovascular problems, and oncological disease spectrum. However, other factors might affect the outcome of patients with COVID-19 [36], such as variable genetic background and epigenetic pre-disposition. All these effectors converge at the level of immune system attenuation.

Since the beginning of the SARS-CoV-2 outbreak, parallels were made with the influenza A virus H1N1 infection, due to its contributions to the mortality of the elderly. Influenza remains a serious global health threat that impacts all countries, with 290,000-750,000 influenza-related respiratory deaths worldwide every year [37].

Senescence defines a stable growth arrest induced when cells reach the end of their replicative potential or are exposed to various stressors, such as infection. Senescent cells accumulate in aging tissues and contribute to the development of age-related disorders [38]. However, it was only in 2011 when evidence was presented showing that the clearance of senescent cells can delay aging-associated diseases [39]. This discovery confirmed senescence as a hallmark of aging.

Like other tissues, the immune system is characterized by the decline of its functions with age (immuno-senescence), reflected not only in increased cancer prevalence, autoimmune and other chronic diseases but also in greater susceptibility to infections [40]. Understood as a gradual deterioration of the immune system brought on by natural age advancement, immunosenescence originates as a disability of T Cells (CD4 as well as CD8 positive) to function correctly [41].

Senescence compromises the ability of CD4+ T cells to correctly activate, differentiate, proliferate, and respond to the H1N1 virus [42]. Aged CD4+ T cells accumulate intrinsic defects that contribute to a reduced helper function during influenza infection [43, 44]. In vivo studies conducted on senescent mice have evidenced low H1N1 influenza-specific antibody titers after influenza infection that reflects the age-related lowered immune response [44].

Viral infections are also known as stressors that can induce senescence in different cell lines. The Dengue virus can cause senescence in endothelial cells [45], and the Measles virus leads to cellular senescence in normal and cancer fibroblasts [46]. Senescent cells can play a role during viral infection by limiting the proliferation of damaged cells. In fact, these cells help to control the viral replication, while in experimental studies, senescence induction restricts the infection in mice [47]. Moreover, the NS1 protein of the avian influenza H7N9 virus can induce growth arrest and cellular senescence in Neuro2a cells [48]. Neurons infected with influenza A virus can respond to the infection by producing oxygen radicals and nitric oxide (NO) [49]. NS1 protein leads to an increased release of NO in Neuro2a cells which causes a reduced proliferation, enlarged cell morphology, an up-regulation of IL-6 and IL-8 as well as increased SA-β-gal activity, all features of senescent cells [48].

Immunosenescence offers insights into the differential resistance of young vs. old individuals, as well as men vs. women, to SARS-CoV-2 infection [50]. The depletion of B lymphocyte-driven acquired immunity is a characteristic of old age, affecting predominantly men [51]. Aging diminishes the upregulation molecules essential for T cell priming and also reduces antiviral interferon (IFN) production by alveolar macrophages and dendritic cells (DCs) [52].

In summary, impairment in number, function, and activation of cells involved in the immune response [53–55] and aging of hematopoietic stem cells [56] are major phenotypes of the immune system associated with immunosenescence (Figure 2). Ultimately, these changes lead to a process termed "inflammaging," where low-grade inflammation is present at an advanced age and is associated with a worsening of chronic progressive medical conditions, such as congestive heart failure [57], and the onset of age-related diseases involving the central nervous system (e.g., Alzheimer's disease) [58]. When the age-associated inflammation persists in the long-term, it may lead to oxidative stress in various tissues, while also triggering organelle dysfunction (e.g., mitochondrial and lysosomal), which could, in turn, increase the cell vulnerability to infection.

Inflammaging: an ally of SARS-CoV-2

An age-related decline in cellular repair mechanisms causes accumulation of damage at genome and proteome levels. This can lead to systemic changes in the immune system and increase pro-inflammatory cytokine production (interferon, interleukin, etc.), resulting in inflammaging [57]. The increase in cytokine production originates from the tissue macrophages, which initiate and regulate the inflammation [59]. Macrophages may, therefore, play significant roles in inflammaging. Some of the cellular hallmarks of aging, such as deregulated nutrient signaling and mitochondrial dysfunction, are also implicated in inflammaging, thus promoting the inflammatory environment [60].

Macrophages are also affected by aging, characterized mainly by the reduced potential for phagocytosis, and a decline in the gut barrier function [61]. Alveolar macrophages (AM) maintain lung homeostasis and play an important role in the influenza infection [62]. In particular, aged AM have a reduced power to control lung damage during influenza infection. During the progress of aging, the number of AM is reduced, leading to a lowered ability for phagocytosis [63]. Previous studies have also shown a decline of innate immune receptor functions and a substantial increase in viral replication efficiency after influenza infection in aged or senescent cells [64]. While the detailed mechanisms remain to be further studied, a reduction of the interferon (IFN) response in senescent cells after

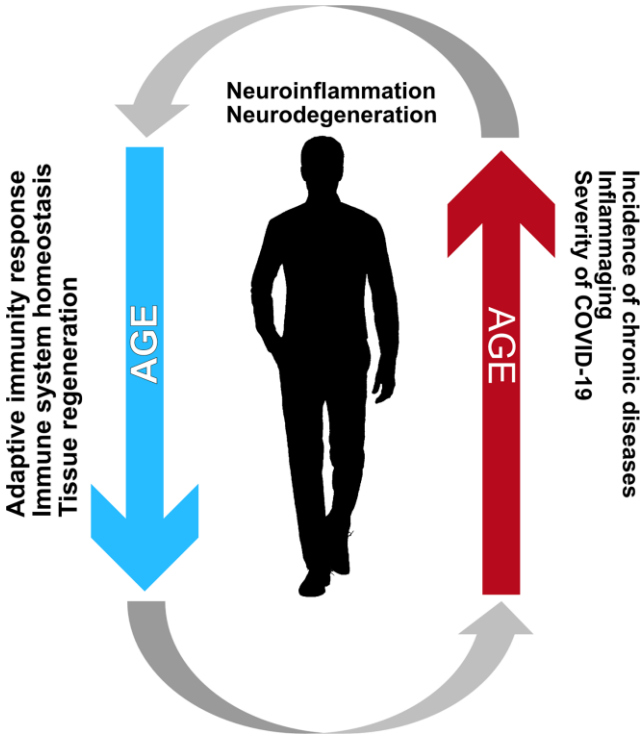

Figure 2. Immunosencescence and inflammaging create a vicious cycle creating an environment favorable for the development of neurodegenerative diseases. Such a relationship between these processes is mainly characteristic of the elderly and is the most likely reason for the increased incidence and adversity of COVID-19 among the elderly.

viral infection may play an important role. Moreover, a significant decrease in percentages and numbers of CD8+ T cells specific for at least one of the dominant epitopes of the influenza virus (influenza A nucleoprotein, NP, epitope) is typical for aged mice [65].

Pro-inflammatory cytokines play an important role in aging processes. The activation and the high levels of inflammatory cytokines such as IL-1, IL-6, TNF, and IFN-gamma are linked with morbidity and mortality in older patients [66]. In particular, IL-6 is a multi-functional cytokine produced in response to tissue damage and infections by multiple cell types [67]. Previous studies demonstrate its critical role in promoting lung tissue inflammation [68] and stimulating viral replication [69]. Moreover, elevated IL-6 is correlated with respiratory failure [10], and high concentrations of IL-6 in the serum is considered one of the hallmarks of severe MERS-CoV infections [70]. Additionally, an increase of IL-6 levels predicts adverse outcomes of COVID-19, underscoring inflammaging as the main ally of SARS-CoV-2 [35, 71]. Moreover, a recent study investigated the occurrence of cytokine storm in COVID-19 patients, also focusing on immunological characteristics of the response to COVID-19. In both mild and severe cases of COVID-19, increased levels of IL-6 are typical, while this is not the case among asymptomatic patients [10].

Inflammaging is also consistent with the gender bias of SARS-CoV-2. The more robust age-dependent activation of the innate pro-inflammatory pathways in COVID-19 is demonstrated in men compared to women [51], which is consistent with a higher rate of inflammaging among men [72]. A different situation among centenarians lends further support to the inflammaging importance for COVID-19 progression. Distinct longevity traits characterize centenarians, anti-inflammatory markers being the most prominent example, likely protecting them against the adverse outcomes of sustained inflammation as well as from the most severe forms of COVID-19 [73, 74].

Another critical factor is the impact of senescence in the lungs. Although COVID-19 shows symptoms across the entire body, the most prominent symptoms are respiratory and those associated with respiratory illness. The lung function tends to decrease with age having decreased alveolar elasticity [75], and increased senescence of epithelial cells and fibroblasts render cells frail to injuries such as the one caused by age-associated inflammation and viral infection [76]. Resident immune cells, most notably neutrophils, are also present in the lungs and are subject to immunosenescence. These cells become less functional due to age-associated chronic exposure to inflammatory cytokines [77], ultimately leading to fibrosis and aberrant tissue regeneration. The senescence phenotype, however, can be controlled by external factors, such as smoking [78], thus increasing the pool variability found in patients from the same age. In summary, the literature reviewed above may hold the key as to why the combination of immunosenescence and inflammaging does not allow an efficient response to the invasion of SARS-CoV-2 and why older individuals with co-morbidity are more prone to adverse outcomes of COVID-19 [79].

Diminished immune functions characterize immunosenescence, and inflammaging leads to a lack of anti-inflammatory modulators. The existing evidence suggests that inflammaging and immunosenescence, taken together, have vital roles in the decline of immune system functions to fight SARS-CoV-2 infection and lead to severe COVID-19 in older subjects (Figure 2).

SARS-CoV-2: a possible tipping point for inflammaging and neurodegeneration

Aging is the most significant risk factor for the development of neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease, or amyotrophic lateral sclerosis (ALS). In PD, inflammation in the central nervous system (CNS), i.e., neuroinflammation, plays a vital role in the severity of the pathogenesis and is considered a key player in nigral cell loss [80].

Neuroinflammation is mainly regulated by glial cells, such as microglia and astrocytes. Microglia are considered the resident macrophages of the brain, therefore representing the first line of immune defense in the CNS. Moreover, they perform clearance of the metabolic waste, damaged cells, and pathogens, thus regulating both the pro-inflammatory and anti-inflammatory response [81]. During pathogenesis, microglia become activated due to cellular damage and the presence of protein aggregates in their surroundings, triggering the production of chemokines and cytokines such as TNF-α, IL-6, IL-1β, IFN-γ and CCL2 [82]. The resulting oxidative stress amplifies the damage to cellular components and further activates neighboring glial cells, thus causing a chronic activation [83]. Moreover, recent studies show that microglia can play a crucial role in defense of olfactory neuronal cells against viral infection [84]. Although data regarding the role of chemokines in SARS-CoV-2 infection is still scarce, it is known that infected epithelial cells upregulate genes encoding multiple chemokines such as CXCL1, CXCL3, CXCL6, CXCL16, and CXCL1. This increases the immune activation and recruitment of immune cells to the infected tissue, thus representing a potential therapeutic target [85].

It has been long established that peripheral inflammation associated with chronic diseases increases the production of cytokines, in particular IL-1β, in the CNS [86]. However, viral infections, such as with H1N1, can cause microglial activation [87]. This, in turn, increases the risk of developing diseases such as PD [88] and may trigger protein aggregation [89]. Another pointer towards neuro-immune crosstalk in neurodegeneration is the fact that nonsteroidal anti-inflammatory drugs also show a protective effect in the case of neurodegenerative diseases [90].

A milestone in the research on mechanisms of neuro-immune crosstalk was the discovery of the brain meningeal lymphatic system that clears proteins and metabolic waste from the cerebrospinal fluid (CSF) [91]. During aging, the lymphatic system becomes impaired due to a reduction in the lymphatic vessel diameter and leads to an increase in waste accumulation in the brain [92]. Such CNS-derived antigens contribute to the neuroinflammatory conditions, and their clearance is essential to counter the inflammation [91]. It is possible that due to peripheral inflammation, not only blood-borne cytokines can enter the brain, causing the detrimental neuroinflammatory effects, but also the immune cells present in the lymphatic system, exposing the brain to a vicious circle increasing its vulnerability to additional injuries.

The available literature on SARS-CoV-2 suggests that the virus may enter the nervous system via the lymphatic circulation [93]. SARS-CoV-2 can infect lymph endothelial cells [94] and, therefore, may use the paranasal lymph vessels to reach the brain. The presence of the virus was confirmed in the neuronal and capillary cells in the frontal lobe of the COVID-19 patients [95], associated with a worsening of neurological symptoms. The convergence of viral load in the nervous system and its relationship with brain lymphatics and microglial reaction against the virus may explain why some patients have prominent neurological symptoms, while others do not appear to experience these at all.

Aging triggers debilitating conditions, such as systemic low-grade inflammation and neurodegeneration. Such conditions can be set off or aggravated by viral infections, as evidenced by the H1N1 infection shown to contribute to PD development. The severity of SARS-CoV-2 infection indicates not only an overwhelming response of the immune system, but the presence of neurological symptoms suggests the connection with the CNS.

Severe neurological symptoms associated with COVID-19 have become increasingly noticeable after SARS-CoV-2 has been detected in the CSF of some patients [24]. A growing number of cases show neurological manifestations in COVID-19 patients, including examples of cerebrovascular disease, Guillain-Barré syndrome, encephalitis, and necrotizing encephalopathy [96]. The neurological symptoms appear in proportion with the severity of SARS-CoV-2 infection: patients with severe cases of COVID-19 show neurological manifestations (45.5%) with a higher incidence relative to the mild cases [97, 98]. The overall number of patients who displayed neurological symptoms is still low compared to respiratory manifestations. Still, the continuing pandemic and the data collected so far predict an increase in the number of neurological diseases that should not be underestimated [98]. It has also been proposed that SARS-CoV-2 infection may disrupt cellular homeostasis, ultimately leading to protein misfolding and, this way, increasing the propensity for the future development of neurodegenerative diseases [99].

This relationship calls for caution and extensive research related to the development of neuro-inflammation and neurodegenerative diseases among COVID-19 survivors.

CONCLUDING REMARKS

Our understanding of COVID-19 is growing by the day due to the increasing amount of clinical data and laboratory studies. The most prominent symptoms are associated with the tissues expressing the ACE2 receptor (airway epithelia and lung parenchyma). Still, the presence of neurological symptoms draws attention to the potential interaction of COVID-19 with the CNS.

Older people and people with co-morbidities are more prone to display severe symptoms of COVID-19 due to cellular senescence in the affected tissues and the immune system. Therefore, in the elderly, SARS-CoV-2 'preys' on the tissue debility and the deficiency of the immune system. The knowledge of immunosenescence and inflammaging provides a potential interpretation of epidemiological data underscoring the elderly as the population most sensitive to COVID-19.

Peripheral inflammation associated with aging and chronic diseases increases the production of cytokines also in the CNS. Similar effects can be triggered by viral infection via microglia activation, promoting protein aggregation, and, in turn, increasing the risk of developing neurodegenerative diseases [99]. Therefore, understanding the triangle between SARS-CoV2, immunosenescence, and inflammaging may shed important light on the molecular underpinnings of COVID-19, and open novel avenues for therapeutic

interventions. These are desperately needed so that our lives can return to the 'normality' we used to know before this pandemic.

AUTHOR CONTRIBUTIONS

All authors contributed in conceiving, discussion and writing of this manuscript.

CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

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