Original Article

Liver Dysfunction and Its Association with the Risk of Death in COVID-19 Patients: A Prospective Cohort Study

Lin Fu[#1,2], Jun Fei[#1], Shen Xu[#1], Hui-Xian Xiang[1], Ying Xiang[1], Biao Hu[1], Meng-Die Li[1], Fang-Fang Liu[1], Ying Li[3], Xiu-Yong Li[4], Hui Zhao*[1] and De-Xiang Xu*[2]

[1]*Second Affiliated Hospital, Anhui Medical University, Hefei, Anhui, China;* [2]*School of Public Health, Anhui Medical University, Hefei, Anhui, China;* [3]*Union Hospital, Huazhong University of Science and Technology, Wuhan, Hubei, China;* [4]*The Second People's Hospital of Fuyang City, Fuyang, Anhui, China*

Abstract

Background and Aims: Coronavirus disease 2019 (COVID-19) is a new respiratory infectious disease caused by severe acute respiratory syndrome coronavirus-2 (commonly known as SARS-CoV-2) with multiple organ injuries. The aim of this study was to analyze COVID-19-associated liver dysfunction (LD), its association with the risk of death and prognosis after discharge. ***Methods:*** Three-hundred and fifty-five COVID-19 patients were recruited. Clinical data were collected from electronic medical records. LD was evaluated and its prognosis was tracked. The association between LD and the risk of death was analyzed. ***Results:*** Of the 355 COVID-19 patients, 211 had mild disease, 88 had severe disease, and 51 had critically ill disease. On admission, 223 (62.8%) patients presented with hypoproteinemia, 151(42.5%) with cholestasis, and 101 (28.5%) with hepatocellular injury. As expected, LD was more common in critically ill patients. By multivariate logistic regression, male sex, older age and lymphopenia were three important independent risk factors predicting LD among COVID-19 patients. Risk of death analysis showed that the fatality rate was higher in patients with hypoproteinemia than in those without hypoproteinemia (relative risk=9.471, $p<0.01$). Moreover, the fatality rate was higher in patients with cholestasis than those without cholestasis (relative risk=2.182, $p<0.05$). Follow-up observation found that more than one hepatic functional index of two-third patients remained abnormal at 14 days after discharge. ***Conclusions:*** LD at early disease stage elevates the risk of death of COVID-19 patients. COVID-19-associated LD does not recover completely by 14 days after discharge.

Citation of this article: Fu L, Fei J, Xu S, Xiang HX, Xiang Y, Hu B, *et al.* Liver dysfunction and its association with the risk of death in COVID-19 patients: A prospective cohort study. J Clin Transl Hepatol 2020;8(3):246–254. doi: 10.14218/JCTH.2020.00043.

Keywords: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2); Coronavirus disease 2019 (COVID-19); liver dysfunction (LD); Hepatocellular; Hypoproteinemia.

Abbreviations: ACE2, angiotensin-converting enzyme 2; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; COVID-19, coronavirus disease 2019; DBIL, direct bilirubin; γ-GGT, gamma-glutamyl transferase; IBIL, indirect bilirubin; LD, liver dysfunction; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TBA, total bile acid; TBIL, total bilirubin.

Received: 10 May 2020; Revised: 11 June 2020; Accepted: 4 August 2020

[#]These authors contributed equally to this work.

***Correspondence to:** De-Xiang Xu and Hui Zhao, Anhui Medical University, 81 Meishan Road, Hefei, Anhui 230032, China. Tel: +86-551-65167923, E-mails: xudex@126.com (DXX), zhaohuichenxi@126.com (HZ)

Introduction

In December 2019, a novel coronavirus-induced respiratory infectious disease was found in Wuhan city of central China.[1] This novel coronavirus was named as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. SARS-CoV-2-induced disease was named as coronavirus disease 2019 (COVID-19) by the World Health Organization. COVID-19 has since become a worldwide pandemic.[2] As of 31 March, 2020, a total of 700,000 people had been infected with SARS-CoV-2 and more than 33,000 patients had died from COVID-19 all over the world.[1]

The study has found SARS-CoV-2 mainly causes infection in humans through droplets or direct contact.[3] Recent studies have shown that the virus is also present in stool. The duration of viral shedding from the feces after negative conversion in pharyngeal swabs was 7 (6-10) days, regardless of COVID-19 severity. However, the transmission infectivity through feces may be less.[4] Recently, angiotensin-converting enzyme 2 (ACE2), expressed mainly in pulmonary epithelial cells and upper respiratory tract epithelium cells, was identified as a receptor of the virus. Moreover, conjunctiva, cardiomyocytes and renal tubular epithelial cells have also been identified as functional receptors for SARS-CoV-2.[5–8] The main symptoms and signs of COVID-19 patients include fever, accompanied by dry cough, dyspnea, diarrhea, fatigue, and lymphopenia.[9–14] While only a few cases of mild COVID-19 patients have died, the risk of death was found to be increased among critically ill patients with intensive care unit admission.[15]

A large number of clinical data have revealed that infection with SARS-CoV-2 not only caused severe acute respiratory syndrome but also multiple organ injuries, including lymphocyte reduction, myocardial dysfunction, and even acute renal failure.[16–18] Previous research found abnormal liver enzymes in patients with COVID-19.[19] Liver injury was more popular in the critically ill COVID-19 patients with intensive care unit admission.[20] Nevertheless, the clinical characteristics of COVID-19-associated liver dysfunction (LD) are rarely described. The prognosis of LD of COVID-19 patients remains unknown. Therefore, the clinical significance of COVID-19-associated LD and its prognosis need to be clarified further.

The objective of the present study was to analyze COVID-19-associated LD, its association with the risk of death and prognosis after discharge. This prospective cohort study from two different regions was performed among 355 COVID-19 patients. LD was compared among different severity of COVID-19 patients. The effects of demographic characteristics and complications on hepatic function were analyzed. The association between LD and the risk of death among COVID-19 patients was evaluated and the prognosis after discharge was tracked.

Methods

Study design and participants

In the present study, 220 patients with confirmed COVID-19 were recruited from Union Hospital of Huazhong University of Science and Technology in Wuhan city. Another 155 patients, who were confirmed to be infected with SARS-CoV-2 were recruited from the Second People's Hospital of Fuyang City in Anhui province. The Second Affiliated Hospital of Anhui Medical University sent a medical team to the Union Hospital of Huazhong University of Science and Technology to care for COVID-19 patients. The Second Affiliated Hospital of Anhui Medical University sent a specialist group to the Second People's Hospital of Fuyang City to guide COVID-19 treatment. All patients were initially detected with real-time RT-PCR assay for SARS-CoV-2 RNA, and the genetic sequence that matched COVID-19 was analyzed and used to confirm infection with SARS-CoV-2. Ten patients with negative COVID-19 detection results and ten patients with incomplete information were excluded in the Union Hospital of Huazhong University of Science and Technology. Ultimately, a total 355 COVID-19 patients were analyzed for this work (Fig. 1).

All COVID-19 patients were clinically diagnosed on the basis of typical clinical manifestations, accompanied by characteristic chest radiology changes. All COVID-19 inpatients were recruited on admission. Demographic and clinical characteristics were collected. Finally, 150 cured patients attended follow-up examination on 14 days after discharge in the Second People's Hospital of Fuyang City; biochemical indexes and routine blood test parameters were again detected.

The present study was approved by the Ethics Committee of Anhui Medical University (2020-5). All COVID-19 patients were eligible for this study. Oral consent was gained from patients or patients' next of kin.

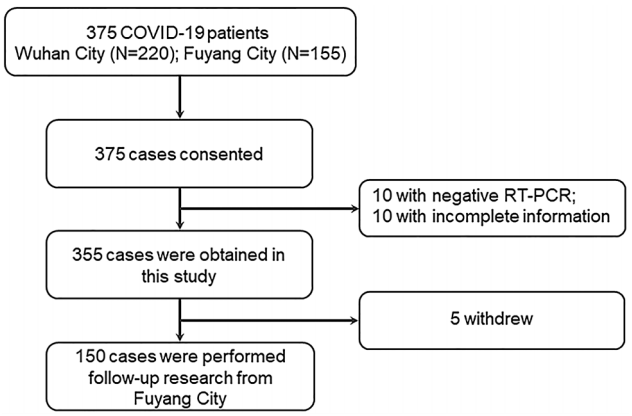

Fig. 1. Flow diagram of recruitment and follow-up research in this cohort study.

Data collection

The medical record of each COVID-19 patient was evaluated on admission. The following data were collected from the electronic medical records: demographic information, preexisting comorbidities, including chronic obstructive pulmonary disease, hepatic disease, cardiovascular disease, hypertension, diabetes mellitus and other disease. Patient's signs and symptoms, and laboratory test results were also collected. The dates of onset, admission and death were recorded. The onset time was defined as the date when any symptom and sign were found.

Laboratory testing

Each patient's pharyngeal swab specimens were subjected to extraction of SARS-CoV-2 RNA by real-time RT-PCR, to detect viral nucleic acid using a COVID-19 nucleic acid detection kit following experimental instructions (Shanghai BioGerm Medical Technology Co Ltd., Shanghai, China). Viral RNA extraction and nucleic acid detection were executed by either the Center for Disease Control and Prevention of Wuhan City or the Center for Disease Control and Prevention of Fuyang City. Hepatic function indexes included: alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT ratio, total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL),total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GGT), total bile acid (TBA), and oxygenation index were examined on admission. All laboratory tests were analyzed by the clinical laboratory of either the Union Hospital of Huazhong University of Science and Technology or the Second People's Hospital of Fuyang City. Moreover, the follow-up study was performed on 14 days after discharge in the Second People's Hospital of Fuyang City. Liver function and chest CT scan were measured among 150 COVID-19 patients.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 software (IBM Corp., Armonk, NY, USA). Categorical variables were expressed with frequencies and percentages. Continuous variables were shown using median. The difference among different severity COVID-19 patients was compared using nonparametric test. Proportions for categorical variables were compared with the chi-square and Fisher's exact tests. Univariable logistic regression modeling between basic disease or different parameters and demise was performed. Moreover, the main risks related with demise were examined using multivariable logistic regression models adjusted for potential confounders. Statistical significance was determined at $p \leq 0.05$.

Results

Demographic and clinical characteristics of COVID-19 patients

The clinical characteristics of 355 COVID-19 patients were analyzed. As shown in Table 1, mild cases, defined as

oxygenation index higher than 300, accounted for 60.3%. Severe cases, whose oxygenation index was from 200 to 300, accounted for 25.1%. Critically ill cases, whose oxygenation index was lower than 200, accounted for 14.6% (Table 1). Five patients' information of the severity was missed. The demographic characteristics of 350 COVID-19 patients were then analyzed. Of the 350 COVID-19 patients, male patients accounted for 53.5% and female patients accounted for 46.5% (Table 2). There were 103 patients younger than 40 years-old, 137 cases aged between 40 and 60 years, and 115 patients older than 60 years-old (Table 2). As shown in Table 2, 145 patients presented with diabetes mellitus, 125 with hypertension, and 16 with hepatic diseases. Finally, blood lymphocytes were analyzed among COVID-19 patients; as shown in Table 2, 61.1% (217/355) patients presented with lymphopenia.

Association between the severity and LD among COVID-19 patients

The association between the severity and LD was analyzed among COVID-19 patients. LD was defined as any of hypoproteinemia markers (albumin and globulin), cholestasis markers (ALP, γ-GGT and TBA) or hepatocellular injury markers (DBIL, IBIL and ALT) outside the normal range. As shown in Table 1, all hepatocellular injury markers, including TBIL, DBIL, IBIL, ALT and AST, were higher in critically ill patients than those of mild cases. By contrast, total protein, albumin and albumin/globulin ratio, three markers of hypoproteinemia, were lower in critically ill patients than those of mild cases. Despite no difference in serum TBA, ALP and γ-GGT, two markers of cholestasis, was observed in critically ill and mild patients. They were higher in critically ill patients than those of mild cases. The association between oxygenation index and hepatic functional indexes was analyzed. As shown in Fig. 2, there was a weakly negative correlation between oxygenation index with ALT, AST, AST/ALT and γ-GGT. By contrast, oxygenation index was weakly and positively correlated with albumin among COVID-19 patients (Fig. 2).

Male elderly COVID-19 patients with lymphopenia are more susceptible to LD

The effects of demographic characteristics on hepatic functional indexes were analyzed. As shown in Table 2, the levels of DBIL, IBIL, ALT, ALP and γ-GGT were higher in males than in females. By contrast, the level of albumin was lower in males than in females. Further analysis showed that the levels of TBIL, ALT, ALP and γ-GGT were higher in patients older than 60 years-old than those of younger patients. By contrast, the level of albumin was lower in patients older than 60 years-old than those of younger patients (Table 2).

The effect of comorbidity on hepatic functional indexes was then analyzed. As shown in Table 2, ALP was slightly increased in COVID-19 patients with hypertension as compared with those without hypertension. Further analysis showed that the levels of ALP and γ-GGT were weakly increased in COVID-19 patients with diabetes as compared with those without diabetes. By contrast, the level of albumin was slightly decreased in COVID-19 patients with diabetes. Of interest, there was no significant association between hepatic functional indexes and comorbidity with hepatic disease (Table 2). Moreover, the association between blood lymphocytes and LD was analyzed among COVID-19 patients. As shown in Table 2, the levels of ALT and γ-GGT were higher in COVID-19 patients with lymphopenia than those without lymphopenia. By contrast, the levels of albumin and globulin were lower in COVID-19 patients with lymphopenia than those without lymphopenia (Table 2).

Table 1. Association between the severity and hepatic function markers among COVID-19 patients

	Mild patients (*n*=211)	Severe patients (*n*=88)	Critically ill patients (*n*=51)
Hepatocellular injury markers			
TBIL (μmol/L)	10.4 (7.5, 14.7)	10.9 (8.0, 16.2)	12.6 (10.5, 17.0)**¶
DBIL (μmol/L)	3.4 (2.6, 4.9)	3.6 (2.4, 5.3)	4.3 (3.0, 6.1)*
IBIL (μmol/L)	7.0 (4.7, 10.0)	7.6 (5.5, 11.0)	8.3 (6.2, 10.8)**
ALT (U/L)	22.0 (14.0, 35.0)	23.0 (15.0, 42.0)	33.0 (19.0, 61.0)**
AST (U/L)	26.0 (20.0, 35.0)	29.0 (23.0, 54.0)**	49.0 (35.0, 80.0)**¶¶
AST/ALT ratio	1.2 (0.9, 1.7)	1.3 (1.0, 1.8)	1.6 (1.1, 2.2)**¶
Hypoproteinemia markers			
Total protein (g/L)	69.9 (64.8, 74.8)	65.0 (59.1, 70.1)**	61.0 (58.8, 65.3)**¶¶
Albumin (g/L)	40.5 (36.8, 44.0)	37.3 (33.7, 40.0)**	34.5 (31.7, 37.4)**¶¶
Globulin (g/L)	29.4 (25.9, 32.9)	27.9 (23.8, 31.4)*	27.3 (23.6, 30.1)
Albumin/globulin ratio	1.4 (1.2, 1.5)	1.3 (1.2, 1.7)	1.2 (1.0, 1.5) **¶
Cholestasis markers			
ALP (U/L)	62.0 (51.0, 77.0)	62.0 (51.0, 71.0)	71.5 (57.8, 102.0)**¶¶
γ-GGT (U/L)	24.0 (15.0, 41.0)	26.0 (18.0, 51.8)	42.5 (26.5, 93.2)**¶¶
TBA (μmol/L)	4.8 (3.0, 7.0)	4.0 (2.5, 7.7)	4.0 (2.2, 5.8)

Five patients' information of the severity was missed. Compared with mild patients, *p<0.05, **p<0.01; Compared with severe patients, ¶p<0.05, ¶¶p<0.01.

Table 2. Effects of demographic characteristics and complications on hepatic function among COVID-19 patients

	Cases, *n*	DBIL (μmol/L)	IBIL (μmol/L)	ALT (U/L)	Albumin (g/L)	Globulin (g/L)	ALP (U/L)	γ-GGT (U/L)	TBA (μmol/L)
Gender									
Male	190	4.2 (3.1, 5.9)	8.1 (5.9, 11.6)	29.0 (18.0, 49.8)	39.0 (35.1, 43.1)	28.3 (24.7, 32.0)	64.0 (53.0, 78.0)	33.0 (21.0, 70.0)	4.8 (3.0, 7.4)
Female	165	2.9 (2.2, 4.1)**	6.5 (4.4, 8.7)**	19.0 (13.0, 28.0)**	38.6 (34.3, 41.3)**	28.7 (25.7, 32.5)	61.0 (49.0, 77.0)**	20.0 (13.0, 35.3)**	4.0 (2.8, 6.9)
Age (year)									
<40	103	3.4 (2.6, 4.6)	6.2 (4.2, 9.3)	12.0 (13.0, 38.0)	42.0 (38.8, 45.8)	29.6 (26.3, 32.9)	58.0 (47.3, 69.8)	20.5 (11.0, 36.0)	4.8 (3.3, 6.9)
41-59	137	3.6 (2.4, 5.4)	7.6 (5.5, 10.6)	24.0 (16.0, 42.0)**	38.8 (35.5, 41.8)**	28.8 (25.2, 32.3)	62.0 (52.0, 75.0)*	30.0 (19.0, 69.0)**	4.8 (3.0, 8.0)
>60	115	4.0 2.7, 5.4)**	8.3 (6.5, 11.3)**	25.0 (17.5, 35.0)**	35.3 (32.3, 39.5)**¶¶	27.1 (23.2, 31.4)**	67.0 (55.0, 80.0)**	28.0 (18.0, 48.7)**	3.9 (2.0, 5.9)*¶
Hypertension									
Yes	125	3.6 (2.6, 5.0)	7.8 (5.8, 10.3)	24.0 (14.0, 42.0)	37.6 (33.4, 40.4)	27.2 (24.1, 30.3)	63.5 (54.0, 82.8)	28.0 (18.0, 63.0)	4.2 (2.6, 7.0)
No	230	3.6 (2.6, 5.0)	7.3 (4.8, 10.6)	23.0 (16.0, 38.0)	39.8 (35.7, 43.3)**	29.2 (25.5, 32.8)**	62.0 (50.5, 75.0)*	26.0 (16.3, 46.0)	4.4 (2.9, 7.0)
Diabetes									
Yes	145	11.4 (8.2, 15.6)	3.5 (2.2, 5.1)	22.5 (15.0, 42.0)	37.3 (33.3, 40.6)	26.5 (22.9, 29.4)	65.0 (54.0, 86.0)	28.5 (19.0, 63.8)	4.4 (2.5, 7.0)
No	210	10.7 (7.8, 15.1)	3.7 (2.7, 5.0)	23.0 (15.0, 38.0)	39.9 (36.2, 43.5)**	30.0 (26.9, 33.3)**	61.5 (50.0, 72.3)**	26.0 (16.0, 42.3)*	4.4 (3.0, 7.0)
Hepatic diseases									
Yes	16	3.4 (2.4, 11.9)	6.2 (4.2, 15.4)	24.5 (17.8, 49.5)	36.7 (27.2, 40.3)	28.1 (24.2, 31.1)	63.0 (53.0, 73.3)	24.5 (16.0, 35.0)	4.9 (2.5, 8.5)
No	339	3.6 (2.6, 5.0)	7.5 (5.3, 10.4)	23.0 (15.0, 39.3)	38.8 (34.8, 42.4)	28.7 (25.0, 32.3)	62.0 (51.0, 78.0)	27.0 (17.0, 50.0)	4.3 (2.8, 7.0)
Lymphopenia									
Yes	217	3.7 (2.4, 5.3)	7.2 (5.0, 10.6)	25.0 (16.0, 45.0)	37.6 (34.1, 40.7)	27.9 (24.1, 31.8)	63.0 (51.0, 79.0)	30.0 (18.0, 68.0)	4.7 (3.0, 7.5)
No	138	3.4 (2.7, 4.3)	7.8 (5.2, 10.3)	21.0 (13.0, 33.5)**	41.2 (37.5, 44.8)**	29.3 (26.1, 32.7)**	62.0 (52.5, 72.5)	25.0 (15.5, 38.5)*	4.2 (2.7, 6.6)

Cases in Sex, compared with "Male" group, *p<0.05, **p<0.01.

Cases in Age, compared with "<40" group, *p<0.05, **p<0.01; Compared with "41-59" group, ¶p<0.05, ¶¶p<0.01.

Cases in Hypertension, compared with "Yes" group, *p<0.05, **p<0.01.

Cases in Diabetes, compared with "Yes" group, *p<0.05, **p<0.01.

Cases in Lymphopenia, compared with "Yes" group, *p<0.05, **p<0.01.

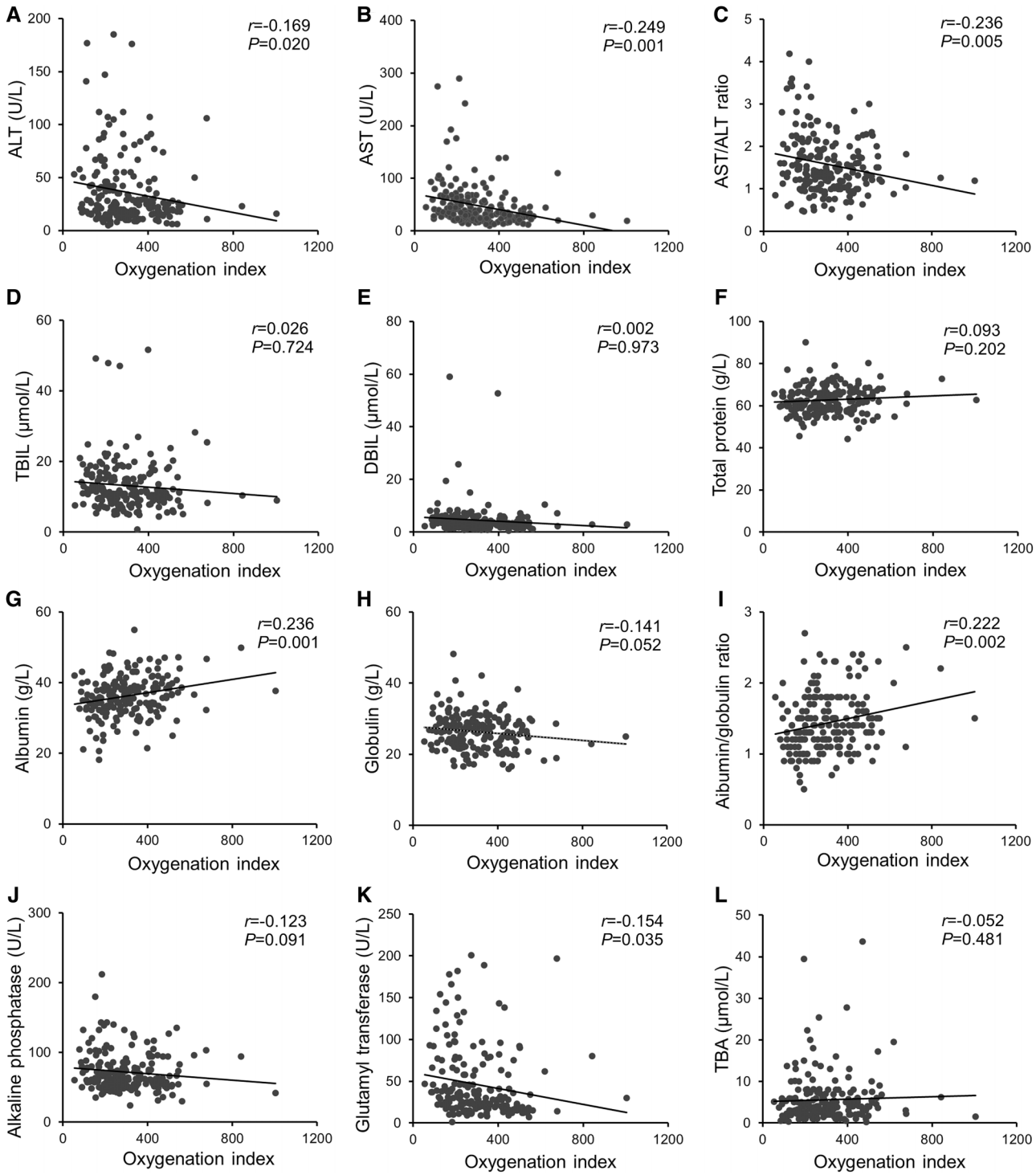

Fig. 2. Association between oxygenation index and hepatic functional indices among COVID-19 patients.

(**A-E**) Correlation between oxygenation index and hepatocellular injury indices was analyzed among COVID-19 patients. (**A**) Serum ALT; (**B**) AST; (**C**) AST/ALT ratio; (**D**) TBIL; (**E**) DBIL. (**F-I**) Correlation between oxygenation index and hypoproteinemia indices was analyzed among COVID-19 patients. (**F**) Serum total protein; (**G**) Albumin; (**H**) Globulin; (**I**) Albumin/globulin ratio. (**J-L**) Correlation between oxygenation index and cholestasis indices was analyzed among all COVID-19 patients. (**J**) Serum ALP; (**K**) γ-GGT; (**L**) TBA.

Multivariable logistic regression was used to analyze risk factors of LD among 355 COVID-19 patients. As shown in Supplementary Table 1, the odds ratios of males were 2.884 (95% confidence interval (CI): 1.812, 4.591; $p<0.001$) for cholestasis and 4.047 (95% CI: 2.367, 6.921; $p<0.001$) for hepatocellular injury, respectively. Further analysis showed that the odds ratios of older age were 1.593 (95% CI: 1.593, 1.979; $p<0.05$) for cholestasis and 3.739 (95% CI: 2.078, 6.727; $p=0.001$) for hypoproteinemia. The association between blood lymphocyte count and LD was analyzed among COVID-19 patients. The odds ratios of lymphopenia were 2.003 (95% CI: 1.241, 3.233; $p=0.004$) for cholestasis, 3.218 (95% CI: 1.969, 5.260; $p=0.001$) for hypoproteinemia, and 2.292 (95% CI: 1.329, 3.953; $p=0.003$) for hepatocellular injury (Supplementary Table 1). The association between comorbidity and LD was analyzed among COVID-19 patients. The odds ratios of diabetes were 1.727 (95% CI: 1.014, 2.941; $p=0.044$) for cholestasis and 1.829 (95% CI: 1.010, 3.312; $p=0.046$) for hypoproteinemia. No significant relationship was observed between LD and comorbidity with hypertension and hepatic disease among COVID-19 patients (Supplementary Table 1).

LD at early stage elevates the risk of death of COVID-19 patients

The effects of LD at the early stage on the risk of death are presented in Table 3. Analyses were adjusted for confounding variables using logistic regression. Age, sex and comorbidities were adjusted. Among 223 COVID-19 patients with hypoproteinemia, 14.3% died on mean day 10.9 after hospitalization. The fatality rate was higher among COVID-19 patients with hypoproteinemia than those without hypoproteinemia (14.3% vs. 1.5%; relative risk=9.471, 95% CI: 2.307, 38.880; $p<0.001$). Among the 151 COVID-19 patients with cholestasis, 13.9% died. The fatality rate was higher among COVID-19 patients with cholestasis than those without cholestasis (13.9 % vs. 6.4%; relative risk=2.182, 95% CI: 1.129, 4.218; $p<0.05$). As shown in Table 3, there was no significant association between hepatocellular injury and the risk of death among COVID-19 patients.

Hepatic functional indexes remain abnormal 14 days after discharge

The prognosis of COVID-19-associated LD was tracked 14 days after discharge. As shown in Table 4, no significant change was observed between hepatic functional indexes on 14 days after discharge and those on admission. Although the percentage of patients with hypoproteinemia was lower on 14 days after discharge than on admission (18.7% vs. 51.9%, $p<0.001$) (Supplementary Table 2), patients with levels remaining below the normal range accounted for 16.7% for albumin, 1.3% for prealbumin and 6.7% for albumin/globulin ratio (Table 4). As shown in Table 4, the percentage of patients with serum ALT elevation was higher on 14 days after discharge than on admission (31.3% vs. 19.5%, $p<0.05$). In addition, patients with serum IBIL elevation was higher on 14 days after discharge than on admission (4.5% vs. 10.0%, $p<0.05$). No significant difference on the percentages of patients with abnormal value for DBIL, TBA, ALP, γ-GGT and AST was observed on admission and 14 days after discharge (Table 4). Further analysis showed no significant difference on the percentages of patients with cholestasis [116 (75.3%) vs. 97 (64.7%)] and hepatocellular injury [60 (39.0%) vs. 53 (35.3%)] between admission and discharge, while the percentages of patients with hypoproteinemia was significantly reduced on 14 days after discharge [28 (18.7%)] as compared with on admission [80 (51.9%)] (Supplementary Table 2). In addition, there was no difference of COVID-19 patients with both cholestasis and hepatocellular injury on admission [90 (58.4%)] and discharge [86 (57.3%)] (data not shown).

Discussion

The present study aimed to analyze COVID-19-associated LD, its association with the risk of death and the prognosis after discharge. The major findings of this study include: (1) LD is more common in critically ill COVID-19 patients; (2) male elderly COVID-19 patients with diabetes and lymphopenia are more susceptible to LD; (3) LD at the early stage elevates the risk of death of COVID-19 patients; and (4) hepatic functional indexes of two-thirds of COVID-19 patients remain abnormal on 14 days after discharge.

Accumulating data demonstrated that SARS-CoV-2 infection caused multiple organ injuries, including myocardial

Table 3. The association between LD and the risk of death among COVID-19 patients

	Cases, *n*	Death, *n* (%)	Relative risk (95% CI)	*p*
Hypoproteinemia				
Yes	223	32 (14.3)	9.471 (2.307, 38.880)	<0.001
No	132	2 (1.5)	1	—
Cholestasis				
Yes	151	21 (13.9)	2.182 (1.129, 4.218)	0.017
No	204	13 (6.4)	1	—
Hepatocellular injury				
Yes	101	14 (13.9)	1.760 (0.926, 3.348)	0.084
No	254	20 (7.9)	1	—

Adjusted for age, sex and comorbidities.

Table 4. Hepatic function markers on admission and after discharge among COVID-19 patients

	On admission (*n*=154)			Discharge (*n*=150)		
Hepatic function markers	Median (Q25, Q75)	Below the range, *n* (%)	Above the range, *n* (%)	Median (Q25, Q75)	Below the range, *n* (%)	Above the range, *n* (%)
Total protein (g/L)	72.6 (68.9, 76.5)	13 (8.4)	5 (3.2)	77.5 (72.8, 80.9)**	6 (4.0)	8 (5.3)
Albumin (g/L)	41.1 (38.5, 44.1)	58 (37.7)	—	45.1 (41.4, 47.4)**	25 (16.7)¶¶	—
Globulin (g/L)	31.8 (28.7, 34.1)	—	5 (3.2)	31.9 (28.7, 34.5)	0	4 (2.7)
Albumin/globulin ratio	1.3 (1.2, 1.5)	31 (20.1)	—	1.4 (1.3, 1.5)	10 (6.7)¶¶	—
Prealbumin (mg/dL)	20.7 (14.6, 28.7)	57 (37.0)	15 (9.7)	35.3 (27.4, 39.7)**	2 (1.3)¶¶	76 (50.7)
ALP (U/L)	62.0 (49.0, 71.0)	—	12 (7.8)	65.0 (56.0, 78.0)	—	14 (9.3)
γ-GGT (U/L)	26.0 (15.0, 42.8)	—	37 (24.0)	30.0 (19.0, 53.0)*	—	48 (32.0)
TBA (μmol/L)	5.0 (3.3, 7.5)	—	20 (13.0)	5.5 (4.0, 7.8)	—	17 (11.3)
ALT (U/L)	23.0 (13.8, 37.0)	—	30 (19.5)	25.0 (15.0, 48.0)	—	47 (31.3)##
AST (U/L)	24.0 (20.0, 31.0)	—	30 (19.5)	22.0 (19.0, 30.0)	—	23 (15.3)
DBIL (μmol/L)	3.9 (2.9, 5.1)	—	8 (5.2)	3.4 (2.6, 4.2)	—	4 (2.7)
IBIL (μmol/L)	6.0 (3.6, 9.7)	—	7 (4.5)	6.8 (4.1, 8.8)*	—	15 (10.0)#

Compared with "Median values" among COVID-19 patients on admission, *p<0.05, **p<0.01; Compared with "Below the range" among COVID-19 patients on admission, ¶p<0.05, ¶¶p<0.01; Compared with "Above the range" among COVID-19 patients on admission, #p<0.05, ##p<0.01.

dysfunction, lymphopenia, and even acute renal failure.[16–18] Moreover, several studies found that SARS-CoV-2 infection was associated with abnormal liver enzymes and liver injury.[20–22] In the present study, we described LD among COVID-19 patients. LD was determined by measuring biochemical indexes, such as ALT, AST, DBIL and IBIL, several hepatocellular injury markers, total protein, albumin and globulin (three protein metabolic indexes), and ALP, TBA and γ-GGT (three markers of cholestasis). Our results showed that 39.6% COVID-19 patients presented with cholestasis, 51.9% with hypoproteinemia and 39.0% with hepatocellular injury on admission. We found that DBIL, IBIL, ALT and AST (four indexes of hepatocellular injury) and ALP and γ-GGT (two markers of cholestasis) were higher in critically ill patients than those of mild cases. By contrast, total protein and albumin were lower in the critically ill patients than those of mild cases. These results provide evidence that LD on admission is associated with the severity of COVID-19 patients.

Several studies found that elderly COVID-19 patients had more severe symptoms and signs than younger cases.[23,24] The present study analyzed the influence of sex and age on COVID-19-associated LD. We showed that the levels of serum TBIL, ALT, ALP and γ-GGT were higher in males than in females. By contrast, serum albumin level was lower in males than in females. Further analysis showed that the levels of TBIL, ALT, ALP and γ-GGT were higher in older patients than younger ones. By contrast, albumin and globulin were lower in older patients than those in younger ones. According to several clinical reports, COVID-19 patients with comorbidities had a worse prognosis.[15,25] Indeed, this study found that 40.8% COVID-19 patients presented with diabetes, 35.2% with hypertension, and 4.5% with hepatic disease. To explore the influence of comorbidities on LD, the present study analyzed hepatic functional indexes among different groups. Our results showed that ALP was slightly higher in COVID-19 patients with hypertension than those without hypertension. In addition, ALP and γ-GGT were weakly higher in patients with diabetes than those without diabetes. By contrast, albumin was slightly lower in patients with either diabetes or hypertension than those without diabetes or hypertension.

Unexpectedly, the comorbidity of hepatic disease did not influence hepatic functional indexes of COVID-19 patients. Lymphocytopenia is one of the important early manifestations during the pathogenesis of COVID-19.[26,27] This study analyzed the influence of lymphocytopenia on COVID-19-associated LD. We found that almost all hepatic functional indexes were worse among patients with lymphocytopenia than without lymphocytopenia. To exclude potential confounding factors, multivariable logistic regression was used to further analyze the impact of sex, age and comorbidities on COVID-19-associated LD. We found that male, older age, comorbidities with diabetes and lymphocytopenia were independent risk factors of cholestasis. In addition, older age, lymphocytopenia and comorbidity with diabetes were independent risk factors of hypoproteinemia. Moreover, male and lymphocytopenia were independent risk factors of hepatocellular injury. Taken together, the present study provides evidence that male sex, older age, and comorbidities of diabetes and lymphocytopenia are major risk factors of LD among COVID-19 patients.

The association between COVID-19 and liver injury is now generally accepted. However, the influence of LD on the prognosis of COVID-19 remains unclear. The present study analyzed the impact of LD on the risk of death in COVID-19 patients. Our results showed that the fatality rate was higher in COVID-19 patients with hypoproteinemia than those without hypoproteinemia. Moreover, the fatality rate was higher in COVID-19 patients with cholestasis than without cholestasis. Our results suggest that hypoproteinemia and cholestasis at the early stage elevate the risk of death of COVID-19 patients. It is especially interesting to consider whether COVID-19-associated LD recovers in a short time after discharge. In the present study, the prognosis was tracked and hepatic function was measured among 150

COVID-19 patients at 14 days after discharge. Unexpectedly, no significant difference on the values of serum DBIL, IBIL, TBA, ALP, γ-GGT and AST was observed between on admission day and 14 days after discharge. Although serum albumin level had rebounded on 14 days after discharge, the level of albumin in 17% COVID-19 patients remained below the normal range. Our results indicate that hepatic functional indexes of two-thirds of COVID-19 patients remain abnormal on 14 days after discharge. Therefore, further follow-up is required to evaluate whether SARS-CoV-2 infection causes permanent liver injury.

The mechanism of which SARS-CoV-2 evokes LD remains unclear. Accumulating data indicate that SARS-CoV-2 infection causes multiple organ injuries, such as lymphopenia, myocardial dysfunction, and acute renal failure.[10,28,29] In the present study, we showed that oxygenation index, an index of respiratory function, was positively correlated with serum albumin. By contrast, there were weakly negative correlations between oxygenation index with ALT, AST, AST/ALT, and γ-GGT among COVID-19 patients. Moreover, clinical study found that respiratory failure was associated with poor prognostic markers of liver failure.[30,31] These results suggest that respiratory failure may contribute, at least partially, to COVID-19-associated LD. Several studies demonstrated that ACE2, as a receptor for SARS-CoV-2, was expressed in cholangiocytes and hepatocytes.[32,33] Moreover, ACE2 was found to be highly expressed in cholangiocytes, which may be the reason for higher levels of serum ALP being observed among COVID-19 patients. Therefore, this study does not exclude that SARS-CoV-2 evokes LD partially through infecting liver directly. It is required to further explore whether human liver is another target organ of SARS-CoV-2 injection.

This study mainly analyzed COVID-19-associated LD, its association with the risk of death and prognosis after discharge. Nevertheless, there are several potential limitations in this research. Firstly, the cases were enrolled from only two different regions rather than from multiple centers in China, so the results may not be generalizable to COVID-19 patients from all over the world. Secondly, the degree of LD was mild in the present study. Although, LD elevated the risk of death, we still could not exclude the possibility that other potential confounders, such as cytokine storm, acute kidney injury, myocardial injury and COVID-19 itself, may induce poor outcome of COVID-19 patients. Several animal experiments may resolve this confusion. Thirdly, the current study is the result of a prospective cohort study. COVID-19-associated LD existed between the admission day and 14 days after discharge. Liver function remained abnormal all along. This research can't exclude whether cytokine storm, side effects of the drugs used during treatment, or other comorbidities induced LD, not just a sequel of COVID-19. Therefore, animal experiments could help to further confirm the results of this investigation and determine the causal relationship. Fourthly, AST is not a specific marker of LD; it also originates from cardiomyocytes. So, AST elevation cannot fully exclude the cause of myocardial injury. Fifthly, SARS-CoV-2 injection also induced acute kidney injury. Lower albumin in older patients may contribute to acute kidney injury and other comorbidities, like diabetes. Moreover, several baseline comorbidities, such as renal impairment, diabetes and malnutrition, may always induce lower albumin. Therefore, albumin decrease cannot completely contribute to LD. *In vivo* experiments can help to explain the cause of lower albumin in future research work.

In summary, this study aimed to investigate COVID-19-associated LD among 355 COVID-19 patients from two hospitals. Our results revealed that COVID-19-associated LD was more common in critically ill patients. In addition, male elderly COVID-19 patients with diabetes mellitus and lymphopenia were more susceptible to LD. We provide evidence that LD at the early stage elevates the risk of death of COVID-19 patients. Importantly, COVID-19-associated LD has not recovered completely 14 days after discharge. Therefore, it is necessary to further evaluate whether SARS-CoV-2 infection causes permanent liver injury.

Acknowledgments

We thank all members of the respiratory and critical care medicine in the Second People's Hospital of Fuyang City and Union Hospital of Huazhong University of Science and Technology for recruiting participants.

Funding

This study was supported by the National Natural Science Foundation of China (Grant Number: 81630084) and the National Natural Science Foundation Incubation Program of the Second Affiliated Hospital of Anhui Medical University (Grant Number: 2019GQFY06).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Designed the research (DXX and HZ), conducted the research (LF, JF, SX, HXX, YX, MDL, FFL, YL, and XYL), analyzed the data (LF and JF), writing the paper and having primary responsibility for final content (DXX and LF). All authors read and approved the final manuscript.

References

[1] WHO. Coronavirus disease (COVID-19) pandemic. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
[2] Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, *et al*. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis 2020;91:264–266. doi: 10.1016/j.ijid.2020.01.009.
[3] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int J Antimicrob Agents 2020;55:105924. doi: 10.1016/j.ijantimicag.2020.105924.
[4] Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, *et al*. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol 2020;92:833–840. doi: 10.1002/jmv.25825.
[5] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, *et al*. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–280.e8. doi: 10.1016/j.cell.2020.02.052.
[6] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281–292.e6. doi: 10.1016/j.cell.2020.02.058.
[7] Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020;367:1444–1448. doi: 10.1126/science.abb2762.
[8] Lange C, Wolf J, Auw-Haedrich C, Schlecht A, Boneva S, Lapp T, *et al*. Expression of the COVID-19 receptor ACE2 in the human conjunctiva. J Med Virol 2020. doi: 10.1002/jmv.25981.

[9] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507–513. doi: 10.1016/S0140-6736(20)30211-7.
[10] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061–1069. doi: 10.1001/jama.2020.1585.
[11] Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, *et al*. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy 2020;75:1730–1741. doi: 10.1111/all.14238.
[12] Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ. 2020;368:m792. doi: 10.1136/bmj.m792.
[13] Liu K, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, *et al*. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl) 2020;133:1025–1031. doi: 10.1097/CM9.0000000000000744.
[14] Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. Lancet Infect Dis 2020;20:689–696. doi: 10.1016/S1473-3099(20)30198-5.
[15] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497–506. doi: 10.1016/S0140-6736(20)30183-5.
[16] Fu L, Fei J, Xiang HX, Xiang Y, Tan ZX, Li MD, *et al*. Influence factors of death risk among COVID-19 patients in Wuhan, China: a hospital-based case-cohort study. medRxiv 2020.03.13.20035329. doi: 10.1101/2020.03.13.20035329.
[17] Xu S, Fu L, Fei J, Xiang HX, Xiang Y, Tan ZX, *et al*. Acute kidney injury at early stage as a negative prognostic indicator of patients with COVID-19: a hospital-based retrospective analysis. medRxiv 2020.03.24.20042408. doi: 10.1101/2020.03.24.20042408.
[18] Xu L, Liu J, Lu M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. Liver Int 2020;40:998–1004. doi: 10.1111/liv.14435.
[19] Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y, *et al*. COVID-19: Abnormal liver function tests. J Hepatol 2020. doi: 10.1016/j.jhep.2020.04.006.
[20] Wijarnpreecha K, Ungprasert P, Panjawatanan P, Harnois DM, Zaver HB, Ahmed A, *et al*. COVID-19 and liver injury: a meta-analysis. Eur J Gastroenterol Hepatol 2020. doi: 10.1097/MEG.0000000000001817.
[21] Mantovani A, Beatrice G, Dalbeni A. Coronavirus disease 2019 and prevalence of chronic liver disease: A meta-analysis. Liver Int 2020;40:1316–1320. doi: 10.1111/liv.14465.
[22] Parohan M, Yaghoubi S, Seraji A. Liver injury is associated with severe coronavirus disease 2019 (COVID-19) infection: A systematic review and meta-analysis of retrospective studies. Hepatol Res 2020. doi: 10.1111/hepr.13510.
[23] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, *et al*. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med 2020;180:1–11. doi: 10.1001/jamainternmed.2020.0994.
[24] Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020. doi: 10.1001/jama.2020.2648.
[25] Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, *et al*. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol 2020;5:1–8. doi: 10.1001/jamacardio.2020.1017.
[26] Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, *et al*. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 2020;130:2620–2629. doi: 10.1172/JCI137244.
[27] Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, *et al*. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020;368:m1091. doi: 10.1136/bmj.m1091.
[28] Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, *et al*. Clinical characteristics of imported cases of coronavirus disease 2019 (COVID-19) in Jiangsu Province: A multicenter descriptive study. Clin Infect Dis 2020;71:706–712. doi: 10.1093/cid/ciaa199.
[29] Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, *et al*. Multiple organ infection and the pathogenesis of SARS. J Exp Med 2005;202:415–424. doi: 10.1084/jem.20050828.
[30] Audimoolam VK, McPhail MJ, Wendon JA, Willars C, Bernal W, Desai SR, *et al*. Lung injury and its prognostic significance in acute liver failure. Crit Care Med 2014;42:592–600. doi: 10.1097/01.ccm.0000435666.15070.d5.
[31] Li J, Fan JG. Characteristics and mechanism of liver injury in 2019 coronavirus disease. J Clin Transl Hepatol 2020;8:13–17. doi: 10.14218/JCTH.2020.00019.
[32] Rajapaksha IG, Gunarathne LS, Asadi K, Cunningham SC, Sharland A, Alexander IE, *et al*. Liver-targeted angiotensin converting enzyme 2 therapy inhibits chronic biliary fibrosis in multiple drug-resistant gene 2-knockout mice. Hepatol Commun 2019;3:1656–1673. doi: 10.1002/hep4.1434.
[33] Paizis G, Tikellis C, Cooper ME, Schembri JM, Lew RA, Smith AI, *et al*. Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut 2005;54:1790–1796. doi: 10.1136/gut.2004.062398.