Dynamic changes of liver function parameters in patients with coronavirus disease 2019: A multicenter, retrospective study

Qing-Lei Zeng (zengqinglei2009@163.com)
The First Affiliated Hospital of Zhengzhou University
Zu-Jiang Yu
The First Affiliated Hospital of Zhengzhou University
Fanpu Ji
The Second Affiliated Hospital of Xi'an Jiaotong University
Guang-Ming Li
The Sixth People's Hospital of Zhengzhou City
Guo-Fan Zhang
The First Affiliated Hospital of Nanyang Medical College
Jiang-Hai Xu
The Fifth People's Hospital of Anyang City
Wan-Bao Lin
Xinyang Central Hospital
Guo-Qiang Zhang
Luoyang Central Hospital
Guo-Tao Li
Luoyang Central Hospital
Guang-Lin Cui
The First Affiliated Hospital of Zhengzhou University
Fu-Sheng Wang
The Fifth Medical Center of Chinese PLA General Hospital, National Clinical Research Centre for Infectious Diseases

Research Article

Keywords: Alanine aminotransferase, Aspartate aminotransferase, Coronavirus disease 2019, Dynamic changes, Liver function, Liver injury, Severe acute respiratory syndrome coronavirus

DOI: https://doi.org/10.21203/rs.3.rs-48131/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: Liver injuries in patients with coronavirus disease 2019 (COVID-19) have been reported, however, the clinical role played by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is obscure.

Methods: In this multicenter, retrospective study, the parameters of liver function tests in COVID-19 inpatients were compared between various timepoints referred to SARS-CoV-2 shedding, and 3 to 7 days before first detection of viral shedding was regarded as reference baseline.

Results: Totally, 70 COVID-19 inpatients were enrolled. Twenty-two (31.4%) cases had self-medications history after illness. At baseline, 10 (14.3%), 7 (10%), 9 (12.9%), 2 (2.9%), 15 (21.4%), and 4 (5.7%) patients already had abnormal rates of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin, and total bilirubin (TBIL), respectively. ALT and AST abnormal rates and levels did not show any significantly dynamic change during the full period of viral shedding (all \( P > 0.05 \)). GGT abnormal rate (\( P = 0.008 \)) and level (\( P = 0.033 \)) significantly increased on day 10 of viral shedding. Meanwhile, no simultaneously significant increases of ALP abnormal rates and levels were observed. TBIL abnormal rates and levels significantly increased on day 1 and 5 of viral shedding (all \( P < 0.05 \)). Albumin abnormal decrease rates increased and levels decreased consistently from baseline to SARS-CoV-2 clearance day (all \( P < 0.05 \)). Thirteen (18.6%) patients had chronic liver diseases, two of them died. The ALT and AST abnormal rates and levels did not increase in patients with chronic liver diseases during SARS-CoV-2 shedding.

Conclusions: The SARS-CoV-2 does not directly lead to elevations of ALT and AST, but may result in elevations of GGT and TBIL, the albumin decreased extraordinarily even SARS-CoV-2 shedding discontinued.

Background

From December 2019, the coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected nearly all the countries globally within the past six months [1]. On March 11, 2020, the COVID-19 has been characterized as a pandemic by World Health Organization (WHO). As of July 1, 2020, a total of 10,357,662 have been infected with SARS-CoV-2 worldwide, with a mortality rate of around 4.9% (508,055 deaths) [1]. However, no effective drugs are clinically approved because of absence of evidence [2], although we preliminarily found that convalescent plasma treatment can discontinue SARS-CoV-2 shedding [3].

Recently, several studies have described the epidemiological and clinical characteristics of patients with COVID-19 in China [4, 5]. Of these studies, liver injury has been reported in 16.1% to 53.1% of patients, which raised the concern of relationship between the SARS-CoV-2 and liver impairment [6]. It is important to note that the details of liver injury occurrence were confusing in these studies. Firstly, the timepoints of liver injury can be occurred before the illness and viral shedding, during various timepoints of illness and viral shedding, and on or after the day of viral clearance. Different timepoints may have various abnormal rates and levels of liver injury. Additionally, many parameters have been employed to reflect liver injury, most of these studies only reported one or two parameters, such as alanine aminotransferase (ALT) or aspartate aminotransferase (AST), which lead to the invisibility of the panorama of liver injury. Furthermore, no dynamic changes of liver function test parameters to reflect the liver injury have been reported. These limitations prevent us to explore the actual relationship of SARS-CoV-2 and liver injury. In current study, we aim to investigate the role of SARS-CoV-2 played for liver injury through a multicenter, retrospective cohort with full continuous data of liver function test parameters and clinical course, especially full duration of viral shedding.

Patients And Methods

Data sources

According to the arrangements by the Chinese government, all COVID-19 patients were admitted centrally to the designated local hospitals. This retrospective, multicenter, observational study includes 6 designated referral hospitals in Henan Province of where near Hubei Province and had the third largest number of COVID-19 patients in China [7]. We retrospectively collected and analyzed the epidemiological, clinical, laboratory, virologic, management, and outcome data on patients with laboratory confirmed SARS-CoV-2 infection from the electronic medical records by using the pre-designed case report form. The COVID-19 patients were enrolled during main epidemic period from January 20 to February 29, 2020 (Before January 19 and after March 1, nearly no and few patients can be identified in Henan Province, respectively); and clinical outcomes were followed up until March 26, 2020.

Definition of laboratory confirmed COVID-19

Laboratory confirmed COVID-19 was diagnosed according to WHO interim guidance [8]. Laboratory confirmation of SARS-CoV-2 infection was done in Centers for Disease Control and Prevention (CDCs) of government, i.e., the Henan Provincial CDC, the CDCs of Xinyang, Nanyang, Anyang, Luoyang cities of Henan province, and Shaanxi Provincial CDC. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) tests for SARS-CoV-2 RNA were performed using nasopharyngeal swabs (Novel Coronavirus PCR Fluorescence Diagnostic Kit, BioGerm Medical Biotechnology)
The procedures of specimen pretreatment, RNA extraction, RT-PCR reaction conditions, and results interpretation were strictly followed the manufacturers’ instructions.

**Main criterion of hospital discharge or recovery of COVID-19 patients**

The criteria of hospital discharge (recovery) were the same as described by Lan et al [9]. Shortly, the criteria of hospital discharge were mainly based on the recovery of symptoms and signs, seroconversion of SARS-CoV-2 RNA, and absorption of lung inflammations (such as ground-glass opacities and/or consolidations). The SARS-CoV-2 RNA negativity should be confirmed for at least two times with an interval of more than 48 hours.

**Inclusion, exclusion, and grouping criteria of current study**

All male and nonpregnant female patients of 18 years of age or older were eligible for inclusion if they had the full liver function test parameters records and completely clinical course data during abovementioned study duration (Figure 1), which indicated that the patients had detailed data from illness to discharge (recovery) or death. Exclusion criteria included patients who were unmet of above-mentioned inclusion criteria, lacked the medical records of relevant self-medication information, alcohol drinking condition, and previous diagnosis of chronic liver diseases. The illness severity of COVID-19 was defined according to the Chinese management guideline for COVID-19 (version 6.0) [10]. Based on the severity and clinical outcomes, the patients in current study were divided into 3 groups, i.e., non-intensive care unit (ICU), ICU, and fatality groups.

**Liver function test parameters and abnormal rates**

The liver function test parameters in current study contain the serum ALT, AST, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin (ALB), and total bilirubin (TBIL). The abnormal rate was defined as the proportion of a liver function test parameter more than upper limit of normal range (apply to ALT, AST, GGT, ALT, and TBIL) or less than lower limit of normal range (apply to ALB only) in a group of patients. The upper limit of normal range for the most important parameter to reflect liver injury, i.e., ALT, was defined as 40 U/L according to the Asian Pacific Association for the Study of the Liver guidelines committee [11].

**Study timepoints**

Using the SARS-CoV-2 shedding as the reference, 6 timepoints were investigated in current study, i.e., 3 to 7 days before first detection of viral shedding (day -[3-7]), the first day of viral shedding (day 1), the fifth day of viral shedding (day 5), the tenth day of viral shedding (day 10), the fifteenth day of viral shedding (day 15), and the day of SARS-CoV-2 clearance (day-clearance). The day -[3-7] was defined as reference baseline.

**Study outcomes**

The study outcomes were abnormal rates and detailed levels of liver function test parameters at various timepoints referred to the viral shedding in different groups of COVID-19 patients, including a separately subgroup of patients with chronic liver diseases. The dynamic abnormal rates and levels of liver function test parameters were investigated to analyze the potential relationship between SARS-CoV-2 and liver injury as well as the potential role of SARS-CoV-2 for patients with chronic liver diseases.

**Statistical analysis**

Continuous and categorical variables were presented as median (interquartile range), mean ± standard deviation, and n (%) where appropriate. Mann-Whitney U test, Wilcoxon Test, Chi-square test, or Fisher’s exact test were used to compare the differences between various subgroups where appropriate. Analyses were carried out using SPSS statistical software, version 25.0 (IBM, Chicago, IL, USA). A *p* value of < 0.05 was set as the threshold for statistical significance.

**Results**

**Demographic and clinical characteristics**

A total of 316 hospitalized, laboratory-confirmed COVID-19 patients were assessed for eligibility (Figure 1), and 70 patients were eventually enrolled according to the inclusion and exclusion criteria. Of them, 30 asymptomatic, mild, and common patients were divided into non-ICU group, 23 severe and critically survived patients in ICU group, and 17 critically non-survived patients in fatality group. The demographic and clinical characteristics of these patients were presented in Table 1.

Totally, the median age was 56.5 (41-73) years, and ages were gradually older from non-ICU to ICU and fatality groups. 65.7% (46) of patients are male. The median viral shedding durations were 9 (6-11.3), 13 (11-16), and 19 (12.5-21) days in non-ICU, ICU, and fatality groups, respectively. The most common comorbidities were hypertension (18, 25.7%), diabetes (13, 18.6%), and cardiovascular diseases (7, 10%). Thirteen (18.6%) patients were previously diagnosed with chronic liver diseases, thereinto, 5 patients had hepatitis B e antigen (HBeAg) negative chronic hepatitis B and 2 of them received entecavir treatment, 5 patients had alcoholic liver disease, 2 had fatty liver disease, and 1 had chronic hepatitis C. Notably, 1 patient
with chronic hepatitis B and 1 patient with fatty liver disease eventually died. It is important to note that 38 (54.3%) patients had the combination of three HBV antibodies, i.e., antibodies to surface antigen (HBsAb), e antigen (HBeAb), and core antigen (HBcAb); more importantly, the percentages of this combination were gradually increased in three groups (non-ICU [33.3%] vs ICU [65.2%], P = 0.001; non-ICU [33.3%] vs fatality [76.5%], P = 0.004). Before visit or admission to hospital, 22 (31.4%) patients had the history of self-medication after illness onset. During hospitalization, symptomatic treatment (62, 88.6%), antivirals (56, 80%), and antibiotics (36, 51.4%) were the most common treatment strategies.

**Dynamic abnormal rates of liver function test parameters between three groups**

The dynamic abnormal rates of liver function test parameters between three groups were presented in Table 2. Notably, a total of 10 (14.3%) and 7 (10%) patients had the ALT and AST of more than upper limit of normal ranges on the day -(3-7), and these abnormal rates did not increase on day 1. Additionally, no significant differences of ALT and AST abnormal rates were observed between the three groups on day -(3-7) and day 1. On day 5, AST abnormal rate increased in fatality group (non-ICU [3.8%] vs fatality [37.5%], P = 0.008; ICU [8.7%] vs fatality [37.5%], P = 0.045); and the same phenomenon was also observed on day-clearance (non-ICU [0] vs fatality [44.4%], P = 0.002; ICU [8.7%] vs fatality [44.4%], P = 0.038). Meanwhile, on day 5, TBIL abnormal rate increased in fatality group (ICU [8.7%] vs fatality [43.8%], P = 0.019); and the same phenomenon was also observed on day 10 (non-ICU [0] vs fatality [69.2%], P = 0.005; ICU [0] vs fatality [69.2%], P < 0.001) and on day-clearance (non-ICU [10%] vs fatality [55.6%], P = 0.009; ICU [8.7%] vs fatality [55.6%], P = 0.010). Although the GGT and ALP abnormal rates accounted for 12.9%-34.1% and 1.4%-10.8% at various timepoints in total patients respectively, no significant differences were found between three groups with exception of ALP abnormal rate significantly increased on day 10 in fatality group (ICU [0] vs fatality [30.8%], P = 0.017). Additionally, no significant differences of abnormal rates for ALB were observed between three groups with exception of abnormal decrease rates increased on day 5 in ICU group (non-ICU [26.9%] vs ICU [56.5%], P = 0.035).

**Dynamic abnormal rates and levels of liver function test parameters in total patients**

Totally, no significant elevations of abnormal rates and levels for ALT and AST were found at various timepoints referred to the viral shedding (all P > 0.05, Table 3 and Fig. 2). Meanwhile, the GGT abnormal rate (P = 0.008) and level (P = 0.033) increased on day 10, and ALP levels elevated on day 10 (P = 0.001) and on day-clearance (P = 0.042) without matched with its abnormal rates (both P > 0.05). Notably, the ALB abnormal rates increased and levels decreased gradually from day -(3-7) to the day-clearance (all P < 0.05). Additionally, the TBIL abnormal rates and levels significantly increased on day 1 and 5 (all P < 0.05), and no its matched significant differences were observed on day 10 and day-clearance.

**Dynamic levels of liver function test parameters in non-ICU and ICU groups**

In non-ICU and ICU groups, no significant elevations were observed for ALT, AST, GGT, ALP and TBIL at various timepoints referred to the viral shedding (all P > 0.05, Fig. 3 and Fig. 4). Notably, the ALB levels decreased gradually from day -(3-7) to the day-clearance (all P < 0.05 with exception of day 10 in ICU group).

**Dynamic levels of liver function test parameters in fatality group**

In fatality group, no significant increases were founded for ALT, AST and GGT at various timepoints (all P > 0.05, Fig. 5). Meanwhile, the ALP increased from the day 5 (P = 0.048) to the day-clearance (P = 0.011), and TBIL increased from day 10 (P = 0.007) to the day-clearance (P = 0.011). Additionally, the albumin decreased on day 1 (P = 0.015) and 5 (P = 0.030) during viral shedding.

**Dynamic changes of liver function test parameters in patients with chronic liver diseases**

Two patients with chronic liver diseases died due to non-liver related reasons. The dynamic changes of liver function test parameters in 13 COVID-19 patients with previously diagnosed chronic liver diseases were presented in Table 4 and Fig. 6. The ALT abnormal rates decreased unexpectedly and gradually from 38.5% (5/13) on day -(3-7) to 8.3% (1/12) on day-clearance, and the ALT levels decreased simultaneously although significant differences were all not found (Fig.6). Meanwhile, the AST abnormal rates and detailed levels had the similar fluctuations with ALT with the exception the significant difference was observed for AST level on the day-clearance (P = 0.038). Additionally, 30.8% (4/13) to 62.5% (5/8) of patients had GGT abnormal rates at various timepoints although no significant differences were observed. Furthermore, the abnormal rates of low albumin increased from 38.5% (5/13) on day -(3-7) to 58.3% (7/12) on the day-clearance, and the detailed levels were also showed the decrease tendency although the difference was not significant on day 10 (P = 0.889). Notably, abnormal rates and levels for ALP and TBIL were steady during the full clinical course. For the 5 patients with chronic hepatitis B and 1 patient with chronic hepatitis C, no viral reactivations or breakthrough were observed during hospitalization, and the liver function test parameters were also steady during the full clinical course of COVID-19.

**Discussion**

The SARS-CoV and the current SARS-CoV-2 have been demonstrated as highly pathogenic human coronaviruses, which can lead to respiratory, intestinal, hepatic, neuronal diseases [12]. The current SARS-CoV-2 shares 79.6%-82% genome sequence similarity to SARS-CoV. It is known that
SARS-CoV and SARS-CoV-2 employ angiotensin-converting enzyme 2 (ACE2) as the receptor for cell entry [13, 14]. The ACE2 was reported to be abundantly expressed on the endothelial cells of the liver, which makes the liver a potential target for SARS-CoV and SARS-CoV-2, although ACE2 expression on bile duct cells is much higher than on liver cells [12]. Indeed, many studies have indicated that patients infected with human coronaviruses may have different degrees of hepatic impairments, not only the previous SARS-CoV [15, 16], but also the current SARS-CoV-2 [4, 5, 17-19].

However, the relationship between SARS-CoV-2 and liver injury is still confusing because no full liver function test parameters and its dynamic changes as well as its comparisons with SARS-CoV-2 shedding were included in previous studies. Commonly, viraemia peaks in the first week after infection in most acute viral diseases including SARS, and patient usually develops a primary immune response by day 10 to 14, which is followed by virus clearance [20]. In the third week, and clinical deterioration is occurred to be the result of inflammatory or hyperimmune attacks rather than direct viral-induced tissue damage [20, 21]. Generally, hepatotropic virus related liver injury always associate with viral replication and its related immunological responses [22]. Therefore, we used the SARS-CoV-2 shedding period as the chronological reference to firstly investigate the potential relationship between SARS-CoV-2 and liver injury in current cohort, and not intend to investigate the subsequently complicated or ambiguous relationship or correlation between liver injury and the outcome of COVID-19 patients.

In current study, 6 liver function test parameters were included. Notably, a total of 14% and 13% of patients had ALT and AST elevations on day -(3-7). Importantly, these abnormal rates did not significantly increase on day 1. More importantly, the detailed ALT and AST levels did not significantly fluctuate from the day -(3-7) to the day-clearance in all groups with the exception of AST abnormal rates increased on day 5 and day-clearance (median of 19 days) in fatality group. These data indicate that ALT the AST elevations were not directly caused by SARS-CoV-2. The ALT and AST elevations before viral shedding may associate with other factors, it was found that 13% and 31.4% of the total patients had previously diagnosed chronic liver diseases and self-medications history after illness, respectively. The AST abnormal increase rates on day 5 and even day-clearance of viral shedding in fatality group may be attributed to inflammatory, hyperimmune attacks, or drugs usage rather than direct SARS-CoV-2-induced tissue damage, because the viral clearance may lead to the discontinuation of injury, and virus related hyperimmune response and drug induced liver injury may last longer even after the viral clearance.

As the diagnostic biomarker for cholangiocyte injury, GGT abnormal rate significantly increased on day 10 (Table 2). Simultaneously, the GGT level significantly increased on day 10 ($P = 0.033$, Fig. 2). Meanwhile, the ALP abnormal rates did not have significantly changes from day -(3-7) to the day-clearance of viral shedding (all $P > 0.05$), although the ALP levels significantly elevated on day 10 and day-clearance. Further analysis found that the fatality group contributed to the significantly elevations of the ALP level (Fig. 5). These data indicate that SARS-CoV-2 may contribute to the cholangiocyte injury. These results support SARS-CoV-2 employ ACE2 as the receptor for cell entry and lead to the injury of bile duct epithelium.

For TBIL (Table 2 and Fig. 2), the abnormal rates significantly increased on days 1, 5, and 10, and no significant difference was found on the day-clearance. Simultaneously, the TBIL levels significantly increased on days 1 and 5, and no significant differences were found on day 10 and day-clearance. Further analysis found that the fatality group contributed to the significantly elevations of the TBIL levels (Fig. 5). Notably, majority of TBIL increases were attributed to the direct bilirubin elevations, which indicates the impairment of excretion for bile duct and the SARS-CoV-2 did not directly lead to the impairment of hepatocytes.

Notably, the ALB abnormal decrease rates increased and levels decreased significantly and simultaneously increased on days 1, 5, 10, and day-clearance. These data strongly demonstrated that the synthetic function of liver was affected severely. It is important to note that the severity degree of ALB abnormal decrease rates and levels did not match its corresponding ALT and AST, which indicate that some other occult factors may be involved to decrease the ALB levels. It may be related to the long-term consumption of disease and the insufficient intake of nutrition and calorie, or even kidney impairment may be involved, future studies are needed.

Unexpectedly, the ALT and AST abnormal rates and levels were all decreased from day -(3-7) to the day-clearance in 13 patients with chronic liver diseases (Table 4 and Fig. 6). This difficult-to-explain phenomenon may be caused by discontinuation of alcohol drinking after illness of COVID-19, because 5 of 13 patients had previously diagnosed alcoholic liver diseases and alcohol abuse. However, this phenomenon just added clinical evidence to indicate that SARS-CoV-2 did not directly broke the hepatocytes. Additionally, no significant dynamic changes were found for GGT, ALP, and TBIL with exception of ALB decreased on day 1, 5, and day clearance. Interestingly, it is also hard to explain why the rate of HBsAb/HBeAb/HBcAb positive combination was significantly higher from non-ICU to ICU and fatality groups (Table 1). Maybe it was accompanying the increase of ages in three groups, because HBV vaccine were unavailable and HBV infection risk were higher in older age patients.

There are several limitations in current study. Firstly, we are unable to analyze the role of drug use (treatment) for the occurrence or deterioration of liver injury during hospitalization, because drug-induced liver injury during the treatment of coronavirus infection may exist [6, 23]. However, in current study, the ALT and AST, regardless of abnormal rates and levels, did not show any significantly dynamic change during the full period of viral shedding, which strongly precluded the affection by the potential treatment for COVID-19. We indeed found more abnormal rates and levels of liver function test parameters in fatality group than non-ICU and ICU groups, which may be partly caused by the more drugs were used in fatality group. Additionally, the serum cytokine levels were unable to be tested to analyze its potential role for liver injury because it was unavailable for
majority of patients. The potential liver injury may also be caused by direct virus-induced cytopathic effects or immunopathology induced by overshooting inflammatory responses, which may produce a large amount of cytokines and it may be the cause of higher rates and levels of liver injury in fatality group in current study [12]. Furthermore, we did not investigate the relationship or correlation between liver injury and the COVID-19 as well as the prevalence rate of liver injury in COVID-19, because we think the relationship between SARS-CoV-2 and liver injury was the original problem. Finally, the SARS-CoV-2 RNA quantitation was not available for patients under the prevailing conditions. Nevertheless, despite of the limitations, it did influence our clinical judgement of SARS-CoV-2 did not directly destroy the hepatocytes, because the ALT and AST abnormal rates and levels did not show any significantly dynamic changes during the full clinical course of COVID-19, including patients in fatality group.

Conclusions

To the best of our knowledge, we firstly take the viral shedding as the reference to investigate the relationship between the SARS-CoV-2 and liver injury, and present the dynamic changes of liver function test parameters before, during, and at the clearance of viral shedding in COVID-19 patients. In conclusion, we found that the SARS-CoV-2 does not directly lead to the elevations of ALT and AST, but it may cause the elevations of GGT and TBIL (mainly direct bilirubin), which reflect the impairment of excretion function of bile duct. Notably, the albumin levels were extraordinarily decreased even the SARS-CoV-2 shedding discontinued. Future large-scale, prospective validation studies for the SARS-CoV-2 infection and liver injury relationship are needed.

Abbreviations

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COVID-19, coronavirus diseases 2019; CRRT, continuous renal replacement therapy; GGT, gamma-glutamyl transferase; HBcAb, anti-hepatitis B core antibody; HBeAb, anti-hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAb, anti-hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TBIL, total bilirubin.

Declarations

Acknowledgments

The authors would like to thank all participants and their families in the study.

Authors’ contributions

Qing-Lei Zeng, Zu-Jiang Yu, and Fanpu Ji contributed equally to this work. Fu-Sheng Wang and Qing-Lei Zeng: study concept and design, analysis and interpretation of the data, and preparation and critical review of the manuscript. All authors contributed to the collection and interpretation of the data and the drafting and critical review of this manuscript. All authors approved the final version of this manuscript.

Funding

This study was supported by The National Natural Science Foundation of China (No. 81970517), Zhongyuan (Henan) Thousand Outstanding Talents Plan (No. ZYQR201912179), and The Key Scientific Research Project of Henan Higher Education Institutions of China (No. 20B320028). The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

All procedures used were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964, as revised in 2008. The study protocol was approved by the Institutional Review Commission of The First Affiliated Hospital of Zhengzhou University and each involved center, and the requirement for informed consent was waived in light of de-identified data of patients.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no financial or non-financial interests with other people or organizations that could inappropriately influence this work.

References

1. WHO. World Health Organization. Coronavirus disease (COVID-19) Situation Report - 163. July 1, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200701-covid-19-sitrep-163pdf?sfvrsn=c202f05b_2. Accessed July 2, 2020.
2. Wang FS, Zhang C. What to do next to control the 2019-ncov epidemic? Lancet. 2020;395(10222):391-3.
3. Zeng QL, Yu ZJ, Gou JJ, Li GM, Ma SH, Zhang GF, et al. Effect of Convalescent Plasma Therapy on Viral Shedding and Survival in Patients With Coronavirus Disease 2019. J Infect Dis. 2020;222(1):38-43.
4. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
5. 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(11):1061-9.
6. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5(5):428-30.
7. Zeng QL, Li GM, Ji F, Ma SH, Zhang GF, Xu JH, et al. Clinical course and treatment efficacy of COVID-19 near Hubei Province, China: A multicentre, retrospective study. Transbound Emerg Dis. 2020; published online 12 June.
8. WHO. World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. January 28, 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed April 3, 2020.
9. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. JAMA. 2020;323(15):1502-3.
10. National Health Commission of the People's Republic of China. Chinese management guideline for COVID-19 (version 6.0). February 18, 2020. http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2/files/b218cfef1bc54639af227f922bf6b817.pdf. Accessed April 5, 2020; In Chinese.
11. Sarin SK, Kumar M, Laiu GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10(1):1-98.
12. Xu L, Liu J, Su M, Yang D, Zheng X. Liver injury during highly pathogenic human coronavirus infections. Liver Int. 2020;40(5):998-1004.
13. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9.
14. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
15. Chau TN, Lee KC, Yao H, Tsang TY, Chow TC, Yeung YC, et al. SARS-associated viral hepatitis caused by a novel coronavirus: report of three cases. Hepatology. 2004;39(2):302-10.
16. Wang JT, Sheng WH, Fang CT, Chen YC, Wang JL, Yu CJ, et al. Clinical manifestations, laboratory findings, and treatment outcomes of SARS patients. Emerg Infect Dis. 2004;10(5):818-24.
17. 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(10223):507-13.
18. 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(10223):497-506.
19. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. 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:m606.
20. Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis. 2005;24(1):44-6.
21. Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet. 2003;361(9371):1767-72.
22. Wang FS, Zhang Z. Host immunity influences disease progression and antiviral efficacy in humans infected with hepatitis B virus. Expert Rev Gastroenterol Hepatol. 2009;3(5):499-512.
23. Cai Q, Huang D, Yu H, Zhu Z, Xia Z, Su Y, et al. COVID-19: Abnormal liver function tests J Hepatol. 2020; In press.

Tables

Table 1 Demographic and clinical characteristics of patients with COVID-19
|                           | Total (n=70) | Non-ICU (n=30) | ICU (n=23) | Fatality (n=17) |
|---------------------------|-------------|----------------|------------|----------------|
| **Age, years**            | 56.5 (41-73)| 46 (31-65.5)   | 57 (44-66) | 73 (63-79)     |
| **Sex, Male**             |             |                |            |                |
| Male                      | 46 (65.7)   | 20 (66.7)      | 13 (56.5)  | 13 (76.5)      |
| **Viral shedding period, days** | 12 (9-16.3) | 9 (6-11.3)    | 13 (11-16) | 19 (12.5-21)   |
| **Chronic comorbidities** |             |                |            |                |
| Hypertension              | 18 (25.7)   | 8 (26.7)       | 7 (30.4)   | 3 (17.6)       |
| Diabetes                  | 13 (18.6)   | 3 (10)         | 5 (16.7)   | 5 (29.4)       |
| Cardiovascular diseases   | 7 (10)      | 2 (6.7)        | 3 (13)     | 2 (11.8)       |
| Chronic kidney disease    | 4 (5.7)     | 20             | 3 (13)     | 1 (5.9)        |
| Respiratory system diseases| 1 (1.4)     | 0 (0)          | 0 (0)      | 1 (5.9)        |
| **Chronic liver diseases**|             |                |            |                |
| Chronic hepatitis B       | 5 (7.1)     | 1 (3.3)        | 3 (13)     | 1 (5.9)        |
| Alcoholic liver disease   | 5 (7.1)     | 5 (16.7)       | 0 (0)      | 0 (0)          |
| Fatty liver disease       | 2 (2.9)     | 0 (0)          | 1 (4.3)    | 1 (5.9)        |
| Chronic hepatitis C       | 1 (1.4)     | 0 (0)          | 1 (4.3)    | 0 (0)          |
| **HBV markers**           |             |                |            |                |
| HBsAg/HBeAb/HBcAb (+)     | 5 (7.1)     | 1 (3.3)        | 3 (13)     | 1 (5.9)        |
| HBsAb/HBeAb/HBcAb (+)     | 38 (54.3)   | 10 (33.3)      | 15 (65.2)  | 13 (76.5)      |
| All HBV markers (+)       | 11 (15.7)   | 6 (20)         | 3 (13)     | 2 (11.8)       |
| Sole HBsAb (+)            | 7 (10)      | 6 (20)         | 0 (0)      | 1 (5.9)        |
| Sole HBeAb (+)            | 4 (5.7)     | 3 (10)         | 1 (4.3)    | 0 (0)          |
| HBsAb/HBeAb/HBcAb (+)     | 3 (4.3)     | 2 (6.7)        | 1 (4.3)    | 0 (0)          |
| HBeAb/HBeA (+)            | 2 (2.9)     | 2 (6.7)        | 0 (0)      | 0 (0)          |
| **Self-medication after illness** | 22 (31.4) | 8 (26.7) | 6 (26.1) | 8 (47.1) |
| Traditional Chinese medicine | 14 (20)  | 6 (6.7) | 4 (17.4) | 4 (23.5) |
| Acetaminophen             | 4 (5.7)     | 2 (6.7)        | 1 (4.3)    | 1 (5.9)        |
| Levofloxacin/Moxifloxacin | 4 (5.7)     | 0 (0)          | 1 (4.3)    | 3 (17.6)       |
| **Treatment during hospitalization** |          |            |          |                |
| Symptomatic treatment     | 62 (88.6)   | 22 (73.3)      | 23 (100)   | 17 (100)       |
| Antiviral§                | 56 (80)     | 20 (66.7)      | 19 (82.6)  | 17 (100)       |
| Antibiotics§              | 36 (51.4)   | 8 (26.7)       | 11 (47.8)  | 17 (100)       |
| Traditional Chinese medicine | 12 (17.1) | 3 (10) | 5 (21.7) | 4 (23.5) |
| Immunglobulin             | 20 (28.6)   | 0 (0)          | 7 (30.4)   | 13 (76.5)      |
| Glucocorticoid            | 17 (24.3)   | 0 (0)          | 5 (21.7)   | 12 (70.6)      |
| High-flow oxygen          | 35 (50)     | 0 (0)          | 18 (78.3)  | 17 (100)       |
| Mechanical ventilation    | 17 (24.3)   | 0 (0)          | 3 (13)     | 14 (82.4)      |
| CRRT                      | 11 (15.7)   | 0 (0)          | 0 (0)      | 11 (64.7)      |

Data are presented as median (interquartile range) or n (%). †Viral shedding period in fatality group was calculated from the beginning of detectable SARS-CoV-2 to discontinuation of viral shedding or to death date even the viral shedding continued at the time of fatality. ‡Antiviral agents mainly included oseltamivir, interferon α (aerosol inhalation), and lopinavir/ritonavir. §Antibiotics mainly included levofloxacin and moxifloxacin for all groups, and meropenem, biapenem, vancomycin, and tigecycline for fatality group. Abbreviations: COVID-19, coronavirus diseases 2019; CRRT, continuous renal replacement therapy; HBeAb, anti-hepatitis B e antibody; HBsAb, anti-hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ICU, intensive care unit.
| Timepoint | Total (n=70) | Non-ICU (n=30) | ICU (n=23) | Fatality (n=17) | p1 | p2 | p3 |
|-----------|-------------|----------------|------------|-----------------|----|----|----|
| **Day 3-7 before viral shedding** | | | | | | | |
| ALT increased (0-40 U/L) | 10 (14.3) | 5 (16.7) | 3 (13) | 2 (11.8) | 1.000 | 1.000 | 1.000 |
| AST increased (0-40 U/L) | 7 (10) | 2 (6.7) | 3 (13) | 2 (11.8) | 0.642 | 0.613 | 1.000 |
| GGT increased (0-58 U/L) | 9 (12.9) | 5 (16.7) | 2 (8.7) | 2 (11.8) | 0.685 | 1.000 | 1.000 |
| ALP increased (40-130 U/L) | 2 (2.9) | 1 (3.3) | 0 (0) | 1 (5.9) | 1.000 | 1.000 | 0.425 |
| ALB decreased (35-55 g/L) | 15 (21.4) | 6 (20) | 4 (17.4) | 5 (29.4) | 1.000 | 0.709 | 0.605 |
| TBIL increased (0-25 μmol/L) | 4 (5.7) | 0 (0) | 2 (8.7) | 2 (11.8) | 0.184 | 0.126 | 1.000 |
| **During viral shedding, on day 1** | | | | | | | |
| ALT increased (0-40 U/L) | 8 (11.4) | 4 (13.3) | 1 (4.3) | 3 (17.6) | 0.374 | 0.692 | 0.294 |
| AST increased (0-40 U/L) | 7 (10) | 3 (10) | 1 (4.3) | 3 (17.6) | 0.624 | 0.653 | 0.294 |
| GGT increased (0-58 U/L) | 15 (21.4) | 8 (26.7) | 3 (13) | 4 (23.5) | 0.384 | 1.000 | 0.432 |
| ALP increased (40-130 U/L) | 1 (1.4) | 1 (3.3) | 0 (0) | 0 (0) | 1.000 | 1.000 | - |
| ALB decreased (35-55 g/L) | 30 (42.9) | 10 (33.3) | 10 (43.3) | 10 (58.8) | 0.450 | 0.089 | 0.337 |
| TBIL increased (0-25 μmol/L) | 12 (17.3) | 5 (16.7) | 3 (13) | 4 (23.5) | 1.000 | 0.850 | 0.432 |
| **During viral shedding, on day 3** | | | | | | | |
| ALT increased (0-40 U/L) | 11/65 (16.9) | 5/26 (19.2) | 2 (8.7) | 4/16 (25) | 0.424 | 0.956 | 0.205 |
| AST increased (0-40 U/L) | 9/65 (13.8) | 1/26 (3.8) | 2 (8.7) | 6/16 (37.5) | 0.584 | 0.008 | 0.405 |
| GGT increased (0-58 U/L) | 14/65 (21.5) | 7/26 (26.9) | 5 (21.7) | 2/16 (12.5) | 0.674 | 0.472 | 0.678 |
| ALP increased (40-130 U/L) | 7/65 (10.8) | 2/26 (7.7) | 1 (4.3) | 4/16 (25) | 1.000 | 0.180 | 0.139 |
| ALB decreased (35-55 g/L) | 29/65 (44.6) | 7/26 (26.9) | 13 (56.5) | 9/16 (56.2) | 0.035 | 0.057 | 1.000 |
| TBIL increased (0-25 μmol/L) | 14/65 (21.5) | 5/26 (19.2) | 2 (8.7) | 7/16 (43.8) | 0.424 | 0.175 | 0.019 |
| **During viral shedding, on day 10** | | | | | | | |
| ALT increased (0-40 U/L) | 8/41 (19.5) | 0/8 (0) | 4/20 (20) | 4/13 (30.8) | 0.295 | 0.131 | 0.681 |
| AST increased (0-40 U/L) | 6/41 (14.6) | 0/8 (0) | 2/20 (10) | 4/13 (30.8) | 1.000 | 0.131 | 0.182 |
| GGT increased (0-58 U/L) | 14/41 (34.1) | 3/8 (37.5) | 7/20 (35) | 4/13 (30.8) | 1.000 | 1.000 | 1.000 |
| ALP increased (40-130 U/L) | 4/41 (9.8) | 0/8 (0) | 0/20 (0) | 4/13 (30.8) | - | 0.131 | 0.017 |
| ALB decreased (35-55 g/L) | 23/41 (56.1) | 5/8 (62.5) | 10/20 (50) | 8/13 (61.5) | 0.686 | 1.000 | 0.722 |
| TBIL increased (0-25 μmol/L) | 9/41 (22) | 0/8 (0) | 0/20 (0) | 9/13 (69.2) | - | 0.005 | <0.001 |
| **On the day of viral clearance** | | | | | | | |
| ALT increased (0-40 U/L) | 7/62 (11.3) | 3 (10) | 2 (8.7) | 2/9 (22.2) | 1.000 | 0.572 | 0.557 |
| AST increased (0-40 U/L) | 6/62 (9.7) | 0 (0) | 2 (8.7) | 4/9 (44.4) | 0.184 | 0.002 | 0.038 |
| GGT increased (0-58 U/L) | 19/62 (30.6) | 9 (30) | 8 (34.8) | 2/9 (22.2) | 0.712 | 0.974 | 0.681 |
| ALP increased (40-130 U/L) | 1/62 (1.6) | 0 (0) | 1 (4.3) | 0/9 (0) | 0.434 | - | 1.000 |
| ALB decreased (35-55 g/L) | 32/62 (51.6) | 13 (21.3) | 14 (22.6) | 5/9 (55.6) | 0.206 | 0.706 | 1.000 |
| TBIL increased (0-25 μmol/L) | 10/62 (16.1) | 3 (10) | 2 (8.7) | 5/9 (55.6) | 1.000 | 0.009 | 0.010 |

Data are presented as n (%) or n/N (%), where N is the total number of cases with available data. Unavailable data in some patients because their viral seroconversion occurred before this timepoints or viruses were not cleared till death. The corresponding normal ranges and units of the liver function test parameters are presented in the parentheses, increased indicates over the upper limit of the normal range and decreased indicates below the lower limit of the normal range. p1: non-ICU vs ICU groups; p2: non-ICU vs fatality; p3: ICU vs fatality. Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COVID-19, coronavirus diseases 2019; GGT, gamma-glutamyltransferase; ICU, intensive care unit; TBIL, total bilirubin.

Table 3 Dynamic abnormal rates and levels of liver function test parameters between various timepoints of viral shedding in total patients with COVID-19.
### Day -(3-7) vs day 1, p2: day -(3-7) vs day 5, p3: day -(3-7) vs day 10, p4: day -(3-7) vs day-clearance.

**Table 4 Dynamic changes of liver function test parameters in 13 COVID-19 patients with chronic liver diseases**

| Parameter | Day -3 to -7 | Day 1 | Day 5 | Day 10 | Day-clearance |
|-----------|--------------|-------|-------|--------|---------------|
| ALT (U/L) | 22 (19-76)   | 21.5 (15.5-67.5) | 23.5 (14.8-73.8) | 24 (11.5-54.5) | 21.5 (17-28.3) |
| > 40 U/L | 5 (38.5) | 4 (30.8) | 3/12 (25) | 2/8 (25) | 1/12 (8.3) |
| AST (U/L) | 32 (20.5-45) | 32 (23.3-39.5) | 23 (17-34.8) | 27 (15-41.3) | 25 (14.8-30.8) |
| > 40 U/L | 4 (30.8) | 3 (23.1) | 2/12 (16.7) | 2/8 (25) | 1/12 (8.3) |
| GGT (U/L) | 50 (29.1-121) | 61 (37-132) | 42.5 (28.3-103) | 78 (33-182.8) | 50 (30.9-93) |
| > 35 U/L | 4 (30.8) | 7 (53.8) | 4/12 (33.3) | 5/8 (62.5) | 4/12 (33.3) |
| ALP (U/L) | 66 (57.5-69.5) | 63 (54.5-81) | 63 (46.3-85.5) | 65.5 (37.5-75.8) | 63 (52-70.5) |
| > 130 U/L | 1 (7.7) | 0 (0) | 1/12 (8.3) | 0/8 (0) | 0/12 (0) |
| ALB (g/L) | 35.1 (33.5-41.5) | 34 (30.4-40.5) | 32.8 (31-37.6) | 36.7 (32.4-37.9) | 33.2 (31.5-36.3) |
| < 35 g/L | 5 (38.5) | 8 (61.5) | 6/12 (50) | 3/8 (37.5) | 7/12 (58.3) |
| TBIL (μmol/L) | 15 (10-19.4) | 10.4 (6.5-20.3) | 12.5 (6.8-24.4) | 8.5 (5.3-20.5) | 10.7 (8.1-16.9) |
| > 25 μmol/L | 0 (0) | 0 (0) | 0/12 (0) | 1/8 (12.5) | 0/12 (0) |

Data are presented as median (interquartile range), n (%), or n/N (%), where N is the total number of cases with available data. 1Unavailable data in 1 patient because his viral seroconversion occurred on day 4. 2Unavailable data in 5 patients because their viral seroconversion occurred before day 10. 3Unavailable data in 1 patient because he did not clear the virus till death. The corresponding normal ranges and units of the liver function test parameters are presented in the parentheses. Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; COVID-19, coronavirus diseases 2019; GGT, gamma-glutamyl transferase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TBIL, total bilirubin.
Figure 1

Fig. 1 Flow diagram of the study enrollment. Abbreviations: COVID-19, coronavirus diseases 2019; ICU, intensive care unit.
Figure 2

Flow diagram of the study enrollment. Abbreviations: COVID-19, coronavirus diseases 2019; ICU, intensive care unit.
Figure 3

Dynamic levels of liver function test parameters in total patients. Data were presented as mean ± standard deviation. D -(3-7): n=70; D 1: n=70; D 5: n=65; D 10: n=41; D Clear: n=62. Abbreviations: D, day; ref., reference.
Figure 4

Dynamic levels of liver function test parameters in non-ICU group. Data were presented as mean ± standard deviation. D -(3-7): n=30; D 1: n=30; D 5: n=26; D 10: n=8; D Clear: n=30. Abbreviations: D, day; ICU, intensive care unit; ref., reference.
Figure 5
Dynamic levels of liver function test parameters in ICU group. Data were presented as mean ± standard deviation. D -(3-7): n=23; D 1: n=23; D 5: n=23; D 10: n=20; D Clear: n=23. Abbreviations: D, day; ICU, intensive care unit; ref., reference.
Figure 6
Dynamic levels of liver function test parameters in fatality group. Data were presented as mean ± standard deviation. D -(3-7): n=17; D 1: n=17; D 5: n=16; D 10: n=13; D 15: n=10; D Clear: n=9. Abbreviations: D, day; ref., reference.
Figure 7
Dynamic levels of liver function test parameters in patients with chronic liver diseases. Data were presented as mean ± standard deviation. D-(3–7): n = 13; D 1: n = 13; D 5: n = 12; D 10: n = 8; D Clear: n = 12. Abbreviations: D, day; ref., reference.