

Dear Professor Dennis A Bloomfield, Professor Bao-gan Peng and Professor Sandro Vento:

Thank you very much for offering us the opportunity to improve our manuscript. We are now submitting the revision in accordance with the reviewers' recommendations. We have addressed all the comments raised by the reviews and the amendments were highlighted in the manuscript. We appreciate the reviewer's time and comments, and hope the revised version will meet the criteria for publication on your journal.

Here are the point-by-point responses to reviewers' query/comment.

For Reviewer #1:

An overview of clinical aspects and mechanisms of liver injury caused by SARS-CoV-2 infection is proposed. In brief, the Authors conclude that liver injury is a common complication in COVID-19 patients and may be due to virus-induced cytopathic effects, immune mediated inflammation, drug toxicity and pneumonia-associated hypoxia. In most cases, liver injury appears as a transient elevation of serum aminotransferases. Furthermore, patients with abnormal liver tests had higher risks of progressing to severe disease. Based on the available data, close monitoring of liver function should be advised in patients with COVID-19, especially in severe cases. Although there are no elements of great originality, this review makes a good review of the literature and provides useful information on the hepatic aspects of COVID 19.

Suggestion & Question 1:

I would suggest reviewing the part where the potential hepatotoxicity of paracetamol is addressed. According to an American Association for Study of Liver Disease document (Malespin MH. Risk of Nonsteroidal Anti-inflammatory Drugs and Safety of Acetaminophen in Patients with Advanced Liver Disease. Clin Liver Dis (Hoboken). 2018 Oct 2;12(3):85-88. doi: 10.1002/cld.737), paracetamol has to be preferred in patients with liver disease over NSAIDs. Could the authors please clarify whether paracetamol causes hepatotoxicity according to a dose-dependent mechanism even in patients with COVID -19? I believe this is very important for clinicians who treat patients with COVID-19. In these patients there

is a need to treat fever, myalgia, malaise and all flu-like symptoms. Paracetamol and NSAIDs are widely used to treat these symptoms. When hypertransaminasemia or signs of liver disease are present, can the clinicians continue to use them? When should they not be used or discontinued in the presence of liver disease? Are the AASLD recommendations applicable to COVID-19 patients with signs of liver disease?

Answer: Thank you for the suggestion. We reviewed the potential hepatotoxicity of paracetamol on page 11, line 311 to 327, which were highlighted in yellow.

Drug-induced liver injury is traditionally classified as idiosyncratic vs. intrinsic (or direct) ^[1]. The occurrence of the former is only related to individual susceptibility, not necessarily related to dose. While the latter causes hepatotoxicity according to a dose-dependent mechanism both in animal model and human body. Acetaminophen (also known as paracetamol, APAP), one of the most widely used analgesic and antipyretic agent, is a classic dose-dependent liver injury drug. In therapeutic doses ($\leq 4\text{g/d}$), acetaminophen can give rise to transient serum aminotransferase elevations in a proportion of subjects, generally starting after 3 to 7 days, and with peak values rising above 3-fold elevated in 39% of persons^[2]. These elevations are usually asymptomatic, which will fade quickly with discontinuation or reduction of dose, and even with full continuous treatment in some cases. In contrast, acetaminophen overdose (generally more than 15 grams) may cause an acute, serious hepatocellular injury. It is reported that APAP is responsible for nearly 50% of acute liver failure in the United States. Hepatic injury often occurs at 24-72 hours after ingestion, with a significant increase in serum ALT and AST (often to above 2000 U/L). While clinical symptoms develop at 48-96 hours, including jaundice, confusion, liver failure, and even death.

In patients with COVID -19, paracetamol is widely used to treat fever, myalgia, malaise and all flu-like symptoms. But it is unclear whether paracetamol causes hepatotoxicity according to a dose-dependent mechanism due to limited evidence. Unlike most of the drugs repositioned for COVID-19 therapy, paracetamol have not yet been evaluated in controlled clinical trials or analyzed through retrospective analyses. There may be two reasons account for this phenomenon. During clinical treatment, paracetamol is often

combined with other hepatotoxic drugs such as antivirals in COVID-19 patients. Even if liver injury occurs, it is difficult to determine which drugs are causative. Furthermore, since paracetamol are available over-the-counter, accurate records about who has taken them, when, and in what doses is often sparse and, if collected from patient memory, would be inherently unreliable. But recently, a 27-year-old healthy African American female with a positive SARS-CoV2 test and acute liver failure secondary to acetaminophen overdose has been reported [3]. She has a remote history of focal segmental glomerular sclerosis. To manage her pain, she ingested >50 tablets of acetaminophen over the 3–4 days preceding presentation. Initial blood work revealed acetaminophen level was 42 µg/ml (upper limit of normal 30 µg/ml) and elevated aminotransferases with alanine transaminase (ALT) of 2791 U/L and aspartate transaminase (AST) of 3202 U/L. Two days after admission, her hepatic synthetic function worsened significantly. Aminotransferases peaked to an AST 9741 U/L and ALT 11322 U/L.

Mechanically, acetaminophen-induced hepatotoxicity is mainly due to the production of quinone imine metabolite. In therapeutic doses, 90%-95% of the APAP is combined with glucuronic acid or sulfuric acid to form a non-toxic substance, which is excreted by the kidney [2]. While the remaining part is metabolized via the cytochrome P450 system to intermediates that can be toxic to the liver and kidney, particularly N-acetyl-p-benzoquinoneimine(NAPQI). Then, this intermediate is rapidly conjugated to reduced glutathione (GSH), detoxified and secreted. But if the glutathione in the liver is depleted by an overdose of acetaminophen, NAPQI will bind covalently with proteins in hepatocytes, resulting in hepatocyte necrosis.

According to AASLD, paracetamol has to be preferred in patients with liver disease over NSAIDs [4]. But whether the AASLD recommendations applicable to COVID-19 is still controversial. The advocates argue that the use of NSAID (even if given for short times and/or associated to antibiotics) for the treatment of fever and nonrheumatic pain could worsen bacterial infections and upregulate angiotensin converting enzyme 2 (ACE2), a target of SARS-COV2[5]. Whereas acetaminophen revealed a dose-dependent reduction in expression of ACE2 in liver, implying reduction in hepatic

susceptibility to the SARS-CoV-2 infection [6]. On the contrary, the opponents argue that the routinely use of paracetamol in COVID-19 at risk population may further worsen the scarcity of GSH, resulting in increased risk of developing severe COVID-19[7].

All in all, paracetamol is a safe and effective first line agent in almost all patients regardless of liver disease etiology [8]. Since NAFLD can sensitize the liver for acetaminophen. Therefore, when administered in such patients, the dose should be controlled especially [9]. In addition, it is worth noting that dose reduction should be warranted in certain severe or decompensated hepatic disease states, particularly if patients are malnourished, are not eating or have a dry weight less than 50 kg [8]. Thresholds for treatment discontinuation in clinical trials suggested by the Food and Drug Administration guidance are [10]:

①ALT or AST >8 ULN

②ALT or AST >5 ULN for more than 2 weeks

③ALT or AST >3 ULN and (TBL >2 ULN or INR >1.5)

④ALT or AST >3 ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

[1] Yu PF, Wu Q, Duan ZP, Chen Y. Research advances in the mechanism of drug-induced liver injury due to paracetamol. *J Clin Hepatol*,2020;9:2108-11

[2] [Anonymous]. Acetaminophen. In, *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*. Bethesda (MD); 2012

[3] Rouphael C, D'Amico G, Ricci K et al. Successful orthotopic liver transplantation in a patient with a positive SARS-CoV2 test and acute liver failure secondary to acetaminophen overdose. *Am J Transplant* 2020; DOI:10.1111/ajt.16330

[4] Malespin MH. Risk of Nonsteroidal Anti-inflammatory Drugs and Safety of Acetaminophen in Patients with Advanced Liver Disease. *Clin Liver Dis (Hoboken)*. 2018 Oct 2;12(3):85-88. doi: 10.1002/cld.737

[5] Micallef, J., Soeiro, T., and Jonville-Béra, A. P. (2020b). Non-steroidal anti-inflammatory drugs, pharmacology, and COVID-19 infection. *Thérapie* 75, 355–362. doi: 10.1016/j.therap.2020.05.003

- [6] Saheb Sharif-Askari N, Saheb Sharif-Askari F, Mdkhana B, Al Heialy S, Ratemi E, Alghamdi M, Abusnana S, Kashour T, Hamid Q, Halwani R. Effect of common medications on the expression of SARS-CoV-2 entry receptors in liver tissue. *Arch Toxicol.* 2020 Dec;94(12):4037-4041. doi: 10.1007/s00204-020-02869-1. PMID: 32808185; PMCID: PMC7430937.
- [7] Sestili P, Fimognari C. Paracetamol-Induced Glutathione Consumption: Is There a Link With Severe COVID-19 Illness? *Front Pharmacol.* 2020 Oct 7; 11:579944. doi: 10.3389/fphar.2020.579944. PMID: 33117175; PMCID: PMC7577213.
- [8] Hayward KL, Powell EE, Irvine KM, Martin JH. Can paracetamol (acetaminophen) be administered to patients with liver impairment? *Br J Clin Pharmacol.* 2016 Feb;81(2):210-22. doi: 10.1111/bcp.12802. PMID: 26460177; PMCID: PMC4833155.
- [9] Michaut A, Moreau C, Robin MA et al (2014) Acetaminophen-induced liver injury in obesity and nonalcoholic fatty liver disease. *Liver Int* 34:171–179. <https://doi.org/10.1111/liv.12514>
- [10] Study of Drug Induced Liver Disease of Chinese Medical Association. Diagnosis and treatment guideline on drug-induced liver injury. *Zhonghua Gan Zang Bing Za Zhi.* 2015 Nov;23(11):810-20. PMID: 27252993.

Suggestion & Question 2:

It would be desirable to provide more details about management of COVID-19 patients with liver disease. Some figures would be welcome to summarize some points addressed both for the pathogenetic aspects and on the treatment.

Answer: Thank you very much for the advice. Details about management of COVID-19 patients with liver disease were presented on page 13, part 4, as well as Table 2 on page 29. And we summarized the pathogenetic aspects in Fig.1 on page 27.

Since there is no complete and systematic data at present, most of the recommendations for management of COVID-19 patients with liver disease are based on expert consensus. By comparing the recommendations of several international guidelines, we summarize the management principles listed as follows:

Table 2 Management of COVID-19 patients with liver disease

Out-patient care	<ul style="list-style-type: none">● Use telemedicine or visits by phone wherever possible. Consider seeing in person only patients with urgent issues and clinically significant liver disease (e.g., jaundice, elevated ALT or AST >500 U/L, or recent onset of hepatic decompensation) [1-3].● Seeing at the fever clinic [1].
Hospital treatment	<ul style="list-style-type: none">● Separate management from non-COVID-19 patients [1,4].● Monitor liver biochemistries regularly, particularly in patients treated with remdesivir or tocilizumab [1].● Avoid ultrasound or other advanced imaging unless it is likely to change management, for example, clinical suspicion for biliary obstruction or venous thrombosis [1].● Hospitalize COVID-19 patients with advanced liver disease as soon as possible [4].
Patients with hepatitis B; hepatitis C	<ul style="list-style-type: none">● Document discussion with patient regarding CLD diagnosis and management [2].● Delay starting DAA therapy until after their recovery from COVID-19 disease if there is no suspicion of advanced liver disease [5].● Continue treatment and provide 90-d supplies for HBV oral antiviral drugs or a full course of DAA medications to complete HCV treatment [5].
Patients with autoimmune liver disease	<ul style="list-style-type: none">● Continue immunosuppressive therapy in stable patients with AIH [5].● Lower the doses of azathioprine or mycophenolate mofetil when patients develop lymphopenia [5].

	<ul style="list-style-type: none"> ● Avoid liver biopsy and start empiric therapy In new patients presenting with features of AIH [5]. ● Avoid high doses of prednisone in AIH patients on corticosteroids [5].
Patients with HCC	<ul style="list-style-type: none"> ● Continue HCC surveillance schedule for high-risk subjects [1]. ● Document discussion of risks and benefits of delaying surveillance with patient [1]. ● Proceed with HCC treatments as appropriate [1]. ● Postpone elective transplant and resection surgery, withhold immunotherapy [2].
Pretransplant and post-transplant patients	<ul style="list-style-type: none"> ● Have low threshold for admitting patients on transplant waiting list diagnosed with COVID-19[1,2]. ● Consider reduction of immunosuppression therapy as appropriate for posttransplant patients with moderate COVID-19 [1,2]. ● Avoid reductions in immunosuppressive therapy in patients with mild COVID-19 disease [1,2].

COVID-19, coronavirus disease 2019; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLD, chronic liver disease; DAA, direct acting antiviral; HBV, hepatitis B virus; HCV, hepatitis C virus; AIH, autoimmune hepatitis; HCC, hepatocellular carcinoma.

[1] FixOK, Hameed B, Fontana RJ, et al. Clinical Best Practice Advice for Hepatology and Liver Transplant Providers During the COVID19 Pandemic: AASLD Expert Panel Consensus Statement. Hepatology, 2020, 72: 287-304.

[2]APASL Covid-19 Task Force, Lau G, Sharma M. Clinical practice guidance for hepatology and liver transplant providers during the COVID-19 pandemic: APASL expert panel consensus recommendations. Hepatol Int, 2020, 14: 415428.

[3]Wong GL, Wong Vw, ThompsonA, et al. Management of Patients with liver derangement during the COVID19 pandemic: an Asia-Pacific position statement. Lancet GastroenterolHepatol, 2020, 5: 776—787.

[4]Boettler T, Newsome PN, Mondelli MU, et al. Care of patients with liver disease during the COVID-19 pandemic: EASL-ESCMID position paper. JHEP Rep, 2020, 2: 100113.

[5] Hamid S, Alvares da Silva MR, Burak KW, Chen T, Drenth JPH, Esmat G, Gaspar R, LaBrecque D, Lee A, Macedo G, McMahon B, Ning Q, Reau N, Sonderup M, van Leeuwen DJ, Armstrong D, Yurdaydin C. WGO Guidance for the Care of Patients With COVID-19 and Liver Disease. J Clin Gastroenterol. 2021 Jan;55(1):1-11. doi: 10.1097/MCG.0000000000001459. PMID: 33230011; PMCID: PMC7713641.

For Reviewer #2:

Suggestion & Question 1:

The authors should clarify whether ACE2 are absent on hepatocytes, Kupffer cells and hepatic stellate cells.

Answer: Thanks very much for your advice. We clarified whether ACE2 are absent on hepatocytes, Kupffer cells and hepatic stellate cells on page 8, line 237 to 240, which are highlighted in yellow.

ACE2 is rarely expressed in hepatocytes, while absent on Kupffer cells and hepatic stellate cells. Chai and his colleagues applied single-cell RNAseq to healthy human liver samples and found that hepatocellular ACE2 expression is low but still detectable (2.6%), with average expression level 20 fold less than the expression level in the cholangiocytes population^[1]. Of note, hepatic ACE2 expression in hepatocytes is significantly increased in both humans and rat upon liver fibrotic/cirrhotic conditions^[2,3]. In addition, ACE2 expression has neither been detected in Kupffer cells nor in quiescent, fibrogenic/activated hepatic stellate cells, suggesting that these cells may be a rather non-permissive host for SARS-CoV-2^[4].

[1] Chai X, Hu L, Zhang Y, et al. Specific ACE2 expression in cholangiocytes may cause liver damage after 2019-nCoV infection[J]. biorxiv, 2020.

[2] Paizis G, Tikellis C, Cooper ME, et al. Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut. 2005; 54:1790-

1796.

[3] Huang Q, Xie Q, Shi CC, et al. Expression of angiotensin-converting enzyme 2 in CCL4-induced rat liver fibrosis. *Int J Mol Med*. 2009; 23:717-723.

[4] Nardo AD, Schneeweiss-Gleixner M, Bakail M, Dixon ED, Lax SF, Trauner M. Pathophysiological mechanisms of liver injury in COVID-19. *Liver Int*. 2020 Nov 15. doi: 10.1111/liv.14730. Epub ahead of print. PMID: 33190346.

Suggestion & Question 2:

Page 5 discuss in more details what is the mechanistic basis of higher AST levels and greater liver injury seen in COVID-19 patients. Why is AST higher than ALT?

Answer: Thanks very much for your question. The reasons why AST is higher than ALT were shown on page 6, line 143 to 153, which were highlighted in yellow.

To our knowledge, there are three possible reasons for this phenomenon. Firstly, given that AST is also distributed in myocardium and skeletal muscle, the AASLD has recommended consideration of myositis or cardiac injury as contributors to the AST elevation^[1]. Secondly, recent data have identified ribosomal proteins as important host-dependency factors for SARS-CoV-2^[2]. Therefore, the virus may directly cause hepatic mitochondrial injury and subsequent AST elevation. Thirdly, AST-predominant aminotransferase elevations have been reported in alcohol-related liver disease, ischemia, and cirrhosis. It is possible that hypoxia as well as metabolic changes such as hepatic steatosis may account for AST dominance in COVID-19 patients^[3,4].

[1] Fix OK, Hameed B, Fontana RJ, Kwok RM, McGuire BM, Mulligan DC, et al. Clinical best practice advice for hepatology and liver transplant providers during the COVID-19 pandemic: AASLD expert panel consensus statement. *HEPATOLOGY* 2020. doi: 10.1002/hep.31281. Epub ahead of print

[2] Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, O'Meara MJ, et al. A SARS-CoV-2-human protein-protein interaction map reveals drug targets and potential drug-repurposing. *bioRxiv* 2020. doi: 10.1101/2020.03.22.002386.

[3] Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with

SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020; 8:475-481.

[4] Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; 8:420-422.

Suggestion & Question 3:

Especially in relation to point#2, discuss the absence of liver failure or bile duct injury in autopsy of COVID-19 patients as reported on Page 8. Liver histological findings on autopsy of patients with COVID-19 should be discussed.

Answer: Thanks very much for your comment. Liver histological findings on autopsy of patients with COVID-19 were discussed in various paragraphs describing the mechanisms of liver injury, which were highlighted in blue.

Histopathological findings of autopsied liver samples have shed light on the pathogenesis of SARS-CoV-2 induced liver injury. Recently, Wang et al^[1] investigated the patterns of liver impairment by electron microscopy and pathological studies in two COVID-19 cases. In this study, typical coronavirus particles were identified in the cytoplasm of hepatocytes. Histologically, massive hepatic apoptosis and binuclear hepatocytes were observed. These findings strongly indicate direct hepatic impairment by SARS-CoV-2 infection. Moderate microvascular steatosis and mild lobular and portal activity detected by Xu et al^[2] was likely due to SARS-CoV-2 infection or drug toxicity. Whereas, another finding has revealed the watery degeneration of some hepatocytes, implying the possibility of hepatic ischemia and hypoxia^[3].

In addition, canalicular cholestasis and mild nuclear pleomorphism of cholangiocytes was presented by Lax et al^[4]. And a 27-year-old female with a positive SARS-CoV2 test and acute liver failure secondary to acetaminophen overdose has been reported^[5]. Therefore, we deleted this part on Page 9 (on Page 8 previously) which was marked with strikethrough.

[1] Wang Y, Liu S, Liu H, Li W, Lin F, Jiang L, Li X, Xu P, Zhang L, Zhao L, Cao Y,

Kang J, Yang J, Li L, Liu X, Li Y, Nie R, Mu J, Lu F, Zhao S, Lu J, Zhao J. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol*. 2020 Oct;73(4):807-816. doi: 10.1016/j.jhep.2020.05.002. PMID: 32437830; PMCID: PMC7211738.

[2] Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; 8:420-2; PMID:32085846 DOI:10.1016/S2213-2600(20)30076-X.

[3] Cai Q, Huang D, Yu H, et al. COVID-19: Abnormal liver function tests. *J Hepatol* 2020; 73:566-74.

[4] Lax SF, Skok K, Zechner P, et al. Pulmonary arterial thrombosis in COVID-19 with fatal outcome: results from a prospective, single-center, clinicopathologic case series. *Ann Intern Med* 2020. Epub ahead of print.

[5] Rouphael C, D'Amico G, Ricci K et al. Successful orthotopic liver transplantation in a patient with a positive SARS-CoV2 test and acute liver failure secondary to acetaminophen overdose. *Am J Transplant* 2020; DOI:10.1111/ajt.16330

Suggestion & Question 4:

Page 8-9. discuss in more details the cytokine-storm mediate liver injury by elaborating on the various players involved and their potential role as that seems the most plausible reason for greater liver injury seen in COVID-19 patients. Also discuss why only a subset of patients are getting liver involvement.

Answer: Thanks very much for your suggestions. We discussed the topic on page 9, line 268 to 292.

In young individuals with an intact innate immune system, the virus is cleared during the initial phase, so they show only mild symptoms ^[1]. However, in the elderly and individuals with underlying chronic diseases, the insufficient viral clearance due to altered innate immune response will lead to cytokine storm, which may trigger a violent attack to the body and cause multiple organ failure including the liver ^[1,2]. This is the reason why only a subset of patients are getting liver involvement.

Cell entry of SARS-CoV-2 depends on binding of the viral spike (S) proteins to cellular ACE2 receptor and on S protein priming by host cell proteases ^[3]. While the virus enters the cells via fusion with the host membrane, its antigen will be recognized by the antigen presentation cells (APC) and then presented to cytotoxic (CD8+) and regulatory (CD4+) T lymphocytes, which initiate an antiviral immune response including inflammatory cytokine production and a weak interferon (IFN) response ^[4]. In particular, SARS-CoV-2 can rapidly activate pathogenic Th1 cells to secrete pro-inflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-6 (IL-6). GM-CSF further activates CD14+CD16+ inflammatory monocytes to produce large quantities of IL-6, tumor necrosis factor- α (TNF- α), and other cytokines ^[5]. Among these cytokines, IL-6 not only can bind to sIL-6R to activate STAT3 in non-immune cells but also can bind to membrane-bound IL-6 receptor (mIL-6R) to lead to pleiotropic effects on acquired and innate immune cells, resulting in cytokine storms ^[6]. Meanwhile, sIL-2R may regulate CD8+ T cells negatively and contribute to lymphopenia through IL-2 signaling inhibition ^[7].

[1] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

[2] Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal* 2020; 10: 102-108 [PMID: 32282863 DOI: 10.1016/j.jpha.2020.03.001]

[3] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020 Apr 16;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052. Epub 2020 Mar 5. PMID: 32142651; PMCID: PMC7102627.

[4] Sahin TT, Akbulut S, Yilmaz S. COVID-19 pandemic: Its impact on liver disease

and liver transplantation. World J Gastroenterol. 2020 Jun 14;26(22):2987-2999. doi: 10.3748/wjg.v26.i22.2987. PMID: 32587443; PMCID: PMC7304105.

[5] Haiming W, Xiaoling X, Yonggang Z, et al. Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus. BioRxiv. 2020

[6] Hu B, Huang S, Yin L. The cytokine storm and COVID-19. J Med Virol. 2020 Jun 27;10.1002/jmv.26232. doi: 10.1002/jmv.26232. Epub ahead of print. PMID: 32592501; PMCID: PMC7361342.

[7] Zhang Y, Wang X, Li X, Xi D, Mao R, Wu X, Cheng S, Sun X, Yi C, Ling Z, Ma L, Ning Q, Fang Y, Sun B, Wu D. Potential contribution of increased soluble IL-2R to lymphopenia in COVID-19 patients. Cell Mol Immunol. 2020 Aug;17(8):878-880. doi: 10.1038/s41423-020-0484-x. Epub 2020 Jun 25. PMID: 32587367; PMCID: PMC7315399.

Suggestion & Question 5:

This review should include discussion on whether COVID-19 patients with liver injury had alcohol-or other drug use disorders?

Answer: Thanks very much for your suggestion. What we know so far is that the COVID-19 patients with elevated ALT levels are more likely to have a history of drinking^[1]. And a series of drugs may increase the risk of hepatic damage, which has been discussed and highlighted in yellow on page 7, line 184 to 187 as well as on page 11, line 333 to 340.

[1] Li L, Li S, Xu M, et al. Risk factors related to hepatic injury in patients with corona virus disease 2019. medRxiv. Available from: <https://www.medrxiv.org/content/10.1101/2020.02.28.20028514v2>.

Suggestion & Question 6:

Is there anything known whether the COVID-19 patients with more severe liver injury were positive for other hepatotrophis viruses', HepC, B, A, E?

Answer: Thanks very much for your question. This question was answered on page 7, line 209 to 228. So far, the answer is still controversial. In a retrospective study, the authors analyzed liver function parameters including ALT, AST, TBIL in COVID-19 patients with or without HBV infection and found no significant differences between the two groups ^[1]. Another study reached a similar conclusion and further proved the longitudinal changes of median values for liver biochemistries were not significantly different between the two groups, either ^[2]. These findings indicate that SARS-CoV-2 will not exacerbate liver injury in patients with HBV co-infection.

However, Lin et al ^[3] drew a completely opposite conclusion. In their cohort, COVID-19 cases with HBV co-infection had higher levels of ALT, AST, TBIL, ALP than the COVID-19 cases without HBV co-infection, showing that inactive HBV carriers with SARS-CoV-2 co-infection are at risk of greater liver injury. In addition, SARS-CoV-2 was reported to induce HBV reactivation, which may cause severe liver injury in patients with co-infection ^[2,4]. In our study, viral hepatitis (hepatitis B and hepatitis C) was much more frequent among patients with liver injury than those without.^[5] Moreover, a case of COVID-19 with Epstein-Barr Virus infection developed acute liver injury, with liver enzymes that were much higher than typically seen solely with COVID-19 infection^[6]. Although the evidence is limited, more attention should be paid to COVID-19 patients with other viral co-infections during clinical treatment.

[1] Chen L, Huang S, Yang J, Cheng X, Shang Z, Lu H, Cheng J. Clinical characteristics in patients with SARS-CoV-2/HBV co-infection. *J Viral Hepat.* 2020. doi: 10.1111/jvh.13362. PMID: 32668494; PMCID: PMC7404861.

[2] Liu J, Wang T, Cai Q, Sun L, Huang D, Zhou G, He Q, Wang FS, Liu L, Chen J. Longitudinal changes of liver function and hepatitis B reactivation in COVID-19 patients with pre-existing chronic hepatitis B virus infection. *Hepatol Res.* 2020 Nov;50(11): 1211-1221. doi: 10.1111/hepr.13553. PMID: 32761993; PMCID: PMC7436737.

[3] Lin Y, Yuan J, Long Q, Hu J, Deng H, Zhao Z, Chen J, Lu M, Huang A. Patients with SARS-CoV-2 and HBV co-infection are at risk of greater liver injury. *Genes Dis.*

2020. doi: 10.1016/j.gendis.2020.11.005. PMID: 33225036; PMCID: PMC7672332.

[4] Aldhaleei WA, Alnuaimi A, Bhagavathula AS. COVID-19 Induced Hepatitis B Virus Reactivation: A Novel Case From the United Arab Emirates. *Cureus*. 2020 Jun 15;12(6): e8645. doi: 10.7759/cureus.8645. PMID: 32550096; PMCID: PMC7296884.

[5] Ming Wang, Weiming Yan, Weipeng Qi, Di Wu, Lin Zhu, Weina Li, Xiaojing Wang, Ke Ma, Ming Ni Dong Xu, Hongwu Wang, Guang Chen, Haijing Yu, Hongfang Ding, Mingyou Xing, Meifang Han, Xiaoping Luo, Tao Chen, Wei Guo, Dong Xi, Qin Ning. Clinical characteristics and risk factors of liver injury in COVID-19: a retrospective cohort study from Wuhan, China. *Hepatology International*, 2020, 14(5), 723-732.

[6] Singh, T., Alameri, A., Rampy, J., Brady III, C., & Guerrero, J. S2658 Two for One: A Case of COVID-19 and Epstein-Barr Virus-Induced Acute Liver Injury. *Official journal of the American College of Gastroenterology* | ACG, 115, S1393.

Suggestion & Question 7:

Funding source is listed as a grant from 2018?

Answer: Yes. The grant titles are: the National Major Science and Technology Special Project on Major New Drug Innovation(2018ZX09733001-002-006) and Natural Science Foundation of Hubei Province(2019CFB328). These grants were not especially for COVID-19 acutely. Due to the suddenness of COVID-19, the authors were authorized to use part of these grants to investigate COVID-19.

Suggestion & Question8:

This review will benefit from some Figures to highlight the potential mechanism outlined in this review.

Answer: Thanks very much for your advice. The potential mechanisms were summarized in Fig.1 on page 27.

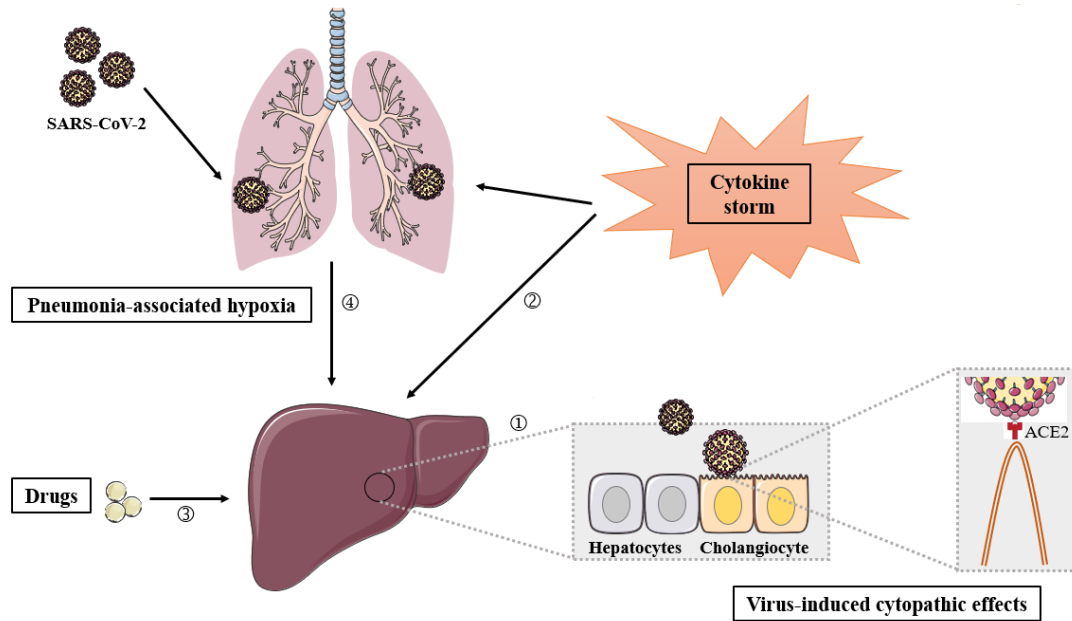


Fig.1 Potential mechanisms of liver injury in patients with COVID-19. 1. SARS-CoV-2 may directly bind to ACE2 positive cholangiocytes to dysregulate liver function. 2. Inflammatory cytokine storm leads to persistent activation of lymphocytes and macrophages that secrete huge amount of inflammatory cytokine, thus contributing to lung as well as liver damage. 3. Drugs including antipyretics, antiviral medications (lopinavir/ritonavir), antibiotics (macrolides, quinolones) and steroids may have potential hepatotoxicity and lead to abnormal liver function. 4. Hepatic ischemia and hypoxia-reperfusion dysfunction induced by complications such as respiratory failure may cause liver damage, especially in critically ill patients.