Cover Letter

November 14, 2022

Re: Revised version of manuscript 80010

Dear Editorial Board and Reviewers,

Thank you for taking the time to assess our review article entitled "Acute liver injury in COVID-19 patients hospitalized in the Intensive Care Unit: Narrative review".

We have addressed all reviewers' insightful comments.

Please see below our detailed response to each comment of every single reviewer. The revision has been carried out in consultation with all co-authors and each author has given approval to the final revised version of the manuscript. Please address all correspondence concerning this manuscript to me at ventoulis@hotmail.com (or alternatively at iventoulis@uowm.gr)

Thank you for reviewing our manuscript. It is our belief that the manuscript is substantially improved after making the recommended edits. We look forward to your final approval.

Sincerely,

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Response to Reviewer 1 (Reviewer's code 03622345)

1- Figure 1: The infogram of pathology of ALI in COVID-19 needs to be elaborative, explainable, simple colors, educational.

We followed the suggestion of reviewer 1 by making slight modifications to the infogram and by adding an elaborative figure legend which provides a comprehensive description of the information presented in the figure.

2- Figure 2. Present the data as infogram instead of bundles, use short informative sentences, and stepwise order. Make the text more precise and remove repetitive information.

We followed the suggestion of reviewer 1 and modified the figure by presenting the data in the form of inforgram with a stepwise order instead of bundles. We have added a detailed figure legend which precisely explains the information presented in the figure.

3- Table 1: Too much data in each line and column, please summaries the contents and mention important data.

We have revised the table in order to make it much briefer. We have summarized data and included only the most important information.

4- Add an explanatory paragraph on: the underlying causes of elevated liver enzymes in COVID-19

The underlying causes of elevated liver enzymes are presented in detail in the section of the text under the general heading ''Pathophysiology''.

5- Add a paragraph on: Clinical course and outcomes following SARS-CoV-2 infection in pre-existing chronic liver diseases.

We have added information about pre-existing chronic liver diseases in the section of the text under the heading ''Predisposing factors for ALI''.

6- Add a paragraph on: Clinical course and outcomes of SARS-CoV-2 infection in liver transplant recipients.

We have added further information about liver transplant recipients in the section of the text under the heading ''Predisposing factors for ALI''.

7- Add a paragraph on: Findings of histology in ALI in COVID-19

We have added information regarding histological findings in the section of the text under the heading ''Liver histopathology in COVID-19: Is there a link with pathophysiological mechanisms in ALI?''. All additions in the text are highlighted in yellow for the reviewers' convenience.

Response to Reviewer 2 (Reviewer's code: 02524651)

In the narrative review "Acute liver injury in COVID-19 patients hospitalized in the Intensive Care Unit", the authors defined ALI and gave existing evidences on the COVID-19 patients hospitalized in ICU, and described the pathophysiology and therapeutic strategy. The authors give many details and is helpful to understand ALI in COVID-19 patients.

We thank reviewer 2 and appreciate the fact that the detailed information provided in our manuscript are considered helpful to understand ALI in COVID-19 patients.

1, From the very beginning to data, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been evolving to different stains. Each strain has different toxicity compared with other strain. So, it is valuable if the author would identify the different effects on ALI of the strains?

Reviewer 2 is correctly highlighting a very important aspect. This concern was also raised by us during the initial preparation of the manuscript so that we would report the different virus strains in the text and in the summarized table, but unfortunately such data are lacking in the included studies. Based on reviewer's 2 insightful comment, we have added the following sentence at the end of the text under the heading ''Direct cytopathic viral effect'': '' Even more so, data regarding the potential discrepancies in the hepatotropism of the different SARS-CoV-2 strains are lacking; hence, it would be intriguing for researchers to investigate the possible different effects that are exerted by different strains of SARS-CoV-2 on the liver and identify any associated variations in the pathophysiology and clinical course of ALI.''

2, In the part of pathophysiology, the authors provide many details. However, the authors focus on the general pathophysiology (for example, septic shock, hypoxia, MV,) , but not the specific relationship between COVID-19-induced pathophysiology and ALI.

The proposed pathophysiological mechanisms that ultimately lead to ALI in the ICU setting are the ones described in detail in the text and summarized in Figure 1. Instead of briefly mentioning putative pathophysiological mechanisms that lead to COVID-19 induced ALI in the ICU, we have comprehensively analyzed the general pathophysiology of every single proposed mechanism that eventually results in ALI, so that it is easy for the reader to become familiar with the underlying pathophysiology and the specific mechanisms that come into play in the ICU setting.

3, In the part of therapeutic strategy, also should focus on the specific COVID-19-related therapy rather than the general therapy.

We would like to thank reviewer 2 for this comment. Unfortunately, there is no specific therapy for COVID-19 induced ALI. The therapeutic strategy is initially preventive and afterwards supportive when ALI occurs. In order to better clarify this, we have included relevant sentences in the abstract (‘’ It should be noted that no specific therapy for COVID-19 induced ALI exists. Therefore, the therapeutic approach lies in preventive measures and is exclusively supportive once ALI ensues.’’), as well as in the text immediately after the heading ‘’Therapeutic strategy’’(‘’It should be emphasized that no specific therapy for COVID-19 induced ALI exists. Therefore, the therapeutic approach initially lies in prevention and is exclusively supportive once ALI ensues.’’).

Response to Reviewer 3 (Reviewer's code: 05230016)

1. The narrative review titled "Acute liver injury in COVID-19 patients hospitalized in the Intensive Care Unit: Narrative review." is interesting and sheds light on pathophysiology of acute liver injury and treatment in COVID-19 patients. However, the abstract needs to be modified to represent a summary than just an introduction of the manuscript.

The observations of reviewer 3 about the abstract have been taken into account. We have revised parts of the abstract and added certain sentences in order to improve coherence and provide a summary of the present narrative review.

2. Further, an objective parameter for assessment of data in form of a table citing the incidence, type of liver injury, occurring in isolation or as part of other digestive symptoms, ethnicity and impact on outcome in all the studies included will greatly improve the quality of the manuscript. With the inclusion of these changes and improvement in clarity of the images provided, the manuscript may be accepted for publication after revision.

We agree with reviewer 3 and have therefore modified Table 1 by highlighting the incidence of acute liver injury and the main outcomes of each study. Furthermore, we followed his/her recommendation and added elaborative figure legends which improve the clarity of the images by providing a comprehensive description of the data presented in the figures.

Response to Reviewer 4 (Reviewer's code: 05451751)

I would like to congratulate the authors team for considering this topic. Acute liver injury is one of the most frequent, but less discussed problem in severe COVID-19 patients. Given the multisystem involvement, and secondary infections, sometimes it is difficult to predict the nature and cause of liver injury in these patients. In this review, the authors discussed the aetiology, pathogenesis and potential implications of COVID-19 outcome based on acute liver injury. The review is well written, which will contribute to the various aspect of the COVID-19 management. I have raised few points which needs further clarification. Please find my response below:

We would like to thank reviewer 4 and appreciate his/her feedback on the particular topic. We tried to address his/her insightful comments and clarify certain points.

1. Line-184….The reference should be cited for these definitions.

Unfortunately, we were not provided with the manuscript version that was sent to the reviewers (the one with the numbered lines), although we requested it several times. So, based on our own calculation of lines, we assume that Line 184 refers to the following sentence: ''Severe liver dysfunction was associated with more severe respiratory failure, as manifested by more frequent development of ARDS and lower values of Horowitz index, namely lower ratio of partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2 ratio)''. The definition of Horowitz index is actually included in the study which is cited at the end of the paragraph as reference 28. Furthermore, based on the reviewer's comment 3, we have additionally placed the citation in the first sentence of the same paragraph, as follows: '' The allegedly first study to explore the incidence, clinical characteristics and outcomes of ALI exclusively in ICU patients with COVID-19 was conducted in Germany and included 72 critically ill patients between March and July 2020[28].''

2. Line – 228, citation should be added.

Again, as mentioned previously, we were unfortunately not provided with the manuscript version that was sent to the reviewers (the one with the numbered lines), although we requested it several times. So, based on our own calculation of lines, we assume that Line 228 refers to the following sentence: ''A similar definition of ALI was employed by Arentz et al who published the first case series of ICU patients with COVID-19 in the USA, dating back to February-March 2020.'' We have indeed placed the citation at the end of this sentence, which now reads as follows: '' A similar definition of ALI was employed by Arentz et al who published the first case series of ICU patients with COVID-19 in the USA, dating back to February-March 2020[31].''

3. References should be appropriately cited at the place where it mentioned first.

We have ensured that the references are also cited in the first relevant sentence where they are first mentioned and not only at the end of several corresponding sentences that refer to the same citation.

4. In paragraph 'DILI' I don't think LMWH is an important factor contributing to DILI. Is there any evidence to support this? Especially in severe COVID-19 patients, where it is invariably used. Since author is discussing DILI in context with COVID-19, so I am more interesting regarding Remdesivir, which is known to cause hepatotoxicity. Is there any data regarding its hepatotoxicity in COVID-19? Similarly what about other drugs like Tocilizumab, Baricitinib which are frequently used in severe COVID-19 disease?

We agree that LMWH is not a common agent that will eventually lead to drug discontinuation, but our purpose is to mention all relevant drugs that are commonly used in the ICU setting and may potentially cause liver injury. DILI due to LMWH has been described as a phenomenon in the literature since decades and, in support of this, we have provided the following references:

118 **Ortiz GX**, Lenhart G, Becker MW, Schwambach KH, Tovo CV, Blatt CR. Drug-induced liver injury and COVID-19: A review for clinical practice. *World J Hepatol* 2021; **13**: 1143-1153 [PMID: 34630881 DOI: 10.4254/wjh.v13.i9.1143]

119 **Gabrielli M**, Franza L, Esperide A, Gasparrini I, Gasbarrini A, Franceschi F, On Behalf Of Gemelli Against Covid. Liver injury in patients hospitalized for COVID-19: Possible role of therapy. *Vaccines (Basel)* 2022; **10**: 192 [PMID: 35214651 DOI: 10.3390/vaccines10020192]

129 **Yang X**, Li N, Guo T, Guan X, Tan J, Gao X, Wu Y, Jia L, Gu M, Hua L, Liu H. Comparison of the effects of low-molecular-weight heparin and fondaparinux on liver function in patients with pulmonary embolism. *J Clin Pharmacol* 2020; **60**: 1671-1678 [PMID: 32639644 DOI: 10.1002/jcph.1686]

130 **Hahn KJ**, Morales SJ, Lewis JH. Enoxaparin-induced liver injury: Case report and review of the literature and FDA adverse event reporting system (FAERS). *Drug Saf Case Rep* 2015; **2**: 17 [PMID: 27747729 DOI: 10.1007/s40800-015-0018-0]

131 **Carlson MK**, Gleason PP, Sen S. Elevation of hepatic transaminases after enoxaparin use: case report and review of unfractionated and low-molecular- weight heparin-induced hepatotoxicity. *Pharmacotherapy* 2001; **21**: 108-113 [PMID: 11191729 DOI: 10.1592/phco.21.1.108.34436]

Yet, we agree with reviewer 4 that LMWH is invariably used in the ICU setting, as already highlighted in the text under the heading ''Therapeutic strategy'' (Besides, the importance of using pharmacologic thromboprophylaxis in critically ill patients cannot be overemphasized. As a matter of fact, thromboprophylaxis is strongly recommended in the recently published surviving sepsis campaign guidelines on the management of adults with COVID-19 in the ICU[120].), as well as in Figure 2. For purposes of better clarity and to avoid any reader's misunderstanding about the role of LMWH, we have modified the initial sentence as follows: '' Heparin-induced hepatotoxicity has been described in the literature, but is generally mild, transient and self-limited and does not

warrant discontinuation of heparin therapy, which is an essential component of COVID-19 treatment[118-119,129-131].''

Furthermore, with regard to antiviral and immunomodulatory therapies, we have included two detailed paragraphs in the main text under the heading '' Drug-induced liver injury (DILI)''. All additions in the text are highlighted in yellow for the reviewers' convenience.

5. Author should highlight the predictor of liver injury in COVID-19. Since it is associated with increased mortality in COVID-19, it will be helpful if author discuss about the risk factors/potential predictor of liver injury in COVID-19. There are few recent reports which highlighted this topic. Mishtaq et al. PMID: 33223215 Wang et al. 2022. https://doi.org/10.1186/s12876-022-02188-y

We totally agree with reviewer 4 and have thus followed his/her suggestion to discuss about risk factors of liver injury. For this reason, we have added one more detailed section in the manuscript under the heading ''Predisposing factors for ALI''. We are also very grateful to reviewer 4 for providing us some references on the particular topic, which we have actually used.

6. The author discuss the risk of Acute liver injury in ICU patients, what about those who recovered from acute illness? Are there any long-term residual liver dysfunction in these patient? Is there any data available to discuss this? (Post-COVID Liver injury)

We appreciate the reviewer's insightful suggestion about post-covid liver injury and agree that it would be interesting and useful to investigate that. However, such a discussion is beyond the scope of our paper. Nevertheless, this topic would be a great suggestion for a future analysis dealing exclusively with post-covid complications.

7. What is the actual incidence of liver injury in COVID-19 ICU patients? What are the incidence of acute liver failure?

Based on the included studies, we have summarized the incidence of ALI in ICU patients in Table 1.

8. Since Liver injury is quite common in critically ill ICU patients, How this liver injury differs in COVID-19 from other infections? Or is it similar to other viral transaminitis? Or bacterial sepsis?

This is a very interesting point that reviewer 4 is highlighting. However, there is still no definite answer to this raised question, since the pathophysiological mechanisms of COVID-19 ALI are putative and remain largely unclear. Triggered by reviewer's 4 insightful comment, we have added the following sentences at the end of the text under the heading ''Direct cytopathic viral effect'':

'' It needs to be emphasized that, although there are several viruses displaying some form of hepatotropism, there may be considerable heterogeneity among them. This could be due to the fact that the immune responses mounted by the host against the virus may differ significantly depending on whether the virus is cytopathic or not, as well as on which immune evasion mechanisms are adopted by the virus, in conjunction with other factors, like impaired immunity or high viral load[92]. Furthermore, the pattern of the observed changes in transaminases during SARS-CoV-2 infection differs from the liver injury pattern of other epidemic viruses, which result in a much steeper curve of aminotransferase elevations owing to massive parenchymal necrosis[18].

Given that the mechanisms of COVID-19 induced ALI still remain largely unclear, our current understanding is limited with regard to the exact pathophysiology behind the liver injury caused by SARS-CoV-2 and how this differs or resembles the effects of other hepatotropic viruses.''

9. Any comment regarding the liver histopathology in these group of patients?

We have added information regarding histological findings in the section of the text under the heading ''Liver histopathology in COVID-19: Is there a link with pathophysiological mechanisms in ALI?''

10. What are the implications of pre-existing liver disease on further liver injury in severe COVID-19?

We have added information about pre-existing liver diseases in the section of the text under the heading ''Predisposing factors for ALI''.

11. Another mechanism of acute liver injury is COVID-19 induced cardiomyopathy and congestive hepatitis which needs to be discussed.

We appreciate reviewer's 4 constructive suggestion about referring to COVID-19 induced cardiomyopathy and have actually added some discussion on this mechanism in the manuscript under the heading ''Hypoperfusion''. The addition in the text reads as follows: ''It is worth mentioning that in the COVID-19 era a specific cause leading to cardiogenic shock may be related to myocardial injury caused by SARS-CoV-2 virus. Cardiovascular involvement in COVID-19 may manifest in the form of myocarditis and pericarditis, but also as arrhythmias, acute coronary syndromes and stress-induced cardiomyopathy. When severe, all of these cardiovascular manifestations may potentially lead to acute heart failure and shock, especially in vulnerable patients with pre-existing cardiovascular disease[47-49].''