

Point-by-point replies

Dear Ze-Mao Gong,

Science Editor, Editorial Office

Baishideng Publishing Group Inc

I greatly appreciate the review process of my manuscript titled “**Changes in cellular proliferation and plasma products are associated with liver failure**” Number ID: **27321**. According to the reviewers’ suggestions, I revised carefully and a native English speaker reviewed and edited this version of the manuscript. All points that need to be reviewed are highlighted with red color font.

Our point-by-point replies to the reviewer’s comments are shown below (in blue color font).

Thank you for your considerations in advance on our manuscript and I hope to hearing a positive reply from you.

Sincerely,

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Reviewer#1:

General comment to authors

This is a very good effort at translational research involving ALF patients. I feel that the cytokine, PBMC and T cell profile which you present in AH [HAV and non-HAV], ALF [HAV and non-HAV] and healthy controls are interesting. The correlation of these factors to severity of liver disease and outcome is interesting too. However, my observation is that these important results and their following discussion have been

diluted by other not so novel results [i.e. TB, AST, and ALT]. These analyses and their accompanying tables and discussions can be removed. Also, the discussion can be made a bit more succinct by limiting the discussion on other authors' work .

Abstract

Line 73: The words "plasma products" is a bit vague. This may be replaced by something like "cytokine profile"

Responses: The corrections were performed.

Introduction

The introduction is very long. It can be summarized better. The difficulty in predicting outcome in ALF can be just 1 sentence. Past proposed use of cytokines and inflammation in ALF can be 2-3 sentences. The paragraph from 141 to 150 emphasizing the importance of HAV is appropriate. The lines 150 - 157 do not add anything to the manuscript.

Responses: Totally agree. The corrections were performed.

Line 129: My suggestion is to remove the phrase "Encephalopathy signals are present". The words "encephalopathy signals" do not mean much. Coagulopathy is also an important factor present in patients of ALF.

Responses: The phrase was removed.

Line 131: Maybe the better way to put this would be "Several studies have proposed non -invasive methods to evaluate the severity of liver damage and death, with little success"

Responses: The corrections were performed.

Line 137: I do not agree with "HE and INR being "gold standard" for "ALF" prognosis". This is a very over-simplified statement. King's College criteria and LIU are complex scores, which include the above parameters amongst others. Even these have been met with limited success. I would suggest removing this statement.

Responses: This statement was removed.

Materials and methods

These can be organized better

A] First explain the subjects [see below]

b] Case definition [Lines 196 – 201]

c] Work-up for each patient [as is been mentioned]

d] All other analysis as has been appropriately described.

Line 174 – 177: please explain the subjects better i.e. 84 subjects = 49 outpatients + 13 inpatients + 22 healthy subjects [this should be mentioned here]

Responses: My mistake. The information about healthy subjects was missing in this paragraph, I wrote the right information carefully.

What about tests for Wilson and other metabolic conditions? Were these performed?

Responses: Actually, Wilson Disease was only investigated if the patient presented past family history. The diagnosis confirmation can be performed by histological findings with liver biopsies, therefore in this study any patients presented Wilson Disease. Other metabolic disorders were investigated if the routine exams (biochemical, haematological, ...) presented alterations. A statement was inserted to explain better the not confirmed diagnosis (cryptogenic disease).

Line 195 – This line is not needed. The only etiologies present were HAV, indeterminate and drug induced.

Responses: This line was removed.

Lines 201 -211 should be in the Result section

Responses: These data were inserted in the Results section as subtitle “**Characterization of the AH and ALF patients**”.

Results

There are some very interesting take away points, which have been diluted by some unnecessary analyses. As mentioned above, please dedicate the first paragraph to describe subjects i.e How many, what etiologies, AH VS. ALF [number], outcome.

Responses: These changes were performed in the Results section as subtitle “**Characterization of the AH and ALF patients**”.

Table 1: Why did 3 patients die in the acute hepatitis group? They had normal INR and no HE.

Responses: There is a mistake, but just on the classification of groups on table 1. The table 1 submitted for the first time was wrong. Thanks for this observation. Three patients with acute HAV infection, INR<1.5 and no coma grade (HE<I) had their samples collected before the evolution to liver failure. They progressed to death before transplant procedure, according medical records. So they will include into ALF group. Therefore, statistical analyses were performed according mentioned above (inserted these three cases in ALF group), and will be carefully checked and any changed was found with this information. The corrections were performed in revised manuscript.

Line 324: The authors do not present evidence to this statement. “Liver inflammatory condition” is a vague phrase. There were no biopsies done to quantify this. The elevated levels of cytokines may be due to “systemic inflammatory condition” [SIRS], seen with ALF rather than “Liver inflammatory condition”. The appropriate heading for the results presented would be “Elevated plasma cytokine levels are seen in acute hepatitis and ALF compared to healthy controls”

Responses: Thank you for this observation. The appropriate heading for this result was inserted.

Again, I think that the results section is very long and there are too many tables. My suggestion would be to remove all analyses involving TB [bilirubin], ALT, and AST. These parameters have been correlated to outcome in many bigger studies in the past, without much conclusive evidence to their use. Hence, my suggestion would be to remove these analyses from lines 332-336, 347-354, 373-380, 389-395.

Responses: I’d appreciate this suggestion. The analyses involving total bilirubin, ALT, and AST were removed from results and information was added in table 1.

Line 338: I think that the appropriate conclusion for results presented is “ Elevated plasma cytokines an... show positive correlation with the degree of liver damage [or hepatocyte loss], as represented by presence of HE or coagulopathy”

Responses: The correction was performed according your suggestion.

I think that Table 3 is unnecessary

Responses: Table 3 was removed

Line 356: I think that the conclusion is “Elevated cytokine levels correlate with outcome in ALF”.

Responses: The correction was performed, including information about mitochondrial DNA results.

Also, Only 10 patients died [7 in ALF group] and 3 in acute hepatitis group. Hence, I feel that this section of results can be summarized better. Again, Table 5 is unnecessary.

Responses: I will rewrite carefully with correct information. The table 5 is removed.

Please do mention the number of survivors. I hope that the healthy subjects were not included in these analyses.

Responses: The number of survivors was included. The healthy subjects were not included in these analyses, just acute liver disease patients, such as self-limited acute hepatitis (AH) and acute liver failure (ALF) patients. This part was carefully rewrite such as suggested.

I think that the results presented in the remainder to the paper are really very interesting [PBMC analyses].

Responses: Thanks. I revised carefully and seriously.

Figure 3 can be a supplementary figure. Please remove figure 4. Please include mtDNA in figure 3.

Responses: The figure 3 was designed for supplementary figure 1, as suggested. Figure 4 was removed and mtDNA analysis was inserted in figure 3. The figures were reorganized along the manuscript.

Discussion

Please remove line 468. Again, I think that the discussion regarding AST, ALT and Tb should be removed. I do not understand the importance of lines 478 – 482 in the discussion.

Responses: Line 468 was removed. The phrase in lines 478-482 "Liver mitochondrial toxicity has also been described as an adverse effect of nucleoside/nucleotide analog therapy in patients who underwent long-term antiviral treatment [38]" should be removed before first submission. My mistake, this sentence does not make sense, so this was removed.

I feel that the phrase describing cytokines in Dengue Hemorrhagic fever is irrelevant and should be removed; or if kept, the reference to current context should be explained better.

Responses: Thanks for this observation. I revised this phrase carefully, and it was removed, because severe dengue fever paper mentioned here does not have any relation with liver alterations. So, this phrase does not make sense for this manuscript.

I think that lines 489-501 describing the work of others do not add much to the discussion. Instead, please discuss about biological relevance of IL 8 and 10. What do you think is the mechanism which leads to poor outcomes in liver injury ALONG with elevations of IL-6 and 8 i.e are these secreted by a particular cell, which is associated with worse liver inflammation in preclinical models?

Responses: Monocytes have been an important role in liver inflammation during acute liver diseases. High levels of IL-6, IL-8 and IL-10 have been associated with macrophages differentiation on the intrahepatic environment and inflammatory status during acute hepatitis and liver failure. In our study, we do not have explored the monocytes profile (CD14 and CD16 markers), but cytokines production by monocytes and those can be activate these cells by antigen presentation, and T cell proliferation were elevated in our ALF group and associated to worst outcome. So, a paragraph about was inserted carefully in Discussion section.

Please remove lines 511-516. These limitations are already known.

Responses: The lines 511-516 were removed.

The paragraph on limitations may be moved to the end of the discussion.

Responses: The limitations were moved to the end of the discussion.

I think that the authors should emphasize the “time effect” limitation of the study. The patients were enrolled 8-12 weeks after the onset of clinical manifestations. Hence, we do not know whether the cytokine profile, impairment of PBMC response to stimulants or the high T reg frequencies would be seen early in the course of the illness. It is very important to predict the course early in the course of ALF. Most of the patients would have met their outcome within 3-4 weeks of onset of illness. A follow-up study which the authors could propose/do is to validate these results early in the course of the illness.

Responses: Actually, the time of sample collection from patients was not well explained. So, the time (minimum and maximum) for blood collection on the first version of this manuscript was considering the first general symptoms, such as headache, for example. It was revised based on medical records, and our group in this revision decided it was better if the time was based on weeks after onset of jaundice and liver enzyme levels for self-limited acute hepatitis. For ALF patients, the sample collection was based on the time after ALF diagnosis and hospital admission. This correction was performed and the new information was included in M&M section, Results section, and Discussion section.

Thank you for the time taken to review our work.

Reviewer#2:

Acute liver injury is very common. The causes of acute liver diseases involve a variety of viruses, toxic drugs, alcohol, metabolic diseases, and severe bacterial infections. They are severely harmful to the health of mankind. Virus infection is the most common cause. Such as, HAV, HBV, HCV, HEV, EBV, CMV et al. In recent years, the acute viral hepatitis caused by HAV and HBV is significantly reduced due to the extensive vaccination. Most HAV infection is self-limited, and acute HBV infection is easy to cause liver failure. In this paper, the authors analyzed and compared clinical biochemistry index and immune index between acute hepatitis and acute liver failure. They want to get an accurate marker to predict the necessity for liver transplantation which is very

important for guiding clinical work. Mitochondrial damage is common in the course of acute liver injury which can predict the severity of liver damage. This study is of great clinical significance.

But there are still some problems in the following aspects:

1. There are many reasons for liver injury, although it may be related to the immune response, but the pathogenesis is not the same. Because the author does not clear the virus, drugs, and other groups, so there may be more confounding factors. Whether the grouping method is reasonable?

Responses: Details about the study population and group definition was unclear in my first submission of this manuscript. Therefore, to better explain the study population groups in my study, I rewrite carefully a paragraph on the M&M section and rewrite the table 1. In the Results section, I wrote carefully a phrase that justify why the parameters analyzed were generalized by clinical condition. Actually, we did not found significant differences when etiologies were compared. Every changes were in red color font along the text.

2. Disease has a development process. Acute hepatitis can still develop into liver failure. In this paper, there are 3 cases of HA group of death. What is the cause of death? Is the development of liver failure and death?

Responses: My mistake. The table 1 submitted for the first time was wrong. Thanks for this observation. Three patients with acute HAV infection, INR<1.5 and no coma grade (HE<I) had their samples collected before the evolution to liver failure. They progressed to death before transplant procedure, according medical records. So they will include into ALF group. Therefore, statistical analyses were performed according mentioned above (inserted these three cases in ALF group), and will be carefully checked and any changed was found with this information. The corrections were performed in revised manuscript.

3. In this paper, the specimens were taken from the cross section, but the time was not clear. The blood samples were collected in the early, middle and late stages. Its comparability is not the same. Need further clarify.

Responses: Actually, the time of sample collection from AH and ALF patients was not well explained in the first submission. This observation was also noted by other reviewer. So, the time (minimum and maximum) for blood collection on the first version of this manuscript was considering the first general symptoms, such as headache, for example. It was revised based on medical records, and our group in this revision decided it was better if the time was based on weeks after onset of jaundice and liver enzyme levels for self-limited acute hepatitis. For ALF patients, the sample collection was based on the time after ALF diagnosis and hospital admission. I revised seriously and corrections were performed in the M&M section, Results section, and Discussion section

Thank you for the time taken to review our work.

Reviewer#3

COMMENTS TO AUTHORS

Interesting findings , though not novel. But the data from Hepatitis A predominant cohort with liver failure.

Thank you for the time taken to review our work.