Dear Editor,

Thank you very much for your letter and advice. We have revised our manuscript, according to reviewers, editorial office's comments and suggestions. We responded point by point to the comments as listed below and had made the changes in the paper. The revisions that we make to the revised manuscript have been highlighted in red in the updated version of the manuscript and we also explained the revisions at last of this letter. We hope that the revised manuscript is acceptable for publication.

Thank you.

With best wishes,

Yours Sincerely,

Zhao-Lian Bian

Response to Reviewers

We would like to thank the reviewers for the constructive comments.

Response to Reviewers #1

Major comments:

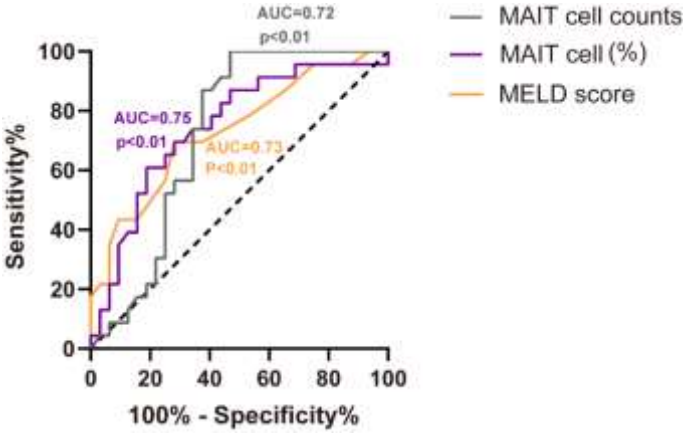
1. The most import disadvantage might be the small sample of patients, which creates queries when it comes from a university institution, why this study is not a multi-center one to avoid bias? why the authors did not recruit more patients in order to have better sample for subgroup analyses?

It is a good suggestion. But because the incidence of HBV related liver failure is decreased. Of course, we try our best to expanded our sample size within a limited time and added more data, and we have updated the relevant data in our manuscript.

2. Why they did not correlate and compare their "biomarker" with the

existing clinical scoring systems Like MELD and CLIF?

Thanks for your suggestion and we agree with your opinion strongly. We have added the data of MELD score in our study, and we also compared the prognostic value of MELD score with MAIT cells which showed in ROC curve. As for the CLIF score, because it is too tedious to be commonly used in clinical, so we did not get the details about the CLIF. In our paper, we added the MELD score which is commonly used in clinical. (Figure 6)



3. This study as the previous referred to by this one just presents a possibility which cannot stratify its results in clinical level safely and soundly. University institution should have higher targets and pave the way towards future not just record existing hypotheses.

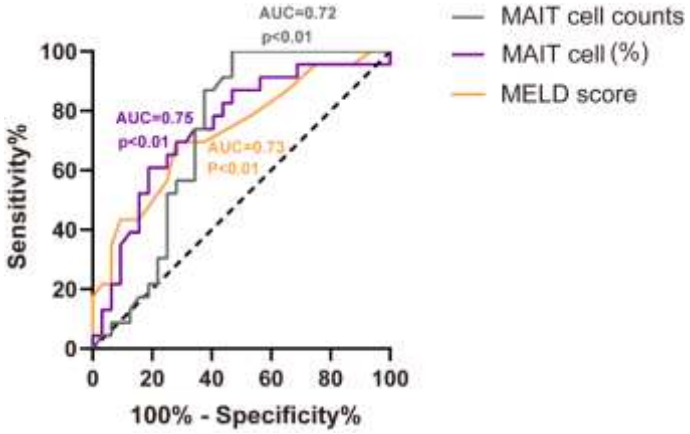
Thanks for your suggestion. Due to the absence of gold standard markers for prognosis of liver failure, it is important to find additional reliable markers for predicting its prognosis, which is still at exploring stage, maybe it is difficult, and we hope that our research will be worthwhile even if it brings certain reference value and ideas. And we will also do further research. Thank you again for your suggestions.

Response to Reviewers #2

Major comments:

I would suggest to add a comparative analysis regarding the prognostic power of circulating mucosal-associated invariant T cells as a marker and clinical scoring systems assessing the severity of the disease as they discussed in the introduction as MELD and CLIF scores.

Thanks for your suggestion and we have added the comparative analysis as suggested in ROC curve (Figure 6), and we added the MELD score which is commonly used in clinical.



The data showed that the proportion of MAIT cells can better predict the prognosis of HBV-related liver failure patients than MELD score and MAIT cell counts.

Response to Reviewers #3

Major comments:

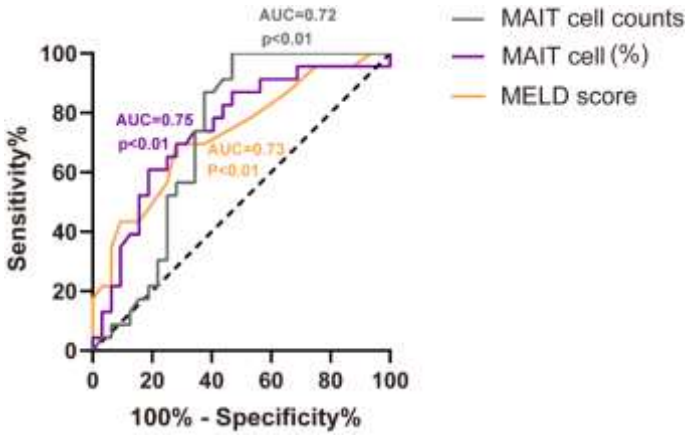
1. First, patients with HBV infection represent a heterogeneous group. Despite the authors stated that they enrolled patients with chronic form of the disease, the SDs of ALT and AST values require some comments.

Thanks for your suggestion and we have showed the SDs of ALT and AST in Table1, and we also added the data in paper as "The levels of alanine aminotransferase (ALT) (HBV-related liver failure *vs* HC: 549.60 ± 686.13 *vs* 16.55 ± 5.43, $P < 0.0001$; HBV-related liver failure *vs* CHB: 549.60 ± 686.13 *vs* 327.52 ± 312.57, $P < 0.05$), aspartate aminotransferase (AST) (HBV-related liver

failure *vs* HC: 487.55 ± 571.49 *vs* 22.95 ± 5.70, P < 0.0001; HBV-related liver failure *vs* CHB: 487.55 ± 571.49 *vs* 176.58 ± 208.19, P < 0.001) were dramatically increased in HBV-related liver failure group compared to HCs and CHB group."(page 9, line 1-9)

2. There are commonly used methods to assess decompensation of the liver function and assess prognosis in patient with chronic liver diseases (like Child-Pugh, MELD score, and others). It would be nice if these methods' results would be shown also to better describe patients' population and show correlation between previously described methods and MAIT cells counting.

Thanks for your suggestion and we have added the data of MELD score in our study, and we also compared the prognostic value of MELD score with MAIT cells as the ROC curve showed (Figure 6).



Child-Pugh classification can judge the liver compensatory ability of patients with liver disease, while MELD score is recognized prognostic indicator for liver failure, so we compared the ROC of percentage of MAIT cell, MAIT cell counts and MELD score.

3. Could you please explain the absence of difference between studied groups by albumin level? This situation is strange, as low albumin concentration is one of the main parameters that are used to establish the

presence of liver failure. Could this be caused by treatment (Albumin infusions)?

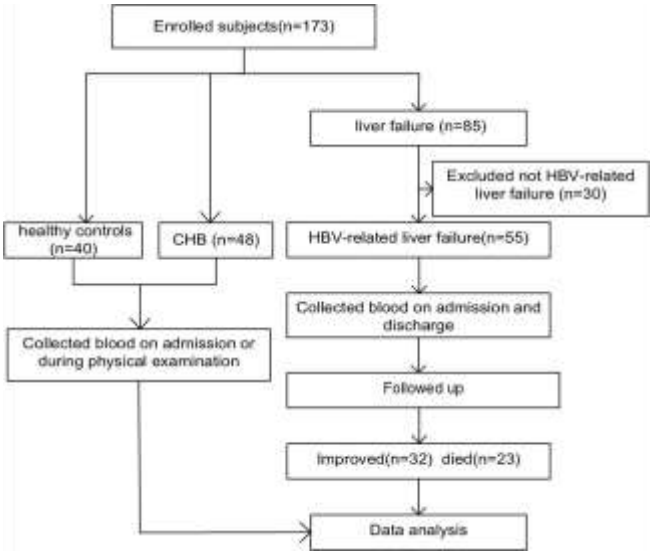
Thanks for your suggestion, because of our mistake, we did not note the difference in the manuscript. In the new version, we update our data and provide information about the difference of albumin level between studied groups, and data showed that the albumin level was significantly decreased in HBV-related liver failure patients (HBV-related liver failure *vs* HC: 31.01 ± 4.06 *vs* 42.98 ± 2.32, $P < 0.0001$; HBV-related liver failure *vs* CHB: 31.01 ± 4.06 *vs* 42.36 ± 3.99, $P < 0.0001$) compared to healthy controls and CHB patients (page 9, line 9-15). We have corrected that in our manuscript and marked in Table1.

4. The "other reasons" of liver failure are not described, please, give more details to ensure that liver failure was not associated with, for example, presence of liver cancer, alcohol intake or something else.

Thanks for your suggestion. We enrolled only HBV-related liver failure patients, and excluded liver failure caused by other reasons including liver cancer, alcohol intake and others.

5. To make the paper closer to the requirements of the good publication practice, please consider to add patients' flow chart and study design graph.

It is an excellent suggestion and we have added the patients' flow chart in our paper. (Figure 1)



6. Please, disclose the information about study registration (required per ICMJE recommendations). At least some of the measurements were made twice.

Thanks for your suggestion. We have downloaded the ICMJE recommendations, read it carefully, and improved our research and manuscript according to per ICMJE recommendations. All experiments were repeated three times.

7. However there is no description in which time points this was made. No data on the mean time of follow-up is provide. Please, consider to add this information to the paper.

Thanks for your suggestion and we have added the time of follow-up in our paper as suggested: "We followed up the 55 HBV-related liver failure patients for 2 to 90 days" (page 11, line 8-9).

8. Taking into the account that patients with end-stage chronic liver disease in most cases have at least some degree of malnutrition and decreased protein synthesis, it may be logical to assume that MAIT cells' decrease in patients with liver failure may be associated with this factor also.

Moreover, it has been described that in case of starvation or energy and protein deficiency, lymphocytes count in peripheral blood is decreased predominantly due to a dramatic fall of the number of CD3+ T-lymphocytes with relatively constant values of B-lymphocytes and null cells. It is clear that decreased protein synthesis and energy deficiency caused by liver failure are associated with poorer prognosis. But is there a need for relatively expensive and not widely available method to detect effects, that may be measured with much simpler tools? Please, consider to add this in the discussion. I disagree with the conclusion provided in the abstract that circulating MAIT cells may play a critical role in the PATHOGENESIS of HBV-related liver failure because it is more likely that on the contrary, liver failure and/or associated protein deficiency affect MAIT cells count.

Thanks for your suggestion and we agree with your opinion, maybe MAIT cells' decrease is associated with malnutrition, decreased protein synthesis and the reduction of lymphocytes, and we showed that the proportion of MAIT cells to $CD3^+$ T lymphocytes was also decreased significantly in patients with HBV-related liver failure, and the ROC curve also suggested that the proportion of MAIT cells is better for judging the prognosis(Figure 6). Therefore, maybe the lower proportion of circulating MAIT cells is more important for HBV-related liver failure patients. As your suggestion, we have corrected the conclusion provided in the abstract as" Circulating MAIT cells may play an important role in the process of HBV-related liver failure, and can be an important prognostic marker." And we also added some explanations in the discussion part. (page 14, line 14-18)

Response to Reviewers #4

Major comments:

I have with interest this manuscript, which concerns the role of circulating mucosal-associated invariant T cells in patients with HBV-related liver failure.

Overall, the paper is well-written and gives to the reader the right perspective on this topic. I believe that this manuscript will be of interest to the readership of WJG.

Thank you for your approval sincerely.

Response to Reviewers #5

Major comments:

1. At results: You have presented data about levels and percentages of MAIT cells in patients with HBV-related liver failure and in CHB patients compared to HCs, however it is clear from your figures that the MAIT cells count and percentages had still significant lower values in patients with HBV-related liver failure when compared to CHB patients. These data are important as it may indicate that liver failure in such patients may represent a more advanced and aggressive state of inflammatory cascade that may be incriminated in the more depressive effect on the status of circulating MAIT cells. You have to demonstrate this issue statistically.

Thanks for your suggestion, we learned and are appreciated with your important opinion that "liver failure in such patients may represent a more advanced and aggressive state of inflammatory cascade that may be incriminated in the more depressive effect on the status of circulating MAIT cells". We have provided more detail information to demonstrate this in our paper as suggested: "Circulating MAIT cells exhibited a significant decrease in HBV-related liver failure patients compared with CHB patients (percentage: 2.00 ± 1.22 *vs* 3.59 ± 0.87%, P < 0.0001; number: 5.47 ± 4.93 *vs* 48.26 ± 15.45, P < 0.0001) (Figure 2B and C)." (page 9, line 26-29). we also added this important opinion in the discussion part (page 13, line 7-9).

2. At table 1, it would be more informative to indicate the statistical significance in liver functions between CHB and HBV-related liver failure

groups.

Thanks for your suggestion and we have added the details in Table1. ([a]P < 0.0001 vs HC, [b]P < 0.05 vs CHB, [c]P < 0.01 vs CHB, [d]P < 0.001 vs CHB, [e]P < 0.0001 vs CHB.)

Clinical parameters	HC	CHB	HBV-related liver failure
Number	40	48	55
Age (yr)	47.05 ± 14.70	41.63 ± 13.08	47.89 ± 11.04
Male, (*n*)	24	27	37
ALT (U/L)	16.55 ± 5.43	327.52 ± 312.57	549.60 ± 686.13[a,b]
AST (U/L)	22.95 ± 5.70	176.58 ± 208.19	487.55 ± 571.49[a,d]
Total bilirubin (μmol/L)	12.48 ± 3.23	25.65 ± 43.43	302.52 ± 144.84[a,e]
Albumin (g/L)	42.98 ± 2.32	42.36 ± 3.99	31.01 ± 4.06[a,e]
Prealbumin (mg/L)	NA	109.17 ± 68.43	174.09 ± 81.47[d]
Cholinesterase (U/L)	NA	6536.11 ± 2543.80	3214.65 ± 1663.35[e]
Prothrombin time (s)	NA	NA	25.44 ± 8.30
International normalized ratio	NA	NA	2.22 ± 0.74
Prothrombin activity (%)	NA	NA	29.87 ± 9.09
Leucocyte ($\times 10^9$/L)	5.77 ± 1.60	5.01 ± 1.30	6.74 ± 4.18[c]
Lymphocyte number ($\times 10^9$/L)	2.64 ± 0.73	1.70 ± 0.60	3.08 ± 5.40
Blood platelets ($\times 10^9$/L)	194.68 ± 41.53	157.52 ± 67.53	97.96 ± 52.06[a,e]
Creatinine (μmol/L)	NA	63.08 ± 13.24	96.69 ± 123.50
hs-CRP (mg/dl)	NA	NA	20.04 ± 29.97
Log (HBV DNA (IU/mL)	NA	6.81 ± 1.33	5.27 ± 1.79[e]

3. At discussion, you mentioned MAIT cells also play a critical role in liver diseases by promoting hepatitis and fibrosis, maintaining intestinal permeability, and responding to biliary epithelium cells. These statements need more clarification to indicate how these cells do with the favor or against these situations.

Thanks for your suggestion and we have added some explanations in the discussion part (page 12, line 18-26). Here, we will also provide some explanations as below:

MAIT cells were reported to be activated in patients with cirrhosis and displayed a proinflammatory profile, the profibrogenic functions of MAIT cells suggested that targeting MAIT cells may constitute an attractive

antifibrogenic strategy during chronic liver injury[1]. And MAIT cells has the antibacterial potency and play a key role as biliary firewall protecting the epithelial lining from translocated bacteria. In patients with alcoholic liver disease, the antibacterial potency of MAIT cells impaired as a consequence of contact with microbial products and microbiota, resulting in the susceptibility to infection[2].

[1] Hegde P, Weiss E, Paradis V, et al. Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver [J]. Nature communications, 2018, 9(1): 2146.
[2] Riva A, Patel V, Kurioka A, et al. Mucosa-associated invariant T cells link intestinal immunity with antibacterial immune defects in alcoholic liver disease [J]. Gut, 2018, 67(5): 918-30.

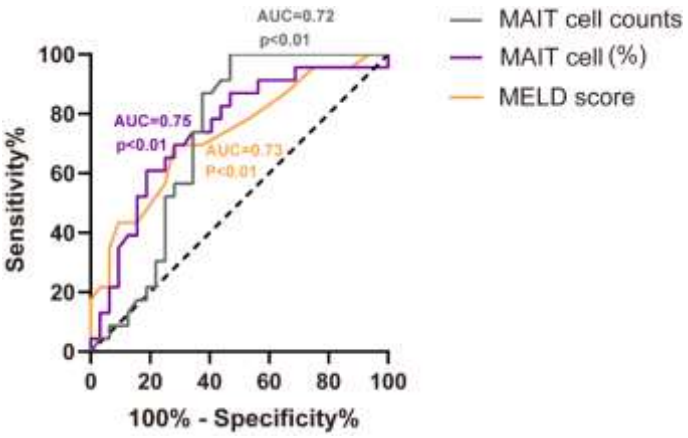
4. It seems that the status of MAIT cells peripherally in circulation may not reflect their counts and percentage centrally inside the liver and hence the functional impact inside the liver may be totally different than the value of the peripheral expression of these cells in circulation. This may represent the phenomena of homing of lymphocytes in general inside the liver at the site injury with its depletion peripherally in the circulation.

We are appreciated with your valuable opinion and we have learned this carefully, we agree with your opinion and we will do further research to better understand the different status of MAIT cells inside the liver and in circulation.

5. Actually, discussion of your findings is lagging behind offering an explanation to the relevance of depleted MAIT cells peripherally on outcome of both CHB and HBV-related liver failure. Furthermore, the prediction needs cutoff values derived from ROC curve studies with sensitivity indices which is not present at your study and is difficult to

perform due to small sample size.

Thanks for your suggestion. We have expanded our sample size and added the ROC curve as below, and data suggested that the proportion of MAIT cells can better predict the prognosis of HBV-related liver failure patients than MELD score and cell counts.



In addition, we would like to explain that, we have not updated the Figure 4 and Figure 7, the reason:

1) Some blood samples can not be collected before death, leading to a less data for pre- and post-treatment.

2) As for the plasma cytokine detection, our reagents have been used up, and due to the epidemic situation of COVID-19, we can't obtain it in a short time. Then the plasma cytokine detection still keeping the original data.

Other figures have been updated with added data.

We have revised our manuscript and required accompanying documents following the list of issues carefully. And we will explain the revisions as below:

STROBE Statement: we have downloaded and completed the 'STROBE Statement—checklist of items' to ensure our manuscript meets the requirements of the STROBE Statement. And we also stated on the

manuscript that the guidelines of the STROBE Statement have been adopted.

Style and format: we have revised the file format, length and page as required.

Abbreviations: we have defined the abbreviations upon first appearance in the Abstract, Key words, Core tip, Main Text, Article Highlights, Figure Legends, and Tables. We didn't use non-standard abbreviations.

Ethics: we have uploaded the Chinese version of the Institutional Review Board's official approval and Signed informed consent form(s) or document(s).

Manuscript organization: Our manuscript has been organized as required.

Title: we have corrected our title as required (marked in red). There are no abbreviations in the title and no more than 12 words.

Running title: we have shorten the running title to no more than 6 words.

Citation: we have added this in our paper as required and highlighted in red.

Introduction, Materials and Methods, Results, Discussion, and Conclusion (optional): we have corrected these as required.

Article Highlights: As required, we have added these in our manuscript and highlighted in red.

References: We have checked carefully to verify the accuracy and completeness of references and for correct in-text citation, and the format has been modified as required.

Figures and Tables: We have checked and corrected the format in the figures and tables, and we will provide the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed; and In consideration of color-blind readers, we didn't use red and green for contrast in vector graphics or images.