# Response to the reviewer comments

Comment 1. Was a sample size calculation done?

Response: Thanks for your question. This is a retrospective study based on the completed clinical trial. The 4-week mortality rate was 19.9% (Of 567 patients conformed the diagnosis of HBV-ACLF, 113 cases was deceased within 4 weeks). An estimated mortality in the high-risk group was 25%. Considering a power of 0.80, 195 subjects are needed for each group to achieve the significance level of 0.05 to detect a relative risk radio of approximately 2.0. Finally, from the total 1059 patients, all the patients (412) who met the inclusion criteria were selected and enrolled in this study.

Comment 2. Being a retrospective study, why was it not extended up to date / recent (study was done from 2012-2014)?

Response: HBV-ACLF is a disease with a high short-term mortality, so usually short-term follow-up such as 4 weeks or 12 weeks can make sense for this disease. Once survival from the short-term, most patients will become stable within 4 weeks. Therefore, our study only focused on the 4-week mortality in these patients.

Comment 3. How was alcohol excluded (based on amount of intake / label as substance user)?

Response: Sorry for my negligence. A history of alcohol intake was identified by the Alcohol Use Disorders Identification Test. The detail (including a citation,[16]) was added (Page 6, Line 13).

Comment 4. Being multicentric, how was IL6 estimation standardized?

Response: We have a unified standard flow for all the centers from the draw of blood, storage, shipment, to the testing, in order to ensure the quality of the study. IL-6 (Elecsys IL-6 kit, electrochemiluminescence immunoassay) were uniformly determined in serum using the Cobas 8000 analyzer. Quantification of IL-6 was added in text (Page 6, Section 5).

Comment 5. How was uniformity assured at times when the IL6 estimation was done? How many times samples were drawn during the course of stay?

Response: All the samples were batched tested with the same method at the only designated testing agency.

The samples were drawn at enrollment and 4 weeks from the enrollment during the course of stay. The detail was added in “method” section (Page 6, Section 5).

Comment 6. Renal 'disfunction' mentioned in tables 1-3 not defined.

Response: Renal dysfunction was defined by serum creatinine levels ranging

from 1.5 to 1.9 mg/dL. The corresponding endnotes have been added to the text (Pages 20-22, Tables 1-3).

Comment 7. What exactly were the infections other than SBP?

Response: Infections other than SBP included respiratory, urinary and digestive infections, as well as sepsis. The detail was supplemented in the text (Page 7, Line 28).

Comment 8. How to account for the discrepancy in mortality between groups A & B with regard to IL 6 values (high IL6 - higher association with death, whereas group A had high IL6 value with low mortality compared to group B with low IL6 value and higher mortality)

Response: In additional to the results shown in the manuscript，I have carried out a statistical analysis to compare the difference of mortality among the 4 groups by chi-square tests, and the result showed no significance (5.0% vs 7.5% vs 11.5% vs 16.7%, P=0.151; 5.0% vs 7.5%, P=0.526). Interestingly, the increasing trend of the mortality rate with the dynamic changes of IL-6 was significantly meaningful by Cochran-Armitage test for trend. The finding in addition indicates dynamic monitoring of IL-6 within 4 weeks could be beneficial for judging the prognosis.

Comment 9. "According to the dynamic changes in IL-6 within 4 weeks, patients were classified into four groups, .." Is it the total number ? Table 3 A+B+C+D = 246 (total 412)

Response: Sorry for the confusion. The dynamic changes in IL-6 within 4 weeks were based on twice IL-6 results at baseline and 4 weeks, therefore, of 335 cases who were survival at 4 weeks, 89 cases without second IL-6 results were excluded, so the total number of 246 in 4 groups corresponds to the patients which have twice IL-6 results at baseline and 4 weeks. The detail was shown in text (Page 7, Line 29) and the corresponding changes have been added to the flow diagram (Page 19). Hopefully, it will help to understand. Besides, the bias due to missing data was discussed in study limitation, which showed that the conclusion is stable (Page 11, Line 20 and Supplementary Table 1).

Comment 10. "IL6 was an independent prognostic factor". Is the data sufficient to reach this conclusion?

Response: In this study, we corrected the potential confounding factors (age, bilirubin, creatinine, INR, and the presence of hepatic encephalopathy and upper gastrointestinal bleeding) according to univariable analysis and clinical correlation, in order to ensure the reliability of the results. Through the result of multivariate analysis, it is reasonable to reach the conclusion that IL-6 was an independent prognostic factor of HBV-ACLF.

Comment 11. Whether level of IL6 responsible for poor prognostic significance

/ outcome determination in ACLF available? Were HBV titre and IL6 co-related?
Response: Based on our study, we could only conclude the association between high level IL6 and the poor prognosis of ACLF. Whether IL-6 was responsible for the poor outcome needs further study.
In this study, we analyzed the level of HBVDNA (log10 IU/ml) between patients with high IL-6 and patients with low IL-6 (Table 2), and the result was not significant (3.1±2.4 vs 3.1±2.2, P=0.825). Besides, IL-6 was reported as a biomarker related with inflammation and tissue homeostasis in liver. Therefore, no evidence showed that IL-6 was co-related with HBV titre currently.

Comment 12. Other study limitations not acknowledged such as multicentre / sample size, etc
Response: Thanks for your suggestion. We analyzed other limitations including selective bias resulting from missing cases which were supplemented in the original section (Page 11). The corresponding details were shown in Supplementary Table 1, which were submitted as supplementary materials. Although the patients were from multi-centers, we have taken some measures to avoid the heterogeneity caused by multi-centers as possible, such as third-party supervision, a unified standard flow for samples collection and test，as well as data management.

Comment 13. References not in appropriate format (author numbers, abbreviated journal name)
Response: Thank you for pointing out this mistake. All the references were corrected according to the Format of WJG for references guidelines by EndNote.