Format for ANSWERING REVIEWERS

December 30, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4559-edited.doc).

Title: HBsAg levels in HBeAg-positive chronic hepatitis B patients with different immune conditions

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 We would like to express our sincere thanks to the reviewers for the constructive and positive comments.Revision has been made according to the suggestions of the reviewer.
Reply to Reviewer 1,
Major points

1. Authors grouped CHB patients to IT group, IC-Mild group, and ACLF group. The rationale of this grouping is not clear. Authors should explain the reasons for this grouping in more detail.

Answer: The primary aim of this paper is to to investigate whether or not dramatic immune response exhibiting acute on chronic liver failure will result in different HBsAg levels comparing with mild immune response in patients with immune clearance phase, which may indicate anti-viral therapy results. In addition, HBsAg levels should be compared with immune-tolerance patients to determine whether or not different characteristics are observed when patients in the immune clearance phase are subdivided according to different immune clearance condition. So three groups of patients were enrolled in this study: patients with mild immune response in the immune clearance phase, IC-Mild group; patients with dramatic immune response in the immune clearance phase and exhibited acute on chronic liver failure, ACLF group; and immune-tolerant patients, IT group.
We have revised the introduction to explain the reason for these three groups of HBeAg-positive patient enrolled in this study in detail.

2. Tables. Explanation or legends of tables is not enough. Authors should describe tables more correctly and reader-friendly.

Answer: We have double-checked the tables then corrected legends according to the suggestion.

Table 1. Just show some mistakes or inappropriate points.
(1) The location of "(median, interquartile range)" is strange. The explanation should be in the table legend and should be described more correctly.

Answer: The location of "(median, interquartile range)" has been switched to the legend of Table 1. and correct the explanation.

(2) "Gender (M/F)" should be "Gender (male/female)".

Answer: Correction has been made in the revised version.

(3) "10" in "log10 IU/mL" should be subscribed.

Answer: Correction has been made in the revised version.

(4) "Total Bilirubin" should be "total bilirubin". Why do authors capitalize only "Total Bilirubin"?

Answer: It is a mistake to capitalize the "Total Bilirubin". Correction has been made in the revised version.

Table 2. (1) Authors described "r" values in the text (page 8, lines 18-20). Authors should add the data in Table 2.

Answer: It may not been expressed clearly about the relation between Table 2 and the text. What we want to express in Table 2 is the "Multivariate linear regression with HBsAg levels" in text(this table has been deleted in revised version according to another reviewer's suggestion). However "r" values in the text (page 8, lines 18-20 in original version)refer to the "r" in Figure 3.

(2) Explain "beta".

Answer: "beta" means "standard coefficients", the explanation has been added to the legend of "Table 2" (this table has been deleted in revised version).

3. Figures. Figure legends are too short and not enough.

Answer: More details about the Figure have been added to the Figure legends in the revised version.

(1) Dots in figures are too weak!

Answer: Dots have been deepened in the revised version.

(2) “10” in “log10 IU/mL” should be subscribed.

Answer: Correction has been made in the revised version.

4. Statistical analysis. Description of “Kruskall-Wallis ANOVA” (page 6, line 3 from the bottom) is wrong. Kruskall-Wallis is a non-parametric statistical analysis method and ANOVA is a parametric method. They are different statistical method!

Answer: In this paper, we used “Kruskall-Wallis test” for statistical analysis which means the non-parametric statistical analysis method. It is a mistake to express it as “Kruskall-Wallis ANOVA” for imitating such expression from one reference paper. We have revised the expression from “Kruskall-Wallis ANOVA” to “Kruskall-Wallis test”.

Minor points There are many careless mistakes through the manuscript. Authors should check and correct them.

Answer: We have double-checked the manuscript and correct some mistakes according to your kindly suggestion. For example, we correct the unit of HBV DNA in table 2 from “log 10 IU/Ml” to “log $_{10}$ IU/mL”.

Some of them are listed below.
Page 2, line 3. Add “(CHB)” after “chronic hepatitis B”.
Answer: Correction has been made in the revised version.

Page 2, line 7 from bottom. Spell out “INR” when it comes out first. And explain INR somewhere in the manuscript. INR is not familiar for readers whose expertise is not in hepatitis.

Answer: INR has been spelled out when it comes out first in revised version. Explanation of INR are given in the revised version.

Page 3, line 10. Remove “infection”.
Answer: Correction has been made in the revised version.

Page 3, line 21. Add a space between “Serum” and “HBeAg”.
Answer: This part has been revised in combination with the suggestion of another reviewer.

Page 5, line 5. Spell out “ULN” when it comes out first. And explain ULN.
Answer: Correction has been made in the revised version. We think may the spell of “ULN” as “upper limit of normal” also explain the meaning of “ULN”.

Page 6, line 8. Remove “and”.
Answer: Correction has been made in the revised version.

Page 6, line 20. Remove “Germany”. It already appeared in line 17.
Answer: Correction has been made in the revised version.

Page 6, lines 20, 21. “if HBsAg level > 250 IU/ml” should be “if the HBsAg levels were more than 250 IU/ml”.
Answer: Correction has been made in the revised version.

Page 6, line 23. “for the samples with HBV DNA > 108 IU/ml” should be “for the samples with > 108 IU/ml HBV DNA level”.
Answer: Correction has been made in the revised version.

Page 7, line 3. “San Diego” should be before “USA”.
Answer: Correction has been made in the revised version.

Page 7, line 4. “P” should be italicized.
Answer: Correction has been made in the revised version.

Page 8, line 16. The subtitle should be “Correlation between serum HBsAg levels and serum HBV DNA levels”.
Answer: Correction has been made in the revised version.

Page 9, line 3. Add “significantly” after “serum HBsAg levels”.
Answer: This part has been revised in combination with the suggestion of another reviewer.

Page 9, line 5. “and” reads “or”.
Answer: This part has been revised according to the in combination with the suggestion of another reviewer.

Page 10, line 10. “triggled” reads “triggered”.
Answer: Correction has been made in the revised version.

Page 10, lines 11, 12. “HLA-class I antigen-restrict cytotoxic T lymphocyte” reads “HLA class I-restricted cytotoxic T-lymphocyte”.
Answer: This part has been revised in combination with the suggestion of another reviewer.

Page 10, line 14. “destroied” reads “destroyed”.
Answer: Correction has been made in the revised version.

Page 10, line 20. Add “be” before “related”.
Answer: Correction has been made in the revised version.

Page 10, line 22. “predominantly” reads “predominant”.
Answer: Correction has been made in the revised version.

Page 11, line 7. “number” reads “amounts”.
Answer: Correction has been made in the revised version.

Page 11, line 8. “Peg-IFN” should be spelled out when it appears first and explain it.
Answer: “Peg-IFN” has been spelled out when it appears. The explanation of “Peg-IFN” was given in the same time.

Page 11, line 14. Remove “of”.
Answer: Correction has been made in the revised version.

Page 11, line 18. “greater” should be after “102 to 105”.
Answer: Correction has been made in the revised version.

Page 12, line 5 from bottom. “crossection” reads “cross-section”.
Answer: Correction has been made in the revised version.

The format of References section should be followed to the format described in Instructions for Authors of WJG.
Answer: Correction has been made in the revised version.

Reply to Review 2.

The authors have investigated the role of quantitative HBsAg in HBeAg-positive IT, IC and ACLF patients. They have shown modest correlation of HBsAg levels with HBV DNA and differences in HBsAg levels in the 3 categories of the patients. The study has a number of shortcomings:

1. The English language needs significant improvement. There are numerous spelling mistakes, and places where the space between words in missing.

Answer: We have double checked the paper and correct spelling mistakes. The paper were also edited by professional English language editing companies before the revised version were submitted.

2. There is no new message from the study – similar studies with similar results have already been published in HBeAg-positive patients. The authors contradict themselves by stating in the Introduction that there are no studies in the IC group while in Discussion, they cite studies in IC patients while comparing their results with those studies.

Answer: Thanks for your kindly suggestion. It may not be expressed clearly in our early version. What we mainly want to express is to investigate whether the HBsAg levels is different in various immune conditions in IC phase. In previous study(reference 10,11 and

12), scientists considered all the patients in IC phase as a whole or ACLF patients were excluded in the study .In this study, we compared the HBsAg levels between patients with mild immune response in IC phase, IC-Mild group; and patients with dramatic immune response ,ACLF group. The result showed the HBsAg level are lower in special group of IC patients, ACLF group. We revised this part in introduction part which will make it much clearer.

3. The majority of the patients in the ACLF group seem to have low platelets (Table 1) and hence likely cirrhotic. As such the statement in the Methodology regarding exclusion of cirrhotics patients is misleading and in my opinion can be removed. I cannot understand the rationale for excluding cirrhosis, particularly since the study does not aim to study the histological stage in relation to HBsAg levels.

Answer: Thanks for your kindly suggestion. We think the low platelet level in ACLF patients may because that liver failure often leads to disseminated intravascular coagulation which presents low platelet even the patients do not have history of chronic liver disease. I try to explain the exclusion the patients with cirrhosis. In our small scale clinical observation, we found that the serum HBsAg levels of HBeAg-positive CHB patients are lower than the patient without cirrhosis. So we exclude cirrhosis patients to reduce the influence of cirrhosis which may indicate another immune condition. If you can kindly agree with our opinion, we are so grateful.

4. Were patients excluded if they had recently received chemo/immunotherapy? This is important since the mechanism for immune response in such patients is externally stimulated, and hence the HBsAg response may potentially be also different. The authors should clearly state whether such patients were included and if so, then I recommend that this category of patients be excluded from the analysis.

Answer: Thanks for your kindly suggestion. This category of patients were excluded and stated in early submitted version（page 6, line 1）.

5. The inclusion exclusion criteria are lacking in comprehensiveness. For instance, were pediatric patients included; HCC; chemo/immunotherapy?

Answer: In this study, pediatric patients were excluded and missed to stated. It has been stated in revised version, the exclusion of cancer (including HCC) and chemo/immunotherapy patients were also stated in revised version.

6. The authors should clarify how patients were determined to have normal ALT (how many recordings?), how many HBV DNA recordings, at what time intervals, etc? It is well known that patients may have fluctuating ALT levels and this may impact on patient categorization. Note that the authors mention that ALT for IC group were <2 ALT. I assume this is a typo and I assume the authors mean to say ALT >2 and <10 ULN.

Answer: For IT and ACLF patients, serum HBsAg and HBV DNA level was determined once at the time of enrollment. For IC-Mild patients, a median of three-week follow-up (minimum two weeks, maximum eight weeks) was provided, serum HBsAg and HBV

DNA level were determined once at the time of enrollment and repeated at least once during the follow-up period. It has been stated in revised version.

7. It is unclear when the serum samples were collected. Were they collected at the time of the 1st visit or subsequently, since this will clarify if patients with ACLF had samples collected during the disease flare?

Answer: Serum samples for the HBsAg and HBV DNA test were collected at same time point at the time of enrollment or follow-up period. It has been stated in revised version.

8. I'm confused about the multivariate analysis – what exactly is the outcome measure here? I assume its HBsAg levels. If so, what level? Clearly then, there should be a cutoff level. If the outcome measure is the predictability to be in a particular category (as defined in the study) based on HBsAg level, then which category was the outcome measure? This entire objective, its methodology, its reporting in the results, the associated table and the discussion should be re-written completely. At present I fail to understand what the authors are aiming to perform.

Answer：It has been deleted in revised version as the suggestion.

9. The statement in the discussion "HBsAg production is significantly correlated with viral replication ($r = 0.30$, $p = 0.09$)." is misleading and incorrect. The result (of the previous study) cited is not significant. This should be changed.

Answer: It has been corrected as "HBsAg production is poorly correlated with viral replication ($r = 0.30$, $p = 0.09$)" in the revised version".

10. I'm unclear why patient categories definitions were chosen from 2 different guidelines (APASL and Chinese). I recommend categorization based on a single guideline in order to maintain uniformity and consistency.

Answer: The categories of guideline of ACLF adopted was switched to APASL guideline on ACLF. All the ACLF patients enrolled in our study accorded with this guideline. The reason we do not use the single guideline is that none of the guidelines cover all the definitions of all three groups in the study.

Replies to Reviewer 3

Authors have performed an interesting and straightforward study. Although sample size is not very large and main limitation comes from being a cross-sectional study, results are of value. However, some points should be addressed in order to improve the manuscript.

Major points –

(1)How did authors exclude other liver diseases? what was the threshold for alcohol consumption to rule out alcoholic liver disease?
Answer: Other liver diseases were excluded combined by biomarker determine and

history of patients. For example biomarker were determined to exclude viral hepatitis, autoimmune hepatitis and infection of HIV etc. Other liver diseases such as alcoholic liver disease, chemo/immunotherapy were excluded by history of patients. The alcoholic liver disease are ruled out according to these criteria, the intake of alcohol 40g/d (male) or 20g/d (female) for more than 5 years or intake of alcohol 80g/d according to Chinese Guidelines for diagnosis and treatment of alcoholic liver disease (2010 update) according to the history of drinking.

(2) Multivariate regression with such a small sample size is well known to be unreliable and prone to overfitting. Thus, it is likely that INR as a predictive factor for HbsAg is just a marker for hepatic failure. I recommend that authors perform multivariate linear regression considering all patients as a whole, in order to analyze which factors predict HbsAg in these kind of patients, but not in every specific subgroup, since sample size does not allow to do that.

Answer: We have tried to make multivariate linear regression according to your kindly suggestion considering all patients as a whole. However the HBV DNA was found as a single independent factor in all these patients. In combination with another review's suggestion, we delete this part.

(3)Discussion is rather long and should be shortened and focused on author's findings. Also reference list.
Answer：Revision has been made according to the suggestion.

(4) Please kindly revise the paper by a native English speaker since there are many mistakes. E.g., "andused" instead of "and has been used", "host's inmune" instead of "host inmune" or "understanting" instead of "understanding", and other minor mistakes and typos.

Answer: Professional language agency have revised the paper before resubmission. All the example mistakes has been corrected in revised version.

(5) Minor points - Please, P (p-value) should be italicized and in upper cases.

Answer: Correction has been made in the revised version.

- Since only ultrasound cannot completely rule out or confirm cirrhosis, authors should state that patients had no clinical or ultrasonographic findings of cirrhosis, instead of "Ultrasound B test was conducted and revealed that all of the patients did not suffer from cirrhosis"

Answer: Correction has been made in the revised version.

- ULN has not been defined. –

Answer: Correction has been made in the revised version.

Figure 3 is not referenced in the text. And figure 2 and "figure 2A" are incorrectly referenced.
Answer: Correction has been made in the revised version.

-Bilirubin levels are expressed in mol/L and mg/dL. Please unify.
Answer: Correction has been made in the revised version. All the bilirubin levels are unified to expressed in mol/L.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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