Answer Letter

15 April 2019

Professor Lian-sheng Ma

EDITOR-IN-CHIEF

World Journal of Gastroenterology

Dear Associate Editor Ma Ruoyu,

On behalf of all authors, I deeply appreciate your consideration of our manuscript entitled "On-treatment Monitoring of Liver Fibrosis with Serum Hepatitis B Core-related Antigen in Chronic Hepatitis B" (Manuscript NO: 46975) for publication in World Journal of Gastroenterology, and the invaluable comments/suggestions from the expertise reviewers. We carefully considered and digested all those comments and have carefully revised our manuscript and made a major revision based on these very valuable comments by you and the reviewers. Revisions in the text are used a different color font to highlight the changes and indicated in our point-to-point response letter to each of the comments. We feel that with the reviewers' inspiration, the re-written MS clarified all the reviewers' comments with significantly improved quality. We sincerely hope this resubmission will meet World Journal of Gastroenterology scientific criteria for publication.

Thank you gain and the reviewers for your support and consideration. Please feel free to contact me at your convenience, if further questions.

We are looking forward to your positive response soon.

Sincerely,

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Point-by-point Response Letter

Responses to the comments by the Reviewer #1

1. The title reflects the main subject of the manuscript, but it is difficult to read and includes a typo. "**Noninvasive" is inconsistently written with and without hyphen throughout the manuscript.**

Response: *Many thanks for your expertise suggestion. We fully agree with the reviewer's suggestions, and Revision has been made as following and in title page of the revised MS.*

On-treatment Monitoring of Liver Fibrosis with Serum Hepatitis B Core-related Antigen in Chronic Hepatitis B

2. **The authors wrote on page 14** "*As described previously, the HBV genotype was strongly associated with the presence of HBV variants(Tseng, Liu et al., Gut, 2015). However, we did not find any independent association between HBV genotype and serum HBcrAg level suggesting that HBV genotype is dependent on other factors.*" **This information is not logical. The genotype of the virus might depend on the geographical issues but can not be affected by any host or virus markers. Content of this part does not make sence and need to be revised**.

Response: *Thanks for your very nice suggestion. We fully agree and have revised this portion and in the page 14 of the revised MS.*

As noted earlier, the HBV genotype might depend on the biogeography, and not be affected by any host or viral proteins, but can influence the progression of liver disease (Halfon, Bourliere et al., J Viral Hepat, 2006, Khatun, Mondal et al., Sci Rep, 2018, Lapalus, Laouenan et al., Liver Int, 2015). Our results confirmed that there was no independent association between HBV genotype and serum HBcrAg level, indicating that HBV-triggered liver injury is mediated by host immune response to viral proteins (Khatun, Mondal et al., Sci Rep, 2018).

3. **The authors wrote on page 14** "*These results suggest that selection of BCP/PC*

mutants affect disease course and explain the HBcrAg level–liver fibrosis dissociation." **This term need to be explained and suggestion should be discussed in more detail, including references linking state of the art literature.**

Response: *We appreciate this important suggestion. We fully agree with the reviewer's suggestions, and have revised this portion and in the page 14, para 15 of the revised MS.*

Interestingly, we found that patients with BCP/PC mutation had a significantly lower serum concentration of HBcrAg when compared with their wild-type counterparts. Furthermore, after treated with entecavir for 72 weeks, the decline of HBcrAg level in patients with BCP mutation was significantly less than that of patients with BCP non-mutation. BCP mutation, which down-regulate HBeAg production, have been reported to be associated with advanced liver disease (Tseng, Liu et al., Gut, 2015). Therefore, selection of BCP/PC mutants can affect the HBcrAg level and explain changes of the HBcrAg level. Indeed, patients with BCP/PC mutants whose the decline of serum HBcrAg level less after treated by entecavir had a lower proportion of the regression of liver fibrosis.

4. ***Again on page 14*** *"Not surprisingly, HBcrAg showed a strong negative correlation with liver fibrosis staging in HBeAg-positive individuals, and positive correlation with that in HBeAg-negative individuals. Results corroborate those of Zhang et al(Zhang, Lu et al., J Virol Methods, 2016). who reported that HBcrAg levels correlated positively or negatively with liver fibrosis staging, irrespective of HBeAg status."***This discussion is insufficient, the HBeAg-related HBcrAg-dependent correlation with fibrosis need to be discussed in more detail!**

Response*: Thanks for your very expertise suggestion. We fully agree with the reviewer's suggestions. Indeed, this discussion is insufficient. References have been updated. The paragraph was revised as follows (added in Page 15, page16 of the revised MS):*

Whether the on-treatment monitoring of liver fibrosis with serum HBcrAg levels is unknown (Mak, Seto et al., Gut Liver, 2019, Mak, Wong et al., Aliment Pharmacol Ther, 2018, Zhang, Lu et al., J Virol Methods, 2016). In our study, the serum of HBcrAg level was significantly higher in treatment-naïve HBeAg-positive patients compared to treatment-naïve HBeAg-negative patients, due to the fact that HBeAg production (one of the components of HBcrAg) was delined after HBeAg seroconversion (Seto, Wong et al., Clin Microbiol Infect, 2014). More importantly, the serum of HBcrAg level showed a strong negative correlation with liver fibrosis staging in treatment-naïve HBeAg-positive individuals, and positive correlation with that in treatment-naïve HBeAg-negative individuals(Zhang, Lu et al., J Virol Methods, 2016), extended our current knowledge and suggested that the serum of HBcrAg level may be able to differentiate between chronic infection and chronic hepatitis(Mak, Seto et al., Gut Liver, 2019). We also found that HBV DNA was undetectable by PCR in 88.37% of patients treated with entecavir for 72 weeks, while HBcrAg was still detectable. Since nucleos(t)ide analogues have no direct inhibitory action on the transcription and translation of viral mRNA(Hsu, Nguyen et al., Aliment Pharmacol Ther, 2019, Wong, Tanaka et al., J Clin Microbiol, 2007), HBcrAg continues to be produced in spite of adequate suppression of viral DNA synthesis. The present study demonstrated that patients with high baseline serum concentration of HBcrAg resulted in a significantly higher regression rate of fibrosis/cirrhosis. Moreover, patients with significantly declined in HBcrAg level were more likely to have a significantly higher regression rate of liver fibrosis and better histologic improvement after treatment week 72. Hence, in nucleos(t)ide analogues treated patients whose HBV DNA has become undetectable by PCR, the use of HBcrAg measurement would be particularly useful for monitoring hepatic histological changes, especially in HBeAg-negative CHB.

5. Are the figures, diagrams and tables sufficient, good quality and

appropriately illustrative of the paper contents? **Figure 1 contains randomized groups treated with RTG or PLC, this need to be clarified. *HBcrAG is a calculated value shown in Figure 5 C and D. These data need to be explained in detail in the text and in the legend.**
Figure legends Fig.1, Fig. 2 and Fig 4, are insufficient. Information for stand-alone interpretation are needed.

Response: *Thanks for your very nice suggestion. We fully agree with the reviewer's suggestions, and have revised this portion and in figure 1 legend of the revised MS.*

Fig. 1 Flow chart of study population.

ETV, entecavir; PLC, placebo; RGT, biejia-ruangan tablet; HCC, hepatocellular carcinoma.

Fig. 2 Correlations of hepatitis B core-related antigen with Ishak fibrosis score and Knodell necroflammation score in HBeAg-positive (A, C) (*n* = 164) and HBeAg-negative (B, D) (*n* = 156) patients. Error bars, interquartile range; expression of measurement units: HBcrAg, log10 kU/mL. HBcrAg, hepatitis B core-related antigen.

Fig. 4 Variables associated with hepatic fibrosis. Odds of Ishak fibrosis score 3–6 by multiple ordinal logistic regression for the whole patient.

HBcrAg, hepatitis B core-related antigen. OR, odds rate; CI, Confidence interval.

6. Are the findings and their applicability/relevance to the literature stated in a clear and definite manner? Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently?

   No, discussion part contains redundant information regarding the results, but insufficient discussion and comparison to current literature.

Response: *Excellent comments! We have modified our manuscript.*

7. More recent literature should be cited, more than 50% of references are 10 years and older.

Response: *thanks you for your excellent comments! The references have been updated.*

8. The manuscript is well, concisely and coherently organized and presented.

However, enumerations within the manuscript make it difficult to read and to understand (i.e. page 7, inclusion criteria; page 13 strengths of this study, …)

Response: *We appreciate your expertise comments. We fully agree with the reviewer's suggestions, and have revised this portion and in the page 7, para 14 of the revised MS.*

Inclusion criteria were enrolled: (1) men or women aged 18-65 years, positive HBsAg for at least 6 months; (2) as for HBeAg-positive CHB, HBV DNA≥20,000 IU/ml and ALT≥2×upper normal limit, as for HBeAg-negative CHB, HBV DNA≥2,000 IU/ml and ALT≥2×upper normal limit, or clinically compensated cirrhosis with detectable serum HBV DNA regardless of HBeAg status and the ALT level at screening before liver biopsy(Anonymous, J Hepatol, 2015, Kim, Kim et al., Hepatology, 2015); (3) a baseline liver-biopsy specimen obtained showing an Ishak fibrosis score stage ≥3 within 4 weeks before enrollment; and (4) nucleos(t)ide analogues treatment naïve or anti-fibrotic therapy within 6 months before enrollment. The exclusion criteria included: co-infection with other virus hepatitis or chronic liver diseases; 2) liver biopsy was inadequate for grading and/or staging; 3) one more variables were missing; and 4) decompensated cirrhosis or history of any concurrent malignancy.

The main strengths of this study were: (1) the size, prospective, multicenter design and 291 patients underwent paired liver biopsy; (2) the nature of study population was relatively homogeneous (i.e., covering a full spectrum of fibrosis stage); (3) central pathological analysis was performed to unify the stage of liver fibrosis; and (4) except for cirrhosis as an observed endpoint, significant and advanced fibrosis was evaluated to prevent spectrum bias.

9. **i.e. the figure 1 represents the study design of a previous published manuscript. Patients are treated with RGT or PLC both groups are not further described or distinguished in the present manuscript.**

Response*: This is a good question. We feel sorry that we were not further described in*

our manuscript, and would like to done it in the revised MS.

This study was a multicenter prospective sub-study of our ongoing clinical trial (NCT01965418). The clinical trail was a prospective, multicenter, randomized, double-blind, placebo-controlled trial conducted in 14 institutions in China to determine the synergistic effects of a long-term (5-year) combination treatment of Biejia-Ruangan (RGT), a Traditional Chinese Medicine, and entecavir (ETV, i.e., the RGT group) on regression of advanced fibrosis or cirrhosis and the occurrence of HCC by comparing to ETV plus placebo (PLC) (i.e., the control group). The present study included 4 institutions of all focused to investigate on-treatment monitoring of liver fibrosis with serum of HBcrAg.

Responses to the comments by the Editor

CrossCheck:

According to the CrossCheck report, the similarity index between this manuscript and the published ones is too high, which doesn't meet the publishing requirements. Please revise the duplicated parts according to the CrossCheck report uploaded by the editor.

***Response*: We have revised and marked them by yellow highlight in the page 6-7 of the revised MS.**

Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).

For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies mentioned in **'The Revision Policies of BPG for Article'**.

Response: **We have provided the language certificate by professional English language editing companies.**

Audio core tip:

In order to attract readers to read your full-text article, we request that the author make an audio file describing your final core tip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information. The accepted formats are mp3 or wma.

Response: **We have made an audio file describing the final core tip and uploaded to the system.**

Please provide the decomposable figure of all the figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. - image files.ppt" on the system. Make sure that the layers in the PPT file are fully editable. For figures, use distinct colors with comparable visibility and consider colorblind individuals by avoiding the use of red and green for contrast.

Please read these four important guidelines carefully and modify your figure(s) accordingly:

First, all submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes.

Second, for line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes.

Third, please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor.

Fourth, in consideration of color-blind readers, please avoid using red and green for contrast in vector graphics or images.

For pictures with multiple parts, please create text box in the upper left corner with uppercase letters A, B, etc. ; please use SmartArt, text box and shape to draw the flowchart directly in PowerPoint;

Response: **We have submited it as ''Manuscript No.46975 - image files.ppt''.**

Your manuscript should be prepared with Word-processing Software, using 12 pt Book Antiqua font and 1.5 line spacing with ample margins.

Response: **We have modified it according to your requirements.**

Please revise and perfect your manuscript according to peer-reviewers' comments. Please upload the required files on the system.

Response: **We have uploaded the required files on the system.**

Please provide the author contributions. Authors must indicate their specific contributions to the published work. This information will be published as a footnote to the paper. See the format in the attachment file-revision policies. The format of this section should be like this:

Author contributions: XXX (family name should be put first in full, followed by middle names and first name in abbreviation with first letter in capital) designed research; XXX performed research; XXX contributed new reagents or analytic tools; XXX analyzed data; XXX wrote the paper. An author may list more than one contribution, and more than one author may have contributed to the same aspect.

Response: We have revised the format of author contributions according to your requirements in the page 3 of the revised MS.

Institutional review board statement:

Response: This manuscript was approved by the institutional review board and uploaded to the system.

Clinical trial registration statement:

Response: The clinical trial have been registered (NCT01965418) and uploaded to the system.

Informed consent statement:

Response: Informed consent statement was uploaded to the system.

Biostatistics statement:

Response: Biostatistics statement was uploaded to the system.

Conflict-of-interest statement:

Response: Authors disclose no any conflict of interest. Conflict-of-interest statement was uploaded to the system.

Data sharing statement:

Response: **No additional data are available.**

CONSORT 2010 statement:

Response: CONSORT 2010 statement was uploaded to the system.

Corresponding author:

Please add your title, such as MD, PhD, Professor, etc.

Please provide **your organization real-name mailbox here:**

Telephone:

Fax:

Response: We have added information of corresponding author according to your requirements in the page 3 of the revised MS

BACKGROUND **(no more than 100 words)** This section should clearly describe the rationale for the study. It should end with a statement of the specific study hypothesis.

AIM: No more than 20 words, and start with "To..."

METHODS: no less than 80 words

RESULTS: no less than 120 words

CONLUSION: no more than 30 words

Response: We have revised abstract according to your requirements in the page 3 of the revised MS.

Core tip: Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Response: We have added core tip according to your requirements in the page 5 of the revised MS.

Please provide all authors' abbreviation names and manuscript title here. *World J Gastroenterol* 2019; In press

Response: We have added **all authors' abbreviation names and manuscript title** in the page 5 of the revised MS.

Please distinguish between the title of the article series. Three levels of subtitles are allowed: (1) First subtitle: All in bold and capital; (2) Second subtitle: All in bold and italic; and (3) Third subtitle: All in bold.

Response: We have revised it according to your requirements.

Statistical significance is expressed as $^{a}P < 0.05$, $^{b}P < 0.01$ ($P > 0.05$ usually does not need to be denoted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used, and a third series of P values is expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$.

Response: We have revised it according to your requirements.

ARTICLE HIGHLIGHTS

Response: We have added the highlight in page16-17 of the revised MS according to your requirements.

Please check and confirm that there are no repeated references!

Please add PubMed citation numbers (**PMID** NOT PMCID) and DOI citation to the reference list and **list all authors.** Please revise throughout. The author should provide the first page of the paper without PMID and DOI.

PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) (Please begin with PMID:) DOI (http://www.crossref.org/SimpleTextQuery/)

(Please begin with DOI: 10.**)

Response: We have revised references in page18-23 of the revised MS according to your requirements.

Figure/table

For figures, use distinct colors with comparable visibility and consider colorblind individuals by avoiding the use of red and green for contrast.

Please don't include abbreviations in the title of the figure/table.

Please explain all the abbreviations in the figure/table legends as full name (abbreviation).

Please explain all the abbreviations of each figure/table under each piece of figure/table legends.

Please don't include any *, #, ...in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters.

Please create another file for all the supplementary figures and tables and name the file as "xxxxx-Supplementary material.pdf"

Response: We have revised figures and tables according to your requirements.