ANSWERING REVIEWERS

September 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13308-review.doc).

Title: Differentiation of Acute and Chronic hepatitis B in IgM anti-HBc positive patients

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

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer 02242399

- Abbreviation list is not complete, such as AFP (alpha fetoprotein) and AHB (acute hepatitis B) should be added.
  Response: Thank you for comments. We added AFP (alpha fetoprotein) and AHB (acute hepatitis B) in abbreviation list (page 3).
- The proportion of IgM anti-HBc positive among CHB patients should be described in the introduction section.
  Response: Thank you for comments. We described the proportion of IgM anti-HBc positivity among CHB–AE patients in the instruction section as you recommended (page 7).
- The values of HBeAg positive S/CO should be added in Table 1rather than only described in the text (page 9, the first paragraph).
  Response: Thank you for comments. We added the S/Co ratio of HBeAg positive patients in Table 1 as you recommended.

(2) Reviewer 0122188

- The levels of IgM anti-HBc are measured using only one kit (Abbot Architect). Therefore, it is not confirmed that the formula (combination of IgM HBcAb titers and HBV DNA levels) is useful in other commercial kits. The authors should revise this point.
  Response: Thank you for comments. We created the formula to prove the combination of IgM anti-HBc and HBV DNA level is better diagnostic parameter than each of them. As you pointed out, the different formula may be

needed in case of using other commercial kit for IgM anti-HBc. In the discussion section, we added your comment in the limitation of this study(page 17).

- Table 1 should be combined with Table 2.
  Response: Thank you for comments. Table 1 showed the comparison clinical features between AHB and CHB-AE groups. Table 2 showed multivariate analysis using variables with a P-value of less than 0.05 on univariate analysis. The combination of Table 1 and Table 2 make big table which might be uncomfortable to see. Therefore, we did not combine them.

- The authors should describe how the formula was made.
  Response: Thank you for comments. The formula (0.2303*IgM anti-HBc – 1.0694*logHBV-DNA) was made by logistic regression. After logistic regression, we obtained AUROC curve of combination using 'lroc' function in STATA. We added this explanation in the result section (page 13).

(3) Reviewer 02453015

- English needs to be improved by native speakers.
  Response: Thank you for comments. We have the manuscript edited by the English language editing companies as you recommended.
- Is the p value one tailed or two tailed?
  Response: Thank you for comments. The *P* value is two tailed.
- It is not clear from which groups the authors got p value in Table 1.
  Response: Thank you for comments. Table 1 showed three groups (total, AHB, CHE-AE). But, actual comparison was done between AHB and CHB-AE groups. First total column is inserted to show just baseline clinical characteristics.
- The clinical HBV history is not clear in both groups. Does it help in diagnosis of AHB or CHB-AE?
  Response: Thank you for comments. The patients of AHB group didn't have history of HBV infection and all patients of CHB-AE group had already diagnosed as CHB in the past. In this study, the presence or absence of previous HBV infection was mostly determined by medical record based on patient's memory and not previous negative result for HBsAg test. This point was mentioned additionally in the discussion section. The clinical information of the previous HBV infection helps in discrimination between AHB and CHB-AE.
- In the last sentence of first paragraph, it should be "Taiwanese".
  Response: Thank you for comments. We corrected the misspelling(page 7).

- Abbreviations in the abstract and the text should be spelled out at their first appearance.

  Response: Thank you for comments. We spelled out abbreviations in the abstract and the text at their first appearance.

(4) Reviewer 02941838

- Introduction (paragraph 1) mentions that CHB-AE and AHB have different prognosis and treatment strategy - this section could also highlight prevention/public health management, eg) acute cases have acquired infection recently and an outbreak could be suspected.

  Response: Thank you for comments. As you recommended, we add the importance of AHB in aspect of an outbreak and public health management (page 7).
- State the prevalence of CHB in Korea, rather than simply 'intermediate'

  Response: Thank you for comments. As you recommended, we expressed the prevalence of CHB in Korea as the percentage (page 8).
- Provide some detail about the sample/cutoff ratio as a diagnostic method eg) how it is determined & where else it is used, for people who manage CHB but are not familiar with this method.

  Response: Thank you for comments. In this study, IgM anti-HBc was done using the chemiluminescent immunoassay on the Abbot Architect (Abbot GmbH, Wiesbaden Delkenheim, Germany). It has a direct relationship between the amount of IgM anti-HBc in the sample and the chemiluminescent reaction measured as relative light units. The presence or absence of IgM anti-HBc in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from a previous manufacture's IgM anti-HBc calibration. According to the product reference, the positivity was defined by a S/CO ratio ≥ 1. We added this explanation in the materials and methods section (page 10). IgM anti-HBc by semi-quantitative or quantitative assay had been suggested as meaningful marker to monitor disease activity of CHB and also reflect the host's active immune response. We added this point in the introduction section (page 7,8).
- Methods: Patients and study design needs more detail - how were patients selected?

  Response: Thank you for question. This study is retrospective case-control study design. During study period, the patients showing clinical presentation of acute hepatitis, defined as elevation of alanine aminotransferase levels more than 10 times the upper limit of normal and IgM anti-HBc positive result were selected. These patients were categorized to AHB or CHB-AE group. The AHB group was defined as patients without history of HBV infection before this episode and with loss of hepatitis B surface antigen (HBsAg) within 6 months after onset of acute hepatitis. All patients of CHB-AE group had already diagnosed as CHB in the past.
- Did study include all AHB and CHB-AE cases during the period?

  Response: Thank you for question. All AHB patients were enrolled, but not all CHB-AE patients. Because IgM anti-HBc is not routine test in CHB patients, IgM anti-HBc was performed in some of CHB-AE patients. Of them, CHB-AE patients with positive result of IgM anti-HBc were enrolled during the study period.
- Did all AHB patients have a previous negative HBsAg test result?

  Response: Thank you for your question. The presence or absence of previous

HBV infection was mostly determined by medical record based on patient's history and previous negative result for HBsAg test was identified in some patients.

- HBsAg loss 6 months after onset could represent resolved CHB - could any of the 'AHB' patients actually have had previously undetected CHB with AE?
  Response: Thank you for your valuable comments. As you mentioned, some cases of AHB group may represent resolved CHB. In this study, we determined the definition of AHB using the clinical criteria, taking the patient's history into account. It is very challenging to exactly classify the AHB and first presentation of CHB-AE patients in clinical settings. Therefore, classification of patients according to our strict criteria might be the best practical approach to determine chronicity. It is likely that a small percentage of CHB patients were classified as being part of AHB in our study. Considering that natural course of CHB, the possibility of HBsAg loss within 6 months after CHB-AE would be extremely rare and the differences in IgM anti-HBc titer and serum HBV DNA levels between two groups would decrease if a subset of the CHB-AE patients had indeed been misallocated into the AHB group. Nevertheless, IgM anti-HBc titer and serum HBV DNA levels remained a significant for differentiation AHB from CHB-AE under multivariate analysis. This suggests that IgM anti-HBc titer and serum HBV DNA levels are indeed discriminating factors between AHB and CHB-AE, although there is limitation in classification of the chronicity based on patient's medical history. We added this point to the discussion section (page 16,17) .
- 2000 IU/mL is relatively high for HBV DNA detection lower limit. Did any patients have undetectable DNA using this assay? If so include in Table 1
  Response: Thank you for comment. Serum HBV DNA levels were measured by the VERANT 3.0 assay (Bayer Healthcare, Tarrytown, NY, USA; lower limit of detection 2,000 copies/mL) or COBAS TagMan PCR assay (Roche, Branchburg, NJ, USA; lower limit of detection 60 copies/mL). The detection lower limit was 2000 copies/mL, not 2000 IU /mL. When 2000 copies/mL was converted to IU/mL, it was approximately 357 IU/mL.
- Methods statement that statistical analyses performed needs clarification - specify which analyses
  Response: Thank you for comments. As you recommended, we changed the statement as follows. The Mann–Whitney *U*-test, chi-squared test, and multiple logistic regression analysis were performed using SPSS, version 16 (page 11).
- Further discussion is needed as to why the retrospective design is a limitation. Eg see point 4 above regarding how patients were selected; also, potential biases because the CHB patients were already receiving clinical management. They may have different clinical characteristics than people with CHB who are unaware/not being managed and then present with AE.
  Response: Thank you for comments. In CHB-AE group, there were three patients (10.3%) receiving antiviral treatment, these all patients were in a state of biochemical breakthrough because of the resistance to antiviral agent. However, there was no difference in biochemical, virological profile and IgM anti-HBc S/CO value comparing other patients without antiviral treatment of

CHB-AE group. As you recommended, we made up for the lack of explanation of the study limitation.

- State p-values in tables rather than 'Not significant'.
  Response: Thank you for comments. We recorded P-value in tables instead of 'Not significant'.

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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