Peer Review File

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Response to Reviewer A

Comment1. Good work by authors on this meta-analysis. In this meta-analysis, the heterogeneity associated with diagnosis of Hepatic steatosis (HS) is not addressed. Mode of diagnosis of HS changes the prevalence and even the outcomes i.e diagnosis based on biopsy or fibroscan or Ultrasound. Authors should try to address the above by first mentioning the diagnostic criteria of hepatic steatosis in the study.

Reply 1: Thank you very much for the valuable suggestion. We agree that the mode of diagnosis of HS might change the prevalence of HS in CHB patients. In order to address the heterogeneity associated with the diagnostic criteria, a subgroup analysis was performed and further interpreted in Supplementary Table 2 (Table S2). Liver biopsy is the gold standard diagnostic method as well as the most commonly used method for HS diagnosis in the studies (57/90). Furthermore, HS defined as 5% or more of hepatocytes affected was the dominant diagnostic criteria (40/52) for HS using liver biopsy (Table S2). HS defined as 5% or more of hepatocytes affected in HS patients with CHB had the closest prevalence rate (35.81%) to the total pooled rate (35%). A subgroup analysis was also conducted to address the heterogeneity associated with diagnosis by the CAP score. However, due to the limited subgroup studies reporting CAP scores, we cannot draw a straightforward conclusion. Also, the subgroup analysis of the impact of diagnostic modes on the outcomes (cirrhosis and fibrosis) was added in Table S4. There was no significant difference between the subgroups (Table S4). The outcomes have no significant relationship with the presence of HS in CHB patients under different

diagnostic modes.

Changes in the text:

(See page 7, lines 107-109): Diagnoses based on biopsy, controlled attenuation parameter (CAP) score, or ultrasound were placed in subgroups and are presented in Table S2.

(See page 9, lines 149-151): We addressed the heterogeneity associated with the diagnostic criteria of HS by performing a subgroup analysis in Supplementary Table 2 (Table S2).

(The content below was added for Table S2): Interpretation of the diagnostic criteria of HS in CHB patients

Liver biopsy: Liver biopsy is the gold standard diagnostic method as well as the most commonly used method for HS diagnosis in the studies (57/90). The prevalence of HS in CHB was also stratified by diagnostic criteria for HS using liver biopsy. HS defined as 10% or more of hepatocytes affected had a lower prevalence rate of HS in CHB patients (20.35%; 95% CI: 17.64–23.20%) than that of HS defined as 5% or more (35.81%; 95% CI: 31.13–40.63%; Table S2). Furthermore, HS defined as 5% or more of hepatocytes affected was the dominant diagnostic criteria (40/52) for HS using liver biopsy.

Controlled attenuation parameter (CAP) score: The lower limit of the CAP score to determine HS in CHB patients was slightly heterogeneous (220–248 dB/m). The subgroup analysis showed that HS defined as CAP ≥ 248 dB/m (42.90%; 95% CI: 38.98–46.87%) had a lower prevalence rate of HS in CHB patients than that of HS defined as CAP ≥ 238 dB/m (57.27%; 95% CI: 45.49–68.64%; Table S2). However, due to the limited subgroup studies reporting CAP scores, we cannot draw a straightforward conclusion to determine the heterogeneity associated with the

diagnostic CAP score.

Abdominal ultrasonography: HS was assessed using criteria including the presence of liver and kidney echo discrepancy, with or without the presence of posterior attenuation of ultrasound beam, vessel blurring, difficult visualization of the gallbladder wall, and difficult visualization of the diaphragm.

(See page 10, lines 172-174): The outcomes (F2-F4 fibrosis and cirrhosis) had no significant relationship with the presence of HS in CHB patients under different diagnostic modes (Table S4).

We deeply appreciate your understanding and hope that you will be satisfied with the current state of this article. Once again, thank you very much for your suggestions.

Response to Reviewer B

This is an interesting and systematic review on a topic that is of clinical importance given the relatively high world wide prevalence of both fatty liver and chronic hepatitis B. The findings are very relevant and I do not have any significant criticisms. My comments are as follows:

Comment 1. The authors state that "Fibroscan" was used to determine hepatic steatosis in a significant number of the studies. This is not technically correct as transient elastography is an assessment of liver fibrosis. The Central Attenuation Parametre study (CAP score) is the study that determines the presence of hepatic steatosis and is a combined feature in the newer model Fibroscan units. This should be corrected.

Reply 1: Thank you for your constructive comment and kind reminder. We are sorry for our improper exposition of the method used to determine hepatic steatosis. Following your suggestion, the improper word “Fibroscan” in the text has been changed to controlled attenuation parameter (CAP) score in our text. Also, the language of our manuscript has been further polished by a native English speaker. Once again, thank you very much for your suggestions.

Comment 2. The authors discuss hepatocellular carcinoma in the Discussion but did not include this as a studied variable in their meta analysis. This needs to be explained (ie. the reasons for not studying HCC).

Reply 2: Thank you for your constructive comment. It is important to recognize the probability that the coexistence of HS may accelerate the progression of liver disease such as liver fibrosis and hepatocellular carcinoma (HCC). In our study, we have included liver fibrosis as a study variable in our meta-analysis. As liver fibrosis is universally recognized as a prelude to HCC (1), we also aimed to discover the relationship between the presence of HCC and hepatic steatosis in CHB patients.

However, only 5 studies reported cases of liver cancer and HS (2-6). Furthermore, the follow-up period of these related cohort studies was heterogeneous, which influences the incidence of major clinical outcomes (HCC) during the follow-up period. Therefore, including this as a variable in the meta-analysis with the current data might cause high heterogeneity and low representativeness. We also included a short section to review the possible relationship between the presence of HCC and hepatic steatosis in the discussion. We hope more large-scale prospective and the cohort studies with confounders controlled will be conducted to further establish the risk of HCC in patients with concomitant HS and CHB. Following your suggestion, we have modified our discussion by adding the concise reasons for not studying HCC in CHB patients with HS through meta-analysis (See page 13, lines 226-230).

Changes in the text:

(See page 13, lines 226-230): Due to the limited studies (n = 5) with cases of liver cancer between the 2 groups, as well as the incomplete data and the complicated confounding factors, we cannot draw a straightforward conclusion from the meta-analysis. We hope that more large-scale prospective and the cohort studies with confounders controlled will be conducted to further establish the aggravated risk of HCC in patients with coexisting HS and CHB.

Comment 3. The authors report that HBeAg negative status was associated with negatively associated with fatty liver disease. Was there any association between HBV viral load and fatty liver disease?

Reply 3: Thank you for your constructive comment. Following your suggestion, we explored the association between HBV viral load and fatty liver; however, the results displayed no significance. Due to the heterogeneity of the data presented, a subgroup analysis was further performed to explore the association between HBV viral load

(DNA > 1000 copies/ml, DNA> 5000 copies/ml) and fatty liver disease. DNA > 1000 copies/ml status and DNA> 5000 copies/ml status were negatively associated with the presence of HS in CHB patients (OR 0.57; 95% CI: 0.31–1.03; OR 0.87; 95% CI: 0.61–1.27). However, the number of studies included in the subgroup analysis was limited (n = 3; approximately 3000 participants were identified), and the result displayed no significance (P >0.05). We also further analyzed the relationship between the quantitative HBV viral load and hepatic steatosis. However, the result also showed no significance (WMD, –0.35 [–0.89 to 0.19]). We have also added some data to Supplementary Table 3 (Table S3).

Changes in the text:

(See page 10, lines 164-166): We further analyzed the relationship between HBV viral load and the presence of HS in CHB patients. However, the result also showed no significance (Table S3).

We deeply appreciate your valuable advice and hope that you will be satisfied with the current state of this article. Once again, thank you very much for your suggestions.

Response to Reviewer C

Comment 1. Hepatic steatosis prevalence is varied depending on the tool used for diagnosis i.e if Ultrasound, liver biopsy, fibroscan or MRI was used

As the authors have failed to define the way diagnosis of Hepatic steatosis was made

Reply 1: Thank you very much for the valuable suggestion. We agree that the mode of diagnosis of HS might change the prevalence of HS in CHB patients. In order to address the heterogeneity associated with the diagnostic criteria, a subgroup analysis was performed and further interpreted in Supplementary Table 2 (Table S2). Liver biopsy is the gold standard diagnostic method as well as the most commonly used method for HS diagnosis in the studies (57/90). Furthermore, HS defined as 5% or more of hepatocytes affected was the dominant diagnostic criteria (40/52) for HS using liver biopsy (Table S2). HS defined as 5% or more of hepatocytes affected in HS patients with CHB had the closest prevalence rate (35.81%) to the total pooled rate (35%). A subgroup analysis was also conducted to address the heterogeneity associated with diagnosis by the CAP score. However, due to the limited subgroup studies reporting CAP scores, we cannot draw a straightforward conclusion.

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References:

1. Zhang J, Lin S, Jiang D, Li M, Chen Y, Li J, et al. Chronic hepatitis B and non-alcoholic fatty liver disease: Conspirators or competitors? *Liver International* (2020) 40(3):496-508. doi: 10.1111/liv.14369.

2. Peleg N, Issachar A, Sneh Arbib O, Cohen-Naftaly M, Braun M, Leshno M, et al. Liver steatosis is a strong predictor of mortality and cancer in chronic hepatitis B regardless of viral load. *JHEP Reports* (2019) 1(1):9-16. doi: 10.1016/j.jhepr.2019.02.002.

3. Lim CT, Goh GBB, Li H, Lim TKH, Leow WQ, Wan WK, et al. Presence of Hepatic Steatosis Does Not Increase the Risk of Hepatocellular Carcinoma in Patients With Chronic Hepatitis B Over Long Follow-Up. *Microbiology Insights* (2020) 13. doi: 10.1177/1178636120918878.

4. Lee YB, Ha Y, Chon YE, Kim MN, Lee JH, Park H, et al. Association between hepatic steatosis and the development of hepatocellular carcinoma in patients with chronic hepatitis B. *Journal of Hepatology* (2018) 68:S503.

5. Cho H, Chang Y, Lee JH, Cho YY, Nam JY, Lee YB, et al. Radiologic nonalcoholic fatty liver disease increases the risk of hepatocellular carcinoma in patients with suppressed chronic hepatitis B. *Journal of Clinical Gastroenterology* (2020) 54(7):633-41. doi: 10.1097/MCG.0000000000001217.

6. Chan AWH, Wong GLH, Chan HY, Tong JHM, Yu YH, Choi PCL, et al. Concurrent fatty liver increases risk of hepatocellular carcinoma among patients with chronic hepatitis B. *Journal of Gastroenterology and Hepatology (Australia)* (2017) 32(3):667-76. doi: 10.1111/jgh.13536.