Responses to Reviewers

To all reviewers and editors,

We noticed that "endotoxemia" is more common than "endotoxinemia" as the term "the presence of endotoxin in blood", so we changed "endotoxinemia" to "endotoxemia" throughout the manuscript.

Reviewer #1 (code 00058872)

Authors are firmly requested to lessen of importance the hypothesis that dosage of LPS is inadequate to justify obtained data. They should emphasize on the basis of their results that the previously evidenced association between endotoxemia and onset or worsening of NAFLD has not been confirmed and thus this study casts serious doubts about this mechanism. In order to reinforce their novel findings, they ought to refer to a recent paper dealing with gut flora modifiers, i.e., Future Microbiol. 2015;10(5):889-902. doi: 10.2217/fmb.15.13. Systematic review on intervention with prebiotics/probiotics in patients with obesity-related nonalcoholic fatty liver disease.

Response

Thank you for this comment. We have added the review as Reference #39 and described it in the Discussion section (indicated in red) to lessen the importance that LPS dosage was inadequate. We also elaborated more on LPS in the study limitations.

Reviewer #2 (code 00004157)

The role of endotoxemia in the pathogenesis of liver damage progression in human nonalcoholic fatty liver disease (NAFLD) is still disputed. In this manuscript, Kitabatake and coworkers examined the association of surrogate biomarkers of endotoxemia, including LPS binding protein (LBP) and EndoCab IgG with histological severity of liver damage in 126 Japanese patients with histological NAFLD. They found that LBP was significantly correlated with severity of steatosis and hepatocellular ballooning, and consistently with aminotransferases levels and inflammatory markers, such as CRP and fibrinogen. Conversely, EndoCab IgG was not associated with liver damage. It is concluded that data do not conclusively support a role of LPS in NASH, and better biomarkers are needed.

This is a well conducted study a relatively large cohort of patients with

serum samples available at the time of liver biopsy. The manuscript is well written and results cautiously interpreted and very well discussed. I have a few comments that I think may be useful to improve the manuscript.

1. Table 1: report results also in patients stratified according to the presence of histological NASH, and correspondent P values.

Response

Thank you for this suggestion. We have revised Table 1.

2. To identify the determinant of circulating LBP concentration, it would be useful to analyze the independent predictors (including both histological and biochemical variables significant at univariate analysis) at multivariate linear regression analysis.

Response

Significant correlations were detected between LBP concentration and 10 clinical parameters shown in Table 3, as well as with steatosis score. Multivariate linear regression analysis using these parameters revealed that FIBG, CRP, AST, and steatosis score were strongly associated with LBP. We have added these results and their related statements to the Abstract, Results, Methods, and Discussion sections as indicated in red letters.

3. It would be important to know whether the association between histological steatosis and inflammation with circulating LBP levels is modified by genetic risk factors for these traits, especially the PNPLA3 I148M variant, which is a major determinant of these traits.

Response

We appreciate this comment. Since we could not examine for *PNPLA3* gene polymorphisms in this cohort, we mentioned this point as a limitation of the study in the Discussion section in red letters. We have also added the results of a previous report (Reference #41) that *PNPLA3* variants did not affect circulating LBP levels in HCV-infected patients.

4. Even if I agree with Authors that measurement of circulating LPS levels may be flawed by several methodological limitations, it would be nevertheless useful to add it to this study, or acknowledge the lack of as a limitation.

Response

We agree with the Reviewer, but would like to stress that several samples had

been stored for more than 1 year and there was a risk of inaccurate LPS measurement. If we measured LPS, we fear these data would have confused our findings and obscured the conclusion. Accordingly, we mentioned this point as a limitation of the study in the Discussion section in red letters.

5. In the core tip it is reported this is the first study to correlate endotoxemia surrogate markers with histological features of NAFLD, whereas previous literature is cited in the discussion.

Response

Thank you for pointing this out. We corrected the statement in the core tip to be more precise as follows: This is the first study simultaneously measuring two surrogate endotoxemia markers, LBP and EndoCab IgG, in biopsy-proven NAFLD patients to assess for relationships with the histological features of NAFLD.

Reviewer #3 (code 02860895)

This report, written by Kitabatake et al, is of an important retrospective study that has been carried out to disclose pathological mechanisms of NAFLD/NASH as well as its diagnosis. Especially, the close relationship between LBP and NASH is very interesting. However, the following two points should be elucidated.

1. Although the study design is similar to that of Wong et al (ref.14), the results are partially and substantially different. The previous study concluded that LBP was related to the degree of steatosis but did not link to NASH. The present study suggested that LBP was significantly higher in NASH than in NAFL. The authors should sufficiently discuss and explain the difference.

Response

We appreciate this comment. Wong et al. (Ref.14) assessed the correlation between endotoxin markers and clinical parameters, such as ALT and liver TG contents as measured by H-MRS, in NAFLD patients. Since these participants did not undergo liver biopsy, accurate diagnosis of NASH was not possible. In contrast, the present study examined the correlation between these endotoxin markers and clinicopathological findings in biopsy-proven NAFLD/NASH patients. Additionally, multivariate linear regression analysis revealed that LBP was associated with steatosis score and CRP/FIBG, suggesting a close link among LBP, steatosis, and acute phase reaction. In order to emphasize the

differences between Wong' study and our own, we have revised the Discussion section, as indicated in red letters.

2. The authors previously reported the usefulness of CK18 in clinical diagnosis of NASH (ref.15). Because many readers will be interested in the potential association between CK18 and LBP, CK18 should be evaluated and the data should be added to Table 3.

Response

We have added the data and their related statements in the Methods and Results sections and in Table 3 in red letters.