Author’s response to reviews

Title: The Role of Small Intestinal Bacterial Overgrowth (SIBO) in Non-alcoholic Fatty Liver Disease (NAFLD) Patients evaluated using Controlled Attenuation Parameter (CAP) Transient Elastography (TE): A Tertiary Referral Center Experience

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Masahide Hamaguchi (Reviewer 1):

Major comments

1. Authors claimed that there is no influence of SIBO in NAFLD development, although the study design was a cross-sectional study. Authors cannot claim the causal relationship of SIBO.

Response: Has been corrected as statistical association measured by prevalence ratio rather than causal relationship.

2. Authors claimed that SIBO plays an important role more in NAFLD patients with obesity. But authors didn't present the role of SIBO in NAFLD.
Response: Has been added to the revised manuscript. The role of SIBO in NAFLD in this study expressed as association in prevalence ratio.

3. Authors examined SIBO in this study. But, they didn't present the definition of SIBO in this study. They just explained as follows; The presence of SIBO if there is an increase of hydrogen concentration ≥20 ppm from baseline within the first 120 minutes. General readers wants to know the detail of measurements.

Response: Has been revised, the full method of subjects preparation and method explained in detailed in methods section.

4. The study subjects are patients in the university hospital. I guess that many of them receive medication and the medication can effect SIBO. However, authors didn't examine the relationship of SIBO with medication.

Response: Our study conforms heavily to “Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus” (https://www.ncbi.nlm.nih.gov/pubmed/28323273) for the sake of uniformity and reproducibility. As stated in the consensus, there is not necessary to stop medication such as Proton Pump Inhibitor (PPI) and to only stop antibiotics at least 4 weeks prior hydrogen breath test.

Benjamin Misselwitz (Reviewer 2):

Major points:

1. This is a largely negative study. The conclusions in the last sentence of the abstract ("NAFLD is a complex disease" and "SIBO plays an important role in patients with obesity") are not supported by the data. Similarly, the last two sentences of the conclusion of the discussion are not supported by the data.

Response: Has been revised in latest manuscript.

2. Nutrition was not assessed (which would impact on microbiota and intestinal transit), data regarding smoking and alcohol consumption are also lacking.

Response: We have added our study limitation regarding nutritional and cigarette smoking assessment, whereas for alcohol consumption we have added a more detailed inclusion criteria regarding significant alcohol consumption.
3. The study argues against the utility of the "SIBO" breath test (the association of obesity and SIBO is not clinically useful since obesity is immediately obvious). However, studying the microbiota seems to hold some promise. This seems to..

Response: Already explained in the discussion and limitation.

4. Multivariate analysis should be strongly considered. Controlling for age, gender might unmask some associations.

Response: We could not perform multivariate analysis in this study due to the terms of confounding factors are not met because risk factors of either SIBO nor NAFLD are within the pathophysiological pathway of both study variables.

5. Some tables should be fused if the same outcome is assessed. For all outcomes, the associations with all risk factors should be calculated. For instance, it is unclear why we do not see the association of epidemiological risk factors and fibrosis degree. It seems, only a selection of the data is presented.

Response: Tables who have same outcome have been fused. Calculations of all risk factors have been added for all outcomes.

6. This is likely not the first paper studying the association of SIBO, obesity, central obesity, NAFLD, Fibrosis and the respective metabolic risk factors. However, the discussion does not attempt to put the data in perspective to the current literature (i.e. compare findings with previous studies).

Response: Relevant studies have been added in the discussions.

7. Most of the significant findings of the study would not be significant after Bonferroni correction (which is a further limitation)

Response: In this study we are not using adjusted p-value for control alpha error. We conducted multivariate analysis by likelihood ratio in logistic regression and discontinue analysis while a statistically significance result is observed. Indeed, it might be our limitation because we only show the data without correction of alpha error. But in fact, it is not necessary needed since clinical findings are more important.

8. On some occasions the language of the paper needs improvement

Response: Some improvements have been made.
Minor points:

1. Line 26, for clarity, metabolic risk factors required or inclusion should be provided

Response: Detailed risk factors have been provided.

2. All exclusion criteria should be better defined (for instance is anti-HBc positivity already a hepatitis B infection?)

Response: we usually confirmed the patients with anti-HBc test when there is suspicion of liver disease and the HBsAg was negative.

3. Some data might be better presented as graphs (for better readability; this is a non-essential suggestion)

Giuliano Ramadori (Reviewer 3): This is a timly paper dealing with the possible microbioma role in fatty liver developemnt and possible progress of liver damage by dibiosis in the small intestine.

1. The definition of NASFLD accept some consumtion of alcoholic beverages. This should be carefully evaluated in the anamnesis. This is a difficult task but it has to be mentioned and discussed.

Response: The inclusion criteria have been made clearer.

2. Also the measurement of "disbiosis is difficult when intestinal fluid culture is not performed.

Response: It has been stated in our third study limitation.

3. Also when clearance efficacy of the RES-System of the liver is not sufficient production of mediators by Kupffer cells and also by hepatocytes become measurable in the serum.

Response: ? (Unclear question from the reviewer).

4. Authors should at least discuss this point in the discussion section if they are not able to perform some measurements.

Response: Unperformed measurements have been mentioned in the study limitations section.
5. As a clinician I must say that bacterial overgrowth in small intestine normally causes clinical symptoms

Response: Clinical symptoms profile of SIBO patients doesn’t reliably correlate with diagnostic tests for SIBO. https://www.mayoclinicproceedings.org/article/S0025-6196(16)30589-4/pdf