Reviewer's report

Title: Elevated plasma copeptin levels identify the presence and severity of non-alcoholic fatty liver disease in obesity.

Version: 0 Date: 25 Jan 2019

Reviewer: Fernando Bril

Reviewer's report:

In the current work, Barchetta et al assessed the role of plasma copeptin to identify the presence of NAFLD and NASH in a cohort of obese individuals. They concluded that plasma copeptin levels were higher in patients with NAFLD and NASH compared to patients without NAFLD and NAFL.

However, there are several issues that should be analyzed more carefully:

1. Data discrepancies: just as an example, note that mean HOMA-IR was 4.5 for obese individuals (n=60), but only 3.6 and 4.1 when divided in no NAFLD (n=28) and NAFLD (n=32). How is it possible that when combining the no NAFLD and NAFLD patients the mean actually goes up by that much? Please provide an explanation. This is observed in several variables.

2. If, as stated in the manuscript, the aim was to explore the relationship between plasma copeptin and the presence/severity of NAFLD and NASH, I would suggest combining Tables 1 and 2 to show patients' characteristics in 3 groups: 1) non-obese (n=60), 2) obese-no NAFLD (n=28) and 3) obese NAFLD patients (n=32). Use ANOVA to compare the 3 groups and Bonferroni's adjustment (or other) for pairwise comparisons.

3. Obese individuals undergoing bariatric surgery and healthy non-obese individuals are 2 different extremes of the obesity spectrum. Regardless of this, we see no difference in many metabolic parameters such as fasting insulin and HOMA-IR. How do authors explain this observation?
4. HOMA-IR of 3.3 (and insulin of 17 uU/mL) is relatively high for non-obese, "metabolically healthy" patients (as stated in the manuscript). Indeed, it is not significantly different from the 4.5 in obese individuals. This is extremely surprising. Please justify and expand on this in the discussion.

5. There were no differences in copeptin in obese vs. non-obese individuals. However, several studies have suggested that copeptin is associated with obesity and insulin resistance. How do authors explain these discrepancies in their results? Were controls not as "healthy" as originally presumed?

6. Patients with NAFLD had lower insulin levels than patients without NAFLD (this by itself is perplexing). Now, FPG was similar in the 2 groups, so it strange than HOMA-IR was slightly higher in the NAFLD group (even when not significantly). Also, we would expect a higher IR in patients with NAFLD. Please explain your interpretation of these results. Why do authors believe that TG levels were not that different between No NAFLD and NAFLD patients? Or SBP and BMI slightly lower in NAFLD? (While due to small sample size these differences are not statistically significant, the direction of their trend is strange).

7. It is unclear to this reviewer why you would present a correlation between a continuous and a dichotomous variable like in Table 2 (e.g. copeptin and NASH yes/no).

8. Provide the information of how many patients of the 32 with NAFLD had NASH and how many had borderline NASH.

9. Provide information regarding diabetes and lipid-lowering medication use.

10. Expand Table 3 to include all quartiles to assess gradual changes.

11. This manuscript only shows an association or higher levels of copeptin in specific subgroups. Authors should try to assess whether these differences are important enough to use copeptin as a biomarker of NASH. What was the sensitivity, specificity, PPV and NPV to diagnose NASH?
12. Power calculation was done between NAFLD and No-NAFLD groups. It should be mention in the power calculation section that because copeptin levels were surprisingly higher in the non-obese group compared to the obese No NAFLD group, the same calculation between NAFLD and non-obese would result in the need of 90 patients per group to have a power of 80%.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:
Acceptable
**Declaration of competing interests**

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

**Statement on potential review bias**

Please complete a statement on potential review bias, considering the following questions:

1. Did you co-author any publication with an author of this manuscript in the last 5 years?

2. Are you currently or recently affiliated at the same institution as an author of this manuscript?

If you can answer no to all of the above, write 'I declare that I did not publish with these authors in the last 5 years and also meet the affiliation criteria'. If your reply is yes to any, please give details below.

I declare that I did not publish with these authors in the last 5 years and also meet the affiliation criteria.
I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.