ANSWERING REVIEWERS

Title: Metabolomics studies identify novel diagnostic and prognostic indicators in patients with alcoholic hepatitis

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Dear Editor,

We would like to thank you for considering our manuscript entitled: "Metabolomics Studies Identify Novel Diagnostic and Prognostic Indicators in in Patients with Alcoholic Hepatitis," for publication in the *World Journal of Hepatology*.

We would like to re-iterate the fact that our research is novel, represents the combined work of all the co-authors, and has not been submitted to any other scientific journal for simultaneous review. It is our hope that this manuscript can help pave the way for novel innovations in understanding, diagnosing, and assessing liver disease. The findings from this paper can be used as a platform for further research into the use of metabolomics to provide a diagnosis and prognosis in patients with liver disease. As such, this paper should be of interest to a broad readership.

This manuscript describes original work and is not under consideration by any other journal. Findings from this study were presented at a plenary session in basic science at Digestive Disease Week in Washington, DC, USA on May 17, 2015. All authors approved the manuscript and this submission.

As follows, please find a point-by-point response to the comments made by reviewers. The corresponding changes are highlighted in yellow in the manuscript.

1. Statistical analysis
   - 1.1 There are several methods the authors used. The description of statistical models need to be better organized. For example, there are several places mentioned test the correlation between levels of compounds and severity of liver disease (First and third paragraph in the section); 'Logistics regression analysis was performed to build a predictive model for AH' came in the middle of the description of correlation analysis
     - This has been better organized.
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   - 1.2 There are a lot of comparison of biomarkers between two groups. Holm-Bonferroni adjustment should be considered in such case.
     - The objective was pattern detection or hypothesis generation and explain that adjustments for multiple comparisons are usually somewhat conservative and

you would miss many potential associations that should be further explored. Even the Holm-Bonferroni correction is quite conservative when the number of tests is large or the tests are not independent.
        - Please see the new reference (#15) which supports this claim.
        - Despite this information, we did perform Holm-Bonferroni adjustment per the suggestion of the reviewer. See Table 7 with the adjusted corrections. In this case only citrulline, phenylalanine, and homocitrulline remain significantly different between the groups.
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    - 1.3 The sample size seems quite small. Did the authors determine the sample size needed for the study or the power?
        - No power calculations were done to determine optimum sample size. Given no power calculations and a small sample size, we are only capable of generating sufficient power for large differences.
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    - 1.4 Did the authors run multivariate regression to determine the independent association between a biomarker and outcome?
        - We did multivariable Cox regression analysis to adjust for MELD, the most important predictor of mortality, and Tyrosine remained significantly associated with mortality (HR=1.1 (1.01, 1.04) for a one unit increase in tyrosine; p=0.002). Also, in Figures 2 and 3 it can be seen that Tyrosine performs better than MELD for prediction of 3 and 6-month mortality and the combination of MELD and Tyrosine (in a multivariable analysis) performs more or less the same as the compound by itself.
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- 2. Results
    - 2.1 There is no results for the correlation between levels of compounds and severity of liver disease
        - Table 5 presents the correlations between biomarkers and liver disease severity for alcoholic hepatitis. There was moderate to strong correlation between several biomarkers and both MELD and Maddrey's scores. Correlations between 0.0-0.3 are considered trivial/low, 0.3-0.5- are considered moderate, 0.5-0.7 are considered high and 0.7-1.0 are considered very high/strong.
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    - 2.2 Groups in Table 2 and table 3 are alcoholic cirrhosis and alcoholic hepatitis. Are those the wrong labeling?
        - This has been fixed.

Please let me know if you have any questions or concerns regarding this revision. Thank you for your consideration.

Kind regards,
Mona Ascha, B.S.