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Professor Lian-Sheng Ma, Company Editor in Chief
World Journal of Gastroenterology

Dear Prof. Ma,

RE: Manuscript ID number: 75445

My co-authors and I would like to thank you for considering our manuscript entitled "Comprehensive evaluation of microRNA as biomarker for the diagnosis of hepatocellular carcinoma" for publication in *World Journal of Gastroenterology*.

We would like to thank the reviewers and editor for their comments. Please find enclosed our revised manuscript with changes tracked, and below our point-by-point response to each comment.

We look forward to receiving your final decision.

Best regards,

Dr Juliane Malik

Reviewer #1 Comments:

1. In brief: The authors have performed case contro study identifying miR-21-5p (miR-21), miR-320a, and miR-186-5 miRNAs to have diagnostic agility in HCC. AUC was 0.87. A combination of miRNAs did not perform better than any single miRNA. Improvement of PIVKA-II performance through combination with miRNAs could not be confirmed in the validation panel. Two putative miRs — put-miR-6 and put-miR-99 — were tested in the training and validation panels, but their expression could only be detected in very few samples and at a low level (cycle threshold between 31.24 and 34.97. Which I belive reflects the real thing and they have honestly shared their results. Generally the etxt is well written. I believe it can be published.

Author response: We thank the reviewer for their positive feedback.

Reviewer #2 Comments:

1. How miRNA combined with PIVKA have a diagnostic relevance while the same miRNA combined with AFP do not have.

Author response: We thank the reviewer for this interesting observation. Indeed, in our cohort, AFP showed better diagnostic performance in all evaluated intended uses compared with miRNAs and even PIVKA-II biomarkers. This observation is in line with data published previously [Piratvisuth T, et al. *Hepatol Commun* 2022;6:679–691. PMID: 34796691 DOI: 10.1002/hep4.1847]. Due to the superior performance of AFP, individual miRNAs only identify the same set of patients and thus have no significant additive value. The performance of PIVKA-II in the same cohort is between 5% and 20% lower than the performance of AFP and thus the miRNAs have higher additive value when combined with PIVKA-II. We have added a statement addressing this point to the Discussion: “We observed that individual miRNAs identified the same set of patients as those identified by AFP, thus the miRNAs did not add any significant value in this case. However, as the performance of PIVKA-II in the same cohort was between 5% and 20% lower than the performance of AFP, the miRNAs therefore had higher additive value when combined with PIVKA-II.”

2. You have mentioned that the three best partners for PIVKA-II were miR-21-5p in 60%, miR-320d in 18% and miR-652 in 16% then The two best miRNA partners for PIVKA-II were miR-652 in 64% and hsa-miR-221 in 26% ??

Author response: Indeed, the reviewer is correct. Depending on the selected intended use and sample subpanel, different miRNAs were selected as best partners for PIVKA-II:

- For differentiation between **all** HCC cases and CLD controls, best partners of PIVKA-II were hsa-miR-21-5p and hsa-miR-320d, selected in 96% and 10% of cross-validations, respectively
- For differentiation between **early** HCC cases and CLD controls, best partners of PIVKA-II were hsa-miR-21-5p, hsa-miR-320d and hsa-miR-652-3p, selected in 60%, 18% and 16% of cross-validations, respectively
- For differentiation between **early** HCC cases and **cirrhosis** controls, best partners of PIVKA-II were hsa-miR-652-3p and hsa-miR-221-3p, selected in 64% and 26% of cross-validations, respectively

We have added bolding to the font for the subgroups in the Result section for clarity.

Taken together, the results indicate that individual miRNAs can display different performance depending on the target (at-risk) population, even within the same disease.

Interestingly, PIVKA-II has a robust and good diagnostic performance in all tested intended uses, and its improvement by the combination with miRNAs was very limited.

Science Editor Comments:

1. This case control study explored microRNA as diagnostic marker for hepatocellular carcinoma, which is an important and significant topic for clinical work and crucial for the early diagnosis of hepatocellular carcinoma. The structure and content of the article are complete, and the references

are complete and new. The authors also describe the limitations of the study well. This manuscript is novel and of some significance to the clinical field, attracting the attention of liver cancer experts.

This manuscript has some guiding significance for the early diagnosis of HCC.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade B (Very good)

Author response: We thank the Editor for this positive feedback.

Company editor-in-chief Comments:

1. I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Author response: We thank the Editor-in-Chief for their positive appraisal of our manuscript.

2. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file.

Author response: Editable figures have been provided as requested. The ROC curves in Figure 3 are not editable as that is the way they were generated.

3. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of

the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

Author response: The tables have been amended to conform with the above requirements.

4. In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the reference source and copyrights. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022.

Author response: All figures are original, and we have added the copyright information to each figure in the PPT.