

## Point-by-Point response

**Name of Journal:** *World Journal of Hepatology*

**Manuscript NO:** 57325

**Manuscript Type:** ORIGINAL ARTICLE

### *Retrospective Study*

#### **Circulating miR-21-5p level has limited prognostic value in patients with hepatocellular carcinoma and is influenced by renal function**

The work by Franck et al. was focused on the evaluation of the prognostic value of circulating miR-21 levels in HCC. Using an European cohort of patients (n=91) with HCC, the authors found that there was no association between serum miR-21 levels and disease etiology, BCLC stage and/or Child-Pugh score. However, a positive correlation of miR-21 levels and AST or miR-122 levels was evident, while serum miR-21 levels negatively correlated with kidney function. Regarding survival, miR-21 serum levels were not found to be correlated with worse survival. Most of the results presented here pinpoint that serum miR-21 levels might not have a prognostic role for HCC. Still, there are some important points that should be addressed in order to confirm these findings.

1 - Please revise the manuscript to correct some grammatical errors and typos;

**Response:** We appreciate the comment. We apologize for the errors, which could be explained partly by the time pressure due submission deadline. The paper has been now extensively revised to correct the language and typos.

2 - Since the authors were not able to reproduce some of the previous findings from other groups (mostly obtained in Asian cohorts), it would be important in figure 1 to include also healthy controls, in order to confirm if miR-21 serum levels are increased in HCC, thus recapitulating the findings from other groups.

**Response:** The comment is well accepted. There are few papers that provided some evidence to diagnostic value of miR-21, but also those with negative results, however, the main data are related to prognostic value of miR-21. Therefore, we aimed to address in this work primarily prognostic value of miR-21 in European cohort. Having seen the substantial variability of miR-21 levels, we do not expect any positive data in our cohort. Furthermore, after completing the analysis, addition of an independent cohort of control subjects would provide potential technical bias in analysis. We will carefully follow the topic on impact of various factor on microRNA as biomarker and hope to address this point in future work. At this stage, we included additional point in discussion to highlight the potential relevance of miRNA level variation also in diagnostic settings.

3 – How were the patients diagnosed? Was the diagnosis confirmed by histology?

**Response:** The diagnosis of HCC was made either using histology or using non-invasive radiological criteria with CT and MRI. We added the explanation in the methods to clarify this aspect.

4 – In methods, the authors mention that miR-21 expression was measured by SYBR Green while miR-122 by the Taqman methodology (which is the most widely used). What is the rationale behind this? Probably measuring miR-21 expression with the Taqman technology would be more reliable.

**Response:** In general, Taqman is the most widely used method. However, since 2010 we have tested numerous of qPCR methods and SYBR Green showed an excellent reproducibility and validity for miR-21 measurements in comparison to TaqMan. For this reason, we already broadly implemented SYBR green method for example for the miRNA determination in stool of patients with colorectal cancer, IBD etc.

5 – In Figures 2-5, please include the r and p values (instead of presenting them in the tables). It is easier to follow when they are accompanied by the figure.

**Response:** We thank the reviewer for this point. The r and p values are included in the revised version.

6 – Was there any correlation between miR-21 serum levels and albumin (hepatocyte function)?

**Response:** As shown in the Table 1 and 2, we did not find any correlation between serum miR-21-5p and other parameters of liver function such as albumin or bilirubin. We added additional sentence in results to underline this statement.

7 – Regarding survival, considering that patients with high AFP display higher levels of miR-21, do patients with high AFP and high miR-21 display worse survival?

**Response:** We kindly appreciate the valuable comment. We have looked at the survival data for different miR-21 groups (miR-21 high and low) and divided the groups based on the AFP levels (median). The data are shown in the figure 5C and D. According to the data, it seems that only AFP is the determinant of the prognosis but not miR-21. In some additional analysis (the data are not included) we also tested various combination of miR-21 and high/low AFP, but also in those analysis primarily the AFP but not miR-21 was predominantly responsible for survival differences.

8 – Throughout all the manuscript, in order to follow the most recent guidelines for mentioning miRNAs, the authors should include the species and the strand of the miRNA being evaluated (e.g. has-miR-21-3p).

**Response:** We appreciate the comment of the reviewer. According to the reviewer's recommendation, we have included complete name in the abstract and main paper and refer to the abbreviation in the subsequent parts. We hope that in this way we comply with the recommendation on reporting of miRNA research.

9 - The authors only evaluated overall survival. What about relapse-free survival (RFS)? Do patients with higher miR-21 levels present more recurrence? Please repeat all the survival analysis for RFS.

**Response:** Unfortunately, we do not have a systematic data on relapse-free survival on this cohort of samples. Overall, this cohort included large proportion of patients with BCLC Stage C and D, which are defined as a palliative group.

10 - Are the serum levels of miR-21 correlated with the development of metastasis?

**Response:** It is an interesting point. From biological perspective, only a subgroup of patients with HCC indeed develop metastasis. Unfortunately, we do not have a systematic data related to development of metastasis for this cohort.

11 - In table 1, please replace <50% and >50% percentile by Low and High. It is easier to follow. Furthermore, regarding pre-treatment of patients, what was the regimen in this case?

**Response:** According to reviewers helpful recommendations, we have simplified the description of the groups. In our study, we included patients with various therapeutic regimens of pretreatment. Some patients received combined therapies before inclusion. Most common pretreatments were hepatic resection, transarterial chemoembolization (TACE) or sorafenib. We added a sentence to the methods and into Table 1 to clarify this aspect.