Format for ANSWERING REVIEWERS

January 24, 2019

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: reviewed manuscript-45419.doc).

Title: Angiogenesis of hepatocellular carcinoma: an immunohistochemistry study

Authors: Decebal Fodor, Ioan Jung, Sabin Turdean, Catalin Satala, Simona Gurzu
Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 45419

Reviewer`s 1 opinion

1. It did not mention its limitations and the question which remains to be solved.
2. Showing the data in the form of table is not so intuitive.
3. The originality of this manuscript is not cleared stated with the lack of efficient literature review.
4. The results presented are too weak to support authors' stating of "it seems that dedifferentiated HCCs developed in non-cirrhotic liver may benefit by anti-COX-2 therapy. The anti-VEGF-A drugs might be used in patients with HCCs developed in patients with cirrhosis, before occurrence of vascular invasion."
5. Flaws exist in authors' writing in terms of grammar and typo.
6. The final conclusion of this study is only for patients with non-cirrhosis, but most of hepatocellular carcinoma is secondary to hepatitis and cirrhosis.
7. Very good paper

Authors' answers

1. The limitations of study were added in red at the end of Discussion
2. To be more comprehensive, we have added supplementary explanation of the tables in the text, in Results. In Discussion, the obtained data were analyzed based on literature data.
3. Supplementary paragraphs were added in blue in Introduction and Discussion, to enlarge the literature review. New titles were added in red in References list
4. We have modified the core tip, in blue. Conclusion was also changed.
5. The grammar was corrected using a professional service system.
6. The final conclusion was changed.
7. Thank you!

Reviewer's 2 opinion

In this paper, the authors proposed that COX-2 could be used as angiogenesis marker and predict benefit from anti-COX-2 therapy.

1. The rational should be addressed for using anti-COX-2 treatment for HCC in Introduction or Discussion.
2. Images in Fig 1 are not representative enough, IHC pictures from low power field should be shown beside the high power images.
3. Good paper.

Authors' answers

1. Thank you for this valuable suggestion! We have added a phrase in green in Introduction and a new title in References list, in green. In Discussion, a paragraph was also inserted, in green.
2. Figure 1 was modified, to be more representative.
3. Thank you!

Reviewer`s 3 opinion

The work is interesting and done well.

1. There are very few photomicrographs for a paper that relies totally on immunohistochemistry data. Please add more photomicrographs . Please show examples of lower power views of regions of note. Also, show examples of : 1. VEGF-A in small tumors without vascular invasion Versus HCC with low levels of VEGF-A. 2. COX-2 in dedifferentiated tumors in non-cirrhotic liver Vs HCC in the absence of premalignant lesions. 3. CD31 in HCC in patients with cirrhosis Versus no cirrhosis. 4. CD105 in tumors without associated hepatitis Versus HCC with low levels of CD105.
2. I made about 20 minor edits to the English and will try to attach that edited file. ; if I cannot, please improve the English.

Authors' answers

1. Figure 1 was modified, to be more conclusive.
2. Thank you for your English correction!

Reviewer`s 3 opinion

In this article, Dr. Gurzu' group has investigated the immunohistochemical aspects of angiogenesis in hepatocellular carcinoma (HCC) through a retrospective cohort study of 50 randomly selected HCC patients. This study provides valuable information in the individualized anti-angiogenesis therapy of HCC. It is a worthy study done by Dr. Gurzu's group, however, some concerns need to be sufficiently addressed before consideration for publication. Major Comments:

1. The purpose of this investigation, based on the title and main part of Introduction, is to retrospectively gather and analyze information on immunohistochemical aspects of angiogenesis in HCC; however, the information in the article on these selected cases is too vague such as the source of the cases, the criteria for inclusion and so on.
2. The article only mentioned that 50 out of 113 cases were randomly selected for analysis, but it did not explain why the sample size was 50. Why they did the random sampling other than using all cases?
3. Based on the small sample size, it is not surprise that most variables have no significance which P value more than 0.05. Still, because the small sample size, it should use Fisher's Exact Test other than Chi Square Test.
4. When authors did the immunohistochemistry assessment, it should review and score by two pathologist and assess the consistency between them.

Authors' answers

1. As this study is a morphologic one, we have included 50 consecutive cases of HCC that were randomly selected. The positive point is that we did not use tissue microarray slides. For this reason, although randomly selected, the full slide evaluation of the tumor assure the reproductibility of the study. We have added this information in Discussion, last part, in red. In Methods, in green, we have added that no preoperative chemo- or radiotherapy was done in any of the selected cases. If you consider that supplementary information should be included, please let us know.
2. As the study was performed on classic slides (not tissue microarray-TMA), we chose to have a representative number. In TMA-based studies, below 100 cases were also used (e.g. titles 26 and 31 from References). This information was included in limitations of the study (last part from Discussion).

3. Thank you so much for this suggestion! We have modified in methods and checked the statistical correlations another time.
4. We have added in Methods, in blue, the contribution of the three pathologists regarding the independent selection of the used area for quantification.

Thank you again for publishing our manuscript in the *World Journal of Hepatology*

Sincerely yours,

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