Dear Editors,

Please find enclosed the revised version of our manuscript NO.:72825 Review, with the new title "**Novel approaches in search for biomarkers of cholangiocarcinoma**" by Lavinia-Patricia Mocan *et al.*, an invited manuscript with ID 03741771 for dr. Tudor Mocan. We thank you and the Editors of the World Journal of Gastroenterology for their positive assessment of our manuscript. We appreciate the constructive comments and we have adapted our manuscript according to the suggestions made by the reviewers. All individual issues have been addressed below **in bold** and throughout our revised manuscript the changes have been marked also **in bold**.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: The manuscript represents a literature review of available and potential diagnostic and prognostic biomarkers in cholangiocarcinoma. The topic is interesting and relevant, and in view of this it is also useful. The review is generally well-written. As a general remark there is quite extensive use of literary, abstract and pompous language which is not always suitable to support the scientific background. This should be addressed.

Response

We thank you very much for the positive comments and helpful suggestions to further improve our manuscript. We agree that throughout our manuscript unsuitable language has been used, which does not always support the scientific background. We have carefully reread our manuscript and this aspect has been addressed.

Furthermore, a number of specific revisions are recommended as follows: Importantly, it would be useful to clarify within the text the applicability of various statements in relation to the different types of cholangiocarcinoma by location, where appropriate. Several statements are

provided in a generalized manner, while they may not be uniformly applicable as such. The authors should make reference to the main predictors of clinical outcomes in patients with cholangiocarcinoma (i.e., resectability, T stage, N stage, margin status, metastasis, etc.). In particular, as the authors make repeated reference to the diagnostic performance of several biomarkers in predicting recurrence, it should be highlighted in the first place that the only treatment with curative intent is radical surgery with clear surgical margins.

Response:

Thank you very much for the helpful suggestions. We agree that we provided some statements in a generalized manner. We have carefully reread our manuscript and addressed the recommended improvements. In terms of prognosis there are some validated tools that are useful in clinical practice. These markers are not specific for CCA but rather apply to all human malignancies. Tumor size and differentiation, vascular involvement, lymph node status, margin status and presence of occult metastasis were all shown to be good predictors for overall survival for both intra and extra-hepatic cholangiocarcinoma. As such, the main predictors of CCA clinical outcomes were added and the only curative treatment was also highlighted.

Few typographic errors throughout the text should be corrected.

Response:

Thank you for highlighting this. We have identified, revised, and corrected the typographic errors throughout the text.

Abstract As a general remark, it appears that the abstract contains a degree of exaggerated language, probably deriving from the fact that the authors do not seem to take into account the fact that cholangiocarcinoma can be intrahepatic, perihilar and distal. As such a number of statements are either unjustifiably generalized or exaggerated. More specifically: ''Cholangiocarcinoma (CCA) is a biliary-derived neoplasia marked by an exceptionally dim prognosis'' - this is a generalized statement which is literally not valid (exceptionally dim prognosis). '' an overwhelming proportion of cases are discovered beyond the moment of curative intent.'' Again, this is a generalized statement and as such it is an overstatement. '' Moreover, the diagnostic process is typically laborious, and histology, the hallmark of any cancer diagnosis, is painstakingly challenging to obtain.'' This is not applicable of all types of cholangiocarcinoma. ''Not least, there is an acute lack of prognostic predictors following the

diagnosis, which further complicates disease management.'' - ''prognostic predictors'' should be changed to either predictors or prognostic factors / biomarkers or similar. -How does the lack of prognostic biomarkers complicate disease management?

Response:

We thank you for giving us the possibility to further improve the abstract of our manuscript. We agree that the abstract appears to contain inadequate language. We have carefully reread and revised the abstract and made the improvements suggested.

Core tip What do the authors mean by "critically most recent"?

Response:

Thank you for highlighting the inappropriate phrasing, which reads now "The current review paper aims to critically analyze the most recent developments in non-invasive cholangiocarcinoma diagnosis and prognosis.**"**

INTRODUCTION ''To this point, CCA lacks definite diagnostic criteria.'' - In the form this statement is provided, it is not valid. There are for instance well-defined histopathological criteria. ''curative intended surgical resection'' - ''surgical resection with curative intend'' is a preferable phrasing. "However, only a dismal 15% of cases are fit for surgery at the initial diagnosis due to advanced stages[6].'' - This statement is inaccurate and controversial. If the authors wish to refer to the limited number of surgical candidates relating to a high percentage of advanced stage at diagnosis (inoperable), this is irrelevant to the patients' fitness. If they wish to refer to the percentage of unfit patients, this is irrelevant to the stage. The provided percentage is lower than the actual for those with potentially resectable disease; moreover, it is not the same for all types of cholangiocarcinoma according to localization. ''Mortality rates are high, and thus the prognosis is abysmal[7],'' - It needs to be clarified whether the authors refer to the general population of patients with cholangiocarcinoma, those with inoperable disease, and whether they refer to all types of cholangiocarcinoma. For instance, resectable cases in general have a much better prognosis. In any case the word "abysmal'' is unsuitable. ''charbohydrate antigen 19-9'' should be ''Carbohydrate antigen 19-9''. (e.g., Fibroblast growth factor receptor 2 (FGFR2) gene - Use punctuation appropriately. ''As an alternative to tissue'' should be ''As an alternative to tissue biopsy'' in this sentence. The authors state: "The current review aims to explore the nascent waters of the non-invasive biomarkers for CCA and provide an evidence-based input to aid clinical decisions and provide grounds for future research …..tumor cells (Figure 1)." I appears

that the part ''and provide an evidence-based input to aid clinical decisions'' is stronger than implied by the conclusions.

Response:

We thank you very much for the very constructive suggestions to further improve the Introduction of our manuscript. We agree with the remarks you highlighted and adjusted our manuscript accordingly. As such, the rather invalid statement with respect to the CCA definite diagnostic criteria was improved with more valid data. The phrasing "curative intended surgical resection" was changed with the more preferred phrasing as suggested. Further, the rather inaccurate and controversial statement "However, only a dismal 15% of cases are fit for surgery at the initial diagnosis due to advanced stages**" was also revised. The phrase "**'Mortality rates are high, and thus the prognosis is abysmal**" was clarified and the word abysmal was replaced. The typographic errors throughout the text were also corrected. We thank also for highlighting the fact that the aim of our manuscript is rather stronger than implied by the conclusions and was also improved.**

PROTEINS "The field of proteomics has gained notoriety" - "notoriety" is unsuitable. "and carcinoembryonic antigen (CAE)" - it should be (CEA). ''There are three protein-based biomarkers …., their levels being measured from serum samples usually by ELISA.'' - The sentence needs to be rephrased. What is the diagnostic sensitivity and specificity of the protein-based biomarkers that the authors discuss? Their value is provided in a very general descriptive manner. Specific numbers should be provided/discussed. ''Being a well-known biomarker, CA125 is currently used primarily on ovarian cancer clinical management'' should be rephrased. "However, various other protein-based biomarkers ……in recent CCA studies (Table 2 and Table 3).'' should be rephrased. Table 3 is not meaningful without outcomes-related columns. Potential protein-based diagnostic biomarkers The description is very vague and does not highlight the potential clinical applicability of these biomarkers. The authors need to be specific rather than using general terms such as ''better results'', ''good value'', ''high diagnostic powers''. ''Out of a protein multimarker panel consisting of serum S100A9, MUC5AC, TGF- β1, Ang-2, and CA19-9, serum levels of TGF- β1 and Ang-2 provided effective prognosis in CCA patients with metastasis and severe cancer[64].'' The phrasing needs to change in any case. Furthermore, what was the ''effective prognosis''? What is ''severe cancer''? ''In tumors of combined HCC and CCA (cHCC-CC),….after resection[72].'' should be rephrased. ''However, future validation studies on large patient cohorts are needed to distinguish false paths from real solutions.'' - ''false paths'' is unsuitable.

Response:

Thank you very much for your valuable comments and helpful suggestions to further improve the Proteins section of our manuscript. We agree that throughout the section the indicated phrasings need revision and we have rephrased all of them according to your constructive advice. Moreover, the specificity and sensitivity of the protein-based biomarkers that we discuss at the beginning of the section have been introduced in the text to reduce the rather descriptive manner of the text and improve the consistence of the manuscript. With respect to Table 3, we thank you very much for the practical suggestion to add an outcome column for the prognostic biomarkers reviewed, which we have now introduced.

METABOLITES ''A panel of four metabolites attained a diagnostic accuracy (HCC vs iCCA) or 99.7%[91].'' - Which ones? ''A combination of serum levels of nine metabolites could discriminate between dCCA and PDAC with a sensitivity of 55.9% and specificity of 89.5%[91]. '' - Which ones?

Response:

We thank you very much for the meaningful suggestions and for giving us the possibility to further improve the Metabolites section of our manuscript. We agree with you that some phrases lack to indicate the metabolites discussed. We have revised the indicated phrasings and completed with the missing parts and further improved our manuscript.

CONCLUSIONS AND FUTURE DIRECTIONS ''However, most available reports are deeply heterogeneous, study protocols are not harmonized, and the number of included patients is still relatively small.'' The authors have made very little reference to the number of patients/samples in the studies they have reviewed.

Response:

Thank you very much for your valuable comments to further improve the Conclusions section of our manuscript. We agree that throughout the sections we have made little reference to the number of patients/samples in the reviewed studies. Now we have introduced throughout the manuscript the missing number of patients involved in the studies reviewed and made also some comments with respect to some study design.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Firstly, thank you for opportunity to review the article. 1. As the author mentioned intrahepatic (iCCA) and extrahepatic (including both: peri-hilar (pCCA) and distal (dCCA)), had different aspects in etiology, molecular alterations, pathogenesis, behavior, and management. The different of biomarkers were not mentioned.

Response:

We thank you very much for the meaningful suggestions and for giving us the possibility to further improve our manuscript. We agree with you that mentioning the differences of CCA and of biomarkers will improve the understanding of our manuscript and we have now made the recommended suggestion throughout the manuscript.

2.AFP was not inclueded. Approximately 10-15% ICC the level of AFP may elevated.

Response:

Thank you very much for highlighting this very important aspect. We have now included the indicated aspect in the Introduction section. Also, AFP was mentioned in the proteins section as a component of a multimarker panel.

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (High priority)

Specific Comments to Authors: Thank you for giving me a chance to review this research regarding the developments in non-invasive cholangiocarcinoma diagnosis and prognosis, and the manuscript describes the circulating nucleic acids, proteomic and metabolomic-derived

biomarkers, extracellular vesicles, and circulating tumor cells in an attempt to outline promising results for future research and clinical use.

My major comments are as follows:

1.In the "INTRODUCTION" Page 4, Paragraph 2, :" The risk factors for iCCA are the same as HCC, including hepatitis…"and" To this point, CCA lacks definite diagnostic criteria "should be corrected. ICCA and HCC are different diseases, and their etiologies are obviously different.. The diagnosis of CCA is difficult, but the diagnostic criteria is clear.

Response:

We thank you very much for the positive comments and for giving us the possibility to further improve our manuscript. We agree with you that throughout the Introduction some unclear statements were made. We have reread our manuscript and the suggested corrections were carefully addressed.

2.It is suggested to number the chapters of the article for easy reading.

Response:

Thank you very much for the helpful suggestions to further improve our manuscript. We agree that the numbering of the chapters will enhance the reading of our review. Thus, we have now added numbers to the chapters as suggested.

3.It seems that some attributives need to be added to the title in order to accurately cover the content.

Response:

Thank you very much for the helpful suggestions to further improve our manuscript. We agree that some attributives are needed to be added to the title in order to accurately cover the content. The title reads now "Novel approaches in search for biomarkers of cholangiocarcinoma".

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This is an review on an important topic. It summarizes a large number of potential biomarker in cholangiocarcinoma (CCA). However this leave the review somehow unfocused. For most of the biomarkers there is no real solid evidence and positive and negative predictive values are not reported. It would be important to clearly state at the beginning the definition of biomarker, that here seems to be very extensive. It should also be clearly stated whether a certain biomarker is supposed to be of diagnostic or prognostic value.

Response:

We thank you very much for the meaningful comments and helpful suggestions to further improve our manuscript. We have identified, revised, and corrected the phrases that appeared to lack solid evidence. Further, the definition of biomarker has been added to the manuscript as suggested. Also, we carefully revised our manuscript and further improved it with respect to your relevant suggestions.

In addition, the authors should go carefully through the manuscript to detect mistakes (e.g. CAE instead of CEA). What is meant by the abbreviation OSN on page 12? What is a cCCA on page 16?

Response:

Thank you for the helpful suggestions to further improve our manuscript. We have identified, revised, and corrected the mistakes and the typographic errors throughout the text.

EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

According to reviewers suggestion, the manuscript could be accepted on WJG after revision.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade C (Good)

(2) Company editor-in-chief:

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. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. 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.

Response:

We thank you very much for giving us the possibility to revise our manuscript and further improve it. We have carefully reread our manuscript, addressed all comments, made all the suggested revisions to further improve our review, and provided all needed files as suggested.

Nevertheless, our manuscript was proofread and corrected by a professional English language editing company and a new language certificate was provided along with the manuscript.

We thank you very much for giving us the possibility to revise our manuscript. We hope that our revision complies with your remarks and comments. We would like to thank you for analyzing our revised manuscript and hope that it will now be acceptable for publication in the World Journal of Gastroenterology.

Sincerely,
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