Dear Editor,

Name of the Journal: World Journal of Gastrointestinal Oncology

Manuscript Number: 88296

Manuscript type: ORIGINAL ARTICLE

Observational study:

Identification of exhaled breath volatile organic compounds to distinguish between pancreatic adenocarcinoma, pancreatic cystic neoplasm, and patients without pancreatic-lesion

Tiankanon K. *et al*. PDAC exhaled breath volatile organic compound

Authors: Tiankanon K., Pungpipattrakul N., Sukaram T., Chaiteerakij R. Rerknimitr R.

We deeply appreciate the time the editors and the reviewers have taken in reviewing our manuscript. We have carefully modified the original manuscript based on these comments and suggestions which we believe have improved the manuscript. The changes are shown in the marked copy (highlighted in yellow). All revisions point-to-point replies and explanations are listed below for easy reference. Following these changes, we hope the revised manuscript can be published in the World Journal of Gastrointestinal Oncology journal.

We itemize our answers as follows;

Reviewer 1:

Useful study. However needs more data to validate the findings.

Response: We were grateful for the reviewer's words of encouragement. We recognize the significance and importance of the validation cohort. However, because there was no study of exhaled breath VOC in PDAC using TD-GC/FAIMS before, we intended to demonstrate a proof-of-concept study for the first phase. We agree that further validation of our study's findings is required before they can be used in clinical practice.

We have added sentences in the Discussion section on page 17 to describe this as a limitation of the study and the future direction of this work as follows:

"*The study could not show if early stage PDAC VOC levels were significantly different than at later stages of the disease and might not be effective as a screening method. A larger longitudinal study with more early-stage PDAC patients is needed*."

Reviewer 2:

The authors aimed to identify exhaled breath volatile organic compounds to diagnose PDAC. They compared the VOCs between patients with PDAC and patients with IPMN or healthy individuals. They found two VOCs, dimethyl sulfide and acetone dimer, were higher expressed in PDAC patients than those in control groups. Then the two VOCs seemed to be good biomarkers with high AUCs. Overall, the study was well organized and well written. Here are also some shortcomings:

1. VOCs in PDAC have been explored by previous studies (e.g., Br J Surg 2018, BMC cancer 2018). How about the difference between the current study and previous ones?

Response: We apologize for not adequately explaining the differences between our study and the previous ones. The method for collecting exhaled breath samples and the method used to identify VOCs are related to the differences in VOC identified. The previous study (Br J Surg 2018) used Bio-VOC breath samples to collect exhale breath, which potentially introduced ambient air into the samples collection more than alveolar air. Ion-molecule reaction mass spectrometry (IMR-MS) was used to profile the VOCs, which is more complex, takes longer time, and may not be suitable for use as a point-of-care technique. Another study from BMC cancer 2018 collected breath samples with the ReCIVA® device, similar to ours. However, the author used gas chromatography-mass spectrometry (GC-MS) to identify VOCs, which differs from the TD-GC/FAIMS method used in our study. The advantage of GC-MS is that it does not require a VOC library like TD-GC/FAIMS to identify the substance. However, TD-GC/FAIMS is far superior in identifying VOC in low concentrations, which we believe is more appropriate in exhale breath samples. We have elaborated on this matter in the Discussion session on page 15-16 as follows:

"The strength of this study compared to previous research is the method used for alveolar air collection and the use of FAIMS in combination with the XGBoost algorithm VOC library for VOC profile analysis. Previous VOC studies employed a variety of methods for collecting breath samples, such as aluminum bags or the Bio-VOC sample device, which potentially introduced ambient air into the samples collection more than alveolar air. We selected the ReCIVA^{TM} breath sample system with software to monitor the real-time progression of the breath sample collection with a pure oxygen gas supply to avoid contamination with confounding gas. We also collected the gas in thermal desorption tubes, which are more stable than aluminum bags.

A second difference compared to previous studies was the use of FAIMS instead of previously used complex analytic techniques for VOC extraction such as gas chromatography-mass spectrometry (GC-MS), ion-molecule reaction mass spectrometry (IMR-MS), or electrical nose. FAIMS, can distinguish VOCs by using specific mobilities in the electrical field of the substances. The advantages of this method include better VOC discrimination performance at low concentrations, which we believe is more appropriate in exhale breath samples, and a shorter runtime (approximately 10 minutes) compared to GC-MS (up to 45 minutes). As a result, it is regarded as a promising method in real clinical practice"

2.1 How about the specificity in disguising PDACs from other ampullary cancers? Sometimes it is hard to do using imaging.

Response: Thank you for the insightful suggestion. Due to the heterogeneity, cell type, and biomarker diversity of peri-ampullary cancers, such as PDAC, distal cholangiocarcinoma, duodenal cancer, or ampullary adenocarcinoma, VOCs produced from different cancers are therefore different. To reduce the heterogeneity of the VOC profiles, we included only PDAC patients in this "proof-of-concept" study to ensure disease group homogeneity. However, we agree that this is an area that should be researched further. We have elaborated on this matter as the limitation of our study in the Discussion section on page 17 as follows:

"Fourth, this is a "proof-of-concept" study. We intended to identify PDAC VOCs from potentially malignant pancreatic lesions like IPMN. Other benign pancreatic tumors with no malignant potential such as serous cystic neoplasm, as well as other peri-ampullary cancers such as distal cholangiocarcinoma, ampullary cancer, or duodenal cancer, were not included in

the comparison group. The specific VOCs that distinguish PDAC from these populations require further investigation"

2.2 However, the acetone dimer level in human secretions have been reported to be higher in a variety of digestive cancers. It seems to be a common biomarker in digestive cancers.

Response: Acetone dimer levels have been found to be higher in a variety of digestive cancers, including colorectal cancer, gastric cancer, HCC, and cholangiocarcinoma. We have elaborated this in the Discussion session. According to our findings, acetone dimers are the only VOC that has a better AUROC for PDAC diagnosis than CA19-9 and can increase the AUROC when combined with CA19-9. We believe that using acetone dimers alone as a biomarker may not be as beneficial as combining them with CA19-9.

3. Regarding IPMN, it is important to distinguish the malignant one from benign ones. Could VOCs do it? How many PDACs were from IPMN in the current study?

Response: Thank you for your insightful comment. We believe there may be a VOC that can differentiate between malignant and benign IPMN. Our study, however, was underpowered due to the small number of malignant IPMN (6 malignant IPMN among 42 IPMN). We believe that more research with a larger number of malignant IPMN patients is needed to answer this question. As suggested, we have included this limitation in our Discussion session on page 17 as follows:

"Secondly, because of the small number of malignant IPMN in our study, a conclusion that the VOCs can distinguish malignant from benign IPMN is problematic. Further research with larger numbers of malignant IPMN is still needed."

Importantly, we were not able to identify which patients developing PDAC from IPMN in our study as almost all patients were referred to our centers for PDAC treatment. We did not have information on the past history whether the patients had pre-existing IPMN prior to PDAC development. However, none of PDAC patients had any cystic component or other pancreatic cystic lesion that represented an area of current or previous IPMN. To make this point clearer, we have included the limitation in the discussion session as follows:

"Third, we cannot conclude which PDAC was developed from IPMN in our study. However, no PDAC patients had any cystic component or other pancreatic cystic lesion that represented an area of current or previous IPMN."

4. Other benign pancreatic tumors should be included.

Response: We appreciate your insightful comments. However, because this is a proof-of-concept study, we did not include other benign lesions without malignant potential, such as serous cystic neoplasm, pancreatic pseudocyst, or wall of necrosis to reduce the heterogeneity of control population. We believe that now that the concept of VOC has been proven, more research is needed to elaborate on the subject. This limitation was also added in the Discussion section page 17, as follow:

"Fourth, this is a "proof-of-concept" study. We intended to identify PDAC VOCs from potentially malignant pancreatic lesions like IPMN. Other benign pancreatic tumors with no malignant potential such as serous cystic neoplasm, as well as other peri-ampullary cancers such as distal cholangiocarcinoma, ampullary cancer, or duodenal cancer, were not included in the comparison group."

5. Any prognostic value of VOCs?

Response: We thank the reviewer for a thoughtful question. Exhaled breath VOCs in HCC was previously demonstrated their feasibility as a non-invasive tool for diagnosis, monitoring of HCC progression, and treatment response. (Sukaram T. et al.; *Scientific Reports* 2022). In that study, the levels of acetone, butane and dimethyl sulfide were significantly altered after treatment. Patients with complete response had a greater decreased acetone level than those with remaining tumor post-treatment (73.38 ± 56.76 vs. 17.11 ± 58.86 ($\times 10^6$ AU, $p = 0.006$).

To the best of our knowledge, the use of VOC in PDAC has primarily been studied for detection and discrimination, but we have yet to come across a specific study for prognosis evaluation.

In our study, dimethyl sulfide level was higher in the metastatic PDAC group than in the localized PDAC group (0.96 vs. 0.92 AU; p = 0.016), as shown in Table 3. This likely implied a prognosis value of VOCs in PDAC. However, more data on symptom-free survival and response to treatment after follow-up is required to confirm these findings. To clarify, we also expanded on this topic in the Discussion section, page 14-15, as follow:

"To the best of our knowledge, no previous study has demonstrated statistical differences in dimethyl sulfide levels between PDAC and non-PDAC populations. In this study, we discovered a correlation between dimethyl sulfide levels and PDAC metastasis status. This finding suggested that dimethyl sulfide could be used as a biomarker for PDAC metastasis. However, more data on symptom-free survival and response to treatment after follow-up is required to confirm these findings."

6. The authors have claimed in the limitation part that there was no external validation. But internal validation could be done.

Response: Thank you for your valuable suggestion. We recognize the significance and importance of the validation cohort. However, because there has never been a study of exhaled breath VOC in PDAC using TD-GC/FAIMS, we plan to conduct a proof-of-concept study in the first phase. Furthermore, because there are no screening tools for pancreatic adenocarcinoma at the moment, the number of patients we see each year is limited. Again, we recognize the significance of internal and external validation phases, and we are currently gathering samples for our next validation phase of research.

EDITORIAL OFFICE'S COMMENTS TO THE AUTHORS:

Science editor:

1 Conflict of interest statement: Academic Editor has no conflict of interest.

2 Academic misconduct: No academic misconduct was found.

3 Scientific quality: The authors submitted a study of identification of specific volatile organic compounds in exhaled breath to distinguish pancreatic ductal adenocarcinoma, pancreatic cystic neoplasm, and patients without pancreatic-lesion. The manuscript is overall qualified.

(1) Advantages and disadvantages: The reviewers have given positive peer-review reports for the manuscript. Classification: Grade C and Grade C; Language Quality: Grade B and Grade B. The study was well organized and well written. However, there are still some issues in the manuscript that need to be revised.

Response: We appreciated the editors' and reviewer's time and insightful comments. We addressed each reviewer's specific point-by-point comments and made the changes to our manuscript that were mentioned above.

(2) Main manuscript content: The author clearly stated the purpose of the study and the research structure is complete. However, the manuscript is still required further revision according to the detailed comments listed below.

(3) Table(s) and figure(s): There are 4 Figures and 4 Tables should be improved. Detailed suggestions for each are listed in the specific comments section.

(4) References: A total of 38 references are cited, including 9 published in the last 3 years.

4. Language evaluation: The English-language grammatical presentation needs to be improved to a certain extent. There are many errors in grammar and format, throughout the entire manuscript. Before final acceptance, the authors must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240.

Response: We apologize for any inconvenience our English may have caused. We have sent the manuscript to the Certified English editing service with whom we have contact. The certification for English editing was provided with the revised manuscript as requested, and we hope that this has made the manuscript more understandable.

5 Specific comments:

(1) Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited, including A,B, arrows, etc. With respect to the reference to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends are incorrectly formatted and require a general title and explanation for each figure. Such as Figure 1 title. A: ; B: ; C: .

Response: All of the graphics, images, and graphs were generated by our team and have never been published elsewhere. These figures and images are not the property of any third party. As suggested, we provided the editors with the editable Power Point files containing all of the editable figures used in the manuscript.

(2) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published, and correctly indicate the reference source and copyrights. For example, “Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]”. And please cite the reference source in the references list. If the author fails to properly

cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

Response: All of the graphics, images, and graphs were generated by our team and have never been published elsewhere. These figures and images are not the property of any third party.

(3) Please don't include any *, #, †, §, ‡, ¥, @….in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as $^aP < 0.05$, $^bP < 0.01$ ($P > 0.05$ usually does not need to be denoted). If there are other series of P values, $^cP < 0.05$ and $^dP < 0.01$ are used, and a third series of P values is expressed as $^eP < 0.05$ and $^fP < 0.01$.

Response: We have removed the symbols in the manuscript and replace them with superscript letters in all the images and tables as suggested.

(4) The structure of Abstract does not meet the requirements. The abstract includes five parts: "Background", "AIM", "Methods", "RESULTS", and "CONCLUSION".

Response: We deeply regret making these errors. We added the Background section and double checked that the abstract format is correct.

(5) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text (and directly before the References).

Response: We apologize to the reviewer for overlooking this section. As requested, we have added the Article highlight at the end of the main text.

(6) Please provide the Biostatistics statement.

Response: Thank you for your valuable suggestion. We double-checked our manuscript with the biostatistician and provided you with the Biostatistics Review Certificate, as requested.

(7) Please provide the Informed consent statement.

Response: We apologize for not clarifying this earlier with the editors and reviewers. Before collecting breath samples, all participants provided verbal and written informed consent. As suggested, we provided the signed consent form with an English translation and highlighted the inform consent statement in the Method section and footnote.

(8) Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. If there is no PMID or DOI, please provide the website address.

Response: We corrected the reference pattern in accordance with the suggestion.

(2) Company editor-in-chief:

- I recommend the manuscript to be published in the World Journal of Gastrointestinal Oncology. The title of the manuscript is too long and must be shortened to meet the requirement of the journal (Title: The title should be no more than 18 words).

Response: We have shortened the title and removed abbreviations to meet the requirement and to make it easier for the editor and readers to understand.

- When revising the manuscript, it is recommended that the author supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply PubMed, or a new tool, the RCA, of which data source is PubMed. RCA is a unique artificial intelligence system for citation index evaluation of medical science and life science literature. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/, or visit PubMed at: https://pubmed.ncbi.nlm.nih.gov/

Response: Thank you for your valuable suggestion. We have revised the manuscript with the latest result.

Again, we would like to thank the editors and reviewers for your valuable comments and suggestions on our manuscript.

Yours Sincerely,

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