

RESPONSE TO EDITOR'S COMMENTS AND PEER-REVIEW REPORT

Name of journal: World Journal of Radiology

Manuscript NO: 45529

Title: Imaging plaque inflammation in asymptomatic cocaine addicted individuals with simultaneous PET/MR Imaging.

Science editor: Jin-Lei Wang

Dear Dr. Wang and Respected Reviewers,

We appreciate the comprehensive feedback throughout the review process and are grateful for the endorsement of our paper for publication in your journal. We agree with a majority of the comments/suggestions, and have modified our claims to depict our findings as accurately as possible. In addition, the manuscript has now been comprehensively edited by co-authors who are native English speakers. We hope that the revised manuscript is clearer in terms of content and appropriate in terms of language, grammar, punctuation, spelling, and overall style. Changes were marked with Strikethrough and Underline.

RESPONSE TO EDITOR'S COMMENTS

1. English Editing Certificate from an editing company is required: Many of the co-authors on this manuscript are native English speakers. They have now reviewed the manuscript for content and edited it for clarity. We now provide an English Editing Certificate from both the first author as well as one of the other authors who is also a native English speaker (Dr. Kaufman). We hope that the clarity of the revised manuscript will satisfy the English language requirements needed for publication of this manuscript in your esteemed Journal.

2. Re-write the author contributions: The author contributions were re-written as follows:

Mani V, Goldstein RZ, Fayad ZA, and Alia-Klein N designed the research; Bachi K reviewed literature and collected drug use data; Mani V and Fayad ZA performed vascular imaging; Mani V, Kaufman AE, and Alie N analyzed imaging data; Bachi K performed statistical analyses; Bachi K, Mani V, Goldstein RZ, Fayad ZA, and Alia-Klein N provided interpretation; Mani V, Kaufman AE, Goldstein RZ, and Fayad ZA provided commentary and manuscript editing; and Bachi K and Alia-Klein N wrote the paper.

3. Provide and upload the approved grant application form(s): The approved grant

application forms have now been provided with this submission.

4. Check/Reframe Study Aim: The study aim was revised as follows:

Page 3: AIM: ~~To assess atherosclerosis in iCUD aiming to quantify markers of early vascular disease.~~ To assess inflammation, plaque burden and plaque composition in individuals with cocaine use disorder aiming to detect markers of atherosclerosis and vascular disease.

5. Write Core Tip/ Summary (>100 words): Core tips were added as follows: **Page 4:**

- Despite undetected clinical signs, cocaine use increases risk of stroke, coronary artery disease and myocardial infarction.
- Simultaneous carotid PET/MR can effectively evaluate vascular inflammation and plaque burden in individuals with cocaine use disorder.
- Cocaine users had increased wall area, comparable to individuals with cardiovascular risk and significantly higher than healthy controls.
- Wall area in cocaine users positively correlated with greater cocaine withdrawal and craving.
- Broader trials are warranted to develop protocols for early detection of cardiovascular risk and preventive intervention in individuals with cocaine use disorder.

6. Add Highlight: Article highlights were added according to the guidelines provided by the editor, including research background, research motivation, research objectives, research methods, research results, research conclusions, and research perspectives.

7. Revise references: the reference list was reformatted as requested.

RESPONSE TO SPECIFIC COMMENTS OF REVIEWER 1 (Reviewer's code: 00227565)

The authors wish to thank the reviewer for his/her thoughtful comments and suggestions. We have attempted to address the concerns raised and hope that the revised manuscript is better suited for publication.

1. The Methodology is sound yet the study sample is small to conclude a general statement:

We agree with the reviewer and are thankful for this feedback. We therefore added to the limitations, the following statement: **Page 20:** "These preliminary ~~findings~~ results should be considered in light of several caveats which limit the generalizability of the findings, including

small sample size, ~~the~~ limited number of women, and the absence of a match on race.” Additionally, in the discussion we state: **Page 21:** “Early detection and preventive intervention protocols will thus await the results of a broader trial.” We further deleted the following concluding remark in the discussion: **Page 21:** “It is clear, however, that vascular damage in iCUD should be considered with the aim of reducing impairment and sudden premature mortality.” Finally, we also modified the concluding remark in the abstract as follows: **Page 3-4:** “These preliminary results show markers of carotid artery disease burden in cardiovascular disease-asymptomatic iCUD. Broader trials are warranted to develop protocols for early detection of cardiovascular risk and preventive intervention in individuals with cocaine use disorder, ~~suggesting that cardiovascular screening and preventive treatment is greatly warranted in this population.~~”

2. The results have to be displayed in a more interesting way for the practicing clinician who has no time to pass through all the data displayed.

We appreciate this suggestion and have therefore added on **Page 17**, MRI images (see **Image 1**) showing plaque burden in a cocaine addicted individual as compared to a healthy vessel in a control subject. We hope these figures will provide the readers with further clarity regarding the results.

3. From the sample and AUC analysis the use of PET-MRI for this purpose is just fair and doesn't rank as a good tool. The reviewer wonders why the authors did not use a more practical and cost-effective tool such as CE-US that would address neo-vascularity as a marker of inflammation, avoid the logistic difficulties of MRI and PET-MRI as well as radiation associated with the use of PET. Furthermore, it will be rapid avoid measuring the background noise and its elimination as the authors do for the PET.

CE-US is indeed a practical and cost-effective tool that could be utilized in clinics to address neo-vascularity once disease is well characterized. This study aimed to examine mechanisms of action of the effects of cocaine use on vascular disease, which to date have not been characterized in asymptomatic cocaine addicted individuals. We therefore used the simultaneous Carotid PET/MR since this is the only method that allows for the precise quantification of inflammatory activity and plaque burden during a single scan, which are not possible to achieve with CE-US.

Notably, this advanced multi-modal imaging used is non-operator dependent, which enhances the reliability and validity.

4. Also some confusion due to poor language has been aroused during my review. For instance, 1. In the methods section: this phrase is perplexing. It needs to be rephrased to be clear “Urine tests on screening day identified drug use (all subjects) and on the two study days pregnancy (women). Measures of alcohol (with an alcohol breathalyzer) and tobacco use (nicotine and Cotinine in blood) were obtained at screening visit).”

The language has been meticulously edited and clarified throughout the manuscript (see details in pages 6 below). In particular, we have revised the noted sentence as follows: **Page 7:** “Drug use was assessed with urine tests in all subjects on screening day and pregnancy was tested in women on screening as well as on imaging visits. In addition, on screening day alcohol use was measured with a breathalyzer and tobacco use was measured by levels of nicotine and cotinine in blood.”

5. 2. In the discussion section: this statement “However, because the FDG inflammation measures did not correlate with the MR measures of plaque burden (nor with cocaine withdrawal or craving symptoms), the FDG measure may not represent the progression of atherosclerosis (indeed it does not indicate the presence of plaque).” Makes the research of non-sense and get us back why you did not use a cost-effective and handy tool like US and CE-US. I recommend addressing these points, working more on the organization, coherence of the work and meticulous English polishing of the manuscript to be palatable for the reader.

The noted incoherent sentence was removed. We clarified the abstract as follows: **Page 19:** The majority of iCUD and controls had carotid FDG-PET signal greater than 1.6 but lower than 3, indicating the presence of mild to moderate inflammation.

We also added to the discussion the following clarification: **Page 19:** “...overall the detected inflammatory levels in both samples were mild to moderate”.

6. Please see response #3 above regarding the choice of PET/MR rather than US or CE-US as the most suitable methodology for addressing the purpose of this study.
7. In addition, the manuscript was meticulously edited for proper English language, grammar, punctuation, spelling, and overall style (see details in pages 6 below).

RESPONSE TO SPECIFIC COMMENTS OF REVIEWER 2 (Reviewer's code: 03366604)

The authors wish to thank the reviewer for his/her thoughtful comments and suggestions. We have attempted to address the concerns raised and hope that the revised manuscript is better suited for publication.

1. The paper reports that FDG-PET showed inflammation in most controls in this study. It suggests that the result may indicate the beginning of an atherosclerosis process in those subjects, but the paper has not explained why this happened to most of the controls and whether a similar result is expected for the general population. Besides, if the result represents the general population, then it seems that PET may not be effective/useful for this particular application.

Thank you for this comment. Though inflammation as measured by 18-F-FDG PET was detected in both the cocaine as well as the control group, as we noted in response to comment #5 of the first reviewer: we added to the discussion the following clarification in **Page 19**: "...overall the detected inflammatory levels in both groups were mild to moderate".

2. Table 1 indicates that 13 of the 14 iCUD subjects were African-American. Though the Discussion explains the difficulty in recruiting controls who match the racial profile, it seems the difference in race can potentially create a bias in the results. The paper has not addressed this issue.

We added to the study limitations, the absence of matching on race by stating on: **Page 20**:

"These preliminary results should be considered in light of several caveats which limit the generalizability of the findings, including small sample size, limited number of women, and the absence of a match on race. Race is very important for cardiovascular disease with African-American individuals showing greater progression of coronary atherosclerosis as compared to Caucasians³⁹. Notably, among African-American men, cocaine was the largest contributor to overdose deaths⁴⁰. Therefore, close matching on race in similar future studies could reduce potential bias in results."

3. There is a big difference in nicotine usage between the iCUD and the control subjects. As mentioned in the Discussion, matching between groups on nicotine use could provide a better approximation of the vascular effects of cocaine usage.

We agree with this concern. As noted in response to query #2 of this reviewer, we added a comment about this study's caveats which limit the generalizability of findings.

4. On page 8, it is mentioned some PET and MRI indices were missing on some subjects. It is not clear to me to what the "indices" are referring.

We clarified the information regarding missing data as follows: **Page 9:** "PET data for one subject, right and left carotid MRI data of one subject, and right carotid MRI data of a third subject were ~~corrupted~~ not analyzable for iCUD."

5. The English writing needs editing to improve readability. Some sentences are hard to understand.

The manuscript was meticulously edited for proper English language, grammar, punctuation, spelling, and overall style.

Some examples of revisions made:

Page 3: "In the absence of clear cardiovascular symptoms, individuals with cocaine use disorder (iCUD) seeking addiction treatment receive mostly psychotherapy and psychiatric pharmacotherapy, with no attention to vascular disease (i.e., atherosclerosis) ~~in the absence of clear symptoms.~~"

Page 3: "~~Addiction questionnaires assessed~~ Drug use and severity of addiction were assessed with standardized instruments."

Page 5: "Cocaine use disorder (CUD), ~~is a long term chronic brain disease, of the brain but it also has multi-determined~~ imparts multiple cardiovascular effects."

Page 5: "Furthermore, cocaine's main vasoactive metabolite benzoylecgonine, a tropane alkaloid, is associated with hematological effects on the vessel and the loss of the endothelium's protective functions ~~itself is a substance that can cause vascular disease mainly through chronic vasoconstriction effects experienced with every use.~~"

Page 7: "~~Urine tests on screening day identified drug use (all subjects) and on the two study days pregnancy (women). Measures of alcohol (with an alcohol breathalyzer) and tobacco use (nicotine and Cotinine in blood) were obtained at screening visit.~~" Was revised as follows: "Drug use was assessed with urine tests in all subjects on screening day and pregnancy was tested in women on screening as well as on imaging visits. In addition, on screening day alcohol use was measured"

with a breathalyzer and tobacco use was measured by level of nicotine and cotinine in blood."