Response to the Reviewer's Queries

Date: June 14, 2022

Manuscript Title: Cell-free mitochondrial DNA quantification in ischemic stroke patients for non-invasive and real-time monitoring of disease severity and outcome

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On behalf of all the authors, I would like to thank all reviewers and editors for their constructive comments, which have helped us refine the manuscript. We are pleased to submit the revised manuscript for your kind perusal. The submission includes the following:

1. Response to reviewers' comments
2. Revised version of the manuscript (all changes are made in track version) **uploaded as Supplementary File** on submission portal
3. Figure as .ppt
4. Audio core tip
5. Copyright license agreement
6. Conflict of interest doc

We hope our responses to the reviewers' comments are satisfactory and believe that the revised manuscript is suitable for publication in your esteemed journal. Kindly find below the response to the reviewers' comments. We have made relevant changes to the manuscript.

S.No	Comments	Response to the Comments
Reviewer: 1		
	In this prospective article, the authors attempted to investigate the role of cf-mtDNA in determine severity and outcome in ischemic stroke patients, but there are many problems that need to be solved.	Thank you for your valuable inputs! Please find the revision and possible explanation for each query.
	(1) Intravenous administration of tissue plasminogen activator (TPA) is the gold standard treatment for acute ischemic stroke within window period, but not all patients benefit from this treatment, and a small number of patients even get worse because of the use of TPA. I want to know the effect of treatment with TPA on these patients. It is important because different treatment results may have a great impact on detection indicators, such as cf-mtDNA concentrations and relative ND1 expression levels in your study.	We agree with the reviewer's point for the variable response with TPA treatment. Our study included only those patients who had positive treatment response with TPA or who were managed in combination with anti-platelet therapy. All other patients who were non-responsive to these treatments were excluded from the study.
	(2) ROC analysis can't describe the correlation between two variables or two groups, but can often be used to evaluate the diagnostic and discriminating efficiency for diagnostic test. Sentences such as "ROC analysis for cf-mtDNA concentration between control and disease at onset showed significant association with almost linear response", "The ROC analysis for cf-mtDNA concentration between disease at onset and 24hrs of treatment showed significant association with 65.84% sensitivity and 55.12% specificity", and so on, are not accurately expressed in my opinion.	Thank you for your input! As suggested, we have revised the relevant findings throughout the manuscript.
	(3) "Patients after 24hrs of treatment didn't show significant difference with patients with onset. Similarly, no significant difference was observed between 24hrs and 72hrs of treatment". Since the intergroup comparison has shown no significant difference, ROC analysis seems to be of little significance.	We agree with the reviewer's suggestion. However, we included ROC analysis for such findings to compare the sensitivity and specificity between the groups compared to others.
	(4) Section "Intergroup analysis and diagnostic significance of cf-mtDNA concentration" is confusing and inconsistent with the Figure, which needs to be clearly explained.	We have revised the findings throughout the manuscript and clarified such findings.

(5) Sentences "Moreover, the values of relative expression of ND1 were comparable in patients at 24hrs (0.4474±0.4784 vs 0.9790±0.2605) and 72hrs (0.4474±0.4784 vs 1.105±0.03871) of treatment with control individuals (p>0.05)" should not be placed in this section, because the comparative analysis of relative expression of ND1 at 24hrs and 72hrs with control individuals has been described in the previous paragraph.	As suggested, we have deleted the sentence.
(6) Sentences "While, no diagnostic significance of ND1 relative expression values was observed between patients at disease onset and 72hrs of treatment and represented only 51.14% sensitivity and 50.28% specificity" is not consistent with the previous expression, and the description of the related figure also should be changed.	As suggested, we have revised the sentence and related description of the figure.
(7) The r value is -0.82 instead of -0.62 in the correlation analysis of circulating cf-mtDNA concentrations between 24hrs and 72hrs of treatment.	Thanks for pointing out the typo error. We have revised the values and cross-checked others accordingly throughout the manuscript.
(8) Why are there two values 0.867 and 0.863 between onset and 24hrs of treatment in the correlation matrix analysis of relative expression levels of ND1?	We have revised and simplified the plot and tables representing p values to avoid confusion.
(9) You don't correlate the patient's disease status including severity and outcome with the quantification of cf-mtDNA and don't apply some research tools for clinical neuroscience such as NIHSS, so you can't convince me to believe the role of cf-mtDNA in determine the severity and outcome in ischemic stroke patients.	We have added data for NIHSS score at baseline and compared with the cf-mtDNA concentration as well as Ct values of ND1. Hope this should give some relevant information for the role of cf-mtDNA in determine the severity and outcome in ischemic stroke patients.

Reviewer: 2	
The authors provide a primitive platform for non-invasive and cost-effective diagnosis and prognosis of patients with AIS using circulating cell-free mitochondrial DNA (cf-mtDNA) quantification and validation. I think that this paper may be precious providing useful data to the literature and adding new evidence, but I have some concerns: Major concerns:	Thank you for your valuable inputs! We have added new evidences as suggested and revised the complete manuscript with more relevant details. Please find the possible explanation for each query.
1. The inclusion and exclusion criteria for subjects in the manuscript are not clearly expressed, which affects the overall quality of the manuscript, please elaborate.	As suggested, we have revised inclusion and exclusion criteria in the manuscript in more detail.
2. The severity of ischemic stroke needs to be evaluated from multiple aspects. Our commonly used evaluation methods are NIHSS score and mRS score. In addition, we often discuss strokes in the anterior and posterior circulations separately. In this manuscript, I do not see a description related to it. If the authors have conducted research in this area, please specify.	We have added data for NIHSS score at baseline and compared with the cf-mtDNA concentration as well as Ct values of ND1. In our study the 80% cases had anterior circulation. As the number of cases in remaining 20% was very less, we couldn't separate the data based on anterior and posterior circulation. Hope this should give some relevant information for the role of cf-mtDNA in determine the severity and outcome in ischemic stroke patients.

Thank you again for consideration of our revised manuscript!

Sincerely,

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