

March,14th, 2023

Answer to the reviewers

Thank you for the time and effort that you have dedicated to providing valuable feedback on our manuscript ***Endoscopic ultrasound-guided fine-needle aspiration pancreatic adenocarcinoma samples yield adequate DNA for next-generation sequencing – a cohort analysis*** (Manuscript NO.: 82959, Observational Study). We are grateful for your insightful comments on our paper. We have been able to incorporate changes to reflect the suggestions you provided. Changes made in the manuscript are marked using track changes. It is our belief that the manuscript is substantially improved after making the suggested edits. Please find below the point-by-point response to each of the issues raised in the peer review report:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Dear Editor, Dear Authors, I read with interest the manuscript entitled “Endoscopic ultrasound-guided fine-needle aspiration pancreatic adenocarcinoma samples yield adequate DNA for next-generation sequencing – a cohort analysis” by Bunduc S et al. This was a well-conducted, relatively large single-center prospective observational study reporting on the efficacy of EUS-FNA by 22 or 25 G needles to obtain adequate DNA for next generation sequencing (NGS) among PDAC patients. Although I consider the manuscript relevant for the research context, I have the following minor comment only: 1) Strengths and limitations: **the non-randomized nature and the absence of a control group (i.e. EUS-FNB) should be mentioned as main limitations of the study.**

Answer for reviewer 1:

Language quality: we agree that especially for non-native English speakers, English polishing is never too much; to this end, **the paper was revised and updated by an authorized entity for English language correctness and appropriateness and a certificate that proves the process was added to the resubmission files**

Specific Comments to Authors : Strengths and limitations: we fully support the view of the reviewer in this matter and we have updated our manuscript accordingly. Please find below the before and after versions of that paragraph.

Before:

Strengths and limitations:

Our study included a high number of samples. Besides measuring the yielded DNA concentration and purity ratios, we were able to successfully perform NGS on subgroup of samples, functionality in the downstream application being a reliable method of sample adequacy evaluation ^[26]. Nevertheless, several limitations should be pointed out: our study was performed in a tertiary gastroenterology center and all involved personnel were experts in their fields (endosonographers, pathologists, biologists). The analysis

was not based on a prior sample size calculation therefore our results must be interpreted with caution especially since 71% of the procedures were performed with one EUS FNA needle type. We did not use 25G needles for our samples therefore our conclusions cannot be extrapolated to all FNA needle sizes. Moreover, the spectrophotometric methods may overestimate the quantity of amplifiable DNA by measuring not only the double stranded fragments, but also single stranded DNA, free and oligonucleotides ^[22].

After:

Strengths and limitations:

Even though relatively similar analyses have been previously reported, our study included a high number of sample^[21,27]. Besides measuring the yielded DNA concentration and purity ratios, we were able to successfully perform NGS on subgroup of samples, functionality in the downstream application being a reliable method of sample adequacy evaluation ^[26]. Nevertheless, several limitations should be pointed out: **1) study design – one of the main limitations of our work – since lack of patients randomization precludes the evaluation of causality between needle size and samples' NGS adequacy;** 2) our study was performed in a tertiary gastroenterology center and all involved personnel were experts in their fields (endosonographers, pathologists, biologists); 3) the analysis was not based on a prior sample size calculation therefore our results must be interpreted with caution especially since 71% of the procedures were performed with one EUS FNA needle type; 4) we did not use 25G needles for our samples therefore our conclusions cannot be extrapolated to all FNA needle sizes; **5) lack of a comparison group comprising samples obtained by EUS-FNB – another main limitation of our study; to this end however we cite the study of Razzano et al. that compared the performance for NGS between FNA, FNB and resection PDAC specimens^[29]. They obtained similar success rates for mutation and amplification analysis between FNA and FNB samples and proposed FNA material as a source for comprehensive molecular testing^[29].** Moreover, the spectrophotometric methods may overestimate the quantity of amplifiable DNA by measuring not only the double stranded fragments, but also single stranded DNA, free and oligonucleotides ^[22].

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Thank you for the opportunity to review the manuscript titled, Endoscopic ultrasound-guided fine-needle aspiration pancreatic adenocarcinoma samples yield adequate DNA for next-generation sequencing – a cohort analysis **The viewpoint of this article is objective and forward-looking.**

Language quality: we agree that especially for non-native English speakers, English polishing is never too much; to this end, **the paper was revised and updated by an authorized entity for English language correctness and appropriateness and a certificate that proves the process was added to the resubmission files.**

Specific comments to authors: We thank the reviewer for their kind and encouraging feedback.

Reviewer #3:

Scientific Quality: Grade D (Fair)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors: This manuscript evaluated the adequacy of pancreatic adenocarcinoma specimens for NGS biopsied by EUS-FNA, and found that EUS-FNA could sample adequate yield DNA for NGS. However, this study **was not innovative enough, and such similar finding has been reported before in a small cohort**. In addition, the English expression had to be polished by native English speaker.

Language quality: we agree that especially for non-native English speakers, English polishing is never too much; to this end, **the paper was revised and updated by an authorized entity for English language correctness and appropriateness and a certificate that proves the process was added to the resubmission files**

Specific comments to authors: We agree with the reviewer's comment that **findings to a certain extent similar to ours have been reported before. However we emphasize the size of our cohort as a strength of the study that comes to support the previous reports**. This confirmation, we consider is **justified taking into account the lack of strong recommendations regarding the methods of EUS TA for comprehensive molecular analysis in PDAC**. Please find the updates on the manuscript below.

Before:

Strengths and limitations:

Our study included a high number of samples.

After:

Strengths and limitations:

Even though relatively similar analyses have been previously reported, our study included a high number of sample^[21,27].