Peer Review File

Article information: https://dx.doi.org/10.21037/gs-22-742

Review A

Comment 1: *First, the title is not accurate and is misleading since it indicates that this is a diagnostic test but the authors did not examine the diagnostic accuracy of endoscopic ultrasound-guided 19G fine-needle aspiration. The authors need to revise the title to indicate the comparisons between heparin group and conventional wet-suction group, as well as the outcomes of interest of this study. Please revise other parts of the paper accordingly.*

Reply 1: Thank you very much for your comments. We have modified the title of this study.

Comment 2: *Second, the abstract is not adequate and needs further revisions. The background did not indicate the clinical needs for this research focus and what the knowledge gaps are in relation to the relative superiority of heparin vs. conventional wet-suction. The methods did not describe the inclusion of subjects, the assessment of the baseline clinical characteristics, and measurements of these outcomes of interest of this study. The results need to present the clinical characteristics and the baseline comparability of the two groups. Please report accurate P values, unless P<0.001. Because of the small sample size of this study, the current conclusion needs to be tone down.*

Reply 2: Your comments are greatly appreciated. We have adjusted the Abstract and given a exact P value in the manuscript if the P value is > 0.05.

Comment 3: *Third, the introduction of the main text has provided clear review and conclusions on the superiority of effect of heparin on improving the structural integrity of the biopsied tissue, so my question is why the authors still examined this clinical question. The authors need to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.*

Reply 3: Thank you very much for your comments. We have modified the Background.

Comment 4: *Fourth, in the methodology of the main text, please correctly describe the clinical research design, sample size estimation and assessment of baseline clinical characteristics. In statistics, please first describe the test of baseline comparability of the two groups. Please indicate the gold diagnosis for the test of diagnostic performance. The authors need to describe the calculation of 95%CIs since the sample is very small.*

Reply 4: Your comments are greatly appreciated. We have added these descriptions to the manuscript.

Review B

The paper titled "Detection value of endoscopic ultrasound-guided 19G fine-needle wet-heparinized suction for pancreatic solid tumors: a randomized controlled trial" is interesting. The results shows that wet-heparinized suction improves the quality of pancreatic solid tumor tissue biopsy obtained by 19G fine-needle aspiration and is a safe and efficient aspiration method in conjunction with MOSE for tissue biopsy. Given the limited number of included cases, more multi-center trials are desired to verify our findings. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There have been many studies on pancreatic cancer. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply: Thank you for your valuable instructions. We have added the following descriptions in the section INTRODUCTION (Page#4, Line 97-101):

① Macroscopic on site evaluation (MOSE) could help improve the diagnosis of pancreatic cancer, while it is poorly studied that how to improve the diagnostic efficiency of MOSE in pancreatic cancer-diagnosis through improving the quality of the samples. Therefore, further exploration regarding this issue would be necessary.

② We have proposed for the first time the use of wet-heparinized suction in processing the

puncture path, in combination with MOSE. We hope this would improve the diagnostic efficiency of MOSE for pancreatic cancer through improving the quality of the samples.

2) It is suggested to increase the correlation between white tissue length and the extracted DNA amount, which may make the whole study more complete.

Reply: Thank you for your valuable comments and instructions. The lack of DNA extraction did affect the completeness of our study. We have tried to add this process following your instructions. However, our technical personnel suggested that further DNA extraction might not be able to obtain accurate results in that the specimens were embedded in paraffin and prepared, and were preserved for a long time so that the DNA might degrade：

① The tissues were fixed using neutral formalin, which might exert a negative effect on the DNA molecules.

② With the prolonging of the fixing, multiple methylene cross-linked bridges were formed between macromolecules, making the DNA molecules more susceptible to random breakage.

③ The tissues needed to be heated during wax dipping and embedding, and part of the DNA molecules were unchained. The residual formalin in the tissues might methylate the unchained the DNA single strand, and the modified DNA single strand would be easy to degrade after cooling.

④ During the DNA extraction process, residual paraffin wax might prevent protease from binding to proteins in the tissues so that the release of the DNA could be affected. As for the association between the length of the tissue strips and the contents of extracted DNA, study by Lin My et al., has demonstrated that the length of the white tissue strips were positively associated with the contents of the extracted DNA. (Tissue Quality Comparison Between Heparinized Wet Suction and Dry Suction in Endoscopic Ultrasound-Fine Needle Biopsy of Solid Pancreatic Masses: A Randomized Crossover Study. Gut Liver. 2023 Mar 15;17(2):318-327. PMID: 36052613) Give this situation, we plan to take into serious consideration your valuable instructions in the following randomized controlled trial that we will conduct with other centers, and perform DNA extraction to ensure the quality of the study.

3) It is suggested that the current research status of pancreatic cancer and precancerous lesions in pancreatic specimens obtained under the guidance of endoscopic ultrasound should be added to the discussion.

Reply: Thank you for your valuable instructions We have added the relevant contents in the manuscript (Page#10, Line 298-302 and 316-320. Page#11, Line 338-343).

4) It is recommended to extend the follow-up time to observe possible adverse reactions.

Reply: Thank you for your valuable recommendation. We have tried to contact with the patients. Finally, we have successfully got in touch with 43 patients or their family members (20 in the heparin group and 23 in the control group). There were 5 patients who lost to follow-up (2 in the heparin group and 3 in the control group). Among these 43 patients, only 2 are still alive after receiving surgery and conventional treatments, and the other patients had died. Their survival time ranged from 3 months to 5 months. All of these patients reported no relevant adverse events after receiving the biopsy. Detailed results of the follow-up have been added in the manuscript (Page#10 and 11, Line 354-357).

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Utility of endoscopic ultrasound-guided fine-needle aspiration in pancreatic cancer patients who failed to obtain a pathological diagnosis in surgical exploration, PMID:35284304". It is recommended to quote the articles.

Reply: Thank you for your valuable instructions. The articles have been cited in the manuscript. (Citation 2, Page#3, Line 80).

6) The number of patient samples in this study is too small, and a large sample study should be added for verification.

Reply: Thank you for your valuable comments. The aim of this study was to preliminarily assess the feasibility and effect of wet-heparinized suction combined with MOSE for pancreatic solid cancers. Therefore, the patients were recruited from one single center, and the recruitment time was short. Strict exclusion criteria were set, leading a small number of participants. We plan to conduct a larger randomized controlled trial with other hospitals to further validate the conclusion of this study.

Reviewer C

1. Your article should also follow CONSORT checklist. Please kindly find the attached checklist for further revisions to your paper. See file "2-CONSORT Checklist.pdf". Please note that your paper would be published together with both STARD and CONSORT checklists. Furthermore, below elements are required for paper following CONSORT checklist:

a. According to the CONSORT checklist, you need to **register the trial** for your study, which is a must. You could check this website out for registration: http://www.chictr.org.cn/index.aspx

Please kindly revise your CONSORT checklist and indicate the relevant registration information at the end of the Abstract.

Here's an example: https://atm.amegroups.com/article/view/56152/html
Reply: Thank you very much for your suggestion. This study strictly followed the CONSORT checklist. We have attached the attachment for you to check; we have registered, and the registration number is ChiCTR2300069324.

b. **Trial Protocol** is required and is a must. Please provide it as a separate file to the editorial office (This could be written in English OR in Chinese). Please note that this protocol would be published together with the CONSORT checklist.

And the protocol statement should be indicated in "Footnote" section in your manuscript.
Reply: Thank you for your valuable comments. We've added it.

c. Your current Figure 1 should be re-structured as the one shown in the template attached (See file "2d_CONSORT 2010 Flow Diagram (Template).doc"). Please check and revise.
Reply: Thank you for your valuable advice. We have supplemented the CONSORT 2010 Flow Diagram (page18/Line518) in the manuscript.

d. **A Table** (**describing baseline characteristics of patients**) should be provided and cited in "Results" section. It is better to be named as Table 1, therefore you should rename the other tables in your paper.
Reply: Thank you for your valuable suggestion. We have supplemented the table of baseline characteristics of patients in the manuscript.(page16/line502-504)

e. Table 2 in CONSORT checklist is for **Abstract**. Thus, please pay attention to the Page/Line number and Section/Paragraph in the checklist. For example, "Section" here should be all filled out with "Abstract". For items not included in Abstract, you could just fill "N/A" instead.

Table 2 Items to include when reporting a randomized trial in a journal or conference abstract

Item	Description	Reported on Page Number/Line Number	Reported on Section/Paragraph
Title	Identification of the study as randomized		
Authors *	Contact details for the corresponding author		
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)		

Reply: Thank you for your valuable comments. We have filled it as required.

2. Please confirm if there is any Acknowledgments - those who contributed to the manuscript, but who do not qualify for inclusion as authors. Indicate all sources of support for the work (list funding/grants in a new paragraph). If there's no funding, please indicate "Funding: None".

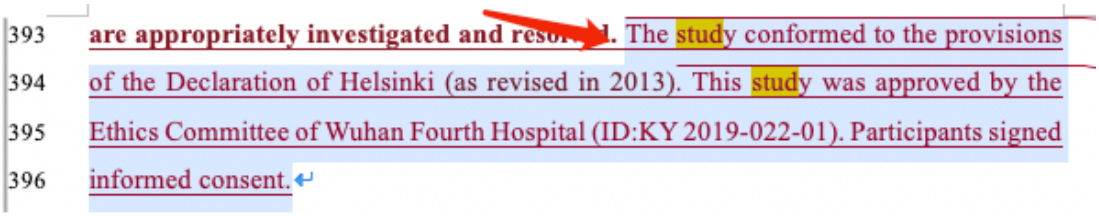
Reply: Thank you for your valuable comments. We have filled it as required.

3. Please define below abbreviations in Abstract.

group had the highest diagnostic performance, with a Youden index of 0.819 (AUC =0.944, 95% CI: 0.857, 1.000, P<0.05). There were no differences in the incidence of

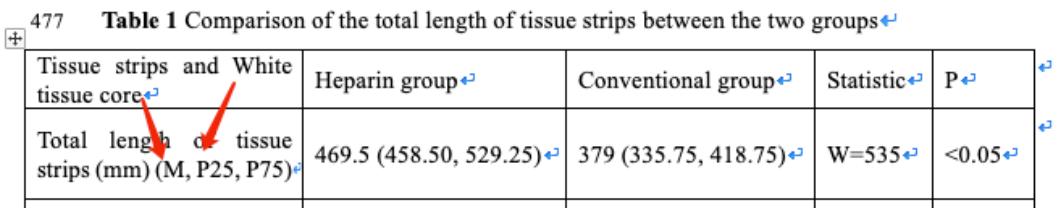
Reply: Thank you for your valuable advice. We have defined these abbreviations in Abstract. (page2/Line57-58).

4. Please also add below statements to Methods section of the main text.


393 are appropriately investigated and reso[illegible]. The study conformed to the provisions
394 of the Declaration of Helsinki (as revised in 2013). This study was approved by the
395 Ethics Committee of Wuhan Fourth Hospital (ID:KY 2019-022-01). Participants signed
396 informed consent.

Reply: Thank you for your valuable comments. We have added the statements to Methods section of the main text.(page4/line116-119)

5. What do M and P stands for? Please define them (provide the full name) in Table 1 footnote.


477 **Table 1** Comparison of the total length of tissue strips between the two groups

Tissue strips and White tissue core	Heparin group	Conventional group	Statistic	P
Total length of tissue strips (mm) (M, P25, P75)	469.5 (458.50, 529.25)	379 (335.75, 418.75)	W=535	<0.05

Reply: Thank you for your valuable comments. We have defined them. (page18/Line 518)

6. Please unify, use numbers or letters only. If they should be letters, please revise Figure 2 and resend us updated one.

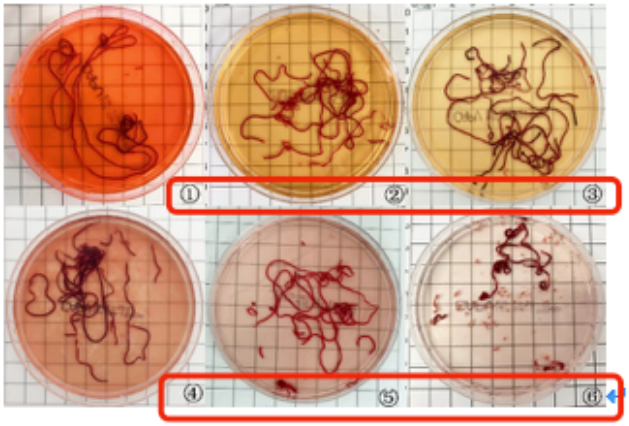


Figure 2 The specimens obtained from the first to third puncture in the heparin group (A-C). The specimens obtained from the first to third puncture in the control group (D-F).

Reply: Thank you for your valuable comments. We have unified it.