COMMENTS TO REVIEWERS:

Manuscript NO: 82711

Title: **Comprehensive review on endoscopic ultrasound-guided tissue acquisition techniques for solid pancreatic tumor**

Andrzej S Tarnawski, DSc, MD, PhD
Editor-in-Chief,
World Journal of Gastroenterology

Dear Dr. Tarnawski:

Thank you very much for inviting us to resubmit our manuscript, titled "Comprehensive review on endoscopic ultrasound-guided tissue acquisition techniques for solid pancreatic tumor." The manuscript ID is 82711.

The reviewers and editor provided excellent suggestions and guidance. We believe that the reviewers' suggestions have helped us to substantially improve the quality of the manuscript. I look forward to working with you and the reviewers to move this manuscript closer to publication in the *World Journal of Gastroenterology*.

The manuscript has been rechecked and the necessary changes have been made in accordance with the reviewers' suggestions (shown in red in the revised manuscript). The responses to all comments have been prepared and attached below. We sincerely hope that the concerns raised by the reviewers and editor have been sufficiently addressed and that the revised manuscript is considered for publication in the *World Journal of Gastroenterology*.

Thank you for your consideration. I look forward to hearing from you.

Sincerely ,
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RESPONSE TO REVIEWER #1:

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This is a clinically important a Review mentioning the EUS-TA procedure. However, there are several issues in this paper that need to be reconsidered.

1. *In P5L24, you stated that stylet-throw-pull method is applicable in EUS-FNA but not EUS-FNB. The reason for this needs to be clearly stated. The following investigation indicates that the stylet slow-pull method may also be effective for FNB.*

Roberto Di, Mitri, Filippo, Mocciaro, Filippo Antonini, et al. Stylet slow-pull vs. standard suction technique for endoscopic ultrasound-guided fine needle biopsy in pancreatic solid lesions using 20 Gauge Procore™ needle: A multicenter randomized trial. Dig Liver Dis ;52(2):178-184, 2020.

Response: Thank you for this pertinent comment. We have now deleted the following text from page 5, line 26 in "Negative pressure during EUS-FNA" subsection, and added the following text to page 10, line 16-19 in "Negative pressure during EUS-FNB" subsection:

"~~However, these theories apply to EUS-FNA, not EUS-FNB.~~"

"Stylet retraction and standard suction technique may be equivalent when using the 20G reverse-beveled needle[62]; however, the stylet retraction technique is considered equally or more useful than the standard suction technique when performing EUS-FNB."

The statement "However, these theories apply to EUS-FNA, not EUS-FNB" implies that "all citations in this subsection are to EUS-FNA, therefore another paper is needed to understand EUS-FNB." We are not suggesting that the stylet slow-pull method is not useful for EUS-FNB. The utility of the stylet slow-pull method for EUS-FNB was described in the section on EUS-FNB, citing a report of Bang et al.

However, we agree that the statement in P5L24 is misleading; therefore, we have deleted it.

2. *In introduction section, you mentioned personalized medicine and precision treatment, and I agree that FNB is important for these. Thus, "New Applications of EUS-FNB in the Era of Personalized Medicine" on P7L15~ can play an important role in this review. Therefore, I think this section should be emphasized, including future prospects.*

Response: Thank you for this pertinent comment. We have now added the following text to page 8 line 7–page 9 lines 13 in "The era of personalized and precision medicine" section. In addition, we have separated the section "The era of personalized and precision medicine".

"Advances have been made in both tissue collection methods and devices, and genetic analysis technology. The most common genomic alterations noted in metastatic pancreatic cancer specimens are in the *KRAS, TP53, CDKN2A,* and *SMAD4* genes. The first genetic analysis reported using EUS-FNA samples was KRAS mutation analysis. This driver mutation is found in more than 90% of pancreatic cancers, and drugs targeting KRAS mutations, such as Sotrasib, may be effective for pancreatic cancer[27,44]. The DNA mismatch repair (MMR) pathway is an important function that identifies and corrects base pair mismatches in DNA. Loss of MMR function leads to elevated microsatellite instability (MSI)-high, making the MSI test useful for evaluating pembrolizumab response. Sugimoto et al. reported that in unresectable pancreatic ductal adenocarcinoma, EUS-FNB with the Franseen 22G needle had a higher success rate in MSI analysis than EUS-FNA (FNB, 88.9% [8/9] vs. FNA, 35.7% [5/14]; P = 0.03)[45].
Targeted genome sequencing (TGS) applies polymerase chain reaction technology to construct and sequence a library of specific regions. Currently available multigene NGS systems, such as MSK-IMPACT (Memorial Sloan Kettering Cancer Center, New York, NY, USA) and FoundationOne CDx (F1CDx; Foundation Medicine, Cambridge, MA, USA), screen several hundred cancer-related genes simultaneously as companion diagnostic tests[46,47]. Whole genome sequencing (WGS) can detect various genomic structural alterations (translocations, inversions, duplications, chromosomal aberrations, chromosomal breaks) by obtaining sequence information of the entire genome, including non-coding regions. WGS may be more useful than TGS, but it is significantly more costly, requires more samples, and its superiority in therapeutic selection has not yet been proven[48]. RNA sequencing is a newly developed technology that can identify fusion genes faster, with greater sensitivity, and more efficiently than DNA panels. It can also detect new genes and genetic mutations.

Agents for actionable molecular changes are still being investigated. For example, evidence suggests trametinib in GNAS alterations[49], sotorasib in KRAS G12C alterations[44], olaparib in germline BRCA1/2 mutation[50], entrectinib in NTRK gene fusion[51], pembrolizumab in MSI-high[52,53], and afatinib in NRG1 fusions[54,55] may be effective treatment options. In malignancies such as pancreatic cancer, it is likely that multiple genomic drivers may be present. A combination of compatible drugs with targeting multiple genomic alterations at once may overcome this; however, evidence is lacking, and further studies are needed[56,57]. The application of precision medicine for treating pancreatic ductal adenocarcinoma has just begun, and further development of genetic testing equipment, drugs, and tissue collection devices is required[27]."

3. *I agree that the ROSE is unlikely to be recommended for diagnostic practice in the era of EUS-FNB. As a method similar to ROSE, there is Macroscopic onsite evaluation (MOSE). A following study reported that MOSE can evaluate the amount of tissue samples obtained by EUS-FNB, and MOSE may play an important role in NGS. Therefore, it is recommended to mention MOSE in this review.*
Junichi Kaneko, Hirotoshi Ishiwatari, Keiko Sasaki, et al. Macroscopic visible core length can predict the histological sample quantity in endoscopic ultrasound-guided tissue acquisition: Multicenter prospective study. Dig Endosc. 34(3):622-631. 2022.

Response: Thank you for this pertinent comment. We have now added the following text to page 11, lines 23–28 in "Rapid on-site pathologist evaluation and macroscopic on-site quality evaluation" section:

"Direct observation of specimens obtained by FNA/B, macroscopic on-site evaluation (MOSE), is more feasible and readily available alternative to ROSE[68,69]. Kaneko et al. showed that the macroscopic visible core (MVC) length and histological sample quantity were positively correlated in EUS-FNB using a 22G Franseen needle. Multivariate analysis showed that MVC length ≥30 mm on MOSE was a significant factor affecting suitability for NGS (odds ratio 6.19; 95% CI 2.72-14.10)[70]."

4. *Regarding needle tract seeding (NTS) after EUS-FNA for patients undergoing distal pancreatectomy for pancreatic cancer, I recommend referring to the following study. A non-negligible NST rate of 3% was observed in the following study. Kei Yane, Masaki Kuwatani, Makoto Yoshida, et al. Non-negligible rate of needle tract seeding after endoscopic ultrasound-guided fine-needle aspiration for patients undergoing distal pancreatectomy for pancreatic cancer. Dig Endosc; 32(5):801-811, 2020.*

Response: Thank you for this pertinent comment. We have now added the following text to page 12, lines 18–23 in "Needle tract seeding" section:

"Yane et al. also reported that the 5-year cumulative needle tract seeding rate, estimated using Fine and Gray's method, was 3.8% (95% CI 1.6-7.8%). They concluded that, although preoperative EUS-FNA for pancreatic body and tail cancers has no negative effect on recurrence-free survival or overall survival, needle tract seeding after EUS-FNA was observed to have a non-negligible rate[75]."

RESPONSE TO REVIEWER #2:

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: This article reviews the role of EUS-TA in pancreatic cancer, e.g. technical aspects, needle caliber, negative pressure, and puncture methods to obtain an adequate specimen in EUS-TA.

1. *It's suggested to supplement some comparisons between FNA and FNB*

Response: Thank you for this pertinent comment. Since we have already discussed factors that influence treatment decisions, such as tissue diagnosis and suitability for precision medicine, we will also include additional information on cost. We have now added the following text to page 7, lines 8–12 in "EUS-FNB for histological diagnosis" section:

"An RCT analysing cost-effectiveness found that pancreatic mass EUS-FNB (two passes without on-site cytopathology evaluation) was more cost-effective than EUS-FNA (number of passes dictated by on-site cytopathology evaluation). Variables with the largest impact were EUS procedure and sedation cost, specimen adequacy, and diagnostic yield associated with EUS-FNB[30]."

2. *It's recommended to ideal display the respective characteristics of FNA and FNB in the forms of Figure or Table.*

Response: Thank you for this pertinent comment. We have now added Table 1 to compare the characteristics of FNA and FNB. We have also added the following text to page 4, lines 24–25 in "EUS-FNA" section:

"We present a table summarizing the respective characteristics of FNA and FNB, focusing on key points (Table 1)."

3. *Please supplement some prospects for the future development of EUS-TA*

Response: Thank you for this pertinent comment. We have now added the following text to page 8 line 7–page 9 lines 13 in "The era of personalized and precision medicine" section:

"Advances have been made in both tissue collection methods and devices, and genetic analysis technology. The most common genomic alterations noted in metastatic pancreatic cancer specimens are in the *KRAS, TP53, CDKN2A,* and *SMAD4* genes. The first genetic analysis reported using EUS-FNA samples was KRAS mutation analysis. This driver mutation is found in more than 90% of pancreatic cancers, and drugs targeting KRAS mutations, such as Sotrasib, may be effective for pancreatic cancer[27,44]. The DNA mismatch repair (MMR) pathway is an important function that identifies and corrects base pair mismatches in DNA. Loss of MMR function leads to elevated microsatellite instability (MSI)-high, making the MSI test useful for evaluating pembrolizumab response. Sugimoto et al. reported that in unresectable pancreatic

ductal adenocarcinoma, EUS-FNB with the Franseen 22G needle had a higher success rate in MSI analysis than EUS-FNA (FNB, 88.9% [8/9] vs. FNA, 35.7% [5/14]; P = 0.03)[45].

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Agents for actionable molecular changes are still being investigated. For example, evidence suggests trametinib in GNAS alterations[49], sotorasib in KRAS G12C alterations[44], olaparib in germline BRCA1/2 mutation[50], entrectinib in NTRK gene fusion[51], pembrolizumab in MSI-high[52,53], and afatinib in NRG1 fusions[54,55] may be effective treatment options. In malignancies such as pancreatic cancer, it is likely that multiple genomic drivers may be present. A combination of compatible drugs with targeting multiple genomic alterations at once may overcome this; however, evidence is lacking, and further studies are needed[56,57]. The application of precision medicine for treating pancreatic ductal adenocarcinoma has just begun, and further development of genetic testing equipment, drugs, and tissue collection devices is required[27]. ”