Guayaquil, November 24, 2021

Jin-Lei Wang
Company Editor-in-Chief
Editorial Office
Baishideng Publishing Group Inc

Dear Editor,

Thank you for revising our article entitled "*Endoscopic ultrasound-guided through-the-needle microforceps biopsy and needle-based confocal laser-endomicroscopy increase detection of potentially malignant pancreatic cystic lesions: A Single-Center Study*" (Manuscript NO.: 70088, Retrospective Cohort Study). Below you will find the revision of the manuscript addressed following the reviewers' and editors' comments.

We would like to express our sincere gratitude to the editorial team and reviewers, as all comments and recommendations were driven constructively and have improved the quality of our article. We have addressed these revisions in a point-by-point manner, and all changes were made within the manuscript using the MS Word track change for your reference.

Kind regards,

Carlos Robles-Medranda, M.D.
Head of the Endoscopy Division
Instituto Ecuatoriano de Enfermedades Digestivas
Guayaquil, Ecuador

Reviewer #1 Comments

Reviewer #1:

Very important topic. detecting malignant potential of pancreatic cystic lesions is very challenging. However, I have some comments:

1. Please, if possible, to describe the malignant criteria for each technique (EUS alone, CEEUS, nCLE, … etc.)

Response to the Reviewer:

Dear Reviewer, thank you for your important suggestion. In the manuscript, we have fully described malignant criteria for endoscopic ultrasound (EUS), contrast-enhanced EUS (CE-EUS), cystoscopy, and confocal laser endomicroscopy (nCLE), as follows:

"Due to sparse cellularity of acquired specimens, several complementary clinical, radiological, and imaging techniques are required to achieve pancreatic cystic lesions (PCLs) definitive diagnosis. PCLs with potential to progress to malignancy mainly include intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN), and neuroendocrine tumors (CNET) with cystic degeneration. Identifying malignancy features for these lesions with EUS, CE-EUS, cystoscopy, nCLE, FNA, and mFB include the following:

- **EUS:** Presenting two out of the three following characteristics was considered as increased risk for malignancy criteria: main pancreatic duct dilation between 5-9 mm (10 mm high risk stigmata for malignancy), PCLs size >3 cm, and mural nodules presence (1,2).
- **CE-EUS:** A thick/hyper-enhancing wall/septum, enhancing solid component within a cyst, or an enhancing mural nodule favors malignancy criterion. Furthermore, there is a radiological correlation between pancreatic duct communication and IPMN diagnosis, but not MCN. Also, main duct type IPMNs hold a higher risk of malignancy transformation than branch duct type IPMNs (up to 68% vs 22%, respectively). MCN may show peripheral calcifications within multilocular septate lesions (1,3).
- **Cystoscopy:** Cloudy fluid and a smooth cyst wall identify MCN, while finger-like projections and a mucin cloud are perceived with IPMN through single-operator cholangioscopy (SOC) (3,4).

§ **nCLE:** Prone to malignancy lesions may depict epithelial or vascular patterns in nCLE (2,4–7). nCLE Epithelial patterns: MCN show epithelial borders with a flat mosaic appearance (single or multiple layers of epithelial bands). IPMN exhibit dark rings and papillary projections. Cystic neuroendocrine tumor (c-NET) portrays a trabecular pattern (fibrous bands separating cells nests). nCLE Vascular patterns: MCN, IPMN and cystic-NET may show a branched pattern; IPMN and MCN may also display a rope-ladder pattern (5).

§ EUS-**fine-needle aspiration (FNA)** and EUS-**micro forceps biopsy (mFB)** are resources for tissue sample extraction. For these techniques, cytology should be assessed in the context of radiological and clinical findings (1,3,4). Low and high-grade **IPMNs'** dysplasia should be distinguished as the latter may easily become invasive. Low-grade IPMN: may resemble normal gastric epithelium. High-grade IPMN may show a cell size ≤12-μm, hypo/hyperchromasia, background necrosis, nuclear irregularity, large single vacuolated cells, and increased nuclear to cytoplasmic ratio (3).

IPMNs histologic examinations exhibit four possible morphologies: *gastric* (columnar cells lining papillae with basally located nuclei rich in apical mucin), *intestinal* (similar morphology to colonic villous adenomas with cigar shaped nuclei and variable apical mucin amount), *pancreaticobiliary* (more complex papillae composed of rounded nuclei cuboidal cells with some prominent nucleoli), and *oncocytic* (complex papillae lined with round cells with granular eosinophilic cytoplasm and prominent central nucleoli) (1,3).

MCNs also display low and high-grade dysplasia features. While bland mucin-containing epithelium honeycomb sheets are seen with low-grade MCNs, a complex papillary structure with smooth nuclear contour mucin-containing cells, inconspicuous nucleoli, and fine chromatin is found in high-grade MCNs. On histologic examination, MCN's show focally flat o cuboidal lining and tall mucin-containing epithelium, with a densely ovarian-type stroma wall that positively stains for progesterone/estrogen receptors, calretinin, and inhibin (1,3).

C-NET aspirate display classic endocrine morphology (pseudorosettes, isolated, and loosely cohesive groups of round/polygonal cells with finely stippled chromatin round nucleus) (3–5,7). Immunostains (chromogranin, CD10, vimectin, and β-catenin cytoplasmic expression) provide a definitive diagnosis (3)."

2. If possible, to add chart based on your study to guide the readers when to use each technique according to the cyst size, type, suspicious malignant potential, … etc)

Response to the Reviewer:

Dear Reviewer, thank you for your important suggestion. All the cited EUS-related techniques are available to perform when malignancy on PCLs is suspected. However, larger cysts (especially >3cm) allow more techniques to assess for malignancy. We have added the following paragraph at the *Endoscopic techniques* sub-section: "*Indication of EUS-related techniques was based on endosonographers discretion. Although more techniques are available to perform on larger cysts (>3cm)*".

3. Adding EUS images, CE-EUS, nCLE will add value to the manuscript.

Response to the Reviewer:

Dear Reviewer, thank you for your important suggestion. We have added **Figure 1**, where we illustrate EUS (1A), CE-EUS (1B), and cystoscopy (1C) malignant criteria in a patient from our study cohort. In **Video 1** and **Video 2** we had illustrated the nCLE malignant criteria.

Reviewer #2 Comments

Reviewer #2:

This is a retrospective study aimed to compare the accuracy of the following EUS and associated techniques for the detection of potentially malignant pancreatic cystic lesions (PCLs): EUS-FNA, contrast-enhanced EUS, EUS guided fiberoptic probe cystoscopy, direct intracystic micro-forceps biopsy and EUS guided needle-based confocal laser-endomicroscopy. They focus on the differential diagnosis of potentially malignant PCLs (MCN, IPMN, neuroendocrine tumors) and non-malignant PCLs (SCN, pseudocysts). However, many readers will be more interested in the differential diagnosis of high-grade dysplasia/adenocarcinoma in nonmalignant PCLs. So, the authors should focus on the accuracy for diagnosing high-grade dysplasia/adenocarcinoma in MCN and IPMN using these modalities.

Response to the Reviewer:

Dear Reviewer, excellent observation. Certainly, there is a high interest of readers about the differential diagnosis of high-grade dysplasia in non-malignant PCLs. However, this study was designed in the context of PCLs assessment with EUS, to estimate EUS (and eventual used related techniques) diagnosability of malignancy considering a 24-months follow-up as gold standard. Your suggestion opens the door to the design of a prospective diagnostic trial to re-analyse histopathological samples of PCLs after discarding malignancy during follow-up (e.g., mucinous cystadenoma). In this hypothetical study, it must be preferable to analyse samples even with immunohistochemistry analysis: TP63, cytokeratin 5, 6, 7, 8, 18, 19, and 20. As a team dedicated to digestive disease research, we will consider this topic in our future trials. We are grateful for your input and wonderful idea. This study limitation that has been described in the manuscript's discussion as follows:

"Finally, as this study was designed in the context of PCLs assessment with EUS, to estimate EUS (and eventual used related techniques) diagnosability of malignancy considering a 24-months follow-up as gold standard, a prospective diagnostic trial to re-analyse histopathological samples of PCLs after discarding malignancy during follow-up may be warranted to further asses the accuracy in diagnosing high-grade dysplasia/adenocarcinoma in non-malignant PCLs (MCN, IPMN) using the studied endoscopic techniques."

Reviewer #3 Comments

Reviewer #3:

Thank you for giving me a chance to review the manuscript entitled "EUS-guided through-the-needle microforceps biopsy and needlebased confocal laser endomicroscopy increase detection of potentially malignant pancreatic cysts lesions during EUS assessment". There are some issues in this study.

1. What is the criteria of diagnosing malignancy by EUS, CE-EUS, Spy Glass, nCLE?

Response to the Reviewer:

Dear Reviewer, excellent question. In the manuscript, we have fully described malignant criteria for endoscopic ultrasound (EUS), contrast-enhanced EUS (CE-EUS), cystoscopy and confocal laser endomicroscopy (nCLE), as follows:

"Due to sparse cellularity of acquired specimens, several complementary clinical, radiological, and imaging techniques are required to achieve pancreatic cystic lesions (PCLs) definitive diagnosis. PCLs with potential to progress to malignancy mainly include

intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN), and neuroendocrine tumors (CNET) with cystic degeneration. Identifying malignancy features for these lesions with EUS, CE-EUS, cystoscopy, nCLE, FNA, and mFB include the following:

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- **Cystoscopy:** Cloudy fluid and a smooth cyst wall identify MCN, while finger-like projections and a mucin cloud are perceived with IPMN through single-operator cholangioscopy (SOC) (3,4).
- **nCLE:** Prone to malignancy lesions may depict epithelial or vascular patterns in nCLE (2,4–7). nCLE Epithelial patterns: MCN show epithelial borders with a flat mosaic appearance (single or multiple layers of epithelial bands). IPMN exhibit dark rings and papillary projections. Cystic neuroendocrine tumor (c-NET) portrays a trabecular pattern (fibrous bands separating cells nests). nCLE Vascular patterns: MCN, IPMN and cystic-NET may show a branched pattern; IPMN and MCN may also display a rope-ladder pattern (5).
- EUS-**fine-needle aspiration (FNA)** and EUS-**micro forceps biopsy (mFB)** are resources for tissue sample extraction. For these techniques, cytology should be assessed in the context of radiological and clinical findings (1,3,4). Low and high-grade **IPMNs'** dysplasia should be distinguished as the latter may easily become invasive. Low-grade IPMN: may resemble normal gastric epithelium. High-grade IPMN may show a cell size ≤12-μm, hypo/hyperchromasia, background necrosis, nuclear irregularity, large single vacuolated cells, and increased nuclear to cytoplasmic ratio (3).

IPMNs histologic examinations exhibit four possible morphologies: *gastric* (columnar cells lining papillae with basally located nuclei rich in apical mucin), *intestinal* (similar morphology to colonic villous adenomas with cigar shaped nuclei and variable apical mucin amount), *pancreaticobiliary* (more complex papillae composed of rounded nuclei cuboidal cells with some prominent nucleoli), and *oncocytic* (complex papillae lined with round cells with granular eosinophilic cytoplasm and prominent central nucleoli) (1,3).

MCNs also display low and high-grade dysplasia features. While bland mucin-containing epithelium honeycomb sheets are seen with low-grade MCNs, a complex papillary structure with smooth nuclear contour mucin-containing cells, inconspicuous nucleoli, and fine chromatin is found in high-grade MCNs. On histologic examination, MCN's show focally flat o cuboidal lining and tall mucin-containing epithelium, with a densely ovarian-type stroma wall that positively stains for progesterone/estrogen receptors, calretinin, and inhibin (1,3).

C-NET aspirate display classic endocrine morphology (pseudorosettes, isolated, and loosely cohesive groups of round/polygonal cells with finely stippled chromatin round nucleus) (3–5,7). Immunostains (chromogranin, CD10, vimectin, and β-catenin cytoplasmic expression) provide a definitive diagnosis (3)."

2. The diagnosability of EUS alone is too low. Would you please describe the reasons for that?

Response to the Reviewer:

Dear Reviewer, excellent question. EUS alone has a 52.9% specificity for detecting potentially malignant pancreatic lesions and a higher inter-observer variability. Considering that in this study three endosonographers performed the procedures, both the low EUS specificity and inter-observer variability are the main reasons of a very low EUS alone diagnosability. This has been detailed in the introduction section of the manuscript as follows: "EUS is the most sensitive diagnostic method for detecting potentially malignant pancreatic lesions, with an 88.5% sensitivity; yet it holds a 52.9% specificity and a higher inter-observer variability. Thus, EUS alone has very low diagnosability capacity. Similarly, a considerable number of PCLs cannot be characterized by CT, MRI or MRCP alone. EUS-guided diagnostics techniques increase EUS accuracy for differentiating PCLs, namely: a) EUS-FNA; b) contrast-enhanced EUS; c) fiberoptic probe cystoscopy (cystoscopy); d) EUS-guided

through-the-needle direct intracystic micro forceps biopsy (mFB); and e) EUS-guided confocal laser endomicroscopy (nCLE).”

3. Is the table 2 analysis for malignant diagnosability?

Response to the Reviewer: Dear Reviewer, excellent question. Yes, it is. Table 2 presents a uni- and multivariate analysis considered observed diagnostic agreement among EUS and EUS-related techniques with 24-months follow up. In a statistical context, the observed diagnostic agreement is a variable with a binomial distribution: malignant or non-malignant. So, table 2 analysis corresponded to malignant but also non-malignant diagnosability. To avoid any misinterpretation, we have considered to rewrite the table 2 caption: “*Table 2. Association between different additional performed techniques vs. a positive observed agreement for malignancy diagnosis among EUS and EUS-related techniques vs. 24-months follow-up. [OR (95% CI; P-value)]”.*

4. The results of ROC curve were shown in Table 3 and Figure 3. The independent variables (EUS alone, EUS+FNA/CE/Cystoscopy, EUS+mFB, EUS+nCLE, EUS+nCLE+mFB) were not continuous variables. How did you make the ROC curve?

Response to the Reviewer: Dear Reviewer, thank you for your question. ROC curve analysis is built from two types of variables: the predicting and the responding variable. The responding variable is also called “*outcome*” or “*endpoint*”. It must always be a discrete variable with a Bernoulli or binomial distribution: the classic dichotomic yes/no. In this study, the responding variable was the 24-months follow-up: malignant/non-malignant. On the other hand, the predicting variable can be either a continuous but also from a polytomous ordinal variable, or even a discrete variable with Bernoulli or binomial distribution. When a ROC curve is drawn from a continuous predicting variable, the result is precisely a soft curve. When a ROC curve is drawn from a discrete predicting variable with ordinal options, the result is a shaped curve. When a ROC curve is drawn from a Bernoulli or binomial predicting variable, the result is an angled curve. In this analysis, as you listed, we have the following predicting variables: EUS alone, EUS+FNA/CE/Cystoscopy, EUS+mFB, EUS+nCLE, EUS+nCLE+mFB. All of them corresponded to binomial variables (malignant/non-malignant). So, as you can see at the figure 4, all the drawn ROC curve are angled. A slightly rounded angle was drawn only for aesthetic purposes.

5. Statistical analysis is too complicated.

Response to the Reviewer: Dear Reviewer, our institutional biostatistician apologizes for the technically explanation of the statistical analysis. As a team, we see this thorough explanation is essential for the best comprehension of how this complex study was carried out.

Science Editor Comments

Science editor:

The manuscript elaborated EUS-guided through-the-needle microforceps biopsy and needle-based confocal laser-endomicroscopy increase detection of potentially malignant pancreatic cysts lesions. I find it a well-structured interesting study. An important question for the author is what is the criteria of diagnosing malignancy by EUS, CEEUS, Spy Glass, nCLE? In addition, a line is required at the bottom of the table. Abbreviations should be avoided in the title.

Response to the editor:

Dear Editor, great question. In the manuscript, we have fully described malignant criteria for endoscopic ultrasound (EUS), contrast-enhanced EUS (CE-EUS), cystoscopy, and confocal laser endomicoscopy (nCLE), as following:

“Due to sparse cellularity of acquired specimens, several complementary clinical, radiological, and imaging techniques are required to achieve pancreatic cystic lesions (PCLs) definitive diagnosis. PCLs with potential to progress to malignancy mainly include intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN), and neuroendocrine tumors (CNET) with cystic degeneration. Identifying malignancy features for these lesions with EUS, CE-EUS, cystoscopy, nCLE, FNA, and mFB include the following:

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  IPMNs histologic examinations exhibit four possible morphologies: *gastric* (columnar cells lining papillae with basally located nuclei rich in apical mucin), *intestinal* (similar morphology to colonic villous adenomas with cigar shaped nuclei and variable apical mucin amount), *pancreaticobiliary* (more complex papillae composed of rounded nuclei cuboidal cells with some prominent nucleoli), and *oncocytic* (complex papillae lined with round cells with granular eosinophilic cytoplasm and prominent central nucleoli) (1,3).

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papillary structure with smooth nuclear contour mucin-containing cells, inconspicuous nucleoli, and fine chromatin is found in high-grade MCNs. On histologic examination, MCN's show focally flat o cuboidal lining and tall mucin-containing epithelium, with a densely ovarian-type stroma wall that positively stains for progesterone/estrogen receptors, calretinin, and inhibin (1,3).

C-NET aspirate display classic endocrine morphology (pseudorosettes, isolated, and loosely cohesive groups of round/polygonal cells with finely stippled chromatin round nucleus) (3–5,7). Immunostains (chromogranin, CD10, vimectin, and β-catenin cytoplasmic expression) provide a definitive diagnosis (3)."

In addition to your excellent suggestion, we have removed abbreviations from the title, as following: "*Endoscopic ultrasound-guided through-the-needle microforceps biopsy and needle-based confocal laser-endomicroscopy increase detection of potentially malignant pancreatic cysts lesions during assessment*".

A line has been added at the bottom of the tables as per advised.

Company Editor-in-chief Comments

Company editor-in-chief:

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastrointestinal Endoscopy, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. Authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content.

Response to the editor:

Dear Editor, thank you for all your observations. We have carefully reviewed thoroughly the manuscript to perform corresponding adjustments.

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endomicroscopy for evaluation of pancreatic cystic lesions: a systematic review and international Delphi consensus report. Endosc Int open [Internet]. 2020 Nov [cited 2021 Dec 14];8(11):E1566–81. Available from: https://pubmed.ncbi.nlm.nih.gov/33140012/