

Dear Editor and reviewers, thanks for considering and reviewing our manuscript, and thanks for your valuable comments. This is a point to point response to your comments; we are hoping that it will satisfy your valuable queries and comments, thanks.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: Role of EUS, EUS-FNA, and Cyst Fluid Tumor Markers in the Diagnosis of Cystic Pancreatic Lesions.

1 Title. The title reflects well the main subject of the manuscript.

2 Abstract. The abstract summarizes and reflects the work described in the manuscript.

3 Key words. The key words reflect the focus of the manuscript. In addition, you may also add such terms as pancreatic cystic neoplasm, MCN, IPMN.

The words “pancreatic cystic neoplasm”, “MCN”, “IPMN” were added to the key words.

4 Background. The authors describe well the background, present status and significance of the study.

5 Methods. The authors mention that the final diagnosis was based on histopathology after surgery (15 patients out of 76 according to table 11) and positive cytopathology.

Q: Could you please specify what do you mean under the term “positive cytopathology”?

It means positive for malignancy, it was changed in the manuscript.

According to recent studies the accuracy of EUS-FNA in pancreatic cystic neoplasms is quite low. On the other hand, the morphologic EUS criteria themselves are not sufficient for final diagnosis of Pancreatic cystic neoplasms (Wu J, Wang Y, Li Z, Miao H. Accuracy of Fukuoka and American Gastroenterological Association Guidelines for Predicting Advanced Neoplasia in Pancreatic Cyst Neoplasm: A Meta-Analysis. *Ann Surg Oncol.* 2019 Dec;26(13):4522-4536. doi: 10.1245/s10434-019-07921-8. Epub 2019 Oct 15. PMID: 31617119). You also report that aspirated material was spread over dry slides for cytopathologic examination.

Q: Could you describe what kind of staining did you use (excluding mucin staining).

The stains used were Diff Quick stain of air dried smears Papanicolaou stain for alcohol fixed smears. The cell blocks were processed to paraffin blocks, and stained with H & E staining.

Q: Could you please specify sensitivity and specificity of your cytopathological examinations?

It was added in the manuscript.

As it is one of the difficult problems in diagnostics of pancreatic cystic lesions.

Q: Was the aspirated fluid sufficient for cytopathologic verification of the cysts?

Yes. As a rule we should evacuate the cyst as complete as possible to decrease the pressure inside it to minimize oozing of fluid from the puncture site, so we have a large volume of fluid. Only few ml is sufficient for chemical analysis, and the rest was sent for cytopathological examination.

Q: How did you define low-grade and high-grade dysplasia in a case of IPMN (excluding postoperative pathological diagnosis)?

According to subjective morphological changes in to high grade and low grade dysplasia.

The references are:

- Hruban RH, Takaori K, Klimstra DS, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol.* 2004;28(8):977-987. doi:10.1097/01.pas.0000126675.59108.80
- Longnecker DS, Adler G, Hruban RH, Kloppel G. Intraductal papillary mucinous neoplasms of the pancreas. In: Hamilton SR, Aaltonen LA, eds. *Pathology and Genetics of tumors of the digestive system.* Lyon, France: LARC press; 2000:237-240. World Health Organization Classification of Tumors.
- Terris B, Ponsot P, Paye F, et al. Intraductal papillary mucinous tumors of the pancreas confined to secondary ducts show less

aggressive pathologic features as compared with those involving the main pancreatic duct. Am J Surg Pathol. 2000;24(10):1372-1377. doi:10.1097/00000478-200010000-00006

You also mention such cyst characteristic as “wall thickness”. *Q: Did you measure it? If “yes”, please specify what values did you use for determining “thick” and “thin” cystic wall? What diameter of main pancreatic duct did you rate as “dilated”?*

Thin cyst fluid is up to 5mm, thick cyst fluid is larger than 5mm. the pancreatic duct is dilated when its diameter is more than 3mm in the head region, 2 mm in the body region and 1 mm in the pancreatic tail.

Q: You mention in text that MPD was dilated in 66 patients, while in table 2 you give the opposite information. Check this point please.

Thanks for your comment. It is corrected.

6. Results. It is very important and useful that the authors investigate this poorly highlighted issue of pancreatic cyst fluid examination, particularly wide range of tumor markers. It's better to use terms **serous cystic neoplasms and mucinous cystic neoplasms instead of cystadenomas according to WHO classifications** to avoid misunderstanding.

It was changed in the manuscript.

This also will allow to use your manuscript in meta-analyses in the future. You unify *malignant and potentially malignant lesions* in one

group. In my working group opinion, it would be better to divide these two groups in order to make proper conclusions, as treatment tactics differ in these groups.

We put potentially malignant and malignant in one group as the management is the same, so that any mucin containing cyst should be surgically removed regardless the degree of dysplasia in the cytopathological examination as it may turn malignant later on. The only exception is asymptomatic side branch (Branched-Duct) IPMN smaller than 3cm in diameter, which is also recommended for follow up of its size by CT or MRI.

7 Discussion. In literature that you cite in discussion part, **neoplastic and non-neoplastic** groups of cysts are given. Think about using the same terms instead of **malignant/ potentially malignant and benign**.

The difference in using the terms “malignant/ potentially malignant and benign” and the terms “neoplastic and non-neoplastic” in the benign neoplastic lesions as serous cystadenoma and lymphangiomas which will be considered benign in the first terms and neoplastic in the second term. Therefore the term malignant/ potentially malignant and benign is more reliable than neoplastic and non-neoplastic groups of cysts

8. Illustrations and tables. *Check please the values for pancreatic duct dilation in table 2 as you propose the opposite in results part of the text.*

Thanks for your comment. It is corrected.

Q: For table 3 it's better to use terms serous cystic neoplasms and mucinous cystic neoplasms according to WHO classifications.

Thanks for your comment. It is corrected.

Q: In table 7 check please the values of glucose and CEA as you mention the opposite in the text.

Thanks for your comment. It is corrected.

Q: In table 11 check please the values for MCN.

Thanks for your comment. It is corrected.

9. Biostatistics. The manuscript meets the requirements of biostatistics. 10 Units. The manuscript meets the requirements of use of SI units. 11 References. The citations are correct. 12 The manuscript organization and presentation are recommended to be slightly revised according to highlighted above questions and issues. The style, language and grammar are accurate and appropriate. 13 Research methods and reporting. The authors prepared the manuscript according to the appropriate research methods. 14 The manuscript met the requirements of ethics. Comments on writing. Cystic pancreatic neoplasms is very relevant subject in clinical practice. The authors performed very interesting, good arranged and useful investigation. Pancreatic cystic fluid has been poorly explored yet. In this study wide range of tumor markers is being estimated. The authors figured out tumor markers that can help in diagnosis of neoplastic pancreatic cysts. Long follow up period makes this investigation reliable. The combination of CEA,

glucose and SPINK1 plus mucin stain are useful in predicting neoplastic nature of pancreatic cysts. Since the diagnostic accuracy of EUS-FNA for pancreatic cystic neoplasms remains low we need additional tools of diagnosis, except cytopathologic examination. Cystic fluid analysis can help in differential diagnosis of pancreatic cystic neoplasms. The limitations of the study and its findings. It's better to use terms serous cystic neoplasms and mucinous cystic neoplasms instead of cystadenomas according to WHO classifications in order to unify terms. This also will allow to use your manuscript in meta-analyses in the future. You unify malignant and potentially malignant lesions in one group. It would be better to divide these two groups, and to use "neoplastic and non-neoplastic" terms instead of "malignant/ potentially malignant and benign" in order to make more specified conclusions. Let me thank the authors for interest in this field and for the great job that has been done. The difference in using the terms "malignant/ potentially malignant and benign" and the terms "neoplastic and non-neoplastic" in the benign neoplastic lesions as serous cystadenoma and lymphangiomas which will be considered benign in the first terms and neoplastic in the second term.

Reviewer #2:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing)

Conclusion: Rejection

Specific Comments to Authors: The study is somewhat novel. The Author gathered as much biochemical information possible to assess which parameters should a clinician rely on to establish the need of resecting a pancreatic cyst. However, I have major concerns as follows:

- The Authors selected the patients to submit to cyst fluid (and EUS) assessment, namely those having cysts greater than 3 cm. The cut-off is arbitrary for each pancreatic cyst considered, and none of the Guidelines recommend surgery for a cyst "only" greater than 3 cm.

Q: What about the cysts smaller than 3 cm?

The few cysts smaller than 3 cm were followed up if asymptomatic and no severe dysplasia or malignant cells in the cytopathological examination.

- The primary outcome is not clear: *did the Authors proposed surgery by default each patient, regardless of the findings obtained, due to have a final histology for confirmation?* This resize also the concept of "comparison" proposed.

Surgery was proposed only in surgically fit patients with high risk stigmata as mural nodules, pancreatic duct dilatation larger than 10mm, obstructive jaundice or suspicious cytopathology, so it was done only in 15 patients.

- The 18 months period proposed is not suffragate by appropriate evidence, even produced by the Authors - *I don't see any demographics*

All our patients were followed up for 18 months and out of them (76 patients) only two died. Follow up course and interventions required were demonstrated in table 11 and 12. No need to repeat their demographic data.

- Tables are too much, some of them should be placed under supplementary material

Thanks for your comment. We will handle this during the submission process.

- Given the title itself proposed, I would expect a more detailed description of the EUS findings, that, nowadays, are the features on whom the decision to perform surgery relies on.

We stressed and concentrated on the mural nodules and pancreatic duct diameter which are the most predictive signs of malignancy. Other features as wall thickness, septations and size are not predictive of malignancy as they occur in both malignant and benign cysts. However we reported many of the EUS features in tables 2 and 9 as wall thickness, loculations, calcifications and turbidity of fluid.

- The Authors should focus on the features associated with pre-malignancy (high-grade dysplasia), that may become curative, rather than on malignancy ones (if a patient has been under surveillance, then probably surgery arrived late)

We included all cases seen in the study period (76 patients) whether benign, potentially malignant or malignant cases. We put the premalignant and malignant cysts in the same category as both were treated by surgical excision if the patients were fit for surgery.

- *The Authors should provide also information on the features associated with "futile/unnecessary" surgery*

The histopathological examination of all 15 patients proved malignant or potentially malignant, so no futile or unnecessary operation were done in our series.

Reviewer #3:

Scientific Quality: Grade D (Fair)

Language Quality: Grade D (Rejection)

Conclusion: Major revision

Specific Comments to Authors: The study explored the role of cyst fluid biomarkers in differential benign and malignant PCLs. However, the study was not wrote in the regular form, which makes the study difficult to read. The result section was not well orgnised, I can't get what I want to know easily. Some comments:

1. These makers have been proved to have little value in predicting malignant PCLs. However, the predictive value was proved in this study. *What are the advantages of the study make the conclusion reliable?*

Thanks for your comments. Our study aimed to evaluate the role of EUS examination of cyst morphology with cytopathological and

chemical analysis and cyst fluid that could improve the differentiation between malignant and benign pancreatic cysts and avoid the unnecessary surgery for a benign pathology. Also, we could evaluate the value of several markers that help in predicting a malignant pancreatic cyst.

2. There were 31 pseudocyst in the study. The median amylase level was only 130 U/L, the data seems unlikelihood.

The study included 76 patients, so there are another 45 non inflammatory cysts including benign neoplastic cysts as serous cysts and lymphangiomas in addition to the mucinous cystadenomas in which the amylase level was very low.

3. Too many table. Some tables can be mixed together.

Thanks for your comment.

We will handle this during the submission process.

4. The study design was more like a retrospective study rather than prospective study.

It is a prospective study, the samples were collected and stored then all markers were done in the same specimens in the same time. This was added to the material and method section.

Thanks again for your valuable comments and the meticulous revision of our manuscript.