Peer Review File
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1st round author response to reviewers' comments

Reviewer A

This is an interesting study using a previously described technique of measuring level of PEI using PABA excretion rate in patients receiving radiotherapy for locally advanced PDAC.

I have a number of questions related to the current study -
1. Study group's PABA excretion rates were not compared with standard investigations for pancreatic exocrine insufficiency (PEI)such as-
a. Faecal elastase 1
b. 72 Hour Faecal fat content

Reply 1: Standard tests for pancreatic exocrine insufficiency (PEI) such as fecal elastase 1 and 72-hour fecal fat content were included in the revision.

Changes in the text:
We have modified our text as advised (see Page 9, lines 143-148)

2. PEI is a well-recognized clinical problem in patients with PDAC. Pancreatic enzyme replacement therapy (PERT) is standard treatment for patients with PDAC with evidence of wt loss and/or steatorrhoea. What is the additional benefit of PABA test?
Reply 2: As the reviewer noted, PERT is beneficial for PDAC patients with weight loss and fatty stools. In fact, patients in this study with decreased PABA excretion rates and symptoms of weight loss and fatty stools were prescribed Lipacreon (Pancrelipase, Viatris Pharmaceuticals). Further studies are warranted to investigate the utility and efficacy of the PABA test.

3. Did authors consider testing PABA levels before and after radiotherapy for locally advanced PDAC? This would clarify how radiotherapy adversely affect PEI in your study cohort.

Reply 3:
We originally intended to test PABA levels before and after radiation therapy, but most patients required medication with Lipacreon and/or acetaminophen, which affect PABA excretion rates. Changes in PABA excretion after radiotherapy is our research interest, but patient benefit is the priority. Therefore, we did not test PABA excretion rates after radiotherapy.

4. PEI Most of control group's PABA excretion rates were not in normal range. Explain why?

Reply 4: Normal values for PABA excretion rates are based on data from healthy volunteers. The control group consisted of post-operative CRC patients who had already received chemotherapy. Previous treatment and the recurrent tumor itself may have influenced PABA excretion rates in the control group.

5. Study groups' CT AP/ MRCP findings on size of main pancreatic duct would further support evidence for PEI.

Reply 5: We also agree with the comment that the CT AP/MRCP findings of the study groups on the size of the main pancreatic duct would further support the evidence for PEI. However, the imaging modalities and protocols varied from patient to patient in this study. Future studies using images obtained with the same imaging modalities and protocols will address the issue raised by the reviewer.

6. What are the rates of pancreatic endocrine insufficiency in your group of patients?

Reply 6:
In the study arm, patients with type 1 diabetes mellitus were excluded from the study, but 10 (41.7%) patients had type 2 diabetes mellitus. We added the rates of pancreatic endocrine insufficiency

Changes in the text 6:
We added the data on the rates of pancreatic endocrine insufficiency (see, page 6, lines 90-93.)

Reviewer B

The presence of pancreatic exocrine insufficiency in PDAC patients has been described in literature. I don't agree with your statement that this is the first manuscript to report this.

PABA test is not universally accept as a valid method to asses PEI.

Reply: We also agree with the reviewer's comment that the presence of pancreatic exocrine insufficiency in PDAC patients has been described in the literature. However, quantitative assessment of pancreatic exocrine function using PABA test in symptomatic patients with locally advanced PDAC has not been reported in the literature. Although the PABA test is not universally used as a measurement method for PEI, it is in fact routinely used in our country as a standard quantitative evaluation method. Since the PABA test is not widely used as a universal test, it is necessary to accumulate the results of studies such as this one. The validity of the PABA test has already been demonstrated by several clinical trials.

Noda, A., Hayakawa, T., Kondo, T. et al. Clinical evaluation of pancreatic excretion test with dimethadione and oral BT-PABA test in chronic pancreatitis. Digest Dis Sci 28,

230–235 (1983). https://doi.org/10.1007/BF01295118

Kodama M, Tanaka T, Seikoh R, et al. A study of exocrine pancreatic function by pancreatic function diagnostant (PFD) on cancer of pancreas and biliary tract. Hiroshima J Med Sci 1983;32(1):19-23.

Lankisch PG, Brauneis J, Otto J, Göke B. Pancreolauryl and NBT-PABA tests. Are serum tests more practicable alternatives to urine tests in the diagnosis of exocrine pancreatic insufficiency? Gastroenterology. 1986 Feb;90(2):350-4. PMID: 3484456.

Reviewer C

The authors present a cross-sectional study of PABA test results among treatment naïve patients with locally advanced PDAC who are preparing to undergo radiation therapy. Patients with colorectal cancer are recruited as controls. The study is straightforward and appears to be more of a pilot study, with a small number of individuals and a primary focus on PABA results rather than other aspects of PDAC care (such as survival, treatment response, etc).

Major

1. The main conclusion seems somewhat overstated. Consider editing to something like "Exocrine pancreatic insufficiency is prevalent among patients with locally advanced PDAC. Therefore, pancreas enzyme replacement therapy should be considered as part of treatment, in addition to anti-tumor therapies." This conclusion is contained in the abstract, key points, and the conclusion of the manuscript and should be rephrased at each occurrence.

Reply: In response to the reviewer's comment, we have changed the conclusion as suggested by the reviewer. This conclusion has been rephrased in the abstract, key points, and conclusion of the manuscript.

Changes in the text:

We have modified our text as advised (see Page 2, lines 34-48, Page 4, line 43, Page 9, lines 150-156).

Minor

1. Absorption of PABA requires hydrolysis of bentiromide by chymotrypsin, but also requires small bowel absorption of PABA. I would include the reference: Dr J.E. Witvliet-van Nierop, et al "exocrine pancreatic and enterocyte function in patients with advanced pancreatic cancer" in Clinical Nutrition 2019. They showed that exocrine function, not enterocyte function, is the determinant of malabsorption in pancreatic cancer.

Reply: In response to the comment, we added the reference and modified our text.

Changes in the text:
We added the reference and modified the test (see Page 8 lines 120-121, reference #5).

2. Page 3, line 83. These subjects were treatment naïve. Recommend changing to say "All patients were planned to receive palliative or radical radiation…"

Reply: In response to the reviewer's comment, we modified the statement.

Changes in the text:
We modified our text as suggested by the reviewer (see Page 6 lines 81-82)

3. Line 85 – "colorectal"

Reply: We corrected the typo.

Changes in the text:
We changed "corolectal" to "colorectal" (see Page 6, line 84).

4. Line 125-126 – these subjects are treatment naïve, so you cannot imply that radiation does or does not alleviate symptoms. Recommend rephrasing as "…locally advanced PDAC have impaired exocrine function suggesting the potential usefulness of pancreatic extract therapy."

Reply: We have rephrased the text as suggested by the reviewer.

Changes in the text:
We have modified our text as advised (see Page 8, lines 129-130)

2nd round author response to reviewers' comments

Reviewer A

I have reviewed the revised manuscript as the authors attempted to answer some of the comments.

Comment 1:
In current study, it lacks clear objectives of the study and it does not examine objectively the proposed method of assessing pancreatic exocrine insufficiency in patients with PDAC pre/post radiotherapy.

Reply 1: The objective of this study was to quantitatively evaluate pancreatic exocrine function in symptomatic patients with non-metastatic locally advanced pancreatic ductal adenocarcinoma prior to radiotherapy (see Page 2, lines 34-36, Page 5, lines 61-63). Whether the PABA test is indeed a feasible biomarker for assessing pancreatic exocrine function before and after radiotherapy is a subject for further research. Clinical trials of quantitative assessment of pancreatic exocrine function using the PABA test

before and after radiotherapy need to take into account confounding factors such as medication, tumor extent of pancreatic cancer, radiotherapy dose and field size. However, this study provides evidence to support the NCCN recommendation and demonstrates that exocrine pancreatic function can be quantitatively assessed in patients who are unable to undergo a stool test.

Reviewer B

Comment 1: This study attempts to assess exocrine pancreatic insufficiency (EPI) via a previously described urinary marker, PABA. An important limitation of this study comprises its small sample size and the use of an alternative test to assess EPI. Small sample size has been adequately addressed by the authors. PABA as a test is a surrogate like fecal elastase-1 and therefore provides indirect information about pancreatic function. I agree with Reviewer A that using fecal elastase in conjunction with fecal fat is more widely studied and accepted as the standard for diagnosing EPI. This should be clarified somewhere.

Reply 1:
We agree that the combination of fecal elastase and fecal fat is more widely studied and accepted as a diagnostic criterion for EPI. However, most patients undergoing radiation therapy have decreased appetite and little or no defecation. We have made this point clear in our discussions. (see Page 9, lines 138-140).

Comment 2: I suggest refining the text in the Highlight Box. For example, the key finding that patients with symptomatic locally advanced pancreatic cancer have impaired pancreatic exocrine function seems too generalized of a statement. It would be apt to switch the current key finding with the second bullet ("Quantitative assessment showed that most patients with symptomatic locally advanced pancreatic cancer had decreased exocrine pancreatic function").

Reply 2: In response to the reviewer's comment, we changed the "Key findings" to "Quantitative assessment showed that most patients with symptomatic locally advanced pancreatic cancer had decreased exocrine pancreatic function" (see, Page 4, Key findings).

Comment 3: In line 129, it is better to add the qualifier that PABA is a potentially useful biomarker *in patients who cannot undergo stool testing*. This helps address Reviewer A's Comment #2.

Reply 3: As the reviewer suggested, we added that PABA is a potentially useful biomarker *in patients who cannot undergo stool testing*. (see, Page 8, line 128).

Comment 4: Can the authors please clarify the diabetic status of patients in the control arm? Also, how was it known that none of the patients in the control arm had CRC – was their PABA or elastase level tested beforehand? A narrative review reports that,

“Autoantibodies against exocrine pancreatic antigens were detected in 77% of patients with type 1 diabetes, but were not detected in any patients with type 2 diabetes.”

1. Singh, V. K., Haupt, M. E., Geller, D. E., Hall, J. A., & Quintana Diez, P. M. (2017). Less common etiologies of exocrine pancreatic insufficiency. *World journal of gastroenterology*, *23*(39), 7059–7076. https://doi.org/10.3748/wjg.v23.i39.7059

2. Taniguchi T, Okazaki K, Okamoto M, Seko S, Tanaka J, Uchida K, Nagashima K, Kurose T, Yamada Y, Chiba T, et al. High prevalence of autoantibodies against carbonic anhydrase II and lactoferrin in type 1 diabetes: concept of autoimmune exocrinopathy and endocrinopathy of the pancreas. Pancreas. 2003;**27**:26–30.

Reply 4: None of the patients in the control group had diabetes mellitus, which was confirmed by their HbA1c test each time they received chemotherapy. No prior PABA or elastase testing is performed in the control patients.

Comment 5: In the discussion, the authors can mention that future studies should investigate differences in the pancreatic exocrine function based on location of the tumor in the pancreas (head vs. body. vs. tail) as there is evidence that enzyme secretion varies at each site.

Reply 5: In response to the reviewer's comment, we mentioned differences in pancreatic exocrine function depending on tumor location. (see, Page 9, lines 143-144).

Comment 6: The following author comments need to be embedded into the discussion as another limitation. “Normal values for PABA excretion rates are based on data from healthy volunteers. The control group consisted of post-operative CRC patients who had already received chemotherapy. Previous treatment and the recurrent tumor itself may have influenced PABA excretion rates in the control group.”

Reply 6: In response to the reviewer's comment, we have added the description in the limitations section of the discussion (see, Page 9, lines 144-147).

Comment 7: It is already established in the literature that there is a high prevalence of exocrine pancreatic insufficiency in patients with unresectable PDAC. As for your study’s conclusions, stating that EPI is prevalent among patients with locally advanced PDAC is too expansive in scope to be generalized from this study’s limited cohort. It can instead be stated that a high index of suspicion should be maintained for EPI because EPI carries clinical importance. Importantly, the National Comprehensive Cancer Network (NCCN) is one organization which already advised patients with pancreatic cancer to be treated with pancreatic enzyme replacement therapy. Therefore, this study provides evidence to support this recommendation.

Reply 7: As the reviewer suggested, we changed the Conclusions. (see, Page 9, lines

150-153).