Round-1

Dear Editor,

Many thanks for the valuable comments by reviewers.
Here you will find, point-by-point our paper changes:

Comments Reviewer 1:

1- Introduction - This sentence is not clear - . Although, both cytology and CEA intracystic fluid dosage are not perfect tools, the first with high specificity but low sensitivity, and the second with an accuracy of 54%

Currently, endoscopic ultrasound (EUS) with fine-needle aspiration (FNA) represents the most accurate procedure to discriminate the nature of the cyst by combining cytology and CEA intracystic fluid dosage; although,CEA specificity of 98% and sensitivity of only 48% and overall accuracy of 79% have been described and, in the absence of an associated solid component, pancreatic cyst fluid from cysts is frequently acellular or paucicellular with resultant low diagnostic yield [5,6].

2-Methodology - Authors state - undetermined PCLs were enrolled. However to reach reference diagnosis - a final diagnosis was reached in 36 (64%) patients by imaging and multidisciplinary team review. This is a major issue with methodology.

Otherwise, all the patients had follow up at 6 months with Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) scan or EUS, and final diagnosis was based on a consensus based of EUS findings plus CEA dosage with at least 12 months follow up.

3- How many EUS-specialists performed the procedure: 5

4- What was the experience of endosonographers in EUS-nCLE (per endosonographer). It is known from prior literature that the rate of acute pancreatitis and adverse events is higher during the first few cases of EUS-nCLE. In a current ongoing multi center US study, the EUS-MDs are required to perform at least 10 EUS-nCLE cases independently prior to enrolling any single patient. Experience in EUS alone doesn’t mean

expertise in EUS-nCLE. EUS-nCLE has two critical aspects - safely and effectively performing nCLE and real time image interpretation or real time high quality image acquisition.

Previous nCLE experience (>15/per operators)

4- The authors need to show the data used for: 1. Interobserver agreeement in reaching a final diagnosis for each case.

The extent of Agreement among raters in nCLE diagnosis was performed with Gwet's Agreement Coefficient (95%CI). Gwet's AC was shown to provide a more stable inter-rater reliability coefficient than Cohen's Kappa. It was also found to be less affected by prevalence and marginal probability than that of Cohen's Kappa, and therefore should be considered for use with inter-rater reliability analysis. For all measures of agreement, the guidance provided by Landis & Koch for the interpretation of κ was used: <0.00, poor; 0.00 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1.00, almost perfect [18,19].

5-What criteria was used to reach a final diagnosis in 36 patients.

The final diagnosis was based on histological analysis of the surgical specimen and/or when FNA results were diagnostic on cell block sections or smears. Otherwise, all the patients had follow up at 6 months with Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) scan or EUS, and final diagnosis was based on a consensus based of EUS findings plus CEA dosage with at least 12 months follow up.

6- Interobserver agreement in reaching nCLE diagnosis. Was there a kappa value with 95% confidence interval.

See point 4

7- Questions regarding adverse events: Can the authors specify the cyst types that resulted in acute pancreatitis/other adverse events.

Six adverse events (10%) were registered: 2 self-limited intracystic bleeding, occurred in one SCA and one IPMN, 3 cases of acute pancreatitis, occurred three IPMN, and 1 abdominal pain in IPMN.

Acute pancreatitis were classified as interstitial oedematous pancreatitis according Atlanta classification [20] and required patients hospitalization; none evolved to infected pancreatic necrosis or Walled-off necrosis (WON).

8-In results the authors state that acute pancreatitis was moderate-severe. However, in discussion they say - The cases of pancreatitis were mild and none of the pancreatitis evolved to walled-off necrosis.
We need more clarity in terms of severity.

See point 7

9-How was post EUS bleeding defined.

See point 7

10-What was classified as significant bleeding? Generally there should be intracystic bleeding but this is not clinically significant and is not necessarily an adverse event.

See point 7

11-The authors should perhaps list post EUS-nCLE pancreatitis as a separate line in the abstract. This is the most significant complication to consider.

See point 7

Comments Reviewer 2:
1-As the data analysed is heterogenous and not many RCTs are included in this study, the authors may add the limitations of this study, to the conclusion itself.

Limitations of our study are: a limited study group population, and the

small numbers of surgical final diagnoses available; this has been frequently described in PCLs studies due to the surveillance approach suggested by various International Guidelines even in lesions with high risk of progression (mucinous cystadenoma and IPMN > 3 cm) [21].

2-Also the authors may indicate the need for further prospective studies and RCTs to evaluate the role of NAC (peri operative chenmotherpy) for a more definitive conclusion. The paper mainly discuss the role of NAC but most of the studies discuss the role of peri operative chemotherapy. This may be clarified by the authors.

I have the feeling that the reviewer has mixed up our study with another one. We don not evaluate the use of NAC and in literature no available RCTs about the use of nCLE as it is considered a relatively new technique

Comments Reviewer 3:

This article demonstrates excellent performance of needle based confocal laser endomicroscopy (nCLE) in diagnosis of indeterminate pancreatic cystic lesions (PCLs). The authors' article caters to the current application of noninvasive diagnostic technology and has important prospective value in clinical practice. However, some major and minor revisions need to be handled before this study can be published.

1. Only 55 patients were included in this study. Such a small sample size seriously affects the reliability and applicability of the research results. It is suggested to **increase several patients' samples.**

All the enrolling centers submitted the study to local ethical committee prior enrollment and received an authorization to proceed with defined end of enrollment and end of the study. As well this a prospective study and it not possible to increase the patients samples.

2. To give better guidance to clinicians, try to discuss **how to reduce the incidence of adverse events**, such as acute pancreatitis , intracystic self-limiting bleeding and cyst infection, during the procedure of nCLE.

We had the feeling that a prolonged examinations of the cyst wall could be related with an increased risk of bleeding or debris that could enhance the risk of acute pancreatitis, however, this was not statistically

significant.

3. Some minor revisions in the contents have to be made.
    3.1. A few mistakes in grammar need to be corrected.
        3.1.1. Line 153, replace “bilio” with “biblio”.
        3.1.2. Line 198, replace “follow” with “followed”.
    3.2. Line 224, the full name of PD, pancreatic duct, should be written for the first time.
    3.3. Line 252 and 303, the full name of WON and GLs respectively should be noted.
    3.4. Line 70, add “, and” between “specificity” and “accuracy”
    3.5. Line 310, add a title for the table.

Corrections have been made

Sincerely
Helga Bernani

Modena 21/6/2021

Round-2

Dear Editor,

Many thanks for the valuable comments by reviewers.
Here you will find, point-by-point our paper changes:

Comment:

- In the abstract - please include only the rate of post procedure acute pancreatitis. If space allows overall adverse events can be listed separately. Also, as shown, overall adverse events can be listed in the main results section. The key issue is post-procedure acute pancreatitis which needs specific mention in the abstract.

-…Post-procedure acute pancreatitis occurred in 5%.

Corrections have been made according reviewer suggestion

Sincerely
Helga Bertani

Modena 23/8/2021