

Appendix Detailed Response to Reviewers' Comments

Reviewer #1

1. *The study is well conducted, however, some concerns needs to be adressed: English language needs major revision from an US native speaker with experience with pancreas diseases and imaging tests.*
RESPONSE: Prof. Alfred Cuschieri provided English language editing of the revised text. We attached the certificate of editing and the acknowledgement at the end of the revised manuscript.

Introduction 7

1. *What about EUS? Authors should at least cite this method which is essential on the follow-up of PCN.*

RESPONSE: We agree with you about the need to mention also EUS in the introduction, as a method useful in the study of some PNC. We added the sentence: *“Other imaging modalities used include Endoscopic Ultrasound (EUS) with or without fine needle aspiration (FNA), trans abdominal ultrasound (US), contrast enhanced US (CEUS) and contrast enhanced-EUS (CH-EUS). EUS is recommended in the current guidelines as an adjunct to the other imaging modalities in the assessment of patients harboring PNC with features identified during the initial investigation or follow-up, which may indicate the need for surgical resection. Despite its accuracy, EUS-FNA, is invasive and thus should be performed only when the results are expected to change clinical management. Although US and CEUS, are included in the Italian consensus guidelines for the diagnostic work-up and follow-up of cystic pancreatic neoplasms¹⁷, this recommendation is not included in the European Evidence Based Guidelines; although CH-EUS is considered for evaluation of mural nodules ¹¹.”.*

Methods

2. *This section is confusing. First authors report: “US scan every six months for the first year and then, for stable disease, annually from the second to the fifth year” and them “A planned MRI was routinely executed every two years for static disease or because of suspicious changes observed on US. Abdominal US was always performed just before the planned routine MRI.”. How the abdominal US was ALWAYS performed just before MRI if MIR is just performed every 2 years?*

RESPONSE: In this series, the MRI was always performed at the time of diagnosis, after the US discover of PNC, and then after two years, unless there were suspicious changes at US follow-up. At the second year, a planned US was preformed anyway just before the MRI. We better clarified this part, by changing the sentence with proper changes as follow: “**After US diagnosis and MRI confirmation, all** scheduled patients had been followed up with a non-conventional surveillance protocol in use in our Unit since **2012**. It consisted of an US scan every six months for the first year and then **in patients with** stable disease, annually from the second to the fifth year. A planned MRI was **performed** routinely every two years for **stable** disease **or at any time when** suspicious changes **were** observed on US. Abdominal US was always performed just before the planned routine MRI **at the second year of follow-up (Figure 1)**.”.

3. *Do you really have consent for abdominal US?*

RESPONSE: We intended that in our Hospital, all patients sign before every Imaging examination, sign an informed consent to authorize the scientific use of the collected data, included US. To clarify, the sentence was reworded as follows in the revised manuscript: “**All patients signed an informed consent to authorize the scientific use of the collected data**.”.

4. *Did you exclude patients that used Contrast enhanced? Does this procedures add costs?*

RESPONSE: Yes, we excluded them because contrast enhanced US (CEUS) was used only in few selected cases of patients with relative surgical indications (which was one of the exclusion criteria), because had shown at the time of the diagnosis septa or cystic wall thickness by B-mode US. It is also true that the use of CEUS would have added costs, although lower respect to the MRI. Both these considerations have been added in the methods and discussion sessions of the revised manuscript with the following sentences: “**CEUS was used only occasionally in few selected patients with relative surgical indications, which had shown at the time of the diagnosis septa or cystic wall thickness by B-mode US. Hence, it was not considered in the present analysis.**” and “**Although CEUS has been proven to be more sensitive than US, it is a more complex procedure to be performed, need of a venous access, of the availability of the contras media and is more time consuming and costly (about double respect to a conventional US)**.”.

Results

5. *Table 1: please specify where were the other lesions. How many lesions were at uncinete process and tail?*

RESPONSE: Done, we modified the table 1 by adding the data requested.

6. *Wirsung medium diameter was 2.6. Where the Wirsung was measure? Head or Body?*

RESPONSE: The Wirsung diameter was the wider reported on the records, regardless of location. We added a proper sentence in the revised text, to better clarify this point: "Retrieved data included baseline **patient's** characteristics, Wirsung **caliber (the widest portion regardless of location)**, PCN size (largest diameter)..."

7. *In the first case that the patient went to surgery, did you not perform an EUS before? Why?*

RESPONSE: Actually, EUS was performed as well, but because of the very distal localization of the lesion, and because of poor acoustic window the needle aspiration could not be performed. A specific sentence was added in the text to better clarify this point: "**After MRI, EUS was also performed, but because of the distal localization of the lesion and poor acoustic window, needle aspiration was not performed.**"

8. *"Overall, the US used in the PCN surveillance showed a sensitivity of 72%, negative predictive value of 94%, an accuracy of 95% and an AUC of a ROC curve of 86% (confidence interval 77 - 94 %; $p < 0.001$) (Figure 2)". This results are considering MRI as a gold standard?*

RESPONSE: Yes, they are. This point was expressed in the "Statistical analysis" section, with the sentence: "To determine the competency of US policy in the surveillance period relative to the gold standard MRI, a ROC test was performed with a calculation of **sensitivity**, negative predictive value, accuracy and Area Under the Curve (AUC). **P value less than 0.05 were considered statistically significant.**"

To better clarify this point, we also completed in the revised manuscript adding the following sentences of the materials and methods: "**In the protocol the US diagnosis was always confirmed with an MRI scan.**"; "**After US diagnosis and MRI confirmation, all scheduled patients had been followed up with a non-conventional surveillance protocol in use in our Unit since 2012. It consisted of an US scan every six months for the first year and then in patients with stable disease, annually from the second to the fifth year.**", and of the results section: "**Considering the MRI as gold standard, US used in the PCN**

surveillance showed a sensitivity of 72%, negative predictive value of 94%, an accuracy of 95% and an AUC of a ROC curve of 86% (confidence interval 77-94 %; $p < 0.001$) (Figure 3).”.

9. *You need to consider the value that you spent and not the value that you should spent if any of the patients needs MRI.*

RESPONSE: We completely agree that a control group would be important to add quality to the manuscript. However, in this article we mainly reported our positive experience with the described simplified US follow-up strategy with a reduced MRI frequency respect to the guidelines, for low risk PNC, in patient with good acoustic window, calculating real cost for this group. Because we did not have a control group, we simulated the cost that would have been sustained for the same patients, in case of rigid application of the current evidence based guidelines, that do not consider the possibility of delay the MRI, by replacing it with US, in the same clinical situation. To clarify how the cost-amount of the virtual control group was calculated, the checks interval, as well as the MR protocol virtually applied (without the contrast media administration), were added in the materials and methods section.

However, we understand that this is a limitation of the study, and we acknowledged it in the discussion session with the sentence: “The retrospective nature of the study may also influence extrapolated costs estimation based on the same cohort of patients undergoing surveillance by an exclusive MRI surveillance.”. In the revised manuscript, to further stress the importance of two real groups to be compared, in order to draw more definitive conclusions, we changed the final sentence as follows: “However, the proposed abdominal US-restricted MRI surveillance protocol need to be evaluated and confirmed by a prospective RCT against the currently recommended MRI-based surveillance with the RCT having both clinical and health economic endpoints.”.

10. *Change figure 3 for the second and real analysis.*

RESPONSE: As just explained, we can't perform a real comparative analysis in the present study, because of the lack of the control group (the study is retrospective and in our center, in the study period, all patients with low risk PNC and with good acoustic window were followed with this US-reduced frequency MRI program). Anyway, we think that the data

reported about the cost that would have been sustained for the same patients, in case of rigid application of the current evidence based guidelines, although with the acknowledged limitations, suggest to better explore the possibility to consider US in selected cases with comparative prospective studies, in order to introduce this choice into future guidelines.

Anyway, we agree with you that the figure could be confusing and we changed it by modifying the two groups "MRI vs US", in "US +Restricted MRI surveillance group vs Exclusive MRI surveillance group".

Discussion

The discussion is great showing that the US follow-up program can be used just in a selected group and with a experience US group.

11. *I just believe that authors should discuss about EUS. Otherwise, it appears that just US and MRI have a role in the management of PCN.*

RESPONSE: thanks for the suggestion. We completely agree that this was a limitation of the study, and we added also a specific comment on EUS in the discussion. The added sentence is the following: "EUS is helpful in resolving PCN with suspicious features, but on its own exhibits modest useful diagnostic performance for these lesions. However when combined with fine-needle aspiration (FNA), the diagnostic yield and accuracy of EUS are increased significantly, but because of the invasive nature of FNA, this combination should be reserved in selected PNC cases with suspicious features on the MRI suggesting need for surgery. Otherwise, because of its invasive nature, EUS with FNA is not suitable or recommended for surveillance follow-up modality¹¹."

Limitations: *Perfect. Well done.*

RESPONSE: Thank you for your appreciation.

Conclusion

12. *Please add US done by an expert physician*

RESPONSE: done

References: - *References are updated. It's good.*

RESPONSE: Thank you for your appreciation.

Reviewer #2

Authors reported on their clinical experience in terms of safety, feasibility and cost efficacy of a follow-up strategy based on abdominal ultrasound (US) with restricting use of Magnetic Resonance Imaging (MRI) every two years and suspicious cases. They concluded that in selected patients with PCN without absolute or relative surgical criteria, abdominal US, could be a safe complementary to MRI, delaying and reducing the numbers of second level examinations and therefore reducing the costs of surveillance.

Abstract

1. *What kind of pancreatic cystic neoplasms (PCN) are considered as without absolute or relative criteria for surgical intervention at the time of diagnosis? Please clarify.*

RESPONSE: In the first sentence of the abstract we generically refer to current guidelines. As method of patients' selection, because of the retrospective nature, we retrospectively included in the study only patients with diagnosis of PCN, that were followed with US in our Institution and that today would not meet absolute or relative surgical criteria, according to the European Evidence Based Guidelines. Patients followed with US, but that today according to European Evidence Based Guidelines would have surgical indication, were excluded. The group is obviously selected, because include only patients with good acoustic window (otherwise the patients would not follow the US follow up and would not have been the US database), and with low risk PCN at the diagnosis (absence of absolute or relative surgical criteria).

In the revised manuscript, to better clarify these points we changed the proper sentences of the abstract and of the materials and methods section respectively as follows: "Patients with pancreatic cystic neoplasms (PCN) **without surgical indication** at the time of diagnosis **according to current guidelines, require lifetime image-based surveillance follow-up.**"; "In patients **with non-surgical PCN at the time of diagnosis, US surveillance** could be a safe complementary to MRI, delaying and reducing the numbers of second level examinations and therefore reducing the costs." and "**The patient cohort for this retrospective study were obtained from our institutional, prospectively collected database and selected as patients with confirmed diagnosis of PCN without absolute or relative indications for surgery according to the current European evidence-based guidelines** ¹¹".

2. *'Mean follow-up period was 25.1 months (\pm 18.2)'. What is the end point of follow-up period? Please clarify.*

RESPONSE: Sorry for missing to report this datum. The end-point of the follow-up period was January 1st, 2018. We changed the proper sentence in the revised text as follows: **"The last date of entrance into the US follow-up for the group of patients included in the study was the 1st January 2017, with the end-point of the surveillance follow-up period was 1st January 2018."**

3. *What kind of PCN are defined as 'increased number of the PCN'?*

RESPONSE: In the 11 patients in which the bi-annual MRI identified evolution of the lesions not identified by US, the evolution was not related to a not US-detected increase of the known PNC or to a not US-detected appearance of worrisome features, but to an increased number of very small (few millimeters), PCN, with a MRI diagnosis of IPMN. We better clarified this point by modifying the proper sentence as follow: **"The bi-annual MRI identified evolution of the lesions not identified by US in only 11 patients with IPMN (5.5%), largely consisting of an increased number of very small PCN (p=0.14)."**

Background

1. *Authors mentioned 'PCN are encountered in as many as 3% of abdominal CT examinations and up to 20 - 45% of MRI scans', why the MRI detection rates are (too) much higher than CT in PCN? Please clarify if there is a bias beyond better sensitivity for cysts using MRI?*

RESPONSE: Based on our clinical experience, we agree with you that 3% vs 20-45% overestimate the real difference of the two examinations. However, these data are the ones reported by the Literature, in particular by the European pancreatic cystic lesion guidelines. The quoted studies supporting the CT pancreatic cyst prevalence result were:

- Chang YR, Park JK, Jang JY, Kwon W, Yoon JH, Kim SW. Incidental pancreatic cystic neoplasms in an asymptomatic healthy population of 21,745 individuals: Large-scale, single-center cohort study. *Medicine (Baltimore)* 2016; 95(51): e5535.
- Laffan TA, Horton KM, Klein AP, et al. Prevalence of unsuspected pancreatic cysts on MDCT. *AJR Am J Roentgenol* 2008; 191:802- 807.

In both these studies the sample was very large (more than 2000 of enrolled subject) and

the population was selected in order to include in the study only subject without known pancreatic focal or systemic diseases. The prevalence reordered was 2.1% and 2.6%.

As to the pancreatic cyst prevalence by MR, the supporting Literature was:

- Farrell JJ. Prevalence, Diagnosis and Management of Pancreatic Cystic Neoplasms: Current Status and Future Directions. Gut Liver 2015; 9(5): 571–589.

The prevalence reported in this study varied from 2% to 38% with an overall prevalence rate of 15%. This datum remanded to previous studies as follows:

- Girometti R, Intini S, Brondani G, et al. Incidental pancreatic cysts on 3D turbo spin echo magnetic resonance cholangiopancreatography: prevalence and relation with clinical and imaging features. Abdom Imaging 2011;36:196-205.

- Zhang XM, Mitchell DG, Dohke M, Holland GA, Parker L. Pancreatic cysts: depiction on single-shot fast spin-echo MR images. Radiology 2002;223:547-553.

- Lee KS, Sekhar A, Rofsky NM, Pedrosa I. Prevalence of incidental pancreatic cysts in the adult population on MR imaging. Am J Gastroenterol 2010; 105:2079-2084.

According to Zhang et al, the prevalence of the pancreatic cyst was 19.6% in a study population of 1444 subject. However, probably the prevalence was overestimated due to the inclusion of patient affected by known pancreatic pathologies, such as acute pancreatic disease.

According to Lee et al, the prevalence was about 13% (population size 616 subjects, all of them without already known pancreatic diseases). This datum is reliable: the higher prevalence of cysts detection compared to CT, was related to the intrinsic MR characteristic to be able to identify static fluid components by T2-weighted sequences.

Finally, Girometti et al. reported prevalence of cystic pancreatic lesions of 44.7%. This was the datum that widens the range of MR pancreatic cyst prevalence. The main bias of this study was a small sample of subject enrolled (152 subjects) which may alter the reliability of the prevalence result.

Based on these considerations, and in agreement with the Reviewer, we decided to change the prevalence of pancreatic cysts incidentally detected by MR into "~~13-19.6%~~ ", by excluding the study of Girometti. The corresponding references were also modified.

2. *'The most common PCN are pre- cancerous lesions' (is this really true?), why these patients should still be considered as 'PCN without absolute or relative surgical criteria'?*

RESPONSE: The sentence would mean that while Serous Cystadenoma do not have oncologic risk, the mucinous PCN (Mucinous Cystoadenoma and IPMN) present a known risk of malignant degeneration, which is the reason why the guidelines indicate the need for surveillance or alternatively of surgical intervention.

We agree with you that the term “pre-cancerous lesions” is probably too strong, and we modified the sentence in the revised manuscript as follow: “The **two** most common PCN lesions **are the** intra ductal papillary mucinous neoplasms (IPMN) and **the** mucinous cystic neoplasms (MCN). **Both are benign, but with an established risk of malignant progression; whereas others are always benign, without any risk for malignant transformation , e.g., serous cystic neoplasm (SCN).**”.

3. *Authors want to use ‘trans-abdominal ultrasound (US) in monitoring PCN’. However, according to the European evidence-based guidelines on pancreatic cystic neoplasms, ‘mural nodes < 5 mm are relative indications for surgical intervention’. Could trans-abdominal ultrasound (TUS) detect mural nodes < 5 mm? How about difficult patients? Please clarify in how many patients the pancreas can be sufficiently evaluated using TUS.*

RESPONSE: In the present manuscript, we only refer to “selected”, “low risk” and “with a good acoustic window” PNC/patients. All the other patients followed a standard frequency protocol with MRI, according to current guidelines.

Indeed, we think that probably the most important take home message is that a lot of little PNC are at very low risk of degeneration thus, following the current guidelines there has been an over-use of not necessary MRI.

We agree with you that detecting a mural nodule <5mm is very difficult with US, but it is also true that the appearance of this findings is very rare for little and non-evolutionary PNC, and in our reported experience never happened that a planned MRI discovered them, after a negative US. We reported this consideration in the discussion session in this sentence: “**As the clinical records of the patients included in the present study, contained no data on mural nodes, the ability of US to detect these changes was not evaluated, although, our view is that with conventional US, the difficulty of distinguishing genuine mural nodes from mucin plugs is substantial.** CEUS can be useful to **enhance** the ability of conventional US to **image and detect changes in** the inner **wall** of pancreatic cyst ²⁷. In some reports ²⁰ CEUS alone has **not been found useful or reliable in documenting suspicious abnormalities that** warrant change in management and consider MRI better for

detecting malignant transformation of IPMN, **such that a detected intra-cystic solid mural node requires inevitably a second level MRI exam, thus limiting utility of CEUS.**"

Regarding difficult patients and US intrinsic limitations they are discussed in the following sentence acknowledging the limitations of the study: "We acknowledge that the study has some limitations. The first is its retrospective nature, which prevented **inclusion** of patients with PCN, who could not be **assessed by US** because of a poor **acoustic window, thereby increasing** the risk of selection bias. However, in literature **cases with poor acoustic window precluding US assessment are not common and** range from 2 to 12% of **cases**³². The retrospective nature of the study may **also** influence extrapolated costs estimation **based on the same cohort of patients undergoing surveillance by an exclusive MRI surveillance.**"

4. *Nowadays, CEUS has been gradually recognized as an easy and comparable imaging technique in diagnosis of pancreatic lesions, why do authors still use the conventional trans-abdominal ultrasound (US) technique? Please clarify.*

RESPONSE: The use of US for the follow-up of PCN has the two main advantages respect to MRI of being very easy to be performed, fast and at low cost. Although CEUS has been proven to be more sensitive than US, it is a more complex procedure to be performed, need of a venous access, of the availability of the contrast medium and is more time consuming and costly (about double respect to a conventional US). Furthermore, CEUS is not panoramic as the MRI, because the various phases must be focused only on a precise target instead of the whole gland.

For these reasons, we think that CEUS is a good diagnostic technique in selected cases, when we have to study a precise finding of a B mode US, as alternative or complementary study to MRI, but it is not a good technique for a routine follow-up.

Following your suggestion, we added in the background a sentence to mention CEUS as other diagnostic tool available to study PCN and amplified the discussion on this point in the proper part: "**Other imaging modalities used include Endoscopic Ultrasound (EUS) with or without fine needle aspiration (FNA), trans abdominal ultrasound (US), contrast enhanced US (CEUS) and contrast enhanced-EUS (CH-EUS).**"

5. *Please clarify the role of Endoscopic Ultrasound.*

RESPONSE: We added the following sentences: “Other imaging modalities used include Endoscopic Ultrasound (EUS) with or without fine needle aspiration (FNA), trans abdominal ultrasound (US), contrast enhanced US (CEUS) and contrast enhanced-EUS (CH-EUS). EUS is recommended in the current guidelines as an adjunct to the other imaging modalities in the assessment of patients harboring PNC with features identified during the initial investigation or follow-up, which may indicate the need for surgical resection. Despite its accuracy, EUS-FNA, is invasive and thus should be performed only when the results are expected to change clinical management. Although US and CEUS, are included in the Italian consensus guidelines for the diagnostic work-up and follow-up of cystic pancreatic neoplasms¹⁷, this recommendation is not included in the European Evidence Based Guidelines; although CH-EUS is considered for evaluation of mural nodules ¹¹.”in the introduction; and “EUS is helpful in resolving PCN with suspicious features, but on its own exhibits modest useful diagnostic performance for these lesions. However when combined with fine-needle aspiration (FNA), the diagnostic yield and accuracy of EUS are increased significantly, but because of the invasive nature of FNA, this combination should be reserved in selected PNC cases with suspicious features on the MRI suggesting need for surgery. Otherwise, because of its invasive nature, EUS with FNA is not suitable or recommended for surveillance follow-up modality ¹¹.” in the discussion paragraph.

Methods

1. *What kinds of ‘PCN without absolute or relative indication to surgery’ were included in their current study and what are the criteria?*

RESPONSE: Please, see the response n.1 to your abstract comment.

2. *What is the gold standard for diagnosis in their current study?*

RESPONSE: The gold standard is the MRI. Please, see response n.9 to comment of the first reviewer to results section.

3. *What are the ultrasound diagnostic standard criteria?*

RESPONSE: The diagnostic US standard criteria for the suspect of PCN are: identification of one or more, partial or completely anechoic area into the pancreatic parenchyma and/or dilation of Wirsung duct>2mm, in absence of clear cause of obstruction. The diag-

nosis need always to be confirmed with the gold standard MRI. We clarified it with the following sentence of the materials and methods paragraph: “The diagnostic US criteria for suspect PNC were the identification of one or more, partial or completely anechoic area within the pancreatic parenchyma and/or dilation of Wirsung duct>2mm, in absence of identifiable cause of obstruction.”.

4. *How to differentiate malignant from benign PCN lesions by ultrasound? Please list criteria and cite studies or US guidelines.*

RESPONSE: Diagnostic criteria for suspect a malignant degeneration are the same for all the diagnostic techniques, although with different sensibility and specificity and are: presence of solid vascularized tissue into the cyst or presence of nodal or distant metastasis. Worrysome findings, that indicate an increased risk, but not automatically the diagnosis of a suspected malignancy are: Wirsung caliper >6mm, PCN size >4cm, presence of mural nodules or wall thickness or septa. The US, albeit with a lower sensibility can describe all of them, with the major limitations in identifying vascularization of solid tissue and or mural nodules. We clarified it with the following sentences of materials and methods paragraph: “Exclusion criteria were suspect or proven malignancy at the time of diagnosis (presence of solid vascularized tissue in the cyst, presence of nodal or distant metastasis at imaging, or positive histopathological findings)¹⁸, PCN with absolute or relative surgical criteria¹¹, clear diagnosis of SCN and absence of diagnostic MRI scan and follow up period less than 10 months.”.

5. *All patients underwent to a US 'every six month' annually. However, a MRI scan was routinely performed every 'two years'. The time intervals of US and MRI are different, how to make the results comparable?*

RESPONSE: At the interval of two years and when MRI was executed on demand as well, the US was always performed just before the MRI, so the comparison has been conducted between the two almost synchronous diagnostic techniques. We reworded the text to make this more clear as follow: “After US diagnosis and MRI confirmation, all scheduled patients had been followed up with a non-conventional surveillance protocol in use in our Unit since 2012. It consisted of an US scan every six months for the first year and then in patients with stable disease, annually from the second to the fifth year. A planned MRI was performed routinely every two years for stable disease or at any time when suspicious

changes **were** observed on US. Abdominal US was always performed just before the planned routine MRI **at the second year of follow-up (Figure 1)**."

6. *How to define 'stable disease' or 'static disease'?*

RESPONSE: we intend stable disease when no changes are detectable between two subsequent follow-up controls. To clarify, we added in the material and methods the following sentence: **"Stable disease was defined as PNC without detectable changes between two subsequent follow up images."**

7. *'The reasons for shortening the imaging interval and advance the MRI consisted of dilatation of main duct of more than 50%, increased size of the cyst 2 mm'. Could (can) MRI detect 'increased size of the cyst 2 mm'? Please clarify the accuracy of the methods to show changes of size.*

RESPONSE: We explain the accuracy of the methods in the specific session of image protocol of materials and methods as follow: **"The size of the voxels and therefore the spatial resolution depends on matrix size, the field-of-view (FOV), and the slice thickness. Moreover, higher magnetic field allows improved resolution. In our study the MR cholangiography matrix was 256x160, the slice thickness/spacing was 2.4/-1.2 mm, and the FOV was about 40. By applying these parameters, the 1.5 Tesla MR device allowed evaluation of variation in dimension of 2 mm. Spatial resolution at 1.5T MR commercial device has been reported in the Literature by S. Arizono et al.²¹ as 1.1x1.0 mm (inplane resolution) and 0.84 mm (minimum slice resolution)."**

8. *How about patients with difficult PCN lesion on ultrasound? Such as those located on the pancreatic tail?*

RESPONSE: These patients cannot be followed by ultrasound, albeit the PCN are at low risk, and so they must follow the standard MRI frequency follow-up. For better clarify this point we added in the discussion session this sentence: **"Even so, there can be no doubt that patients/PNC locations with poor acoustic window cannot be safely followed by US. In our opinion, the standard exclusive MRI surveillance is needed for the follow-up of these patients."**

Results

1. *'Two hundred patients with 261 PCN'. How many patients have single lesions and how many have multiple lesions? Are those multiple lesions always the same?*

RESPONSE: We added these information in the results session. We added this data with the following sentence: "At diagnosis 140 patients (74.5%) had a single PNC and 51patients had multiple PNCs (25.5%), the multiple PNCs being referred to IMPNs. The median number of cysts was 2 (range 2-5).".

2. *'Overall, the US used in the PCN surveillance showed a sensitivity of 72%, negative predictive value of 94%, an accuracy of 95% and an AUC of a ROC curve of 86%'. What are the US diagnostic standard mentioned here? How about that of MRI?*

RESPONSE: We reported the requested criteria in materials and methods and results section respectively, adding these sentences: "Specifically, the sensitivity, negative predictive value and accuracy refer to the ability of US to detect changes in PNC, with respect to the gold standard MRI at two years. The diagnostic criteria evaluated for this analysis are the same for both US and MRI. These are detected increase of the number of PCN (detection of new anechoic areas not identified in previous examination; increased of size PCN >2mm; increase of Wirsung caliber >50%. In this series, no patients developed mural nodules or thickness of the wall of PCN, hence, these were not included in the analysis."; "In the present study, the follow up surveillance program did not identify development of mural nodules or thickness of the wall either by US or MRI".

Discussion

1. *Why did authors only consider 'US can be considered an alternative method to follow PCN'? How about EUS? CE-(E)US? Please clarify.*

RESPONSE: The use of US for the follow-up of PCN have the two main advantages respect to MRI of being very easy to be performed, fast and at low cost. Although EUS and CH-EUS has been proven to be more sensitive than US, they are more complex and invasive procedures to be performed, need of a venous access, anesthesia, availability of the kontras medium and are more time consuming and costly. For these reasons, we think that EUS and CH-EUS are good diagnostic tools in selected cases, as alternative or complimentary study to MRI, but they are not good for a routine follow-up.

We agree with you about the need to better clarify this point and, following your suggestion, we added in the background and in the discussion, sentences to mention EUS and CH-EUS as other

diagnostic tool available to study PCN, and amplified the discussion on this point in the proper part, as follows: “Other imaging modalities used include Endoscopic Ultrasound (EUS) with or without fine needle aspiration (FNA), trans abdominal ultrasound (US), contrast enhanced US (CEUS) and contrast enhanced-EUS (CH-EUS). EUS is recommended in the current guidelines as an adjunct to the other imaging modalities in the assessment of patients harboring PNC with features identified during the initial investigation or follow-up, which may indicate the need for surgical resection. Despite its accuracy, EUS-FNA, is invasive and thus should be performed only when the results are expected to change clinical management. Although US and CEUS, are included in the Italian consensus guidelines for the diagnostic work-up and follow-up of cystic pancreatic neoplasms¹⁷, this recommendation is not included in the European Evidence Based Guidelines; although CH-EUS is considered for evaluation of mural nodules ^{11.}”; “EUS is helpful in resolving PCN with suspicious features, but on its own exhibits modest useful diagnostic performance for these lesions. However when combined with fine-needle aspiration (FNA), the diagnostic yield and accuracy of EUS are increased significantly, but because of the invasive nature of FNA, this combination should be reserved in selected PNC cases with suspicious features on the MRI suggesting need for surgery. Otherwise, because of its invasive nature, EUS with FNA is not suitable or recommended for surveillance follow-up modality ^{11.}” and “The use of US as part of the surveillance protocol in the follow-up of patients with PCN has certain advantages: ease of performance by fully trained ultrasonographers, fast, widespread availability and low cost...Although CEUS has been proven to be more sensitive than US, it is a more complex procedure to be performed, need of a venous access, of the availability of the contrast media and is more time consuming and costly (about double respect to a conventional US). Furthermore, CEUS is not panoramic as the MRI, because the various phases must be focused only on a precise target instead of the whole gland.

For these reasons, we think that CEUS is a good diagnostic technique in selected cases, when we have to study a precise finding of a B mode US, as alternative or complimentary study to MRI, but it is not a good technique for a routine follow-up.”.

2. *What is the definition of ‘target US’? Guided by MRI or CT?*

RESPONSE: We mean "...an abdominal US for established PNC ...", and we reworded the proper sentence in the manuscript accordingly.

3. *What are the ultrasound features of 'the development of new cysts and small mural nodes'? Please explain with certain figures.*

RESPONSE: We added the US features for the development of new cysts and small mural nodes in discussion section: "When detectable the US features indicative of the development of new cysts and small mural nodes include appearance of new anechoic areas in the pancreatic parenchyma and the appearance of a solid iso/iper-echoic component inside the anechoic cystic area ²⁶."

As in the present series, no patients developed mural nodules or thickness of the wall of PCN, we did not include in the manuscript figures related to this feature. If the reviewer would like anyway to see these images, although not included in the manuscript, we can attach, those of patients with US features indicative of mural nodules or thickness of the wall of patients with PCN, which having absolute or relative criteria at time of diagnosis, underwent to surgery and thus, they were not included in the present study.

On the contrary, because we registered in the presented series, appearance of new PNC, we included in the revised manuscript an example image (Figure 2).

4. *What kind of 'small new PCN' could be detected or diagnosed by conventional ultrasound?*

RESPONSE: Conventional US can detect all kind of small new PCN as new anechoic areas into the pancreatic parenchyma. After the detection, only the MRI or alternative more invasive techniques, can characterize them with a more precise diagnosis of nature. We added the sentence in materials and methods: "The development of new PCN is diagnosed by conventional US as a new anechoic area into the pancreatic parenchyma."

5. *What might be the potential risk or disadvantages of 'delaying the routine MRI imaging'? Please discuss in details.*

RESPONSE: We specified the potential risk or disadvantages of delaying the routine MRI in Discussion section: "The main potential risk of delaying the MRI imaging routine could be to miss an early recognition of worrisome features, but this has never been found in this study. Furthermore, even if this happened, the additional risk seems to be very low. In fact, relative indications for surgery according to European evidence-based guidelines¹¹, are not

an expression of degeneration but only of increased risk, which is estimated in about 5.7% in patients with one relative indication for surgery ²⁸”.

Figures 1. Figure 2

1. *ROC analysis showed the accuracy of US', please indicate the time of ultrasound in this figure. Did they only include MRI performed at 2 year after diagnosis?*

RESPONSE: No, they didn't. Here are included all the 2 years MRI, and those performed “on demand” during the follow-up. We modified the figure legend 3 as follows: “ ROC analysis showed the accuracy of US in the follow up respect to gold standard MRI performed at 2 year after diagnosis **and those performed “on demand” during the follow-up.**”.