POINT BY POINT RESPONSE TO REVIEWERS

Reviewer 1 (# 00008599):

Excellent, detailed review giving a great insight into this topic. Many institutions including us switched for solid lesions to FNB making ROSE not necessary and providing better tissue, yield, less passes and higher accuracy. For cystic lesions FNA can be used (not discussed in review). A 2.2% seeding rate with FNA of pancreatic lesions (Ref. 6) appears to be the exception. Only a few case reports have seeding of FNA of pancreatic lesions. Please run a spell check - there are many space issues.

AUTHORS ANSWER

Thanks for the comments.

- For cystic lesions FNA can be used (not discussed in review)
  Currently, cystic lesions are biopsied with FNA needles. However, the review is focused only on solid pancreatic lesions sampling.

- A 2.2% seeding rate with FNA of pancreatic lesions (Ref. 6) appears to be the exception. Only a few case reports have seeding of FNA of pancreatic lesions.
  The cited work on seeding (Ref.6), although a bit dated, is one of the few methodologically correct published study on this topic and therefore it remains a reference study, also because the process to methodologically verify the seeding percentage of a pancreatic biopsy (echoendoscopic or percutaneous) in everyday practice can be very difficult.

- Please run a spell check - there are many space issues.
  We performed the spell check and we corrected the text.

Reviewer 2 (#00505466):

The authors provide a comprehensive review on EUS-guided sampling of solid pancreatic masses. The review is too extensive and exhausting for the reader. The length should be definitely considerablyreduced. Some other comments follow below. In the abstract, I would suggest changing ‘the difficulty in obtaining a core tissue with intact histological architecture’ in the ‘the inability of obtaining a core tissue with intact histological architecture’. Please explain the acronyms EUS, RCT, RR, etc at first appearance in the text. In the paragraph on the role of ROSE, I would suggest replacing pathologist by cytopathologist, since in some countries pathology and cytology are different specialties.

‘Early data from three meta-analysis demonstrated that ROSE was associated with a statistically significant improvement in the adequacy rate [15; 18-19].’ Please provide some data. ‘Moreover, high quality studies recently published reported convertible conclusions [22].’ Please replace ‘convertible’ with ‘conflicting’. ‘A meta-analysis published in 2016 compared EUS-FNA with and without ROSE, including RCT, with a ...’. Please change ‘RCT’ in ‘RCTs’. I could imagine that the limited EUS-FNA passages with ROSE decreases procedure related morbidity. Are there any such data in the RCTs and meta-analyses? I would suggest to note the confidence interval (CI) in a different way. For example [RR 0.98 (CI: 0.91-1.06), (I2= 51%)] instead of [RR 0.98 (0.91, 1.06), (I2= 51%)]. ‘... but, in the absence of ROSE, FNB was associated with better diagnostic adequacy (P = 0.02).’ please provide RR and CI. ‘The authors [28] concluded that FNB without ROSE can supply EUS-FNA with ROSE without loss of diagnostic accuracy.’ Please replace ‘supply’ by ‘replace’ or ‘substitute’.. The authors provide only sample data from the last two FNB needle types. Please provide such data as well for the previous needle type so that some comparison is possible. ‘Nevertheless, the most recently introduced FNB-needle is the Acquire™ needle …’. It seems to me too much advertising and I would suggest omitting ‘nevertheless’. Since it is supposed to be a literature review, references should be noted at the last paragraph of page 6, the first paragraph of page 7, the first paragraphs in the section on the evolution of the needles, the first paragraphs on use and types of suction, the first paragraph on the use of Stylet, etc. The authors note ‘Up to now, the literature evidences do not support a strong superiority of FNB over FNA.’, but in the data following this phrase FNB seems to be superior in some studies. Is rephrasing of this opinion necessary here and in the conclusions? I would suggest collecting the data on ‘Type of EUS-FNB needles’ and ‘FNB vs. FNB needles’ in one heading, or to rephrase the second subtitle. ‘Many studies have reported a correlation between EUS-FNA accuracy and lesion size.’ References are required. I would suggest to change the header ‘Discussion’ in such as ‘Practical recommendations’, since during the previous sections there is already lots of data discussions. In figure 3, ‘istitute’ should be ‘institute and the acronyms (AIP, ADK) should be explained in the legend. Editing of the manuscript is definitely needed. The references should be note in a consistent way and according to the Journal’s guidelines.

AUTHORS ANSWER

Thanks for all the comments.

- The review is too extensive and exhausting for the reader. The length should be definitely considerably reduced.
  According to the suggestion, we reduced the length

- In the abstract, I would suggest changing 'the difficulty in obtaining a core tissue with intact histological architecture' in the 'the inability of obtaining a core tissue with intact histological architecture'.
  We replaced it, according to the suggestion (page 2, line 5-6).

- Please explain the acronyms EUS, RCT, RR, etc at first appearance in the text.
  We explained all the acronyms in the text and in the legend (pages 2; 4, lines 2; 4; page 6, line 22 and page 7, line 24)

- In the paragraph on the role of ROSE, I would suggest replacing pathologist by cytopathologist.
  We replaced it, as suggested (page 6, line6; 7)

- Early data from three meta-analysis demonstrated that ROSE was associated with a statistically significant improvement in the adequacy rate [15; 18-19].' Please provide some data.
  We added numerical data (page 6, line 11-13)

- 'Moreover, high quality studies recently published reported convertible conclusions [22].' Please replace 'convertible' with 'conflicting'.
  We replaced it, as suggested (page 6, line 21)

- 'A meta-analysis published in 2016 compared EUS-FNA with and without ROSE, including RCT, with a ...'. Please change 'RCT' in 'RCTs'.
  We changed it, as requested (page 6, line 32)

- I could imagine that the limited EUS-FNA passages with ROSE decreases procedure related morbidity. Are there any such data in the RCTs and meta-analyses?
  No difference was reported in terms of complications related to the number of passes in RCTs and meta-analysis. We added this consideration (page 6, line 27-28)

- I would suggest to note the confidence interval (CI) in a different way. For example [RR 0.98 (CI: 0.91-1.06), (I2= 51%)] instead of [RR 0.98 (0.91, 1.06), (I2= 51%)].'... but, in the absence of ROSE, FNB was associated with better diagnostic adequacy (P = 0.02).' please provide RR and CI.
  We changed the way to note the CI, as suggested, and we provided RR and CI where requested (page 7, line 25 and page 7, line 29)

- 'The authors [28] concluded that FNB without ROSE can supply EUS-FNA with ROSE without loss of diagnostic accuracy.' Please replace 'supply' by 'replace' or 'substitute'.
  We replaced the verb, as suggested (page 8, line 2)

- The authors provide only sample data from the last two FNB needle types. Please provide such data as well for the previous needle type so that some comparison is possible.
  We added more data and references (page 9, line 3-4)

- Nevertheless, the most recently introduced FNB-needle is the Acquire™ needle …'. It seems to me too much advertising and I would suggest omitting 'nevertheless'.
  We removed the word, as requested (page 10, line 1)

- Since it is supposed to be a literature review, references should be noted at the last paragraph of page 6, the first paragraph of page 7, the first paragraphs in the section on the evolution of the needles, the first paragraphs on use and types of suction, the first paragraph on the use of Stylet, etc.
  According with the journal style, we collected all the references at the end of the text and we noted each reference number in the text, when cited

- The authors note 'Up to now, the literature evidences do not support a strong superiority of FNB over FNA.', but in the data following this phrase FNB seems to be superior in some studies. Is rephrasing of this opinion necessary here and in the conclusions?
  Although the literature evidences did not support a strong superiority of FNB over FNA, most recent studies showed a trend in favor of the FNB, especially without ROSE, in terms of specimen adequacy with fewer needle passes. We rephrased the sentence, accordingly (page 14, lines 25-27)

- I would suggest collecting the data on 'Type of EUS-FNB needles' and 'FNB vs. FNB needles' in one heading, or to rephrase the second subtitle.
  We rephrased the second subtitle, as suggested (page 17)

- Many studies have reported a correlation between EUS-FNA accuracy and lesion size.' References are required.
  We added the references (page 18, line 17)

- I would suggest to change the header 'Discussion' in such as 'Practical recommendations', since during the previous sections there is already lots of data discussions.
  Thanks for the precious comment, we changed it (page 18)

- In figure 3, 'istitute' should be 'institute and the acronyms (AIP, ADK) should be explained in the legend.
  We corrected in "Institute" and explained the acronyms.

- Editing of the manuscript is definitely needed. The references should be note in a consistent way and according to the Journal's guidelines.
  We edited the references according to the Journal's guidelines

Reviewer 3 (# 03475362):

This manuscript clarified the dilemma of EUS-guided sampling of solid pancreatic masses. Author mentioned the various condition of EUS-guided sampling following the part such as ROSE, technical aspects of EUS-FNA or FNB, different sampling techniques (standard suction, stylet slow-pull, wet suction method), the different type of needle gauge, shapes (bevel type, shark head, franseen needle), and so on.

Minor: 1. In page 4 line 5, author should cite Hikichi's paper (Hikichi et al. J Gastroentrol. 2009) in which authors firstly clarified the role of ROSE in EUS-FNA.

2. In page 20, author should use appropriate technical term as pancreatic NETs to pan-NENs according to the latest WHO classification of pancreatic tumors.

3. In page20 line30, author should change EUSFNA to EUS-FNA.

4. In figure, reviewer could not notice the good specimen for histological evaluation. Please change the figure or attach an arrow to point out the specimen.

AUTHORS ANSWER

Thanks for the comments.

- In page 4 line 5, author should cite Hikichi's paper (Hikichi et al. J Gastroentrol. 2009) in which authors firstly clarified the role of ROSE in EUS-FNA.
  We cited it and modified the references (Ref 12, page 4, line 24-25), accordingly.

- In page 20, author should use appropriate technical term as pancreatic NETs to pan-NENs according to the latest WHO classification of pancreatic tumors.
  We changed NETs in pan-NENs, as suggested (page 20, line 22-23).

- In page20 line30, author should change EUS FNA to EUS-FNA.
  We corrected it (page 20, line 24)

- In figure, reviewer could not notice the good specimen for histological evaluation. Please change the figure or attach an arrow to point out the specimen.
  We changed the figure with a better one (Fig 4).

Reviewer 4 (#00183086):

This is an interesting article with regard to EUS-guided sampling of solid pancreatic masses. Nevertheless, neither the scientific quality nor the structure of the manuscript meets the criteria of publication in your distinguished journal. All the sections as well as the sub headings should be re-arranged. Grammatical errors should be corrected. Newly published articles should also be included.

AUTHORS ANSWER

Thank you for your comment.

We re-arranged sections and subtitles also by using the suggestions of the other reviewers, we corrected the grammatical errors and we added some references.

Reviewer 5 (#03727100)

Dear authors, thank you for the precise report about EUS-FNA and EUS-FNB. The report is well written, however, There are a few points to be considered. Major point ・The manuscript is too wordy to understand. Would you please make the tables to describe the

detail of each study? The most suitable needle in each situation should be described in each chapter. Minor points ・ Could you add the citation that first describe the word "ROSE" (Hikichi et al. J Gastroenterol 322-328; 2009)?. In "the role of ROSE" section, what time should we puncture to overcome the situation without ROSE? ・ How much the histological diagnosabiliey of the Shark Core needle is? ・ Would you add the reference that describe the differentiation of difficulty in each pancreatic part?

AUTHORS ANSWER

Thanks for your comments

- Would you please make the tables to describe the detail of each study?
  We added a detailed table (Table 1 and 2), as suggested

- The most suitable needle in each situation should be described in each chapter
  Please, refer to the flow chart (Fig 3)

- Could you add the citation that first describe the word "ROSE" (Hikichi et al. J Gastroenterol 322-328; 2009)?
  We added the references (Ref number 12).

- In "the role of ROSE" section, what time should we puncture to overcome the situation without ROSE?
  In case of pancreatic masses, when ROSE is unavailable, current ESGE guidelines suggest (low quality evidence, weak recommendation) to perform three to four needle passes with the FNA needle or two to three passes with the FNB needle. We modified the text accordingly to this observation (page 8, line 3-5) and we added the reference (Ref number 30).

- How much the histological diagnosabily of the Shark Core needle is?
  The use of Shark Core needle was found to have a histological yield of 87%. We added it in the text (page 9, line 26-27)

- Would you add the reference that describe the differentiation of difficulty in each pancreatic part?
  We added the references describing the different difficulty in each pancreatic part (Page, line 19-20)

Reviewer 6 (#00034177)

This paper reports FNA needle vs FNB needle. This paper findings are very important for endosonographers. This paper itself is well written. But, this topics is not so new. Question 1. Please mention or clarify the definition of FNB needle. This is very important. 2. 19G FNA needle is really FNA needle? 3. Aspiration(negative pressure) effects the sample quality. Please mention this point.

AUTHORS ANSWER

Thanks for the comments.

- Please mention or clarify the definition of FNB needle. This is very important.
  FNB-needles were specially designed to obtain a core specimen with preserved tissue architecture. The specimen fragments are not lost or consumed during cell block centrifugation or specimen sectioning. Therefore, the histological architecture can be retained in most of specimens. The FNB needles are the ideal sampling method for solid masses, like subepithelial lesions of the gastrointestinal tract, lymph nodes, pancreatic and non-pancreatic lesions (such as liver parenchyma), because they maintain the tissue integrity in the specimen. In particular, a large volume of tissue with preserved architecture is preferred in some pancreatic diseases like lymphoma and autoimmune pancreatitis, as FNB allows for immunohistochemical testing. We modified the text accordingly (page 7, lines 9-15)

- 19 G FNA needle is really FNA needle?
  Although the 19 G needle is not specifically designed to obtain a core with preserved tissue architecture, its large caliber often allows to collect samples with large volumes of tissue, useful for histological examination. We already mentioned this possibility (page 13, line 26-27).

- Aspiration (negative pressure) effects the sample quality. Please mention this point.
  We already mentioned this point (page 10 line 32 and page-11, line 1-3).