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Tissue acquisition in pancreatic cystic lesions

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ABSTRACT

Despite the progress achieved by scientific research in recent years, pancreatic cystic lesions (PCLs) remain a challenging clinical problem. A significant percentage of benign PCLs are still wrongly sent to surgery, with all the related risks of a high number of surgery-related complications and mortality. Diagnosis of the type of PCL, and risk stratification for malignancy are essential for a correct management of these lesions. Several guidelines have identified some clinical and morphological aspects suggesting the need for more accurate exams. Endoscopic ultrasound fine needle aspiration (EUS-FNA) of cystic fluid for cytology is the advised method of tissue acquisition in several guidelines, and the most used technique around the world. However sensitivity and adequacy of this technique are limited by the low amount of cells dispersed in cystic fluid. Alternative techniques have been tested to target the cystic walls in an attempt to obtain microhistologic specimens in order to augment the probability of obtaining an adequate diagnostic sample.

The aim of this review is to offer a critical overview of the existing literature on tissue acquisition in PCLs, and emphasize advantages and disadvantages of each technique, and unclear areas that need to be investigated with future research.

1. Introduction

In recent years, pancreatic cystic lesions (PCLs) have become a trending topic in the fields of gastroenterology, surgery, pathology, and radiology. This is clear if one looks at the number of yearly scientific publications. An example is the fact that in 2016 the number of scientific articles published under the term "pancreatic cyst" was 303, while in the 1980s the number was about a third of that. Cystic lesions are becoming progressively more common because of the increase of the average life span in the general population, and also because there is a higher rate of prescriptions for abdominal imaging exams, with more developed technological devices. At present, the estimated prevalence, variable with the imaging technique used, is about 15% (2–38%) [1]. With current knowledge, the differential diagnosis of pancreatic cysts can be considered a probabilistic puzzle of epidemiological, clinical, radiological, endoscopic, cystic fluid analysis, and cytological features. There have been many recent attempts, particularly in the cyto-histology field, to establish a better diagnosis. Our aim in this review is to summarize the current literature on tissue acquisition techniques in PCLs.

2. Current knowledge

Cystic neoplasms of the pancreas are a biologically heterogeneous group of lesions, characterized by both a different epidemiological distribution and a different malignant potential, ranging from completely benign to clearly malignant [2]. Because of this malignant potential, in most PCLs for which there is no surgical indication, there must be a periodic follow-up program [3]. Considering the high prevalence of PCLs, the number of radiological and endoscopic ultrasound (EUS) tests that must be performed annually is very high, with great costs for health systems, and a congestion of waiting lists [4]. Despite worldwide efforts to improve the management of these patients, the number of patients undergoing surgery for benign or low/intermediate-grade dysplasia, remains high, though less with respect to the past [5,6]. It is thus necessary to find a method for discriminating degenerated or potentially evolutionary lesions from other PCLs.

EUS with/without fine needle aspiration (FNA) has a central role in distinguishing different types of PCLs. This exam, in fact, allows endoscopists to evaluate the cyst from a morphological point of view, which in some rare cases can be diagnostic alone (e.g.,

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microcystic lesions with a stellar-shaped calcified center in SCAs, or communication with pancreatic duct and finger-like or clubber-like dilation of secondary pancreatic duct-in-branch-duct IPMNs). Furthermore, with FNA a number of different types of analysis, both on the cystic fluid and on the cystic wall can be done. Despite the large number of publications concerning the analysis of cystic fluid to discriminate various types of PCLs using CEA dosage, amylase dosage or the evaluation of the presence of mucus, none of these methods shows sensitivity/specificity high enough to be considered the definitive diagnostic test regarding the type of lesion [7–9]. Moreover, there is no test among these that could identify benign from malignant lesions. Promising results have been achieved with cyst fluid molecular analysis [10], and also with innovative techniques, such as confocal laser endomicroscopy (CLE) [11]. Both these techniques have shown encouraging results, but currently, because of the high costs and a required high degree of expertise, they are still not widespread. Furthermore, we have still some obstacles to overcome before these techniques become widely used, such as the lack of agreement on which should be the best molecular biology tests to perform, or problems with the definition of malignant criteria among different PCLs, or, again, the sub-optimal inter-observer agreement in CLE field.

All the published international guidelines [3,12–14] suggest using EUS-FNA as one of the principal diagnostic methods in the approach to PCLs, particularly in those lesions with "worrisome features" or in which the diagnosis remains unclear. None of the proposed tests on the cystic fluid has been able to eliminate the need for a traditional diagnosis based on a cytopathological tissue analysis of the PCL. Cytohistological study allows us both to identify the type of PCL, and to establish the presence of an advanced lesion (such as severe dysplasia or invasive cancer) and so, in this way, to decide whether either surgery or follow-up is necessary. Cytohistology remains, for this reason, essential for better managing these types of lesions.

We are now going to analyze different tissue acquisition techniques in PCLs.

3. Cytology of cystic fluid

Cytology of cystic fluid was the first proposed technique for the diagnosis of PCLs, and is still widely used and suggested in current guidelines as the principal tissue acquisition technique in these lesions.

In 2002, in a study by Sedlack et al. [15], a comparison was made between diagnostic possibilities of EUS morphologic aspects vs cytology of cystic fluid's diagnostic possibilities to identify malignant or potentially malignant lesions, with partially unexpected results. The sensitivity and specificity of the morphological aspects were 81% and 57%, respectively, with 72% diagnostic accuracy, while cytology of cystic fluid had a sensitivity of 27%, and a specificity of 100%, and diagnostic accuracy 55%. In 2004, an historic article by Brugge et al. [9] was published, in which the diagnostic performance of cytology on cystic fluid was analyzed. The main aim of the study was not the classification of malignant neoplastic lesions, but the discrimination between mucinous and non-mucinous lesions. In this case, the sensitivity in finding mucinous lesions was 34.5%, with an 84% specificity, and an overall diagnostic accuracy of 51%. Nevertheless, the discouraging datum was that cytology could identify only 22% of malignant neoplastic lesions.

In 2005, a pooled analysis including 12 studies, with a total of 450 patients, was undertaken by van der Waaij et al. [8] In this study, in addition to having measurements of amylase, CEA and CA 19-9 concentration on cystic fluid, the diagnostic power of cytology was evaluated; the combined sensitivity on detection of SCA was about 38%, of MCN about 45%, and of malignant lesions 48%.

Then, in 2008, Maker et al. [16] showed that cytology of cystic fluid had a sensitivity, specificity, positive predictive value, and negative predictive value of 28%, 100%, 100%, and 18%, respectively. Cytology positivity could establish the right diagnosis in 100% of cases, but among operated patients (29 patients) 67% of negative results and 92% of non-diagnostic were associated with malignant or premalignant pathology. Similar discouraging results, in terms of sensitivity/specificity/accuracy, were obtained by the Dutch group of de Jong et al. [17] in 2011. In this study in all the EUS-guided cyst aspirations performed, only 31% of the samples had adequate cellularity for analysis. In 2013, a monocentric study [18] was published with the purpose of evaluating the diagnostic accuracy of cytology obtained with EUS-FNA in differentiating benign from malignant lesions. In this study, sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 63%, 100%, 100%, 85%, and 88%, respectively.

With the attempt to bring some order to all these widely contrasting studies, two meta-analyses were published. In one of these, in 2010, by Thosani et al. [19], data from 11 studies, with a total number of 937 patients, were analyzed. Cytology for the identification of mucinous cysts was estimated at 63% overall sensitivity, and 88% overall specificity. As claimed by the authors, a 63% overall sensitivity is probably overestimated, and this is probably due to the so-called "verification bias," which is the type of bias created by the fact that only a few of all the patients included in the various studies had a definitive diagnosis with a confirmatory test (e.g., histology subsequent to surgery). In the other meta-analysis [20], in 2014, two different groups of patients were analyzed: in Group 1 the diagnostic accuracy of cytology in the identification of malignant lesions (high grade dysplasia, carcinoma in situ or invasive carcinomas) was evaluated, with sensitivity and specificity values of 51% and 94%, respectively; in Group 2, potentially malignant lesions (intraductal papillary mucinous tumor, cystic islet cell tumor, solid pseudopapillary tumor) were evaluated, with sensitivity and specificity of 52% and 97%, respectively.

It is clear that cytology on cystic fluid obtained by EUS-FNA has median diagnostic accuracy and sensitivity values of less than 50%. The problem is likely related to the low number of neoplastic cells in cystic fluid. Furthermore, EUS-FNA of cystic fluid allows us to obtain only a cytological specimen, with all the problems related to this fact. Certainly a greater expertise of cytologists is a possible solution, as is the attempt by some groups to find methods for improving diagnostic performance. Pitman et al. [21], for example, proposed using high-grade atypia as a marker for severe dysplasia of PCLs. In only a few centers is an expert pancreatic cytologist present, and the lack of neoplastic cells in cystic fluid, independent of local expertise, remains an unsolvable problem.

As a result, attempts were made to improve tissue acquisition by the sampling of the cystic wall, where a greater concentration of neoplastic cells can be found. Dedicated devices have been tested, such as EchoBrush, or EUS-FNA/B (biopsy) of the cystic wall using different devices, such as standard needles, Tru-Cut needle, ProCore needle or through-the-needle biopsy (EUS-TTNB).

In Fig. 1 principal devices studied to perform EUS-FNA/B, brushing or biopsy on PCLs are summarized.

4. Sampling the cystic wall

4.1. Brushing

In 2009, a preliminary study [22] was published regarding the feasibility of brushing (EchoBrush™, Cook Medical, Bloomington, USA) on different types of abdominal lesions, such as solid pancreatic lesions, cystic pancreatic lesions or lymph nodes. In this study,

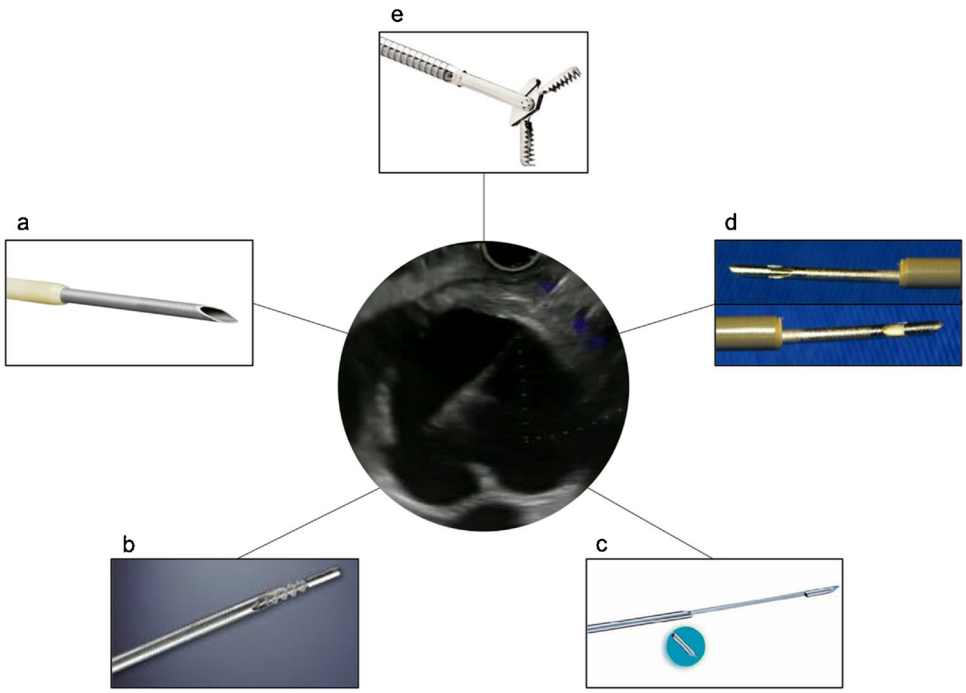

Fig. 1. Devices studied to perform tissue acquisition on PCLs: standard 22G needle (a); brushing device (b); Tru-Cut 19G needle (c); FNA/B needle with side-fenestration (d); micro forceps (e).

diagnostic adequacy was obtained in only 50% of PCLs, showing a very slight improvement compared with cytology. After this, in 2010, three articles on brushing techniques in PCLs were published [23–25]. In the study by Sendino et al. [23], very different results with respect to cytology with EUS-FNA were obtained, with a 73% diagnostic adequacy vs 36%; moreover, with this technique, 50% of mucinous cells vs 18% of EUS-FNA were identified. Al-Haddad et al. [24] reported that brushing is more likely to provide an adequate mucinous epithelium specimen (62%) than standard FNA (23%), with an 8% rate of adverse events. Similar results were obtained by Lozano et al. [25]; in fact, diagnostic material was obtained in 40/47 patients (85.1%) undergoing EchoBrush™, compared with 53/80 patients (66.3%) undergoing EUS-FNA. But what really has not permitted the routine use of brushing in clinical practice is the high number of adverse events [23,24]. In fact, in these studies, about 8%–10% of patients had post-procedural complications, such as acute pancreatitis and post-brushing bleeding (in one case, a retroperitoneal bleeding, with subsequent death).

4.2. FNA/B of cystic wall

Several studies with different types of biopsy needles to sample the cystic wall have been published.

The first study published was done using the 19-gauge Tru-Cut biopsy device (Quick-Core®; Wilson-Cook, Winston-Salem, NC), which was thought capable of obtaining a larger sample of tissue than a 22-gauge needle. This is a very small series which included only 10 patients [26]. In 3 of the 10 patients, the FNB with the Tru-Cut needle was not diagnostic, and there were no complications in any of the patients. The Tru-Cut needle was very stiff, and hard to use, especially in angled positions; in fact, it is no longer made.

In 2012, a study by Hong et al. [27] was published, in which the difference between cytology/CEA of cystic fluid vs FNA of cystic wall performed with a standard 22-gauge FNA needle (Cook Medical, Winston- Salem, NC) was prospectively evaluated.

The technique used was to introduce the standard 22-gauge needle inside the cyst and then, after aspiration and decompression of the cyst, if possible, to puncture the far wall of the cyst, moving the needle back and forth through the wall to sample epithelium.

Cellular material adequate for cytological evaluation was reported in 81% of cases with the 22-gauge needle. The difference in terms of diagnostic performance, that is the percentage of diagnosis of PCLs made by FNA of the cystic wall that was not obtained by cytology/CEA on cystic fluid, was about 29%. However, in this study, the specimens obtained were always cytological. In contrast to brushing, the percentage of adverse events reported with this technique was consistently lower (1.45% vs 10%). Even if no more studies were conducted on this technique, sobering results of cytology of cystic fluid induced many endoscopists to use it with the hope of increasing the diagnostic performance in sampling. Unfortunately, no more data were published to help us learn how much this technique can improve diagnosis of PCLs with respect to cytology on cystic fluid.

Another study, by Barresi et al. [28], utilizing a 22-gauge Echo Tip Ultra FNB needle, featuring ProCore® reverse bevel technology, with side fenestration (Cook Medical, Ireland), was published.

The technique described was to penetrate the PCL with the needle, and to completely aspirate the cystic fluid; when the cystic walls collapsed on the needle, the aspiration was continued for about 30 s to allow tissue walls to enter the side fenestration of the needle. The needle was then moved 3–4 times about 5–10 mm back and forth within the lesion to obtain tissue.

In this study, diagnostic adequacy was about 65%, 46.1% was also adequate for a histological exam (or micro-histologic, defined as a small specimen consisting of stroma + surface epithelium). Taking in account the subgroup of patients with a solid component in the PCL or with a malignant lesion, adequacy increased to 94.4% and 100%, respectively. On the other hand, in the subgroup of patients without solid components in the PCL, diagnostic adequacy was about 37%, very similar to cytology of cystic fluid. Mild complications were observed in 3.3% of patients, while no severe complications were reported. Even if the ProCore® needle obtained better results with respect to EUS-FNA on cystic fluid, with the advantage of obtaining in about half of the cases a

micro-histological specimen, in which immunohistochemistry can be easily performed, the results in PCLs without solid components were disappointing.

No more studies on this type of needle have been published.

Despite better results of the above-mentioned studies with respect to cytology of cystic fluid, we are still far from having adequate specimens in almost all of FNA/B performed. For this reason, in recent years, new techniques have been studied and developed to increase diagnostic power in PCLs.

4.3. Endoscopic-ultrasound-guided biopsy of cystic wall using a micro forceps

In 2010, Aparicio et al. [29] did a pilot study utilizing a 0.8-mm micro forceps designed for PolyScope® (Lumenis Surgical, USA) through a 19-gauge EUS needle in two patients with pancreatic cystic lesions. Since then, several case reports of different micro forceps, for a total of 14 cases have been published [30–36]. In most of these studies, a specifically developed micro forceps for cystic lesions was used: the Moray micro forceps (Moray™ micro forceps, US Endoscopy, Mentor, Ohio, USA) [32–36]. This technique was called by different authors either EUS-guided micro forceps biopsy (MFB) or EUS-through-the-needle biopsy (EUS-TTNB), and these initialisms, at present, have to be considered equivalent; thus, we use both in this review.

Recently, two retrospective studies using the Moray™ micro forceps have been published: one monocentric study [37] on 27 patients (first author Mittal) on technical feasibility, diagnostic yield, and safety of MFB for PCLs, and one multicentric study [38] on 42 patients (first author Basar) comparing the tissue acquisition and diagnostic tissue yield of micro-forceps biopsy with cystic fluid cytology.

The study by Mittal reported a technical success in all cases, and a diagnostic yield of 88.9%. Furthermore, this study showed that MBF drastically changed the diagnosis in 26% of patients, providing diagnoses otherwise not suggested by traditional cytology or cyst fluid CEA. However, cytology provided a diagnosis of mucinous cyst in 4/27 cases (14.8%) (one with high-grade dysplasia) not detected by MBF.

The study by Basar showed a tissue acquisition yield of 90.4% vs a EUS-FNA tissue (fluid) acquisition yield of 88.1%. In this study, the diagnostic tissue yield was evaluated at 3 levels: the ability of differentiation between mucinous/non-mucinous cysts; detection of high-risk for malignancy; and specific cyst type diagnosis. To differentiate between mucinous/non-mucinous cyst the diagnostic cytology yield was 47.6% (20/42), while MFB histological yield was 61.9% (26/42) (p = .188). The percentage of cysts at high risk for malignancy by cytology was 54.7% (23/42), and MFB was 71.5% (30/42) (p = .113). However, the ability of MFB to provide a specific cyst type diagnosis was 35.7% (15/42), and cytology was 4.8% (2/42) (p = .001). Moreover, when specific diagnostic results with MFB histology were compared with cytology results, none of the 6 patients with IPMN, none of the 3 patients with SCN, and neither of the 2 patients with MCN could be diagnosed. Furthermore, surgical histology was concordant with MFB in 6 of 7 (85%) patients, and with cytology in 1 of 7 (15%) patients. Therefore, the diagnostic yield of the MFB for a specific type of cyst was significantly higher than the diagnostic cytology yield, and concordance with surgical histology is very high for MFB and low for cytology. Though statistically not significant, the MFB histology increased the number of mucinous/non-mucinous diagnostic cyst results in a moderate proportion of patients (from 20 to 26/42 patients), and increased the detection of low-grade epithelium in a moderate proportion of patients (from 21 to 27/42 patients).

A single center, retrospective study [39] from one of the centers involved in the multicenter study by Basar (first name Zhang), on 48 patients, substantially confirmed the results reported by the multicenter study.

Another retrospective, multicentric study [40] on 56 patients, coordinated by our institute, was recently published. The results are concordant with the published results, with a technical feasibility of 100%, a diagnostic yield of 83.9%, and specimens were considered adequate for histological diagnosis in 83.9%. EUS-TTNB diagnostic yield resulted significantly higher than cytology fluid (83.9% vs 36.1%, $p < 0.0001$). Furthermore, in the 15 operated patients with adequate EUS-TTNB (12 patients) concordance for type of lesions was 11/12 (91.6%), while concordance for histologic severity of lesion was 9/12 (75%).

With respect to adverse events, the Mittal and Zhang studies reported no complications, while the Basar study reported only two adverse events, one aspecific abdominal pain and one intracystic hemorrhage. In the study by our group, adverse events were 16%, of which 12% were intracystic hemorrhage (and 3 aspecific abdominal pain). However, all adverse events were considered mild because they resolved spontaneously without any treatment.

Intracystic hemorrhage is relatively common even when performing a standard FNA in PCLs and, to date, only one case requiring transfusion has been reported [41]. Future studies are needed to verify whether this complication could have a clinical significance or has to be considered simply a side-effect of EUS-TTNB in PCLs.

Regarding seeding, we do not have specific data on EUS-TTNB. The PIPE study [42] concluded that EUS-FNA in PCLs does not cause this feared complication. EUS-TTNB is done by maintaining the needle inside the PCL, so it likely has the same low probability of causing seeding as EUS-FNA. Future studies will have to assess this point.

In every reported study, broad-spectrum antibiotics were administered to all patients before needle puncture of the cyst, and, in most patients, it was continued for 3–5 days post procedure.

In Table 1 the principal characteristics of the four published studies on EUS-TTNB (or MBF) are reported. It is interesting to note that there are relevant differences between the studies in adequacy results of cytology of cystic fluid. The absence of expert cytologists on pancreatic lesions could result in a larger difference between the adequacy of EUS-TTNB and cytology on cystic fluid in favor of the former.

An advantage of EUS-TTNB (or MBF) is that the specimens obtained are micro-histologic, having a stroma covered with an epithelial lining and, therefore, an architecturally intact piece of tissue from the target lesion. These aspects make the diagnosis easier for the pathologist and facilitates ancillary techniques such as immunohistochemistry both on epithelium and on the stroma, which can be useful for a diagnosis of mucinous cystic neoplasm, and which is impossible to obtain with cytology of cystic fluid, which cannot capture stroma. In any case, before or after EUS-TTNB it is possible to collect cystic fluid, for both biochemical and molecular biology analysis and for cytology, which can be associated with EUS-TTNB to improve diagnosis, as reported in the study by Mittal.

Regarding disadvantages, obviously, it is possible to sample only the opposite wall of the PCL with respect to the point of entrance of the 19G needle used to pass the micro forceps. Furthermore, due to the inhomogeneous distribution of dysplasia inside the PCLs, it is possible that the dysplasia observed in the specimens obtained with the micro forceps does not represent the real dysplasia inside the cyst. These aspects, associated with the not infrequent possibility that some PCLs have a denuded epithelium [43], make it difficult to obtain adequate specimens, and so it is advised to perform several passages on the cystic walls to augment the possibility of obtaining a diagnostic sample, and representative of the real dysplasia inside the lesions.

However, in general, EUS-TTNB (or MBF) is feasible in most patients and seems to have a high diagnostic yield, without sig-

Table 1
Principal characteristics of the four published studies on endoscopic-ultrasound-guided biopsy of cystic wall using a micro forceps.

Study	Number of patients (n)	Design of study	Type of study	Feasibility (%)	Diagnostic yield (%)	Histologic yield (%)	Adverse events (%)	Diagnostic yield of micro-forceps vs CFC (%)	Concordance of EUS-TTNB with surgical histology [%(total no. of patient who underwent surgery)]
Barresi et al. [40]	56	Retrospective	Multicenter	100	83.9	83.9	16	83.9 vs 36.1 (p < 0.001)	91.6 (15) *(type of lesion)* 75 (15) *(histologic severity)*
Basar et al. [38]	42	Retrospective	Multicenter	90.4[a]	61.9 *(mucinous vs nonmucinous)* 71.5 *(degree of dyslpasia)* 35.7 *(specific type of cyst)*	90.4	4.7	61.9 vs 47.6 (p = .18) *(mucinous vs nonmucinous)* 71.5 vs 54.7 (p = .11) *(degree of dyslpasia)* 35.7 vs 4.8 (p = .001) *(specific type of cyst)*	85 (7)
Mittal et al. [37]	27	Retrospective	Monocenter	100	88.9	88.9	0	88.9 vs 84.6 (p = .65)	66.7 (3)
Zhang et al. [39]	48	Retrospective	Monocenter	NA	75	NA	0	75 vs 72.9 (p = .81) *(diagnostic yield)* 58.3 vs 60.4 (p = .94) *(mucinous vs nonmucinous)* 50 vs 18.8 (p = < 0.001) *(specific diagnosis)*	NA

CFC, cytology on cystic fluid.

[a] The only available feasibility value for this study is the "tissue acquisition yield" referred to the ability to collect tissue for analysis and calculated as the number of patients with tissue obtained by MFB.

nificant adverse events. However, the relatively small sample size of these, and the absence of a surgical histology for most of the patients are the limits of these studies. The problem of the absence of a surgical histology is a common problem in the study of diagnosis of PCLs because only few cysts need surgery. Studies with a longer follow up could help, but will require many years, and are difficult to conduct.

Surely, prospective studies on EUS-TTNB or MFB are needed, but the retrospective studies published had promising enough results to justify future studies with this novel tissue collection device.

5. Conclusions

Despite the high number of published articles on this topic, diagnosis and risk stratification for malignancy in PCLs remain an issue that must be resolved. A significant percentage of benign PCLs are still wrongly sent to surgery, with all the related risks of a high number of surgery-related complications and mortality. EUS tissue acquisition techniques in last few years have become more precise. EUS-TTNB (or MBF) seems to be, at the moment, the most promising technique to improve pre-surgical diagnosis of these insidious lesions. However, as for all tissue acquisition methods, we must await the results of future prospective studies to decide on its indications, and to establish whether EUS-TTNB should be included in guidelines, alone or in association with other techniques, such as molecular biology or confocal laser endomicroscopy.

Conflict of interest

None declared.

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