REVIEW

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Needle-based confocal laser endomicroscopy in pancreatic cystic tumors assessment

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Abstract

Pancreatic cystic tumors (PCT) are relatively common findings in general population due to the widespread use of cross-sectional imaging. PCT can be benign, with premalignant potential or malignant, a different management being applied for each type: benign cysts are usually referred for follow-up (based on imaging), while premalignant or malignant lesions should be surgically resected. The aim of this review is to describe the latest imaging technique that could be used for PCT diagnosis and to establish its clinical impact. Endoscopic ultrasound (EUS) is generally used to evaluate a pancreatic mass and to identify its characteristics. It offers a good visualization of the lesion. When combined with fine needle aspiration and cystic fluid analysis, the diagnosis potential is increased, although its accuracy for differentiating benign and malign tumors remains modest. EUS-guided needle-based confocal laser endomicroscopy (nCLE) is a new imaging technique that uses a miniprobe thin enough to be passed through a 19G needle. It provides *in vivo* images of the pancreas at a cellular level, offering the possibility to assess any changes that might have occurred. Several studies have shown that nCLE is feasible to use for PCT evaluation, imaging criteria being established with 100% specificity for intraductal papillary mucinous neoplasms (IPMN) and serous cystadenoma (SCA). Regarding the safety, more studies are needed. EUS-guided nCLE appears to be a new imaging technique that provides encouraging results for differential diagnosis between mucinous/non-mucinous cysts.

Keywords: needle-based confocal laser endomicroscopy (nCLE), pancreatic cystic lesions, endoscopic ultrasound (EUS).

Introduction

Pancreatic cystic tumors (PCT) are relatively common findings in general population due to the widespread use of cross-sectional imaging, being incidentally discovered most of times [1]. They are difficult to diagnose based only on imaging features [2], which may be problematic when it comes to a clinical decision. The incidence of PCT is 2.6–20% during imaging examination, 2.4–13.5% of patients discover they have an incidental tumor on magnetic resonance imaging (MRI) and 1.2–2.6% on computerized tomography (CT), while 24% are discovered on necropsy [3, 4]. In most cases, PCT are asymptomatic.

It is very important to make a differential diagnosis between all types of PCT and to classify them as benign lesions, cysts with premalignant potential or malignant tumors, because their clinical management is different. Sometimes this could be a great challenge because some tumors have imaging characteristic that are not defining for any diagnosis. When discovered early, curative surgery could be an opportunity for premalignant or malignant lesions, a final pathological diagnosis being obtained after resection [2]; generally, benign cysts are referred for imaging follow-up, based on endoscopic ultrasound (EUS) and MRI/MRCP (magnetic resonance cholangiopancreatography).

Pancreatic cysts can be classified into several categories ranging from benign to malignant, as:

- benign lesions: pseudocysts, congenital or retention cysts;
- borderline or premalignant lesions: intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN) and serous cystadenoma (SCA), representing more than 90%;
- malignant lesions: cystadenocarcinoma.

Premalignant lesions are known to be indolent for a period, after which they can progress to cystadenocarcinoma (excepting SCA) [5, 6].

Pancreatic cancer (PC) is one of the top 10 deadliest solid malignancies, with only 4% rate for five-year survival [7]. It grows and spreads rapidly, being often diagnosed in its late stages due to the lack of early signs and symptoms [8]. The median period survival for non-metastatic disease is 6–10 months, while for metastatic disease it is 3–6 months [9]. Although 10% of patients can have curative surgical resection when diagnosed with PC, only 20% of them survive for five years, despite well-performed surgery with clear resection margins. The precursor lesions for PC are represented by IPMN, MCN and pancreatic intraepithelial neoplasia (PANIN).

A strong correlation between both pancreatic cysts and PC and age was noticed, being mostly identified in the elderly [9, 10].

Management of pancreatic cystic lesions

The first step for the management of a new discovered PCT is to decide whether the tumor is inflammatory (pseudocyst) or a cystic neoplasm (cystadenocarcinoma,

IPMN, MCN or SCA) [11]. Every entity has its own morphological characteristics:

- pseudocysts: changes in the pancreatic parenchyma, without nodules or septations;
- SCA: multiple or microcystic lesion, with calcification in the central area, with honeycomb appearance;
- IPMN: connected to the main pancreatic duct or side branch ducts;
- MCN: generally macrocystic, with calcification, thick wall and septations [12].

In the past, clinicians decided that mucin-containing cystic neoplasms were malignant or potentially malignant and should be referred for surgical resection, while serous cystic neoplasms could be managed conservatively as they were considered benign [13]. This is still available today, but due to the advances in understanding biology and histology, now it is known that many mucinous tumors are indolent and may become invasive cancer after decades. *Sendai Consensus Guidelines* published in 2006 recommend surgical resection for MCN and main duct (MD)/mixed duct types (MT) IPMN, while side branch (SB) IPMN can be managed conservatively [14, 15]. Some criteria for branch duct (BD)-IPMN such as symptomatic, size larger than 3 cm, solid component, MD dilated over 6 mm, positive cyst fluid are indications for resection.

The *American Gastroenterological Association* (AGA) [16] recommends MRI surveillance for one year in patients with PCT less than 3 cm and without dilated MD or solid component, and then every two years if no change in size and characteristics, for a total of five years. If at least two high-risk imaging criteria are present, such as dilated MD, solid component or size larger or at least 3 cm, then the lesion should be examined with EUS-guided fine-needle aspiration (FNA). If cytology on EUS–FNA is positive for malignancy, a combination of high-risk features are described on imaging, or a solid component is associated with MD dilatation (confirmed both on MRI and EUS), then the patient should be referred for surgery, although the resection benefit should be considered since the major morbidity is 30% and postoperative mortality is 2% [17].

The aim of this review is to describe the latest imaging technique (EUS-guided needle-based confocal laser endomicroscopy – nCLE) that can be used for PCT diagnosis and to establish its clinical impact.

Endoscopic ultrasound

Endoscopic ultrasound (EUS) is widely used for PCT diagnosis because it provides fine details of a focal pancreatic mass, either cystic or solid. The transducer is placed very close to the lesion, enabling a very precise visualization of its structural component (Figure 1). The very high spatial resolution helps the examiner to see any predictive signs of malignancy. EUS can determine the resectability for cystic tumors if malignancy is present, which is usually revealed by various changes, including mural nodules, solid masses, internal septations, lymphatic metastasis or vascular invasion [12, 18].

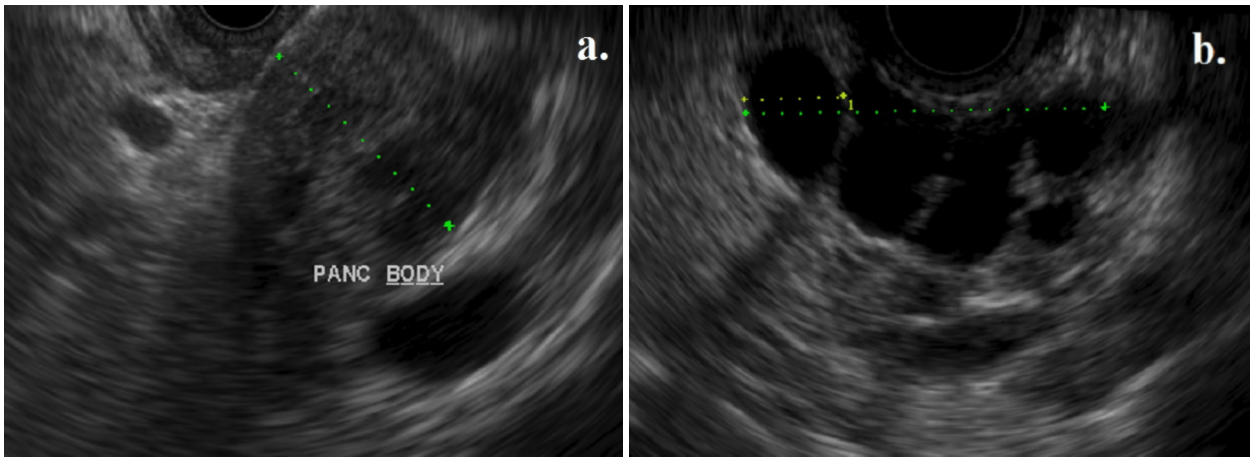

Figure 1 – ***EUS: (a) Pancreatic mass with cystic component in the pancreatic body; (b) Macrocystic lesion in the head of the pancreas.***

During EUS, it is possible to aspirate the cystic fluid or solid components by using the FNA technique (Figure 2). The sample can be used for cytological and histological analysis, tumor markers detection, extracellular mucin, biochemical and molecular analysis. The most frequent complication when using this technique is pancreatitis; other complications reported are infection (rare due to antibiotic prophylaxis), bleeding or pain. Generally, EUS–FNA has a favorable risk/benefit ratio [19]. FNA does increase EUS sensitivity, offering the possibility to differentiate pseudocysts, mucinous cysts and serous ones, each lesion having its own characteristics: mucin is stained by columnar epithelial cells and appears in MCN and IPMN, and glycogen is stained by cuboidal cells in SCA [12]. A recent study showed that EUS–FNA and the far wall repeated puncture of the cyst increases cytology efficiency by 37% when compared to simple FNA fluid analysis [20].

The cystic fluid analysis offers the possibility to use cytology and tumor marker levels in addition to EUS imaging [21]. The most accurate tumor marker used to distinguish mucinous from non-mucinous PCT is carcinoembryonic antigen (CEA), with low levels being observed in non-mucinous lesions [22]. If amylase is present in a cyst, this indicates the communication with the MD, as seen usually in IPMN; a low level of amylase could also indicate a pseudocyst. Low levels of carbohydrate antigen (CA) 19-9 have been associated with SCA or simple cysts, showing 98% specificity, very low sensitivity (19%) and modest accuracy (46%) [23].

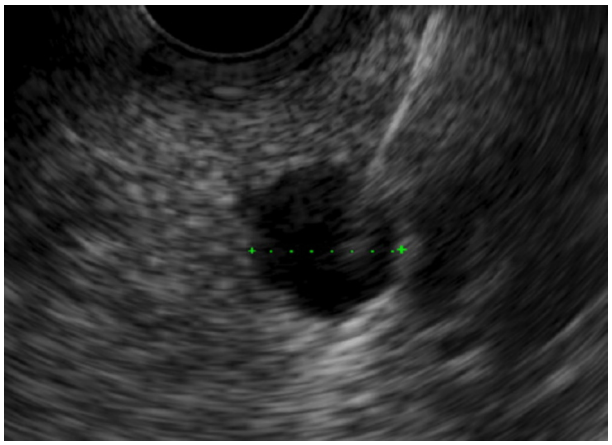

Figure 2 – ***EUS-FNA performed with AQ-Flex™ 19G needle from a cystic lesion located in the head of the pancreas.***

PCT diagnosis is increased and mucinous lesions are easier to differentiate from non-mucinous ones based on the combination of EUS imaging with different cystic fluid analysis as cytology, tumor markers level, amylase and DNA (deoxyribonucleic acid).

EUS-FNA is an invasive technique, having several limitations as: it is operator dependent, lesions smaller than 3 cm may be difficult to sample [18], non-diagnostic cytology or limited on-site cytological evaluation can impair results. Some of the complications that might occur are pancreatitis, abdominal pain or intracystic bleeding [22].

Regarding EUS morphology, it showed modest accuracy (51%) in a study aimed to differentiate mucinous from non-mucinous lesions [24], whilst in another study that used EUS morphology to differentiate all types of PCT, the accuracy was 73% [25].

Although EUS combined with cyst fluid analysis offer advantages for PCT diagnosis, the accuracy for differentiating between benign and malign lesions or between mucinous and non-mucinous tumors remains modest [26].

Needle-based confocal laser endomicroscopy

Recently, new imaging techniques have been investigated for analyzing and differentiating between morphological characteristics of pancreatic tissue [26]. One such technique is represented by confocal laser endomicroscopy (CLE). CLE is an endoscopic technique developed to obtain images with very high resolution and magnification from the mucosal layer of the gastrointestinal (GI) tract. The images are obtained *in vivo* and real-time, offering the possibility to visualize microscopic structures of the tissue at a cellular level [27].

The principle of CLE is that a low power laser light (488 nm) is focused on the tissue at a specific depth and that tissue layer will reflect back a fluorescent light, which will pass through a pinhole. A detection system captures the reflected light (scattered light at other geometric angles that is focused out of the pinhole, will be excluded from detection, which will increase CLE spatial resolution) and sends the imaging signal to a computer that will transform it into a grey scale image; the image obtained represents one specific plane. Having in the same focal plane both the illumination and collection systems is what defines the term "confocal". In order to obtain a high-quality image, a contrast agent is used (which will enable the fluorescent light). Usually, 2.5 mL of 10% Fluorescein sodium is intravenously administered, while Acriflavine, Cresyl violet or Tetracycline are topically administered through a spraying catheter. By using contrast agents, the resolution obtained is similar to traditional histology images [28, 29].

Currently, there are two types of CLE systems, dedicated endoscopes that have integrated CLE systems (currently discontinued) and probe-based systems. The endoscope-based CLE (eCLE – Pentax, Tokyo, Japan) has the endomicroscopy device integrated into the endoscope tip. The probe-based CLE (pCLE – Cellvizio, Mauna Kea Technologies, Paris, France) is actually a miniature probe, represented by a bundle of optical fibers, that pass through the working channel of conventional endoscopes [27, 30, 31].

Recently, a new pCLE has been developed, that is thin enough to be passed through a 19G needle (AQ-Flex™ 19 – Figure 3). The principle of nCLE is to visualize organs that are adjacent or within the GI or respiratory tracts, using a miniprobe that generates *in vivo* histology from solid organs as pancreas or liver. It provides dynamic images at a rate of 12 frames/second, the imaging depth being 40–70 mm [32]. The nCLE miniprobe has a diameter of 0.85 mm, a lateral resolution of 3.5 μm and a field of view of 320 μm. It is actually represented by a bundle of 10 000 optical fibers. The AQ-Flex™ 19 miniprobe enables nCLE images under EUS guidance. Using this technique, the endoscopist can study any pathological entity in its natural environment at the same time when the functional imaging is performed [33].

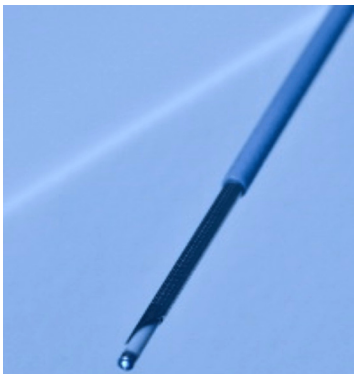

Figure 3 – ***AQ-Flex™ 19G needle.***

nCLE limitations are represented by the same complications that might occur when using EUS–FNA or related to the contrast agent used (intravenously administered Fluorescein sodium) that can determine mild adverse effects as diffuse rash, mild epigastric pain, nausea or vomiting, injection site erythema or rare serious adverse events as anaphylaxis, shock or seizure [27].

Sometimes, the fluid aspirated during EUS–FNA does not have a good quality to be analyzed and further provide a precise diagnosis, due to a small number of cancerous cells, sampling errors or gastrointestinal contamination; in this case another diagnosis technique is needed. Lately, several studies have been performed in order to assess nCLE utility in pancreatic cystic lesions diagnosis. Researchers have tried to develop and establish diagnosis criteria based on nCLE images.

One of the studies that aimed to evaluate nCLE feasibility during EUS–FNA for pancreatic lesions was carried out in 2011 by Konda *et al.* The team examined 18 patients (16 cysts and two masses) that had an indication for EUS–FNA for a pancreatic lesion. In 17/18 procedures, technical feasibility was achieved, with sufficient image quality in 10 cases from 17 examinations. Although two cases with pancreatitis that required hospitalization were reported, the study's conclusion was that nCLE is technically feasible for pancreatic cysts evaluation, but more studies are needed, due to the complications that occurred [34].

In 2013, Konda *et al.* performed the INSPECT study, which aimed to assess both safety and nCLE diagnostic potential for differentiating cysts' types. Eight referral centers participated to the study, performing nCLE in patients with PCT. The study had two stages, the first one defined descriptive terms for the visualized structures (a gastrointestinal pathologist reviewed the cases to identify a correlation between nCLE and histology) and the second stage assessed if the defined criteria could identify different types of pancreatic cystic neoplasms (PCNs) as adenocarcinoma, IPMN or mucinous cystic adenoma. PCNs were associated with the presence of epithelial villous structures (p=0.004), with high specificity of 100%, a medium sensitivity of 59%, negative predictive value of 50% and high positive predictive value of 100%. From 66 patients initially enrolled in the study (57 of them being available for the review), the complication rate was 9%, including pancreatitis, abdominal pain and intracystic bleeding. The conclusion of the study was that nCLE can detect with high specificity PCN, but it is limited by the low sensitivity; regarding the procedure safety, further evaluation is required. Also, the INSPECT study's authors concluded that if villous structures (the finger-like projections) are visualized on a PCL, then the IPMN diagnosis can be confirmed, even if cytology did not confirm it [35].

In another study carried on 11 patients with a pancreatic mass (four solid and three cystic) or celiac/mediastinal lymph nodes (LN), Giovannini *et al.* tried to establish descriptive criteria for nCLE images in order to correctly diagnose the pancreatic masses or LN and predict malignancy. All patients underwent EUS to stage the pancreatic mass or to diagnose any malignant LN. Benign IPMNs were found to be characterized by finger-like projections; adenocarcinoma had irregular vessels and vascular leakage, large dark clumps, which represented groups of malignant cells. Ultra-thin straight bright grey bands have been described in SCA. For benign/malignant LN were found similar imaging features. All the results obtained appear to be very encouraging, making nCLE feasible during the EUS examination [36].

In the pilot study known as CONTACT 1 that included 31 patients with undiagnosed solitary PCL, nCLE successfully confirmed the diagnosis of benign pancreatic cysts in 100% of patients. In this study, the diagnosis of benign PCL was established based on a superficial vascular network identified as the criterion, which was seen only in SCA (expert pathologists confirmed that a homogeneous and diffuse capillary network is observed only in SCA). The nCLE diagnosis was correlated with the pathological diagnosis, with a specificity of 100%, a sensitivity of 69%, negative predictive value of 82%, positive predictive value of 100% and an accuracy of 87%. The study's conclusion was that the new nCLE criterion seems to be highly specific for SCA diagnosis [37].

A multicenter trial (DETECT) tried to determine the feasibility and also the safety of combined through the needle EUS-guided imaging techniques (nCLE and cystoscopy) and to identify any specific imaging characteristics for mucinous/non-mucinous cysts, in order to improve PCT diagnosis. Thirty patients with pancreatic cysts have participated in the study. The cysts were firstly evaluated during cystoscopy (a SpyGlass® fiber-optic probe was used, Boston Scientific), followed by nCLE examination. Specific features were identified for mucinous cysts, including dark rings and papillary projections on nCLE and mucin on cystoscopy. Based on the addition of the CEA tumor marker (from the fluid analysis) with values more than 192, the diagnosis of mucinous cysts could be predicted with a specificity of 100%. The final diagnosis was established based on surgical pathology or clinical presentation and CT/MRI, EUS findings, fluid analysis and cytology. nCLE alone had 100% specificity, 80% sensitivity, 80% negative predictive value and 100% positive predictive value. nCLE sensitivity for mucinous cysts was 80% while for cystoscopy it was 90%; the combination of the two techniques had a sensitivity of 100%. The two through-the-needle imaging techniques identified specific features that might predict the diagnosis for mucinous/non-mucinous cysts; they appear to be feasible to use for pancreatic cysts diagnosis. Regarding the safety for these procedures, more studies are required due to the complication rate that might occur (7% – two patients were reported with pancreatitis after the procedure) [38].

A recent pilot study published in 2015 aimed to evaluate nCLE diagnostic potential, efficacy and safety showed encouraging results. The CONCYST study is proposing to recruit a number of 60 patients with indeterminate PCL that require EUS–FNA. The results to the date were obtained from the evaluation of 21 patients that underwent EUS–nCLE with conscious sedation. In 91% of cases, nCLE findings and the final diagnosis (based on cytology, imaging and multidisciplinary team) were correlated, instead in only 71% of cases cytology alone and the final diagnosis were correlated. One case of mild pruritus, which appeared immediately after the procedure, was registered (probably related to the contrast agent). The study's conclusion was that nCLE performed under conscious sedation seems to be safe and nCLE imaging findings are more sensitive that the EUS–FNA, regarding indeterminate PCL diagnosis. More studies are needed to confirm these results [39].

Multiple clinical studies have identified pathognomonic signs with a specificity of 100% for IPMN and SCA, which can be clearly seen on nCLE images. If a pancreatic cyst has a villous structure (finger-like projections) then it is an IPMN (Figure 4); if a superficial vascular network is visualized which is seen only in one type of pancreatic lesion, then the lesion has to be a SCA (Figure 5).

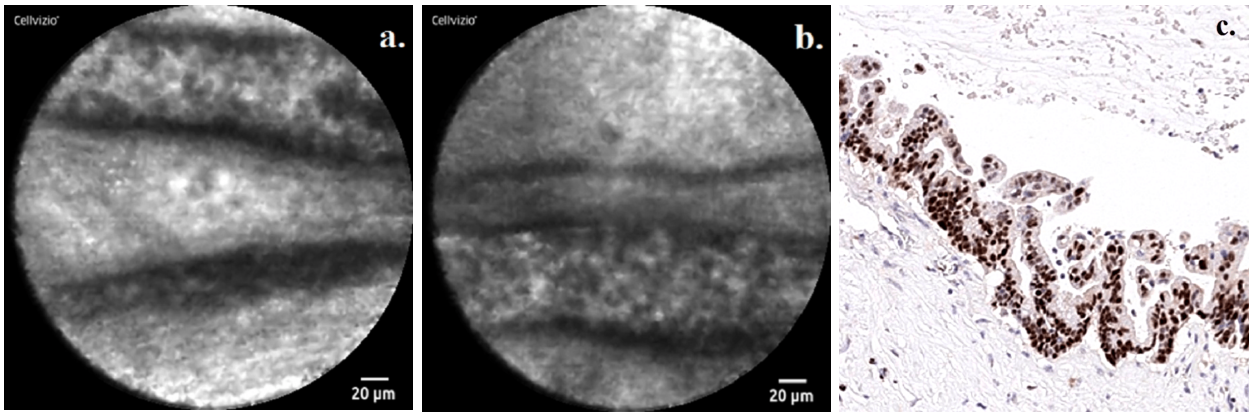

Figure 4 – ***(a and b) nCLE: IPMN with villous structure; (c) Histology (HE staining, ×100): IPMN with finger-like projections aspect (villous structure).***

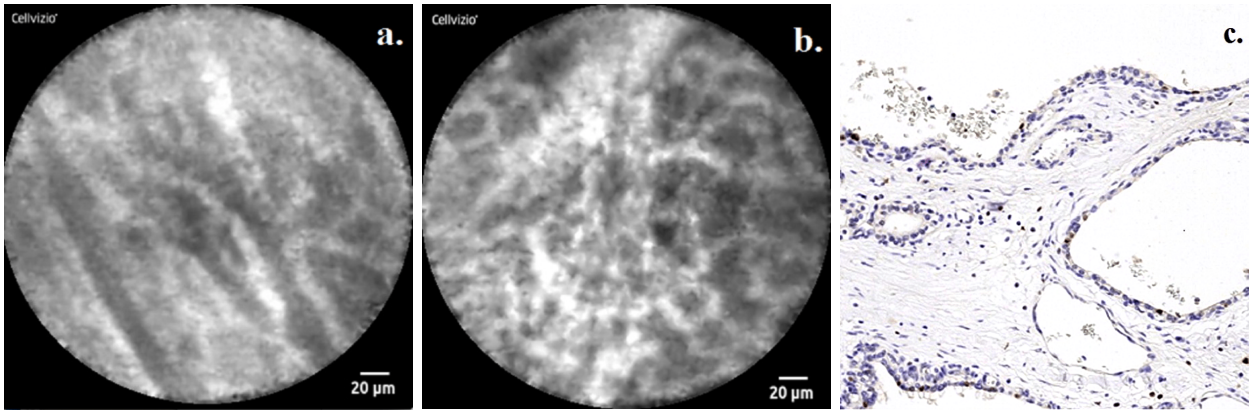

Figure 5 – ***(a and b) nCLE: SCA with vascular superficial network; (c) Histology (HE staining, ×100): SCA with capillary network.***

Conclusions

PCT are increasingly diagnosed in general population due to the widespread use of cross-sectional imaging techniques, being usually discovered incidentally. PCT are classified as benign, premalignant or malignant lesions, each type having a different clinical management.

Currently, EUS–FNA is the gold standard for diagnosing PCT, although it has some limitations. When combined with cystic fluid analysis EUS–FNA diagnosis potential is increased. The need for an accurate diagnosis is crucial for patients' lives, because it can make the difference between unnecessary or live-saving surgery.

CLE is a novel optical technique that enables *in vivo* histology-like images, offering the possibility to visualize the mucosa of the GI tract at a microscopic level and to assess any modification that might have occurred. Recently, a miniprobe thin enough to be passed through a 19G needle has been developed to allow the examination of solid organs beyond the GI tract, as the pancreas. nCLE can be used when tissue is difficult to obtain with conventional EUS–FNA.

At this moment, nCLE cannot replace EUS–FNA diagnosis potential, being mostly used on research studies. Different studies have shown that nCLE is a feasible technique to use for PCL assessment, encouraging results being obtained. Diagnosis criteria that can be used to make a differential diagnosis between mucinous/non-mucinous cysts has been identified; villous structures are 100% specific for IPMN while a superficial vascular network is 100% specific for SCA. Regarding nCLE safety, several cases with post-procedure pancreatitis have been reported, which suggest that more studies are needed.

As a final conclusion, nCLE appears to be a new imaging technique that promises to improve EUA–FNA diagnostic accuracy; further studies have to be performed in order to sustain the present results.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

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