Risk Stratification of Pancreatic Cysts with Confocal Laser Endomicroscopy

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Title: Risk Stratification of Pancreatic Cysts with Confocal Laser Endomicroscopy

Short title: CLE and Pancreatic Cysts

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**Conflicts of Interest:**

Ritu R Singh, Abhilash Perisetti, Kumar Pallav, Saurabh Chandan, Mariajose Rose De Leon: None.


**Ethical Statement:** The study did not require the approval of an institutional review board.
**Abstract**

In the modern era of high-quality cross-sectional imaging, pancreatic cysts (PC) are a common finding. The prevalence of incidental PC detected on cross-sectional abdominal imaging (such as CT scan) is 3-14% which increases with age, up to 8% in those 70 years or older. While PCs can be precursors of future pancreatic adenocarcinoma, imaging modalities such as CT scan, MRI, or endoscopic ultrasound with fine-needle aspiration (EUS-FNA) are suboptimal at risk stratifying the malignant potential of individual cysts. An inaccurate diagnosis could potentially overlook premalignant lesions, which can lead to missed lesions, unnecessary surveillance, or cause significant long-term surgical morbidity from unwarranted removal of benign lesions.

Although current guidelines recommend an endoscopic ultrasound (EUS) or MRI for surveillance, they lack the sensitivity to risk stratify and guide management decisions. Needle-based confocal laser endomicroscopy (nCLE) with EUS-FNA, can be a superior diagnostic modality for pancreatic cysts with sensitivity and accuracy exceeding 90%. Despite this, a significant challenge to the widespread use of nCLE is the lack of adequate exposure and training among gastroenterologists for the real-time interpretation of images. Better understanding, training, and familiarization with this novel technique and the imaging characteristics can overcome the limitations of nCLE use improving clinical care of patients with PC. Here, we aim to review the types of CLE in luminal and non-luminal gastrointestinal disorders with particular attention to the evaluation of PCs. Furthermore, we discuss the adverse events and safety of CLE.
**Key words:** Confocal; Endomicroscopy; Pancreatic neoplasm; Intraductal papillary mucinous neoplasm (IPMN).
Introduction

Confocal laser endomicroscopy (CLE) (also known as an optical biopsy) is a novel diagnostic technique that utilizes laser-beam to obtain *in vivo* real-time pictures of the tissues that mimic histological images.[1, 2] CLE has been used in diagnosing luminal gastrointestinal disorders, like Barrett’s esophagus, colorectal neoplasms, and biliary strictures.[3-6] There is increasing evidence to support the diagnostic accuracy of CLE in pancreatic cysts (PC) with better characterization of the cysts, and thus, indirect prediction of their malignant potential.[7-9] These studies have shown exceptional accuracy of CLE in the diagnosing the subtype of PC (exceeding 90%), with high sensitivity and specificity.

According to recent literature, 2.5 to 13% of patients undergoing abdominal imaging (computed tomography, CT or magnetic resonance imaging, MRI) for unrelated indications have incidental detection of PC, depending on the imaging modality used and the mean age of the population studied.[10, 11] The prevalence increases with age and can be found in up to 40% of people older than 70 years.[12, 13] Although most PCs are incidental, they are not always benign. PC can be a harbinger of future pancreatic ductal adenocarcinoma (PDAC) with the 5- to 10-year risk of malignant transformation being 5-8% in retrospective cohort studies. The highest risk is associated with intraductal papillary mucinous neoplasm (IPMN) greater than 1.5 cm in diameter.[14, 15] Consequently, the discovery of a pancreatic cyst imposes tremendous stress on the patient and the treating physician given the possibility of underlying pancreatic cancer or a precursor lesion.
Mortality in patients with PDAC (the third leading cause of cancer-related deaths) is increasing and attributed to late clinical presentation, lack of early detection strategies, complex pathobiology, and limited therapeutic options.[16] There is no cost-effective surveillance strategy to identify this tumor at a curative stage. Most PDAC is thought to arise from PC, and IPMN is considered a precursor lesion in many cases. The increasing use of cross-sectional abdominal imaging has frequently resulted in the identification of PC. However, these imaging modalities have limited accuracy in characterizing the PCs and in predicting their malignant potential. Current society guidelines endorse the use of Endoscopic ultrasonography (EUS) guided fine needle aspiration (FNA) for diagnosing suspicious or high-risk PC.[12, 17-19] However, the yield is limited by the paucity of cells in the aspirated fluid. The sensitivities of EUS morphology (approximately 50%) and cytology (about 60%), while superior to cross-sectional imaging alone, are suboptimal to make appropriate decisions whether to operate or continue surveillance for PC.[20, 21] Although a low carcinoembryonic antigen (CEA) (<5 ng/ml) can confidently rule out and high CEA (>800 ng/ml) rule in mucinous cysts, the sensitivity is poor at approximately 50%.[20, 21] CLE can complement EUS in better characterization of the PC and simplify the surveillance scheme. Large-scale prospective multicenter studies are underway to confirm the encouraging role of CLE over guideline-directed imaging and biomarker-based diagnostic strategy in PC.

Here, we attempt to provide a comprehensive review of the role of CLE in the diagnosis and characterization of PC, associated challenges, and the limitations to its incorporation into routine clinical practice.

Literature search
We followed preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines for reporting this review (Figure 1). We performed a literature search on Embase (Ovid), Medline (PubMed), Scopus, and the Cochrane library using the keywords ('confocal microscopy', 'endomicroscopy', 'pancreatic cyst', 'pancreatic cystic lesion'). There was no language or year of publication restriction. The search was performed on September 18, 2020 and yielded 271 results. We selected 33 publications and abstracts for review after removing the duplicates and excluding the review articles and irrelevant studies (figure 1). Furthermore, any relevant study published until final submission of the manuscript was included.

1. **Confocal Laser Endomicroscopy (CLE)**

   Although CLE is a novel technique, confocal scanning microscopy was conceptualized and designed back in 1950s to visualize neuronal networks.[22] It was not until the 90’s that this technique was gained use in gastroenterology for examining intestinal epithelial proliferation.[22, 23] Since then, CLE has been investigated for use in various luminal disorders of the gastrointestinal (GI) tract, including Barrett’s esophagus, colorectal neoplasm, and biliary strictures, and non-luminal GI disorders like pancreatic cysts.[1]

   **1.1 Technical aspects**

   CLE involves intravenous injection of fluorescein followed by tissue illumination by low-frequency laser light at a selected tissue depth. The fluorescence is captured through a pinhole of the endomicroscope with its probe positioned against the tissue of
interest. The high-resolution images are taken within a few minutes of the dye injection.\cite{1, 24} ‘Confocal’ refers to the alignment of illuminated and reflected light in the same plane so that light returning through the pinhole is selectively detected, and the scattered light beams are rejected.\cite{2} Thus, tissue images with high spatial resolution and architectural details are obtained.

1.2 Types of CLE

**Probe-based CLE**

GastroFlex\textsuperscript{TM}, CholangioFlex\textsuperscript{TM}, and ColoFlex\textsuperscript{TM} are the probe-based CLE used for gastroesophageal, pancreaticobiliary, and colonic diseases, respectively. They utilize a mini probe that is introduced through the working channel of the endoscope using a catheter (table 1). GastroFlex and ColoFlex performed during upper endoscopy and colonoscopy, respectively, are passed through the channel of standard endoscopes. The CholangioFlex probe has a smaller caliber and can be introduced during endoscopic retrograde cholangiopancreatography. Each probe has 10 to 20 uses. A laser scanner and a confocal processor are integrated with the probe for live image processing. Probe-based CLE has a fixed focal length and scans in a single plane.

**Needle-based CLE (nCLE)**

While probe-based CLE works well for the luminal structures, the access is limited in extraluminal tissue within the pancreas. A needle-based probe was developed for CLE (nCLE) of pancreatic cysts and masses.\cite{25} nCLE utilizes a miniprobe, AQ flex\textsuperscript{TM}
miniprobe that is used with EUS and is loaded on a 19 gauze (g) needle. A fluorescent dye is injected intravenously followed immediately by the acquisition of endomicroscopic images. After identification of the target cyst via EUS, the needle with the probe is used to puncture the cyst with gentle engagement. The probe is advanced slightly beyond the needle tip which provides a blunt end (the probe) to contact the wall of the pancreatic cyst to generate images for interpretation by the endoscopist. This is repeated multiple times to obtain images at various angles. Maneuvering of the needle is minimized to lower the risk of pancreatitis. Following the acquisition of the images, the probe is withdrawn. Still and movie images can be captured and saved for future review. At this point, the cyst can be aspirated for cytology and molecular analysis. The needle is then removed from the cyst.

1.3 CLE in luminal disorders

The characteristic findings of Barrett’s esophagus with dysplasia in CLE include dark spots and irregular structures that correspond to goblet cells and distorted capillaries, respectively. A prospective randomized study demonstrated improved sensitivity of probe-based CLE compared to white light endoscopy alone in high-grade dysplasia and early esophageal adenocarcinoma. CLE with targeted biopsies can improve the diagnostic yield of Barrett’s esophagus with fewer biopsies compared to random biopsies. However, CLE did not improve the detection of residual lesions following endoscopic treatment of dysplastic esophageal mucosa, as shown in a multicenter randomized trial.
Probe-based CLE has improved sensitivity in detecting colonic polyps compared to chromoendoscopy and narrow-band imaging. Moreover, it can increase the accuracy of detection of small polyps, thus potentially reducing the need for histopathology."³, ²⁸"

Another area among colonic diseases where CLE has a potential role is, detecting intra-epithelial neoplasm in patients with inflammatory bowel disease; however, the results are conflicting without the clear advantage of CLE in improving the diagnostic yield."⁵"

Intestinal metaplasia in gastric mucosa demonstrates dark spots also seen with Barrett's mucosa. Irregular capillaries, in addition to the dark disorganized features seen on CLE, are characteristic of dysplasia and can predict high-grade dysplasia or early gastric cancer with a sensitivity of approximately 90% and close to 99% specificity and accuracy."²⁹"

CLE has also demonstrated improvement in the diagnosis of indeterminate biliary strictures with high sensitivity and negative predictive value approaching 100% with >90% accuracy."³⁰, ³¹"

1.4 CLE in non-luminal disorders

Needle-based CLE (nCLE) utilizes a needle probe during EUS-FNA or fine needle biopsy (FNB) to obtain real-time images of the inner wall of the cyst. The commonly used FNB needle is 19 gauge. The role of CLE in PC is the characterization and subclassification of various pancreatic cystic lesions so that their malignant potential is determined accurately to guide surveillance and definitive therapy. Cross sectional imaging (CT scans and MRI) characteristics of PC are not specific for individual cyst types. These traditional radiologic modalities can misclassify a lesion leading to unnecessary morbidity of surgical intervention or provide a false reassurance
with the future discovery of invasive pancreatic cancer. Thus, there is a need for a test that can differentiate PC types to facilitate a cost-effective surveillance strategy. To reliably depend on this novel technique for such critical decisions, it must be reproducible with minimum interobserver and intra-observer variability.

Distinctive features on nCLE can aid in the classification of PC types with high sensitivity and accuracy. In a retrospective analysis, nCLE significantly increased the change in management (by 43%), discontinuation of surveillance (by 32%, \( p<0.05 \)), and referral for surgery (by 10%). Prospective studies have shown substantial to complete interobserver agreement for SCA and pseudocysts and less optimal agreement for discriminating mucinous cyst types (IPMN and MCN). However, differentiation between mucinous and non-mucinous lesions has been achieved with confidence. This narrows the number of patients who require surgery and surveillance and can remarkably reduce the cost of care. INSPECT was a pilot study that assessed the safety of nCLE and practicality in differentiating PC subtypes. The sensitivity and specificity were 59% and 100%, respectively. Nine percent of patients experienced mild to moderate adverse effects, including acute pancreatitis and abdominal pain. This study raised questions about the sensitivity of the test; however, subsequent studies demonstrated optimal sensitivity arguing that there is a learning curve and need to maintain proficiency and experience as an operator in order to apply nCLE to clinical practice.

Krishna et al. demonstrated an excellent correlation between in vivo nCLE images with ex-vivo histopathologic findings in PC. They also defined the characteristic nCLE features of individual cyst types. However, the sample size was small (\( N=10 \)) for
each cyst type, limited to two to three.\textsuperscript{[35]} A multicenter, prospective validation study involving 71 patients with conclusive nCLE (91\%) provided high sensitivity, specificity, and accuracy of 95\%, 100\%, and 97-99\%, respectively, for the diagnosis of mucinous lesions and serous cystadenomas. The primary endpoint of the area under the curve for all types of lesions was approximately 0.98. The procedure was well tolerated, with one patient experiencing acute pancreatitis and one had non-serious bleeding in the cyst.\textsuperscript{[9]}

A single-center prospective study evaluated 65 patients for the accuracy of nCLE compared to cytology or CEA with surgical histopathology as the reference. The accuracy for nCLE for mucinous cysts was significantly higher than cytology or CEA (97\% [95\% CI 89-100\%] versus 71\% [95\% CI 58-81\%]). Furthermore, serous cystadenoma was diagnosed with an accuracy of 97\%. Moreover, it distinguished the PC subtypes (SCA, cystic NEN, and SPN) with utmost precision with 100\% sensitivity, specificity, and accuracy.\textsuperscript{[8]} In a large retrospective study (N=206), nCLE significantly improved differentiation of indeterminate mucinous cysts into branch duct IPMN (BD-IPMN) (p=0.002) and MCN (p=0.001). While a significant number of patients avoided surveillance or surgery, there was no difference in the number of proposed surgeries.\textsuperscript{[34]}

Probe-based CLE has been used in the diagnosis of liver nodules and is to correlate with pathology with high sensitivity and specificity.\textsuperscript{[36]} Other solid intraabdominal structures including the peritoneum, lymph nodes, and adrenal glands have been evaluated with CLE during oncologic surgery.\textsuperscript{[37]}

2. **Confocal Endomicroscopy for Pancreatic Cysts**
PC can be broadly classified based on the presence of mucin, communication with the pancreatic duct (PD), and its malignant potential. Mucinous lesions include intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic adenomas. Most of the non-mucinous cysts are serous cystadenomas and pseudocysts, and rarely cystic neuroendocrine tumors (NET) and solid pseudopapillary tumors. IPMN’s arise from the main or branch pancreatic duct and have a predilection for the head of the pancreas. The vast majority are main duct IPMN. Cystic fluid stains may be positive for mucin and usually have high CEA and amylase content. Communication with the main pancreatic duct (main duct IPMN) renders a high malignant potential. Other high-risk features of PC include the presence of a solid core, mural nodule, and dilatation of the main PD. MCN’s arise more often in the pancreatic body or tail and lack communication with the PD. Low cyst fluid amylase can differentiate them from IPMN. They are precancerous with the highest malignant potential being in those with peripheral “eggshell” calcification.

Serous cystic neoplasms are benign non-mucinous cysts more commonly located in the body or tail of the pancreas and lack communication with the PD. The cyst fluid is low in CEA and amylase. Cystic NET of the pancreas account for less than 1% of all PCL (10-17% of pancreatic NET). They are usually located in the tail of the pancreas and do not contain CEA or amylase. Solid pseudopapillary tumors are the least common PC, and can arise in any part of the pancreas. They are characterized by mixed solid and cystic components and low CEA and amylase. Although indolent in nature, they have malignant potential and rarely metastasize. Pseudocysts are
inflammatory cysts, a consequence of pancreatitis, and can be found in any part of the pancreas. They lack mucin, have high amylase content, and have low CEA.

2.1 Endomicroscopic features of individual cyst types

Three patterns on needle-based CLE (nCLE) differentiate most of the PC types with high specificity\(^\text{[33]}\)

- Epithelial features with papillae and epithelial bands are characteristic of mucinous cysts (Figure 2D) with an almost complete interobserver agreement (Fleiss \(k\) value 0.81).
- Trabecular pattern identifies cystic NEN with substantial reproducibility (Fleiss \(k\) value 0.78).
- Fern pattern of vascularity is distinctive of SCA (Figure 2A).

3. Current society guidelines for the diagnosis and surveillance of PC

Treatment and surveillance strategy for PC endorses identification of premalignant cysts (IPMN and MCN) followed by the characterizing of high-risk features to guide further management. Characterizing PC is often challenging, reflected in current guidelines where the approach bases on “suspected” cyst type. The guidelines recommend MRI or EUS for this purpose which classifies a significant proportion of PC as undefined. CLE can classify mucinous PC with sensitivity, specificity, and accuracy exceeding 90%.\(^\text{[8]}\) Current guidelines do not endorse routine use of CLE for
the diagnosis and classification of PC due to a lack of large-scale prospective studies and need for potential updates to their recommendations. Table 2: Sensitivity, specificity, and accuracy of CT, MRI, EUS±FNA cytopathology, CEA, CA 19-9 in differentiating cyst type.

4. Safety of EUS guided confocal microscopy

Use of a 19 guage needle with a diameter of greater than one mm (1.07 mm) to puncture the cyst during EUS guided nCLE potentiates the risk of local complications. Adverse events have been reported in 3-9% of procedures, the most common being acute pancreatitis, less commonly, intra-cystic bleeding, transient abdominal pain, and rarely cyst infection.\[7-9, 38\] None of the adverse events have been reported as severe. Post-procedure pancreatitis episodes are usually mild and reported in approximately 3% (1.3% to 6.6%) of patients undergoing EUS-nCLE.\[7, 8, 24, 39\] Krishna et al reported five cases (3.5% of 144) of mild acute pancreatitis, and four of the five events of acute pancreatitis occurred during the first 25 nCLE procedures. This indicates that there is a learning curve with reduced rate of adverse events with experience. However, occurrence of acute pancreatitis had no relation with the mean duration of the procedure. Furthermore, all the episodes of acute pancreatitis occurred with the transgastric approach of needle puncture.\[8\] In the largest multicenter, prospective study from France, Napolean et al reported a lower rate of post-procedure pancreatitis (1.3%). The difference in the rate of post-procedure pancreatitis could be possibly accounted by the endosonographer’s experience. Adverse events associated with nCLE for PC are listed in table 3. The overall rate of adverse events reported for EUS-FNA is
approximately 5% (95% CI 1.84–3.62%), and the rate of acute pancreatitis is about 2.0% (95% CI 0.55%–3.81%) which is comparable to that of EUS-nCLE.

5.1 Does nCLE impact management decisions for PC?

The combination of nCLE with EUS-FNA provides an almost perfect diagnostic test for PC with sensitivity, specificity, and positive and negative predictive values approaching 100%.[40] Two retrospective studies have looked at the impact of nCLE in the management of PC. Both studied solitary cysts, and the overall change in management of patients was 28% and 43%. Surveillance rates fell significantly by 35-38%, and the rate of SCA surveillance reduced from 40% to 5%.[32, 34]

5.2 Is there any other endoscopic modality to complement nCLE?

EUS with microforceps biopsy

A Moray microforceps (MFB) is introduced through the 19 gauze FNA needle to obtain a biopsy from the PC wall and mural nodule. MFB has been shown to improve the diagnostic yield in combination with EUS-FNA. The rate of tissue acquisition with MFB is approximately 90% with a diagnostic accuracy of 68-75%.[32, 41] A systematic review (mostly including retrospective studies) comprising a pooled analysis of over 500 patients showed improvement in diagnostic yield (OR 4.79, p=0.007) compared to FNA cytology.[42] In a retrospective analysis comparing the addition of MFB to FNA and nCLE, the use of MFB and nCLE led to discontinuation of surveillance (in 11%) and
avoidance of surgery (in 25%). The diagnostic yield of nCLE was the highest (84%) and improved (93%) in combination with MFB and cytology; however, the improvement in the diagnostic yield was statistically insignificant raising questions on the value of adding MFB in setting of nCLE and FNA.\(^\text{[32]}\)

Adverse events were reported in 8-9% of patients undergoing MFB.\(^\text{[41, 42]}\) Most common adverse events were mild acute pancreatitis and intracystic bleeding which were managed conservatively. The serious adverse event was rare, with approximately 1% of patients reporting severe acute pancreatitis.\(^\text{[42]}\) While MFB has the potential to be a candidate to complement nCLE in the diagnosis of PC, the advantage of the procedure over nCLE alone is yet to be confirmed in prospective studies.

### 5.3 Role of artificial intelligence

Artificial intelligence (AI) has the potential to ease the complexity of image interpretation in CLE, and probably overcome the inter- and intra-observer variability that exists. However, there is limited data on the utilization of AI in nCLE for PC. In a post-hoc analysis of a single-center prospective study, Machicado et al examined nCLE videos of 35 patients with pathologically confirmed IPMN to develop computer aided diagnosis algorithms.\(^\text{[43]}\) The algorithms improved the sensitivity (83% versus 56%) and accuracy (83% versus 68 to 74%) for the detection of high grade dysplasia and adenocarcinoma in IPMN compared with both the Fukuoka and AGA guidelines. Nonetheless, these results are yet to be replicated in other prospective studies. There are upcoming efforts to collect data and incorporate AI to improve the performance and
reproducibility of probe-based CLE. New generations of the processor and software aim to facilitate these advances in near future.

6.0 Challenges and limitations

6.1 Learning curve

As with any novel technique, there is a learning curve with CLE. This includes familiarizing with the device, understanding how to generate high quality images, and the recognizing and interpreting characteristic CLE images of PC subtypes (mucinous and non-mucinous). Competency in the CLE techniques are being attained through continuing medical education and/or advanced endoscopy fellowships. There is likely variation in learning curves for various applications of CLE. A prospective, double-blind review of probe-based CLE images of colorectal neoplasms concluded that the technique and image interpretation of images is learned promptly. The training was provided with 20 CLE images of known colorectal neoplasms, followed by an assessment of obtaining high-quality images. The accuracy of image acquisition improved with the increasing number of lesions examined and plateaued over 61 to 76 lesions (accuracy of 86%).[43] This data provides limited yet good evidence that endoscopists can rapidly familiarize themselves with the technique of pCLE and interpret the images with high accuracy through training and supervision. How these correlates to nCLE in PC evaluation is unknown? Studies are underway to better assess the learning curve for nCLE in PC, at this time, the use of nCLE for PCs is limited to highly trained endosonographers at academic centers, and the importance of specialized training cannot be overemphasized.
6.2 Interobserver agreement and intraobserver reliability

The technical success of nCLE has been reported to be greater than 90% in most of the published studies.\[32, 39, 44\] However, there appears to be inconclusive evidence for the reliability and interobserver agreement on imaging features of nCLE for diagnosing PC. The inter- and intra-observer agreement for recognizing nCLE features of SCA has been reported as nearly perfect (k 0.83, 95% CI 0.71-0.91).\[33\] Likewise, the interobserver agreement for mucinous cysts was substantial (k 0.72, 95% CI 0.52-0.87) in a study of six unblinded investigators.\[45\] However, when 15 nCLE videos were reviewed by six endoscopists at different centers the accuracy between observers was 46% (range 20% to 67%).\[46\]

Intraobserver reliability for interpreting CLE images appears to be higher among experienced observers.\[47\] A retrospective analysis of institutional EUS database demonstrated high intraobserver reliability in recognizing nCLE patterns of PC among six blinded international endosonographers. They reviewed 29 EUS nCLE videos in two phases at 2-week interval to allow wash-out. The intraobserver reliability for recognizing epithelial patterns ranged from 0.82 (for trabecular pattern) to 0.91 (for papillae and epithelial bands), and 0.85 for fern pattern of vascularity.\[33\] This supports the need for adequate training and maintenance of proficiency when utilizing this advanced modality for clinical practice in PC evaluation.

6.3 Other limitations

The nCLE can provide in vivo microscopic images of the cyst wall; however, further study of the cyst is limited by the lack of a sample for molecular analysis. This
limitation can be overcome by combining FNA or MFB with EUS and nCLE. It is important to note that CLE images are limited to the mucosal layer and are unable to provide information on deeper layers. The inability to examine the deep layers can be crucial in cysts with a mural nodule or solid component where biopsy may be needed. In these cases, nCLE may have specific limitations which require further study.

Conclusions

Needle-based CLE provides real-time microscopic visualization of pancreatic cyst and can be a promising endoscopic modality for the diagnosis and risk stratification of these lesions. Proof of principle of this novel approach has been demonstrated in some early studies with notable follow up studies supporting the use of nCLE in PCs. The next steps will be to validate these early results in large prospective trials. There remain challenges to overcome before widespread adoption of the technology. This includes familiarization and training with the technology among endoscopists beyond tertiary academic centers with large pancreatic cyst programs, need to overcome interobserver variability and cost. Furthermore, early evidence suggests a potential role of AI in nCLE to diagnose PC whereby it can enhance accuracy and support AI standardization of the process of real-time image interpretation. Looking forward, improvement in endomicroscopic technology with higher quality images, improved proficiency with the technology, additional standardization of image patterns with AI supporting the ability to further differentiate mucinous cysts can support the applicability of nCLE as a safe and acceptable diagnostic modality which may someday become a standard of care in select pancreatic cysts.
References


22 Memoir on inventing the confocal scanning microscope. *Scanning*; 10: 128-138


### Table 1. Confocal Microprobes and their technical features.

<table>
<thead>
<tr>
<th>Types of probes</th>
<th>GastroFlex™</th>
<th>AlveoFlex™</th>
<th>CholangioFlex™</th>
<th>AQ-Flex™ 19</th>
<th>ColoFlex™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compatible Operating channel</td>
<td>≥ 2.8 mm</td>
<td>≥ 1.9 mm</td>
<td>≥ 1.0 mm</td>
<td>≥ 0.9 mm</td>
<td>≥ 2.8 mm</td>
</tr>
<tr>
<td>Length</td>
<td>3 m</td>
<td>3 m</td>
<td>4 m</td>
<td>3 m</td>
<td>4 m</td>
</tr>
<tr>
<td>Resolution</td>
<td>1 µm</td>
<td>3.5 µm</td>
<td>3.5 µm</td>
<td>3.5 µm</td>
<td>1 µm</td>
</tr>
<tr>
<td>Field of view</td>
<td>240 µm</td>
<td>600 µm</td>
<td>325 µm</td>
<td>325 µm</td>
<td>240 µm</td>
</tr>
<tr>
<td>Observation depth</td>
<td>50-55 µm</td>
<td>0-50 µm</td>
<td>40-70 µm</td>
<td>40-70 µm</td>
<td>55-65 µm</td>
</tr>
</tbody>
</table>

### Table 2. Major gastrointestinal society guidelines for the management and surveillance of Pancreatic Cysts.

<table>
<thead>
<tr>
<th>Society</th>
<th>Year updated</th>
<th>Indications for considering surgery</th>
<th>High risk features for further testing</th>
<th>Recommended test if high risk feature</th>
<th>Surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACG guidelines</td>
<td>2019</td>
<td>Symptoms (jaundice) attributed to PC. A solid component within the cyst</td>
<td>• Size &gt;3 cm</td>
<td>EUS with FNA</td>
<td>IPMN and MCN: EUS or MRI at 6 months to 2-year intervals depending on the cyst size. Asymptomatic non-mucinous cysts: No follow up needed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Rapid growth, &gt;3 mm/year</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Main PD diameter &gt;5 mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>European guidelines</td>
<td>2018</td>
<td>Absolute: jaundice, main PD ≥10 mm, mural nodule ≥5 mm or a solid content within the cyst. Relative: acute pancreatitis, main PD 5-10 mm, mural nodule &lt;5 mm, cyst size ≥4 cm.</td>
<td>NA</td>
<td>NA</td>
<td>IPMN and MCN: 6-monthly follow up with MRI or EUS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revised Fukuoka guidelines</td>
<td>2017</td>
<td>• Surgically fit patients with high-risk features.</td>
<td>“Worrisome features”</td>
<td>EUS for patients with “Worrisome features”</td>
<td>If no “Worrisome features” or “High-risk features”: EUS or MRI. Consideration for surgery in younger patients with cyst size &gt;2-3 cm.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Size &gt;3 cm, MPD 5-9 mm, mural nodule &lt;5 mm, thickened/enhanced wall, lymphadenopathy, elevated serum CA</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 19-9 or</td>
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</tbody>
</table>
rapid growth of cyst >5 mm in 2 years.

“High-risk features”
- Jaundice in the presence of a cyst in the head of pancreas,
- Main PD >10 mm or,
- mural nodule ≥5 mm.

<table>
<thead>
<tr>
<th>AGA guidelines</th>
<th>2015</th>
<th>Solid component and Dilated main PD ± concerning EUS feature</th>
<th>size ≥3 cm Dilated main PD or solid component</th>
<th>EUS and FNA One high risk feature without concerning EUS feature: Annual MRI followed by every other year. No high-risk feature: Annual MRI for 5 years</th>
</tr>
</thead>
</table>

¶ To avoid prolonged follow up, † without absolute or relative indication for surgery. ACG American college of gastroenterology, PC pancreatic cyst, PD pancreatic duct, EUS endoscopic ultrasound, FNA fine-needle aspiration, IPMN intraductal papillary mucinous neoplasm, NA not available, MCN mucinous cystic neoplasm, MRI magnetic resonance imaging, AGA American gastroenterology association.

Table 3. Adverse events associated with Confocal Laser Endomicroscopy for Pancreatic Cysts.

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design</th>
<th>N</th>
<th>Adverse effect; N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krishna et al, 2020</td>
<td>Prospective</td>
<td>144</td>
<td>Acute Pancreatitis; 5 (3.5%)</td>
</tr>
<tr>
<td>Napolean et al, 2019</td>
<td>Multicenter, prospective</td>
<td>206</td>
<td>Acute pancreatitis; 2 (1.3%)</td>
</tr>
<tr>
<td>Keegan et al, 2019</td>
<td>Retrospective cohort</td>
<td>100</td>
<td>Acute pancreatitis; 2 (2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Infected cyst; 1 (1%)</td>
</tr>
<tr>
<td>Nakai, et al, 2015</td>
<td>Prospective feasibility</td>
<td>30</td>
<td>Acute pancreatitis; 2 (6.6%)</td>
</tr>
<tr>
<td>Napolean et al, 2015</td>
<td>Multicenter, prospective</td>
<td>31</td>
<td>Acute pancreatitis; 1 (3.2%)</td>
</tr>
<tr>
<td>Konda et al 2013</td>
<td>Multicenter, pilot</td>
<td>66</td>
<td>Acute pancreatitis; 2 (3.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intracystic bleeding, 2 (3%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transient abdominal pain, 1 (1.5%)</td>
</tr>
</tbody>
</table>

N number of patients.
Figure Legends

**Figure 1.** Search strategy per preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines.

**Figure 2.** Confocal laser Endomicroscopy (CLE) and endoscopic ultrasound (EUS) images of pancreatic cysts.

A. CLE image of serous cystadenoma with “Fern pattern” of vascularity.

B. EUS of the above serous cystadenoma demonstrating cystic lesion composed of micro and macrocystic components.

C. CLE finding of the IPMN with papillary finger-like projections.

D. EUS of an Intraepithelial papillary mucinous neoplasm (IPMN) showing anechoic cystic structure with one internal septation.
271 records identified through database search
- Embase (Ovid)
- MEDLINE (PubMed)
- Scopus
- The Cochrane Library

185 studies screened

90 studies irrelevant

95 full-text studies assessed for eligibility

62 studies excluded
- 36 duplicate abstracts/publications
- 14 review articles
- 4 Wrong patient population
- 4 case report
- 3 Wrong intervention
- 1 commentary

86 duplicates removed.

33 studies included.