Original Article

Diagnosis of Pancreatic Cystic Lesions by Virtual Slicing: Comparison of Diagnostic Potential of Needle-Based Confocal Laser Endomicroscopy versus Endoscopic Ultrasound-Guided Fine-Needle Aspiration

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Abstract

Background: Pancreatic cystic lesions are often challenging entities for diagnosis and management. EUS-FNA diagnostic accuracy is limited by paucicellularity of cytology specimens and sampling errors. Needle-based confocal laser endomicroscopy (nCLE) provides real-time imaging of the microscopic structure of the cystic lesion and could result in a more accurate diagnosis. **Aims and Objectives:** To determine the diagnostic utility of *in vivo* nCLE and EUS-FNA in the diagnosis and histologic characterization of pancreatic cystic lesions (PCL). **Materials and Methods:** All patients diagnosed with PCL who had undergone nCLE and FNA over a 10-year period within a major urban teaching hospital were included in this study. All gastroenterology reports of the nCLE images and corresponding pathologist findings from the EUS-FNA were collected and compared with, a final diagnosis prospectively collected from clinicopathological and imaging data. **Results:** A total of *n*=32 patients were included in this study, which consisted of *n*=13 serous cystadenoma (SCA), *n*=7 intraductal papillary mucinous neoplasms (IPMN), *n*=2 mucinous cystic neoplasms (MCN), *n*=3 well-differentiated neuroendocrine tumors, *n*=2 cysts, *n*=2 benign pancreatic lesions, *n*=1 adenocarcinoma, *n*=1 gastrointestinal stromal tumor (GIST) and *n*=1 lymphangioma. The overall diagnostic rate was higher in nCLE (87.5%) vs. EUS-FNA (71.9%) While the diagnostic accuracy of nCLE and EUS-FNA were comparable in characterization of benign vs. malignant lesions, the nCLE diagnosis demonstrated higher accuracy rate in identifying mucinous cystic neoplasms compared to EUS-FNA. **Conclusion:** nCLE is a useful companion diagnostic tool for pancreatic cystic lesions and could assist the cytopathologist to better triage the sample for required ancillary testing and treatment planning. The combination of nCLE and EUS-FNA may be especially helpful in reducing the proportion of cases categorized as non-diagnostic.

Keywords: Advanced imaging technique, confocal laser endomicroscopy, probe-based confocal laser endomicroscopy, pancreatic cystic lesion

Introduction

Studies have shown an increase in the number of detected pancreatic cysts over the decade, largely due to the improved quality of cross-sectional imaging.[1] Solitary pancreatic cystic neoplasms (PCNs) include a range of histopathologic types which, when combined with factors such as size, associated solid component, and pancreatic duct dilation, can be used to determine the malignant potential of the lesion.[2] While surgical resection is recommended for cystic lesions with significant malignant potential (such as mucinous cystic neoplasm [MCN] and

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Table 1: General features and imaging appearance of the most common pancreatic cystic lesions

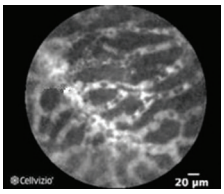
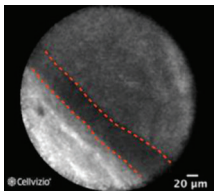
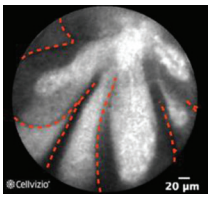
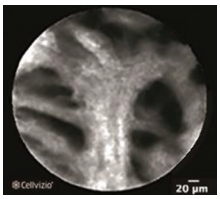
	SCA	Mucinous neoplasm	IPMN	Cystic neuroendocrine
Age	Usually 6th decade	Variable, usually 5th to 7th decade	7th decade	Usually 5th to 6th decade
Gender	Female>Male	Almost exclusively female	Female=Male	Female=Male
Typical pCLE imaging characteristics				
	Fern pattern	Epithelial band	Finger-like papillary projections	Trabecular pattern
Malignant Potential	Negligible	Low	Main duct: High Branch-duct: low to moderate	Moderate to high
Treatment	Resect if symptomatic	Resection	Closely monitor or resect Resection and post-resection surveillance	Resection and post-resection surveillance

*Image courtesy: Mauna Kea Technologies, Paris, France

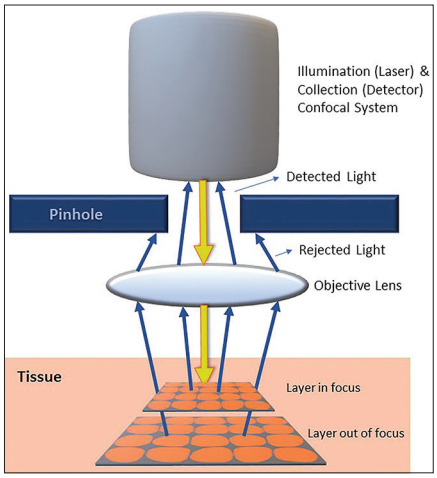


Figure 1: This image illustrates how the emitted light originating outside of the focal plane will be blocked by the pinhole before reaching the detector

intraductal papillary mucinous neoplasm [IPMN] with high-grade dysplasia), simple surveillance is recommended for cysts with low risk of malignant transformation (e.g., serous cystadenomas [SCAs]).[3,4] Table 1 summarizes the demographic distribution, typical imaging and cytological findings, malignant potential, and common treatment method used for the most common PCNs.[5,6]

Confocal laser endomicroscopy (CLE) is based on illuminating the lesion with a low-power laser through a pinhole and then detecting the reflected fluorescent light from the tissue. The laser is focused at a specific depth, and the reflected light is then refocused onto the detector by the same lens. The out-of-focus light from above and below the depth of interest is blocked, improving optical resolution and contrast. As illumination and detection systems are at the same focal plane, they are termed "confocal" [Figure 1]. The tissue is scanned in the horizontal and vertical planes, which allows three-dimensional reconstruction of images. The probe-based CLE (needle-based CLE [nCLE]) system includes a flexible fiber-optic bundle with a fixed focal length that only scans in a single plane. Cellvizio Confocal Miniprobes™ (Mauna Kea Technologies, Paris, France) is created for gastrointestinal (GI) applications [Figure 1].

Current studies demonstrate that imaging modalities such as computed tomography, magnetic resonance imaging, and endoscopic ultrasound (EUS) can accurately classify the premalignant lesions in the majority of cases; however, a significant number of lesions cannot be characterized by any diagnostic technique including EUS-fine-needle aspiration (FNA).[7,8] New imaging methods are needed to classify these lesions in a more precise manner. CLE, performed at the time of EUS-FNA, has been introduced to improve the diagnostic accuracy and management of indeterminate pancreatic cystic lesions (PCLs) by providing real-time imaging of the internal structure of such lesions.[9]

The benefits of adding nCLE are two-fold. First, by imaging the architecture of the intact cyst, it may be possible to better characterize the cyst type. The second benefit of nCLE is its markedly superior ability to target abnormal areas and reduce the sampling of the surrounding unremarkable tissue. An increasing number of studies reported the diagnostic accuracy of nCLE between 80% and 95%.[10] Specifically, in the paucicellular cases, nCLE results provide pathologists with key information, allowing them to accurately classify the cases in the absence of sufficient cell yield. Taken together the combination of nCLE and EUS-FNA could reduce the number of nondiagnostic cases and improve the classification of borderline cases, allowing for better triaging for ancillary testing and treatment planning.

Objective

To determine the diagnostic utility of *in-vivo* nCLE and EUS-FNA in the diagnosis and histologic characterization of PCLs.

Methods

All patients (n = 32) who had undergone nCLE combined with EUS-FNA for further classification of cystic pancreatic

lesions between January 1, 2007, and December 31, 2018, were included in this study. The gastroenterologist interpretation of nCLE image review, including the location of the lesion, type of approach, number of passes, and the size of needle, was abstracted from procedure notes in the electronic medical records. Patients with solid pancreatic masses and biliary lesions were excluded from the study. All the corresponding and subsequent follow-up cytology and surgical reports were extracted from the laboratory information system, and slides were collected for review. The cytologic material was processed as (1) direct smears (Diff-Quick and Papanicolaou stains), (2) ThinPrep preparations (Papanicolaou stain), or (3) formalin-fixed paraffin-embedded cell blocks. All the cytology diagnoses utilized the guidelines for pancreaticobiliary cytology from the Papanicolaou Society for Cytopathology published in June 2014 [Table 2].[11] The present study was approved by the institutional review board of the medical center.

The final diagnosis was established using the following hierarchy of information abstracted from the patient record: surgical resection, subsequent core biopsy, cell block prep of FNA sample, cyst fluid results (carcinoembryonic antigen levels and amylase levels), or follow-up imaging studies, collected from 12 to 36 months after the initial diagnosis date. Cases classified as malignant or those with malignant potential included the following histology types: neuroendocrine, IPMN, MCN, or adenocarcinoma. Cases classified as benign included SCA, pancreatitis, pseudocyst, low-grade GI stromal tumor (GIST), and lymphangioma. If no tissue findings were obtainable, the final diagnosis was made on the basis of clinical follow-up and the absence of malignant features or metastasis.

Table 2: The Papanicolaou Society of Cytopathology System for Reporting Pancreaticobiliary Cytology

I. Nondiagnostic	
II. Negative (for malignancy)	Benign pancreatic tissue
	Pancreatitis (Acute, Chronic, Autoimmune)
	Pseudocyst
	Lymphoepithelial cyst
	Splenule/accessory spleen
III. Atypical	
IV. Neoplastic	
A. Benign	Serous cystadenoma
	Lymphangioma
B. Other	Well-differentiated neuroendocrine tumor
	Intraductal papillary mucinous neoplasm, all grades of dysplasia
	Mucinous cystic neoplasm, all grades of dysplasia
	Solid-pseudopapillary neoplasm
V. Suspicious (for malignancy)	
VI. Positive or Malignant	Ductal adenocarcinoma of the pancreas and its variants
	Cholangiocarcinoma
	Acinar cell carcinoma
	Poorly differentiated neuroendocrine carcinoma
	Metastatic malignancy

Results

During the study period, 32 patients had undergone nCLE and EUS-FNA biopsy. The study included 20 women (62.5%) and 12 men (37.5%). The patients' ages ranged from 26 to 83 years (mean, 65.6 years; median, 71.0 years). Pancreatic cysts were found more commonly within the head (n = 11, 34.3%), body (n = 9, 28.1%), and tail (n = 8, 25.0%). Less commonly, the lesions were found in the neck (n = 3, 9.3%) and involving the head, body, and tail (n = 1, 3.1%). The lesion size varied from 0.7 to 146 cm^2 (mean, 15 cm^2; median, 5.5 cm^2). The aspirated fluid volume ranged from scant to 250 ml. The majority of cases were biopsied through a transgastric approach (n = 21, 63.6%) and 19G needle (n = 23, 69.6%). The histologic findings were available for 18 cases (56.2%), and clinical follow-up data were used for 14 cases (43.7%). The histologic specimens included 12 cell block preparation (37.5%), 3 needle biopsy samples (9.3%), and 3 distal pancreaticoduodenectomy specimens (9.3%). The sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each diagnostic technique, EUS-FNA and nCLE, as demonstrated in Table 3. The nondiagnostic cases were excluded from the statistical analysis with the exception of the calculation of nondiagnostic rate.

The first patient was a 56-year-old woman with an initial presentation of a 2-cm cystic lesion in the neck of pancreas. The outside aspirate showed benign pancreatic tissue which was not representative of the described lesion. The repeat FNA of the lesion at our institution demonstrated scant, bland-appearing epithelial cells. During 5-year clinical follow-up, the cyst increased in size to 6 cm, involving the head and neck of the pancreas, and nCLE findings reported as cystic neuroendocrine tumor (NET). However, the subsequent FNA specimen displayed SCA. The original nCLE recording was reviewed by another gastroentrologist, blinded to the final diagnosis, and interpreted as NET. The second false-positive case was a 65-year-old woman with a 2.7-cm cystic lesion in the tail of the pancreas. The nCLE imaging was interpreted as cystic NET; however, the FNA sample demonstrated a SCA. The review of the initial nCLE recording showed a poorly visible epithelial and vascular pattern.

We found only one false-negative case within our study group [Table 4]. The patient was a 77-year-old woman with a 4-cm solid, cystic lesion in the body of the pancreas. The nCLE findings described as nonspecific changes consistent with chronic pancreatitis. The EUS-FNA specimen showed pancreatic ductal adenocarcinoma and the distal pancreatectomy confirmed the diagnosis. The nCLE criteria were described as dark cell aggregates of irregular size and shape with irregular borders and irregular vessels with leakage of fluorescein for adenocarcinoma. In nCLE images of chronic pancreatitis, residual glands appear as regular structures of identical size on a grey background of fibrotic tissue.[12] However, the lesion appeared as relatively irregular dark

aggregates on nCLE sequence, and the vessels were poorly visualized due to the faint contrast of the background.

Within our study group, there were four cases that were nondiagnostic using nCLE technique, with a nondiagnostic rate of 12.5%. The cytologic findings of EUS-FNA were consistent with that of SCA (n = 2), cystic NET (n = 1), and GIST (n = 1). No further intervention was pursued in these cases. Figure 2 illustrates the success rate of each method in establishing the final diagnosis of different entities such as SCA (n = 13), IPMN (n = 7), and cystic NET (n = 3). The nCLE demonstrated the highest diagnostic accuracy in detecting IPMNs (n = 7, 100%) compared to EUS-FNA (n = 3, 42.8%). In differentiating SCA, nCLE proved to have slightly lower diagnostic accuracy rate (n = 9, 69.2%) than EUS-FNA (n = 10, 76.9%). In neuroendocrine cystic lesions, EUS-FNA showed a higher diagnostic accuracy rate (n = 3, 100%).

Discussion

Our study suggests that nCLE can detect PCN with diagnostic accuracy comparable to EUS-FNA [Table 3], with a nominally higher sensitivity and lower specificity. The EUS-FNA had a two-fold higher nondiagnostic rate compared to nCLE. This report also demonstrated that the combination of nCLE with EUS-FNA could result in perfect diagnostic accuracy (specificity = 100%, sensitivity = 100%, and nondiagnostic rate = 0%) in our small series of study set. In our study, nCLE demonstrated substantially better performance than EUS-FNA in the interpretation of IPMNs.

Table 3: Diagnostic accuracy of needle-based confocal laser endomicroscopy, endoscopic ultrasound with fineneedle aspiration, and combined techniques in differentiating pancreatic cystic lesion

Dx Technique	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Non-Dx (%)
nCLE	91.7	87.5	84.6	93.3	12.5
EUS-FNA	80.0	92.3	88.9	85.7	28.1
Combined	100.0	100.0	100.0	100.0	0.0

PPV: Positive predictive value, NPV: Negative predictive value

The primary aim of our study was to demonstrate the value of nCLE as an advanced imaging modality and its benefits to pathology practice. The GI specialist community has shown increasing interest in conducting studies on the diagnostic potential of this technology as a replacement for tissue examination. To our knowledge, this is the first report in a pathology journal to compare the diagnostic performance of nCLE to demonstrate its clinical utility in the pathology practice. The focus of our study was to present the advantages of nCLE in patient management as an adjunct technique to EUS-FNA, not as a replacement.

A literature search in PubMed identified 17 articles reporting on nCLE diagnostic accuracy in PCLs.[13-16] Most of them were focused on nCLE performance in differentiating mucinous from nonmucinous cysts. The reported sensitivity and specificity ranged from 59% to 100% (mean, 88.4%; median, 95%) and 82% to 100% (mean, 95.2%; median, 100%), respectively. The lower specificity shown by our study is potentially attributed to the fact that most of these studies evaluated the performance of nCLE as a combined nCLE-EUS and not as a stand-alone technique.

The major challenge in the evaluation of PCNs is identifying lesions with the malignant potential to avoid subjecting patients to unnecessary surgery. Cysts with moderate-to-high malignant potential include MCN, IPMNs, and cystic NETs. Serous cystic tumors bear little malignant potential and can be followed with surveillance imaging. nCLE is an emerging technology that could significantly improve the characterization of these lesions and potentially reduce the number of unnecessary surgeries.

The diagnosis and grading of PCN can be challenging.[17-20] The nCLE provides images similar to a low-magnification tissue section with intact architectural pattern. This information could be applied in conjunction with morphologic features to improve the diagnostic accuracy of cytology samples. Diagnosis of IPMNs by the examination of only cytology samples can be challenging specifically in cases associated with low-to-intermediate-grade dysplasia. Some of the contributory

Table 4: Accuracy of diagnostic categorization of pancreatic cyst lesions using needle-based confocal laser endomicroscopy compared to final diagnosis

Final diagnosis	nCLE							
	Non-diagnostic	Benign	Atypical	Neoplastic benign	Neoplastic other	Suspicious for malignancy	Positive or malignant	Total
Non-diagnostic	0							0
Benign		4						4
Atypical			0					0
Neoplastic: benign	3	1		9	2			15
Neoplastic: other	1				11			12
Suspicious for malignancy						0		0
Positive or malignant		1					0	1
Total	4	6	0	9	13	0	0	32

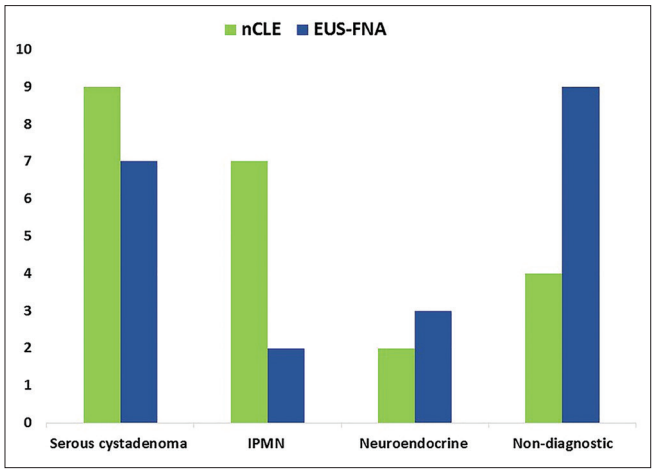


Figure 2: Diagnostic rate of needle-based confocal laser endomicroscopy versus endoscopic ultrasound-fine-needle aspiration in establishing the final diagnosis of each subtype of pancreatic cystic lesion. The last group shows the nondiagnostic results of two methods

factors are paucicellularity, and the loss of architectural pattern in cytology samples results in difficulty in the differentiation of neoplastic mucin from GI contamination. The result of the confocal study for such cases will be vital in determining the management plan. The confocal examination could also assist the pathologist with determining the ancillary testing, such as the essential immunostains with the highest diagnostic yield in paucicellular cell blocks. For example, the diagnostic accuracy of FNA in SCA is often limited due to scant cellularity of loose fragments of cuboidal cells with a notable absence of atypia and mitosis. The nCLE findings of SCA would compel pathologists to preserve the minute fragments of bland-appearing cells on cell block for confirmatory immunostains instead of exhausting the material by ordering deeper sections in search for malignant ductal cells.

The main limitation of nCLE in patient care is its inability to provide tissue for testing of prognostic and therapeutic molecular markers. nCLE also does not penetrate beyond the mucosa due to physical limitation and cannot be used in the diagnosis of submucosal invasions.[21] Other barriers of the adoption are lack of awareness of technology, availability, physician interpretation training, and financial considerations.[9,22] Our findings suggest a need for pathologists to become competent in the interpretation of nCLE sequences. As pathologists are already familiar with the architectural pattern, they could correlate the virtual *in-vivo* biopsy of nCLE images to the corresponding microscopic features more accurately. We also suggest including the nCLE interpretation in the pathology report as an important component of the evaluation and management plan.

Conclusion

Combining the nCLE with EUS-FNA can significantly increase the diagnostic yield (100%) and accuracy of characterization of PCNs and should be considered as the standard of care for PCLs. Further studies of a larger number of nCLE specimens are required to determine the cost-effectiveness of this technology in the management of PCLs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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