—Review Article—

Can endoscopic ultrasound-guided needle-based confocal laser endomicroscopy replace fine-needle aspiration for pancreatic and mediastinal diseases?

Jintao Guo, Manoop S. Bhutani[1], Marc Giovannini[2], Zhaoshen Li[3], Zhendong Jin[3], Aiming Yang[4], Guoqiang Xu[5], Guiqi Wang[6], Siyu Sun

Endoscopy Center, Shengjing Hospital of China Medical University, Shenyang, Liaoning Province, [3]Department of Gastroenterology, Changhai Hospital, Navy Medical University, Shanghai, [4]Division of Gastroenterology, Chinese Academy of Medical Sciences and Peking Union Medical College Hospital, Beijing, [5]Departments of Gastroenterology, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang Province, [6]National Cancer Center/ Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; [1]Department of Gastroenterology, Hepatology and Nutrition-Unit1466, UT MD Anderson Cancer Center, Houston, Texas, USA; [2]Endoscopy Center, Institute Paoli-Calmettes, Marseille, France

ABSTRACT

New applications of confocal laser endomicroscopy (CLE) have been developed, such as needle-based CLE (nCLE) for pancreatic masses, pancreatic cystic tumors, and lymph nodes. nCLE is feasible during endoscopic ultrasound (EUS) examination, and preliminary results are very encouraging and suggest this technology may be used in future as a useful adjunct in cases of inconclusive EUS-guided fine-needle aspiration. The aim of this paper is to give an update in this new technology and to define its place in the diagnosis of pancreatic masses and mediastinal diseases.

Key words: Confocal laser endomicroscopy, endoscopic ultrasound, mediastinal disease, pancreatic cystic tumor, pancreatic mass

INTRODUCTION

Endoscopic ultrasound (EUS) is an excellent tool for imaging the gastrointestinal tract and surrounding structures. EUS-guided fine-needle aspiration (EUS-FNA) has become the standard for tissue sampling in a variety of masses and lymph nodes within and around the gastrointestinal tract, providing further diagnostic and staging information.[1-9] Confocal laser endomicroscopy (CLE) is a novel endoscopic method that enables imaging at a subcellular resolution during endoscopy and provides an optical biopsy. Endoscopists can use CLE to examine the gastrointestinal tract, connective tissue, mucosal cell structure, and have access to real-time histological imaging. The technique

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Address for correspondence

Dr. Sun Siyu, The Shengjing Hospital of China Medical University, No. 36, Sanhao Street, Shenyang 110004, Liaoning Province, China. E-mail: sunsy@sj-hospital.org

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is known as "noninvasive optical biopsy" or "cell computed tomography scans."[10-12] CLE was originally used to observe gastric mucosal lesions, make differential diagnoses of colonic polyps, assess Barrett esophagus, and diagnose ulcerative colitis. A new procedure that has been developed in the past few years is needle-based confocal laser endomicroscopy (nCLE), which involves a mini-CLE probe that can be passed through a 19-gauge needle during EUS-FNA. This enables the real-time visualization of tissues at a microscopic level and has the potential to further improve the diagnostic accuracy of EUS-FNA.[13-16]

In 2010, Becker *et al*.[17] published the first application of nCLE in a porcine model and since this technique has been used to diagnose solid pancreatic masses and pancreatic cystic neoplasms (PCN), and evaluate malignant lymph nodes. The aim of this article is to review the evolving role of EUS-guided nCLE in imaging various structures of the digestive tract wall in terms of its significance, adverse events, limitations, and implications.

MATERIALS AND METHODS

We performed a PubMed search with following keywords: Needle-based confocal laser endomicroscopy. Among the identified PubMed results, we included the original research papers and cases series (at least three cases) that reported the use of nCLE. The search was done on April 6, 2017. We also searched the bibliography of the included papers for additional titles. We excluded non-English papers, reviews, and papers that were unrelated to the issue at hand or were published only as abstracts. In cases of multiple papers from a single center, duplicates were excluded. The literature consisted of a limited number of reports, most of which were small case series.

The technique of nCLE in these studies was as follows: EUS was performed with a linear array echoendoscope (Penta × 3670UT; Pentax Corporation, Tokyo, Japan). Patients received 2.5 mL of 10% fluorescein intravenously during nCLE. Confocal miniprobe (AQ-flex Cellvizio Technology, Mauna-Kea Company, France) with 0.632-mm diameter was preloaded into a 19-gauge EUS needle (Cook Medical, Bloomington, IN, USA) and locked into position with 2 mm exposed beyond the tip. The target lesion was punctured with the needle positioned at its center. The probe was advanced and locked, and image acquisition began in the target lesion. After image acquisition was complete (generally after 6–8 min), the locking device was subsequently released, and the AQ-Flex miniprobe was removed from the FNA needle.

NEEDLE-BASED CONFOCAL LASER ENDOMICROSCOPY IN SOLID PANCREATIC MASSES

Pancreatic adenocarcinoma remains one of the leading cause of cancer deaths.[18] Surgery is the only potentially curative treatment; nevertheless, surgical resection of pancreatic cancer is still associated with higher risk and mortality. Thus, it is important to diagnose benign and malignant solid pancreatic masses before surgery.[19] EUS-FNA is still an important means to determine benign and malignant lesions of the pancreas. Numerous retrospective and prospective studies have been published regarding the diagnostic performance of EUS-FNA. These series have reported diagnostic accuracies ranging from 62% to 96%.[20] In total, EUS-FNA does not allow the pathological diagnosis of pancreatic masses to be made in 8%–25% of cases.[21] For cases with high clinical suspicion of pancreatic cancer, if the first EUS-FNA results are negative, there arises a dilemma for the diagnosis. nCLE is an alternative method for the diagnosis of pancreatic cancer.

Although the current studies on nCLE diagnoses of pancreatic solid lesions are limited, they have all shown a high accuracy rate. In the study by Kongkam *et al*.,[22] 22 patients were recruited, and EUS-nCLE yielded satisfactory images in all patients during the first EUS procedure. The study yielded diagnoses of benign and malignant solid pancreatic lesions (SPLs) in 3 and 19 patients, respectively. The accuracy rate of EUS-nCLE was 90.9% (20/22). One patient in their study experienced self-limiting and nonlife-threatening hemorrhage. The patient was admitted to the hospital for 2 days for close observation and was discharged uneventfully. In the study by Giovannini *et al*.,[23] forty patients were evaluated by EUS-FNA combined with nCLE to diagnose pancreatic masses. nCLE criteria were described for adenocarcinoma (dark cell aggregates, irregular vessels with leakages of fluorescein) [Figure 1], chronic pancreatitis (residual regular glandular pancreatic structures), and neuroendocrine tumor (NET) (black cell aggregates surrounded by vessels and fibrotic areas). These criteria correlated with the histological features of the corresponding lesions. In the validation review, a conclusive nCLE result was obtained in 75% of cases (96% correct). The conclusion of the study was nCLE could help to rule out malignancy after a previous inconclusive EUS-FNA. No complications occurred during their study. Fluorescein was well tolerated by all patients, with no complications identified following the procedure.

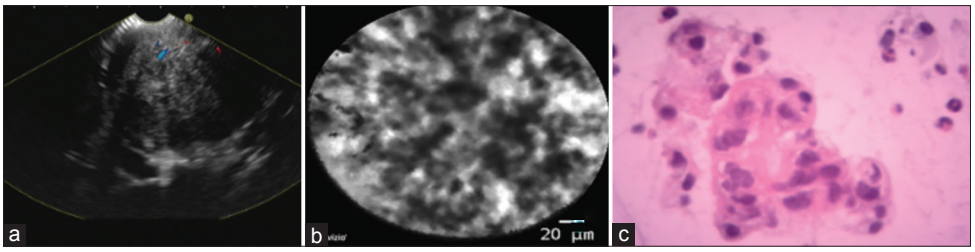

Figure 1. Pancreatic adenocarcinoma. (a) Endoscopic ultrasound showed a solid mass in the pancreatic head. (b) Needle-based confocal laser endomicroscopy: Dark cell aggregates are of irregular size and shape with irregular borders. (c) Histological diagnosis cell block: Tumoral glands

Table 1. Baseline characteristics of included studies on the topic of needle-based confocal laser endomicroscopy in diagnosis of solid pancreatic lesions

Study	Publication	Study design	Country	The accuracy rate of EUS-nCLE	Final diagnosis for pancreatic cancer	Patient (*n*)	Pancreatic cancer (*n*)	Sex (male/ female)	Age (mean in years)	Size (mm)
Kongkam *et al*., 2016[22]	Full text	Prospective study	Thailand	90.9% (20/22)	Histology with or without disease progression within a 12-month follow-up period	22	19	14/8	62.7±12.8	36.0±10.9
Giovannini *et al*., 2016[23]	Full text	Retros pective study	France	85% for adenocar cinoma, 97% for net, and 91% for chronic pancreatitis	Final diagnosis was based on EUS-FNA histology and follow-up at 1 year	32	23	18/14	65	31

EUS: Endoscopic ultrasound, FNA: Fine-needle aspiration, nCLE: Needle-based confocal laser endomicroscopy

Baseline characteristics of the included studies regarding nCLE in the diagnosis of SPLs are presented in Table 1.

NEEDLE-BASED CONFOCAL LASER ENDOMICROSCOPY IN PANCREATIC CYSTIC LESIONS

Pancreatic cysts are increasingly being recognized due to the widespread use of cross-sectional imaging. PCN, including intraductal papillary mucinous neoplasm and mucinous cystadenoma, are considered premalignant lesions that require consideration for definitive surgical treatment or ongoing surveillance. Conversely, pseudocysts and serous cysts are considered benign and have none to very low potential for malignancy, respectively. The management algorithm for these different lesions is complex and takes into account the suspected cyst type, location, size, and patient characteristics. Cross-sectional imaging, EUS, and fluid analysis, including cytology, fluid characteristics, chemistry, and tumor markers are currently relied on the attempt to make a diagnosis. However, the current diagnostic methods do not allow an accurate differentiation between the various types of cysts.

There are some studies focused on differentiating between the various types of pancreatic cysts using nCLE [Figure 2]; their overall accuracy rate was between 46% and 95%. In the study from Konda *et al*.,[13] 66 patients underwent nCLE imaging, and the presence of epithelial villous structures based on nCLE was associated with PCN ($P = 0.004$) and provided a sensitivity of 59%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 50%. These preliminary data suggested that nCLE has a high specificity for detecting PCN, but may be limited by low sensitivity. Two cases of pancreatitis occurred, giving a rate of pancreatitis for this study of 3% (95% Confidence interval 0.4%–10.5%). In the study from Nakai *et al*.,[24] they combined cystoscopy using a through-the-needle fiber optic probe combined with nCLE under EUS guidance to diagnose PCN. The sensitivity of cystoscopy was 90% (9/10), and that of

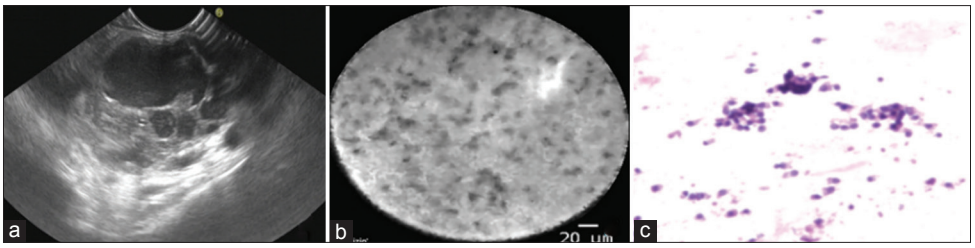

Figure 2. Mucinous cystic neoplasm. (a) Endoscopic ultrasound showed a pancreatic cystic lesion in the pancreatic tail. (b) Needle-based confocal laser endomicroscopy showing a flat mosaic appearance with epithelial borders. (c) Histological diagnosis: Mucinous cystic neoplasm

nCLE was 80% (8/10), whereas the combination was 100% (10/10) in 18 high-certainty patients. Postprocedure pancreatitis developed in two patients (7%) requiring 4–5 days of hospitalization, but without intensive care unit care or interventions. In the study by Karia *et al*.,[25] 15 unidentified nCLE video clips of PCLs were sent to six interventional endoscopists at five institutions. The mean accuracy of the observers was 46%, with the lowest being 20% and highest 67%. The mean accuracy was lower than the other studies. It may associate to limited cases of patients and learning curves. In the study by Kadayifci *et al*.,[26] the sensitivity, specificity, and diagnostic accuracy of the findings of epithelial structures by nCLE were 66%, 100%, and 80%, respectively, for a mucinous cyst diagnosis. In the study from Krishna *et al*.,[27] nCLE was performed in 49 participants, and a definitive diagnosis was available in 26 patients. The overall sensitivity, specificity, and accuracy for diagnosing mucinous PCL were 94%, 82%, and 89%, respectively. In their other study,[28] six endosonographers (nCLE experience >30 cases each) blinded to all clinical data, reviewed nCLE images of PCLs from 29 participants with surgical (n = 23) or clinical (n = 6) correlation. The overall sensitivity, specificity, and accuracy for the diagnosis of mucinous PCL were 95%, 94%, and 95%, respectively. The combination of nCLE imaging with EUS-FNA and cyst fluid tests may contribute to the differential diagnosis of PCN.

Baseline characteristics of the included studies regarding nCLE for the diagnosis of pancreatic cystic lesions are presented in Table 2.

NEEDLE-BASED CONFOCAL LASER ENDOMICROSCOPY IN EVALUATING MALIGNANT LYMPH NODES

nCLE has emerged as a promising technology that allows real-time optical biopsies at the time of EUS. To date, its most studied use has been in the assessment of pancreatic cysts, but there have also been early reports of its use in the lymph nodes.[17]

In the study from Benias *et al*.,[29] 28 consecutive patients who underwent EUS staging of malignancy or assessments of enlarged lymph nodes were included. All 28 patients successfully underwent nCLE during EUS without adverse events. There were 17 cases of carcinoma, four lymphoid malignancies, and seven benign lymph nodes. The conclusion of their study was that nCLE of lymph nodes at the time of EUS is feasible and safe. Dark pleomorphic cells were readily identified in all malignant lymph nodes, which correlated with tumor cells seen on histology [Figure 3].

Limitations of needle-based confocal laser endomicroscopy

EUS-FNA and nCLE have been established to be very safe, with overall adverse event rate ranging from 0% to 2.5%.[30] However, there is also potential for adverse events such as bleeding, perforation, infection, and concern for peritoneal seeding from malignant cystic neoplasms. For patients with coagulopathy, EUS-FNA and nCLE are not recommended. What's more, the price of nCLE is expensive. It is limited the scope of application.

CONCLUSION

Currently, there have been more reports regarding the use of nCLE for diagnosing pancreatic cystic lesions than any other clinical situation. Using nCLE for the differential diagnosis of pancreatic cystic lesions is advantageous, as the overall accuracy rate is high. Although studies focused on nCLE diagnoses of pancreatic solid lesions are limited, they have all shown a higher accuracy rate. Moreover, nCLE can guide EUS-FNA to a certain extent, improving the accuracy of FNA. Although it is unlikely that nCLE will replace EUS-guided FNA cytology for pancreatic masses and lymph nodes, recent developments reviewed above

Table 2. Baseline characteristics of included studies on the topic of needle-based confocal laser endomicroscopy in diagnosis of pancreatic cystic neoplasms

Study	Publication	Study design	Country	The accuracy rate of EUS-nCLE (in diagnosis of cystic neoplasia)	Final diagnosis	Patient (*n*)	Sex (male/female)	Age (mean in years)	Size (mm)
Konda *et al*., 2013[13]	Full text	Retrospective study	United states	71%	Clinical factors, cross-sectional image findings, EUS findings and images, and cyst fluid results, and follow-up imaging studies ranging from 10 to 22 months, if available	31	15/16	59.7	31.8
Nakai *et al*., 2015[24]	Full text	Prospective feasibility study	United states	89%	Surgical pathology; image findings on EUS, CT, and MRI, as well as fluid analysis and cytology on EUS	30	9/21	72	30.1
Karia *et al*., 2016[25]	Full text	Retrospective study	United states	46%	Any positive results after surgery or further clear evidence of malignancy by other imaging or diagnostic methods	15	10/5	66.6	25.3
Kadayifci *et al*., 2017[26]	Full text	Retrospective study	United states	83%	Surgical histopathology the cyst fluid test results and EUS imaging findings	18	8/10	65.4	34.2
Krishna and Lee, 2016[27]	Full text	Retrospective study	United states	89%	Surgical histopathology, cytology, and consensus review at a multidisciplinary tumor board meeting	26	10/16	54.8±12.6	31.7±12.8
Krishna *et al*., 2017[28]	Full text	Retrospective study	United states	95%	Surgical histopathology multidisciplinary tumor board meeting	29	13/16	53	31.6

EUS: Endoscopic ultrasound, nCLE: Needle-based confocal laser endomicroscopy, MRI: Magnetic resonance imaging, CT: Computed tomography

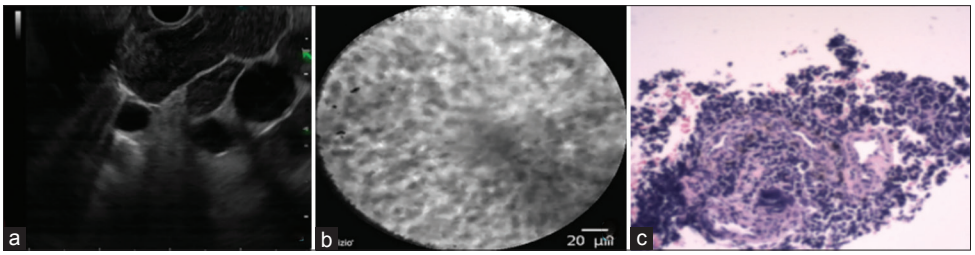

Figure 3. Mediastinal adenocarcinoma. (a) Endoscopic ultrasound showed a solid mass in the mediastinum. (b) Needle-based confocal laser endomicroscopy was forming glandular structures. (c) Histology of fine-needle aspiration confirmed the diagnosis of mediastinal adenocarcinoma

suggest that it is likely to evolve as a useful adjunct to FNA for diagnosis during EUS.

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Conflicts of interest
There are no conflicts of interest.

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