Needle-based confocal laser endomicroscopy

Marc Giovannini
Department of Gastroenterology and Endoscopy, Paoli-Calmettes Institute, Marseille, France

ABSTRACT

New applications of confocal laser endomicroscopy were developed as pCLE in the bile duct and nCLE for pancreatic cystic tumors, pancreatic masses and lymph nodes. The aim of this paper would be to give you an update in this new technology and to try to define its place in the diagnosis of cystic and solid pancreatic masses. The material used was a 19G EUS-needle in which the stylet was replaced by the Confocal mini-probe. The mini-probe (0.632 mm of diameter) is pre-loaded and screwed by a locking device in the EUS-Needle and guided endosonographically in the target. Regarding pancreatic cystic lesion, the presence of epithelial villous structures based on nCLE was associated with pancreatic cystic neoplasm (IPMN) \( P = 0.004 \) and provided a sensitivity of 59%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 50%. A superficial vascular network pattern visualized on nCLE was identified in serous cystadenomas. It corresponded on pathological specimen to a dense and subepithelial capillary vascularization. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of this sign for the diagnosis of SCA were 87%, 69%, 100%, 100%, and 82%, respectively. In pancreatic adenocarcinomas, nCLE found vascular leakage with irregular vessels with leakage of fluorescein into the tumor, large dark clumps which correspond to lumps of malignant cells. These criteria correlate with the histological structure of those tumors which are characterized by tumoral glands, surrounded by fibrosis in case of fibrous stroma tumor. Neuroendocrine tumors showed a dense network of small vessels on a dark background, which fits with the histological structure based on cord of cells surrounded by vessels and by fibrosis. nCLE is feasible during a EUS examination; these preliminary results are very encouraging and may be used in the future in case of inconclusive EUS-FNA.

Key words: Confocal laser endomicroscopy (CLE), endoscopic ultrasound (EUS)
Barrett’s esophagus and ulcerative colitis. Since the first visible neoplastic changes in epithelial cancers occur at a cellular level, these imaging techniques may allow for earlier diagnosis and treatment. In addition, CLE may allow for targeted biopsies of abnormal mucosa, thereby decreasing the number of biopsies required to diagnose dysplasia or neoplasia while increasing diagnostic yield.

During the last 5 years, new applications of CLE were developed as probe-based confocal laser endomicroscopy (pCLE) in the bile duct and needle-based confocal laser endomicroscopy (nCLE) for pancreatic cystic tumors, pancreatic masses, and lymph nodes. The aim of this paper would be to give you an update in this new technology and to try and define its place in the diagnosis of cystic and solid pancreatic masses and in the lymph nodes staging.

**MATERIAL AND TECHNIQUE**

Confocal endomicroscopy consists of focusing a laser beam onto the plane of interest and filtering the returned light by means of a small pinhole that rejects out-of-focus light. The illumination and detection systems are in the same focal plane and are termed “confocal.” After passing the pinhole, the fluorescent light is detected by a photodetection device transforming the light signal into an electrical one that is recorded by a computer. All detected signals from the illuminated spot are captured and measured. The gray-scale image created is an optical section representing one focal plane within the examined specimen. Because confocal images depend on fluorescence, a fluorescent dye (contrast agent) is required to make the objects visible. The contrast agents can be applied systemically (fluorescein and tetracycline) or topically (acriflavine and cresyl violet) by using a spraying catheter. For nCLE, intravenous fluorescein sodium (10%) was mainly used.

The material used was a 19G endoscopic ultrasound (EUS) needle in which the stylet was replaced by the Confocal mini-probe [Figure 1] (AQ-flex Cellvizio Technology, Mauna-Kea company, France). The mini-probe (0.632 mm diameter) preloaded and screwed by a locking device in the EUS-needle was guided endosonographically in the target then the mini-probe was pushed under EUS guidance into the lesion. The intratumoral confocal endomicroscopy examination started after intravenous injection of 2.5 mL of fluorescein.

**nCLE AND PANCREATIC CYSTIC LESION**

The first multicenter study was the INSPECT study,[4] the primary aim of the INSPECT study is to develop descriptive image interpretation criteria and a classification of nCLE findings in pancreatic cysts through a review of prospectively obtained nCLE videos from proven malignant and benign cases, and to propose nCLE diagnostic criteria for predicting malignant cysts. Secondary aims include assessing procedure-related adverse events, technical feasibility of nCLE, and developing a first atlas of nCLE images in pancreatic cysts. A total of 66 patients underwent nCLE imaging and images were available for 65 patients, 8 of whom were subsequently excluded due to insufficient information for consensus reference diagnosis. The presence of epithelial villous structures based on nCLE was associated with pancreatic cystic neoplasm [intraductal papillary mucinous neoplasm (IPMN)] ($P = 0.004$) and provided a sensitivity of 59%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 50% [Figure 2]. The overall complication rate was 9% and included pancreatitis (1 mild case, 1 moderate case), transient abdominal pain ($n = 1$), and intracystic bleeding not requiring any further measures ($n = 3$). These preliminary data suggested that nCLE has a high specificity in the detection of IPMN, but may be limited by a low sensitivity.

The aim of the second multicenter study published[5] was to define the criteria of serous cystadenoma (SCA) and to try to differentiate mucinous from serous pancreatic lesion using nCLE. The differential diagnosis of solitary pancreatic cystic lesions is
frequently difficult. A total of 31 patients with a solitary pancreatic cystic lesion of unknown diagnosis were prospectively included at three centers. EUS-FNA was combined with nCLE. The final diagnosis was based on either a stringent gold standard (surgical specimen and/or positive cytopathology) or a committee consensus. Six nonblinded investigators reviewed nCLE sequences from patients with the most stringent final diagnosis, and identified a single feature that was only present in SCA. The findings were correlated with the pathology of archived specimens. After a training session, four blinded independent observers reviewed with a separate independent video set, and the yield and interobserver agreement for the criterion were assessed. A superficial vascular network pattern visualized on nCLE was identified as the criterion. It corresponded on pathological specimen to a dense and subepithelial capillary vascularization only seen in SCA [Figure 3]. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of this sign for the diagnosis of SCA were 87%, 69%, 100%, 100%, and 82%, respectively. Interobserver agreement was substantial ($\kappa = 0.77$). This new nCLE criterion seems highly specific for the diagnosis of SCA. The visualization of this criterion could have a direct impact on the management of patients by avoiding unnecessary surgery or follow-up.

The last study was a uncenter trial and combine nCLE with an EUS guided cystoscopy (DETECT study). The goal of this study was to assess the feasibility, safety, and diagnostic yield of the combination of cystoscopy and nCLE in the clinical diagnosis of pancreatic cystic lesion. Thirty patients were included. The procedure was technically successful with the exception of 1 probe exchange failure. In 2 patients (7%), postprocedure pancreatitis developed. Specific features associated with the clinical diagnosis of mucinous cysts were identified: Mucin on cystoscopy and papillary projections and dark rings on nCLE. The sensitivity of cystoscopy was 90% (9/10), and that of nCLE was 80% (8/10), and the combination was 100% (10/10) in 18 high-certainty patients. The combination of dual through-the-needle imaging (cystoscopy and nCLE) of pancreatic cysts appears to have strong concordance with the clinical diagnosis of pancreatic cyst.

nCLE: PANCREATIC SOLID MASSES AND LYMPH NODES

The first study published was a feasibility study, and the goal was to develop descriptive image interpretation criteria and a classification of nCLE findings in pancreatic masses and lymph nodes through a review of prospectively obtained nCLE videos from proven malignant and benign cases, and to propose diagnostic criteria for predicting malignancy. Eleven patients underwent EUS for the staging of a pancreatic mass (three cystic and four solid) or for the diagnosis of celiac and/or mediastinal LN ($n = 4$). Benign intraductal IPMN was characterized by the aspect of finger-like projections that correspond to the villous changes of intestinal IPMN type. In pancreatic adenocarcinomas, EUS-nCLE found vascular leakage with irregular vessels with leakage of fluorescein into the tumor, large dark clumps that correspond to humps of malignant cells. Inflammatory lymph nodes were characterized by the presence of diffuse small cells into a homogeneous stroma with a normal vascularization. At the opposite, nCLE showed in malignant lymph node glandular structures with dark cells, large dark clumps, and an important neo-vascularization with huge leakage of fluorescein.

During the United European Gastroenterology Week (UEGW) meeting in 2014, a larger series of nCLE

Figure 2. nCLE aspects of IPMN (finger-like projections)

Figure 3. Vascular network typical of a serous cystadenoma
in solid pancreatic masses was presented. Thirty-four patients with a pancreatic mass of unknown nature were included prospectively. The localization of the pancreatic masses was: Head (17 cases), body (12 cases), and tail (6 cases). The mean size was 30 mm. The puncture of the mass was done in all cases with a 19G puncture needle with the nCLE probe preloaded. After examination of the track of the puncture by nCLE, aspiration was done in the same track to compare images and histological results. No complication occurred during the nCLE procedure or the puncture. A definitive histological diagnosis was obtained in 30/34 patients: Adenocarcinoma (21 cases), fibrous stroma adenocarcinoma (1 case), neuroendocrine tumor (NET) (4 cases), pseudopapillary tumor, (1 case) chronic pancreatitis (3 cases—diagnosis confirmed by a 1-year follow-up). To go further, nCLE sequences were re-visualized by two gastroenterologists and two pathologists to compare, for each type of lesion, their findings to the pathology specimen. During this review, normal pancreas shows an aspect of coffee beans corresponding to the histological structure of acinus [Figure 4]. Adenocarcinomas showed dark cells that aggregates with pseudoglandular aspects and straight hyperdense elements more or less thick [Figure 5]. These criteria correlate with the histological structure of those tumors that are characterized by tumoral glands, surrounded by fibrosis in case of fibrous stroma tumor. NETs showed a dense network of small vessels on a dark background, which fits with the histological structure based on cord of cells surrounded by vessels and by fibrosis. Chronic pancreatitis showed residual acinus, which corresponds to the pancreatic regression. This preliminary classification of nCLE images obtained in pancreatic masses could help in the differentiation of adenocarcinomas and NETs [Figure 6] and between malignant tumors from normal pancreatic tissue.

**CONCLUSION**

nCLE is feasible during a EUS examination; these preliminary results are very encouraging and may be used today for the pancreatic cyst diagnosis and in the future in case of inconclusive EUS-FNA for solid pancreatic masses.

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Nil.

**Figure 4.** Normal pancreatic tissue (coffee bean aspect: Normal acinus)

**Figure 5.** Pancreatic adenocarcinoma: nCLE aspect (a) and histology correlation (b)

**Figure 6.** Pancreatic NET: Network of vessels around humps of small and round malignant cells

**Conflicts of interest**

There are no conflicts of interest.

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