Appraisal of EUS-guided needle-based confocal laser endomicroscopy in the diagnosis of pancreatic lesions: A single Chinese center experience

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ABSTRACT

Background and Objectives: In the recent years, EUS is one of the routine procedures in the diagnosis of pancreatic diseases. EUS-guided needle-based confocal laser endomicroscopy (nCLE) is a novel minimally invasive imaging technique in diagnosis of pancreatic diseases. The pilot researches provided us some preliminary findings and conclusions with small samples, low rate of pathological correspondence. The aim of this current study was to evaluate the diagnostic efficacy of EUS-guided nCLE in solid pancreatic lesions (SPLs) and pancreatic cystic lesions (PCLs) based on large samples. The date was obtained on nCLE imaging findings and high rate of correlation with pathology. Material and Methods: Patients enrolled in the study were underwent EUS-nCLE to achieve the nCLE images and diagnosis. Comparing with the final diagnosis, including surgical histopathological results or cyto-/histopathology through FNA, the efficacy and accuracy of nCLE in diagnosis in solid and cystic pancreatic lesions were evaluated. In other cases, clinical diagnoses were achieved based on the combination with clinical history, image findings and fluid analysis and cytology, by 3 independent committee members strongly agreed with a concordant diagnosis. Results: Totally 172 patients were enrolled into the study. The overall rate of final diagnosis was about 65% while 50% in cystic lesion. The mean sensitivity, specificity, negative predictive value, positive predictive value and accuracy of the nCLE in diagnosis of PDAC is 90.3%, 89.5%, 93.3%, 85.0% and 90.0% respectively. The efficacy and accuracy of pancreatic cystic lesions were very satisfying and some additional nCLE signs were found, including “black aggregates of cells, forming as gland-like structure, surrounding by fibro and vessels” in neuroendocrine tumors (NETs); “black columnar protrusions near vascular area” in the pseudopapillary solid tumor (SPT); macrophage in tuberculosis (TB) and small aggregate of black regular cells maybe corresponds to ovarian-like stroma in mucinous cystadenoma (MCN). In the study, 20 (11.6%) patients suffered complications, including symptomatic (5.2%) and asymptomatic (6.4%). Conclusions: nCLE observation could improve characterization of indeterminate cysts, or confirm the EUS impression, when cytological confirmation is missing. The technique may deliver information to better guide our clinical decisions.

Key words: EUS, nCLE, pancreatic cystic lesions, solid pancreatic lesion
BACKGROUND

In recent years, EUS is one of the routine procedures in the diagnosis of pancreatic diseases. With the fine-needle aspiration (FNA), the cytology acquisition has improved the efficiency of diagnosis significantly in solid pancreatic lesions (SPLs). The sensitivities and specificities of EUS-FNA for the diagnosis of pancreatic cancer were 85%–92% and 96%–98%, respectively. However, EUS-FNA has limitations that include sample error, limited availability of an on-site cytopathologist, additional risk with multiple attempts, and nondiagnostic samples. Furthermore, the accurate diagnosis in pancreatic cystic lesions (PCLs) still poses a challenge for physicians and surgeons, despite applying a combination of advanced imaging technique, FNA cytological examination, and fluid analysis. Hence, the overall management remains suboptimal with unnecessary repeated follow-up or surgery performed.

EUS-guided needle-based confocal laser endomicroscopy (nCLE) is a novel minimally invasive imaging technique, which can be placed into the pancreatic cyst through the 19G fine-needle puncture and provides us the microscopic image of the inner wall of the cyst or parenchyma tissue of the solid tumor, with 1000 times enlargement. Since 2011, EUS-nCLE has been applied in the diagnosis of pancreatic neoplasms and has become the focal spot in the field in this decade year. The pilot researches of nCLE provided us some preliminary findings and conclusions with small samples, such as superficial vascular network (SVN) in serous cystadenoma (SCA) and finger-like projection in intraductal papillary mucinous neoplasm with low rate of pathological correspondence. The aim of this current study was to evaluate the diagnostic efficacy of EUS-guided nCLE in SPLs and PCLs based on large samples. The date was obtained on nCLE imaging findings and high rate of correlation with pathology. In addition, some new imaging findings were summarized in the study, which may be defined as potential diagnostic criteria.

MATERIALS AND METHODS

Patients

Patients presenting for EUS observation of pancreatic lesions were recruited from Huashan Hospital, Shanghai, China, participating in the study. Patients were provided informed consent for the study. The inclusion criteria were (1) ≥18 years, (2) suspending pancreatic lesions, and (3) plan to perform EUS procedure. The exclusion criteria were (1) patients with pregnant, breastfeeding, or renal insufficiency; (2) allergy to fluorescein; and (3) nCLE image blurring and difficult to recognize. The protocol was reviewed and approved by the institutional review board of the hospital.

Devices and procedure

In the study, EUS (SU-7000 or SU-9000; FUJIFILM, Tokyo, Japan) was used for the EUS observation and following procedure of nCLE. A prototype probe (AQ-Flex 19; Mauna Kea Technologies, Paris, France) was used for nCLE. This nCLE probe has 10,000 optical fibers, a diameter of 0.85 mm, a field of view of 320 μm, a lateral resolution of 3.5 μm, a length of 4 m, and a focal depth of 40–70 mm. Before the procedure, the fibers were profited within a 19-G FNA needle (EchoTip; Cook Medical, Bloomington, Ind) with advancement of the probe tip approximately 2 mm beyond the needle tip. The probe position was then secured using a locking device that attaches the probe at a specific length to the inlet of the biopsy channel.

After the routine examination of EUS, the FNA needle was inserted under EUS guidance. Then, the probe was positioned to extend no more than 2 mm from the tip of the needle, and 2.5 mL of 10% fluorescein was injected simultaneously. The needle was moved using “Fanning Technique” to observation in different positions in solid lesions or different areas of the cyst wall. After nCLE image acquisition, FNA was performed for cytology in the SPLs, while the fluid acquisition was performed in the PCLs for quantitative tumor markers and amylase.

Needle-based confocal laser endomicroscopy diagnosis

nCLE diagnosis was established based on the previous original studies, INSPECT, DETECT, CONTACT Phase I and II, and others. For example, the criteria for diagnosing malignant SPLs was dark clumps with or without dilated vessels; the criteria for diagnosing SCA was SVN, the finger-like papillary projection, and so on. During the study, we reviewed all the images to correct the nCLE diagnosis and concluded that some additional potential imaging may help in making a diagnosis.

Final diagnosis

The final diagnosis was established by the surgical histological specimen in those patients who underwent resection of the pancreatic lesion. In
those patients who did not undergo surgery, the final diagnosis was confirmed by positive FNA cytology. In those patients without positive histo-/cytological results, clinical diagnosis was established by a diagnostic committee, including two endoscopists, two surgeons, and one radiologist, based on combination with clinical history, image findings on EUS, computed tomography (CT), and magnetic resonance imaging, as well as fluid analysis and cytology. Clinical diagnoses were defined as high certainty if three independent committee members strongly agreed with a concordant diagnosis. Otherwise, the diagnosis was defined as uncertain.

Statistical analysis

The Fisher's exact test was used for the assessment of the association between imaging findings and clinical diagnosis. Accuracy (AC), sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) with a 95% confidence interval were calculated. $P < 0.05$ was considered statistically significant. A Bonferroni adjustment was performed for multiple testing for associations of imaging findings and clinical diagnosis.

RESULTS

A total 172 patients were enrolled in the study from Huashan hospital from October 2016 to October 2018. Table 1 provides general clinical characteristics of the cases. The cases included 50 solid lesions and 122 cystic/mixed cases. Sixty lesions were in the head and uncinate of the pancreas, 102 in the distal pancreas, and 6 cases were multiple lesions in pancreas. The other 4 cases were diffuse lesion in the pancreas. The overall rate of final diagnosis was about 65% (112/172). As to cystic lesions, 50% (61/122) patients were diagnosed by surgical histopathology and 6% (7/122) patients were diagnosed by the FNA cytology.

Solid lesions

In the study, 88% (44/50) of SPLs were confirmed by the final diagnosis, including 23 surgical histopathologies and 21 FNA cytologies. We had pancreatic ductal adenocarcinoma (PDAC), solid pseudopapillary tumor (SPT), neuroendocrine tumor (NET), tuberculosis (TB), and other rare cases. EUS-nCLE produced satisfactory images for all SPL patients. EUS-nCLE was diagnosed as PDAC in 31 patients, while benign/borderline SPLs in 19 patients. Thirty-one PDAC patients were confirmed based on 16 surgical histopathologies, 14 FNA cytologies, and 1 clinical diagnosis. The 19 benign/borderline SPLs included 7 surgical histopathological diagnoses, 7 FNA cytological diagnoses, and 5 clinical diagnoses.

In most PDAC cases, the nCLE imaging showed similar findings, which describes as dark clumps of cells larger than 40 μm with or without dilated irregular vessels larger than 20 μm with fluorescein leakage. The dark clumps of cells appeared in the 28 PDAC patients, while the dilated vessels appeared in 22 patients. Both images appeared simultaneously in 19 patients [Figure 1]. The mean SN, SP, NPV, PPV, and AC, for the nCLE in the diagnosis of PDAC was 90.3%, 89.5%, 93.3%, 85.0%, and 90.0%, respectively.

Within the other 19 cases, we had 14 definite diagnoses and 5 clinical diagnoses, including 5 NETs, 3 SPTs, 3 accessory spleens, 3 autoimmune pancreatitis (AIP), 1 chronic pancreatitis (CP), 2 lymphomas, 1 gastrointestinal stromal tumor, and 1 TB.

In the NET cases, we observed black aggregates of cells, forming as gland-like structure, surrounding by fibrovascular. In SPT cases, we found black columnar protrusions near the vascular area. In AIPs and CP cases, massive fibrous areas were observed with scattered cells or glands. In the TB case, black huge cells, may corresponds to macrophage, were found easily, mixing with vesicular adipocytes [Figure 1].
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Cystic lesions

Totally, we have 122 cases of PCLs and mixed type of lesions. The categories and diagnostic methods of cases in the study were listed in Table 2.

We had 38 SCAs based on the final and clinical diagnosis. The typical nCLE images of SCA are called fern-like SVN, which was observed in 81.6% (31/38) of the cases [Figure 2]. Surgical resection was performed in 36.8% (14/38) of the SCA patients. According to the pathological results, nCLE achieved the correct diagnosis in 10 cases, while nCLE was misdiagnosed in two cases and inclusive in two cases. The diagnostic efficacy and AC of nCLE in SCA is listed in Table 3.

Thirty one cases were diagnosed with IPMN. The typical nCLE images of IPMN were described as finger-like structure, which was micropapillary projection composed of two dark, parallel epithelial borders with a bright vascular core [Figure 2]. Among them, 45.2% (14/31) of the cases went to surgical resection. Comparing with the histopathological diagnosis, 92.9% (13/14) of the cases reached a consensus based on the nCLE diagnosis. We also had 18 cases of mucinous cystadenomas (MCA), and 83.3% (15/18) cases were performed the surgical resection. We had 1 misdiagnosis and 1 inclusive case. The diagnostic criteria of nCLE in MCA was described as dilated vessels (\(\phi \geq 15\) \(\mu\)m) and/or a single black epithelial border [Figure 2].

Table 2: Overall and diagnosis distribution of cystic lesions

| Characteristics          | n=122 |
|--------------------------|-------|
| Final diagnosis achieved, n |       |
| Surgery                  | 61 (50.0%) |
| FNA                      | 8 (6.6%)  |
| Clinical agreement       | 50 (41.0%) |
| Inclusive                | 3 (2.4%)  |

| Diagnosis, n | Total | Operation | FNA | Surveillance |
|--------------|-------|-----------|-----|--------------|
| SCN          | 38    | 14        |     | 24           |
| MCN          | 18    | 15        | 3   |              |
| IPMN         | 31    | 14        | 17  |              |
| IPMN with malignancy | 3    | 2        | 1   |              |
| Pseudocyst   | 9     | 3         | 1   |              |
| SPT          | 7     | 6         | 1   |              |
| PDAC         | 4     | 2         | 3   | NA           |
| NET          | 3     | 3         | 1   | 0            |
| Tuberculosis | 1     |           | 1   |              |
| Other        | 3     | 2         |     | 1            |
| Inclusion    | 5     |           | 5   |              |
|              |       |           |     |              |

Table 3: Efficacy of nCLE in diagnosis of different PCLs

| Type     | n   | SN (%) | SP (%) | PPV (%) | NPV (%) | AC (%) |
|----------|-----|--------|--------|---------|---------|--------|
| SCA      | 38  | 89.5   | 100    | 100     | 95.5    | 96.7   |
| MCN      | 15  | 86.7   | 98.1   | 86.7    | 98.1    | 94.3   |
| IPMN     | 31  | 96.7   | 100    | 100     | 98.9    | 99.2   |
| Overall  | 122 | 94.3   | 98.1   | 100     | 94.3    | 96.7   |

SN: Sensitivity; SP: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; AC: Accuracy; MCN: Mucinous cystic neoplasm; IPMN: Intraductal papillary mucinous neoplasm; nCLE: needle-based confocal laser endomicroscopy; PCLs: Pancreatic cystic lesions.
We had 9 cases of pseudocysts in the study, only 33.3% cases had pathological diagnosis, while the others were diagnosis based on the clinical information. The nCLE images showed lots of dark and bright particles suspending in cystic fluid, meanwhile without vascular structure or cells on the wall [Figure 2].

As to other mixed cases, the nCLE image characteristics of the solid parts helped in making a diagnosis, which is similar with the SPLs such as SPT, NET, and PDAC.

**Complication**

In the study, 20 (11.6%) patients suffered complications, including symptomatic and asymptomatic. Pancreatitis ($n = 6$, 4 mild + 1 moderate + 1 severe) occurred with symptoms; 2 fluid leakage or collection were detected by CT scan; and bleeding result in hematoma appeared in 1 patient. Asymptomatic complications were usually found during surgery including intracystic bleeding ($n = 4$), subserosal hematoma ($n = 3$), fluid collection ($n = 2$), and peripancreatic necrosis ($n = 1$).

**DISCUSSION**

Pancreatic lesions vary in the clinic, from the lethal malignant tumor to asymptomatic serous cyst. The appropriate treatment depends on the accurate diagnosis. With the advanced imaging techniques, most pancreatic lesions can be recognized. With the EUS-FNA biopsy, the diagnostic AC of SPLs has increased significantly from 62% to 96%, but it still leaves 8%–25% uncertain pathological diagnosis. On the other hand, the accurate diagnosis of PCLs poses a tough challenge to physicians and surgeons. Cross-sectional imaging, EUS, and fluid analysis are currently performed to make a diagnosis. However, the diagnostic AC of PCLs is far from satisfying.

Since 2010, EUS-nCLE has been used to observe the pancreas in models and been proved to be feasible to diagnose pancreatic diseases. Giovannini et al. first described the nCLE, SPL, and PDAC, which show the leakage of fluorescein from irregular vessels in the tumor and large dark clumps representing groups of malignant cells in nCLE images. Afterward, Kongkam et al. indicated the similar signs of malignant SPLs while “find white fibrous bands and normal acinar cells” in benign SPLs. In our study, the similar signs were observed in all the PDAC patients in nCLE images, which diagnostic AC is about 90%. However, EUS-FNA with cyto-/histopathological examination was proved to be satisfying in the diagnosis in SPLs. nCLE did not show certain advantage to FNA in SPLs diagnosis, which can be a supplement when FNA achieves negative result in some inclusive cases. Furthermore, the descriptions of other SPLs, such as NET, were reported based on small samples. We also found some signs that may help in the diagnosis of
The last thing we have to mention is about the complications of nCLE. Previous papers proved that the puncture in cystic lesion will cause more complications than in solid tumors, and the miniprobe of confocal laser could only pass through 19G needle, not the common 22 and 25G. If we define the complication is symptomatic events, the rate is 5.2%, which is similar with previous studies. Furthermore, there still have some complications without any obvious symptoms, we would like to name them potential complications, not delay the following treatment, and only could be found during the operation. Hence, we must notice that the incidence of complications in EUS-nCLE is higher than that in EUS-FNA.

CONCLUSION

The utilization of nCLE in pancreatic cysts is still at the very beginning period; some of our patients really benefit from this technique. Confocal observation could improve the characterization of indeterminate cysts or confirm the EUS impression, when cytological confirmation is missing. The technique may deliver information to better guide our clinical decisions.

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Conflicts of interest

There are no conflicts of interest.

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