

Needle-based confocal laser endomicroscopy of pancreatic cystic lesions: a prospective multicenter validation study in patients with definite diagnosis

Authors

Bertrand Napoleon¹, Maxime Palazzo², Anne-Isabelle Lemaistre³, Fabrice Caillol⁴, Laurent Palazzo⁵, Alain Aubert², Louis Buscaïl⁶, Frédérique Maire², Blandine Mialhe Morellon⁷, Bertrand Pujol¹, Marc Giovannini⁴

Institutions

- 1 Département de Gastroentérologie, Hôpital Privé Jean Mermoz, Ramsay GDS, Lyon, France
- 2 Département d'Endoscopie Digestive, Hôpital Beaujon, Clichy, France
- 3 Département de Biopathologie, Eurofins Biomnis, Lyon, France
- 4 Unité d'Échoendoscopie et d'Endoscopie d'Oncologie Digestive, Institut Paoli Calmettes, Marseille, France
- 5 Clinique du Trocadéro, Paris, France
- 6 Service d'Hépatogastroentérologie, Hôpital Rangueil, Toulouse, France
- 7 Cabinet d'Anatomo-cyto-pathologie, Technipath, Limonest, France

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Corresponding author

Bertrand Napoleon, MD, Département de Gastroentérologie, Hôpital Privé Jean Mermoz, 4 rue Jacqueline Auriol, 69008 Lyon, France

Fax: +33-4-78742655

dr.napoleon@wanadoo.fr

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ABSTRACT

Background Needle-based confocal laser endomicroscopy (nCLE) enables observation of the inner wall of pancreatic cystic lesions (PCLs) during an endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). This study prospectively evaluated the diagnostic performance of nCLE for large, single, noncommunicating PCLs using surgical histopathology or EUS-FNA cytohistopathology as a reference diagnosis.

Methods From April 2013 to March 2016, consecutive patients referred for EUS-FNA of indeterminate PCLs without evidence of malignancy or chronic pancreatitis were prospectively enrolled at five centers. EUS-FNA and nCLE were performed and cystic fluid was aspirated for cytohistopathological and carcinoembryonic antigen (CEA) analysis. The diagnostic performance of nCLE was assessed against the reference standard and compared with that of EUS and CEA. This study was registered on ClinicalTrials.gov (NCT01563133).

Results 206 patients underwent nCLE and 78 PCLs (mean size 40 mm, range 20–110 mm) had reference diagnoses (53 premalignant and 25 benign PCLs). Post-procedure pancreatitis occurred in 1.3% of the patients. nCLE was conclusive in 71 of the 78 cases (91%). The sensitivities and specificities of nCLE for the diagnosis of serous cystadenoma, mucinous PCL, and premalignant PCL were all ≥ 0.95 (with 95% confidence interval from 0.85 to 1.0). The AUROC was significantly larger for nCLE than for CEA or EUS.

Conclusions nCLE had excellent diagnostic performance that surpassed that of CEA and EUS for the diagnosis of large, single, noncommunicating PCLs. The nCLE procedure should be considered in patients with indeterminate PCLs to ensure a more specific diagnosis.

Introduction

Pancreatic cystic lesions (PCLs) are being incidentally discovered more frequently due to the increased use of computed tomography scans (CTs) and magnetic resonance imaging (MRI) [1, 2]. PCLs may carry malignant potential and should therefore be evaluated carefully [3]. Whereas no surveillance is required for asymptomatic benign PCLs such as pseudocysts or serous cystadenomas, surgical resection is considered for lesions with malignant potential, such as mucinous cystadenomas, branch-duct intraductal papillary mucinous neoplasms (BD-IPMNs), and cystic neuroendocrine neoplasms [4].

Currently, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) with cystic fluid analysis remains the most accurate nonsurgical procedure for diagnosing the nature of PCLs [5–7]. Although cytology has good specificity, its sensitivity is low [7]. With a cutoff of 192 ng/mL, cyst fluid carcinoembryonic antigen (CEA) is considered more accurate than cytology and EUS for differentiating mucinous from nonmucinous PCLs [5]. Low levels of CEA (<5 ng/mL) are considered specific for differentiating between premalignant and benign cysts [8]. Nevertheless, because of the limited diagnostic accuracy of these methods, patient management remains suboptimal [9–11], and unnecessary repeated follow-up procedures or surgery may be performed. The consequences are important, both for patients in terms of mortality and morbidity [12, 13], and for health care systems supporting the costs of inappropriate treatments.

Confocal laser endomicroscopy is a powerful novel imaging technique that has already demonstrated efficiency for real-time *in vivo* microscopic imaging of luminal or ductal structures [14]. Needle-based confocal laser endomicroscopy (nCLE) enables the observation of the inner wall of pancreatic cysts during an EUS-FNA procedure [15–17]. The feasibility and safety of EUS-FNA with nCLE in patients with PCLs were previously evaluated and validated in two pilot studies [15, 16] and confirmed in an international multicenter study [17]. Several subsequent studies have described the correlation between nCLE and histological features, and comprehensive nCLE criteria have been established for the characterization of the most frequent types of PCL (serous and mucinous cystadenomas, BD-IPMN, neuroendocrine neoplasm, and pseudocysts) [18–20]. A recent study [21] confirmed excellent interobserver and intraobserver agreement for the nCLE diagnosis of PCL. However, no large prospective series have yet evaluated the diagnostic performance of these criteria [17, 18, 20, 22–25].

This study was the second phase of the CONTACT clinical investigation. The primary aim of the study was to prospectively validate the specificities of previously identified nCLE criteria for the diagnosis of noncommunicating single cysts in a large multicenter cohort of patients, using surgical histopathology or EUS-FNA cytohistopathological analysis as a reference standard. The secondary aims were to compare the diagnostic performance of nCLE with that of EUS and CEA analysis for differentiating mucinous from nonmucinous PCLs and premalignant from benign PCLs, and to evaluate the technical aspects and safety of nCLE.

Methods

Study population

From April 2013 until March 2016, patients were prospectively and consecutively screened for eligibility by nine investigators across five French centers (Hôpital Privé Jean Mermoz in Lyon, Clinique du Trocadéro in Paris, Institut Paoli Calmettes in Marseille, Hôpital Beaujon in Clichy, and Hôpital Rangueil in Toulouse). All participating investigators received training in nCLE image interpretation delivered during a 1-day workshop by the coordinating endoscopist (B.N.) and pathologist (A-I.L.). All patients provided written consent to participate in the trial after receiving comprehensive information from the investigators. The study was approved by French authorities (ethics committee and French agency for security for drug and health products). The CONTACT study was registered on ClinicalTrials.gov (NCT01563133).

The inclusion criteria were: age ≥ 18 years; ability to give informed consent; a single PCL identified with CT and/or MRI without evidence of communication with the main pancreatic duct; cyst size ≥ 20 mm, and with at least one cavity larger than 13 mm (to allow for CEA and cytohistopathology analyses); scheduled for an EUS-FNA procedure. The exclusion criteria were: multiple or communicating cysts on CT and/or MRI; known allergy to fluorescein contrast agent; pregnancy; previous EUS-FNA procedure performed within the past 3 months; chronic calcifying pancreatitis; presence of criteria for malignancy (cyst containing solid masses or mural nodules, distant metastases, ascites, and vascular infiltration); and other common contraindications for EUS-FNA.

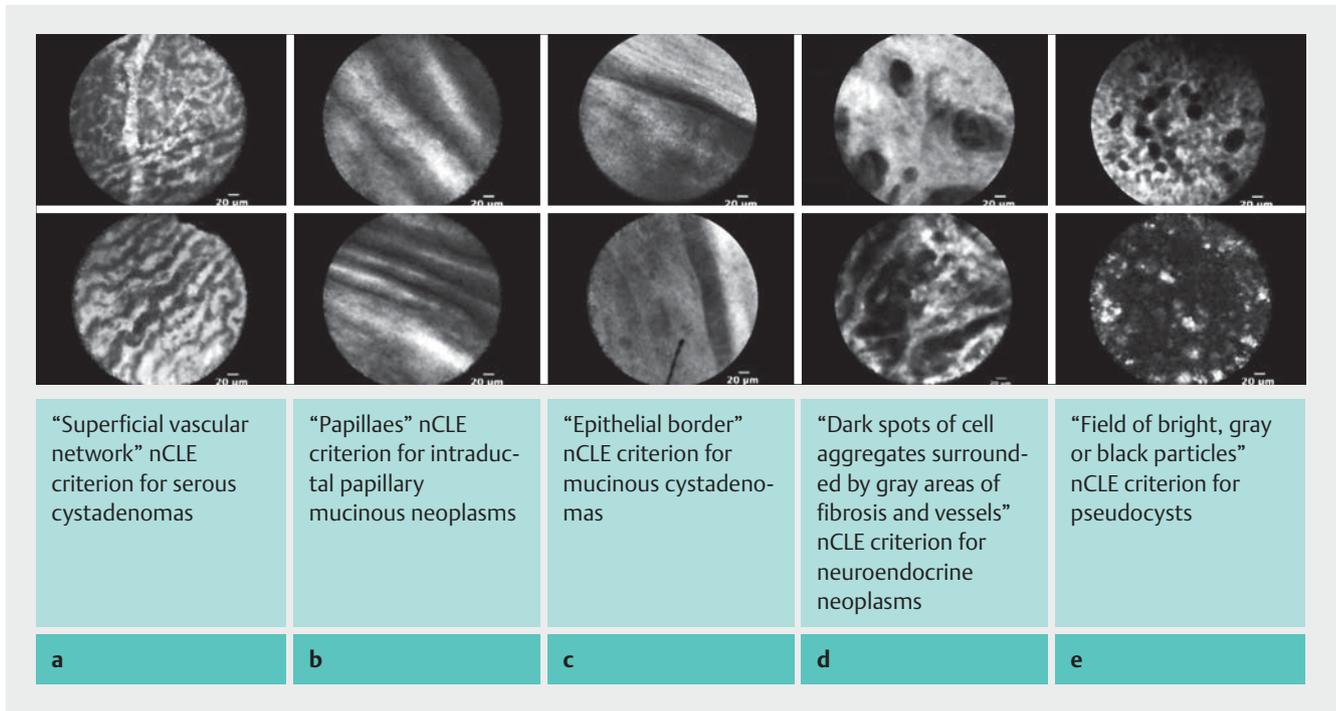
To ensure the highest level of the reference standard used for the evaluation of nCLE diagnostic performance, only PCLs with diagnoses derived from either surgical histopathology or EUS-FNA cytohistopathology were included in the performance analysis.

The number of patients included in the present study was derived from the analysis of 43 PCLs evaluated in the first phase of the CONTACT study [18].

- The prevalence of three major PCL subtypes including serous cystadenomas, neuroendocrine neoplasms, and mucinous cystic lesions (BD-IPMN and mucinous cystadenoma) were 30% (95% confidence interval [CI] 16%–43%), 4% (95%CI 1%–9%), and 25% (95%CI 12%–37%), respectively.
- The proportions of PCLs with reference standards for the three major PCL subtypes, namely serous cystadenomas, neuroendocrine neoplasms, and mucinous cystic lesions were 53%, 100%, and 100%, respectively.

It was assumed that nCLE would be conclusive in at least 75% of the procedures.

For the main objective, a specificity of 85% for the three major subtypes was defined as the primary endpoint. Using a marginal error of 10% and a significance level of 5%, a sample size of 174 with a range of 145–216, reflecting the prevalence range for PCLs, was identified to ensure the inclusion of 70 PCLs with reference diagnoses (range 59–87). Therefore, 216



► **Fig. 1** Example images of the needle-based confocal laser endomicroscopy (nCLE) criteria for the diagnosis of pancreatic cysts. **a** Serous cystadenomas with the “superficial vascular network” criterion. **b** Intraductal papillary mucinous neoplasms with the “papillae” criterion. **c** Mucinous cystadenomas with the “epithelial border” criterion. **d** Neuroendocrine neoplasms with the “dark aggregates of cells surrounded by gray areas of fibrosis and vessels” criterion. **e** Pseudocysts with the “field of bright, gray or black particles” criterion.

PCLs had to be included in CONTACT2 to validate a minimal specificity of 85% for the three major PCL subtypes, taking into account the prevalence range, PCL proportions with reference standards, and a minimal conclusive nCLE procedure rate of 75%.

The secondary aims were to compare the diagnostic performance of nCLE with that of CEA and EUS for differentiating mucinous from nonmucinous PCLs and premalignant from benign PCLs. Our endpoint was the area under the receiver operating curve (AUROC).

Procedure

EUS-FNA and nCLE procedures were performed as previously described [15–18]. Puncture procedures were performed after EUS morphology-based diagnoses were obtained. The investigators had a choice of 19-G needles (Cook Medical Echo Tip Ultra–Cook Medical Inc., Bloomington, Indiana, USA; Boston Scientific Expect Flexible–Boston Scientific Corp., Marlborough, Massachusetts, USA). Prophylactic antibiotic therapy was systematically administered. An intravenous injection of contrast agent (2.5 mL of 10% fluorescein) was administered between 30 seconds and 2 minutes prior to nCLE imaging [26]. nCLE videos of cystic inner structures were systematically recorded. It was recommended to limit the nCLE examinations to 10 minutes and to stop as soon as the typical nCLE criteria were identified. The time from insertion of the needle into the cyst to nCLE probe extraction (nCLE procedure duration), nCLE image quality,

and the ease of insertion, manipulation, and removal of the probe were also recorded.

At the completion of nCLE acquisition, cystic fluid and sometimes cystic wall fragments were aspirated. When confocal miniprobe extraction from the needle was not possible, patients underwent a second puncture with the same needle to retrieve cyst fluid. Samples were split between cytohistopathological and CEA analyses. If the collected cystic fluid was insufficient to perform both analyses (<1 mL), the cytohistopathological examination was given priority over the CEA analysis.

Adverse events such as pancreatitis, bleeding, perforation, infection, and allergic reaction to fluorescein were recorded. Post-procedural monitoring was conducted via phone call to the patients by each center at 24 hours and 1 week after the procedure.

PCL classifications

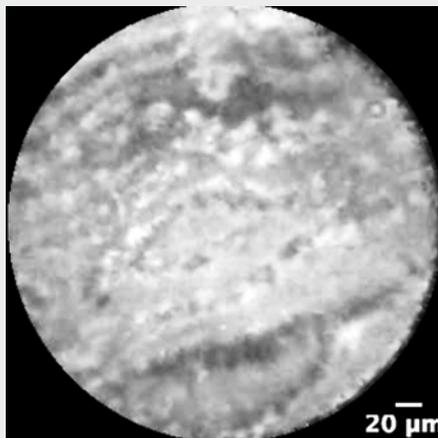
Two classifications of PCLs were used routinely [27]: 1) benign (including serous cystadenoma, pseudocyst, and congenital pancreatic cysts) vs. premalignant PCLs (mucinous cystadenoma, BD-IPMN, cystic neuroendocrine neoplasm, cystic solid pseudopapillary neoplasm, and cystic lymphoma), and 2) mucinous (mucinous cystadenoma and BD-IPMN) vs. nonmucinous PCLs (serous cystadenomas, neuroendocrine neoplasm, pseudocyst, congenital pancreatic cyst, cystic solid pseudopapillary neoplasm, and cystic lymphoma).

EUS diagnoses

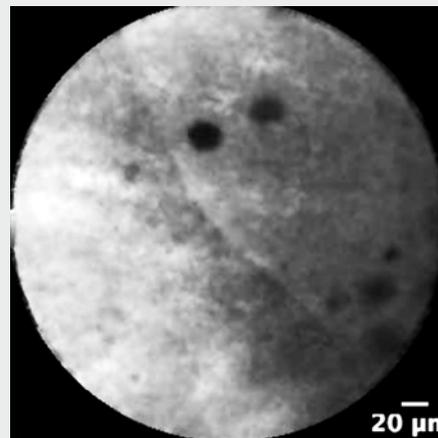
During standard EUS procedures, PCLs were described and assessed based on their localization, dimensions, and characteristic features (wall thickness, presence of septation, number of cavities, and calcification). Diagnoses were prospectively provided by the endoscopist at the end of the EUS procedure and the diagnostic performance was calculated according to the two classifications defined above.

nCLE diagnoses

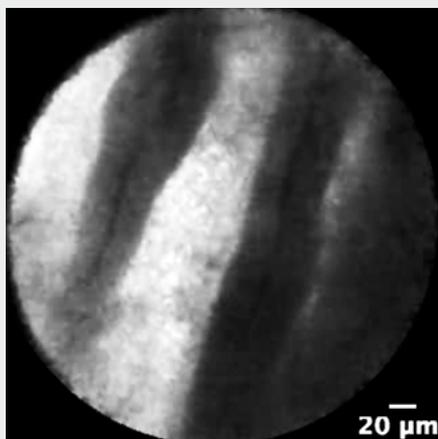
The diagnosis of serous and mucinous cystadenomas, pseudocyst, BD-IPMN, and neuroendocrine neoplasm was based on the observation of the previously published criteria of the INSPECT study [17] and the first phase of the CONTACT study [18, 20] (► **Fig. 1**, ► **Video 1**, ► **Video 2**, ► **Video 3**, ► **Video 4**, ► **Video 5**). When both the “epithelial border” and “papillae” nCLE criteria were observed, the diagnosis of an indeterminate mucinous lesion was made. In the absence of any of these criteria, the nCLE diagnosis was considered to be inconclusive. The nCLE di-



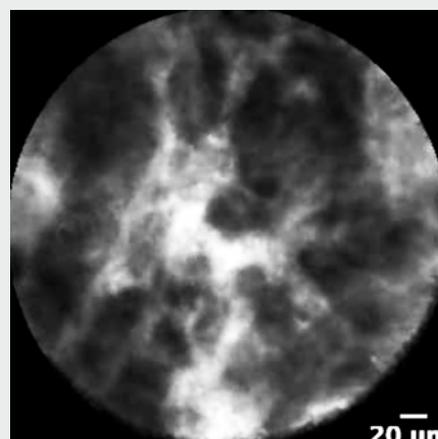
► **Video 1** Needle-based confocal laser endomicroscopy of a serous cystadenoma with the “superficial vascular network” criterion.
Online content viewable at:
<https://doi.org/10.1055/a-0732-5356>



► **Video 3** Needle-based confocal laser endomicroscopy of a mucinous cystadenomas with the “epithelial border” criterion.
Online content viewable at:
<https://doi.org/10.1055/a-0732-5356>



► **Video 2** Needle-based confocal laser endomicroscopy of an intraductal papillary mucinous neoplasm with the “papillae” criterion.
Online content viewable at:
<https://doi.org/10.1055/a-0732-5356>



► **Video 4** Needle-based confocal laser endomicroscopy of neuroendocrine neoplasms with the “dark aggregates of cells surrounded by gray areas of fibrosis and vessels” criterion.
Online content viewable at:
<https://doi.org/10.1055/a-0732-5356>

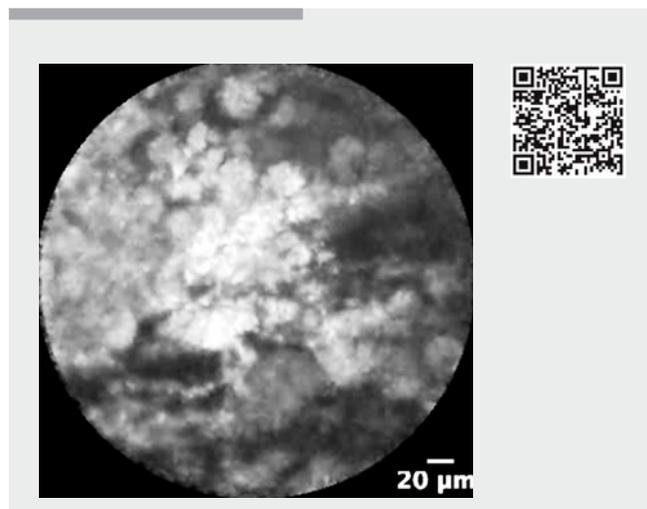
agnoses were prospectively provided by the endoscopists at the end of the nCLE procedures using slow-motion review if needed. The endoscopist who assessed the nCLE was not blinded to the EUS images.

CEA analysis

The diagnostic performance of cyst fluid CEA was assessed using previously published cutoff values [5, 8]: a cutoff value of 192 ng/mL was used for differentiating mucinous from non-mucinous PCLs [5], and a cutoff value of 5 ng/mL was used for differentiating benign from premalignant PCLs [8].

Reference standard diagnosis

The reference standard diagnosis was based on cytohistopathological results from FNA samples collected just after the nCLE procedure or on surgical histopathological results in patients who underwent surgery. Initial cytopathological analyses of FNA samples were performed at each center. When a conclusive diagnosis was made by the local pathologist, and to ensure a reliable reference diagnosis, two pathologists (A-I.L and B.M.M), who were blinded to the patient's medical history and procedural outcomes, reviewed the cytohistopathological samples to confirm the diagnosis. If at least one of the two pathologists was uncertain or disagreed about the diagnosis, the cyst was considered not to have a reference diagnosis and was excluded from the analysis. The definitive diagnosis of serous cystadenoma, mucinous cystadenomas, IPMN, indeterminate mucinous lesions, and neuroendocrine neoplasm were based on cytohistopathological criteria described in the first phase of the CONTACT study [20]. The diagnoses of pseudocysts and rare PCLs were only determined from surgical specimens. For all patients, the final choice of surgery was determined by the referring doctors.



Video 5 Needle-based confocal laser endomicroscopy of pseudocysts with the “field of bright, gray or black particles” criterion.

Online content viewable at:
<https://doi.org/10.1055/a-0732-5356>

Statistical analysis

IPMNs, mucinous cystadenomas, and indeterminate mucinous lesions were pooled in the overall group of mucinous lesions in the diagnostic performance analysis. The accuracy, AUROC, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios (LR+, LR-) were calculated on the conclusive diagnoses for the different index tests (nCLE, CEA, and EUS) with 95%CI using the standard diagnosis (defined above) as a reference. A likelihood ratio >1 indicates that the index test result is associated with the presence of disease whereas a likelihood ratio <1 indicates that the result is associated with the absence of the disease. For both differential diagnoses of mucinous vs. nonmucinous PCLs and malignant vs. benign PCLs, the diagnostic performances of CEA, EUS, and nCLE were compared using the DeLong and DeLong statistical method [28] with surgical histopathology or cytohistopathology as the reference diagnosis.

nCLE performance was assessed both in “per-protocol” and “intention-to-diagnose” statistical analyses. In the intention-to-diagnose analysis, indeterminate nCLE diagnoses were considered false negatives compared with the definitive diagnosis and false positives compared with the other possible diagnoses.

P values of <0.05 were considered statistically significant. We used the R-package version 3.3.2 with the pROC package version 1.9.1 to perform the statistical analysis and the DeLong and DeLong analysis [28].

Results

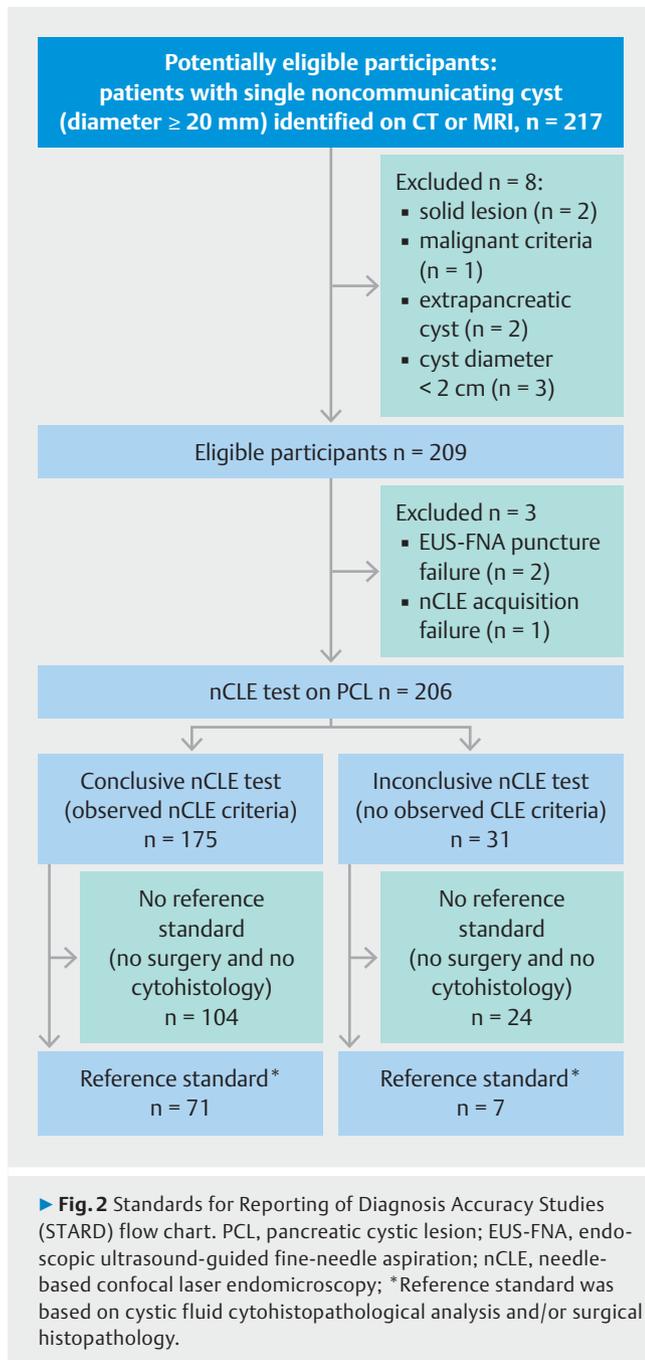
Patient population

A total of 217 patients diagnosed with a single noncommunicating PCL identified on CT or MRI was potentially eligible, and 11 patients were excluded (► **Fig. 2**). Of the 206 remaining patients, 175 patients (85%) had a conclusive nCLE examination. After centralization and review, two blinded pathologists agreed on cytohistopathological diagnoses in 61 PCLs, of which 22 were resected. Surgical histopathological and cytohistopathological analyses were concordant in all of these 22 cases. Based on the decisions of the referring doctors, 17 other patients with negative cytohistopathology underwent surgery. The final number of included PCLs with reference diagnoses was 78 (39 resected cysts + 39 PCLs with EUS-FNA cytohistopathological diagnoses).

The demographic and clinical data of the 78 patients are summarized in ► **Table 1**. There were 53 premalignant lesions including 44 mucinous lesions (19 mucinous cystadenomas, 14 BD-IPMNs, and 11 indeterminate mucinous lesions), 7 neuroendocrine neoplasms, 2 rare premalignant cystic tumors (1 cystic solid pseudopapillary neoplasm and 1 cystic lymphoma), and 25 benign lesions including 22 serous cystadenomas, 2 pseudocysts, and 1 congenital pancreatic cyst.

Diagnostic performance of nCLE

Data on technical feasibility and safety of nCLE are reported in ► **Supplementary Table e2** (available online). Of the 78 PCLs with reference diagnoses, there were 7 inconclusive nCLE le-



sions (9%) with the following reference diagnoses: 2 serous cystadenomas, 4 mucinous lesions, and 1 pseudocyst (► **Fig. 2**, ► **Table 3**). The conclusive nCLE diagnoses were compared with the final diagnoses (► **Table 3**, ► **Supplementary Fig. e3** – available online). Because of the low number of included pseudocysts, the performance of nCLE could not be accurately evaluated for this PCL type. nCLE diagnostic performance is shown in ► **Table 4** and ► **Supplementary Table e5** (available online).

In the per-protocol analysis, a sensitivity of 0.95 and specificity of 1.0 were obtained for the diagnosis of serous cystadenoma and for the diagnosis of mucinous vs. nonmucinous PCLs (► **Table 4**). A sensitivity of 1.0 for the diagnosis of neuroendocrine neoplasms was associated with a 0.95 specificity, because

► **Table 1** Patient and cyst characteristics.

Patients (n = 78)	
Age, mean (range), years	57 (28–81)
Male, n (%)	26 (33)
Pre-existing conditions, n (%)	
▪ Previous EUS-FNA	23 (29)
▪ Acute pancreatitis	9 (12)
Symptoms, n (%)	
▪ No symptoms	57 (73)
▪ Nonspecific abdominal pain	19 (24)
▪ Cholestasis	2 (3)
Cyst characteristics (n = 78)	
Location, n (%)	
▪ Uncinate	4 (5)
▪ Head	22 (28)
▪ Neck	8 (10)
▪ Body	26 (33)
▪ Tail	18 (23)
Size, mean (range), mm	40 (20–110)
Main pancreatic duct dilation, n (%)	8 (10)
Number of cavities, n (%)	
▪ 1	32 (41)
▪ 2	6 (8)
▪ 3–9	23 (29)
▪ ≥ 10	17 (22)
Wall thickness ≥ 1 mm, n (%)	19 (24)
Presence of calcification, n (%)	7 (9)
Intracystic CEA concentrations, n/N (%)	
▪ > 192 ng/mL	24/55 (44)
▪ < 5 ng/mL	20/55 (36)
EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; CEA, carcinoembryonic antigen.	

neuroendocrine neoplasm criterion was observed in one cystic solid pseudopapillary neoplasm, one cystic lymphoma, and one pseudocyst (► **Table 3**). In two mucinous lesions and one case of serous cystadenoma, the observation of a “field of bright, grey or black particles” without other nCLE criteria led to the misdiagnosis of a pseudocyst.

In the intention-to-diagnose analysis, sensitivity, specificity, PPV, NPV, accuracy, and AUROC ranged from 0.79 to 0.94 for serous cystadenoma diagnosis and from 0.84 to 0.93 for mucinous lesions (► **Supplementary Table e5**, available online).

In the subgroup of 23 patients who had undergone an inconclusive EUS-FNA procedure in the past, nCLE was conclusive in

► Table 3 Needle-based confocal laser endomicroscopy diagnoses vs. reference diagnoses based on surgical histology or endoscopic ultrasound-guided fine-needle aspiration cytohistopathology.

Final diagnoses	n	Surgery	nCLE-based diagnoses (19 SCA, 38 ML, 10 NEN, 4 PC)		Inconclusive nCLE criteria
			True positive	Misdiagnosis	
SCA	22	2	19	1 PC	2
ML	44	26	38	2 PC	4
NEN	7	7	7	0	0
PC	2	2	1	1 NEN	0
CSPPN	1	1	0	1 NEN	0
CL	1	0	0	1 NEN	0
CPC	1	1	0	0	1
Total	78	39	65	6	7
ML ¹	44	26	38	2 PC	4
Non-ML	34	13	31	0	3
Total	78	39	69	2	7
Premalignant ²	53	34	47	2 PC	4
Benign ³	25	5	21	1 NEN	3
Total	78	39	68	3	7

nCLE, needle-based confocal laser endomicroscopy; SCA, serous cystadenoma; ML, mucinous lesion (); NEN, neuroendocrine neoplasm; PC, pseudocyst; CSPPN, cystic solid pseudopapillary neoplasm; CL, cystic lymphoma; CPC, congenital pancreatic cyst.

¹ ML = indeterminate mucinous lesions + mucinous cystadenomas + branch-duct intraductal papillary mucinous neoplasms.

² Premalignant lesions = ML + NEN + CSPPN + CL.

³ Benign lesions = SCA + PC + CPC.

91% of patients (21/23) and established a correct diagnosis in all of them (21/21).

Comparisons of nCLE, CEA, and EUS morphology

The AUROCs for nCLE, CEA, and EUS morphology are compared in ► **Table 6** and ► **Supplementary Fig. e4** (available online).

The CEA level could not be obtained in 23 patients (29%, 23/78, ► **Table 6**). The CEA level was >192 ng/mL and <5 ng/mL in 44% (24/55) and 36% (20/55) of patients, respectively. The CEA level was <5 ng/mL for 13 serous cystadenomas and 7 premalignant lesions (5 neuroendocrine neoplasms, 1 mucinous lesion, and 1 cystic solid pseudopapillary neoplasm).

EUS morphology of the cyst was inconclusive in 41 cases (53%, 41/78). The presumptive EUS diagnoses in the remaining 37 were: 22 premalignant lesion (21 mucinous lesions and 1 neuroendocrine neoplasm) and 15 benign lesion (13 serous cystadenomas and 2 pseudocysts). Among the 17 PCLs that exhibited more than 10 cavities without thickened walls, the final diagnoses were premalignant in 41% of the cases (6 mucinous lesions and 1 cystic lymphoma).

The AUROC for nCLE for discriminating between mucinous and nonmucinous lesions was significantly larger than the AUROC for CEA using the 192 ng/mL cutoff value ($P < 0.01$) and the AUROC for EUS morphology ($P < 0.05$) (► **Supplementary Fig. e4a**, available online). The AUROC for nCLE for differentiating premalignant from benign PCLs was also significant-

ly larger than the AUROC for CEA ($P < 0.05$), using a 5 ng/mL cutoff value, whereas it was not significantly larger than the AUROC for EUS morphology (► **Supplementary Fig. e4b**, available online).

Discussion

A very high specificity of nCLE criteria for the diagnosis of cystic tumors has been reported in several studies [17–20]. However, these studies were not prospective validation studies and relied on small numbers of cysts with confirmed diagnoses by surgery or EUS-FNA cytohistopathological analysis. The primary aim of the second phase of the CONTACT trial was to validate nCLE criteria specificities for the diagnosis of PCLs in a large prospective trial. We only included PCLs with no obvious diagnosis (noncommunicating single cysts without criteria of advanced malignancy or chronic pancreatitis), and we used a reference diagnosis based on surgical histopathology or EUS-FNA cytohistopathological analysis. In the group of 78 patients with reference diagnoses, the performance of nCLE was evaluable for serous cystadenomas, mucinous lesions, and neuroendocrine neoplasms. A very high accuracy of nCLE was confirmed (≥ 0.95) for these PCL types and was associated with a high proportion of lesions with conclusive nCLE (71/78). A perfect specificity (1.00) was also confirmed for benign serous cystadenomas [20] and for the overall group of mucinous lesions [17]. For ser-

Table 4 Needle-based confocal laser endomicroscopy (nCLE) for the diagnosis of various types of pancreatic cystic lesion in 71 patients with conclusive nCLE result (per protocol analysis).

	TP	FP	TN	FN	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)	AUROC (95%CI)	LR+ (95%CI)	LR- (95%CI)
SCA (n=20)	19	0	51	1	0.95 (0.85-1)	1 (1-1)	1 (1-1)	0.98 (0.94-1)	0.99 (0.97-1)	0.98 (0.96-1)	Infinity	0.05 (0.0-0.15)
ML ¹ (n=40)	38	0	31	2	0.95 (0.88-1)	1 (1-1)	1 (1-1)	0.94 (0.86-1)	0.97 (0.93-1)	0.98 (0.94-1)	Infinity	0.05 (0.0-0.12)
NEN (n=7)	7	3	61	0	1 (1-1)	0.95 (0.80-1)	0.70 (0.36-1.0)	1 (1-1)	0.96 (0.92-1)	0.98 (0.94-1)	21.3 (5.9-36.8)	0.0 (0.0-0.0)
Premalignant lesions ² (n=49)	47	1	21	2	0.96 (0.90-1)	0.95 (0.87-1)	0.98 (0.94-1)	0.91 (0.80-1)	0.96 (0.92-1)	0.96 (0.92-1)	20.6 (15.0-26.3)	0.05 (0.0-0.14)

TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value; AUROC, area under the receiver operating curve; LR+, positive likelihood ratio; LR-, negative likelihood ratio; CI, confidence interval; SCA, serous cystadenoma; ML, mucinous lesions; NEN, neuroendocrine neoplasm.

¹ ML = indeterminate mucinous lesions + mucinous cystadenoma + branch-duct intra-ductal papillary mucinous neoplasm.

² Premalignant lesions = mucinous lesions + NEN + cystic solid pseudopapillary neoplasm + cystic lymphoma.

ous cystadenomas, the sensitivity was 0.95, which was higher than that previously reported [20] and consistent with the pathological data on serous cystadenoma vascularization, indicating that a superficial vascular network is always present in these tumors [18]. All these results exceed the previously reported performance of nCLE, while the mean nCLE procedure duration was decreased [20]. This is probably due to the learning curve and the experience of the users improving since the pilot studies.

The pattern described in neuroendocrine neoplasms was highly sensitive but less specific than previously published data, as it was observed in other types of premalignant PCLs such as cystic solid pseudopapillary neoplasms or cystic lymphoma. Because of the low number of neuroendocrine neoplasms (n=7), these results should be interpreted with caution and further evaluation needs to be performed.

The pattern described in pseudocysts [18, 20] was observed in four cysts (two mucinous lesions, one serous cystadenoma, one pseudocyst), and this criterion was absent in the second case of confirmed pseudocyst. This is not surprising because inflammatory cells can be found in the cystic fluid of cystic tumors following infection or bleeding. More data will be needed to evaluate the performance of this criterion.

AUROC comparison between nCLE, CEA, and EUS demonstrated that nCLE was the most efficient method for determining the diagnosis of PCLs. In our series, EUS was conclusive in only 53% of PCLs, which was lower than previously described, whereas its accuracy and specificity were increased. In this trial, endosonographers favored an accurate diagnosis rather than a systematic answer. CEA titration was only possible on 71% of the PCLs, highlighting the problem of collecting enough fluid for diagnostic tests in the absence of a large cavity and/or in case of thick or hemorrhagic cystic fluid [29]. With a cutoff value of 192 ng/mL, the diagnostic performance of CEA to differentiate mucinous from nonmucinous lesions corresponded to previously reported results [5,7]. The specificity of CEA levels <5 ng/mL for diagnosing benign cysts was lower than the previously reported specificity (0.83 vs. 0.95) [8]. This reflects the fact that no cases of BD-IPMNs and neuroendocrine neoplasms were present in the previously published meta-analysis [8].

The study confirmed the safety of nCLE in a large cohort. Acute pancreatitis occurred in 1.3% (1/78) of the patients as in standard EUS-FNA procedures [29].

The impact of nCLE on patient management was not evaluated in this study; nevertheless, some points can be highlighted. nCLE was conclusive in 91% of the patients who had already undergone an inconclusive EUS-FNA in the past (21/23) and was 100% accurate (21/21). Only 4.5% of the patients with confirmed serous cystadenomas (1/22) underwent surgical resection due to a doubtful diagnosis, whereas 36% of patients with serous cystadenoma usually undergo unnecessary resection due to diagnostic uncertainty [30]. This confirms that nCLE may be cost-effective, as recently reported in a French health economic evaluation [31].

The study has some limitations. First, we limited our performance analysis to pathologically confirmed pancreatic cysts because multidisciplinary review boards based on patient med-

Table 6 Needle-based confocal laser endomicroscopy, cyst fluid carcinoembryonic antigen, endoscopic ultrasound morphology and their combination for the diagnosis of: **a** mucinous vs. non-mucinous pancreatic cystic lesions; and **b** benign vs. premalignant pancreatic cystic lesions.

a													
	n	TP	FP	TN	FN	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)	AUROC (95%CI)	LR+ (95%CI)	LR- (95%CI)
nCLE	71	38	0	31	2	0.95 (0.88-1)	1 (1-1)	1 (1-1)	0.94 (0.86-1)	0.97 (0.93-1)	0.98 (0.95-1)	Infinity	0.05 (0.0-0.12)
CEA > 192 ng/mL	55	23	1	20	11	0.68 (0.52-0.83)	0.95 (0.86-1)	0.96 (0.88-1)	0.65 (0.48-0.81)	0.78 (0.67-0.89)	0.81 (0.70-0.92)	13.9 (9.4-18.4)	0.34 (0.18-0.5)
EUS morphology	37	19	2	11	5	0.79 (0.63-0.95)	0.85 (0.65-1)	0.91 (0.78-1)	0.69 (0.46-0.91)	0.81 (0.68-0.94)	0.82 (0.68-0.95)	5.11 (3.3-6.9)	0.25 (0.07-0.42)
b													
	n	TP	FP	TN	FN	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)	AUROC (95%CI)	LR+ (95%CI)	LR- (95%CI)
nCLE	71	21	2	47	1	0.95 (0.87-1)	0.96 (0.90-1)	0.91 (0.80-1)	0.98 (0.94-1)	0.96 (0.90-1)	0.96 (0.90-1)	22.8 (13.5-32.2)	0.05 (0.0-0.14)
CEA < 5 ng/mL	55	13	7	33	2	0.87 (0.69-1)	0.83 (0.63-1)	0.65 (0.41-0.89)	0.94 (0.83-1)	0.84 (0.74-0.94)	0.85 (0.75-0.95)	4.9 (2.7-7.1)	0.16 (0.0-0.35)
EUS morphology	37	10	5	22	0	1 (1-1)	0.81 (0.67-0.96)	0.67 (0.43-0.90)	1 (1-1)	0.86 (0.75-0.97)	0.91 (0.90-1)	5.3 (2.4-8.4)	0.0 (0.0-0.0)

TP, true positive; FP, false positive; TN, true negative; FN, false negative; PPV, positive predictive value; NPV, negative predictive value; AUROC, area under the receiver operating curve; LR+, positive likelihood ratio; LR-, negative likelihood ratio; CI, confidence interval; nCLE, needle-based confocal laser endomicroscopy; CEA, carcinoembryonic antigen; EUS, endoscopic ultrasound.

ical history, EUS examinations, biochemical measurements, and 3-years of follow-up would not have provided the same level of confidence. This induced a higher prevalence of premalignant lesions in resected PCLs and did not allow for assessment of the diagnostic performance of nCLE for pseudocysts. Second, because of ethical considerations that prevented systematic pancreatic resections, cytohistopathological analyses of cystic fluid were used as the reference standard in 50% of patients with confirmed diagnoses. This should be discussed, as the specificity of this analysis was only 0.93 (0.67–1) in a large meta-analysis [7]. Nevertheless, we only included patients with diagnoses based on a centralized review performed by two blinded pathologists. The relevance of this process for increasing the specificity was confirmed by the perfect correlation between EUS-FNA cytohistopathological diagnoses and surgical histopathology in the 22 cases in which the results of both tests were available. Third, we did not compare nCLE with the pretest likelihood. Nevertheless, as nCLE was performed between the EUS and EUS-FNA procedures and analyses, this would have required a different study design. Fourth, nCLE and EUS were performed by the same endoscopist and therefore nCLE diagnosis may have been biased by the EUS findings. Finally, DNA-based markers or the string sign test were not assessed in the study and compared with nCLE, as these findings were published only after the start of the study [32–34].

Conclusion

Our study demonstrated a very high sensitivity and specificity of nCLE criteria for the diagnosis of single, noncommunicating, pancreatic cystic tumors. The diagnostic performance of nCLE significantly surpassed that of EUS and CEA titration for differentiating mucinous from nonmucinous lesions and benign from premalignant PCLs. The routine addition of nCLE to standard EUS-FNA procedures could potentially impact patient management and provide a significant economic benefit. This needs to be precisely evaluated in the near future.

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Competing interests

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