Endoscopic Ultrasonography: Role of EUS sampling in Cystic Lesions

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Introduction

Pancreatic cystic lesions (PCLs) show a wide spectrum of demographical, morphological, and histological characteristics. The diagnosis and discrimination of these lesions are very important because of the risk for concurrent or later development of malignancy. From the clinical standpoint, the distinction is mostly needed between mucinous [intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs)] and nonmucinous [pseudocysts and serous cystic neoplasms (SCNs)] cysts. Cross-sectional imaging tests and endoscopic ultrasound (EUS) alone are sometimes ineffective for accurately distinguishing between benign/malignant or mucinous/nonmucinous cystic lesions. In fact, none of the diagnostic modalities are uniformly effective in all cases. Nevertheless, EUS–fine-needle aspiration (FNA) is currently the most helpful procedure for distinguishing the type of cysts and, thus, managing the patient (Fig. 11.1). International consensus guidelines from 2012 for the management of IPMNs and MCNs of the pancreas recommended cyst fluid analysis for evaluation of small branch-duct (BD) IPMNs without "worrisome features" in centers with expertise in EUS-FNA and cytological interpretation [1] (Table 11.1). The diagnostic success of EUS-FNA cyst aspiration depends on the preparation of patients and instruments, technical and procedural factors, and the expertise of a dedicated team as well as the location, size, and characteristics of the target lesion. Therefore, each step of the procedure should be carefully planned and executed with the entire team.

EUS-FNA of PCLs requires extra care compared to solid lesions. Before proceeding with EUS-FNA, a complete diagnostic EUS should be performed to evaluate the lesion and adjacent structures for selection of the optimal needle tract. The procedure itself is generally safe with a low complication rate, but the possible risks and benefits should always be evaluated carefully before the intervention. The expectations for the result of the FNA should be a change in diagnostic algorithm, a decision for a specific treatment and follow-up, or to dispense from invasive treatments. Including sedation, the patient is prepared for the procedure similarly to other endoscopic interventions. EUS-FNA complications such as

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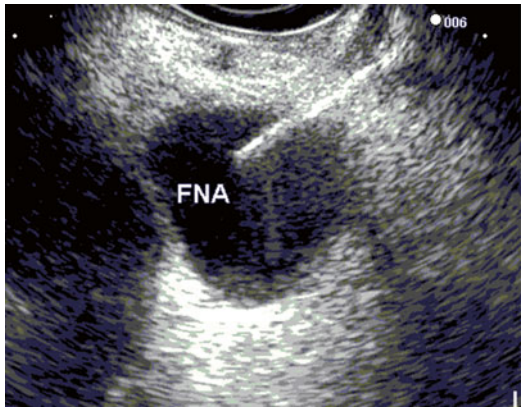

Fig. 11.1 A 25-mm-diameter, thin-walled, anechoic, unilocular, nonseptated, cystic lesion in the tail of the pancreas. The needle is inside the cyst for aspiration

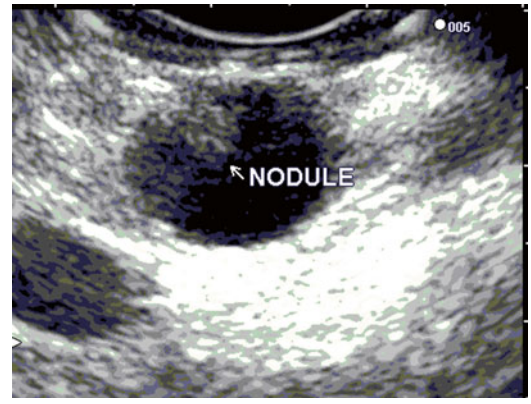

Fig. 11.2 A 20-mm-diameter anechoic cystic lesion with an internal nodule in the pancreas body. The interpretation of this cyst is a side-branch IPMN. The aspiration of the nodule is suggested for cytological evaluation

Table 11.1 "High-risk stigmata" and "worrisome features" of IPMN on cross-sectional imaging

High-risk stigmata	Worrisome features
Obstructive jaundice in a patient with cystic lesion of the head of the pancreas	Cyst > 3 cm
	Thickened/enhancing cyst walls
Enhancing solid component within cyst	Nonenhancing mural nodule
Main pancreatic duct >10 mm in size	Main pancreatic duct size of 5–9 mm
	Abrupt change in caliber of pancreatic duct with distal pancreatic atrophy
	Lymphadenopathy

infection, bleeding, and pancreatitis have been reported more frequently with cystic lesions compared to solid masses. Multiple passes into the cyst may also increase the risk of infection. The aspiration of all cyst contents may minimize the risk of infection and maximize the diagnostic yield. A prophylactic antibiotic is usually recommended for patients undergoing FNA of pancreatic cysts. Tumor seeding has been reported in mucinous cystic lesions located in the body and tail of the pancreas after EUS-FNA [2]. However, a recent comparative study could not find any difference in the frequency of peritoneal seeding in patients undergoing resection of IPMN after EUS-FNA [3]. If there is a solid component inside the cyst which increases the suspicion of malignancy, it should be aspirated for cytological analyses (Fig. 11.2). Usually a 22-gauge (G) needle is most appropriate for cyst aspiration; however, a 25-G needle may also be used for small (<2 cm) nonmucinous cysts or for cases requiring a transduodenal approach. The minimum size of cyst to obtain an adequate sample for analysis is not certain and might be dependent on the location, viscosity of fluid, and size of each component in multilobular cysts. The aspirated cyst fluid volume correlates significantly with cyst size and a minimum size of 1.5 cm is needed for successful analysis [4].

After EUS-FNA, cyst fluid is routinely evaluated for gross appearance, amylase and carcinoembryonic antigen (CEA) levels, and cytology [5]. *KRAS* and *GNAS* genetic mutation analyses have been shown recently to help distinguish mucinous lesions and IPMNs in selected cases. Recently, some metabolomic-derived novel cyst fluid biomarkers have also been identified which have potential clinical utility for differentiating mucinous from nonmucinous pancreatic cysts. No single test diagnoses PCLs with 100 % accuracy, and different EUS-FNA–based tests are combined to obtain the best result. The combination of EUS-FNA test results with clinical findings and imaging features may determine the cyst type in a majority of the patients. This chapter will review the role of EUS sampling in PCLs based on the recent advances in diagnostic tests.

Gross Appearance of Fluid

The aspirated cyst fluid can be visually inspected for its color and viscosity. A highly viscous, thick fluid is the first clue that the cyst is likely IPMN or MCN. The viscosity of cyst fluid may be tested simply at the bedside by a string test. A drop of fluid is stretched slowly between the thumb and index finger until its disruption. A string length of more than 3.5 mm is a strong finding for a mucinous cyst [6]. The fluid is usually thin and clear in SCNs. Because of the vascular nature, aspirants may sometimes be bloody in SCNs. However, this is not a specific finding since all aspirates might be bloody due to traumatic puncture of the cyst wall. Pseudocyst fluid is usually thin, opaque, sometimes hemorrhagic, and may contain inflammatory debris.

Biochemical Analyses of Pancreatic Fluid

Carcinoembryonic Antigen

The epithelium of the cyst wall may produce a variety of tumor markers and chemical substances which are often used in diagnostic testing. Cyst fluids have been evaluated to date for different tumor antigens including CA 19-9, CA 72-4, CA 15-3, CA 125, and CEA [7]. These markers were found elevated in some cases of malignant or mucinous cystic lesions, but only CEA was determined as a useful marker to distinguish mucinous from nonmucinous PCLs. A high concentration of CEA reflects the presence of a mucinous epithelium and is observed in both IPMNs and MCNs. Nonmucinous cysts including pseudocysts and serous cystic neoplasms do not include a mucinous epithelium and should have relatively low levels of CEA. Particularly, low cyst fluid CEA is seen in SCNs. A cutoff CEA level of 192 ng/mL has a sensitivity of 73 %, specificity of 84 %, and accuracy of 79 % for differentiating mucinous from nonmucinous pancreatic cystic lesions in a multicenter series consisting of patients who underwent surgical resection [8]. Among all the cyst fluid diagnostic parameters, CEA concentration alone was the most accurate test for the diagnosis of cystic mucinous neoplasms in the same study. Depending on the assay method, 0.5–1 mL of fluid is needed for the CEA analyses. American College of Gastroenterologists' Guidelines recommended CEA as the first test to do if minimal fluid is acquired during aspiration [5].

Despite considerable overlap, CEA is useful in order to distinguish mucinous from nonmucinous cysts. A meta-analysis of 450 patients from 12 studies reported that a CEA level > 800 ng/mL was 98 % specific but only 48 % sensitive for the diagnosis of mucinous cyst [9]. A CEA level < 5 ng/mL was 98 % specific for a serous cystadenoma but the sensitivity was only 19 %. This study clearly showed that increasing the cutoff value of CEA for support of a mucinous cyst or decreasing it to support a nonmucinous cyst will have a negative effect on sensitivity. Another meta-analysis of 12 published studies showed that the pooled sensitivity and specificity of CEA for differentiate mucinous versus nonmucinous cystic lesions was 63 % and 88 %, respectively [10]. Table 11.2 summarizes the diagnostic results of fluid CEA analyses in various published studies [8, 11–24].

The reported CEA cutoff levels are assay-specific and may change according to manufacturer. Besides, different cutoff values were used in clinical studies which affect the sensitivity, specificity, and diagnostic accuracy rate of CEA for differentiation of a mucinous cyst. Clinical studies are often carried out in groups of patients who underwent surgical resection since there is not a gold standard for diagnosis of mucinous cyst in a clinical setting. As a result, these published series usually consist of patients with MCNs and BD-IPMNs with high-risk stigmata or worrisome features. Cyst fluid CEA levels do not differentiate IPMNs from MCNs or benign IPMNs from malignant; however, some studies demonstrate that higher CEA levels are more likely in high-grade MCNs and IPMNs. Fluid CEA levels correlated with low-, moderate-, and high-grade IPMNs as well as degrees of dysplasia (1261 ng/mL vs. 7171 ng/mL vs. 10,807 ng/mL, respectively) [25]. However, CEA levels were signifi-

Table 11.2 The diagnostic value of fluid CEA for differentiation of mucinous cysts in several studies

Author (year)	Patient (*n*)	Cyst diagnosis	Cutoff (ng/mL)	Sensitivity (%)	Specificity (%)	Diagnostic accuracy (%)
Brugge (2004)	111	SP	192	75	83	79
Shami (2007)	43	SP	300	64	92	76
Sreenarasimhaiah (2009)	20	Cx and SP	192	66	78	75
Khalid (2009)	76	SP	192	64	83	68
Snozek (2009)	442	Cx and SP	30	79	73	77
Sawhney (2009)	84	Cx and SP	192	82	100	84
Morris-Stiff (2010)	47	SP	192	93	43	N/A
Nagula (2010)	97	SP	192	73	65	70
Cizginer (2011)	154	SP	109.9	81	98	85
Park (2011)	124	SP	200	60	93	72
Rogart (2011)	75	Cx	192	55	97	74
De Jong (2012)	18	SP	192	44	100	72
Chai (2013)	52	Cx and SP	192	62	89	76
Talar-Wojnarowska (2013)	52	Cx and SP	45	92	64	71
Al-Haddad (2014)	48	SP	192	63	62	62
Kadayifci and Brugge[a] (2014)	243	Cx and SP	192	49	97	65
Kadayifci and Brugge[a] (2014)	243	Cx and SP	50	77	87	80

SP surgical pathology, *Cx* clinical diagnosis
[a]Unpublished data

cantly lower (462 ng/mL) in cysts with invasive carcinoma; the possible explanation was that fewer cells with intact tight-junctions and less CEA were available at the luminal surface for release into the cyst fluid. In another study including 66 patients, the median CEA level was significantly higher in patients with MCNs than IPMNs (2844 ng/mL vs. 574 ng/mL) [17].

In clinical practice, the most common cysts encountered are those that do not meet criteria for a surgical resection; these cysts are the greatest challenge for early diagnosis and follow-up. Therefore, the lower cutoff level of CEA (less than 192 ng/dL) may be more helpful to increase sensitivity and diagnostic accuracy of fluid CEA level without a significant decrease in specificity. The sensitivity, specificity, and diagnostic accuracy of fluid CEA (>192 ng/mL) level for mucinous differentiation was 49 %, 97 %, and 65 %, respectively, in the evaluation of 243 cyst patients in our database (Table 11.2). A lower CEA cutoff level (>50 ng/mL) increased the sensitivity to 77 % and the diagnostic accuracy to 80 %, but decreased the specificity to only 87 %. The median CEA level of mucinous cysts was 400 ng/mL in patients who underwent surgical resection but only 160 ng/mL for those who followed up without surgery. The lower CEA cutoff level improved the sensitivity and diagnostic accuracy of CEA, especially in IPMN patients who did not have a surgical indication. On the basis of these data, we think a lower fluid CEA cutoff level than 192 ng/mL might be more helpful for the diagnosis of IPMN in a clinical setting.

Amylase

Cyst fluid amylase level is also a useful marker for the differential diagnosis of pancreatic cysts. Its presence in cyst fluid is often used as an indicator of a communication between a cystic lesion and the ductal system. Amylase-rich fluid is uniformly found in pancreatic pseudocysts and the concentration is not expected to be less than 250 U/mL. Due to connectivity to the pancreatic ductal system, amylase levels may also be elevated in IPMNs. It is always low in serous cysts and in the majority of MCNs. The sensitivity of fluid amylase (>250 U/mL) for differentiation of a pseudocyst is very

high (96–100 %); however, the specificity is not good since it is also elevated frequently in IPMNs. In a recent analysis of 139 patients with IPMN, we have detected that fluid amylase was elevated (>250 U/mL) in 76 % of cases. Even MCNs, which have no connection with the pancreatic ductal system, have an elevated amylase level, and the utility of fluid amylase to differentiate IPMNs from MCNs is not clear [26].

Cytology

Cytological examination of cyst fluid alone is often nondiagnostic to characterize cyst type due to the low cellularity of the aspirated fluid [27]. However, a multimodal approach combining the patient's history, clinical findings, imaging features, cytology, special stains, and cyst fluid analyses can improve the overall cytological interpretation. The collaboration between the endoscopist and cytopathologist is one of the main factors that may determine the outcome of EUS-FNA. The aspirated fluid during EUS-FNA is examined cytologically for degenerative debris, inflammatory cells, epithelial cells, granolocytes, histiocytes, extra-cellular mucin, mucinous epithelium with cytoplasmic mucin and atypical/malignant cells. The aim of cytological analyses is to differentiate between serous and mucinous cysts, to distinguish pseudocysts from neoplastic cysts, and to detect malignancy in patients with mucinous cysts.

Cytological findings of a pseudocyst may be affected by infectious complications. An uncomplicated pseudocyst fluid is generally thin, nonmucoid, and discolored and may consist of only scattered histiocytes. However, an infected cyst may be purulent, mucoid-appearing fluid and contain acute and chronic inflammatory cells, histiocytes, and hemosiderin-laden or foamy macrophages [26]. The presence of granulocytes in the aspirated fluid is suggestive of an acute infection. Pseudocysts do not have an epithelial lining and are surrounded by inflammatory cells and histiocytes. If there is any cytological evidence of epithelial cells within the cyst fluid, this should raise the suspicion of a cystic neoplasm rather than a pseudocyst [26].

The fluid aspirated from SCNs is usually very scant in volume and includes few intact cells. Many cases are interpreted as nondiagnostic because of insufficient cellularity. Intact cell clusters are composed of bland cuboidal cells with round central to slightly eccentric nuclei and scant finely vacuolated but nonmucinous cytoplasm [26]. The cells from SCNs can be stained with periodic acid–Schiff (PAS) without diastase for the presence of glycogen. Because there is no mucin in serous cysts, mucicarmine staining should be negative. The yield of cytology with EUS-FNA is poor for SCNs.

Mucinous lesions may be diagnosed with the presence of mucin-producing epithelial cells on cytologic analysis. Mucin can be demonstrated by mucicarmine staining and PAS with diastase in nearly half of mucinous cysts. Direct smears of thick and viscous cyst fluid may be reflected on the slide as thick sheets of colloid-like mucin that covers much of the slide [26]. If mucin is present, it is important to assess if the mucin originates from the cyst lining or represents a contaminant (gastric/duodenal secretions). Degenerated inflammatory cells and histiocytes within the mucin provide added support that the mucin is from the cyst. Cyst fluid cytology is rarely sufficiently diagnostic to distinguish IPMN from MCN, and it is usually reported as "mucinous cyst." The accuracy of cytology alone in differentiating mucinous from nonmucinous cysts was 58 % in a multicenter cooperative pancreatic cyst study [8]. The cytological findings detected in common pancreatic cysts are summarized with other EUS-FNA tests (Table 11.3).

Cytology is the most accurate test for the detection of malignancy in patients with mucinous cysts, and a "positive" or "malignant" diagnosis is generally 100 % specific [26]. In addition, the presence of high-grade epithelial atypia in the cyst fluid analysis has an accuracy of 80 % to predict malignancy and detects 30 % more cancers in small BD-IPMN than the presence of "worrisome features" [28]. Based on these results, new high-risk factors proposed for BD-IPMN include a rapidly increasing cyst size and high-grade atypia rather than "positive" cytology [1]. The reported sensitivity and diagnostic accuracy of cyst fluid cytology for malignant IPMNs is

Table 11.3 Endosonography–fine needle aspiration findings of common pancreatic cysts

Parameters	Pseudocyst	SCNs	MCNs	IPMNs (MD and BD)
Gross examination	Thin, clear or brown to green, nonmucinous, sometimes hemorrhagic	Clear and thin, may be hemorrhagic	Thick, viscous mucus	Thick, viscous mucus
Biochemistry	CEA concentration very low, amylase and lipase concentrations usually high	CEA and amylase concentrations very low	CEA concentration usually high	CEA concentration usually high, amylase concentration may be high
Cytology	Degenerative debris, inflammatory cells, histiocytes, no epithelial cells	Usually acellular and nondiagnostic, small cluster of cells with bland cuboidal morphology, glycogen stain positive, mucin negative	Mucinous epithelial cells with varying degrees of atypia, colloid-like mucin, mucin stains positive	Colloid-like mucin, mucin stains positive mucinous epithelial cells with varying degrees of atypia, sparsely cellular
DNA analyses			*KRAS* mutation (+) (14 %)	*GNAS* mutation (+) (60 %) *KRAS* mutation (+) (60 %)

approximately 75 % and 86 %, respectively [26]. EUS-guided FNA of mural nodules was superior to EUS alone (75 % vs. 61 %) for the diagnosis of malignancy in IPMNs [29]. The reported diagnostic accuracy for a solid pseudopapillary neoplasm (SPN) based on cytology and immunohistochemistry is 65 % [30]. Aspirated cyst fluid may display necrotic debris for SPNs.

DNA Analysis

Certain DNA mutations may serve as molecular markers for the diagnosis of mucinous cysts. DNA is extracted and amplified from epithelial cells that have been exfoliated into the cyst cavity. A multicenter trial, which is referred to as the PANDA study, showed that pancreatic cyst fluid *KRAS* mutation is highly specific (96 %) for mucinous cysts but the sensitivity is only 45 % [13]. *KRAS* is an early oncogenic mutation in the adenoma–carcinoma sequence and can be detected in patients with low-grade pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma. The presence of a *KRAS* mutation cannot distinguish a benign from malignant mucinous cyst. However, the PANDA study demonstrated that high-amplitude *KRAS* mutation followed by allelic loss was the most specific marker (96 %) for malignancy. DNA analysis diagnosed malignancy in all cases where cytology with FNA was negative [13]. *KRAS* mutation had a specificity of 100 % and a sensitivity of 54 % in a more recent study of the same group [31]. *KRAS* mutation was detected in 43 of 63 mucinous cysts (68.3 %), in which diameters were equal or less than 3 cm, in another series [32].

The *KRAS* mutation added value to cytology and CEA in the same series and a diagnosis was made by molecular analysis in 20 patients (31.7 %) when either cytology was unsatisfactory, or CEA was not elevated. The sensitivity of *KRAS* mutation to detect mucinous cysts has been found between 8 and 50 % in some other studies and the value of *KRAS* mutation and CEA combination to differentiate a mucinous cyst was inconsistent among these studies [12, 15, 22, 24]. We have found the sensitivity, specificity, and diagnostic accuracy of *KRAS* mutation to be 58 %, 100 %, and 70 %, respectively, in the analysis of 281 patients with pancreatic cysts (unpublished data). The *KRAS* mutation alone did not offer a more diagnostic test than cyst fluid CEA; however, the combination of both tests improved

the diagnostic accuracy significantly. The role of *KRAS* mutation for a malignant transformation in mucinous cysts, or to predict patients with a high risk of malignancy, is not clear and needs further prospective studies with long-term follow-up.

A recent study demonstrated that the *GNAS* mutation detected in cyst fluid can separate IPMN from MCN but, similar to *KRAS* mutations, does not predict malignancy [33]. The absence of a *GNAS* mutation also does not correlate with a diagnosis of MCN because not all IPMNs will demonstrate a *GNAS* mutation. A *GNAS* mutation was present in 66 % of IPMNs and either *KRAS* or *GNAS* mutations were identified in 96 % of IPMNs [33]. Furukawa et al. performed whole-exome sequencing for primary IPMN tissue and analyzed 17 somatic mutations [34]. They found *GNAS* mutation in 48 of 118 patients (40.7 %) but none of the 32 patients with pancreatic ductal adenocarcinoma. We analyzed *GNAS* mutation in 80 patients with PCLs, including 49 IPMNs, and found the sensitivity, specificity, and diagnostic accuracy as 61 %, 100 %, and 75 %, respectively (unpublished data). The combination of *GNAS* with CEA has also improved the diagnostic accuracy in this series.

The DNA analysis, overall, provides a new insight into the molecular pathogenesis, diagnosis, and management of mucinous cysts. However, most of the studies have been done with a limited number of patients and by a retrospective analysis of cyst databases. Moreover, there are still many queries awaiting a response. The role of molecular analysis to identify high-risk or malignant cysts, the association of IPMN histological subtype with mutational frequency, the importance of type of mutation, and the clonality in the diagnosis and management are not clear yet. Cyst fluid DNA analysis recently has been commercially available (Pathfinder TG; RedPath Integrated Pathology, Inc, Pittsburgh, PA). However, the routine use of DNA analyses does not have strong evidence yet and high cost may be a limitation for widespread usage. Nevertheless, it has the potential to improve the diagnosis in cases in which imaging modalities, the cyst fluid CEA level, and cytology are indeterminate for type differentiation. Future studies will better define the impact of DNA analysis on the diagnostic and prognostic stratification of mucinous cysts and especially in IPMNs.

Novel Tests for Cyst-Type Differentiation

To identify the novel cyst fluid biomarkers, a recent study used a metabolomics approach to identify uniquely expressed metabolites in different pancreatic cyst types [35]. A total of 506 metabolites were detected in the cyst fluids and compared between nonmucinous and mucinous cysts. They identified glucose and kynurenine to be differentially expressed between nonmucinous and mucinous pancreatic cysts. Metabolomic abundances for both were significantly lower in mucinous cysts compared with nonmucinous cysts and the ROC curves for glucose and kynurenine was 0.92 and 0.94, respectively. Neither metabolite could differentiate premalignant from malignant cysts. The clinical utility of these biomarkers will be addressed in future studies.

The cyst fluid's interleukin-1β concentration has been shown to be higher in malignant IPMN than in benign IPMN in a preliminary study including 40 patients with IPMN [36]. It has been proposed as a potential biomarker for differential diagnosis of benign and malignant cysts; however, confirmation is needed in larger clinical studies.

Several microRNA expressions, protein-based biomarkers, proteomic analyses, and glycoproteomics in cyst fluid are under investigation to develop new biomarkers for differentiation of mucinous or malignant cysts in some pilot studies [37].

Combination of Tests for Mucinous Differentiation

Cyst fluid CEA level and *KRAS*/*GNAS* mutations have a very good specificity but low sensitivity in differentiating mucinous from nonmucinous cystic lesions. The cytology alone is also highly specific in describing high-grade atypia and malignant cysts but insensitive for benign/malignant and cyst

type differentiation. Therefore, there is no single test accurate enough for characterization of cyst type in every case. A combination of tests to improve the sensitivity and diagnostic accuracy of mucinous differentiation has been investigated in different studies. The combination of cytology and fluid CEA did not provide additional diagnostic accuracy in the cooperative cyst study, and CEA alone was more accurate than combining tests [8]. The combination of fluid CEA and cyst mucin obtained the best sensitivity to determine mucinous lesions in a retrospective data analysis [38].

The combination of the presence of atypical (not malignant) epithelial cells on cytological evaluation or with a CEA value of >2500 ng/mL improved the sensitivity and accuracy for the detection of malignancy and invasion in patients with small BD-IPMNs [39]. This approach was even better than the recommended management algorithm including evaluation of patient symptoms, positive cytology, dilated main pancreatic duct > 6 mm, or the presence of a mural nodule in the cyst wall as detected by radiological studies [40].

The combination of DNA mutation analysis with CEA and cytology may potentially improve the sensitivity and diagnostic accuracy for mucinous differentiation. Sawhney et al. found a 100 % sensitivity for diagnosing mucinous cysts with the combination of CEA and *KRAS* mutation [15]. Their study was limited to 19 patients and the CEA level did not correlate well with the quantity of DNA. The combination of molecular analysis with cyst fluid CEA and cytology resulted in higher mucinous cyst diagnostic performance than either one of its individual components in another recent study [24]. A volume-based protocol using different components of the specimen has been proposed to be able to optimize diagnostic yield in pancreatic cyst fluids [22]. The protocol used minimal cyst fluid volumes for the analysis of CEA, *KRAS* analyses, and cytology, thus optimizing the use of the often scant cyst fluid volumes obtained during aspiration. They demonstrated that the supernatant is comparable to the neat fluid and cell block material for CEA and KRAS testing. *KRAS* mutation testing increased the diagnostic yield when combined with cytology and CEA analysis. As mentioned above, the combination of *GNAS* or *KRAS* with CEA has also improved the diagnostic accuracy of CEA in our series. These studies shows that, in practice, a combined approach of molecular tests with CEA level has potential to improve the sensitivity and diagnostic accuracy of cyst fluid analysis.

Limitations of EUS-FNA and New Methods to Improve the Diagnostic Yield

Even though EUS-FNA is technically an easier procedure for the experienced endoscopist, the puncture of the cyst wall may not be possible due to an unfavorable location or an unavoidable intervening blood vessel. In a prospective study of 143 patients who underwent EUS for a cyst aspiration, FNA could be performed in 128 (90 %) of them [41]. Cyst fluid sent for cytology provided adequate cellular material in 31 % of patients and sufficient fluid for biochemical analysis was obtained in 49 % of the cases in the same study. Complications occurred in three patients (2.4 %). Several studies have also reported the accuracy of EUS-FNA cytology between 20 and 50 % in PCLs [8, 42, 43]. These results showed that overall diagnostic value of EUS-FNA of PCLs is still limited and new methods are needed to improve the yield of FNA.

A through-the-needle (19-G) new cytologic brush system was compared with standard FNA cytology in ten consecutive patients with PCLs in a preliminary study [44]. In seven of ten patients, brush cytology was superior to conventional FNA cytology in terms of cellularity and detection of diagnostic cells. However, there were one major and one minor intracystic bleeding in this study. The same authors, recently, reported the result of EUS brush cytology to assess intracellular mucin on cytobrushing specimens in 37 patients and compared it with EUS-FNA for the diagnosis of suspected mucinous PCLs [45]. Cytobrushings were more likely to detect intracellular mucin than the EUS-FNA, but complications occurred in three patients. The same method was applied in

30 patients in another prospective study and failed technically in eight cases [46]. Brush cytology provided a cellular diagnosis in 20 of 22 cases (91 %). The EUS brushing was superior to the aspirated fluid for detecting diagnostic cells (73 % vs. 36 %) and mucinous cells (50 % vs. 18 %), but again complications occurred in three patients. The EUS brushing showed promising results to improve the diagnostic yield of cytology in preliminary studies; however, the usage of 19-G needle might be a problem in some cases and more studies are needed to demonstrate safety.

To improve the diagnostic yield of material obtained from FNA, cyst wall puncture with a 22-G needle after fluid aspiration was evaluated in 69 PCLs [47]. Cellular material from cyst wall puncture was adequate for cytological assessment in 56 cysts (81 %), and 4 malignant cysts were diagnosed using this technique. Cytology showed a mucinous epithelium in one third of cysts whose CEA level was <192 ng/mL. Only one episode of mild and self-limited pancreatitis was detected as a complication.

Confocal laser endomicroscopy (CLE) is a novel imaging technology that uses low-power laser to obtain in vivo histology of the gastrointestinal mucosa. Recently, a CLE miniprobe has been developed for use during EUS-FNA to visualize the cyst wall and epithelium directly through a 19-G FNA needle. The technical feasibility of this probe was shown and the preliminary studies of pancreatic cystic lesions revealed some important cyst wall findings to differentiate mucinous and nonmucinous cysts. The presence of epithelial villous structures was associated with IPMNs, with 59 % sensitivity and 100 % specificity, in a recent study [48]. The superficial vascular network criterion, which corresponded to a dense and subepithelial capillary vascularization in pathological specimens, was associated with a serous cystadenoma with 100 % specificity and 63 % sensitivity. In spite of these promising findings, further studies are needed to ascertain the contribution of CLE for the differential diagnosis of IPMNs. In a preliminary study, we successfully visualized the cyst wall with miniprobe CLE during EUS-FNA in 17 cases and confirmed IPMN in 9 and SCN in 2 patients (Fig. 11.3).

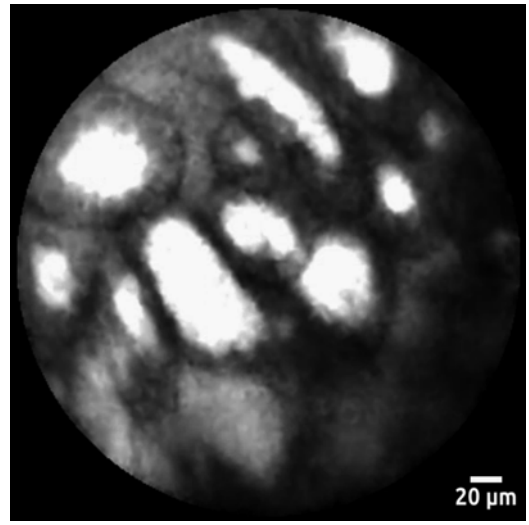

Fig. 11.3 Confocal laser endomicroscopy of a patient with IPMN. Epithelial villous structures were detected on cyst wall consistent with IPMN

Optical coherence tomography (OCT) is an interferometric technique that typically uses near-infrared light and allows noninvasive micron-scale cross-sectional imaging of biological tissues by measuring their optical reflections. Ex vivo OCT of freshly resected pancreatectomy specimens demonstrated that mucinous cysts could be differentiated from nonmucinous cysts with high sensitivity (>95 %), specificity (>95 %), and almost perfect interobserver agreement. A special OCT probe designed for placement through a 19-G FNA needle has been developed for cyst wall imaging [49].

Direct pancreatic cystoscopy and intracystic biopsy through a 19-G needle with a SpyGlass fiber optic catheter was feasible in a pilot study including two patients [50]. Both cysts were considered to be mucinous cystoadenomas, because mucinous-like cylindric epithelium without cellular atypia was observed. Histological examination of biopsies obtained from the cyst wall confirmed the diagnosis.

The diagnostic value of these novel methods have not been confirmed with adequately powered studies yet, but preliminary results show that they have a significant potential to improve the diagnostic yield of EUS-FNA and may be predictive for the malignant potential of PCLs.

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