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Diagnostic performance of endoscopic ultrasound-guided fine-needle aspiration for cystic and non-cystic pancreatic neuroendocrine tumors

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ABSTRACT

Background and study aims Pancreatic neuroendocrine tumors (P-NENs) are rare tumors with malignant potential. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has been shown to be superior to other imaging methods in preoperative localization and diagnosis of P-NENs. The objective of this study was to describe the EUS features of non-metastatic cystic and non-cystic P-NENs seen at a referral center and to evaluate the performance of EUS-FNA in diagnosis of P-NENs.

Patients and methods All patients with histologically confirmed, non-metastatic P-NENs, which underwent EUS-FNA prior to surgical resection at the Moffitt Cancer Center between Jan 2005 and Dec 2012 were included. Clinical, endoscopic and pathologic information was abstracted from electronic medical records.

Results Thirty-nine patients, all with non-functional P-NENs, were included in this study. Thirteen tumors were cystic and 26 were solid. Among the cystic tumors, 50 % were partly cystic and partly solid, and 50 % were fully cystic. The cystic tumors were more commonly seen at the body/tail, and the solid tumors were more uniformly distributed. Fluid could be aspirated from 50 % of the cystic tumors, all with a carcinoembryonic antigen level <192 ng/mL. With surgical pathology as the gold standard, overall sensitivity of EUS-FNA in diagnosing cystic tumors was 62.5 %, and for solid tumors, 95 % ($P<0.03$).

Conclusions EUS-FNA is much more sensitive in diagnosing solid P-NENs than cystic PNETs. Our results indicate that EUS-FNA may have higher sensitivity for diagnosis of cystic P-NENs than the reported sensitivity of EUS-FNA for all pancreatic cystic tumors.

Introduction

Neuroendocrine tumors (NENs) describe a heterogeneous group of tumors with a wide range of morphologic, functional, and behavioral characteristics. Pancreatic neuroendocrine tumors (P-NENs) are subset of NENs and represent a small percentage of all pancreatic tumors (1.3 %) but their incidence is rising [1]. Incidence of PNETs may be significantly underestimated in tumor registries, including the Surveillance, Epidemiology and End Results (SEER) program, which include only malignant neoplasms. Most P-NENs are solitary, well-demarcated and well-differentiated neoplasms. Multifocal tumors are rare and should always raise suspicion of multiple endocrine neoplasia 1 (MEN1) or von Hippel Lindau syndrome (VHL).

Based on WHO classification (2017), P-NENs have been classified as: (1) well-differentiated PanNENs: pancreatic neuroendocrine tumors (PanNETs); (2) poorly differentiated PanNENs: pancreatic neuroendocrine carcinomas (PanNECs); or (3) mixed neuroendocrine: non-endocrine neoplasm [2]. Most P-NENs are solid tumors, although cystic variants have been described as well, which can be misdiagnosed as mucinous or serous cystadenomas of the pancreas [3].

When possible, surgical resection of localized P-NENs is recommended; hence, preoperative localization is essential. Based on several studies, computed tomography (CT) has reported sensitivity of 64 % to 82 % in diagnosis of P-NENs. Their sensitivity decreases further when lesion size is < 2 cm [4, 5]. Somatostatin receptor scintigraphy (SRS) is useful in non-insulinoma P-NENs with an overall reported sensitivity of 58 % to 86 %, whereas in insulinomas, its use is limited because of the lower density of somatostatin receptors [6, 7]. (68) Ga-DOTATATE positron emission tomography (PET/CT) is a new modality for replacing octreotide scan for small PNETs as long as they express somatostatin receptors. Sadowski et al. 2016 showed that (68) Ga-DOTATATE PET/CT detected P-NENs in 65.2 % of patients with negative biochemical testing but positive carcinoid symptoms. In the 65.2 % of patients with detected lesions, 40 % of the lesions were not even detected by CT/magnetic resonance imaging (MRI) and PET imaging studies [8]. EUS has been shown to be superior to other imaging methods in preoperative imaging and localization of P-NENs [4, 9]. EUS-FNA was first described for cytological confirmation of P-NENs in 2002 [9]. Several additional retrospective series describing the endosonographic features and usefulness of FNA in diagnosis on P-NENs have been published [2, 10, 11].

However, data are limited on diagnostic performance of EUS-FNA when comparing solid and cystic P-NENs. The sensitivity of EUS FNA assessed against a surgical gold standard is reported in few studies. To our knowledge, the largest study addressing this issue was published in 2010 [12], including 68 patients with P-NENs who underwent EUS-FNA for diagnosis, with a sensitivity of 87 %. There is also a relative dearth of studies describing chemical analyses of fluid aspirated from cystic P-NENs via EUS-FNA. Therefore, we performed a retrospective single-center study to assess sensitivity of EUS-FNA in diagnosing cystic and non-cystic P-NENs, and also to describe the EUS features of non-metastatic, cystic and solid P-NENs.

Patients and methods

We conducted a retrospective chart review of all cases of P-NENs between January 2005 and December 2012 seen at the Moffitt Cancer Center. The study was conducted with the approval of the Institutional Review Board (IRB#00001124). A database of all the P-NENs seen at our institution during that interval was used as the primary source. From this database, we identified all cases of patients with histologically confirmed, non-metastatic P-NENs who underwent EUS prior to surgical resection. This time frame was chosen to ensure that the results reflected the outcome expected from the current state of EUS technology.

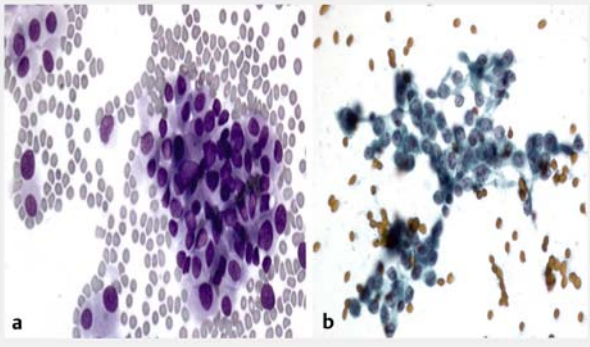
Electronic medical records of these patients were reviewed. The following clinical information was recorded: age, sex, presence of symptoms (i. e. weight loss, abdominal pain, flushing, diarrhea, jaundice, hypoglycemic episodes), and if presence of a P-NEN was suspected based on the symptoms, or pre-EUS radiological imaging.

EUS examination

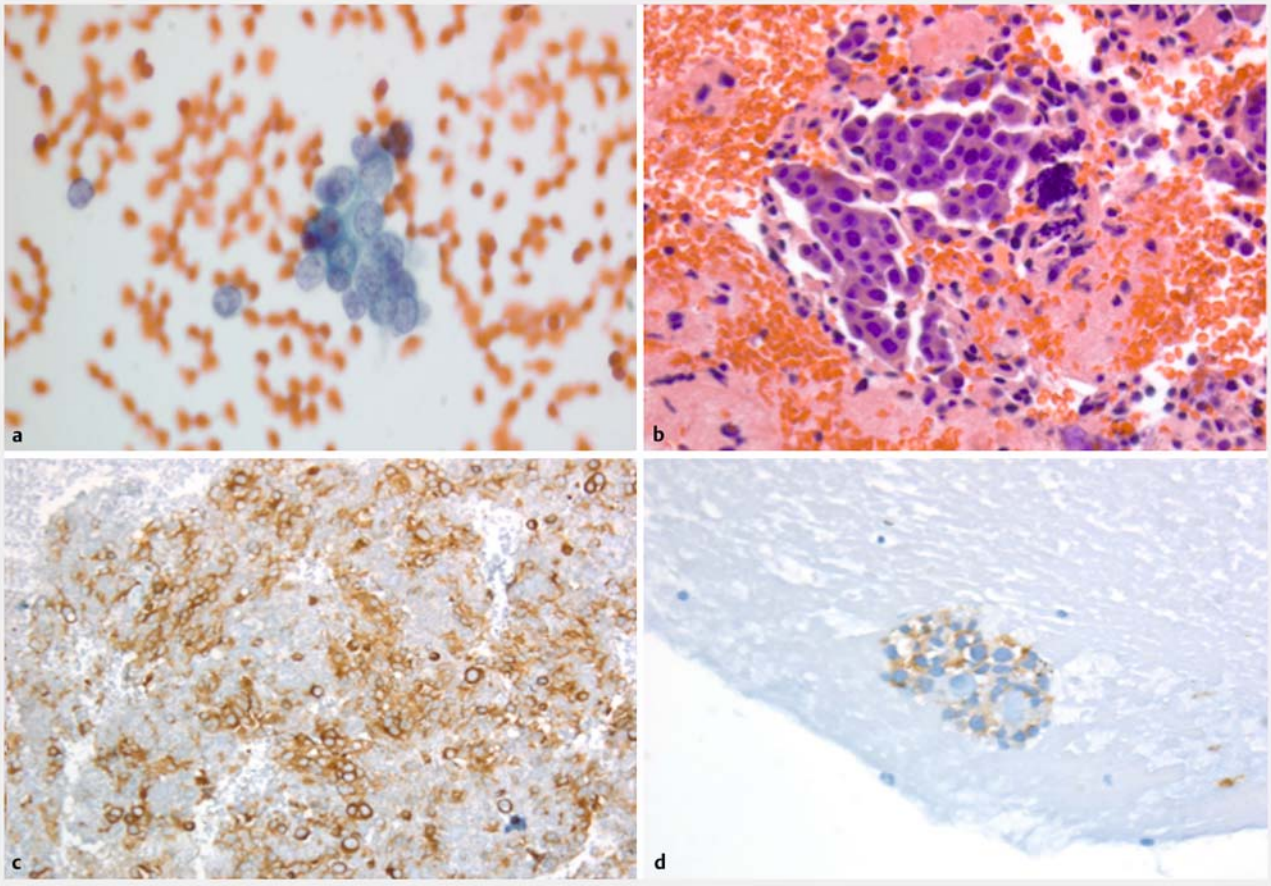
All procedures were performed by three experienced endosonographers after informed consent was obtained. The endosonographers had between 5 and 10 years' experience and had completed 3000 to 7000 EUS procedures. EUS was performed with an Olympus GF-UC140PAL5 curvilinear echo endoscope (Olympus America, Inc, Center Valley, Pennsylvania, United States). A cytopathologist was usually available on-site for preliminary interpretations. Cook Medical Echo Tip Ultra 25 G or 22 G needles were used for FNA and 22 G needle used for fluid aspiration in cystic tumors. The suction technique was preferentially used for EUS-FNA and fanning technique only in the case of solid lesions. Per policy, if EUS-FNA was performed on a cystic lesion, the patient received one dose of intravenous antibiotics (ampicillin/sulbactam or ciprofloxacin) followed by 3 to 7 days of oral antibiotics (amoxicillin/clavulinic acid or ciprofloxacin). Details of the EUS-FNA examinations were abstracted from the dictated reports, available from the electronic medical records.

Cytologic examination

Cytomorphological diagnosis of a P-NEN was considered based on the appearance of monomorphic cells with an eccentrically located, moderately large, round to oval nucleus with finely stippled and uniformly dispersed chromatin (▶ **Fig. 1a**, ▶ **Fig. 1b**, ▶ **Fig. 2a**, ▶ **Fig. 2b**). Immunohistochemical staining of chromogranin and synaptophysin was performed at the discretion of the pathologist (▶ **Fig. 2c**, ▶ **Fig. 2 d**). Each cytopathology and surgical pathology (▶ **Fig. 3a**, ▶ **Fig. 3b**) specimen was reviewed by one of three experienced gastrointestinal pathologists. If



▶ **Fig. 1** Pancreatic neuroendocrine tumor, solid lesion. **a** Smears are hypercellular, composed of a monomorphic population of neoplastic cells with round to oval nuclei and slightly eccentric cytoplasm, Diff Quik 60X. **b** Nuclei have finely distributed, salt-and-pepper chromatin. Papanicoloau, 60X.



▶ **Fig. 2** Pancreatic neuroendocrine tumor, cystic lesion. **a** Small groups of monomorphic cells and single cells with scant, amphophilic cytoplasm are identified on the cytospin. Nuclei are round to oval and the chromatin is finely distributed, with a salt and pepper pattern. Papanicolaou, 60X. **b** The cellblock shows single cells. Hematoxylin and eosin, 40X. **c** The neoplastic cells express synaptophysin and **d** chromogranin. Peroxiadase, 40X.

fluid was aspirated, fluid analysis included cytospin analysis, amylase and carcinoembryonic antigen (CEA) levels.

Statistical analysis

Surgical pathology was used as gold standard for EUS-FNA sensitivity analysis. Stata 10.0 software was used for statistical analysis. For analysis purposes, continuous variables were expressed as means and standard deviations, and dichotomous variables were expressed as simple proportions with or without 95 % confidence intervals (CI). Continuous variable associations were assessed with an unpaired *t* test. The association between categorical variables and malignancy was assessed with the Fisher exact test. $P < .05$ was considered significant.

Results

Patient population

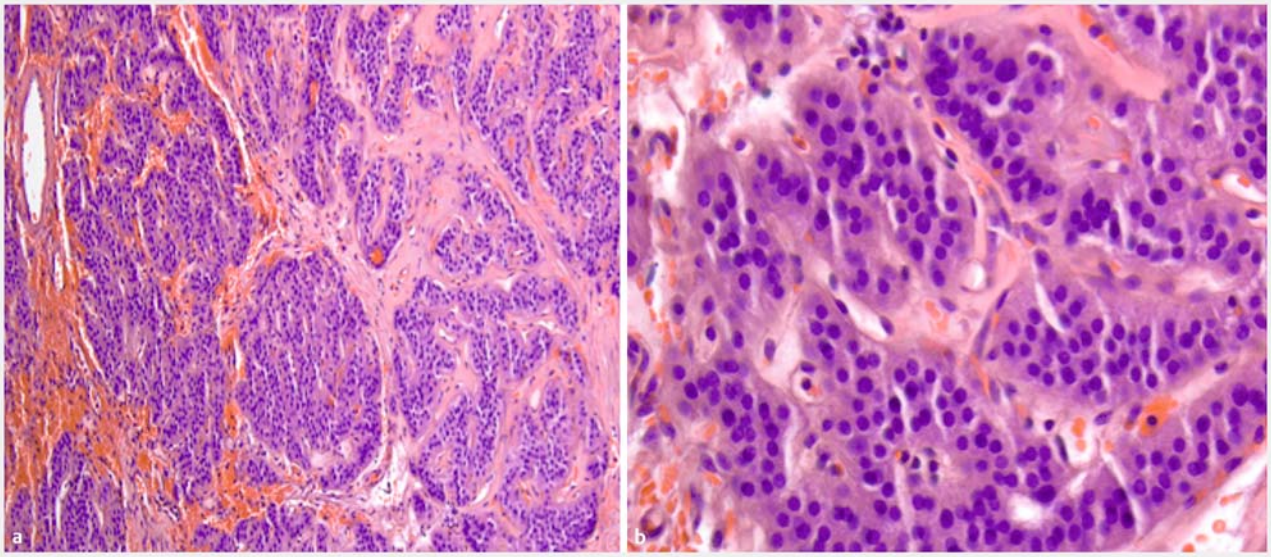
Thirty-nine patients met our inclusion criteria, 26 with solid P-NENs and 13 with cystic P-NENs. There was a higher preponderance of females in the cystic P-NEN group (▶ **Table 1**).

Clinical features

The majority of patients in our study had either no symptoms or non-specific symptoms (▶ **Table 1**). Most of the patients had some form of imaging performed prior to EUS, CT being the most common modality. All the patients in the solid P-NEN group had an abnormal imaging result on pre-EUS imaging, with a solid lesion being seen in 96 % of cases. Within the cystic P-NEN group, 84.6 % of cases had an abnormal imaging result. A cystic component to the lesion was picked up in only 53.8 % of cases (▶ **Table 1**)

Endosonographic features

Cystic tumors were more commonly seen in the body and tail of the pancreas whereas solid tumors were uniformly distributed throughout the pancreas ($P < 0.02$). There was no significant difference in size between cystic and solid tumors. The main pancreatic duct and rest of the pancreatic parenchyma were normal-appearing in a majority of the cases (91.7 % and 94.1 %, respectively). The majority of the solid P-NENs were well demarcated (66.7 %). The echotexture of the solid P-NENs was mostly hypoechoic or heterogeneous. The cystic component



▶ **Fig. 3** Pancreatic neuroendocrine tumor, resection. **a** Low-power image shows monomorphic neoplastic cells arranged in a trabecular and gyriform pattern surrounded by vascular, fibrous stroma. Hemaotxylin and eosin, 10X. **b** Neoplastic cells have round, uniform nuclei with salt and pepper chromatin and fine granular, eosinophilic cytoplasm. Hemaotxylin and eosin, 40X.

▶ **Table 1** Patient demographics and clinical characteristics.

	Solid P-NEN N = 26	**Cystic P-NEN N = 13**	***P***
Age mean (SD)	56.4 (13.1)	63.8 (10.5)	0.1
Sex, %	M, 30.8 F, 69.2	M, 61.5 F, 38.5	0.06
Symptoms, n (%)	Asymptomatic 9/26 (34.6) Nonspecific Sx 15/26 (61.5) Specific Sx 2/26 (7.6)	Asymptomatic 8/13 (61.5) Nonspecific Sx 5/13 (38.5) Specific Sx 0/13	0.2
Pre-EUS radiology, n (%)	CT 23/26 (92) MRI 1/26 (4) US 1/26 (4) None 1/26	CT 12/13 (92.3) MRI 1/13 (7.8)	0.7
Radiological* diagnosis, n (%)	No Lesion 0/25 Cystic lesion 0/25 Solid Lesion 24/25 (96) Solid/Cystic lesion 0/25 Fullness 1/25 (4)	No Lesion 2/13 (15.4) Cystic lesion 7/13 (53.8) Solid Lesion 2/13(15.4) Solid/Cystic lesion 2/13 (15.4) Fullness 0/13	NA

P-NEN, pancreatic neuroendocrine neoplasms; SD, standard deviation; M, male; F, female; Sx, symptoms EUS, endoscopic ultrasound; CT, computed tomography; MRI, magnetic resonance imaging; US, ultrasound
* Radiological diagnosis for solid P-NEN was available for only 25 patients.

of the cystic P-NENs was most commonly anechoic. Among the cystic tumors, 50 % were partly cystic and partly solid, and 50 % were fully cystic. Vascular involvement was seen in only one solid P-NEN, and in none of the cystic P-NENs (▶ **Table 2**).

FNA results

FNA was performed for 23 of 26 of solid P-NENs (▶ **Table 3**). Most of the operators used a 25G needle. A diagnosis could be obtained in two to five passes in the majority of cases, yielding an overall sensitivity of 95 % (19/20) for EUS-FNA for solid P-NENs when compared with surgical pathology. Among the cystic P-NENs, FNA of the solid component was performed in five

▶ **Table 2** Endosonographic features of P-NENs.

	Solid P-NEN N = 26	Cystic P-NEN N = 13	*P*
Location, n (%)	Head 6/26 (23.1) Neck 5/26 (19.2) Body 5/26 (19.2) Tail 4/26 (15.4) Uncinate 6/26 (23.1)	Head 2/13 (15.4) Neck 1/13 (7.7) Body 3/13 (23.1) Tail 7/13 (53.8) Uncinate 0/11	0.1
Size Mean (SD)	23 (11.7) mm	22.3 (12.1) mm	0.7
Margins[1], n (%)	Well-demarcated 17/24 (70.8) Poorly demarcated 7/24 (29.16)	NA	NA
Echotexture[2], n (%)	Hypoechoic 15/26 (57.7) Hyperechoic 1/26 (3.9) Heterogeneous 8/26 (30/8) Isoechoic 1/26 (3.9) Hypo with Anechoic Features 1/26 (3.9)	Anechoic 10/13 (76.9) Anechoic with debris 1/13 (7.7) Hypoechoic 2/13 (15.4) Septations 1/13 (7.7) Mural Nodules 2/13 (15.4)	NA
PD Dilation	0/26	0/13	NA
Extra lesional parenchyma	Normal-appearing 26/26	Normal-appearing 13/13	NA
Vascular involvement	1/26	0/13	NA

P-NEN, pancreatic neuroendocrine neoplasm; SD, standard deviation; NA not applicable; PD, pancreatic duct
[1] Margins were identified in 24 of 26 patients for solid P-NENs.
[2] For the cystic P-NENs, only the echotexture of the cyst is reported among the partly cystic P-NENs

▶ **Table 3** Fine-needle aspiration results of solid P-NENs (FNA performed on 23/26 solid P-NENs).

Needle used, n (%)	19G – 1/23 (4.3) 22G – 4/23 (17.4) 25G – 18/23 (78.3)
Passes made, n (%)	2 – 5 passes – 17/23 (73.9) 6 – 9 passes – 6/23 (26.1)
Cytology results, n (%)	P-NEN 22/23 (95.6) Non-Diagnostic 1/23 (4.3)
Overall sensitivity of EUS-FNA compared with surgical pathology*, n (%)	19/20 (95)

P-NEN, pancreatic neuroendocrine neoplasm; FNA, fine-needle aspiration; EUS, endoscopic ultrasound
* Surgical pathology available for 20 of 23 solid P-NEN patients, of whom only 19 were confirmed solid P-NENs.

▶ **Table 4** Fine-needle aspiration results of cystic P-NENs (N = 13).

FNA of solid component	5/13
FNA of systic component[1]	9/13
Needle used	Solid – 25 G Cystic – 22 G
Passes done	Solid – 3 to 7 Cystic – 1 to 2
Cytology solid, n (%)	P-NEN – 3/5 (60) Carcinoma – 1/5 (20) Non-diagnostic 1/5 (20)
Cytology cystic, n (%)	P-NEN 6/9 (66.7) MCN 1/9 (11.1) No diagnostic 2/9 (22.2)
Fluid amount	4.9 ± 3.6 cc
Fluid CEA (n = 4)	6.6 ± 12.1 < 192 in call cases
Fluid amylase (n = 4)	203 ± 180 WNL in all cases
Overall sensitivity of EUS-FNA compared with surgical pathology[2], n (%)	5/8(62.5)

P-NEN, pancreatic neuroendocrine neoplasm; MCN, mucinous cystic neoplasm; FNA, fine-needle aspiration; CEA, carcinoembryonic antigen; WNL, within normal limits
[1] One case had both solid and cystic component sampled
[2] Surgical pathology was available for eight of 13 cystic P-NEN cases only, of which only five were confirmed cystic P-NENs.

of 13 cases, and cystic component in nine of 13 cases, with a 25G needle and a 22G needle, respectively. One case had both solid and cystic component biopsied (▶ **Table 4**). Cytological diagnosis of P-NENs was obtained from three of five cases (60 %) from the solid component, and six of nine cases (66.7 %) from the cystic component. Surgical pathology was available for eight of 13 cystic P-NEN cases only. Of them, only five were confirmed cystic P-NENs, yielding an overall sensitivity of EUS FNA for cystic P-NENs of 62.5 % (5/8). Fluid could be aspirated from 50 % of the cystic tumors, all with a CEA level < 192 ng/mL, and a normal amylase level. Mean amount of fluid aspirated was 4.9 cc. No adverse events were encountered in our study population.

Discussion

Diagnosis of P-NENs is difficult with standard imaging techniques [4, 10, 12, 13]. EUS- FNA has emerged as a promising and highly accurate tool in imaging, diagnosing, and staging P-NENs. A majority of the P-NENs in most of the published series have been non-functioning P-NENs, which matches our experience. The EUS features of solid P-NENs were consistent with other major series [9], with most of them being well demarcated, with a normal-appearing pancreas, and a majority presenting without vascular involvement. The echotexture of the solid P-NENs was mostly hypoechoic or heterogeneous. In our study, the solid P-NENs had a relatively uniform distribution in the pancreatic head versus body or tail region, which is consistent with other series.

The EUS features of cystic PNETs have not been very well characterized in the literature. The largest series on cystic P-NENs had 50 patients [14]. All of them were non-functioning P-NENs, which is again similar to other series. The majority of the cystic P-NENs in our series were in the body or tail region, and their cystic component was anechoic with no vascular involvement. Based on these EUS features, the cystic P-NENs could be mistaken for mucinous cystadenomas or side branch IPMN of the tail region.

EUS-FNA again proved to be highly sensitive for diagnosing solid P-NENs, and it could establish the diagnosis in 22 of 23 cases (95 %), which is consistent with other series [9, 12]. For cystic P-NENs, it could establish diagnosis in six of nine cases (66.7 %), and it required FNA of both the solid and cystic components. This is the first assessment of the sensitivity of EUS-FNA for cystic P-NENs, to the best of our knowledge. Another important, and new piece of information is that CEA and amylase levels in the fluid aspirated from these cystic tumors was normal in all cases, helping to distinguish them from mucinous cystic neoplasms. Serous cystadenomas are the only other cystic neoplasms of the pancreas, which have similar fluid analysis findings,

Conclusion

In summary, we present experience with EUS-FNA at a large National Cancer Institute-designated cancer center for diagnosing solid and cystic P-NENs. Sensitivity of EUS-FNA for solid PNETs is excellent, but is lower for cystic P-NENs. Often FNA of the solid and cystic P-NENs is required to establish the diagnosis. We recommend that cystic P-NENs be considered in differential diagnosis of cystic neoplasms of the pancreas, especially if the CEA level is below the cutoff of 192 ng/mL.

Competing interests

None

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