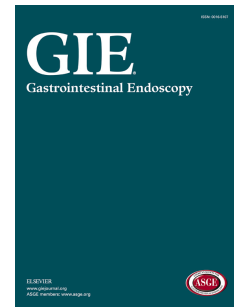


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Sonographic and cyst fluid cytological changes after EUS-guided pancreatic cyst ablation

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Running Title: Sonographic and cytological changes following EUS-FNA

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Abstract

Background and Aims: The effect of EUS-guided pancreatic cyst ablation (PCA) on sonographic morphology and cyst fluid cytology is unknown. The aim of this study is to evaluate morphological, cytological and change in cyst fluid DNA after PCA.

Methods: In a prospective single center study, consecutive patients with suspected benign 10 to 50 mm pancreatic cysts underwent baseline EUS-FNA and EUS-PCA followed 2 to 3 months later by repeat EUS, cyst fluid analysis and possible repeat PCA. Surveillance imaging after ablation was performed at least annually and classified as complete (CR), partial (PR), or persistent with <5%, 5% to 25%, and 25% of the original cyst volume, respectively.

Results: 36 patients underwent EUS-PCA with ethanol alone (n = 8) or ethanol and paclitaxel (n = 28) and CR occurred in 19 (56%). After EUS-PCA, EUS showed an increase in wall diameter in 68%, decreased number of septations in 24%, increased debris in 24%, loss of mural nodule or novel calcification in 21%, and alteration of fluid viscosity in 48%. Follow-up cytology showed increased epithelial cellularity in 27%, loss or decreased cellular atypia in 15%, and increased or appearance of macrophages in 24% and inflammatory cells in 15%. Post-ablation DNA amount increased and quality decreased in 71% each. Between the CR and non-CR patients, there was no significant difference in frequency of sonographic or cytological features. In the CR group, mean DNA quantity was significantly increased after ablation ($p=0.023$) without a change in quality ($p=0.136$).

Conclusions: EUS-PCA induces morphological and cytological changes of the pancreatic cysts none of which appear to predict overall imaging-defined response to ablation.

INTRODUCTION

Asymptomatic or symptomatic pancreatic cystic lesions (PCLs) are frequently diagnosed with the widespread of cross-sectional diagnostic modalities such as computed tomography (CT) or magnetic resonance imaging (MRI). These cysts range from inflammatory (pseudocyst) or benign (serous cyst adenoma, SCA) lesions to premalignant (mucinous cystic neoplasm [MCN] or intraductal papillary mucinous neoplasm [IPMN]) or malignant cysts. The management of pancreatic cysts is principally based on accurate identification of related symptoms and malignant potential. Symptomatic or premalignant cysts often require surgical resection, yet surgical resection or enucleation is associated with high perioperative morbidity (20% to 40%) and mortality rate (~ 2%).¹⁻⁵ Therefore, EUS-guided pancreatic cyst ablation (EUS-PCA) with ethanol and/or paclitaxel has been investigated for non-

operative treatment of PCLs in patients potentially at high risk for or averse to surgery.^{6,7} Cyst ablation with ethanol and paclitaxel leads to a complete (<5% of original cyst volume) or partial (5%-25% of original cyst volume) image-defined response in 60%-70% of patients and may lead to elimination of baseline cyst fluid DNA mutations.^{6,7} However, the effect of ablation on cyst sonographic morphology, cyst fluid cytology and the quality and quantity of cyst fluid DNA is unknown. The primary aim of this single center prospective study was to evaluate changes of cyst fluid cytology and sonographic morphology after EUS-PCA with ethanol lavage alone or combined with paclitaxel injection. The secondary aim was to evaluate the qualitative and quantitative alteration of DNA after ablation.

PATIENTS AND METHODS

Study population

This is a single-center prospective study on consecutive patients who underwent pancreatic cyst ablation at Indiana University Health Hospital over a 10-year period. This study was approved by the Institutional Review Board at Indiana University Health Hospital, and all patients signed informed consent before enrollment (Clinical-Trials.gov identifiers NCT00233038 and NCT01643460). Patients considered for ablation were at least 18 years of age and referred for evaluation of a pancreatic cyst detected by previous cross-sectional imaging that measured 10 to 50 mm in diameter and contained 5 or fewer septations. Most patients treated had cysts that met criteria for surgical resection yet surgery was either refused by the patient or the patient was regarded as unfit for surgery by the referring physician or surgeon.⁸ Cysts were not considered for treatment if any of the following criteria were present: pregnancy, high risk for respiratory failure due to deep sedation with propofol (American Society of Anesthesiology class IV or V), acute pancreatitis and pancreatic necrosis, ascites, portal hypertension, suspicious malignancy including pancreatic cancer, and coagulopathy (international normalized ratio >1.5, activated partial thromboplastin time >50 seconds, platelet count <50,000/ μ L, use of antiplatelet medications or anticoagulants that could not be discontinued).

Study design

Baseline demographics, symptoms and radiographic data were recorded in all patients. Before ablation, EUS morphology (i.e. septations, cyst wall thickness, presence of nodules) and maximal 2-dimension cross-sectional diameter were recorded. Cyst fluid aspiration was then performed and the quantity, viscosity and color of fluid were documented. The sample was sent for cytology in all patients and carcinoembryonic antigen (CEA) and molecular analysis (RedPath Integrated Technologies) in selected patients. Patients underwent initial ablation with saline solution or ethanol alone (as part of a randomized trial) from 2004 to 2009⁹ or ethanol plus paclitaxel (in a prospective cohort study)⁷ from 2009 to 2014. After index ablation, all patients underwent follow-up EUS 2 to 3 months later for assessment of any interval changes in sonographic morphology of the treated cyst. During this first follow-up EUS during the years 2004 to 2009, diagnostic EUS was followed by FNA for cytology and finally an index or second ethanol lavage (depending on initial randomization). For patients treated initially with ethanol and paclitaxel from 2009 to 2014, the first follow-up EUS consisted of diagnostic

EUS, repeat EUS-FNA for cytology in all patients and molecular analysis (when possible) and finally repeat cyst ablation in patients with an initial suboptimal response. In all patients (regardless of initial ablation regimen), repeat CT, MRI, or EUS was performed 3 to 6 months later and then annually to assess for size change from ablation or possible recurrence. Repeat EUS-FNA in previously ablated cysts was performed on a case-by-case basis. All 3-dimensional CT or MR images for baseline and follow-up assessment were interpreted by a single radiologist.

Cyst fluid aspiration and lavage process

Details regarding the process of cyst ablation have been described elsewhere.^{7,9} Briefly, a curvilinear-array echoendoscope (Olympus GF-UC140P-AL5; Olympus America Inc., Center Valley, Pa, USA) was used to puncture the cyst via transgastric or transduodenal route using a single pass of a 22-gauge needle (EchoTip Ultra; Cook Endoscopy Inc., Winston-Salem, North Carolina; or Expect, Boston Scientific America, Natick, Mass, USA). After near total collapse of the cyst, 100% ethanol was injected through needle into the cyst using the same volume as that initially aspirated. After lavage of the cyst contents repeatedly for 3 to 5 minutes, the lesion was nearly completely drained of fluid in all patients. After 2009, with the needle still within the cyst, paclitaxel (Bedford Laboratories, Bedford, Ohio, USA) at a concentration of 2 mg/mL (supplied as 6mg/ mL and diluted 1:2 with normal saline solution) was injected into the cyst (using a volume equal to that initially aspirated from the cyst) and left in place.

Cytology slide evaluation

Cytology slides from all baseline and post-ablation FNA specimens were prepared by both an air-dried modified Diff-Quik stain and a wet-fixed modified Pap stain. Slides were retrieved and reviewed for each patient in random order by a single, blinded cytopathologist for the amount (none, few, moderate, excessive) of mucin, inflammatory cells, macrophages, amount (acellular, hypocellular, cellular) and atypia (none, mild to moderate, severe) of epithelial cells and cellular debris in the entire slide sample. When more than one post-ablation sample was obtained, only the first sample obtained after ablation was compared to the baseline sample.

Study definitions

Baseline and post-ablation cyst volume were evaluated by 2-dimensional (linear EUS) or 3-dimensional (CT or MRI) measurements. Two-dimensional cyst volume was measured using the formula $4/3\pi r^3$, where r represents radius of the maximal cyst by linear EUS image. Three-dimensional volume was calculated by the simplified formula $d1 \times d2 \times d3 / 2$, where $d1$, $d2$, and $d3$ represent the maximal diameters in the axial, coronal, and sagittal planes, respectively.¹⁰ Changes in cyst size measured by axial CT or MRI after ablation were defined as complete response (CR), partial response (PR), or persistent with <5%, 5% to 25%, and >25% of the original cyst volume, respectively.^{6,9} Cysts were classified according to available information including cyst fluid analysis (cytology, amylase, CEA, baseline DNA data, and viscosity) and the presence of communication with

the main pancreatic duct. Viscosity was classified as thin, slightly viscous and highly viscous based on visual inspection of both the fluid aspirated in the syringe and fluid expressed on the microscope slide during in-room cytology preparation. In current study, cyst fluid CEA >192ng/mL was considered to be an MCN or IPMN and when analyzed, a cyst fluid amylase >800U/L was considered suggestive of IPMNs or pseudocysts.^{11, 12} Both cyst fluid CEA < 192ng/mL and cyst fluid amylase < 800U/L was suggestive of a serous cystic neoplasm.¹³ If cyst fluid analysis was not compatible with these criteria, a clinical diagnosis was rendered based on the available information. Adverse events were classified according to the published criteria.¹⁴

Cyst fluid DNA mutational analysis

Molecular analyses were performed by laboratory personnel who were blinded to clinical and management features as well as any prior molecular analysis on an individual patient. DNA was extracted from 200 μ L of pancreatic cyst fluid (Qiagen, Valencia, Calif, USA) and quantified by ultraviolet-visible spectrophotometry (NanoDrop, Wilmington, Del, USA). DNA amplifiability was then determined by quantitative polymerase chain reaction (PCR; iCycler; BioRad, Hercules, Calif, USA).¹⁵ ¹⁶ Cycle threshold is measured in DNA quality (a parameter of degree of DNA strand degradation) and is measured and quantified by PCR on the DNA with primers. The critical cycle threshold (Ct) value means a critical point, number of cycle where the DNA suddenly becomes visible. If Ct value is ≤ 27.5 , it is categorized as good quality DNA, however, if Ct value >27.5, poor quality DNA. Optical density (OD) was used as a measure of DNA quantity at 260/280 wavelength.

Statistical analysis

EUS morphological and cytological changes before and after PCA were evaluated and results were compared between CR and non-CR (PR and persistent) patients. Continuous variables were described as means \pm standard deviation. The Fisher exact test and linear by linear association were used to compare categorical parameters between the 2 groups (CR vs non-CR). The Wilcoxon signed rank test was applied for nonparametric statistics regarding DNA analysis (DNA quantity and quality) between baseline and post-ablation. Differences with a *P* value less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 16 (SPSS, Inc., Chicago, Ill, USA)

RESULTS

Baseline characteristics and study algorithm

Between October 2004 and July 2015, 36 patients (mean age 69.1 ± 12.2 years, 24 female) underwent cyst ablation. Baseline demographics, symptom, imaging and clinical diagnosis are summarized in Table 1. Of the 36 patients, 22 (61.1%) cysts were found in the body and tail. Median follow-up (time from initial EUS-PCA to final CT, MRI, or EUS) was 22.3 months (range 3.0-119.9). The mean original 3-dimensional CT or MRI and 2-dimensional EUS cyst volume were 10.1 ± 10.3 mL

(range 0.5-38.3) and 12.1 ± 11.2 mL (range 0.5-41.6), respectively. Eleven (30.6%) patients were symptomatic before treatment. Median cyst fluid CEA (n=33) and amylase (n=27) were 444 ng/mL (range 0-156,600) and 162 U/L (range 5-327,297), respectively. Presumed clinical diagnosis were 16 (44.4%) MCN, 14 (38.9%) branched IPMN, 5 (13.9%) SCA and 1 pseudocyst. The schematic algorithm for cyst ablation and genetic evaluation of the pancreatic cyst fluid is illustrated in Figure 1 and Figure 2.

Pancreatic cyst ablation and adverse events

Of the 36 patients, EUS-PCA was performed with ethanol alone in 8 (22%) and a combined ethanol lavage with paclitaxel injection in 28 (78%) (Fig. 1). A second and third ablation were performed in 17 (47%) and 1 (3%), respectively. Repeat ablation was not performed in remaining 18 (50%) patients (Fig. 1) due to acute pancreatitis (n=3), decreased cyst size (n=11), decreased cyst size with increased internal debris (n=1), pseudocyst formation at gastric wall (n=1), markedly increased internal debris (n=1) and refusal (n=1) after the first ablation. Except for 2 patients who did not receive follow-up 3-dimensional cross-sectional imaging (CT or MRI), follow-up imaging study in 34 (94%) demonstrated a median volume change of -97% (range -100% to +220%) compared with baseline (Fig. 3). By study definition, a complete response, partial response and non-response were achieved in 19 out of 34 (56%), 7 (21%) and 8 (23%), respectively. Including follow-up examinations, a total of 54 ablations were performed with 9 (17%) procedure-related adverse events including abdominal pain in 4 (7%), pancreatitis in 4 (7%) and intracystic hemorrhage in 1 (2%). All 4 patients with pancreatitis required hospitalization for 6 to 8 days and were discharged without further interventions.

Sonographic change and cytological change after ablation

Post-ablation EUS examinations were performed in 34 patients with follow-up cross-sectional imaging. The development of any sonographic alterations between baseline and any post-ablation examinations are shown in Table 2. After EUS-PCA, follow-up EUS showed an increase in cyst wall diameter in 23 out of 34 (68%) (Fig. 4A, B). Eight (24%) patients had decreased number (n=3, 9%) or loss of septations (n = 5, 15%) (Fig. 5A-D) whereas more septations were noted in 2 (6%). Intracystic debris developed in 8 (24%) (Fig. 6A, B). After ablation, there was a disappearance of mural nodule in 5 (15%), development of novel mural calcification in 1 (3%), and both mural nodule loss and development of calcification in 1 (3%) (Fig. 7A-C). No difference in sonographic changes was present between complete responders compared to those with a partial or no response. Similar results were obtained when comparing the persistent group to the CR + PR groups.

Cytological changes between pre-ablation and post-ablation specimens in 34 patients are summarized in Table 3. Median interval between baseline and first post-ablation cytology was 3.3 months (range 1.9-30.1). Twenty-four patients had 1 specimen whereas 10 had 2 or more. Follow-up cytology after PCA showed overall increased epithelial cellularity in 9/34 (27%). Cellular atypia after ablation was eliminated or decreased in 5 (15%) and increased or newly developed in 3 (9%). Microscopically, an increase of debris was observed in 12 (36%), and an increase or new appearance of macrophages in 8 (24%) and inflammatory cells in 5 (15%). Grossly, there was alteration of cyst

fluid viscosity in 14 (48.3%) after ablation. No difference in cytological changes was present between complete responders compared to those with a partial or non-response. Similar results were obtained when comparing the persistent group to the CR + PR groups, except viscosity change ($p=0.013$).

Molecular DNA analysis

Baseline, pre-ablation cyst fluid DNA evaluation in 20 patients who underwent ablation with ethanol and paclitaxel analysis (Fig. 2) showed a mean DNA quantity (OD) and quality (Ct value) of 60.4 ± 239.6 ng/uL (range 1.6-1,078.0) and 29.3 ± 2.8 (range 24.3-36.8), respectively. In three, post-ablation DNA analysis was not available because of failed amplification ($n=1$), 1 patient who refused the test ($n=1$) and in 1 who was lost follow-up ($n=1$). For the remaining 17 patients, mean post-ablation DNA quantity and quality were 35.8 ± 60.6 ng/uL (range 1.5-255.4) and 27.1 ± 2.9 (range 23.8-32.9), respectively. When classified by imaging response, post-ablation DNA amount increased in 12 out of 17 (70.6%), including 10 of 12 (83.3%) in the CR group, whereas overall post-ablation DNA Ct value decreased in 12 of 17 (70.6%) patients, including 9 of 12 (75%) in the CR group. For the CR group, mean DNA quantity was significantly increased after ablation (44.8 ± 70.7 vs 6.7 ± 9.5 , $p=0.023$), but no change in quality ($p=0.136$) (Table 4).

DISCUSSION

EUS-PCA with ethanol alone or in combination with paclitaxel has emerged as a safe and feasible alternative to surgery in the management of benign cystic lesions.^{6, 9, 17} Previous studies have evaluated response to ablation principally by results of cross-sectional imaging or surgery performed after ablation.^{6, 9, 17} However, the sonographic and cytological changes after pancreatic cyst ablation have not been evaluated.

In the current study, we found that ablation was associated with an increase in cyst wall diameter in 68% of patients. We hypothesize this increase results from epithelial denuding, fibrosis and chronic inflammation of the wall that has been reported in patients undergoing surgery after pancreatic cyst ablation.^{6, 9} It is likely that an ablative agent may activate unknown mediators that cause an inflammatory response and resultant damage to the epithelial lining cells of cystic wall.¹⁸⁻²⁰ Histological examination of surgical specimens of thyroid nodules after percutaneous ethanol injection also showed irreversible fibrous change, hemorrhage, and granulation tissue formation in the central lesion.^{21, 22} The observed cytological changes in the cyst fluid in a minority of patients after ablation (increased cellularity, inflammatory cells, macrophages) have also been described after ablation of hepatic and thyroid cysts and likely reflect cystic wall destruction.^{23, 24} The plausible cellular mechanism likely reflects a cascade of inflammation induced by the ablative agent which changes cellular elements of the cyst by mobilizing inflammatory cells and macrophages.^{20, 23}

Additional sonographic changes noted included a decreased number or loss of septations in 39%, increased internal debris in 24% and loss of mural nodule loss or calcification in 21%. Increased intracystic debris may be a combination of lysed blood cells, sloughed lining cells, contaminated cells

and mucin from ablation of cyst epithelium.^{23, 24} Loss of visible nodules likely results of destruction of mucin adherent to the cyst wall and less likely treatment of an epithelial nodule. The treatment of septated cysts were first reported by Oh et al.²⁵ These authors postulated that the loss or decreasing numbers of the ablated septae may be affected by the number of needle punctures, thickness of septae, and the size of locules. Interestingly, nine patients in our study showed increased numbers of septae after ablation. The reasons for this finding are not clear but may reflect a post-inflammatory response to ablation. We found no differences in the frequency of sonographic or cytological features assessed between patients with a complete and incomplete response to ablation.

We found that pancreatic cyst ablation increased the quantity and decreased the quality of cyst fluid DNA sampled after ablation with ethanol combined with paclitaxel. Furthermore, there was a significant increase of the OD value in CR group without any difference in observed Ct. These findings may reflect epithelial cell turnover after ablation. However it is also possible that the observed changes may reflect one or both ablative agents alone or the release of DNA from the influx of inflammatory cells or blood lysates in the ablated cyst fluid. The observed alterations in DNA quality and quantity may support a previous observation that mutant cyst fluid DNA may be eliminated with pancreatic cyst ablation.⁷

Abdominal pain, pancreatitis, and intracystic hemorrhage are most important adverse events related to the cyst ablation. A series of previous studies have reported that overall procedure-related adverse events have included pancreatitis ranging from 3% to 10% and abdominal pain in up to 13% of patients, which concurs with this study.^{7, 9, 17, 25, 26} In 54 ablations, procedure-related adverse events included abdominal pain (7%), pancreatitis (7%) and intracystic hemorrhage (2%). In particular, a total of 4 pancreatitis patients had full recovery without further interventions and no case of serious adverse events such as venous obliteration or thrombosis occurred in this study.²⁶

The current study is the first to describe sonographic and cytological changes after pancreatic cyst ablation. Furthermore, pathology slides and most cross sectional radiographs were reviewed by a single cytopathologist and radiologist, respectively. However, our study has several limitations. First, 2 different ablative regimens were used for the study population and may have led to different outcomes. Second, DNA changes were only observed for patients treated with ethanol and paclitaxel. Therefore, the effect of ablation with ethanol alone on cyst fluid DNA cannot be assessed. Third, the sample sizes are limited with only 34 patients with follow-up imaging and 17 with post ablation molecular DNA analysis. A final limitation of this study is that surgical or histological samples of the treated cysts were not obtained because most patients responded to endoscopic treatment alone.

In conclusion, EUS-PCA induces morphological and cytological changes of the pancreatic cysts which appear to reflect ablation of cyst wall epithelium. However, none of which appear to predict overall imaging-defined response to ablation.

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Legends of figures

Figure 1. Study profile for EUS guided ablation for pancreatic benign cysts

EUS, endoscopic ultrasound

Figure 2. Profile for DNA analysis before and after pancreatic cyst ablation in 20 patients

*Neither follow-up CT nor DNA analysis in this patient was done. *EUS*, endoscopic ultrasound

Figure 3. Percent change in cyst volume in 32 patients with follow-up imaging after pancreatic cyst ablation

Figure 4.

A, Linear EUS in a 54-year-old woman, demonstrating a single 24mm X 20mm cyst with thin cystic wall measured ~1mm in the head/neck junction of the pancreas. The clinical diagnosis was mucinous cystic neoplasm. The patient underwent an ethanol lavage combined with paclitaxel. **B**, A follow-up EUS 3 months later demonstrated a single 15mm X 8mm cyst. The outer wall of the lesion was homogenously thickened measuring 3.5mm.

Figure 5.

A, Linear EUS in a 77-year-old woman, demonstrating a 32mm X 20mm cyst in maximal cross-sectional diameter with multiple, thin septa in the body/tail of the pancreas. The clinical diagnosis was branched intraductal papillary mucinous neoplasm. **B**, The largest locule downstream measuring 19mm X 16mm (**arrows**) was treated with an ethanol lavage combined with paclitaxel using a 22 gauge needle. **C**, A follow-up EUS 3 month later demonstrated that an 11mm X 8mm downstream cyst previous treated was smaller than previous study. Another 14mm X 11mm upstream (**arrows**) was ablated with same regimen. **D**, A follow-up EUS 14 month later demonstrated a 5mm X 5mm cyst at body.

Figure 6.

A, Linear EUS in a 65-year-old woman, demonstrating a single 18mm X 10mm cyst without internal debris within the fluid-filled cavity in the body of the pancreas. The clinical diagnosis was branched intraductal papillary mucinous neoplasm. The patient underwent an ethanol lavage combined with paclitaxel. **B**, A follow-up EUS scan 4 month later demonstrated a single 14mm X 11mm cyst. The outer wall of the lesion was thick and there was abundant internal debris (**arrows**) within the fluid-filled cystic cavity most likely representing necrosis. Therefore, no additional ablation was performed due to nearly complete internal necrosis. SA, splenic artery

Figure 7.

A, Linear EUS in a 64-year-old woman, demonstrating a 26mm X 18mm nonseptated cyst in the uncinata pancreas. A mural nodule (**arrow**) measuring 5mm X 6mm was present along the medial wall of the cyst. The clinical diagnosis was IPMN. The patient underwent an ethanol lavage combined with paclitaxel. **B**, A follow-up linear EUS 3 month later demonstrated a 16mm X 10mm cyst with thick wall measuring 2.4mm. There was a mural nodule (**arrow**) within the cyst measuring 3mm X 3mm. Second ablation was done by the same regimen. **C**, A follow-up radial EUS 9 months later demonstrated an 11mm X 8mm cyst with disappearance of mural nodule.

TABLE 1. Baseline demographics and imaging characteristics of 36 patients with pancreatic

cysts treated with ethanol and/or paclitaxel

Variables	Results (n=36)
Age (years)	69.1 ± 12.2
Mean ± SD	
Gender	
Female	24 (67)
ASA class	
I	3 (8.3)
II	26 (72.2)
III	7 (19.4)
Location	
Body, tail	22 (61.1)
Head, neck, uncinata	14 (38.9)
Symptomatic cyst	11(31)
Follow-up duration (months)	
Median (range)	22.3 (3.0-119.9)
CT volume (mL)	
Mean ± SD	10.10±10.29
Range	0.50-38.26
EUS maximal diameter (mm)	
Mean ± SD	25.8 ± 8.7
Range	10-43
Volume (mL)	12.07 ± 11.20 (0.52-41.63)
Cystic fluid CEA (ng/mL)	
Median (range)	444 (0-156,600)
Cystic fluid Amylase (Units/L)	
Median (range)	162 (5-327,297)
Presumed diagnosis	
Branched IPMN	14 (38.9)
MCN	16 (44.4)
SCA	5 (13.9)
PC	1 (2.8)

The values are presented as mean ± SD, median (range) or numbers (%). *IPMN*, intraductal papillary mucinous neoplasm; *MCN*, mucinous cyst neoplasm; *SCA*, serous cyst adenoma; *PC*, pseudocyst.

TABLE 2. Sonographic change of pancreatic cysts after EUS-guided pancreatic cyst ablation in

34 patients

Sonographic change	CT response*			P value
	Total (n=34)	CR ¹ (n=19)	Non-CR ¹ (n=15)	
Wall thickness				
Increase	23 (67.6)	14 (73.7)	9 (60.0)	0.316
Decrease or no change	11 (32.4)	5 (26.3)	6 (40.0)	
Septations				
Decrease or loss [†]	8 (23.5) ²	4 (21.1)	4 (26.7)	0.660
Increase	2 (5.9)	1 (5.3)	1 (6.7)	
None	24 (70.6)	14 (73.7)	10 (66.7)	
Internal debris				
Increase or newly formed	8 (23.5)	3 (15.8)	5 (33.3)	0.236
Loss	2 (5.9)	1 (5.3)	1 (6.7)	
None	24 (70.6)	15 (78.9)	9 (60.0)	
Mural nodule				
Loss or calcification [‡]	7 (20.6)	3 (15.8)	4 (26.7)	0.119
Persistent	4 (11.8)	1 (5.3)	3 (20.0)	
None	23 (67.6)	15 (78.9)	8(53.3)	

The values are presented as number (%).

*Two cases were not shown because follow-up CT was not performed. CT response was defined as CR (complete response) and non-CR if cyst size after ablation is <5% or ≥5% of the original cyst volume, respectively.

[†]Of 8 patients, there were decreased numbers of septations (n= 3) and loss of septations (n= 5)

[‡]Of two calcifications, one patient had loss of mural nodule and novel calcification, another one had appearance of calcification.

TABLE 3. Cytological change of pancreatic cysts after EUS-guided pancreatic cyst ablation in 34 patients

Cytological change	CT response*			P value
	Total (n=34)	CR (n=19)	Non-CR (n=15)	
Cellularity of cyst epithelium				
Increase	9 (26.5)	7 (36.8)	2 (13.3)	0.234
Decrease	9 (26.5)	4 (21.1)	5 (33.3)	
No change	16 (47.1)	8 (42.1)	8 (53.3)	
Cyst epithelium atypia†				
Newly develop	3 (8.8)	2 (10.5)	1 (6.7)	0.644
Loss	5 (14.7)	3 (15.8)	2 (13.3)	
None	26 (76.5)	14 (73.7)	12 (80.0)	
Internal debris				
Increase or develop	12 (35.3)	7 (36.8)	5 (33.3)	0.772
Decrease or loss	6 (17.6)	2 (10.5)	4 (26.7)	
None	16 (47.1)	10 (52.6)	6 (40.0)	
Macrophages				
Increase or develop	8 (23.5)	4 (21.1)	4 (26.7)	0.482
Decrease or loss	5 (14.7)	2 (10.5)	3 (20.0)	
None	21 (61.8)	13 (68.4)	8 (53.3)	
Inflammatory cells				
Increase or develop	5 (14.7)	3 (15.8)	2 (13.3)	0.555
Decrease or loss	3 (8.8)	0	3 (20.0)	
None	26 (76.5)	16 (84.2)	10 (66.7)	
Viscosity of cyst fluid				
Increase	5 (17.3)	1 (6.2)	4 (30.8)	0.228
Decrease	9 (31.0)	6 (37.5)	3 (23.1)	
No change	15 (51.7)	9 (56.2)	6 (46.2)	

The values are presented as number (%).

*Two cases were not shown because follow-up CT was not performed. CT response was defined as CR (complete response) and non-CR if cyst size after ablation <5% and ≥5% of the original cyst volume, respectively.

†All cases were mild atypia

TABLE 4. The change of cyst fluid DNA quantity and quality after EUS-guided pancreatic cyst

ablation in 20 patients.

Variable	CT response*		
	Complete (n=12)	Partial (n=2)	Persistent (n=5)
DNA quantity (OD)†, mean ± SD, ng/μL			
Baseline	6.7±9.5	540.8±759.8	8.8±6.6
Post Ablation	44.8±70.7	14.4	13.8±11.8
P value¶	0.023	-	0.715
Change of DNA quantity after ablation (n, %)			
Increase	10 (83.3)	0	2 (40.0)
Decrease	2 (16.7)	1	2 (40.0)
n/a‡	-	n/a	n/a
DNA quality, mean ± SD, Ct value§			
Baseline	29.4±2.2 (25.9-34.0)	26.9±3.7 (24.3-29.5)	30.3±4.0 (25.8-36.8)
Post ablation	26.8±3.0 (24-33)	31.0	27.5±2.1 (25-30)
P value¶	0.136		0.273
Change of DNA Ct value after ablation (n, %)			
Increase	3 (25.0)	1 (50.0)	1 (20.0)
Decrease	9 (75.0)	0	3 (60.0)
n/a‡	-	n/a	n/a

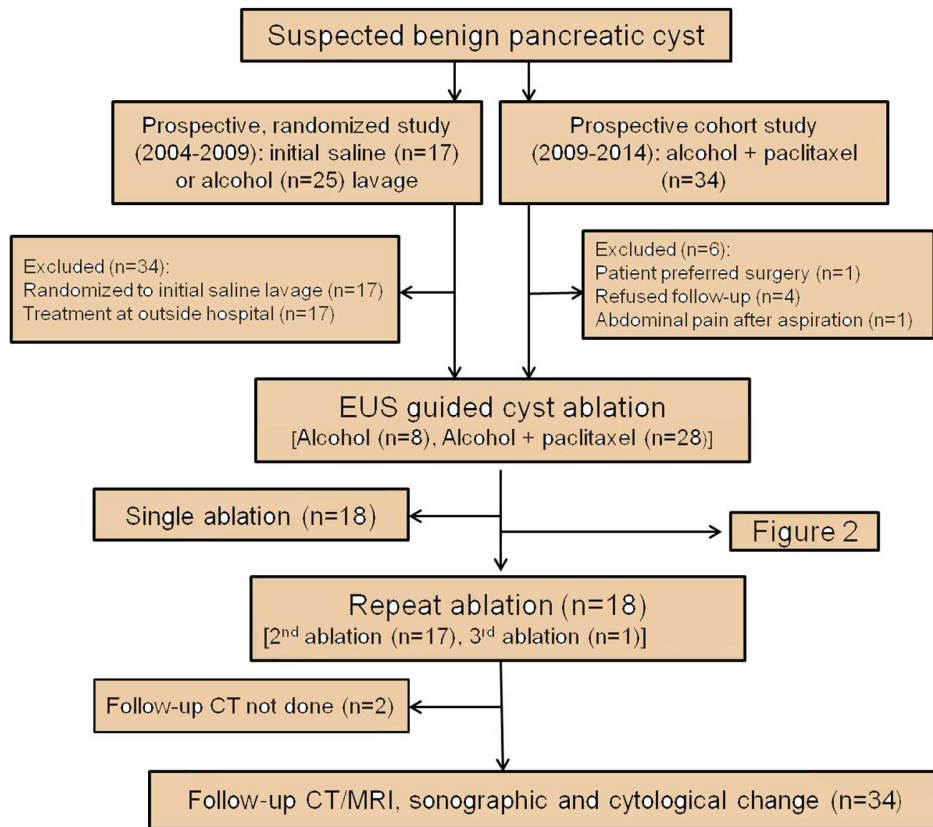
*One case was not shown here because both follow-up CT and post-ablation DNA were not performed. Complete: if CT volume reduction \geq -95%; Partial: -75 ~ -95%; persistent: < -75%

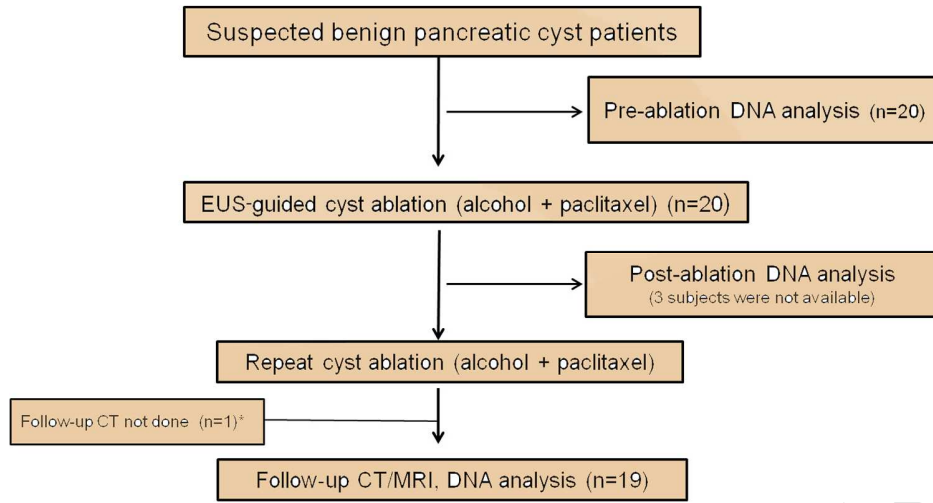
†OD is measure of DNA amounts at 260/280 wavelength

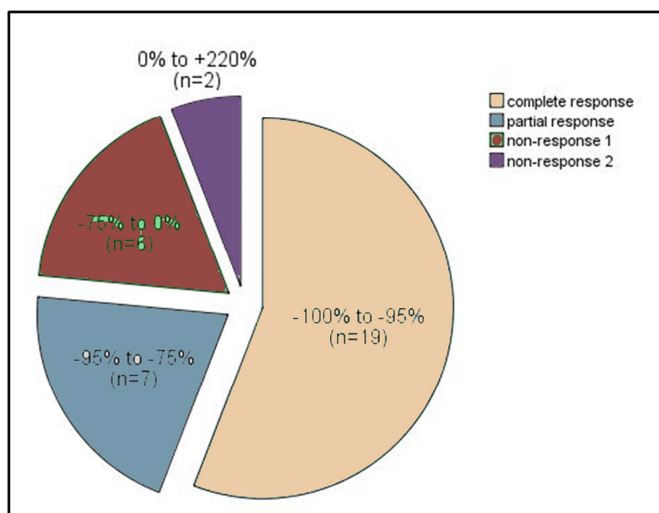
‡Postablation DNA analysis was not available (1 partial response, 1 persistent)

§Cyclic threshold (Ct) is a measure of DNA quality and amplifiability

¶P value is calculated by Wilcoxon signed rank test









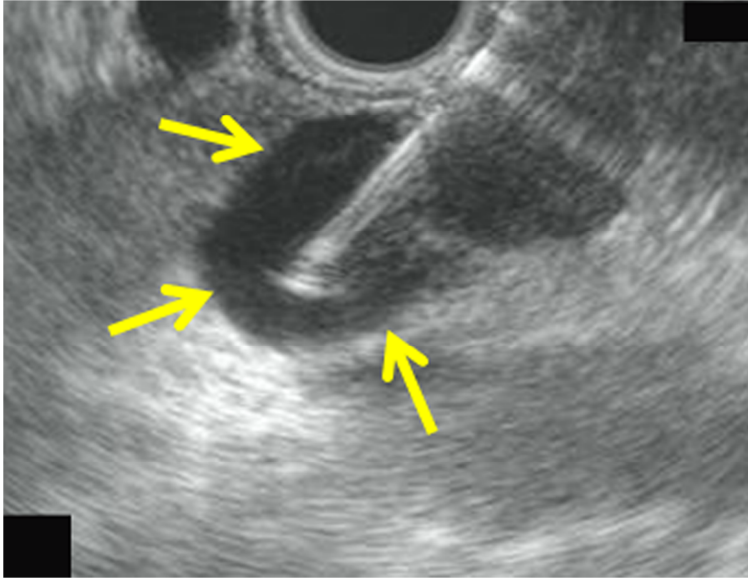
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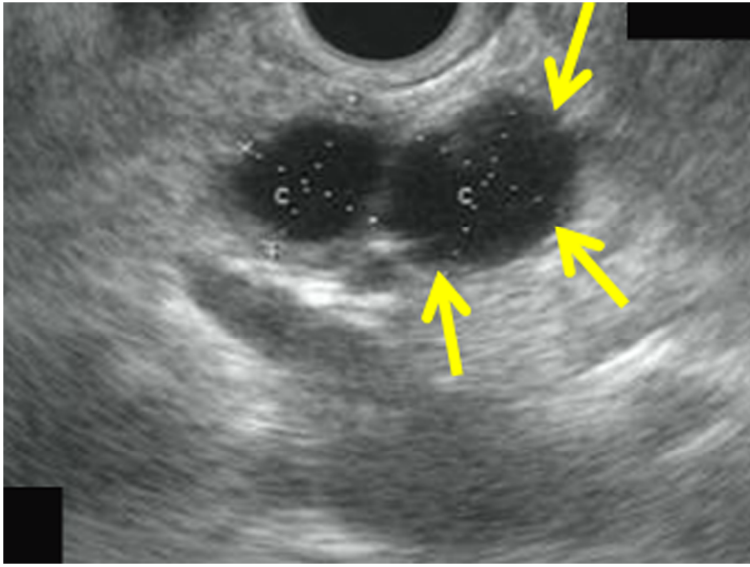
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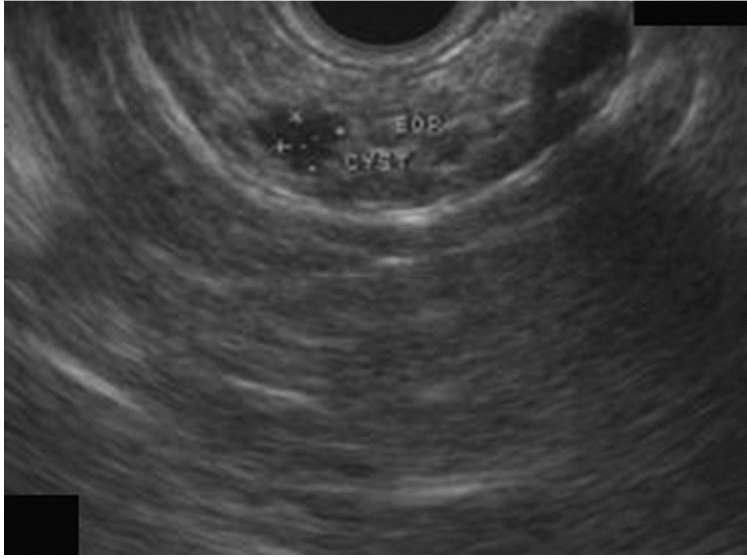
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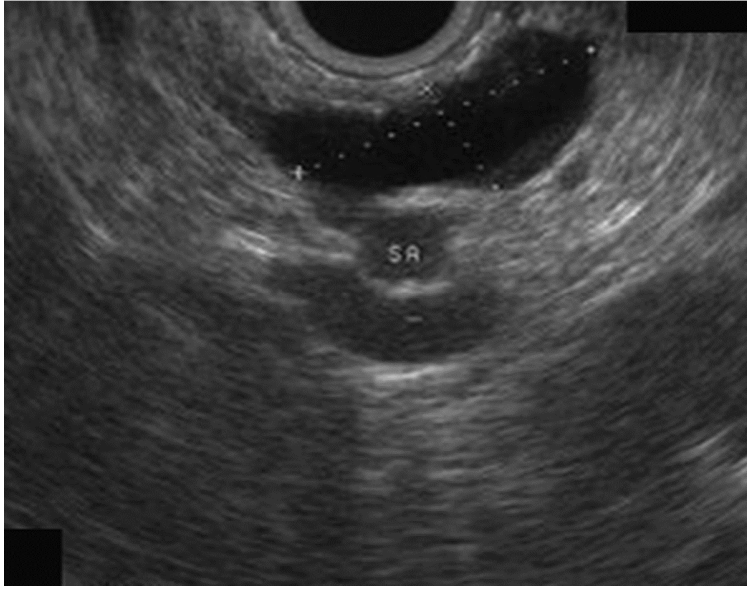
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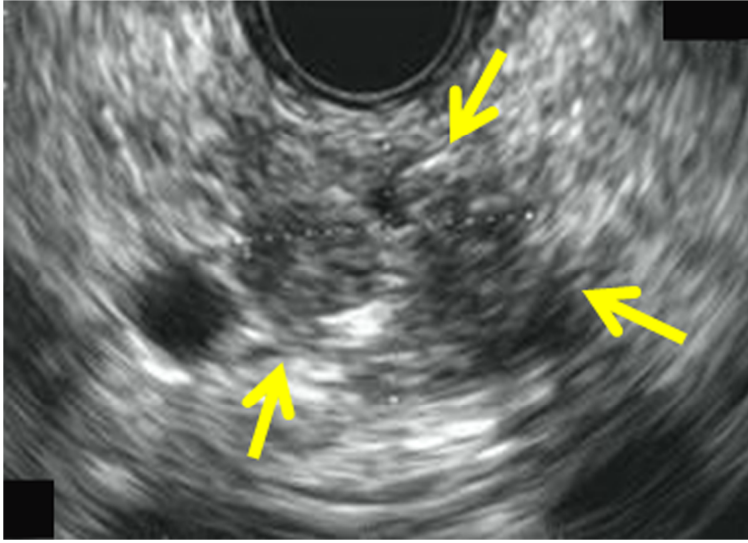
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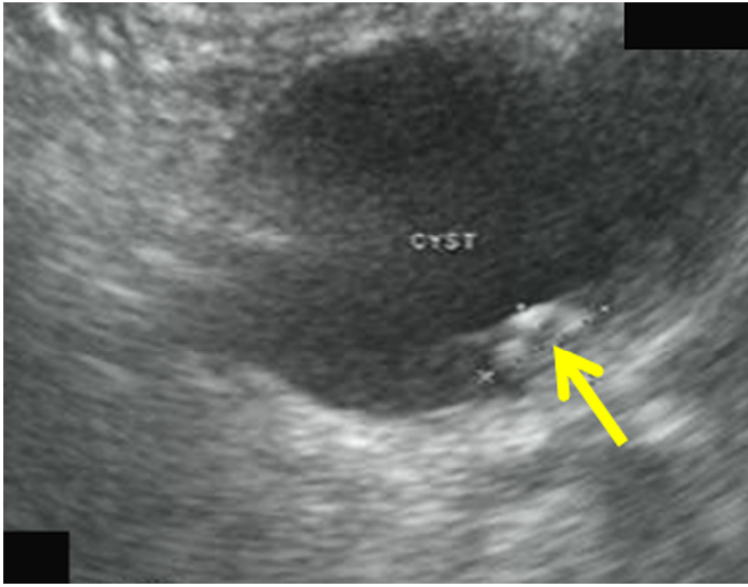
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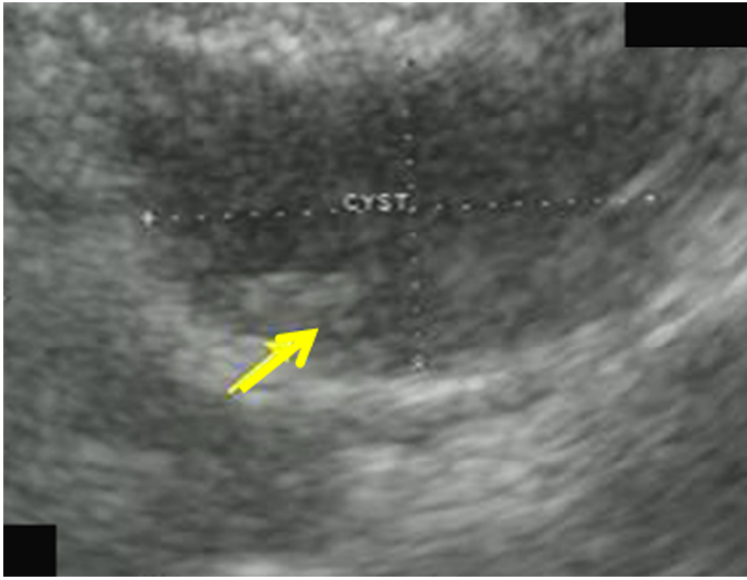
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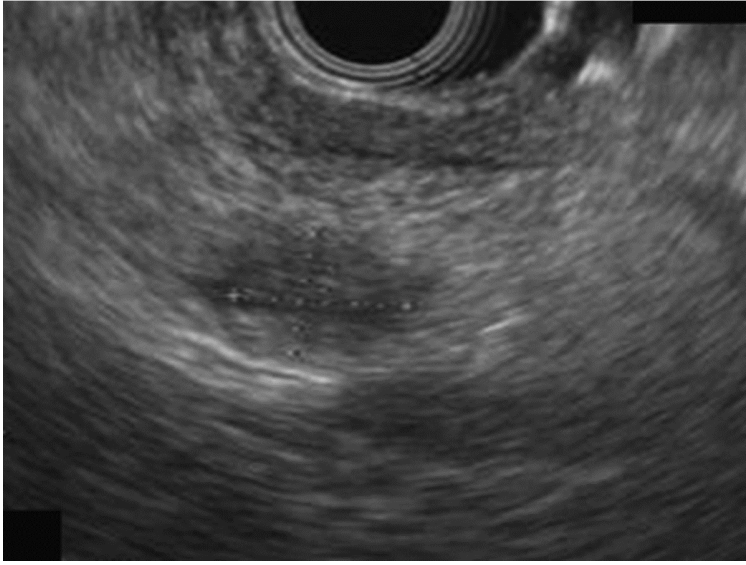
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Abbreviation:

EUS, endoscopic ultrasound; PCA, pancreatic cyst ablation; PCL, pancreatic cystic lesion; CT, computed tomography; MRI, magnetic resonance imaging; EUS-PCA, EUS-guided pancreatic cyst ablation; EtOH, ethanol; PTX, Paclitaxel; SCA, serous cyst adenoma; MCN, mucinous cystic neoplasm; IPMN, intraductal papillary mucinous neoplasm; CEA, carcinoembryonic antigen; CR, complete response; PR, partial response; PCR, polymerase chain reaction; Ct, cycle threshold; OD, optical density