의학석사 학위논문

초음파 내시경 유도하 바늘 관통 생검의 임상적 유용성, 합병증 및 합병증의 위험인자 평가

Clinical outcomes, complications, and risk factors for comorbidities of EUS-guided through-the-needle biopsy in pancreatic cystic lesions in a large cohort

울 산 대 학 교 대 학 원

의 학 과

이 호 승

초음파 내시경 유도하 바늘 관통 생검의 임상적 유용성, 합병증 및 합병증의 위험인자 평가

지 도 교 수　　송 태 준

이 논문을 의학석사 학위 논문으로 제출함

2024 년　2 월

울 산 대 학 교　대 학 원

의　　학　　과

이 호 승

이호승의 의학석사학위 논문을 인준함

심사위원 오 동 욱 인

심사위원 송 태 준 인

심사위원 이 재 훈 인

울 산 대 학 교 대 학 원

2024 년 2 월

Abstract

Background and aims: The incidence of pancreatic cysts has been increasing in recent years. However, accurately distinguishing between these cyst types using current diagnostic methods remains challenging. Surgical intervention for cysts without a precise diagnosis can pose unnecessary risks. Therefore, additional diagnostic approaches such as EUS-guided through-the-needle biopsy (TTNB) have garnered attention. While EUS-TTNB has shown promise in diagnosing pancreatic cysts, concerns have arisen regarding its safety due to adverse events (AEs). This study aims to evaluate the effectiveness, diagnostic yield, performance, and clinical utility of EUS-TTNB, as well as investigate adverse events and the factors associated with them.

Methods: This study analyzed prospectively collected EUS-TTNB records from Asan Medical Center in Seoul, South Korea, focusing on patients who underwent EUS-TTNB for pancreatic cyst diagnosis from January 2019 to September 2023.

Results: Of the 301 patients who underwent EUS-TTNB, tissue was successfully obtained in 300 cases, resulting in a 100% technical success rate. The diagnostic yield of EUS-TTNB was 80%, and when compared with surgical pathology, a high accuracy rate was observed (87.5%, 42/48). Its clinical usefulness led to changes in the diagnosis and subsequent management for 49 patients. (16%, 49/301). Adverse events occurred in 19% of cases, with acute pancreatitis (14%, 42/301) being the most common complication. The rate of moderate to severe acute pancreatitis cases, which occasionally required procedures or led to extended hospital stays, was significant. In multivariable analysis, IPMN diagnosis was identified as a significant risk factor for acute pancreatitis. (OR 4.69 [95% CI 2.21 - 9.96]; $p < 0.001$)

Conclusions: EUS-TTNB demonstrated high technical and diagnostic success rates, offering clinical utility by altering diagnoses and influencing therapeutic decisions. However, the

procedure carries a risk of complications, especially acute pancreatitis, with IPMN diagnosis being a significant risk factor. Therefore, careful consideration and patient selection are essential when deciding to perform EUS-TTNB, weighing the benefits of accurate diagnosis against the associated risks. Further research is needed to prevent severe pancreatitis following EUS-TTNB.

Key Words: EUS-TTNB, Post-TTNB pancreatitis, risk factors of post TTNB complication

Table of contents

Table

Introduction

Pancreatic cancer, which tragically has a 5-year survival rate of only about 9%, can develop from certain specific types of pancreatic cysts known to undergo malignant transformation.[1-3] Some pancreatic cysts, such as serous cystic neoplasms (SCNs), are benign and do not require immediate intervention. But, mucinous cysts, such as mucinous cystic neoplasms (MCNs) and intraductal papillary mucinous neoplasms (IPMNs), can become malignant, requiring continuous monitoring for potential progression.[1] The malignancy risk varies: MCN (10–40%), branch duct IPMN (6.1–37.7%), and main duct IPMN (11–81%).[4-8] Also, the incidence of pancreatic cysts has been reported to be increasing in recent years, due to aging, improved imaging modalities, increased screening and awareness.[9-13] Differential diagnosis of pancreatic cysts is important, however, remains challenging at present.[14-19] The diagnosis of pancreatic cysts is typically achieved through a comprehensive approach that combines various methods such as imaging modalities (CT or MRI), analysis of cystic fluid (CEA, amylase/lipase, glucose), cytology.[5-7, 19] The accuracy rates for distinguishing the particular type of pancreatic cysts range from 40% to 95% for MRI and from 40% to 81% for CT imaging.[5, 14] Cytology only exhibited a low sensitivity (42–63%), and even though it displayed a high specificity(88–95%), in the diagnosis of pancreatic cysts.[20-22] Similarly, the cyst fluid CEA level can distinguish between mucinous and non-mucinous cysts but with limited accuracy (a sensitivity of 61% and specificity of 77%). Compared to surgical pathology, this results in a 39% chance of misdiagnosing MCN cases.[16] Surgical pathology is considered the gold standard for diagnosis and definite treatment for mucinous pancreatic cysts or malignancy that require surgery, but the complication rate is considered high. Pancreatic surgery has been associated with high morbidity (approximately 30–40%) and

mortality (less than 2% in high volume centers).[6, 23, 24] Performing surgery without a precise diagnosis can lead to an unnecessary surgical intervention, despite the significant risks involved. A recent multi-center and prospective study conducted on 1,190 patients who underwent surgery for pancreatic cysts revealed that 88% of the cases were found to be benign.[23, 25, 26] Consequently there is a need for additional diagnostic approaches such as EUS-guided through-the-needle biopsy (TTNB), cyst fluid DNA mutational analysis, EUS-guided needle-based confocal laser endomicroscopy(EUS-nCLE).[27-41] Several studies, including meta-analyses, have consistently demonstrated that EUS-TTNB is superior to traditional techniques when evaluating pancreatic cysts. Reports highlight the efficacy of EUS-TTNB, underlining its safety due to the infrequent occurrence of serious complications.[36, 38-41] However, recent concerns have surfaced regarding the safety of EUS-TTNB due to a significant number of observed severe adverse events (AEs).[27, 42-44] In particular, a recent prospective study conducted at a single center reported an AE rate of 10%, which included a fatal case of post-TTNB pancreatitis.[37, 42] Furthermore, in a retrospective multicenter study recently conducted by Facciorusso et al., three fatal cases were reported following TTNB.[43] This study also analyzed predictors of adverse events, recommending a risk classification system. These findings collectively suggest that caution should be exercised in the implementation of EUS-TTNB, emphasizing the importance of evaluating safety concerns in conjunction with its effectiveness. The objective of this study is to (1) assess the efficacy, diagnostic yield, performance (including the ability to distinguish between mucinous and non-mucinous cysts), and clinical utility of EUS-TTNB, and (2) investigate the adverse events and their associated risk factors.

Materials and Methods

Patients and data collections

We analyzed the prospectively collected EUS-TTNB records from Asan Medical Center in Seoul, South Korea, focusing on patients who received EUS-TTNB for PCLs diagnosis from January 2019 to September 2023. We re-evaluated those patients that had been included in our center's previous retrospective study with specific indication and exclusion criteria, particularly 45 patients were previously included in the earlier study (45 patients from Asan Medical Center). EUS-TTNB was conducted based on the assessment of endosonographers when the potential malignancy of PCLs remained undetermined. Generally, the cases included were one of the following: 1) cyst size larger than 3cm, 2) cyst with main pancreatic duct dilatation, 3) those with increased size or changing shape upon follow-up observation, 4) presence of mural nodules or septal thickening, 5) when the potential malignancy of PCLs remained undetermined, and 6) when endosonographers deemed additional tests necessary for indeterminate cysts. As a result, the outcomes of EUS-TTNB were expected to alter the surveillance and/or therapeutic strategies for PCLs. Endosonographers determined the need for EUS-TTNB during EUS assessment sessions, considering clinical, radiologic factors, and EUS imaging, such as cyst size, the rate of cyst growth, serum CA19-9 levels, and EUS-derived cyst characteristics like size, wall thickness, and the presence of mural nodules. Criteria for exclusion included being younger than 20 years of age, being pregnant, having cystic lesions outside the pancreas, strong indications of pancreatic cancer from CT and/or MRI results and tumor markers, or a PCL measurement less than 2 cm on the EUS imaging, presence of significant coagulopathy, inability to discontinue anticoagulant/antiplatelet as necessary, and having recent pancreatitis within the last six months.

We collected information on demographic details, imaging from CT scans and MRI, evaluation of cystic fluid and/or cytology through EUS-FNA, histology findings, and details from EUS documents, which included the shape, location, dimension, frequency of needle insertions into PCLs, and biopsy count for every patient. Additionally, we scrutinized medical logs for vital statistics, pain scale evaluations, and laboratory assessments post-procedure to ascertain any potential complications. Follow-up data were obtained from electronic charts and follow-up visits.

Procedure

Two skilled endosonographers (D.W.S., T.J.S.) performed procedures using a standardized approach. After sedating patients and administering antibiotics, they used an echoendoscope to evaluate the characteristics of the cyst. When performing EUS-TTNB, the needle was carefully inserted through either the stomach or duodenum, with Doppler imaging ensuring no blood vessels were harmed. They punctured PCLs with a 19-gauge needle and introduced a microforcep (Moray microforceps; US endoscopy, Mentor, Ohio, USA) to target specific cyst regions, if possible, particularly notable features like mural nodules. Tissues, once captured and verified, were stored in formalin. After processing, a pathologist reviewed them. The tissue collection process sometimes required several attempts, and the cyst fluid was later analyzed cytologically in line with the Papanicolaou Society's standards.

Outcomes and definitions

In this research, we primarily assessed technical success, diagnostic outcomes, clinical usefulness, complications, and factors associated with these complications of EUS-TTNB.

Additionally, not only did we investigate the factors affecting the diagnostic yield, but we also compared the surgical pathology thought to be a definite diagnosis, the diagnosis from EUS-TTNB, and the preliminary diagnosis derived from conventional diagnostic methods such as imaging techniques, EUS morphology and cystic fluid analysis. And we conducted a subgroup analysis to assess diagnostic accuracy, which included patients who had undergone surgical procedures. Furthermore, we analyzed the diagnostic outcomes as the number of procedures increased. Technical success referred to the successful acquisition of tangible samples via EUS-TTNB. The term diagnostic yield described the attainment of samples substantial enough for a pathological review to discern the specific categories of PCLs. Insufficient samples for a pathological review were labeled as diagnostic failure. EUS morphology pertained to the distinct PCL classifications determined through features observed on EUS image and assessed by endosonographers. The criteria for adverse events followed the definitions set by the American Society for Gastrointestinal Endoscopy(ASGE) lexicon.[45] The preliminary diagnosis of cyst types was derived from a combination of EUS morphology, imaging results, and cyst fluid analyses, including CEA and amylase/lipase levels. When EUS findings aligned with imaging data, specific PCL categorizations were made. Otherwise, CEA and amylase/lipase concentrations, with CEA levels exceeding 192 ng/mL indicating mucinous cysts, played a decisive role. In cases of diagnostic uncertainties due to misalignments between EUS and imaging data, endosonographers relied on integrated diagnostic findings. The definitive diagnosis was surgical histology when it was accessible. For patients who didn't undergo surgery, the final diagnosis was determined using a blend of findings from cross-sectional imaging/EUS and the cystic fluid cytology/analysis and EUS-TTNB results. The exploration into causes behind diagnostic inaccuracies took into account

various PCL features and procedural details, including the total biopsy samples obtained and the specific lesions targeted. Moreover, we investigated risk factors for the occurrence of adverse events, and these risk factor categories were determined based on our clinical experience. These factors included patient age, the number of needle insertions, the total number of biopsy samples taken, and the presence of IPMN diagnoses. The diagnostic accuracy trend for EUS-TTNB was analyzed as the procedural experience increased.

Statistical analysis

For statistical evaluation, the research sample is characterized using means with standard deviations and median values (interquartile range) for continuous data, or using percentages for categorical data. To contrast categorical data, either Pearson's $\chi 2$ test or Fisher's exact test was employed. Meanwhile, to compare continuous data, we utilized either the Student's t-test or the Mann–Whitney U test. The baseline factors, which we determined based on our clinical experience and their potential prognostic impact on the adverse event rate, were analyzed using both univariate and multivariate logistic regression analysis. All statistical analyses were performed using R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria). P-values <0.05 were considered statistically significant.

Results

Baseline Characteristics of Study Participants

From an initial pool of 304 patients in the database, 3 with extra-pancreatic lesions were excluded, leaving 301 participants in this research. The baseline characteristics can be found in **Table 1**. The participants had a median age of 62.0 years (interquartile range, 48–64). Among them, 42% (126/301) were male and 58% (175/301) were female. Median cyst size was 35mm (interquartile range, 29–43). In terms of location, 37% (112/301) were in the head, 6% (19/301) in the uncinate process, 25% (75/301) in the body, and 32% (95/301) in the tail. Carcinoembryonic antigen (CEA) levels in the cystic fluid were ≥192 ng/mL for 50% (152/301) of the cases. EUS imaging revealed 13% (40/301) with mural nodules and 77% (232/301) with septations. And 21% (62 out of 301) of the patients underwent surgical resection following the EUS-TTNB procedure. On average, patients were followed up for 299 days (interquartile range, 138—581).

Table 1. Baseline Characteristics of the enrolled patients

Characteristic	**Total** (n = 301)
Age	62.0 (48–64)
Sex, M:F	126 (42):175(58)
Size (mm)	35 (29–43)
Location	
Head	112 (37)
Uncinate process	19 (6)
Body	75 (25)
Tail	95 (32)
Carcinoembryonic antigen level of cystic fluid	
≥192 ng/mL	152 (50)
<192 ng/mL	95 (31)
Unknown	54 (19)
EUS image	
Mural nodule	40/301 (13)
Septation	232/301 (77)
Surgical resection	62/301(21)
Follow-up duration, days	384 (138–581)

NOTE. Data are presented as number of patients (%) unless otherwise indicated.

EUS-TTNB Procedure Outcomes and Complication

Table 2 provides a comprehensive summary of the outcomes and adverse events associated with the EUS-TTNB procedure. Of the 301 patients who underwent EUS-TTNB, tissue was successfully acquired in 300 cases, indicating a tissue acquisition yield of 100%. The mean number of needle passages was 1.7 with a standard deviation of 1.2, and the mean number of biopsy samples was 3.6 (± 1.2) per patient. The diagnostic yield of the procedure, or the percentage of patients in which a pathologic diagnosis was made post biopsy, stood at 80% (241 out of 301 patients). And the diagnostic yield of EUS-TTNB showed a tendency to gradually increase as the practitioner's experience accumulated (p=0.03, **Supplement 3**). Out of 301 patients, 58 (19%) experienced adverse events (**Table 2**). Among the study participants, acute pancreatitis emerged as the most prominent complication. Specifically, 3 patients (1%) faced a transient fever that subsided within a couple of days. A cyst hemorrhage was identified in 13 patients (4%). Importantly, during the EUS session, all these hemorrhages halted spontaneously, as evidenced through Doppler imaging. And, there were no indications of a significant drop in hemoglobin levels or overt bleeding, underscoring their minimal clinical significance. When detailing acute pancreatitis cases (**Table 3**), as per the guidelines of the American Society for Gastrointestinal Endoscopy (ASGE), 29 patients had a mild form. These mild cases were all effectively managed and improved within a few days with conservative treatments such as intravenous (IV) Hartman solution hydration. Patients with a moderate form underwent interventions like Endoscopic Retrograde Pancreatic Drainage (ERPD) and experienced an extension of their hospital stay by 4 to 10 days beyond what was initially anticipated. Those diagnosed with severe symptoms faced even longer hospital durations, surpassing the expected period by over 10 days. One individual with a

particularly severe case of pancreatitis underwent EUS-pseudocyst drainage due to extensive peripancreatic fluid accumulation. Also, there were no events of perforation or GI bleeding reported in the study.

Table 2. Summary of procedural outcomes and adverse events of EUS-TTNB

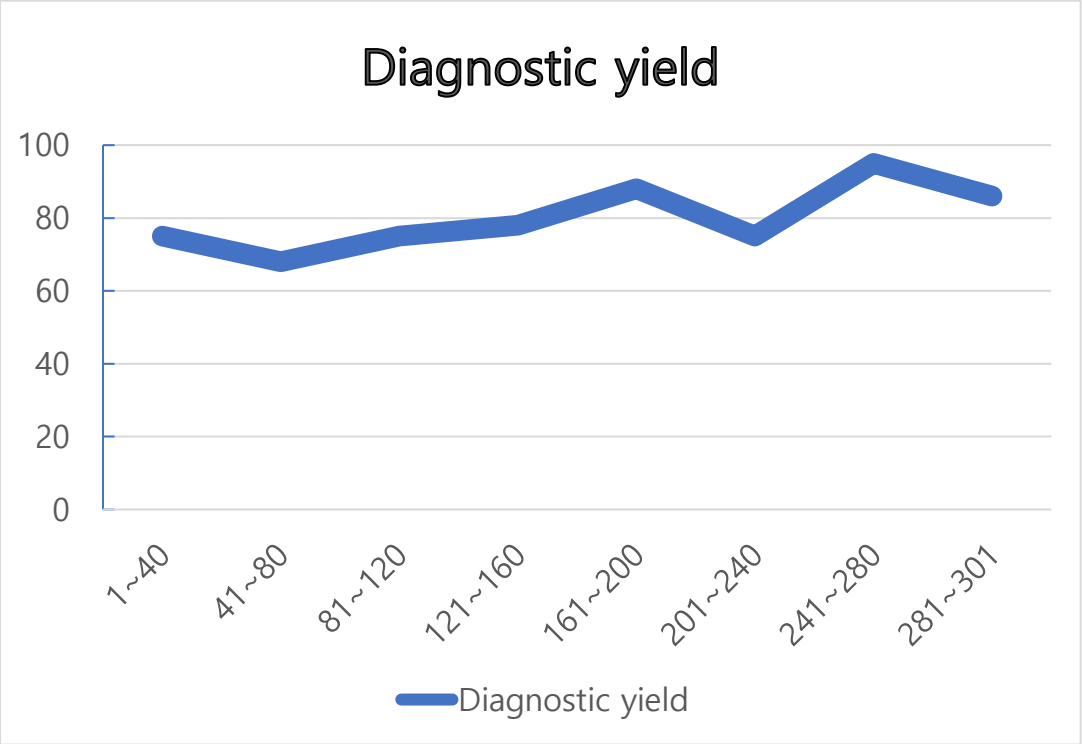
Outcomes	**Total** (n = 301)
Tissue acquisition yield	300/301 (100)
No. of needle passages	1.7 ± 1.2
No. of biopsy samples	3.6 ± 1.2
Diagnostic yield	241/301 (80)
Adverse events	58/301 (19)
Fever	3 (1)
Perforation	0 (0)
Bleeding	0 (0)
Cyst hemorrhage	13 (4)
Acute pancreatitis	42 (14)
mild	29
Moderate	8
Severe	5

NOTE. Data are presented as number of patients (%) unless otherwise indicated.

Table 3. Details of the adverse events

Type of adverse events	Severity			Timing. days	Management
	Mild	Moderate	Severe		
Fever	3	0	0	≤14 (n=3)	Conservative treatment
Perforation	0	0	0	-	-
Bleeding	0	0	0	-	-
Cyst hemorrhage	13	0	0	Intra-procedural	Conservative treatment
Acute pancreatitis	29	8	5	≤14 (n=42)	Mostly conservative treatment, one severe patient received EUS-drainage

Supplement 3. Trend between the number of EUS-TTNB procedures (experience) and diagnostic yield



There is a trend of increasing diagnostic yield as the number of cases increases (P value=0.03).

Diagnostic yield of EUS-TTNB and related factors

The diagnostic yield of EUS-TTNB was recorded at 80%, with tissues obtained from 241 out of 301 individuals. Factors such as the presence of cyst septation, size (based on a 4cm criterion), and the location of the pancreatic cyst did not display statistically significant differences in influencing the diagnostic outcome (**Table 4**). However, a statistically significant enhancement in diagnostic yield was observed when the number of biopsy samples taken was three or more (83.4% vs 50%, $p < 0.010$).

Table 4. Comparison of diagnostic outcomes based on septation presence, lesion size, number of needle insertions per individual, and count of biopsy specimens per patient

	Diagnostic yield	95% CI (%)	P value
Septation			0.798
Yes	187/232 (81)	75.0 – 85.1	
No	54/69 (78)	67.1 – 86.3	
Size			0.399
≥ 40 mm	93/112 (83)	75.0 – 88.8	
< 40 mm	148/189 (78)	71.8 – 83.5	
No. of biopsy samples			<0.001
≥3	226/271 (83.4)	78.5 – 87.3	
<3	15/30 (50)	33.1 – 66.8	
Location			0.465
Head	88/112 (79)	70.0 – 85.1	
Uncinate process	15/19 (79)	56.6 – 91.4	
Body	57/75 (76)	65.2 – 84.2	
Tail	81/95 (85)	76.7 – 91.0	

NOTE. Data are presented as number of patients (%) unless otherwise indicated.

Pathologic results of EUS-TTNB

The pathological outcomes of the EUS-TTNB procedures are displayed in **Table 5**. Among the 241 total diagnoses, IPMN emerged as the predominant diagnosis, detected in 112 patients or 46% of cases. Within the IPMN category, 81 patients were diagnosed with low-grade dysplasia (LGD), 6 had high-grade dysplasia (HGD), and 25 cases were unspecified in terms of dysplasia severity. Following IPMN, MCN was diagnosed in 40 patients, representing 17% of the total. Of these, 16 were categorized with low-grade dysplasia, a single case had high-grade dysplasia, and 23 remained unspecified. SCN was found in 60 patients, which is 25% of the total diagnoses. Other less prevalent diagnoses include solid pseudopapillary neoplasm (SPN) in a single patient, lymphoepithelial cyst in 5 patients, lymphangioma in 3, epidermoid and dermoid cyst in 4, retention cyst and neuroendocrine tumor each in 7 patients. Poorly differentiated carcinoma and pseudocyst were each diagnosed in one patient.

Table 5. Pathologic results of EUS-TTNB

Diagnosis	**Total** (n = 301)
Intraductal papillary mucinous neoplasm	112 (46)
Low grade dysplasia	81
High grade dysplasia	6
Unspecified	25
Mucinous cystic neoplasm	40 (17)
Low grade dysplasia	16
High grade dysplasia	0
Unspecified	24
Serous cystadenoma	60 (25)
Solid pseudopapillary neoplasm	1 (0)
Lymphoepithelial cyst	5 (2)
Lymphangioma	3 (1)
Epidermoid and dermoid cyst	4 (2)
Retention cyst	7 (3)
Neuroendocrine tumor	7 (3)
Poorly differentiated carcinoma	1 (0)
Pseudocyst	1 (0)

NOTE. Data are presented as number of patients (%) unless otherwise indicated.

Comparing initial diagnosis, diagnosis incorporating EUS-TTNB, and diagnosis reflecting surgical pathology

A comparative analysis of diagnoses, including initial assessments, post-EUS-TTNB evaluations, and final conclusions after incorporating surgical pathology findings, is presented in **Table 6**. Notably, there appears to be a gradual decrease in the number of cases diagnosed as IPMN as we move from the preliminary diagnosis with 146 cases (49%), to 139 cases (46%) after EUS-TTNB, and finally 133 cases (44%) when incorporating surgical pathology. Conversely, the diagnosis for SCN seems to have increased, moving from an initial 57 cases (19%) in the preliminary stage, to 71 cases (24%) post-EUS-TTNB, and settling at 73 cases (24%) after factoring in surgical pathology findings. Likewise, the diagnoses for lymphoepithelial cyst, epidermoid and dermoid cyst, and retention cyst also demonstrated an upward trajectory as the evaluation process progressed from initial assumptions to the final surgical pathology-reflected conclusions.

Significantly, the "indeterminate" category, which represents cysts that couldn't be distinctly classified even after synthesizing findings from CT/MR, EUS morphology, and EUS-fluid aspiration results, saw a reduction in the diagnosis reflecting EUS-TTNB. This highlights the utility of EUS-TTNB in offering more definitive diagnoses. Nonetheless, in the diagnosis that incorporated both the influence of EUS-TTNB and surgical histology, 11 cases remained categorized as "indeterminate". These instances weren't subjected to EUS-TTNB or surgery, but rather, they are currently under ongoing observation.

Table 6. Comparison of diagnosis reflecting the results of EUS-TTNB, diagnosis incorporating surgical pathology, and preliminary diagnosis

	Preliminary diagnosis	Diagnosis reflecting EUS-TTNB	Diagnosis incorporating surgical pathology
Intraductal papillary mucinous neoplasm	**146 (49)**	**139 (46)**	**133 (44)**
Mucinous cystic neoplasm	**57 (19)**	**49 (16)**	**51 (17)**
Serous cystadenoma	**57 (19)**	**71 (24)**	**73 (24)**
Solid pseudopapillary neoplasm	**3 (1)**	**1 (0)**	**1 (0)**
Lymphoepithelial cyst	**2 (1)**	**5 (2)**	**5 (2)**
Lymphangioma	**3 (1)**	**3 (1)**	**3 (1)**
Epidermoid and dermoid cyst	**3 (1)**	**5 (2)**	**5 (2)**
Retention cyst	**0 (0)**	**7 (2)**	**6 (2)**
Neuroendocrine tumor	**5 (2)**	**7 (2)**	**7 (2)**
Pseudocyst	**4 (1)**	**2 (1)**	**2 (1)**
Malignancy	**1 (0)**	**1 (0)**	**3 (1)**
Colloid carcinoma arsing from IPMN, HGD	**-**	**0 (0)**	**2 (1)**
Ductal carcinoma arsing from IPMN, HGD	**-**	**0 (0)**	**1 (0)**
Poorly differentiated carcinoma	**-**	**1 (0)**	**0 (0)**
Polycystic kidney disease	**0**	**0 (0)**	**1 (0)**
Indeterminate	**20 (7)**	**11 (4)**	**11 (4)**

NOTE. Data are presented as number of patients (%) unless otherwise indicated.

The Clinical Utility of EUS-TTNB: Changes in Diagnosis

A subgroup analysis was conducted on the 241 individuals out of 301 who underwent EUS-TTNB. Post-EUS-TTNB diagnosis alterations were observed in 49 individuals (**Supplement 1**). Remarkably, 25 of these 49, initially labeled with non-mucinous cysts or as indeterminate, were later identified to have mucinous or solid tumors after undergoing EUS-TTNB. Such reclassifications can significantly influence the therapeutic direction, highlighting the procedure's meaningful impact. On the other hand, 6 out of the 49 had their diagnoses switched from mucinous cysts, solid tumors with cystic degeneration, or indeterminate to non-mucinous cysts. In such cases, actions taken based on the preliminary diagnosis might have led to unnecessary examinations or possibly invasive treatments such as surgery. This demonstrates the potential alterations in therapeutic decisions and the essential role of EUS-TTNB in ensuring diagnostic precision. Also, within the 49 individuals who displayed diagnostic changes post-EUS-TTNB, there were 12 cases where the classification of mucinous cyst shifted within its category, such as from IPMN to MCN or vice versa.

Supplement 1. Patients whose diagnosis changed through the results of EUS-TTNB from preliminary diagnosis

Patient	Cystic Carcinoembryonic antigen ≥192 ng/mL	Cystic amylase/ lipase (U/L)	CT/magnetic resonance imaging	EUS morphology	EUS cytology	Preliminary diagnosis	EUS-TTNB
1	Yes	1422/6716	MCN	MCN	NA	MCN	IPMN, LGD
2	No	196,700/NA	IPMN	IPMN	Negative for malignancy	IPMN	MCN, LGD
3	Yes	9261/NA	Pancreatic cancer	MCN	Negative for malignancy	MCN	IPMN, HGD
4	Yes	5/NA	Pancreatic neuroendocrine tumor	SPN	Nondiagnostic	SPN	Lymphoepithelial cyst
5	Yes	70/NA	MCN	MCN	Negative for malignancy	MCN	IPMN, LGD
6	Yes	338/478	IPMN	SCA	Negative for malignancy	IPMN	Lymphoepithelial cyst
7	No	171/727	SCA	SCA	Atypical	SCA	IPMN, LGD
8	Yes	258/40	Pseudocyst	Pseudocyst	Negative for malignancy	Pseudocyst	Lymphoepithelial cyst
9	No	213,870/ 1,233,300	IPMN	IPMN	Negative for malignancy	IPMN	SCA
10	Yes	51,540/200,260	IPMN	IPMN	Atypical	IPMN	MCN, LGD
11	No	404/NA	IPMN	IPMN	Negative for malignancy	IPMN	SCA
12	No	832/NA	MCN	IPMN or SCA	Atypical	Indeterminate	SCA
13	Yes	88/NA	MCN	MCN	Atypical	MCN	IPMN, LGD
14	Yes	1900/NA	SCA	SCA	NA	SCA	IPMN, LGD
15	NA	NA/NA	MCN	Pseudocyst or lymphoepithelial cyst	Negative for malignancy	Indeterminate	MCN, no identified dysplasia
16	NA	NA/NA	Epidermoid cyst	Solid tumor with cystic degeneration	Negative for malignancy	Indeterminate	Epidermoid cyst
17	NA	NA/NA	MCN	Lymphoepithelial cyst	NA	Indeterminate	SCA
18	Yes	23/7	MCN	MCN	Negative for malignancy	MCN	IPMN, LGD
19	Yes	11/18	IPMN	IPMN	Negative for malignancy	IPMN	Retention cyst
20	NA	NA/NA	IPMN or SCA	IPMN	NA	IPMN	SCA
21	No	150/160	IPMN	IPMN	NA	IPMN	SCA

Patient	Cystic Carcinoembryonic antigen ≥192 ng/mL	Cystic amylase/ lipase (U/L)	CT/magnetic resonance imaging	EUS morphology	EUS cytology	Preliminary diagnosis	EUS-TTNB
22	No	89/49	SCA	Lymphoepithelial cyst	NA	Indeterminate	NET
23	Yes	36/242	IPMN	MCN	Negative for malignancy	MCN	IPMN, LGD
24	NA	NA/NA	IPMN	Pseudocyst or abscess	Negative for malignancy	Indeterminate	NET
25	No	265,670/762,330	IPMN	IPMN	Negative for malignancy	IPMN	MCN, no identified dysplasia
26	No	66/27	MCN	MCN	Negative for malignancy	MCN	NET
27	No	476/2067	MCN	MCN	Negative for malignancy	MCN	Retention cyst
28	No	431/1084	IPMN	IPMN	Negative for malignancy	IPMN	SCA
29	No	116,220/491,210	Pseudocyst	Pseudocyst	Negative for malignancy	Pseudocyst	Retention cyst
30	No	189/554	IPMN	IPMN	Negative for malignancy	IPMN	SCA
31	No	132,690/655,440	SCA	IPMN	Negative for malignancy	Indeterminate	MCN, no identified dysplasia
32	No	3/4	MCN	MCN	Negative for malignancy	MCN	Lymphoepithelial cyst
33	No	20,200/71,200	IPMN	MCN	Negative for malignancy	Indeterminate	Epidermoid cyst
34	Yes	13,027/61,962	IPMN	IPMN	Negative for malignancy	IPMN	Retention cyst
35	NA	NA/NA	MCN	MCN	Negative for malignancy	MCN	IPMN, LGD
36	Yes	513/2541	MCN	MCN	Atypical	MCN	Retention cyst
37	No	97/157	IPMN	IPMN	Negative for malignancy	IPMN	SCA
38	No	437,200/749,960	IPMN	IPMN	Negative for malignancy	IPMN	MCN, LGD
39	NA	NA/NA	IPMN	IPMN	NA	IPMN	Pancreatic cancer
40	No	9519/14,786	MCN	MCN	Negative for malignancy	MCN	SCA
41	No	110/362	MCN	Lymphoepithelial cyst	NA	Lymphoepithelial cyst	SCA
42	No	552/1363	MCN	MCN	Atypical	MCN	SCA
43	No	454/200	IPMN	IPMN	Negative for malignancy	IPMN	SCA
44	No	301/1075	SCA or IPMN	IPMN or MCN	Negative for malignancy	Indeterminate	SCA
45	No	5356/2397	IPMN	IPMN	Atypical	IPMN	SCA
46	Yes	21,104/63,850	MCN	NET	Negative for malignancy	NET	IPMN, LGD
47	No	164/206	IPMN	IPMN	Negative for malignancy	IPMN	Retention cyst
48	Yes	130/131	MCN	MCN	NA	MCN	Retention cyst
49	No	6089/32,177	MCN	MCN	NA	MCN	IPMN, LGD

Comparative analysis of EUS-TTNB results and surgical pathology

A total of 62 patients underwent surgery, and a meticulous analysis of their surgical and pathological outcomes was performed. Histology results from EUS-TTNB were available for 48 of these patients (**Supplement 2**). In 42 of these cases, the cyst type identified in EUS-TTNB matched the surgical pathology. One case showed a poorly differentiated carcinoma in EUS-TTNB, but was identified as IPMN, HGD in surgical pathology. In three cases, IPMN was detected in EUS-TTNB, but they were identified as MCN in surgical pathology. Another case had a retention cyst according to EUS-TTNB, but was identified as SCN in surgical pathology. Lastly, there was a case where the EUS-TTNB indicated IPMN, LGD, but the surgical pathology revealed it to be polycystic kidney disease (PKD).

Supplement 2. Details of preliminary diagnosis, EUS-TTNB, and surgical pathology

Patient	Cystic Carcinoembryonic antigen ≥192 ng/mL	Cystic amylase/ lipase (U/L)	CT/magnetic resonance imaging	EUS morphology	EUS cytology	Preliminary diagnosis	EUS-TTNB	Operation type	Operation pathology
1	Yes	232/NA	MCN	MCN	Atypical	MCN	MCN, unspecified	LA-distal pancreatectomy	MCN, LGD
2	Yes	295/NA	MCN	MCN	Negative for malignancy	MCN	MCN, LGD	Robot assisted distal pancreatectomy	MCN, LGD
3	No	196700/NA	IPMN	IPMN	Negative for malignancy	IPMN	MCN, LGD	Robot assisted distal pancreatectomy	MCN, LGD
4	Yes	171/NA	MCN	MCN	Atypical	MCN	MCN, unspecified	Robot assisted distal pancreatectomy	MCN, LGD
5	Yes	70/NA	MCN	MCN	Negative for malignancy	MCN	IPMN, LGD	LA-distal pancreatectomy	MCN, LGD
6	Yes	59431/NA	MCN	MCN	Negative for malignancy	MCN	MCN, unspecified	LA-distal pancreatectomy	MCN, LGD
7	No	40659/NA	MCN	MCN	Negative for malignancy	MCN	MCN, LGD	LA-distal pancreatectomy	MCN, LGD
8	NA	NA/NA	mixed IPMN	mixed IPMN	Atypical	IPMN	IPMN, unspecified	Open PPPD	Colloid carcinoma arising from IPMN, HGD
9	Yes	46409/192960	mixed IPMN	mixed IPMN	Atypical	IPMN	IPMN, unspecified	LA-distal pancreatectomy	IPMN, HGD
10	No	171/727	SCA	SCN	Atypical	SCN	IPMN, LGD	Open PPPD	Ductal carcinoma arising from IPMN, HGD
11	No	191/985	IPMN	mixed IPMN	Non diagnostic	IPMN	IPMN, LGD	Open PPPD	IPMN, LGD
12	Yes	87/NA	mixed IPMN	IPMN	Negative for malignancy	IPMN	Inadequate specimen	Open PPPD	IPMN, HGD
13	NA	NA/NA	MCN	SPN	NA	SPN	SPN	LA-distal pancreatectomy	SPN
14	Yes	258/40	Pseudocyst	Pseudocyst	Negative for malignancy	Pseudocyst	Lymphoepithelial cyst	LA-distal pancreatectomy	lymphoepithelial cyst
15	Yes	187/833	MCN	MCN	Atypical	MCN	MCN, LGD	Robotic assisted distal pancreatectomy	MCN, LGD
16	No	38670/183310	IPMN	IPMN	NA	IPMN	IPMN, LGD	Open PPPD	IPMN, LGD
17	NA	6859/2468	IPMN	IPMN	Atypical	IPMN	IPMN, unspecified	LA-PPPD	IPMN, HGD
18	No	26369/NA	IPMN	IPMN	Negative for malignancy	IPMN	IPMN, unspecified	LA-distal pancreatectomy	IPMN, LGD
19	NA	NA/NA	MCN	Pseudocyst or lymphoepithelial cyst	Negative for malignancy	Indeterminate	MCN, unspecified	Robotic assisted distal pancreatectomy	MCN, LGD
20	NA	NA/NA	Epidermoid cyst	Solid tumor with cystic degeneration	Negative for malignancy	Indeterminate	Epidermoid cyst	LA-distal pancreatectomy	Epidermoid cyst
21	Yes	2210/8270	IPMN	IPMN	Atypical	IPMN	IPMN, LGD	Robot assisted pancreatoduodenectomy	IPMN, LGD
22	Yes	117/507	Indeterminate	MCN	Negative for malignancy	MCN	Fibrous stroma	Robotic assisted distal pancreatectomy	MCN, LGD
23	Yes	16/83	IPMN	IPMN	NA	IPMN	IPMN, unspecified	Robotic assisted PPPD	IPMN, HGD
24	Yes	19850/97820	IPMN	IPMN	NA	IPMN	IPMN, LGD	Open PPPD	IPMN, LGD
25	No	NA/NA	IPMN	IPMN	NA	IPMN	IPMN, unspecified	Open PPPD	IPMN, HGD
26	NA	NA/NA	IPMN	IPMN	NA	IPMN	Benign pancreatic tissue only	Open PPPD	IPMN, LGD
27	No	28/48	IPMN or SCA	SCN	NA	SCN	Tissue insufficient for diagnosis	LA-distal pancreatectomy	SCN
28	NA	NA/NA	IPMN	IPMN	NA	IPMN	Non-neoplastic pancreatic parenchyma	LA-distal pancreatectomy	IPMN, HGD
29	No	23/7	MCN	MCN	Negative for malignancy	MCN	IPMN, LGD	LA-distal pancreatectomy	PKD
30	NA	NA/NA	IPMN	IPMN	NA	IPMN	Tiny non-specific pancreas tissue only	Open PPPD	IPMN, LGD

31	No	423/3366	IPMN or SCN	IPMN or SCN	NA	SCN	Tissue insufficient for diagnosis	LA-PPPD	SCN
32	Yes	196800/965900	MCN	MCN	Negative for malignancy	MCN	MCN, unspecified	Open distal pancreatectomy	MCN, LGD
33	NA	NA/NA	SCN	SCN	NA	SCN	SCN	Open distal pancreatectomy	SCN
34	No	170040/950250	IPMN	IPMN	NA	IPMN	Fibrous tissue	Open distal pancreatectomy	IPMN, HGD
35	NA	NA/NA	IPMN	IPMN	Non diagnostic	IPMN	Atypical cells, unknown significance	LA-distal pancreatectomy	SCN
36	Yes	29/54	MCN	MCN	Non diagnostic	MCN	MCN, unspecified	LA-distal pancreatectomy	MCN, LGD
37	No	101990/197970	IPMN	IPMN	Negative for malignancy	IPMN	Dilated pancreatic duct and atrophic pancreatic parenchyma	Open PPPD	IPMN, LGD
38	NA	NA/NA	IPMN	IPMN	NA	IPMN	IPMN, LGD	Open PPPD	IPMN, LGD
39	Yes	83/164	MCN	MCN	Negative for malignancy	MCN	Fibrous wall with hemosiderin-laden macrophages	LA-distal pancreatectomy	MCN, LGD
40	NA	NA/NA	NET	NET	NA	NET	NET	Robotic assisted distal pancreatectomy	NET
41	Yes	1946/3576	MCN	MCN	Negative for malignancy	MCN	MCN, unspecified	LA-distal pancreatectomy	MCN, LGD
42	NA	NA/NA	Pancreatic cancer or NET	NET with cystic degeneration	NA	NET	NET	LA-PPPD	NET
43	No	89/49	SCN	Lymphoepithelial cyst	NA	Indeterminate	NET	LA-distal pancreatectomy	NET
44	NA	NA/NA	NET with cystic degeneration	NET	Negative for malignancy	NET	NET	Robotic assisted PPPD	NET
45	Yes	19307/143030	IPMN	IPMN	NA	IPMN	IPMN, LGD	LA-distal pancreatectomy	IPMN, LGD
46	Yes	37090/38640	MCN	Lymphoepithelial cyst or pseudocyst	Negative for malignancy	MCN	Fibrous cystic wall lined by mucinous epithelium	LA-distal pancreatectomy	MCN, LGD
47	No	73549/467320	Pseudocyst or MCN or SCN	MCN	NA	MCN	MCN, LGD	LA-distal pancreatectomy	MCN, LGD
48	NA	NA/NA	IPMN	Pseudocyst or Abscess	Negative for malignancy	Indeterminate	NET	LA-distal pancreatectomy	NET
49	Yes	214/312	IPMN	IPMN or MCN	NA	IPMN	IPMN, unspecified	Open distal pancreatectomy	MCN, LGD
50	No	211/1261	IPMN	IPMN	NA	IPMN	IPMN, HGD	Robotic assisted pancreatoduodenectomy	MCN, LGD
51	NA	NA/NA	NET	NET or epidermoid cyst	NA	NET	NET	Open distal pancreatectomy	NET
52	No	129360/609680	IPMN	IPMN	NA	IPMN	Negative for malignancy	Open total pancreatectomy	IPMN, LGD
53	Yes	96/255	MCN	MCN	Atypical	MCN	MCN, HGD	LA-distal pancreatectomy	MCN, LGD
54	NA	NA/NA	mixed IPMN	IPMN	NA	IPMN	Tissue insufficient for diagnosis	Open PPPD	IPMN, HGD
55	No	116220/491210	Pseudocyst	Pseudocyst	Negative for malignancy	Pseudocyst	Retention cyst	Open distal pancreatectomy	SCN
56	No	71/283	IPMN	IPMN	Negative for malignancy	IPMN	IPMN, HGD	Robot assisted pancreatoduodenectomy	IPMN, HGD
57	Yes	3165/7333	IPMN	IPMN	Negative for malignancy	IPMN	IPMN, unspecified	Open PPPD	IPMN, LGD
58	NA	NA/NA	MCN	MCN	Negative for malignancy	MCN	IPMN, LGD	LA-distal pancreatectomy	MCN, HGD
59	NA	NA/NA	IPMN or malignant IPMN	IPMN or malignant IPMN	NA	IPMN	Poorly differentiated carcinoma	Open PPPD	IPMN, HGD
60	Yes	1024/3925	mixed IPMN	IPMN	Atypical	IPMN	IPMN, HGD	Open total pancreatectomy	Colloid carcinoma arising from IPMN, HGD
61	Yes	4012/10376	MCN	MCN	Atypical	MCN	MCN, unspecified	LA-distal pancreatectomy	MCN, LGD
62	Yes	194/1005	Pseudocyst	MCN	Negative for malignancy	MCN	MCN, unspecified	Distal pancreatectomy	IPMN, LGD

Analysis of risk factors for complications of EUS-TTNB

Out of 301 patients in the study, 58 experienced adverse effects, with acute pancreatitis being the most prevalent (**Table 2**). Most cases were mild and managed with conservative treatments, but more severe forms led to extended hospital stays and additional interventions. Thus, identifying predictive factors of these adverse events, especially acute pancreatitis, is essential for improving patient management and outcomes.

In the univariate analysis, being 70 years of age or older was statistically significant in relation to adverse events (odds ratio [OR] 3.41 [95% CI, 1.43 - 8.13]; p = 0.005). IPMN diagnosis also proved statistically significant, with an OR of 2.76 ([95% CI, 1.52 - 5.03]; p < 0.001). However, factors such as being male, undergoing needling two or more times, and obtaining four or more biopsy samples weren't statistically meaningful. In the multivariable analysis, only IPMN diagnosis showed a significant statistical difference (Adjusted OR: 2.42 [95% CI 1.30 - 4.50]; p = 0.005). Age 70 or older did not reach statistical significance in this analysis but showed a trend (Adjusted OR 2.40 [95% CI, 0.97 - 5.91]; p = 0.057) (**Table 7**).

In the assessment of specific risk factors linked to the occurrence of acute pancreatitis after EUS-TTNB (**Table 8**), the univariate analysis identified certain critical relationships. It was noted that individuals aged 70 and above (OR 3.57 [95% CI 1.42 - 8.98]; p = 0.006), as well as male subjects (OR 2.30 [95% CI 1.18 - 4.47]; p = 0.014), and those with a diagnosis of IPMN (OR 4.69 [95% CI 2.21 - 9.96]; p < 0.001), showed a statistically significant increase in the risk of experiencing acute pancreatitis post-TTNB. In multivariable analysis, the presence of IPMN remained a crucial and significant risk factor (Adjusted OR 4.17 [95% CI 1.93 - 9.03]; p = 0.0003). The age factor, particularly being 70 or older, exhibited a tendency

but did not reach a level of definitive statistical significance (Adjusted OR 2.15 [95% CI 0.83 - 5.60]; p = 0.11). Conversely, variables such as the frequency of needling and the number of biopsy samples procured did not display a meaningful impact on the probability of developing acute pancreatitis subsequent to EUS-TTNB.

Table 7. Risk factors of adverse effect after TTNB

Characteristics	Univariate analysis			Multivariable analysis		
	OR	95% CI	*P* value	Adjusted OR	95% CI	*P* value
Age ≥ 70 years	3.41	1.43 – 8.13	0.005	2.40	0.97 – 5.91	0.057
Male sex	1.51	0.85 – 2.68	0.16			
Needling ≥ 2	0.95	0.51 – 1.77	0.86			
Biopsy ≥ 4	0.96	0.54 – 1.70	0.88			
IPMN	2.76	1.52 – 5.03	<0.001	2.42	1.30 – 4.50	0.005

Note. OR, odds ratio; CI, Confidence interval;

Table 8. Risk factors of acute pancreatitis after TTNB

Characteristics	Univariate analysis			Multivariable analysis		
	OR	95% CI	*P* value	Adjusted OR	95% CI	*P* value
Age ≥ 70 years	3.57	1.42 – 8.98	0.006	2.15	0.83 – 5.60	0.11
Male sex	2.30	1.18 – 4.47	0.014			
Needling ≥ 2	0.79	0.38 – 1.66	0.53			
Biopsy ≥ 4	1.20	0.62 – 2.31	0.58			
IPMN	4.69	2.21 – 9.96	<0.001	4.17	1.93 – 9.03	0.0003

Note. OR, odds ratio; CI, Confidence interval;

Discussion

The detection of pancreatic cysts has been increasing due to the aging population and the advancement in diagnostic technologies.[9-13] There are various types of PCLs, each with different malignant potentials, and the importance of accurate diagnosis has been highly emphasized due to significant morbidity and mortality associated with surgery.[19, 24, 25, 27] However, the absence of a golden standard diagnostic method has led to the existence of multiple guidelines, and there is a state of inconsistency in the criteria for diagnosis, follow-up observation, and treatment.[5-7, 17] A recent study by van Huijgevoort and colleagues examined 145 patients with IPMN among 247 who underwent surgery for pancreatic cystic lesions. The study compared the effectiveness of the AGA (American Gastroenterological Association), IAP (International Association of Pancreatology), and European guidelines in recommending surgery for these cases. The AGA guidelines suggested surgery for a significantly lower percentage of patients with advanced neoplasms compared to the IAP and European guidelines (27% vs. 94% and 96%, respectively). Additionally, the AGA guidelines recommended surgery for fewer patients without advanced neoplasms (8.6%) compared to the IAP (83%) and European guidelines (76%). Thus, while the AGA guidelines may overlook some advanced neoplasms, the IAP and European guidelines, although superior in detecting advanced neoplasms, may lead to more unnecessary surgeries.[17] However, the diagnostic methods used thus far have fallen short in narrowing this gap.[5-8, 27] Traditional cystic fluid analysis (CEA and amylase) has demonstrated low sensitivity (63-75%) and specificity (62-88%) in discriminating mucinous cysts.[8, 27, 46, 47] Previous studies have reported that CH-EUS (contrast

harmonic endoscopic ultrasound) has value in characterizing mural nodules and identifying malignant potential, but it is an indirect method, and studies comparing it with other techniques are scarce.[48] Additional research and reports are being made on cystic fluid analysis (such as glucose), molecular biomarker study, EUS-nCLE, and EUS-TTNB.[27-29, 33, 36, 40, 49] Especially, EUS-TTNB, which allows for obtaining tissue to confirm pathology as in the standard diagnosis of other organ diseases, has been presented as a promising method.[29] However, due to the novelty of the procedure and the challenging techniques conducted by experienced endosonographers, and being performed in limited institutions, there has been an absence of large-scale studies from single institutions. The available studies have been small-scale or have compiled heterogenous groups.[39, 41-44] In this regard, we have analyzed the diagnostic utility, complications, and risk factors of complications of EUS-TTNB on a large scale in a single institution with consistent indications and operators.

To the best of our knowledge, our study is the largest single-center study ever published concerning the analysis of diagnostic yield, clinical usefulness, adverse events (AE), and related risk factors. We were able to find several important findings in this study. Firstly, EUS-TTNB exhibited high technical (100%) and diagnostic yields (80%), as well as clinical usefulness. When correlated with surgical pathology, it showed high accuracy (87.5%, 42/48, **supplement 2**). From the perspective of clinical usefulness, the diagnosis actually changed for 49 patients through EUS-TTNB (16%, 49/301). Twenty-five were found to have pancreatic cysts such as mucinous, solid tumors with cystic degeneration, and indeterminate cysts reclassified as non-mucinous cysts; six were reclassified from non-mucinous cysts and indeterminate cysts to mucinous cysts. For the former cases, this suggests a reduction in unnecessary examinations and, notably, unnecessary surgeries that come with high comorbidity

and mortality.[6, 23, 25] Conversely, for the latter, it indicates a reduction in the underdiagnosis of cysts that require further aggressive examinations or invasive treatments. This confirms that EUS-TTNB has the advantage of providing a pathologic diagnosis, offering critical information that can change clinical practice. Secondly, it is known that EUS-TTNB has a higher risk of postoperative complications compared to other procedures like EUS-aspiration.[37, 42-44] Commonly occurring complications include intra-cystic bleeding, pancreatitis, abdominal pain, and fever, with rare occurrences of perforation. Particularly, there is significant concern regarding pancreatitis as a complication following EUS-TTNB. A recent single-center study involving 101 patients showed an adverse event (AE) rate of 9.9% (10 patients) after undergoing EUS-TTNB, with nine confirmed cases of acute pancreatitis, and one resulting in death.[37, 42] Another multicenter retrospective study reported three fatal cases, with two resulting from post-TTNB pancreatitis and one from septic shock.[43] In line with this, our research also confirmed a considerable occurrence of adverse events (58 out of 301 cases, 19%), with acute pancreatitis being the most prevalent (42 out of 301 cases, 14%). A notable number of patients developed moderate to severe acute pancreatitis (13 out of 42 cases). While most improved with conservative treatment, their hospital stay significantly extended, with severe cases exceeding the expected duration by more than 11 days. One patient required interventions such as EUS-guided drainage due to complications like necrotizing pancreatitis, fluid collection, and obstructive GI symptoms. Efforts are necessary to prevent these complications and identify high-risk groups before the procedure. A recent study suggested that multiple needle passes in IPMN cases are a significant risk factor, and it has been recommended to avoid EUS-TTNB in cases of IPMN, especially in the case of typical IPMN.[43] Our study's analysis of risk factors also highlighted the diagnosis of IPMN as the most significant, while age showed a trend,

though not statistically significant, and needle passes were not notably associated. The mechanisms behind post-TTNB pancreatitis remain unclear. However, it is crucial to actively consider common preventive measures, such as hydration with Ringer's lactate solution, rectal NSAIDs, and ERCP with ERPD insertion, especially before performing procedures on patients with significant risk factors. When executing EUS-TTNB, a cautious and selective approach is advisable in cases where there is a possibility of IPMN. It is cautiously proposed to consider the implementation of EUS-TTNB in situations where 1) the diagnosis is unclear with the possibility of different types of cysts and/or the possibility of malignancy, 2) the likelihood of a typical IPMN is low, 3) the cyst remains undefined despite the evaluation of results from various tests, and 4) there is a need to make a decision regarding surgery. In all of these situations, it seems advisable to proceed with the procedure when the benefits outweigh the risks, taking into account the potential for adverse events. Thirdly, in an actual clinical setting, the diagnosis of pancreas cysts has been conducted through various means such as the patient's age, gender, and other clinical settings, along with imaging tests like CT/MR, EUS, EUS-aspiration, or cytology, but it remains quite challenging.[14-19] A recent network meta-analysis reported a sensitivity of 91% and specificity of 97% in diagnosing mucinous pancreatic cystic lesions through EUS-TTNB, and for malignant pancreatic cysts, it showed a sensitivity of 97% and specificity of 95%.[29] Additionally, another study showed high sensitivity and specificity in diagnosing mucinous cysts and IPMN by integrating NGS through EUS-TTNB (Sensitivity: 83.7–87.2%, Specificity: 81.8–84.6%).[28] However, some previous studies raised questions regarding the clinical impact of EUS-TTNB.[27, 42, 43] It was stated that there might not be significant meaning in typical cases of IPMN, considered a strong risk factor for post-EUS-TTNB pancreatitis, and because TTNB samples are tiny, making it difficult to fully reflect the

whole cyst, and diagnoses may be underestimated. However, the advantages of EUS-TTNB include the ability to directly obtain biopsy specimens, as well as determining the grade of dysplasia. In this study, compared to the whole cyst in surgical pathology (**supplement 3**), it showed high accuracy (87.5%, 42/48), especially in distinguishing between mucinous and non-mucinous cysts [Sensitivity: 1.00(IQR, 0.93–1.00), Specificity: 0.92(IQR, 0.62–1.00)]. Compared to surgical pathology, in cases diagnosed as MCN by EUS-TTNB (**supplement 4A**), the pathologic dysplasia grade seemed to match relatively well. However, in one case where HGD was identified in EUS-TTNB, the surgical pathology confirmed it as LGD. Moreover, many cases came out as unspecified in EUS-TTNB grades (9/15). Similarly, for cases diagnosed as IPMN by EUS-TTNB (**Supplement 4B**), the trend of pathologic dysplasia grade seemed to match, but the diagnoses were more heterogeneous. A total of three cases were confirmed as colloid carcinoma in surgical pathology, and one case as polycystic kidney disease, and four cases as MCN (3 LGD, 1 HGD). Also, in these cases, many came out as unspecified in EUS-TTNB grades (8/21), presumably due to the small tissue size making it difficult to discern pathologically.

Our study has several advantages. In this research, as it is a single-institution study, there can be consistency in case selection, reference standards, procedural methods, operators, and pathological diagnoses. Also, we studied over 300 patients, and more than 60 of these patients underwent surgery, allowing us to compare surgical pathology with other data. Furthermore, the cases encountered in actual practice were included, making it practical. Additionally, we have been able to provide a somewhat adequate answer to the issue regarding the appropriate number of needle passes in relation to diagnostic yield and adverse events. Also, concerning histology, although the tissue size is small, it was confirmed that it possesses adequacy in

diagnosis when compared to the entire tissue.

However, there are still several limitations. First, although we conducted research through the prospective cohort we have collected, it is a retrospective study and has inherent limitations. Secondly, while there is the advantage of having two endosonographers from a single center performing the procedure, considering that access to and experience with pancreatic cysts can vary significantly between different centers, there could be selection bias or performance bias. Furthermore, we were able to identify the high proportion of severe adverse events and their risk factors. However, it seems that further research is needed to determine methods of prevention for these occurrences.

Conclusion

In conclusion, EUS-TTNB is a procedure with a high technical success rate and diagnostic yield, and it has clinical utility. However, there is a substantial risk of complications, with acute pancreatitis being the most common and crucial, and it can be severe or even fatal. The most critical risk factor is IPMN, and in high-risk cases, procedures should be decided cautiously and selectively. The procedure carries a risk of adverse events and should therefore be considered for patients when the benefit of obtaining an accurate diagnosis outweighs the associated risks. Additionally, further research is needed to determine the appropriate indications and usage of EUS-TTNB, and to uncover the causes of post-TTNB severe pancreatitis, its mechanisms, and strategies for its prevention and treatment.

Supplement4A. Comparison of TTNB and surgical pathology when MCN was diagnosed in TTNB

		Surgical pathology		
		MCN, low grade dysplasia	MCN, high grade dysplasia	IPMN, low grade dysplasia
TTNB*	MCN, low grade dysplasia	5	0	0
	MCN, high grade dysplasia	1	0	0
	MCN, unspecified	8	0	1

Table4B. Comparison of TTNB and surgical pathology when IPMN was diagnosed in TTNB

		Surgical pathology					
		IPMN, low grade dysplasia	IPMN, high grade dysplasia	Colloid carcinoma arising from IPMN	Polycystic kidney disease	MCN, low grade dysplasia	MCN, high grade dysplasia
TTNB	IPMN, low grade dysplasia	6	0	1	1	1	1
	IPMN, high grade dysplasia	0	1	1	0	1	0
	IPMN, unspecified	2	4	1	0	1	0

Reference

1. Brugge WR, Lauwers GY, Sahani D, et al. Cystic Neoplasms of the Pancreas. New England Journal of Medicine 2004;351:1218-1226.
2. Oyama H, Tada M, Takagi K, et al. Long-term Risk of Malignancy in Branch-Duct Intraductal Papillary Mucinous Neoplasms. Gastroenterology 2020;158:226-237.e5.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
4. Nagtegaal ID, Odze RD, Klimstra D, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology 2020;76:182-188.
5. Pancreas ESGoCTot. European evidence-based guidelines on pancreatic cystic neoplasms. Gut 2018;67:789-804.
6. Scheiman JM, Hwang JH, Moayyedi P. American gastroenterological association technical review on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. Gastroenterology 2015;148:824-48.e22.
7. Tanaka M, Fernández-del Castillo C, Kamisawa T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. Pancreatology 2017;17:738-753.
8. van Huijgevoort NCM, Del Chiaro M, Wolfgang CL, et al. Diagnosis and management of pancreatic cystic neoplasms: current evidence and guidelines. Nat Rev Gastroenterol Hepatol 2019;16:676-689.
9. Klibansky DA, Reid-Lombardo KM, Gordon SR, et al. The clinical relevance of the increasing incidence of intraductal papillary mucinous neoplasm. Clin Gastroenterol Hepatol 2012;10:555-8.
10. Kromrey ML, Bülow R, Hübner J, et al. Prospective study on the incidence, prevalence and 5-year pancreatic-related mortality of pancreatic cysts in a population-based study. Gut

2018;67:138-145.

11. Laffan TA, Horton KM, Klein AP, et al. Prevalence of unsuspected pancreatic cysts on MDCT. AJR Am J Roentgenol 2008;191:802-7.

12. Lee KS, Sekhar A, Rofsky NM, et al. Prevalence of Incidental Pancreatic Cysts in the Adult Population on MR Imaging. Official journal of the American College of Gastroenterology | ACG 2010;105:2079-2084.

13. Moris M, Bridges MD, Pooley RA, et al. Association Between Advances in High-Resolution Cross-Section Imaging Technologies and Increase in Prevalence of Pancreatic Cysts From 2005 to 2014. Clin Gastroenterol Hepatol 2016;14:585-593.e3.

14. Udare A, Agarwal M, Alabousi M, et al. Diagnostic Accuracy of MRI for Differentiation of Benign and Malignant Pancreatic Cystic Lesions Compared to CT and Endoscopic Ultrasound: Systematic Review and Meta-analysis. J Magn Reson Imaging 2021;54:1126-1137.

15. Thornton GD, McPhail MJW, Nayagam S, et al. Endoscopic ultrasound guided fine needle aspiration for the diagnosis of pancreatic cystic neoplasms: A meta-analysis. Pancreatology 2013;13:48-57.

16. Gaddam S, Ge PS, Keach JW, et al. Suboptimal accuracy of carcinoembryonic antigen in differentiation of mucinous and nonmucinous pancreatic cysts: results of a large multicenter study. Gastrointest Endosc 2015;82:1060-9.

17. van Huijgevoort NCM, Hoogenboom SAM, Lekkerkerker SJ, et al. Diagnostic accuracy of the AGA, IAP, and European guidelines for detecting advanced neoplasia in intraductal papillary mucinous neoplasm/neoplasia. Pancreatology 2023;23:251-257.

18. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, et al. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. Gastroenterology 2004;126:1330-1336.

19. Ardeshna DR, Cao T, Rodgers B, et al. Recent advances in the diagnostic evaluation of pancreatic cystic lesions. World J Gastroenterol 2022;28:624-634.

20. Gillis A, Cipollone I, Cousins G, et al. Does EUS-FNA molecular analysis carry additional value when compared to cytology in the diagnosis of pancreatic cystic neoplasm? A systematic review. HPB (Oxford) 2015;17:377-86.

21. Thosani N, Thosani S, Qiao W, et al. Role of EUS-FNA-based cytology in the diagnosis of mucinous pancreatic cystic lesions: a systematic review and meta-analysis. Dig Dis Sci 2010;55:2756-66.

22. Wang QX, Xiao J, Orange M, et al. EUS-Guided FNA for Diagnosis of Pancreatic Cystic Lesions: a Meta-Analysis. Cell Physiol Biochem 2015;36:1197-209.

23. Allen PJ, D'Angelica M, Gonen M, et al. A selective approach to the resection of cystic lesions of the pancreas: results from 539 consecutive patients. Ann Surg 2006;244:572-82.

24. Oh HC, Kim MH, Hwang CY, et al. Cystic lesions of the pancreas: challenging issues in clinical practice. Am J Gastroenterol 2008;103:229-39; quiz 228, 240.

25. Henn J, Wyzlic PK, Esposito I, et al. Surgical treatment for pancreatic cystic lesions-implications from the multi-center and prospective German StuDoQ|Pancreas registry. Langenbecks Arch Surg 2023;408:28.

26. Valsangkar NP, Morales-Oyarvide V, Thayer SP, et al. 851 resected cystic tumors of the pancreas: A 33-year experience at the Massachusetts General Hospital. Surgery 2012;152:S4-S12.

27. Canakis A, Lee LS. State-of-the-Art Update of Pancreatic Cysts. Dig Dis Sci 2022;67:1573-1587.

28. Pflüger MJ, Jamouss KT, Afghani E, et al. Predictive ability of pancreatic cyst fluid biomarkers: A systematic review and meta-analysis. Pancreatology 2023.

29. Li SY, Wang ZJ, Pan CY, et al. Comparative Performance of Endoscopic Ultrasound-Based Techniques in Patients With Pancreatic Cystic Lesions: A Network Meta-Analysis. Am J Gastroenterol 2023;118:243-255.

30. Rift CV, Melchior LC, Kovacevic B, et al. Targeted next-generation sequencing of EUS-guided through-the-needle-biopsy sampling from pancreatic cystic lesions. Gastrointest Endosc 2023;97:50-58.e4.

31. Machicado JD, Chao WL, Carlyn DE, et al. High performance in risk stratification of intraductal papillary mucinous neoplasms by confocal laser endomicroscopy image analysis with convolutional neural networks (with video). Gastrointest Endosc 2021;94:78-87.e2.

32. Krishna SG, Hart PA, DeWitt JM, et al. EUS-guided confocal laser endomicroscopy: prediction of dysplasia in intraductal papillary mucinous neoplasms (with video). Gastrointest Endosc 2020;91:551-563.e5.

33. Facciorusso A, Buccino VR, Sacco R. Needle-based confocal laser endomicroscopy in pancreatic cysts: a meta-analysis. Eur J Gastroenterol Hepatol 2020;32:1084-1090.

34. Eiterman A, Lahooti A, Krishna SG. Endosonographic diagnosis of advanced neoplasia in intraductal papillary mucinous neoplasms. World J Gastroenterol 2020;26:3201-3212.

35. Cheesman AR, Zhu H, Liao X, et al. Impact of EUS-guided microforceps biopsy sampling and needle-based confocal laser endomicroscopy on the diagnostic yield and clinical management of pancreatic cystic lesions. Gastrointestinal Endoscopy 2020;91:1095-1104.

36. Cho SH, Song TJ, Seo DW, et al. Efficacy and safety of EUS-guided through-the-needle microforceps biopsy sampling in categorizing the type of pancreatic cystic lesions. Gastrointest Endosc 2022;95:299-309.

37. Kovacevic B, Kalaitzakis E, Klausen P, et al. EUS-guided through-the-needle microbiopsy of pancreatic cysts: Technical aspects (with video). Endosc Ultrasound 2020;9:220-224.

38. Facciorusso A, Del Prete V, Antonino M, et al. Diagnostic yield of EUS-guided through-the-needle biopsy in pancreatic cysts: a meta-analysis. Gastrointestinal Endoscopy 2020;92:1-8.e3.

39. Tacelli M, Celsa C, Magro B, et al. Diagnostic performance of endoscopic ultrasound through-the-needle microforceps biopsy of pancreatic cystic lesions: Systematic review with meta-analysis. Dig Endosc 2020;32:1018-1030.

40. Westerveld DR, Ponniah SA, Draganov PV, et al. Diagnostic yield of EUS-guided through-the-needle microforceps biopsy versus EUS-FNA of pancreatic cystic lesions: a systematic review and meta-analysis. Endosc Int Open 2020;8:E656-e667.

41. Rift CV, Scheie D, Toxværd A, et al. Diagnostic accuracy of EUS-guided through-the-needle-biopsies and simultaneously obtained fine needle aspiration for cytology from pancreatic cysts: A systematic review and meta-analysis. Pathol Res Pract 2021;220:153368.

42. Kovacevic B, Klausen P, Rift CV, et al. Clinical impact of endoscopic ultrasound-guided through-the-needle microbiopsy in patients with pancreatic cysts. Endoscopy 2021;53:44-52.

43. Facciorusso A, Kovacevic B, Yang D, et al. Predictors of adverse events after endoscopic ultrasound-guided through-the-needle biopsy of pancreatic cysts: a recursive partitioning analysis. Endoscopy 2022;54:1158-1168.

44. Crinò SF. Through-the-needle microforceps biopsy: a powerful tool but for selected patients. Endoscopy 2021;53:53-54.

45. Cotton PB, Eisen GM, Aabakken L, et al. A lexicon for endoscopic adverse events: report of an ASGE workshop. Gastrointest Endosc 2010;71:446-54.

46. Al-Haddad M, DeWitt J, Sherman S, et al. Performance characteristics of molecular (DNA) analysis for the diagnosis of mucinous pancreatic cysts. Gastrointest Endosc 2014;79:79-87.

47. Anand N, Sampath K, Wu BU. Cyst features and risk of malignancy in intraductal papillary

mucinous neoplasms of the pancreas: a meta-analysis. Clin Gastroenterol Hepatol 2013;11:913-21; quiz e59-60.

48. Lisotti A, Napoleon B, Facciorusso A, et al. Contrast-enhanced EUS for the characterization of mural nodules within pancreatic cystic neoplasms: systematic review and meta-analysis. Gastrointest Endosc 2021;94:881-889.e5.

49. Smith ZL, Satyavada S, Simons-Linares R, et al. Intracystic Glucose and Carcinoembryonic Antigen in Differentiating Histologically Confirmed Pancreatic Mucinous Neoplastic Cysts. Am J Gastroenterol 2022;117:478-485.

국문요약

배경 및 목적: 최근 몇 년 동안 췌장 낭종의 발생률이 증가하고 있다. 그러나 현재의 진단 방법을 사용하여 이러한 낭종 유형을 정확하게 구분하는 것은 여전히 어려운 상황이다. 정확한 진단 없이 낭종에 대한 수술적 치료는 불필요한 합병증 및 때로는 치명적인 결과를 초래할 수 있다. 따라서 EUS(내시경 초음파)를 사용한 바늘 경유 생검(TTNB)과 같은 추가 진단 접근법이 주목을 받고 있다. 최근 EUS-TTNB는 췌장 낭종을 진단하는 데 있어 유망한 결과를 보였지만, 부작용(Adverse Events, AEs)으로 인해 안전성에 대한 우려가 제기되고 있다. 이 연구의 목적은 EUS-TTNB의 효과성, 진단적 능력 및 임상적 유용성을 평가하고, 부작용과 그것과 관련된 요인을 조사하는 것이다.

방법: 이 연구는 서울 아산병원에서 전향적으로 수집된 EUS-TTNB 기록을 분석하였으며, 2019년 1월부터 2023년 9월까지 췌장 낭종 진단을 위해 EUS-TTNB를 받은 환자에 중점을 두고 있다.

결과: EUS-TTNB를 받은 301명의 환자 중 300명에서 성공적으로 조직이 얻어졌으며, 이로 인해 100%의 기술적 성공률이 확인되었다. EUS-TTNB의 진단율은 80%였고, 수술 병리 검체와 비교하였을 때 높은 정확율이 관찰되었다 (87.5%, 42/48). 또한 그 임상적 유용성에 관해서는 EUS-TTNB를 통해서, 49명의 환자에 대한 진단과 그 치료 계획의 변경을 초래하였다. (16%, 49/301). 부작용은 19%의 사례에서 발생하였으며, 급성 췌장염(14%, 42/301)이 가장 흔한 합병증으로 확인되었다. 중등도에서 심한 급성 췌장염 사례의 비율은, 치료나 병원 체류 기간의 연장을 필요로 하는 것이었으며, 다변량 분석에서, IPMN(췌관내 유두상 점액 낭종) 진단은 급성 췌장염의 중요한 위험 요인으로 식별되었다. (OR 4.69 [95% CI 2.21 - 9.96]; $p < 0.001$)

결론: EUS-TTNB는 높은 기술적 및 진단적 성공률을 보였으며, 진단을 변경하고 치료 결정에 영향을 주는 임상적 유용성을 보여주었다. 그러나, 이 방법은 특히 급성 췌장염과 같은 합병증의 위험을 가지고 있으며, 췌관내 유두상 점액 낭종 진단은 중요한 위험 요인으로 확인되었다. 따라서, EUS-TTNB를 수행할지 결정할

때 정확한 진단의 이점과 관련된 위험을 고려하여 신중한 고려와 환자 선택이 필요할 것으로 생각이 된다. 향후 특히나, EUS-TTNB 후 심한 췌장염을 예방하기 위해 추가 연구가 필요하다.