

Randomized controlled trial comparing a conventional needle and a novel needle for endoscopic ultrasound (EUS)-guided histology of peripancreatic masses

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Abstract

Introduction: Cytological study of samples obtained by Endoscopic ultrasound (EUS)-guided fine-needle aspiration (EUS-FNA) allows for recognition of clear signs of malignant transformation. However, certain neoplasms can be difficult to diagnose without histological analysis. Recently, a novel EUS-guided fine needle biopsy (EUS-FNB) needle was developed to increase tissue acquisition. This study set out to investigate the usefulness of this novel EUS-FNB needle (NEFN) in terms of obtaining a proper histology compared with a conventional EUS-FNA needle (CEFN).

Methods: This investigation was a prospective, single-blind, randomized study in a single academic hospital. Primary outcome was the acquisition rate of an appropriate and sufficient specimen for histologic assessment. Secondary outcomes were diagnostic yield of peripancreatic masses using a CEFN and a NEFN. Furthermore, we assessed the feasibility of determining *K-ras* mutation status according to needle type.

Results: The study enrolled 56 consecutive patients. Technical success rates were 96.6% (28/29) for the CEFN and 100% (27/27) for the NEFN ($P = 1.000$). No complications occurred during or after the procedure in either needle group. An adequate sample for cytologic diagnosis was obtained in 89.7% (26/29) of patients in the CEFN group vs 96.3% (26/27) of patients in the NEFN group ($P = .612$). For histologic diagnosis, a sample with a biopsy adequacy score of 2 or more was obtained in 41.4% (12/29) of CEFN-acquired samples vs 88.9% (24/27) of NEFN-acquired samples ($P < .001$). *K-ras* mutation analysis using histologic specimens was possible in 13 (44.8%) CEFN-acquired samples and 25 (92.6%) of NEFN-acquired samples. This difference was significant ($P < .001$).

Conclusions: The present study suggests that the NEFN is an effective and reliable alternative compared to a CEFN in terms of tissue acquisition rate and quality of histologic sampling.

Abbreviations: ASA = American Society of Anesthesiologists, CEFN = conventional EUS-FNA needle, CI = confidence interval, CT = computed tomography, EUS = endoscopic ultrasound, FNA = fine-needle aspiration, FNB = fine needle biopsy, *K-ras* = Kirsten Rat Sarcoma Viral Oncogene Homologue, MRI = magnetic resonance imaging, NCCN = National Comprehensive Cancer Network, NEFN = novel EUS-FNAB needle, PCR = polymerase chain reaction, PDAC = pancreatic ductal adenocarcinoma, ROSE = rapid on-site evaluation.

Keywords: aspiration, biopsy, endoscopic ultrasound, fine-needle aspiration, pancreas

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Key Points

- Previous studies that compared conventional FNA needles and second generation FNB needles did not show consistent results, but rather, were inconclusive.
- Use of the novel EUS-FNAB needle resulted in a significantly superior yield of adequate histological tissue samples than the conventional EUS-FNA needle in peripancreatic masses.
- The present study suggests that a novel EUS-FNAB needle is an effective and reliable alternative compared to a CEFN in terms of tissue acquisition rate and quality of histologic sampling.

1. Introduction

Endoscopic ultrasound (EUS)-guided fine-needle aspiration (EUS-FNA) facilitates a more accurate diagnosis of a solid tumor through the collection of cytological material. Thus, this is fast becoming a key technique for the diagnosis of peripancreatic masses. Although diagnostic accuracy is generally high on a cytological basis for pancreatic ductal adenocarcinoma (PDAC), certain neoplasms such as neuroendocrine tumors, metastatic tumors, and lymphomas need to be diagnosed through histological analysis because the architecture of the core tissue needs to be determined or immunohistochemical analysis needs to be performed.^[1] Sometimes, distinguishing an inflammatory lesion caused by reaction and regeneration from a well differentiated neoplasm based solely on cytological evaluation can be difficult.^[2] Moreover, the National Comprehensive Cancer Network (NCCN) guidelines recommend molecular profiling of tumor tissue in pancreatic cancer and they suggest that knowledge of potentially actionable mutations including Kirsten Rat Sarcoma Viral Oncogene Homologue (*K-ras*) mutations can change clinical management.^[3] For these reasons, it is now well established that obtaining tissue samples for histological examination during EUS has theoretical and practical advantages over cytology alone.^[4] Recently, a novel EUS-guided fine needle biopsy (EUS-FNB) needle was developed to increase tissue acquisition and therefore diagnostic yield.^[5]

This new needle is a 3-plane symmetric needle with Franseen geometry to maximize tissue capture and minimize fragmentation. However, previous studies that compared conventional FNA needles and second generation FNB needles did not show consistent results, but rather, were inconclusive. Some researchers suggested that it is not necessary to distinguish between FNA needles and FNB needles because diagnostic accuracy, or acquisition of core specimen rate, does not differ according to needle classification type.^[6,7] Therefore, for a newly designed FNB needle to be universally applicable, it is important to determine whether or not its tissue acquisition rate is superior to that of a conventional FNA needle.

Our aim in this prospective study was to compare the performance of a conventional EUS-guided FNA needle and the novel EUS-guided FNB needle described above with regard to diagnostic accuracy, yield of core tissue, and ability to perform *K-ras* molecular analysis.

2. Materials and methods

2.1. Study design

This prospective, randomized, controlled trial was carried out in a single center with collaboration between the gastroenterology and pathology departments from July 2017 to December 2019. All consecutive patients with peripancreatic tumors, which were diagnosed with computed tomography (CT) or magnetic resonance imaging (MRI), were enrolled at Samsung Changwon Hospital (Changwon, South Korea). Peripancreatic mass was defined as pancreatic and peripancreatic lymph nodes (including lymph node station numbers 7–14) or tumors behind the head of the pancreas, dorsal and lateral to the superior mesenteric artery. These patients were randomized 1:1 using an online randomization tool accessible on site for sampling peripancreatic masses with a 22-gauge novel EUS-FNAB needle (NEFN group, Acquire, Boston Scientific Corporation, Natick, MA) or a 22-gauge conventional EUS-FNA needle (CEFN group, Expect, Slimline, Boston Scientific Corporation, Natick, MA). Patients and pathologists, but not the endosonographer, were blinded to needle assignment. The study protocol and consent were approved by the Ethics Committee of Samsung Changwon Hospital (SCMC 2017–05–006) and the study was conducted in accordance with the principles of the Declaration of Helsinki. This study was registered at the Clinical Research Information Service (CRIS) (registration number: KCT0002495). This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (NRF-2017R1C1B5018085).

2.2. Objectives

The primary objective of this study was to compare the overall diagnostic yield of peripancreatic masses between NEFN and CEFN groups. The secondary objective was to compare the quality of histologic specimens between NEFN and CEFN groups by evaluating the following 2 factors:

1. acquisition rate of a sufficient and adequate sample for histological evaluation and
2. possibility of achieving sufficient quality and quantity tissue for molecular analysis of *K-ras* mutations.

2.3. Inclusion and exclusion criteria

This prospective study recruited patients who met the following inclusion criteria: age over 18 years; patients with solid peripancreatic masses ≥ 1 cm measured with CT or MRI; patients who required EUS-guided tissue sampling and had an accessible peripancreatic mass; patients who could provide informed consent. We excluded patients who met any of the following criteria: predominantly cystic lesion on CT or MRI; absolute contraindications for procedural sedation; coagulation disorders (prothrombin time-international normalized ratio > 1.5 , activated partial thromboplastin time < 50 seconds); platelet count $< 50,000/\text{mm}^3$; history of acute pancreatitis in the preceding 4 weeks; evidence of systemic infection; pregnancy; or inability to undergo an endoscopic approach.

2.4. EUS-FNA procedure and interventions

All patients were initially sedated using midazolam (2–5 mg) with meperidine 25 mg administered intravenously with appropriate cardiopulmonary monitoring. Propofol was additionally administered and titrated in 10 mg boluses to a steady state of sedation after an initial dose of 0.5 mg/kg (American Society of Anesthesiologists (ASA) classification I-II and age ≤ 70) or 0.25 mg/kg (ASA classification $>III$ or age >70 years). Room air was used for insufflation during EUS-guided procedures. A curved linear array of echoendoscopes (GF-UCT260; Olympus Medical Systems Corp) was employed. EUS-FNA procedures were carried out by a single experienced endoscopist (KMK: had previously performed more than 200 EUS-FNA procedures). When the peripancreatic mass was targeted under EUS surveillance, the endoscopist selected 1 of 2 needle types according to the randomized allocation into CEFN or NEFN groups. To eliminate technical biases, the same EUS-FNA technique was used in both groups. To summarize, after avoiding intervening vessels using color Doppler, the needle was advanced into the target lesion. A 22-gauge needle was used in all cases regardless of assigned group. The stylet was withdrawn slightly before needle puncture, then the stylet was removed using a slow-pull capillary technique.^[8] Four needle punctures and passes were performed routinely. In the first pass, suction was not applied during aspiration and tissue acquisition occurred. Subsequently, continuous suction of 5-, 10-, and 15-ml was applied in the second, third, and fourth needle passes, respectively. The needle was moved forward and backward for 5 strokes at 4 different locations within the lesion using a fanning maneuver.^[9] After performing each pass, the needle sheath was removed with no aspiration. Next, the needle contents were ejected onto a slide using an air-filled syringe and further expressed using a stylet. Because rapid on-site evaluation (ROSE) was not available at the study facility, these contents were macroscopically checked for the presence of suspicious core tissue on the slide. If present, the tissue was fixed in formalin. If not present, the remaining cytological sample, after separating out tissue, was smeared on a slide and immediately placed in absolute alcohol.

2.5. Pathological assessment

For cytological evaluation, smears were stained with Papanicolaou stain. For histologic evaluation, a formalin-fixed specimen was embedded in paraffin, and then prepared in hematoxylin and eosin. If necessary, immunohistochemical staining was performed for optimal diagnosis. All cytological and histological specimens were evaluated by 1 experienced pathologist (HWL), who was blinded to FNA or FNB needle type and clinical information. Cytological and histological diagnoses were categorized as inadequate sample for diagnosis, benign epithelial cell only, inflammatory cell/abscess, atypical epithelium, papillary neoplasm, suspicious of PDAC, PDAC, NET, lymphoma, or metastatic cancer. Inadequate sample for diagnosis was defined as a sample that had no or very few target cells. In this study, lesions with a pathologic report that described any adenocarcinoma, suspicious adenocarcinoma, NET, or metastatic cancer were categorized as malignant/premalignant. On the contrary, samples with pathologic reports of benign epithelial cells, inflammatory cell/abscess, and atypical epithelium were categorized as benign. The quality of specimens obtained by EUS-FNA was assessed based on the following 3 criteria: quantity of cytological and histological material, degree of gastrointestinal

tract contamination, and bloodiness. The quantity of smear and tissue was estimated using a scoring system described in a previous report.^[10] A cytology score of 0 was defined as insufficient cytological material for interpretation, while scores of 1 and 2 represented sufficient cytological material for limited or adequate interpretation, respectively. A histology score of 0 was defined as insufficient histological material for interpretation, a score of 1 was defined as sufficient material for limited interpretation, and scores 2 and 3 indicated sufficient material adequate interpretation with low (total histological material <1 per 10 high-power fields) or high quality (total histological material >1 per 10 high-power fields), respectively. The degrees of GI tract contamination and bloodiness in specimens were evaluated using the scoring system reported by Kudo et al.^[7] Briefly, GI tract contamination or bloodiness on cytology and histology were scaled according to the following percentages of GI or blood contamination in the stain: low (25% of the slide), moderate (25–50%), or high (50%).

2.6. Analysis of *K-ras* mutations

The adequacy of tissue acquisition according to needle type was evaluated by determining whether there was sufficient quality and quantity tissue for *K-ras* mutation analysis. The ability to perform *K-ras* analysis was determined by a pathologist after careful examination of the tissue sample. We employed polymerase chain reaction (PCR) coupled with direct sequencing to test for *K-ras* mutation in specimens. PCR is relatively simple to perform and can provide rapid diagnosis with good sensitivity.^[11] Histological samples for molecular study were formalin-fixed paraffin-embedded fresh specimens. The Cobas (Roche) assay uses a CE-IVD marked TaqMelt PCR assay to detect the presence of 19 *K-ras* mutations in codons 12, 13, and 61 from just 100 ng of DNA extracted from formalin-fixed paraffin-embedded samples.^[12]

2.7. Final diagnosis

In cases where surgery was performed, resected specimens were analyzed, and the final diagnosis recorded in the database. Peripancreatic masses were considered to be benign if the pathology of the resected specimen was benign or if there was spontaneous resolution or no change in radiologic findings for at least 9 months of follow-up. Conversely, specimens were considered to be malignant if the pathology of the resected specimen or EUS-FNA diagnosis based on histologic examination was positive for malignancy, or if there was deterioration of radiologic findings or clinical course.

2.8. Data collection and outcome parameters

Pre-procedure data for patients with a peripancreatic mass from laboratory, radiologic, and endoscopic databases included demographics, symptoms at presentation, laboratory parameters, comorbidities, needle puncture site, and peripancreatic mass characteristics including location and size. Mass size was determined based on the longest dimension of the peripancreatic mass measured by EUS imaging. Technical success of obtaining macroscopic core tissue was defined as the presence of visible suspicious tissue.

All procedure-related complications including pancreatitis and bleeding were graded according to consensus criteria.^[13] A diagnosis of procedure-related pancreatitis was made based on the presence of typical abdominal pain and a serum amylase level

more than three-fold higher than the normal value. Diagnostic yield for a malignant/premalignant lesion was determined based on accuracy, sensitivity, and specificity, which were calculated using the final diagnosis. Accuracy rate was defined as the number of true positives and true negatives divided by the total number of analyzed peripancreatic masses.

2.9. Statistical analysis

Statistical analyses were carried out using SPSS, version 22 (IBM Corporation Armonk, NY), MedCalc for Windows version 4.2 (MedCalc Software, Mariakerke, Belgium), and R version 3.1.0 (Vienna, Austria; <http://www.R-project.org>). We assumed that histological analysis would be possible in 40% of samples obtained using a 22-gauge CEFN based on a previous report.^[14] We assumed that use of an NEFN would afford a tissue acquisition rate of 80% for histologic analysis. Sample size calculation based on a two-tailed test was performed with a type I error rate of 0.05 and a power of 80% to detect a difference in tissue acquisition rate for histologic analysis between CEFN and NEFN groups. The calculated target sample size was 56 (28 in each group). Statistical analyses were performed using the χ^2 test or the Fisher exact test for categorical variables and the Student *t* test (or Mann–Whitney test, if a nonparametric test was appropriate) for continuous variables. Continuous variables are expressed as means and standard deviations, and dichotomous variables are expressed as simple proportions with 95% confidence intervals (CI). A *P* value < .05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

A total of 60 patients with peripancreatic masses were screened for inclusion between July 2017 and December 2019. Of these, 4 were excluded from the study because a predominantly cystic portion was found on MRI (*n* = 2), they had a coagulation disorder (*n* = 1), or a history of acute pancreatitis within 4 weeks before the EUS-FNA procedure (*n* = 1). The remaining 56 patients were randomized to undergo EUS-guided tissue sampling with an NEFN (*n* = 27) or CEFN (*n* = 29). (Fig. 1). Their demographic details and

peripancreatic mass characteristics are shown according to the 2 different needle types in Table 1. Mean patient age was 68.5 ± 9.8 years and the male/female ratio was 5.5:4.5. Fourteen patients (25.0%) had a lesion in the pancreatic head, 33 (58.9%) in the body/tail, and the other 9 (16.1%) had lymph node or non-pancreatic masses. All 56 peripancreatic masses were visible on EUS and technically accessible. Based on measurements from EUS images, the mean mass diameter was 3.14 ± 1.12 mm. Twenty one patients had single or multiple medical comorbidities including hypertension, diabetes mellitus, liver cirrhosis, chronic kidney disease, or cardiovascular disease. Antiplatelet medications were used by 5 (8.9%) patients. There were no significant difference in age, gender, biopsy site, mass size, puncture route, comorbidities, or antiplatelet use between patients in the NEFN and CEFN groups. No significant differences in laboratory findings were found between the 2 groups. Procedure-related complications of pancreatitis occurred in 1 (3.7%) of 26 patients in the NEFN group. No patient in the CEFN group had procedure-related complications. There were no subjects eliminated after randomization occurred. Follow-up data were obtained for all 56 patients without loss. Therefore, approach to intention-to-diagnose analysis is possible and complete outcome data are available for all randomized subjects. Final diagnoses were confirmed from EUS-guided tissue acquisitions for 32 cases and from surgically resected specimens for 14, and the remaining 10 cases were verified based on radiologic and clinical follow-up. Final diagnoses were malignant or premalignant masses in 25 (92.6%) and 27 (93.1%) patients in the NEFN and CEFN groups, respectively. Among malignant or premalignant masses, PDAC was the most common at a frequency of 66.1% (37/56), followed by metastatic cancer with a frequency of 19.6% (11/56).

3.2. Quality of cytological and histological samples

Cytological and histological sample qualities of both groups are detailed in Table 2. Macroscopic core tissue acquisition by EUS-FNA was successful in 27 (100%) and 26 (89.7%) patients in the NEFN and CEFN groups, respectively. There was no significant difference between the 2 groups regarding technical success of macroscopic core tissue acquisition (*P* = .237). Quality was evaluated in 56 cytologic samples and 53 histologic samples

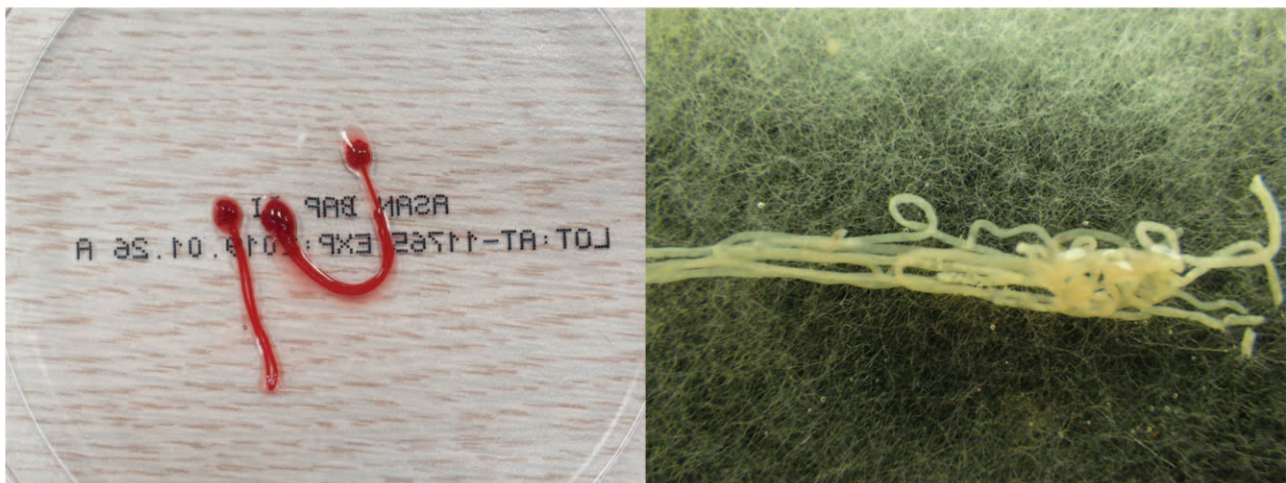


Figure 1. Representative macroscopic tissues obtained with 22-gauge novel EUS-FNB needle (NEFN, Acquire, Boston Scientific Corporation, Natick, MA).

Table 1**Clinical and demographic characteristics according to needle type.**

Variable	NEFN group (n=27)	CEFN group (n=29)	All (n=56)	P value
Age, y, mean ±SD	67.6 ±10.1	69.4 ±9.7	68.5 ±9.8	.477
Male, n (%)	16 (59.3%)	15 (51.7%)	31 (55.4%)	.766
Site of biopsy, n (%)				.552
Pancreatic head	6 (22.2)	8 (27.6)	14 (25.0)	
Pancreatic body/tail	16 (59.3)	17 (58.6)	33 (58.9)	
Lymph node/nonpancreatic mass	5 (18.5)	4 (13.8)	9 (16.1)	
Size of mass, cm, mean ±SD	3.14 ±1.03	3.13 ±1.21		.956
<20 mm, n	5 (18.5)	4 (13.8)		.725
≥20 mm, n	22 (81.5)	25 (86.2)		
Needle puncture route				.642
Transgastric	20 (74.1)	24 (82.8)	44 (78.6)	
Transduodenal	7 (25.9)	5 (17.2)	12 (21.4)	
Major coexisting disease, n (%)				
Hypertension ^a	8 (29.6)	8 (27.6)	16 (28.6)	1.000
Diabetes mellitus ^b	9 (33.3)	8 (27.6)	17 (30.4)	.860
Liver cirrhosis ^c	2 (7.4)	1 (3.4)	3 (5.4)	.604
Chronic kidney disease	1 (3.7)	1 (3.4)	2 (3.6)	1.000
Cardiovascular disease	2 (7.4)	2 (10.3)	4 (7.1)	1.000
Antiplatelet medication	3 (11.1)	2 (6.9)	5 (8.9)	.664
Laboratory findings				
ALP, IU/L	156.8 ±181.8	205.6 ±211.6		.360
AST, IU/L	74.3 ±125.7	54.8 ±62.6		.472
ALT, IU/L	64.5 ±137.9	72.0 ±105.5		.820
GGT, IU/L	209.1 ±486.4	200.4 ±298.2		.937
Total bilirubin, mg/dL	1.0 ±1.3	2.1 ±3.7		.156
Amylase, U/L	69.9 ±48.3	71.1 ±41.2		.919
Lipase, U/L	88.4 ±153.2	81.5 ±128.1		.855
CA 19-9, U/L	679.8 ±1560.8	742.9 ±1558.6		.882
CEA, ng/mL	4.9 ±4.1	92.6 ±302.9		.130
Platelet count (×10 ³ /μL)	241.9 ±88.8	234.6 ±93.3		.765
HbA1c (%)	6.1 ±1.1	6.4 ±1.8		.438
Procedure-related complications	1 (22.6%)	0 (54.5%)		.482
Pancreatitis	1 (3.7)	0 (38.6%)		
Bleeding	0 (3.7)	0 (0.0)		
Final diagnosis	0 (0%)	2 (4.5%)		
Malignant/Premalignant, n (%)	25 (92.6)	27 (93.1)	52 (92.9)	1.000
PDAC	17 (63.0)	20 (69.0)	37 (66.1)	
IPMN	0 (0.0)	1 (3.4)		
Metastatic cancer	6 (22.2)	5 (17.2)	11 (19.6)	
Neuroendocrine tumor	2 (7.4)	1 (3.4)		
Benign, n (%)	2 (7.4)	2 (6.9)		1.000
Chronic pancreatitis	1 (3.7)	0 (0.0)		
Groove pancreatitis	1 (3.7)	0 (0.0)		
Pancreatic abscess	0 (0.0)	2 (6.9)		

All of the results are presented as numbers (%) or means ±SD.

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate transaminase, CA 19 = carbohydrate antigen 19-9, CEA = carcinoembryonic antigen, GGT = gamma glutamyl peptidase, IPMN = intraductal papillary mucinous neoplasm, PDAC = pancreatic ductal adenocarcinoma, SD = standard deviation.

(NEFN: 27 cytologic, 27 histologic; CEFN: 29 cytologic, 26 histologic samples). Most cytological samples (83.9%, 47/56) provided sufficient quality material for adequate cytological interpretation with no significant differences between the 2 needle groups ($P = .541$). There was no significant difference between the 2 groups in terms of degree of bloodiness or GI contamination when maximum negative pressure was applied to obtain a 1.5 ml sample (bloodiness and GI contamination, $P = .765$ and $.509$, respectively). However, with regard to the quality of the histological samples, specimens obtained with the NEFN were significantly more appropriate for histological analysis than those obtained with the CEFN ($P < .001$). In particular, the proportion

of samples with a quality score ≥ 2 , in other words samples where adequate histological interpretation was possible, was higher at 88.9% (24/27) in the NEFN group than 41.4% (12/29) in the CEFN group ($P < .001$). Aside from overall sample quality, the extent of sample bloodiness and GI contamination was not significantly different between the 2 groups (bloodiness and GI contamination, $P = .197$ and $.556$, respectively). *K-ras* analysis of histologic specimens was possible in 67.9% (38/56) of all patients. The ratio of tissue samples suitable for *K-ras* analysis was significantly higher in the NEFN group than the CEFN group (92.6% (25/27) vs 44.8% (13/29), respectively, $P < .001$). All *K-ras* mutations were codon 12 mutations and were detected in 17

Table 2
Comparison of the quality of cytologic and histologic specimens between the 2 needle groups.

Variable	NEFN group (n=27)	CEF group (n=29)	P value
Quality of cytologic sample			
Cellularity, highest score of the slides obtained in each case, n (%)			.541
0: insufficient material for cytologic interpretation	0 (0.0)	0 (0.0)	
1: sufficient material for limited cytologic interpretation	3 (11.1)	6 (20.7)	
2: sufficient material for adequate cytologic interpretation	24 (88.9)	23 (79.3)	
Bloodiness at negative pressure suction with 15 ml, n (%)			.765
Minimal	20 (76.9%)	24 (82.8%)	
Moderate	4 (15.4%)	4 (13.8%)	
Significant	2 (7.7%)	1 (3.4%)	
GI contamination, at negative pressure suction with 15 ml, n (%)			.509
Little (<25% of the slide)	24 (92.3%)	27 (93.1%)	
Moderate (25–50%)	0 (0.0%)	1 (3.4%)	
Abundant (>50%)	2 (7.7%)	1 (3.4%)	
Quality of histologic specimen			
Initial technical success, macroscopic for histology	27 (100.0%)	26 (89.7%)	.237
Sample quality, histology			<.001
0: insufficient material for histological interpretation	0 (0.0)	7 (24.1)	
1: sufficient material for limited histologic interpretation	3 (11.1)	10 (11.1)	
2: sufficient material for adequate histological interpretation, a low-quality sample	3 (11.1)	5 (17.2)	
3: sufficient material for adequate histological interpretation, a high-quality sample	21 (77.8)	7 (24.1%)	
Overall histologic sample quality \geq grade 2	24 (88.9)	12 (41.4)	<.001
Bloodiness at negative pressure suction with 15 ml, n (%)			.197
Minimal	15 (65.2)	16 (84.2)	
Moderate	6 (26.1)	1 (5.3)	
Significant	2 (8.7)	2 (10.5)	
GI contamination, at negative pressure suction with 15 ml, n (%)			.556
Little (<25% of the slide)	21 (91.3)	19 (100.0)	
Moderate (25–50%)	2 (8.7)	0 (0.0)	
Abundant (>50%)	0 (0.0)	0 (0.0)	
Tissue sample suitable for <i>K-ras</i> analysis	25 (92.6)	13 (44.8)	<.001

All of the results are presented as number (%).
 GI = gastrointestinal.

(73.9%) of 23 PDAC samples that met the threshold for *K-ras* mutation analysis. None of the remaining 19 suitable non-PDAC samples had *K-ras* mutations.

3.3. Diagnostic accuracy

With regard to the diagnosis of malignant/premalignant masses, NEFN showed a sensitivity and specificity of 96% and 100%, respectively, with a positive and negative predictive value of 100% and 67%, respectively (Table 3). In comparison, CEFN showed a sensitivity and specificity of 85% and 100%, respectively, with a positive and negative predictive value of 100% and 33%, respectively.

Table 3
Overall diagnostic accuracy according to needle type.

Covariate	CEF group % (95% CI)	NEFN group % (95% CI)
Sensitivity (range)	100 (85.75–100.00)	100 (85.18–100.00)
Specificity (range)	66.67 (9.43–99.16)	33.33 (4.33–77.72)
Positive predictive value (range)	96 (79.65–99.90)	85.19 (66.27–95.81)
Negative predictive value (range)	100 (15.81–100.00)	100 (15.81–100.00)
AUC (range)	0.83 (0.64–0.95)	0.67 (0.50–0.81)
Accuracy	96.30 (81.03–99.91)	86.21 (68.34–96.11)

AUC = area under the curve.

One (3.7%) diagnostic error was identified in the NEFN group and 4 (13.8%) in the CEFN group. Accuracy of histological and cytological analyses was 96.30% (95% CI, 81.03–99.91) and 86.21% (95% CI, 68.34–96.11) in the NEFN and CEFN groups, respectively. Considering both histologic and cytologic interpretation, there was no significant difference in overall diagnostic accuracy for peripancreatic masses between the 2 needle groups ($P = .355$).

4. Discussion

Our primary objective in the study was to compare the performance of 2 needle types, NEFN and CEFN, for the diagnosis of peripancreatic masses. In our randomized controlled study, we found that use of an NEFN provided a significantly higher quality histology specimen than a CEFN. While a CEFN can help in accurate cytological interpretation for peripancreatic masses, it is less appropriate for obtaining high-quality core tissue for *K-ras* mutations analysis. Previous research has established that conventional EUS-FNA needle types are among the best diagnostic tools for pancreatic and adjacent structures.^[15,16] However, we often encounter circumstances that require not only a cytology specimen but also subsequent histologic evaluation or further ancillary tests such as molecular tests to detect specific gene mutations. The use of ancillary testing is still largely in the experimental phase, but these tests can help distinguish well-

differentiated malignant cells from reactive benign hyperplasia.^[17] Various genetic abnormalities, such as mutations in *K-ras*, *p53*, *p16*, and *DPC4* have been demonstrated in PDAC. Although the main driver mutations in pancreatic cancers are in *K-ras*, there are numerous other potentially actionable mutations that can be identified by molecular profiling.^[3] In addition, molecular profiling in certain cancers has identified potential actionable drug targets, prompting efforts to clinically validate biomarkers to guide therapeutic decision-making and enrollment in clinical trials.^[18] Obtaining sufficient tissue is the first step in making these ancillary tests available. To date, debate continues about the best strategies for maximizing the amount of tissue taken. Even though various devices labelled “EUS-FNB needles” have been developed, there is no consensus as to what specific needle type is ideal. Furthermore, it is still questionable whether this broad and innovative category of tools that implies a biopsy function is worthy of the name “EUS-FNB needle.” The NEFN used in this study has a Franseen design with a crown-tip with 3 cutting edges. A preliminary study suggested that this three-symmetric surface geometry can theoretically result in acquisition of more tissue due to a longer insertion length and area.^[5] However, at first glance, it has a somewhat overly sharp edge that may cause bleeding during FNA and use of this unique needle with 3 cutting edges beyond the conventional needle is not yet universally recommended. Therefore, there is a need for prospective, randomized controlled studies to determine the effectiveness and safety of this needle in comparison with conventional needles. In our study, the most important clinically relevant finding was that NEFN can provide higher quality histologic samples with maintenance of safety during the procedure. From a clinical standpoint, obtaining sufficient cytologic specimen plays a minor role, while high histological quality is crucial for employing specific staining procedures or molecular analysis of specific cell types that are potentially important for biomarker identification. Many EUS-guided FNA techniques to increase specimen cellularity and to decrease blood or GI contamination to achieve higher diagnostic accuracy have also been evaluated.^[19] Kin et al showed that a slow-pull technique without suction provides optimal cellularity in EUS-FNA for pancreatic solid masses.^[20] This finding, however, conflicts with the findings of previous studies that suggested that high negative pressure suction could provide adequate cellularity in EUS-FNA for solid masses.^[21,22] In this study, we first performed EUS-FNA using the capillary method without suction, followed by 5 ml, 10 ml, and 15 ml of applied negative pressure to eliminate any confounding factors associated with these technique differences. Even with 15-ml of negative pressure, there was no significant blood or GI contamination compared to the slow-pull technique without suction. Moreover, we did not observe any tendency for histological sample adequacy to improve as negative suction pressure increased. This finding broadly suggests that the role of suction during EUS-FNA is unclear and may vary according to situation depending on the characteristics of the target lesion. Furthermore, the diagnostic accuracy of EUS-FNA could be affected by the presence of ROSE, but this is not available in every center, including ours.^[23] To overcome this, we routinely performed 4 needle passes for all peripancreatic masses and applied 4 different negative pressure levels for each needle pass. The Fanning maneuver is also routinely used because it is now well recognized from a variety of studies that this technique can decrease the minimum number of needle passes necessary for establishing a diagnosis and increases

the probability of diagnosis achievement on the first needle pass when ROSE is available. We detected *K-ras* mutations in 73.9% of patients with PDAC. This proportion of *K-ras* gene mutations in our patient cohort is consistent with that reported in other studies.^[17,24] *K-ras* oncogene encodes a 21 kDa membrane-bound guanosine triphosphate (GTP)-binding protein. Mutation in this gene most commonly occur in codon 12, but also occasionally in codons 13 and 61, and leads to impaired GTPase activity, resulting in much higher proliferation rates of cells expressing these mutant forms.^[25] Diagnosis based on EUS-FNA may be inconclusive and can contradict the clinical diagnosis in up to 20% of PDAC cases.^[26] Factors such as insufficient, very well differentiated, crushed, necrotic, or degenerated samples are possible reasons for this difficulty. In cases where there is doubt about the presence or absence of malignancy, objective findings provided by *K-ras* mutation analysis could aid in the diagnosis.^[27] Bournet et al evaluated the effect of *K-ras* mutation analysis on the differential diagnosis of pancreatic solid masses through a multicenter retrospective study of 186 EUS-FNA cases.^[28] They reported that about 15% of cases with a false-negative pathologic diagnosis were correctly diagnosed when *K-ras* mutation analysis was performed. *K-ras* mutations are extremely rare in pancreatic inflammation and other pancreatic tumors except for PDAC.^[29] In our study, *K-ras* mutations in codon 12 were detected in 17 of 23 patients with PDAC, and in none of the 19 patients with non-PDAC lesions. Interestingly, despite normal levels of CA 19-9, almost 40% (5/12) of PDAC patients had a *K-ras* mutation. This observation suggests that *K-ras* gene abnormalities may help differentiate PDAC from inconclusive FNA cases. The implications of *K-ras* mutation analysis that we have identified highlight the importance of obtaining high quality histologic samples by EUS-FNA. Recently, many personalized models that help guide precision oncology have emerged.^[30] Concurrently, the MicroRNA panel has also been proposed for use, as biomarkers for personalized medicine in PDAC, as has *K-ras* mutation assay.^[31] We suggest that identifying a microRNA signature using core-biopsy samples enables miRNA-based therapeutics in PDAC patients. An important limitation of our study is that the endoscopic practitioner who participated in the study was not blinded to needle type. This could make our findings less generalizable because there might have been bias in the EUS-FNA technique used and how the tissue was handled. However, there was no significant difference in overall diagnostic accuracy between the 2 needle types. This may explain the lower correlation between better histologic specimens and needle type. Second, this study is limited by the lack of histological information on the total core tissue area in each scanned slide. Notwithstanding these limitations, we elucidated the clinical value of NEFN in the acquisition of histologic core tissue samples. The present study is also important because it is one of few studies to prospectively compare the newly designed 22G Franseen-tip needle and the standard Chiba-tip needle in patients with peripancreatic masses.

5. Conclusion

Although both needle types demonstrated similar diagnostic accuracy with comparable safety, use of the NEFN resulted in a significantly superior yield of adequate histological tissue samples than the CEFN in peripancreatic masses. Moreover, the NEFN used in this study facilitates ancillary tests such as *K-ras* mutation analysis. Our results suggest that use of an NEFN can potentially

obviate the need for on-site cytopathologic examination and therefore be a more versatile diagnostic tool in centers where ROSE is not available. We are optimistic that NEFN will become a standard EUS-FNA tool when reliable quality histological samples are required in patients with peripancreatic masses.

Author contributions

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