Prospective study of EUS-guided tissue acquisition with a 20G core biopsy needle with a forward bevel for solid pancreatic mass

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Abstract
There is a growing need for tissue collection for immunostaining and genetic testing. Recently, several fine-needle biopsy needles are commercially available for endoscopic ultrasound-guided tissue acquisition.

This prospective historical controlled study evaluates a 20G core biopsy needle with a forward bevel for solid pancreatic masses larger than 15 mm in diameter. The primary endpoint was the accuracy of histological diagnosis. The secondary endpoints included technical success rate, sample adequacy for histology, cytological diagnostic accuracy, and adverse events.

Seventy consecutive patients were enrolled between January and October 2017. We achieved technical success in all cases regardless of the puncture sites or the endosonographer's experience. The final diagnoses were neoplasms in 67 patients (95.7%); pancreatic cancer in 65 patients, neuroendocrine neoplasm in 1, and malignant lymphoma in 1) and benign lesions in 3 patients (4.3%; autoimmune pancreatitis in 2 patients and mass-forming pancreatitis in 1). The obtained specimens were adequate for histological evaluation in all cases and the histological accuracy was 91.4% (95% confidence interval, 82.3–96.8%, P < .05) with the sensitivity and specificity of 91.0% and 100%, respectively. The cytological diagnostic accuracy was 95.7% and all patients were accurately diagnosed by combining cytological and histological examinations. As for adverse events, an asymptomatic needle fracture occurred in 1 case (1.4%).

This 20G core biopsy needle with a forward bevel showed a high accuracy of histological diagnosis for solid pancreatic masses.

Abbreviations: CI = confidence interval, EUS-FNA = endoscopic ultrasound-guided fine needle aspiration, FNB = fine needle biopsy, G = gauge, ROSE = rapid on-site evaluation.

Keywords: EUS-FNB, histological evaluation, pancreatic mass, prospective study

1. Introduction
Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is the standard method for pathological diagnosis of pancreatic mass lesion.\(^[^1,2]\) EUS-FNA has a pooled sensitivity of 91% (range, 82%–99%) and a pooled accuracy of 83% (range, 74%–92%)\(^[^1,6]\) but some aspirated specimens are too small to allow for a histological diagnosis, to distinguish inflammation from well-differentiated neoplasia, and to diagnose certain diseases such as neuroendocrine neoplasms that require immunohistochemical analyses.\(^[^7–9]\)

To overcome this problem, several types of fine-needle biopsy (FNB) needles have become commercially available. These needles are designed to collect more tissue and to obtain higher histological diagnostic rates. Some needles have improved the tip structures, while others can remove more tissue from their lateral grooves. An Echo Tip ProCore HD Ultrasound Biopsy Needle (Cook Medical, Winston-Salem, NC), which was an early model of FNB needle, was designed to remove tissue from the lateral groove, and it has a reverse bevel attached to the lateral groove of the shaft. This bevel hooks and cuts the tissue and traps it into the needle during the pulling-back motion. However, meta-analyses have shown no differences in tissue acquisition for histology between this FNB core needle and the conventional FNA needle.\(^[^10–12]\)

To improve the tissue acquisition and the histological diagnostic yield of this core needle, the FNB needle was improved...
with a forward bevel instead of a reverse one. As a needle usually moves faster when pushed than when pulled, the bevel cuts the tissue more efficiently. Thereby, this FNB needle should be able to obtain a better specimen for histological diagnosis. Thus, we conducted a prospective historical controlled study to evaluate the histological yield of this FNB needle with a forward bevel for solid pancreatic masses.

2. Patients and methods

2.1. Study design

This was a single-center prospective historical controlled study and approved by the institutional review board of Japanese foundation for cancer research (Study registration; UMIN000025738). We conducted the study in accordance with the principles of the Declaration of Helsinki and written informed consent was obtained from all patients. Inclusion criteria were as follows:

1. patients older than 20 years, and
2. consecutive patients with a solid pancreatic mass larger than 15 mm in diameter requiring EUS-guided tissue acquisition for histological confirmation.

Exclusion criteria were:

1. patients in whom the endoscopic approach was difficult due to prior gastroduodenal surgery,
2. patients using daily anti-coagulants,
3. patients with platelet counts <50,000/mm³,
4. and patients with prothrombin time-international normalized ratio >1.5.

2.2. EUS-guided tissue acquisitions

We performed EUS-guided tissue acquisitions using a convex linear-array echoendoscope (GF-UCT 260; Olympus Optical, Tokyo, Japan) under moderate sedation with a combination of intravenous midazolam and pethidine. We hospitalized patients undergoing the procedures. The endosonographers included 6 “experts” with experience in more than 100 cases, and 5 “trainees” with less than 100 cases. We evaluated an Echo Tip ProCore HD Ultrasound Biopsy Needle 20 gauge (Cook Medical, Winston-Salem, NC). This FNB needle has a lateral 5-mm opening in length at the shaft 2 mm away from the top and a forward bevel at the bottom of the lateral groove. The needle is equipped with a nitinol stylet covered by a protective metal spiral sheath.

After visualizing the target lesion endoscopically, we measured the diameter of the lesion on the puncture line to confirm the eligibility criterion of a minimum diameter of 15 mm. We set this definition due to the needle structure: if the tumor is too small, the needle tip will penetrate the tumor when attempting to take a specimen from the side hole. We performed each puncture with 20 strokes while applying suction with a 10-mL syringe (Fig. 1A and B). We adopted rapid on-site evaluation (ROSE) in this study. The punctures were repeated at least twice until the ROSE identified the appropriate sample. Endosonographers chose to use a different needle such as a conventional 22 gauge (G) FNA needle (EZ Shot 3 Plus; Olympus Medical, Tokyo, Japan) or another type of 22G FNB needle (Acquire Endoscopic Ultrasound Fine Needle Biopsy Device; Boston Scientific, Natick, MA) whenever a sample could not be obtained after multiple punctures with the first needle. Moreover, the “expert” physician attending the procedure decided on the timing to switch from “trainee” to “expert” during a procedure.

2.3. Tissue processing and the ROSE procedure

After each puncture, a 10-mL saline-filled syringe was used to express the sample from the needle into a petri dish. Then, another glass slide was used to spread a part of the specimen onto a glass slide to prepare a smear specimen. Both slides were wet-fixed with an alcohol-based fixing solution, and one of the slides was stained with modified Papanicolaou stain (double dilution Gill’s hematoxylin) for ROSE. The cytologist evaluated the findings immediately and reported whether the sample was adequate for cytological diagnosis. Later, the glass slides were stained with standard Papanicolaou stain (Fig. 1C). The remaining specimen was placed in 7% formalin and was processed for histological examination (Fig. 1D and E). We did not use cell blocks to evaluate cells in this study. We performed pathological evaluations for every specimen obtained by each puncture individually.

2.4. Final diagnosis

In the operated patients, the final diagnosis was made by histological assessment of the resected specimen. In the patients with unresectable tumors, the final diagnosis was made by the cytological or histological assessment obtained after EUS-guided tissue acquisition, but we checked the clinical course of each patient to confirm its consistency to the diagnosis. In patients with no pathological evidence of malignancy, a final diagnosis was made after a follow-up of at least 12 months, confirming the absence of a progression by imaging assessment.

2.5. Outcome parameters

The primary endpoint of this study was the accuracy of the histological diagnosis. The secondary endpoints were technical success rate, sample adequacy for histology, accuracy of cytology, and the adverse events. We defined diagnostic accuracy as the sum of true-positive and true-negative results divided by the total number of patients, and technical success as the successful puncture of the lesion. In addition, we evaluated sample adequacy for histology according to the rate at which the obtained samples were available for histological diagnosis (including immunohistochemical staining if needed). We defined adverse events as any postprocedural events in a lexicon of endoscopic adverse events.13

2.6. Sample size calculation and statistical analysis

We used an accurate binomial test to calculate the required sample size. According to the previous randomized control studies and meta-analyses, the threshold for the histological accuracy rate with a standard FNA or FNB needle with a reverse bevel was 77%. We assumed the diagnostic accuracy of histology with this needle to be 90%. To achieve a statistical power of 0.80 for exact binomial tests with a 2-sided type-I error of 0.05, our minimum sample size was 60 patients. Since we expected some patients to be nonevaluable, we considered enrolling 70 patients.

We expressed out results as numbers and percentages for categorical variables or as medians and ranges for continuous
variables. For the analysis of overall diagnostic yield, Clopper & Pearson exact confidence interval was used for binary outcome. We considered a $P < .05$ as statistically significant. We used the SAS software version 9.4 (SAS Institute, Cary, NC) to perform all the statistical analyses. The final clinical data obtained with follow-ups until March 2019.

3. Results
Between January 2017 and October 2017, seventy patients were enrolled in this study. Table 1 shows the patient characteristics. The median tumor size was 22 mm and the mass lesions were located in the pancreatic head in 23 patients (32.9%). The final diagnoses were neoplasms in 67 patients (95.7%; pancreatic

Figure 1. A typical case of pancreatic cancer confirmed by EUS-FNB. (A) CT scan image of pancreatic cancer, (B) EUS image of EUS-FNB, (C) Papanicolaou staining of specimens obtained by EUS FNB, (D) Core specimens obtained by single puncture. (E) Hematoxylin-Eosin staining obtained by EUS-FNB.
cancer in 65 patients, neuroendocrine neoplasm in 1, and malignant lymphoma in 1) and benign lesions in 3 patients (4.3%; autoimmune pancreatitis in 2 patients and mass-forming pancreatitis in 1).

Table 2 shows the details of the punctures. The puncture site was the stomach in 51 patients (72.9%), the first portion of the duodenum in 10 patients (14.3%), and the second portion of the duodenum in 9 patients (12.9%). We achieved technical success in all cases regardless of the puncture sites or the experience of the endosonographers. Eighteen procedures (25.7%) were performed by “trainees.” The median number of punctures was 3 (range; 2–8). Four or more punctures were needed in 11 patients (15.7%). Although 4 patients (5.7%) required the use of additional 22G FNA or FNB needle because of undetermined ROSE results during the procedure, the specimens obtained from the first ProCore needles could be evaluated for histological examination and all of them were accurately diagnosed as malignancies afterward.

The primary endpoint of histological accuracy was 91.4% (95% confidence interval, 82.3%–96.8%), which was significantly higher than the historical control of 77% (Table 3), and the sensitivity and specificity were 91.0% and 100%, respectively. The histological sensitivity increased with the number of punctures in malignant cases; the sensitivity values were 71.6%, 89.6%, 91.0%, and 91.0% after 1st, 2nd, 3rd, and 4th punctures, respectively (Fig. 2). In terms of the histological adequacy of the samples, all specimens were evaluable for histological examination. After standard histological examination, immunohistochemical staining was required for 5 patients and evaluable in 4 with adequate specimen; acinar cell carcinoma, neuroendocrine neoplasm, malignant lymphoma, and autoimmune pancreatitis in each one. Although routine staining for Programmed cell Death1 (PD1)/ Programmed cell Death1-Ligand 1 (PD-L1) was not performed because of the study period, microsatellite instability was examined in 4 patients with long survivor after 2019 and microsatellite instability examination was successful in all of them.

The cytological sensitivity, specificity, and overall accuracy were 95.5%, 100%, and 95.7%, respectively, and all of the patients were accurately diagnosed by combining cytological and histological examinations.

| Table 1 | Patient characteristics. |
|---------|--------------------------|
|         | n = 70                   |
| Age, median (range), yrs | 68 (36–87)              |
| Gender, male, n (%)    | 43 (61.4)                |
| Tumor size, median (range), mm | 22 (15–42) |
| Tumor location, n (%)  | Head 23 (32.9)           |
|                      | Body 36 (51.4)           |
|                      | Tail 11 (15.7)           |
| Treatment, surgery, n (%) | 8 (11.4)         |
| Final diagnosis, n (%) | Pancreatic cancer 65 (92.9) |
|                      | Adenocarcinoma 63 (90.0) |
|                      | Adenocarcinoma 1 (1.4)   |
|                      | Acinar cell carcinoma 1 (1.4) |
|                      | Neuroendocrine neoplasm 1 (1.4) |
|                      | Malignant lymphoma 1 (1.4) |
|                      | Autoimmune pancreatitis 2 (2.9) |
|                      | Mass-forming pancreatitis 1 (1.4) |
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|                      | Autoimmune pancreatitis 2 (2.9) |
|                      | Mass-forming pancreatitis 1 (1.4) |

| Table 2 | The details of puncture. |
|---------|----------------------------|
|         | All patients (n=70) | Malignant (n=67) | Benign (n=3) |
| Puncture site, n (%) | Stomach 51 (72.9) | 50 (74.6) | 1 (33.3) |
| First portion of the duodenum 10 (14.3) | 9 (13.4) | 1 (33.3) |
| Second portion of the duodenum 9 (12.9) | 8 (11.9) | 1 (33.3) |
| Technical success, n (%) | 70 (100) | 67 (100) | 3 (100) |
| Number of punctures, n (%) | 2 33 (47.1) | 32 (47.8) | 1 (33.3) |
| 3 26 (37.1) | 26 (38.8) |
| 4 or more* 11 (15.7) | 9 (13.4) | 2 (66.7) |
| Number of punctures to achieve diagnosis, n (%) | 1 48 (71.6) |
| 2 12 (17.9) |
| 3 1 (1.5) |
| 4 or more 0 (0) |

* The maximum number of punctures was 8.

| Table 3 | Overall diagnostic yield. |
|---------|----------------------------|
|         | Cytology | Histology | Cytology and histology |
|         | 95%CI | 95%CI | 95%CI |
| Sensitivity | 0.96 | (0.87–0.99) | 0.91 | (0.82–0.97) | 1.00 | (0.95–1.00) |
| Specificity | 1.00 | (0.99–1.00) | 1.00 | (0.99–1.00) | 1.00 | (0.99–1.00) |
| PPV | 1.00 | (0.99–1.00) | 1.00 | (0.99–1.00) | 1.00 | (0.99–1.00) |
| NPV | 0.50 | (0.12–0.88) | 0.33 | (0.07–0.70) | 1.00 | (0.95–1.00) |
| Accuracy | 0.96 | (0.80–0.99) | 0.91 | (0.82–0.97) | 1.00 | (0.95–1.00) |

CI = confidence interval, NPV = negative-predictive value, PPV = positive-predictive value.
An adverse event occurred in 1 case (1.4%). The patient experienced a needle fracture during the procedure. In this patient, the mass in the pancreatic head was very hard, and the puncture was repeated three times from the first portion of the duodenum. Although the needle fragment remained in the pancreas, the patient experienced no related symptoms.

4. Discussion

In this prospective study, the 20G EUS-FNB needle with a forward bevel confirmed a significantly higher histological accuracy (91.4%) for pancreatic mass lesions than that of the historical control. The technical success and sample adequacy for histology were 100% and cytological diagnostic accuracy was 95.7%. This high diagnostic yield was achieved even though 25.7% of the procedures were performed by trainees. The diagnostic sensitivity reached a plateau after the third puncture. Only one adverse event (1.4%) of needle fracture was occurred.

There were several previous reports that have examined this 20G FNB needle. A retrospective study comparing two types of ProCore needles (20G with forward bevel and 22G with reverse one) revealed that the microcore collection rate was significantly higher in the 20G needle with forward bevel than that in 22G needle with reverse bevel (92.6% vs 49.5%; \( P < 0.01 \)). A large multicenter randomized controlled trial comparing the diagnostic yields of the 20G ProCore needle with forward bevel and the 25G standard FNA needle also showed the superiority of the 20G ProCore needle in terms of tissue collection rates and diagnostic accuracy. However, the histological yield was 77% in the 20G ProCore needle which was slightly lower than our result (91.4%). An observational study comparing between the 20G ProCore needle and the 22G Acquire Endoscopic Ultrasound Fine Needle Biopsy needle (Boston Scientific Corporation, MA) for solid pancreatic masses confirmed that the histological diagnosis was 92% in the 20G ProCore needle.

In the present study, EUS-guided tissue acquisition was technically successful in all cases regardless of the puncture site or the experience of the endosonographers. The other study comparing the 19G, 22G, and 23G ProCore needle with reverse bevels showed a lower maneuverability of the 19G needle, especially when the punctures were performed from the duodenum. The newly designed coil-spring sheath of the 20G ProCore needle reduces the friction between the echoendoscope and the needle, and may explain the puncture successes in all our cases (even when the scope was extremely bent). Additionally, our study included 18 procedures (25.7%) performed by trainees, as compared to the previous multicenter randomized controlled study with expert endosonographers with life-time performances >1000 EUS-FNA procedures. Even so, we achieved a high technical success rate and an overall diagnostic accuracy. Therefore, it can be concluded that this needle could be used for general use even for the beginners of EUS-guided tissue acquisition.

Our study revealed the histological sensitivities after 1st, 2nd, 3rd, and 4th puncture were 71.6%, 89.6%, 91.0%, and 91.0%, respectively, and the sensitivity reached a plateau after the third puncture. Previous randomized controlled study reported that the sensitivities after the first and third punctures were 75% and 89%, respectively. In addition, their tissue collection rate after the first puncture of the 20G ProCore was 61% and the collection rate after the 1st to 3rd punctures was 77%. Since the tissue collection rates did not increase significantly after the 4th puncture, they concluded that a good tissue sample can be obtained by puncturing only 3 times. Based on these results, 3 puncture might be enough to gain the sample when using 20G ProCore needle with forward bevel.

We experienced a rare complication of needle fracture. The puncture was performed from the duodenum and was repeated 3 times to obtain the core sample. It is conceivable that the durability of the needle may be reduced in the side-hole portion. The area of the side-hole of this needle is larger than that of the previous needle with the side-hole. Therefore, it cannot be denied that the needle may be vulnerable at this site. Therefore, it is necessary to be careful about multiple punctures to avoid the risk of needle fracture. In this respect, it was considered to be a very significant that the diagnostic yield reached a plateau with 3 punctures in this study. However, the puncture should be completed with a smaller number especially when the puncture is performed in situations where the needle is likely to be overloaded.

Recently, other types of FNB needles have been introduced for EUS-guided tissue acquisition, including the fork-tip needle and...
FNB needle for EUS-guided tissue acquisitions.

Success and it may be thought as one of the standard arm study with an intermediate number of cases. In order to study. Finding an optimal method for evaluating the amount of needle may help avoid tumor seeding. Third, some of the resectable, cytological con
of the small pancreatic cancer lesions smaller than 15mm are
bevel is located 8 mm away from the top of the needle, this needle
comparing these FNB needles are now in progress
20G ProCore and 22G Acquire needles found no difference in

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In conclusion, we conducted a single-center prospective study to evaluate the 20G core biopsy needle with a forward bevel. This FNB needle has a high histological diagnostic yield with a high technical success and it may be thought as one of the standard FNB needle for EUS-guided tissue acquisitions.

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