

Evaluating the Minimal Specimens From Endoscopic Ultrasound-Guided Fine-Needle Aspiration in Pancreatic Masses

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Abstract: Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has become one of the most useful diagnostic modalities for the diagnosis of pancreatic mass. The aim of this study was to investigate the role of analyzing the minimal specimens obtained by EUS-FNA for the diagnosis of solid masses of pancreas.

This study consisted of retrospective and prospective analyses. The retrospective study was performed on 116 patients who underwent EUS-FNA of solid masses for cytological smear, histological analysis, and combined analysis including immunohistochemical (IHC) staining. In the prospective study, 79 patients were enrolled to evaluate the quality and accuracy of EUS-FNA histological analysis and feasibility of IHC staining.

The final diagnoses of all patients included pancreatic cancer ($n=126$), nonpancreatic cancer ($n=21$), other neoplasm ($n=27$), and benign lesions ($n=21$). In our retrospective study, the combined analysis was more sensitive than cytological analysis alone ($P < 0.01$). The overall sensitivity of cytology, histology, and combined analysis was 69.8%, 67.2%, and 81.8%, respectively. In the prospective analysis, 64.2% of all punctures were helpful for determining the diagnosis and 40.7% provided sufficient tissue for IHC staining. Histological analysis was helpful for diagnosis in 74.7% of patients. IHC staining was necessary for a definite diagnosis in 11.4% of patients, especially in the cases of nonmalignant pancreatic mass.

Histological analysis and IHC study of EUS-FNA specimens was useful for the accurate diagnosis of pancreatic and peripancreatic lesions. Combined analysis showed significantly higher sensitivity than cytology alone because IHC staining was helpful for a diagnosis in some patients.

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Abbreviations: EUS-FNA = Endoscopic ultrasound-guided fine-needle aspiration, EUS-TCB = EUS-guided trucut biopsy, GIST = gastrointestinal stromal tumor, IHC = Immunohistochemical.

INTRODUCTION

Approach to the patients with pancreatic masses can be very challenging, and EUS-FNA for differential diagnosis of a pancreatic or peripancreatic mass is often challenging. Pancreatic ductal adenocarcinoma is the most frequently observed; however, pancreatic masses can be lymphomas, other metastatic tumors, cystic tumors, tuberculosis, and chronic pancreatitis as well. Therefore, an accurate histologic diagnosis is critical in determining the prognosis and optimal treatment.^{1,15}

In the past, directly obtaining tissues of pancreatic or peripancreatic masses was difficult before surgery because of the abundant amount of vessels around the mass. To solve this problem, EUS-FNA was introduced in the early 1990s for the diagnosis of pancreatic and gastrointestinal tumors.^{16,18} Since then, EUS-FNA has become an important method for differential diagnosis of pancreatic masses or nearby nonpancreatic masses.³ EUS-FNA, which is traditionally performed with a 19-, 22-, or 25-gauge needle, has been shown to be a technically safe, minimally invasive, and accurate method for tissue sampling.^{2,4-7} However, the diagnostic yield of EUS-FNA depends on the location, size, and features of tumors or other tissues and on several technical factors such as type of needle, biopsy technique, and material processing technique.⁹ Specific diagnosis of certain tumors could require more than a cytological analysis. In these cases, a 19-gauge needle trucut biopsy (TCB) is traditionally used because it provides a larger tissue specimen for analyzing tissue architecture and IHC staining.^{9,11,12,14} However, EUS-TCB (EUS-guided trucut biopsy) has certain limitations. For example, some sites or small lesions (<2 cm) are not suitable, and more complications may occur than with EUS-FNA. Therefore, to improve the diagnostic accuracy for solid pancreatic tumors, there have been attempts to obtain histology using 22- and 25-gauge needles instead of EUS-TCB.^{8,11,13} These studies have reported that combined cytology and histology is useful for the differential diagnosis of solid pancreatic masses. However, these studies were limited because the histological analysis of tissue samples did not include IHC staining for differential diagnosis of solid pancreatic tumors with very similar or overlapping cytological and histological characteristics. Therefore, the aim of the present study was to investigate the role of analyzing the minimal specimens obtained by EUS-guided 22-gauge FNA needle and EUS-guided 25-gauge FNA needle for the diagnosis of pancreatic or peripancreatic solid masses.

PATIENTS AND METHODS

Retrospective Analysis

We retrospectively reviewed the medical records of patients who underwent EUS-FNA to evaluate solid pancreatic or peripancreatic lesions from January 2009 to July 2010 at Samsung Medical Center, Seoul, Korea. This study was approved by the Institutional Review Board of Samsung Medical Center. EUS-FNA was performed in 337 consecutive patients to discriminate between malignant and benign conditions and to determine a specific diagnosis. We selected 116 patients for whom both cytological and histological analyses were performed. Medical records were reviewed to note the lesion location, size, FNA needle type used, cytopathologic results, final diagnosis, occurrence of complications, and clinical features. If a patient underwent surgery, final diagnosis was based on surgical pathology. If surgery was not performed, we checked whether there was a definite pathologic diagnosis that was consistent with clinical findings, and this was considered the final diagnosis. If neither approach yielded a definite diagnosis, we made a diagnosis based on a biopsy of another site or on clinical follow-up for at least 1 year. In cases with cytologic and/or histologic analysis, but without a clear diagnosis, we additionally performed IHC staining on tissue specimens.

Prospective Analysis

To validate and evaluate the quality and diagnostic accuracy of histological analysis including the feasibility of IHC staining, we prospectively enrolled 79 patients with solid pancreatic or peripancreatic lesions. The flowchart of study design is shown in Figure 1.

Endoscopic Technique and Specimen Preparation

All EUS-FNA procedures were performed under conscious sedation by 1 experienced endosonographer (K. H. Lee). A linear EUS (GF-UE160-AL; Olympus, Tokyo, Japan) with a ProSound SSD 5000 processor (Aloka, Wallingford, CT) was used in all cases. Echotip needles (22- or 25-gauge;

Wilson-Cook Inc., Winston-Salem, NC) were used, allowing several passes under direct endosonographic visualization to obtain sufficient cellular aspirate for cytologic analysis. The choice of needle diameter was based on the circumstances at the time. A transgastric approach was performed on lesions in the body or tail of the pancreas, and a transduodenal approach was used for lesions in the head or uncinate process. For tissue retrieval, the stylet was introduced into the needle or the needle was inserted with a 5 to 10 cc air-filled syringe. The extruded material was placed onto glass slides for primary gross inspection. After a part of EUS-FNA specimen was smeared on the slide, aspirates were fixed with 96% ethanol for cytological analysis. The remaining specimens were placed into 10% formalin solution for histological analysis. Figure 2A showed histologic core specimen in a formalin vial and 2B showed gross aspirated specimen from the pancreatic mass. The acquired samples were prepared and examined by the pathology department.

Pathologic Preparation and Assessment of Specimens

After alcohol fixation, the slides were stained with standard Pap stain. The sample for histological evaluation was processed using the standard protocol of a formalin-fixed paraffin-embedded block for H&E staining with further evaluation by IHC staining according to the pathologist's judgment.

The quality of slides was categorized as inadequate or adequate. The histological sample was considered adequate if a core tissue specimen from the target tissue was obtained, and the cytological sample was adequate if it contained sufficient target tissue cells for inspection. The cytological and histological findings were classified as inconclusive, nondiagnostic, atypical cell, strongly suspicious, or positive for the diagnosis of specific malignancy. We considered it a definite diagnosis if the results of cytological or histological analysis were strongly suspicious or positive for specific malignant tumors or consistent with benign or with some other neoplasm pathology for nonmalignant tumors. We considered diagnosis helpful if the results of cytological or histological analysis indicated atypical cells in pancreatic cancer or if they indicated spindle cells in gastrointestinal stromal tumor (GIST).

Statistical Analysis

Noncontinuous variables were analyzed by Fisher's exact test or chi-square test and continuous variables were analyzed by 2-sample T-tests or 001Wilcoxon signed rank test. In the adequacy and accuracy of cytological analysis, histological analysis, and the combination, methods were compared using the McNemar χ^2 test. The Bonferroni correction was used to correct *P*-values because we performed multiple tests to analyze subgroups (pancreas versus nonpancreas, malignancy versus nonmalignancy, pancreas versus nonpancreas, and 22 gauge versus 25 gauge). We analyzed the factors that influenced the accuracy of histological analysis using a multiple logistic regression model. *P* < 0.05 was considered statistically significant and all analyses were performed using STATA 11 (StataCorp LP, TX).

RESULTS

Retrospective Data Analysis

A total of 116 solid masses from 116 patients were identified from the pancreas (*n* = 93), from the gastrointestinal

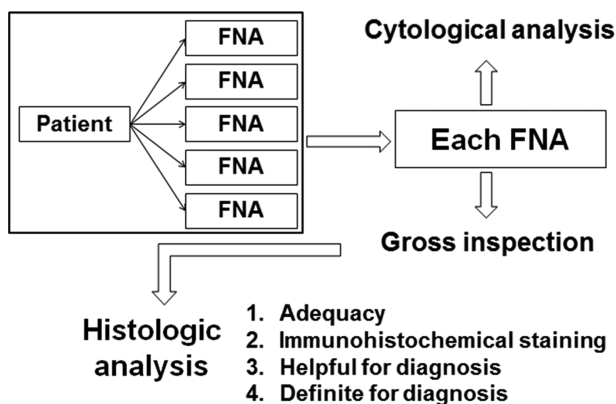


FIGURE 1. The prospective study design. The patients underwent 4 to 5 needle punctures during EUS-FNA. Histological analysis was performed in available cases after gross observation. Tissue adequacy, the immunohistochemical (IHC) staining, and diagnostic accuracy were assessed in each tissue obtained by EUS-FNA. EUS-FNA = endoscopic ultrasound-guided fine-needle aspiration, IHC = immunohistochemical.

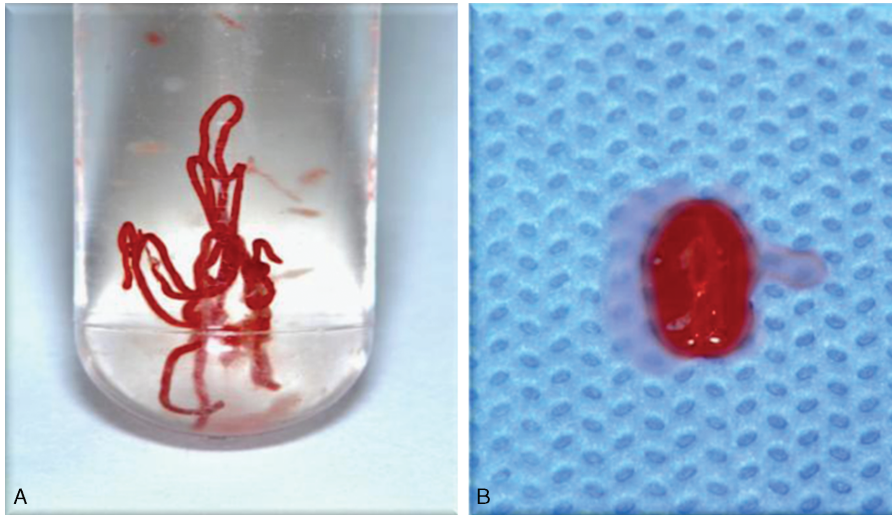


FIGURE 2. Gross image of a core of pancreatic tissue obtained by EUS-FNA. (A) Panel A shows histologic core specimen in a formalin vial. (B) Panel B shows gross aspirated specimen from the pancreatic mass. EUS-FNA = endoscopic ultrasound-guided fine-needle aspiration.

tract (n = 10), and from adjacent structures including the lymph node, biliary tract, and retroperitoneum (n = 13). The overall size of the masses was 36 ± 16.6 mm (range 8–210 mm). Of the 116 patients, information on needle type was lacking for 3. When we analyzed the data of the remaining 113 patients, 117 needles were used; 22-gauge needles were used for 63 patients, 25-gauge needles for 46 patients, and both needles for 4 patients. The mean number of needle passes was 3.5 ± 0.9 (range 2–5). Table 1 shows the patients characteristics at baseline. The most common diagnosis was pancreatic cancer (76/116, 65.5%). Fourteen other malignant tumors included cholangiocarcinoma (n = 1), Ampulla of Vater cancer (n = 1), gall bladder cancer (n = 3), common bile duct cancer (n = 1), lymphoma (n = 4), and metastatic cancers (n = 4). Sixteen other neoplasms included GIST (n = 5), neuroendocrine tumor (n = 5), SPEN (n = 3), schwannoma (n = 1), desmoid tumor (n = 1), and inflammatory pseudotumor (n = 1). Six benign lesions were tuberculosis (n = 2), hamartoma (n = 2), and autoimmune pancreatitis (n = 2). Four lesions were not diagnosed.

The overall diagnostic accuracy rates for cytological, histological, and combined analysis were 69.8% (95% CI, 61–78%), 67.2% (95% CI, 59–75%), and 81.8% (95% CI, 75–89%), respectively (Table 2). Although there was no difference in diagnostic accuracy between cytological analysis and histological analysis, the overall diagnostic accuracy for combined analysis was significantly higher than that for cytological analysis (*P* < 0.001).

We performed the subgroup analysis of cytology, histology, and combined method based on pancreas versus nonpancreas, malignant lesion versus nonmalignant lesion, pancreatic cancer versus nonpancreatic cancer, and 22-gauge needle versus 25-gauge needle. In the pancreatic lesions, the diagnostic accuracy of cytology and combined analysis were 77.4% and 84.9%, respectively (*P* < 0.01). In nonpancreatic lesions, the diagnostic accuracy of cytology and combined analysis was 39.1% and 69.6%, respectively (*P* = 0.01). When the final diagnosis was malignant, the diagnostic accuracy of combined analysis (86.7%) was not significantly different from that of cytological analysis (80.0%). However, the diagnostic

TABLE 1. Patient Characteristics at Baseline (n = 116)

Sex (M:F)		1.27: 1.0
Age (mean ± standard deviation, SD)		59.6 ± 12.2
Location	Pancreas	93 (80.2%)
	Other sites*	23 (19.8%)
Size of mass (mean ± SD, mm)	All	36.2 ± 16.6
	Malignancy	37.3 ± 14.6
	Borderline	33.9 ± 22.5
	Benign	30.1 ± 21.1
Type of needle	22G	63 (54.3%)
	25G	46 (39.6%)
	Both	4 (3.4%)
	No information	3 (2.7%)
Number of needle passes		3.5 ± 0.9
Final diagnosis	Malignancy	90 (77.6%)
	Pancreatic cancer	76
	Nonpancreatic cancer†	14
	Other neoplasms‡	16 (13.8%)
	Benign§	6 (5.2%)
	Unknown	4 (3.4%)
Gold standard of diagnosis	Surgery	25
	Biopsy at another site	7
	EUS-FNA and follow-up	77
	Clinical follow-up	7

SD = standard deviation.

*Gut wall, biliary tract, lymph node (intra-abdominal or mediastinal).

†Cholangiocarcinoma, Ampulla of Vater cancer, gall bladder cancer, common bile duct cancer, lymphoma, and metastatic cancers (colon, lung, and kidney).

‡GIST, neuroendocrine tumor, SPEN, schwannoma, desmoid tumor, and inflammatory pseudotumor.

§Tuberculosis lymphadenopathy and autoimmune pancreatitis.

TABLE 2. Diagnostic Accuracy of Cytological, Histological, and Combined Analyses

	Cytology	Histology	Combined	P Value*
Overall cases	69.8%(81/116)	67.2%(78/116)	81.8%(95/116)	<0.001
Pancreatic	77.4%(72/93)	68.8%(64/93)	84.9%(79/93)	<0.01
Nonpancreatic	39.1%(9/23)	60.8%(14/23)	69.6%(16/23)	0.010
Malignant	80.0%(72/90)	70.0%(63/90)	86.7%(78/90)	0.093
Nonmalignant	34.6%(9/26)	57.7%(15/26)	65.4%(17/26)	0.016
Pancreatic cancer	84.2%(64/76)	75.0%(57/76)	92.1%(70/76)	0.062
Nonpancreatic cancer	42.5%(17/40)	52.5%(21/40)	62.5%(25/40)	0.016
22G	68.2%(43/63)	68.3%(43/63)	84.1%(53/63)	0.002
25G	73.9%(34/46)	67.4%(31/46)	80.4%(37/46)	0.250

*Result from the comparison of cytology and combined analyses.

accuracy of combined analysis for nonmalignant masses (65.4%) was significantly higher than that of cytological analysis (34.6%) ($P=0.016$). Similarly, there was no significant difference in the diagnostic accuracy between cytological and combined analysis in pancreatic cancer; however, the diagnostic accuracy of the combined analysis was significantly higher than that of cytological analysis in nonpancreatic cancer or with a 22-gauge needle (Table 2).

In 16.4% of patients (19/116), IHC staining was performed according to the judgment of the pathologist to determine or discriminate the histological type of the solid mass (Table 3). Among these 19 patients, 8 were diagnosed by histological analysis combined with IHC staining, but not by cytological analysis. Four GISTs were diagnosed by IHC for CD-117 (c-KIT), 1 Schwannoma by IHC for S-100 and CD-117 (c-KIT), and 3 neuroendocrine tumors by IHC for chromogranin and synaptophysin. Figure 3 showed pancreatic neuroendocrine tumor diagnosed by EUS-FNA with cytology smear (Figure 3A) and histopathologic examination using immunohistochemical staining of chromogranin and synaptophysin as well (Figure 3B and C). Figure 3A showed smears from EUS-FNA of pancreas and cytology specimen had moderate cellularity with rosette formation and many single cells with peripherally placed nuclei. Also, acquired tissue specimen from EUS-FNA was evaluated and H&E staining as well as immunostaining with chromogranin and synaptophysin was performed (Figure 3B and C). H&E staining from the tissue sample of EUS-FNA

showed well-differentiated pancreatic neuroendocrine tumor, and it has nested growth pattern (Figure 3B). In Figure 3C, immunostaining revealed positive expression of synaptophysin in tumor tissue. In addition, normal control of EUS-FNA cytology samples were also shown in supplementary Figure 1, <http://links.lww.com/MD/A990> and the control samples have been validated in pathology department of our center. Immunohistochemical staining results for each patient can be found in supplementary Table 1, <http://links.lww.com/MD/A990>.

Prospective Data Analysis

We tried to analyze 387 punctures of 79 patients. From a total of 79 patients, 50 were diagnosed with pancreatic cancer. From among the remaining patients, 7 had nonpancreatic cancers, 13 had other neoplasms, and 9 had benign diseases. In addition, 22-gauge needles were used for 48 patients (60.7%) and 25-gauge needles were used for 31 patients (39.3%). Four needle punctures were sufficient to obtain tissue specimens in 8 patients, and 5 needle punctures were enough to obtain tissue specimen in 71 patients (Table 4). The tissue specimens were transferred to the pathologist after the endoscopist evaluated the appropriate tissue specimen for histological analysis. Of the total 387 punctures, 62.8% (243/387) could be transferred to the pathologist. Of the transferred tissue specimens, 64.2% (156/243) were sufficient for a helpful diagnosis, and 40.7% (99/243) were appropriate tissues specimens for IHC staining. From 79 patients, 5.1% of tissue specimens were not transferred to the pathologist. In total, 74.7% were helpful for making a diagnosis and 65.8% (52/29) were sufficient for performing IHC staining (Table 5). Pathologists were examined EUS-FNA aspirates and evaluated the adequacy of the specimen.

Based on the helpful diagnosis, the overall diagnostic accuracy rates for cytological, histological, and combined analysis were 92.4% (73/79), 74.7% (59/79), and 93.7% (74/79), respectively. There was no difference in the diagnostic accuracy between cytological analysis and combined analysis ($P=0.50$). However, for definite diagnoses, the diagnostic accuracy of combination analysis (74.7%) was significantly higher than that of cytological analysis (59.5%). From among all patients, 9 cases (11.4%) needed IHC staining for definite diagnosis. The cases diagnosed by IHC staining were neuroendocrine tumors ($n=2$), metastatic cancers ($n=2$), SPEN ($n=2$), leiomyosarcoma ($n=1$), schwannoma ($n=1$), and desmoid tumor ($n=1$).

TABLE 3. Number of Cases With Additional Diagnostic Information from IHC Staining

	Final Diagnosis	Number
Malignant tumors	Pancreatic cancer	5
	Lymphoma	1
	Metastatic colon cancer	1
	Metastatic lung cancer	1
Borderline tumors	GIST	4
	PNET	4
	Solid pseudopapillary tumor	2
	Schwannoma	1
	Total	19

IHC Staining = immunohistochemical staining.

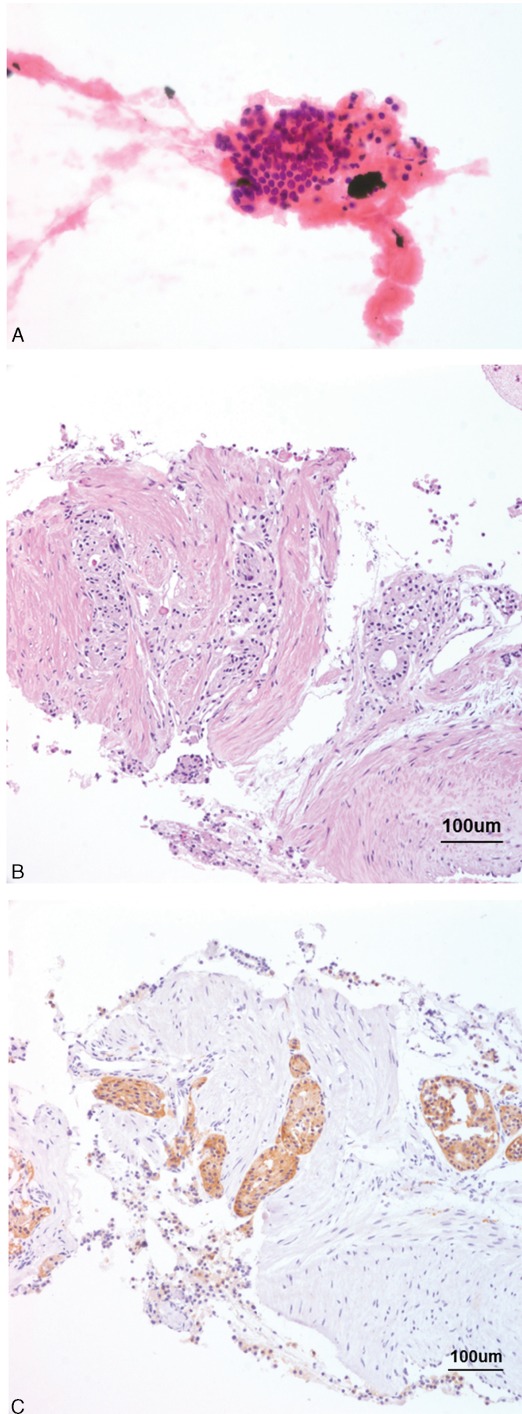


FIGURE 3. Pancreatic neuroendocrine tumor diagnosed by EUS-FNA. (A) Smears from EUS-FNA of pancreas shows moderate cellularity with rosette formation (Papicolaou stain; magnification $\times 400$). (B) H&E staining from the acquired tissue sample of EUS-FNA shows well-differentiated pancreatic neuroendocrine tumor, and it has nested growth pattern (H&E $\times 100$). Architecture and the structural relationship between tumor cell and connective tissue are shown. (C) Immunostaining (brown color) revealed positive expression of synaptophysin in tumor tissue ($\times 100$). EUS-FNA = endoscopic ultrasound-guided fine-needle aspiration, H&E = hematoxylin and eosin.

TABLE 4. Prospectively Enrolled Patient Characteristics at Baseline

	Number (%)
Sex (M:F)	49:30
Age (median \pm SD, years)	59.3 \pm 12.1
Needle (22G:25G)	48:31
Number of puncture	
Four	8 (10.1)
Five	71 (89.9)
Location	
Head and neck	39 (49.4)
Body and tail	40 (50.6)
Final diagnosis	
Pancreatic cancer	50 (64)
Non pancreatic cancer*	7 (8.9)
Other neoplasm [†]	13 (16.5)
Benign [‡]	9 (11.4)

SD = standard deviation.

*Metastatic adenocarcinoma (n = 3), renal cell carcinoma (n = 1), leiomyosarcoma (n = 1), metastatic lung cancer (n = 1), and gall bladder cancer (n = 1).

[†]Serous cystic neoplasm (n = 5), SPEN (n = 2), GIST (n = 1), desmoid tumor (n = 1), schwannoma (n = 1), and neuroendocrine tumor (n = 3).

[‡]Reactive lymphadenopathy (n = 4), chronic pancreatitis (n = 3), and tuberculosis lymphadenopathy (n = 2).

Using a logistic regression model, we investigated the factors that influenced the histologic diagnosis. In the univariate analysis, statistical significance was observed for malignant lesion versus benign lesion (OR = 5.33, $P = 0.003$, 95% CI 1.78–15.99) and the number of tissue transfers to pathologists (OR = 1.90, $P = 0.008$, 95% CI 1.19–3.03) (Table 6). In the multivariate analysis, we observed the statistical significance of malignant lesion versus benign lesion (OR = 7.85, $P = 0.001$, 95% CI 2.25–27.5) and the number of tissue transfer to pathologists (OR = 2.23, $P = 0.003$, 95% CI 1.31–3.78). Also in the multivariate analysis of factors influencing IHC staining, female gender (OR = 3.17, $P = 0.036$, 95% CI 1.08–9.35) and the number of tissue transfers to pathologists (OR = 1.52, $P = 0.05$, 95% CI 1.00–2.30) were statistically significant.

TABLE 5. The Histologic Results by EUS-FNA

	Specimen (%)	Patients (%)
Transferred aspirates	243/387 (62.8)	75/79 (95)
Adequate aspirates	114/243 (46.9)	52/79 (65.8)
IHC possible aspirates	99/243 (40.7)	52/79 (65.8)
Helpful for diagnosis	156/243 (64.2)	59/79 (74.7)
Definite diagnosis	94/243 (38.7)	40/79 (50.6)

EUS-FNA = endoscopic ultrasound-guided fine-needle aspiration. Definite diagnosis: the results of cytological or histological analysis were strongly suspicious or positive for specific malignant tumors or consistent with benign or other neoplasm pathology for nonmalignant tumors. Helpful diagnosis: the results of cytology or histology indicated atypical cells in pancreatic cancer or spindle cells in GIST.

TABLE 6. Factors Influencing Histological Diagnosis

	Odds Ratio (CI)	P Value
Age	1.02 (0.98–1.07)	0.259
Sex (male vs female)	2.20 (0.71–6.87)	0.172
Location (pancreas vs other site)	0.79 (0.29–2.19)	0.652
Mass size (cm)	1.14 (0.76–1.72)	0.522
Diagnosis (benign vs malignant)	5.33 (1.78–16.0)	0.003
Needle type (22 gauge vs 25 gauge)	0.96 (0.43–2.17)	0.92
Number of needle passes	1.90 (1.19–3.03)	0.008

CI = confidence interval.

DISCUSSION

EUS-FNA has been shown to be a safe, minimally invasive, and accurate method for tissue sampling of suspicious lesions in or around the pancreas.^{3,16,18} A tissue specimen obtained by EUS-FNA using 22- or 25-gauge needles is commonly used only for cytological analysis because most pancreas cancers can be diagnosed with cytological analysis. However, specific diagnosis of certain tumors such as lymphomas, stromal tumors, neuroendocrine tumors, and metastatic tumors may require more than cytological analysis.^{9,11,12,14} For this purpose, 19G TCB is used for histological analysis, but technical difficulties limit its clinical application. Therefore, we evaluated the diagnostic benefit and feasibility of histological analysis for specimens obtained by 22- and 25-gauge needles from the pancreatic or peripancreatic solid masses combining with the IHC staining. We could observe that the combined analysis improved the diagnostic accuracy when compared with cytological analysis alone. Moreover, IHC staining was useful in difficult cases whose diagnoses were inconclusive based on cytological analysis alone. Our results were similar to those of recent studies on the diagnostic accuracy of histologic-cytologic analysis of specimens obtained by 22-gauge guided EUS-FNA for diagnosis of pancreatic masses.^{11,14} Lee et al also reported a well-designed study that they approached in 3 different ways for EUS-FNA specimens; on-site cytologic examination, conventional cytologic examination and IHC staining, and this 3-step approach had significantly increased accuracy compared to conventional cytologic examination alone.¹⁰ That being said, we have found out the multiple combined approaches may increase the accuracy of diagnosis for pancreatic solid mass.

In addition, we also compared accuracy based on the needle gauge. The combined analysis with a 22-gauge needle alone was more accurate than that of other methods in our retrospective results. However, the prospective results did not identify needle gauge as a factor influencing the histological diagnosis. We evaluated which cases were useful for performing additional histological analysis. The retrospective results demonstrated that the benefit of combined analysis was more pronounced in nonpancreatic cancer and nonmalignant cases. However, the prospective results demonstrated that the benefit of combined analysis was more beneficial in nonmalignant cases alone. The difference in needle type or benefit of combined analysis between the retrospective and the prospective result might be caused by differences in the disease population. Therefore, a large-scale randomized controlled study is required to determine the difference between both groups.

In a previous study, Voss et al evaluated the feasibility and diagnostic accuracy of EUS-guided fine needle biopsy (EUS-FNB) in patients with solid pancreatic masses. The overall diagnostic accuracy by EUS-FNB was 74.4%, and EUS-FNAB was useful for diagnosing solid pancreatic masses due to IHC feasibility.¹⁷ However, this study did not comment on the results of IHC in solid pancreatic masses. Other similar studies have not evaluated the feasibility of IHC staining.^{8,11} Our prospective study demonstrated that 64.2% of histologic analyses were helpful for diagnosis and 40.7% of samples were sufficient for IHC staining based on the number of needle passes. In terms of each patient, 74.7% of histological analyses were helpful for diagnosis and 65.8% were suitable for analysis by IHC staining. These results demonstrate that at least 50% of samples could be analyzed by IHC staining alone without an additional procedure if the histologic analysis was routinely performed as part of the EUS-FNA procedure. Our results demonstrate that the diagnostic accuracy of cytological analysis was low in nonpancreatic cancers or nonmalignant cases. The combined analysis showed higher diagnostic accuracy. It is thought that cytological analysis is sufficient for diagnosis of most pancreatic cancers, whereas histological analysis followed by IHC staining is often required for diagnosis of nonmalignant lesions and/or nonpancreatic cancers such as stromal, neuroendocrine, and solid pseudopapillary tumors. This is especially useful for a solid mass lesion that is likely to be something other than pancreatic cancer.

This study is the first to evaluate the additional benefit of diagnosis using combined cytologic-histologic analysis and IHC staining in peripancreatic and pancreatic specimens obtained by EUS-FNA. Additionally, we prospectively evaluated the feasibility of histological analysis by IHC staining in all patients. Although our study showed a large set of retrospective and prospective data as well, there are some limitations of this study. First of all, we did not have onsite pathologist, and therefore, we could not only estimate the adequacy of the samples but also depended on our experts' hands. The second, we only included solid mass of pancreas and did not include cystic tumors. The role of EUS-FNA in pancreas cystic tumors may become significantly important in the process of clinical decision making to distinguish malignant ones from the benign ones. These mentioned subjects should be further investigated with more cases in the EUS-FNA procedure.

Here, we would like to address that the diagnostic accuracies of cytology and combined analysis of specimens obtained by EUS-FNA may not be significantly different in pancreatic cancer; however, combined analysis of specimens for solid masses showed significantly higher sensitivity than that of cytology alone, especially in nonpancreatic cancers. In cases that were nondiagnostic according to cytologic and histologic analysis, additional IHC staining of tissues obtained by EUS-FNA could be helpful for the diagnosis of nonpancreatic cancer.

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