High-resolution endoscopic ultrasound imaging and the number of needle passages are significant factors predicting high yield of endoscopic ultrasound-guided fine needle aspiration for pancreatic solid masses without an on-site cytopathologist

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Abstract
Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is the accurate diagnostic method for pancreatic masses and its accuracy is affected by various FNA methods and EUS equipment. Therefore, we aimed to elucidate the instrumental and methodologic factors for determining the diagnostic yield of EUS-FNA for pancreatic solid masses without an on-site cytopathology evaluation.

We retrospectively reviewed the medical records of 260 patients (265 pancreatic solid masses) who underwent EUS-FNA. We compared historical conventional EUS groups with high-resolution imaging devices and finally analyzed various factors affecting EUS-FNA accuracy.

In total, 265 pancreatic solid masses of 260 patients were included in this study. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of EUS-FNA for pancreatic solid masses without on-site cytopathology evaluation were 83.4%, 81.8%, 100.0%, 100.0%, and 34.3%, respectively. In comparison with conventional image group, high-resolution image group showed the increased accuracy, sensitivity and specificity of EUS-FNA (71.3% vs 92.7%, 68.9% vs 91.9%, and 100% vs 100%, respectively). On the multivariate analysis with various instrumental and methodologic factors, high-resolution imaging (P = 0.040, odds ratio = 3.28) and 3 or more needle passages (P = 0.039, odds ratio = 2.41) were important factors affecting diagnostic yield of pancreatic solid masses.

High-resolution imaging and 3 or more passes were the most significant factors influencing diagnostic yield of EUS-FNA in patients with pancreatic solid masses without an on-site cytopathologist.

Abbreviations: EUS-FNA = endoscopic ultrasound-guided fine needle aspiration, NPV = negative predictive value, PPV = positive predictive value.

Keywords: accuracy, endoscopic ultrasound-guided fine needle aspiration, pancreatic cancer, pancreatic neoplasms

1. Introduction
Pancreatic solid masses may be benign or malignant lesions. Pancreatic ductal adenocarcinoma constitutes most pancreatic malignancies and is associated with an overall 5-year survival rate of 1.2% to 6.0%,[1,2] its incidence has steadily increased over the past 30 years.[3] Because a pathologic confirmation is important for differential diagnosis and optimal therapeutic strategy,[4,5] Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) has been used for diagnosis of pancreatic solid masses.[6–8] EUS-FNA has a reported sensitivity of 54% to 95%, a specificity of 71% to 100%, and an accuracy of 85% to 90%.[6,8–12] The foundation for the diagnostic accuracy of EUS-FNA is obtaining adequate tissue, and it could be influenced by several variables, including the size of the lesion, location of the lesion, needle gauge, needle type, use of a styllet and suction, number of needle passes, the endosonographer’s skill and experience, and on-site cytopathology evaluation.[13,14]

Previous studies reported factors influencing the diagnostic yield of EUS-FNA for pancreatic solid masses.[13–15] However, they did not consider high-resolution imaging modalities reflecting the advancement of imaging technology. Because the diagnostic accuracy of EUS-FNA is inevitably affected by various FNA methods and EUS equipment such as newer scope and ultrasound generator, we aimed to elucidate various factors influencing the diagnostic yield of EUS-FNA for pancreatic solid masses without on-site cytopathology evaluation.
2. Materials and methods

2.1. Patients

We retrospectively reviewed the medical records of 260 patients (265 pancreatic solid masses) who underwent EUS-FNA at the Gachon University Gil Medical Center, Incheon, Korea, a tertiary referral medical center, from May 2011 to December 2015. We reviewed data to dates starting from 1 year after our hospital actively began to perform EUS-FNA, because the skills of endosonographers and cytopathologists influence the diagnostic yield of EUS-FNA for pancreatic solid masses. The inclusion criteria were as follows: age of >18 years, pancreatic solid mass identified by the investigational modalities, and follow-up of >12 months in patients with a benign result on EUS-FNA. The exclusion criteria were as follows: coagulopathy (international normalized ratio of >1.5 or platelet count of <50,000/mm^3), pancreatic cystic mass, nonpancreatic site (i.e., lymph node or wall thickening), presence of intervening blood vessels, and altered gastrointestinal anatomy. This study was approved by the Institutional Review Board of the Gachon University Gil Medical Center (GAIRB 2015-181).

2.2. EUS-FNA procedures

EUS-FNA procedures were performed using a standardized method in patients who were under conscious sedation with intravenous midazolam and propofol. All procedures were carried out using a linear array echoendoscope (GF UCT2000; Olympus Medical Systems, Tokyo, Japan) connected to an ultrasound scanning system (EU-C2000; Olympus Medical Systems) by 2 endosonographers each having performed >500 procedures. New video processors were used from November 2013 onward (PENTAX-HI VISION Preirus with EG-3870UTK; Pentax Japan, Tokyo, Japan and EU-ME2 Premier Plus with GF-UCT180; Olympus Medical Systems), allowing for the attainment of high-resolution images (Fig. 1). We divided the conventional image group and high-resolution image group for pancreatic solid masses by the attainment of high-resolution images in November 2013. The needle size was chosen to fit the situation randomly by endosonographer. A standard 19-, 22-, or 25-G FNA device (EchoTip; Cook Medical, Bloomington, IN) was employed for EUS-FNA. A 22- or 25-G fine needle biopsy device (EchoTip ProCore; Cook Medical) with a reverse bevel at the tip of the needle was employed for EUS-FNA. The capillary (slow pull) technique was employed for EUS-FNA mostly. In some cases, we applied suction technique during EUS-FNA in order to increase the quantity of the FNA sample. Pancreatic head masses were approached from the duodenum, whereas pancreatic body and tail masses were accessed from the stomach. The adequacy of obtained specimens is judged by the presence of macroscopic material without cytopathologist, and the puncture is repeated until adequate specimens are obtained. After the masses were punctured by the needle, the stylet was withdrawn and the needle moved backward and forward within the masses 10 to 15 times per pass. The needle was then removed. The aspirated specimen was expressed onto slides by reinsertion of the stylet within the needle and air flushing, if needed.

2.3. Preparation, analysis, and cytopathologic evaluation of specimens

Part of the acquired specimen was smeared onto glass slides and stained with Papanicolaou stain. The remaining material was processed into 2 or 3 wells of a tissue tray, fixed with 10% neutral buffered formalin solution, and embedded in paraffin before histologic analysis. The endosonographers conducted all preparation of specimens. The remaining samples were stained with hematoxylin and eosin and periodic acid–Schiff reagent. In...
In addition, immunohistochemical analyses were performed on some histologic specimens. A cytopathologist in the pathology department evaluated the adequacy of samples and made a diagnosis through histologic analysis.

2.4. Diagnosis

The cytopathologic results were graded as follows: definite for malignancy, suspicious for malignancy, atypical, benign, and nondiagnostic for malignancy without rapid on-site evaluation. The category “positive for malignancy” included both definite and suspicious for malignancy, whereas the category “negative for malignancy” included atypical, benign, and nondiagnostic for malignancy. Inadequate results were considered nondiagnostic for malignancy. The final diagnoses of the patients were determined according to the comprehensive findings of EUS-FNA, surgical pathology, other pathologic examinations such as ultrasound-guided biopsy, and clinical and radiological follow-up compatible with the diagnosis. If benign lesions were contemplated, the patients were followed up for at least 12 months. The diagnostic yield of EUS-FNA (accuracy, sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) was evaluated by comparing the EUS-FNA results with the final diagnosis.

2.5. Statistical analysis

The baseline characteristics of EUS-FNA are expressed as the mean (with standard deviation) or median (with interquartile range and full range) for continuous data and as frequency and proportion for categorical data. The analysis of factors influencing EUS-FNA results was undertaken using univariate and multivariate logistic regression analysis. A P-value of <0.05 was considered statistically significant.

3. Results

We investigated 273 patients (278 pancreatic solid masses) who had undergone an EUS-FNA procedure during the study period. Thirteen patients (13 pancreatic solid masses) were excluded: 7 patients due to pancreatic solid masses with cystic portion, 2 due to aspiration of a lymph node, 2 due to aspiration of a thickened mass of lesion in mm, mean ± SD (range): 64.5 ± 13.1 (26–91) mm. One hundred sixty-two patients were analyzed.

Of the 260 patients, 141 were male and 119 were female. The head of the pancreas was the most common location (48.3%) of pancreatic solid masses. The mean size of the masses was 13.1 years. Among the 260 patients, 141 were male and 119 were female. The head of the pancreas was the most common location (48.3%) of pancreatic solid masses. The mean size of the masses was 13.1 years. Among them, 242 (91.3%) were diagnosed as malignant, 23 (8.7%) as benign, including 22 adenocarcinomas, 17 neuroendocrine tumors, 1 metastatic tumor, and 2 intraductal papillary mucinous neoplasms. Twenty-three (8.7%) masses were diagnosed as benign, including 17 inflammatory masses from pancreatitis and 6 cases of autoimmune pancreatitis.

The malignant or benign status of the EUS-FNA diagnoses and final diagnoses are summarized in Table 2. The mean age of the patients was 64.5 ± 13.1 years. Among the 260 patients, 141 were male and 119 were female. The head of the pancreas was the most common location (48.3%) of pancreatic solid masses. The mean size of the masses was 35.2 ± 16.4 mm (range, 8–116 mm). One hundred fifty (56.6%) masses were evaluated using EUS-FNA with the new video processor that offers high-resolution imaging. The mean number of needle passes was 3.3 ± 0.9. The head of the pancreas was the most common location (48.3%) of pancreatic solid masses. The mean size of the masses was 35.2 ± 16.4 mm (range, 8–116 mm). One hundred fifty (56.6%) masses were evaluated using EUS-FNA with the new video processor that offers high-resolution imaging. The mean number of needle passes was 3.3 ± 0.9. EchoTip and ProCore needles were used to obtain specimens from 150 (56.6%) and 115 (43.4%) masses, respectively. Specimens were taken from 163 (61.5%) masses with a 19- or 22-G fine needle and from 102 (38.5%) masses with a 25-G fine needle. Tissue biopsies were performed for 132 (49.8%) masses.

The final diagnoses of the 265 masses are shown in Table 2. Among them, 242 (91.3%) were diagnosed as malignant, including 222 adenocarcinomas, 17 neuroendocrine tumors, 1 metastatic tumor, and 2 intraductal papillary mucinous neoplasms. Twenty-three (8.7%) masses were diagnosed as benign, including 17 inflammatory masses from pancreatitis and 6 cases of autoimmune pancreatitis.

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respectively. Considering differential results between imaging modalities, we conducted univariate and multivariate analyses to elucidate methodological and instrumental factors affecting diagnostic yield in pancreatic solid masses with EUS-FNA, as shown in Table 4. In the univariate analysis, the number of needle passes (P = 0.003), needle size (P = 0.001), needle type (P = 0.001), high-resolution imaging (P < 0.001), and coexistence of tissue biopsy (P < 0.001) were statistically significant. However, age, sex, size of the mass, coexistence of pancreatitis, and location of the lesion were not significant. High-resolution imaging (P = 0.040), odds ratio = 3.28 and 3 or more needle passes (P = 0.039, odds ratio = 2.41) were independent factors affecting diagnostic yield in the multivariate analysis.

4. Discussion

In the present study, the sensitivity, specificity, accuracy, PPV, and NPV of EUS-FNA in the diagnosis of pancreatic solid masses were 81.8%, 100.0%, 83.4%, 100.0%, and 34.3%, respectively. These results are not significantly different from those of previous studies reporting the sensitivity (78–91%), specificity (75–100%), and accuracy (78–95%) of this procedure.¹⁴⁻¹⁷ EUS-FNA has recently become a standard modality for the diagnosis of pancreatic solid masses. Previous studies that analyzed the accuracy of EUS-FNA for pancreatic masses and lymph nodes have reported an accuracy ranging from 71% to 98%.¹⁻⁹,¹⁰,¹³,¹⁸⁻¹⁹ Other recent studies have been conducted to optimize the diagnostic yield of EUS-FNA; they showed that several factors influenced the diagnostic yield, such as the needle size, needle type, combination of cytology and histology, location of the lesion, existence of an on-site cytopathologist, and skill of the endosonographer.²⁻²³

Newer endoscopic systems obtain high-definition images with enhanced resolution due to advancement of imaging technology and endoscopic technology. Due to its high spatial resolution, EUS with high-resolution imaging can visualize the pancreas in more detail and more completely than can EUS with previous imaging. Consequently, EUS-FNA with high-resolution imaging may be able to improve the targeting and tissue sampling of pancreatic masses. To best my knowledge, there has been no report about the determining the relationship between imaging quality and diagnostic yield of EUS-FNA. That is why we compared the results of EUS-FNA between conventional and high-resolution image groups. We found that high-resolution image group has the increased accuracy, sensitivity, and specificity of EUS-FNA. Also, recent studies revealed that needle

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**Table 3**

Baseline characteristics and the diagnostic yield between conventional image group (n = 115) and high-resolution image group (n = 150).

|                          | Conventional image group | High-resolution image group | P       |
|--------------------------|--------------------------|----------------------------|---------|
| Age (mean±SD range)      | 64.9±12.1 (26–91)        | 64.2±13.8 (26–91)          | 0.065   |
| Sex (male/female)        | 64:51                    | 77:70                      | 0.619   |
| Location of lesion, n (%) (access route) | 0.164                     |                            |         |
| Head (transduodenal)     | 51 (44.3)                | 77 (51.3)                  |         |
| Body/tail (transgastric) | 64 (55.7)                | 73 (48.6)                  |         |
| Size of lesion in mm, mean±SD (range) | 39.7±17.5 (9–100)        | 31.8±14.7 (8–116)          | 0.011   |
| No. of passes, mean±SD (range) | 3.2±1.0 (1–5)            | 3.3±0.8 (1–6)              | 0.333   |
| Needle type (ProCore vs EchoTip) | 5:110                    | 110:40                     | <0.001  |
| Size of needle, n (%)     |                          |                            |         |
| 19/22 G                  | 49 (42.6)                | 114 (76.0)                 | <0.001  |
| 25 G                     | 66 (57.4)                | 36 (24.0)                  |         |
| Coexistence of tissue biopsy, n (%) | 22 (19.1)                | 110 (73.3)                 | <0.001  |
| Final diagnosis (malignant/benign) | 106:9                    | 136:14                     | 0.666   |
| Diagnostic yield, % (n)  |                          |                            |         |
| Accuracy                 | 71.3 (82/115)            | 92.7 (139/150)             |         |
| Sensitivity              | 68.9 (73/106)            | 91.9 (125/136)             |         |
| Specificity              | 100 (9/9)                | 100 (14/14)                |         |

S = gauge, no. = number, SD = standard deviation.

One hundred forty-seven patients were analyzed.

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**Table 4**

Factors influencing the diagnostic accuracy of EUS-FNA for pancreatic mass lesions (univariate analysis and multivariate analysis).

|                      | Odds ratio (95% CI) | P     | Odds ratio (95% CI) | P     |
|----------------------|--------------------|-------|--------------------|-------|
| Age (≥70 vs <70)     | 1.27 (0.65–2.48)    | 0.482 |                   |       |
| Sex (male vs female) | 1.48 (0.77–2.83)    | 0.251 |                   |       |
| Mass size (≥20 vs <20 mm) | 1.25 (0.51–3.07) | 0.622 |                   |       |
| Coexistence of pancreatitis | 1.83 (0.68–4.92) | 0.226 |                   |       |
| No. of passes (≥3 vs 1–2) | 3.01 (1.41–6.46) | 0.003 | 2.41 (1.04–5.58) | 0.039 |
| Needle size (19/22 G vs 25 G) | 3.09 (1.59–6.03) | 0.001 | 0.38 (0.03–3.43) | 0.439 |
| Needle type (ProCore vs EchoTip) | 3.59 (1.65–7.81) | 0.001 | 0.87 (0.26–2.95) | 0.821 |
| High-resolution image | 5.09 (2.44–10.61) | <0.001 | 3.28 (1.06–10.2) | 0.040 |
| Coexistence of tissue biopsy | 4.19 (1.97–8.90) | <0.001 | 1.96 (0.73–5.29) | 0.185 |
| Location (body/tail vs head) | 1.72 (0.89–3.31) | 0.137 |                   |       |

CI = confidence interval, EUS-FNA = endoscopic ultrasound-guided fine needle aspiration, G = gauge, no. = number.
size, needle shape, pass number and FNA method were related to high diagnostic yield of EUS-FNA.\textsuperscript{14,23–25} Therefore, we conducted multivariate analyses to elucidate methodological and instrumental factors affecting diagnostic yield of EUS-FNA. In the multivariate analysis, we demonstrated that two important factors for determining diagnostic yield of EUS-FNA in pancreatic solid masses. One is a high-resolution image and the other is needle pass number (≥3 or more). Previous studies have investigated the optimal number of needle passes for a higher diagnostic yield of EUS-FNA for pancreatic solid masses without an on-site cytopathologist. Erickson et al\textsuperscript{24} performed a large study and found that 5 to 6 passes were used. Suzuki et al\textsuperscript{25} showed that 4 needle passes were sufficient for acquiring core tissue. A more recent study reported that 1 to 3 needle passes were optimal for EUS-FNA for pancreatic lesions without an on-site cytopathologist.\textsuperscript{26} In most institutions, a cytopathologist is not available to routinely participate in the EUS-FNA procedure. In this situation, previous studies reported that 3 or more passes were needed to acquire an adequate sample without an on-site cytopathologist.\textsuperscript{23,25} This result is consistent with the present study. However, a cytopathologist who offered adequate diagnosis attended the EUS-FNA procedure, this could decrease the optimal number of needle passes and provide higher cytologic yield of EUS-FNA.

The present study is limited by its retrospective design and different competence levels of pathologists and endosonographers over time. As time passed, they became more expert at their work, and there was a possibility that unskillful performance might be related to lower diagnostic yield of EUS-FNA in the early conventional image period. Although 2 endosonographers independently experienced more than 100 cases of EUS-FNA in their training periods, in order to minimize this limitation, we censored the data of 1 year after our hospital actively began to perform EUS-FNA.

In conclusion, high-resolution imaging and 3 or more passes were the most significant factors influencing the diagnostic yield of EUS-FNA in patients with pancreatic solid masses without an on-site cytopathologist. To improve the diagnostic yield of EUS-FNA for pancreatic solid masses, it is most important to observe lesions closely, target lesions adequately, and use high-resolution imaging and appropriate needle passage. A prospective study is necessary to elucidate various factors influencing the diagnostic yield of EUS-FNA for pancreatic solid masses without an on-site cytopathologist.

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