ORIGINAL ARTICLE

A Triple Approach for Diagnostic Assessment of Endoscopic Ultrasound-Guided Fine Needle Aspiration in Pancreatic Solid Masses and Lymph Nodes

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Abstract

Background and Aims Endoscopic ultrasound (EUS)-guided fine needle aspiration (FNA) has been becoming the standard tool for acquiring pancreatic lesion tissue. However, a single cytologic or histologic evaluation is not satisfactory for diagnosis. In this study, we evaluated the diagnostic yield of EUS–FNA for pancreatic solid masses and intra-abdominal lymph nodes using a triple approach.

Methods This study included patients undergoing evaluation for a solid pancreatic mass ($n = 59$) or intra-abdominal lymph nodes ($n = 16$) using EUS–FNA with a 22- or 25-gauge (G) needle, respectively. The specimens from each pass were analyzed by on-site cytology using Diff-Quick stain, cytology using Papanicolaou stain, and histology with immunohistochemical (IHC) staining.

Results A total of 75 patients (49 males; mean age; 63.7 years) were included. The median number of needle pass for diagnosis of malignancy was 2.0, and there was no technical failure. The diagnostic accuracies with on-site cytology, cytology using Papanicolaou staining, and histology were 70.7, 80.0, and 80.0 %, respectively. The diagnostic accuracy using a triple approach was significantly greater than cytology using Papanicolaou staining alone (94.7 vs. 80.0 %; $p = 0.007$). In patients with malignant lesions, cytology identified 12 of 71 (16.9 %) malignant lesions that were not diagnosed by histology using IHC, and histology identified six (8.5 %) malignant lesions that were not diagnosed by cytology.

Conclusion On-site cytopathologic evaluation combined with cytologic and histologic analysis with IHC stain for one-pass specimen is considered to be able to increase the overall accuracy of EUS–FNA in pancreatic solid masses and lymph nodes.

Abbreviations

EUS	Endoscopic ultrasound
EUS–FNA	EUS-guided FNA
G	Gauge
IHC	Immunohistochemical
GI	Gastrointestinal
CT	Computed tomography
MRI	Magnetic resonance imaging
H&E	Hematoxylin and eosin
NET	Neuroendocrine tumor
SPEN	Solid and papillary epithelial neoplasm
CI	Confidence interval
ERCP	Endoscopic retrograde cholangiopancreatography
SD	Standard deviation
FNB	Fine needle biopsy

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Introduction

Endoscopic ultrasound (EUS) and EUS–guided fine needle aspiration (FNA) are essential tools in the accurate

diagnosis and staging of gastrointestinal (GI) and certain non-GI malignancies. However, the diagnostic accuracy of EUS–FNA varies [1–6] based on several factors, including the presence of an on-site cytopathologist, the endosonographer technique, and location and character of the lesion [7–9]. Limitations of cytologic analysis also affect the accuracy of EUS–FNA, since cytologic analysis alone may not distinguish inflammation from well-differentiated neoplasia. Furthermore, certain neoplasms, such as lymphoma and stromal tumors, are difficult to diagnose without histologic samples that provide immunohistochemical (IHC) information about tissue architecture and cell morphology [2, 10–12].

These limitations have been overcome in previous studies that described methods to optimize the accuracy, efficiency, and quality of EUS–FNA specimens [8, 13, 14]. Based on these results, we evaluated the diagnostic yield of EUS–FNA for pancreatic solid masses and intra-abdominal lymph nodes using a triple approach comprised of simultaneous cytopathologic and histologic evaluations with on-site examination.

Patients and Methods

Patients

Patients who underwent EUS–FNA for solid pancreatic mass or intra-abdominal lymph nodes at the SoonChun-Hyang University Hospital in Bucheon, Korea, a tertiary referral medical center, from January 2011 to January 2012 were identified retrospectively from a prospectively collected endoscopy database. Solid pancreatic masses or intra-abdominal lymphadenopathy was confirmed in all patients by at least a single investigational modality, such as computed tomography (CT), magnetic resonance imaging (MRI), or EUS. The inclusion criteria were patients with pathological data to make a diagnosis or to guide management decision, patients older than 18 years, and patients with the ability to provide informed consent. The exclusion criteria were coagulopathy (international normalized ratio > 1.5 [0.85–1.25] or platelet count < 60,000/mm^3 [150,000–450,000/mm^3]) and the inability to sample the lesion because of the presence of intervening blood vessels or altered GI anatomy. Written informed consent was obtained from all enrolled patients.

EUS–FNA

All procedures were performed in a standardized method by two experienced investigators using a linear array echoendoscope (GF-UCT240; Olympus Medical Systems, Co., Ltd., Tokyo, Japan) in patients who were under conscious sedation. FNA was performed using a standard 22- or 25-gauge (G) FNA device (Echotip; Wilson-Cook Medical, Winston-Salem, NC, USA). FNA was performed either from the duodenum using a 25-G needle for a pancreatic mass in the head/uncinate and in lymph nodes around the pancreatic head, portal vein, inferior vena cava, superior mesenteric artery, and the uncinate process, or from the stomach using a 22-G needle for a pancreatic mass in the body/tail, lymph nodes in celiac axis, and upper peri-aortic nodes [14]. After puncturing the lesion, the stylet was removed, and the needle was moved back and forth 15–20 times using a fanning method that positioned the needle at different areas within the mass. For a pancreatic solid mass, suction was applied using a 10-mL syringe and released before the needle was withdrawn from the lesion. For lymph nodes, either suction was not applied, or 5-mL suction was applied. The aspirated specimen was expelled onto a glass slide by reinsertion of the stylet and flushing with air, if needed.

Preparation and Analysis of Specimens

The part of obtained material was smeared onto three to five glass slides for on-site analysis and cytology with Papanicolaou stain after physical dissection. The residual material was placed into two-to-three wells of a tissue tray with formalin for histologic analysis (Fig. 1).

On-site Evaluation

Three to five slides were air-dried and stained with Diff-Quick (International Reagents Co., Ltd., Kobe, Japan) for immediate on-site interpretation. After each pass, the cytopathologist determined whether the sample was adequate for diagnosis of malignancy.

Cytologic and Histologic Evaluations

The remaining sample was prepared for cytologic and histologic analyses, regardless of the on-site diagnosis. Three to five slides were stained with alcohol and prepared using Papanicolaou cytological stain to describe the cellularity and to diagnose malignancy in each specimen. The part of material was formalin-fixed and embedded in paraffin for histologic analysis. Sections were stained with hematoxylin and eosin (H&E) and periodic acid–Schiff reagent. IHC analyses were performed for all cases with adequate histologic specimens. P53, CEA, and Ki-67 were used for diagnosis of adenocarcinoma, and CD 56, synaptophysin, and chromogranin were used for neuroendocrine tumor (NET). In patients suspected with solid and papillary epithelial neoplasm (SPEN), vimentin and beta-catenin were stained. For histologic analysis, a cytopa-

Fig. 1 Triple approach. The specimen obtained from each pass was divided into thirds for on-site cytology using Diff-Quick staining, cytology using Papanicolaou staining, and histology using hematoxylin and eosin (H&E) with immunohistochemical staining
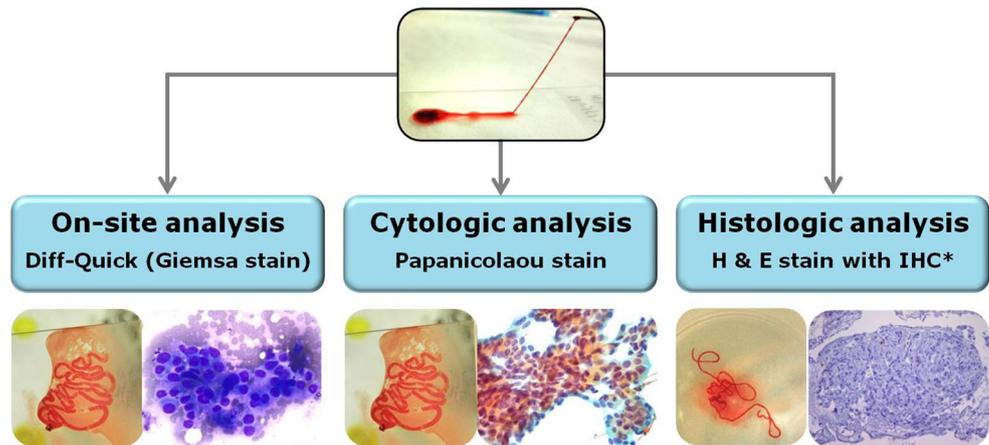
thologist evaluated the adequacy of the core sample and diagnosis for malignancy.

Completion of Procedure

A maximum passes were performed until malignancy was established by on-site analysis. If adequate cellularity was achieved in patients in whom malignancy was not established within three passes, the procedure was completed. If adequate cellularity was not established, one or two additional passes were performed until adequate cellularity was identified. In patients for whom no diagnosis was established or who did not show adequate cellularity within five passes, additional passes were made, as clinically indicated, at the discretion of the endosonographer, as clinically indicated, but the results of evaluation were not included in final analysis.

Classification of Results

On-site diagnosis was categorized as non-diagnostic, negative, atypical, or positive for malignancy according to the malignant features reported previously [15], and only specimens that were positive for malignancy were categorized as such. Cytologic and histologic diagnoses were categorized as non-diagnostic, negative, atypical, suspicious, or positive for malignancy. Specimens that were considered positive or suspicious for malignancy were categorized as positive for malignancy, whereas specimens that were considered benign, indeterminate, or atypical were categorized as negative for malignancy. Positivity for malignancy "in combination" was defined as positivity by one or more of on-site cytology, cytology with Papanicolaou stain, and histology with ICH. The obtained specimen was classified as adequate for cytological examination if it contained cells from the target organ optimal for histologic examination.

Standard of Reference for Final Diagnosis

A final diagnosis of a malignant or benign tumor was based on one of the following methods:

1. Histologic diagnoses from other sources, such as endoscopic biopsy during duodenoscopy or endoscopic retrograde cholangiopancreatography (ERCP), surgery with biopsy or resection, biopsy of any metastatic lesion;
2. Obvious positive histologic or cytologic findings obtained from EUS–FNA combined with follow-up data compatible with progressive tumor disease;
3. If lesions were considered benign, clinical follow-up of at least 12 months with negative-repeated imaging and/or biopsy results and a clinical course compatible with benign disease.

Outcome Parameters

The primary outcome was diagnostic yield (sensitivity, specificity, and accuracy) of each method and the triple approach for diagnosing solid pancreatic masses or intra-abdominal lymph nodes. The secondary outcomes were to evaluate technical failure, complications, adequate sample, and the median numbers of pass necessary to establish diagnosis. The diagnostic accuracy was defined as the ratio of the sum of true-positive and true-negative values divided by the number of lesions. Technical failure was defined as a needle malfunction before a diagnosis was reached. Complications were defined as any post-procedure event attributable to EUS–FNA. Excessive bleeding at the site of puncture, hypotension, and perforation were documented. For patients with abdominal pain, serum amylase and lipase levels were initially measured, and an abdominal CT scan was performed if the symptoms persisted. Acute pancreatitis was defined as abdominal pain associated with

nausea or vomiting coupled with a three fold elevation of serum amylase or lipase. All patients were followed for at least 12 months.

Statistical Analysis

The sample size was estimated on the basis of a diagnostic accuracy rate of 78.0 % for cytological analysis of EUS–FNA for diagnosis of a pancreatic solid mass [13]. Assuming a technical failure rate of 5 % for EUS–FNA, which correlated to a 17 % difference in diagnostic accuracy and 80 % power, the sample size was estimated to be 67 patients. The test statistic used was the two-sided likelihood ratio test, and the significance level of the test was 0.0500. Normally distributed variables are presented as means with standard deviations (SDs) and as medians with ranges. Sensitivity, specificity, and overall accuracy were also calculated. Statistical comparisons were performed using the χ^2 test or Fisher's exact test for categorical variables. $p < 0.05$ in a two-tailed test was considered a statistically significant difference. All statistical analyses were performed using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 75 patients (49 males; mean age, 63.7 years) were included in the study. A pancreatic tumor was found in 59 (78.7 %) patients and a lymph node lesion in 16 (21.3 %) patients. The median pancreatic tumor size and lymph node size were 37 mm (range, 15–74 mm) and 23 mm (range 8–37 mm), respectively. The demographic and pancreatic tumor characteristics of the patients are shown in Table 1.

The final diagnoses, as well as the methods of confirming diagnoses, are listed in Table 2. Fifty-one (86.4 %) of the fifty-nine pancreatic mass lesions were adenocarcinoma, one was a NET, four were metastasis, and three were non-specific inflammatory masses. In lymph nodes, 15 (93.7 %) were metastatic lymphadenopathy, and one was tuberculosis.

Technical Outcome

A 22-G needle was used for a transgastric approach to sample in 36 patients (48.0 %), and a 25-G needle was used for a transduodenal approach in 39 patients (52.0 %). The median number of needle pass for malignancy diagnosis was 2.0, and there was no technical failure. EUS–FNA yielded samples adequate for on-site cytologic analysis in 73 (97.3 %) patients, while a preliminary diagnosis was established in 50 (68.5 %) of 73 patients. There were

Table 1 Patient demographics and pancreatic tumor characteristics

Characteristics	$n = 75$
Mean age [year (SD)]	63.7 (12.2)
Sex [n (%)]	
Male	49 (65.3)
Female	26 (34.7)
Size of mass on EUS (mm)	
Pancreas	
Mean (SD)	35.9 (11.2)
Median (range)	37 (15–74)
IQR	27–42
Lymph node	
Mean (SD)	23.1 (9.5)
Median (range)	23 (8–37)
IQR	14.5–33
Location [n (%)]	
Pancreas	59 (78.7)
Head/uncinate	33 (55.9)
Body/tail	26 (44.1)
Lymph nodes	16 (21.3)
Periduodenal	5
Peripancreatic	3
Celiac	3
Porto-carval	2
Others	1

IQR interquartile range, *SD* standard deviation

Table 2 Final diagnosis and standard of reference for defining malignant and benign lesions

Final diagnosis, n (%)	$n = 75$	Standard of reference		
		Operation	Other tissue diagnoses	Follow-up[b]
Pancreas	59 (78.7)			
Adenocarcinoma	51 (86.4)	5	15	31
Neuroendocrine tumor	1 (1.7)	0	0	1
Metastases	4 (6.8)	0	3	1
Inflammatory masses	3 (5.1)	1	0	2
Lymph nodes	16 (21.3)			
Metastases[a]	15 (93.7)	1	6	8
Tuberculosis	1 (6.3)	0	1	0

[a] 11 adenocarcinoma, 1 neuroendocrine tumor, 3 others

[b] Cytology or histology findings with definite proof of malignancy plus follow-up data compatible with progressive tumor disease

sufficient samples for cytology using the Papanicolaou stain in 74 (98.7 %) and for histology in 63 (84.0 %) patients. Mild pancreatitis was a minor complication

Table 3 Technical characteristics and outcomes of EUS–FNA

Characteristics	n = 75
Access route [n (%)]	
Transgastric	36 (48.0)
Transduodenal	39 (52.0)
Number of needle passes for diagnosis	
Mean (SD)	2.1 (0.12)
Median (range)	2.0 (1–5)
IQR	2.0–3.0
Technical success [n (%)]	75 (100)
Adequate specimen [n (%)]	
On-site cytology	73 (97.3)
Cytology with Papanicolaou stain	74 (98.7)
Histology with IHC	63 (84.0)
Complications[a] [n (%)]	1 (1.3)

EUS–FNA endoscopic ultrasound–fine needle aspiration, *IHC* immunohistochemical stain, *IQR* interquartile range, *SD* standard deviation

[a] Pancreatitis, mild

observed in one patient (1.3 %) and was managed conservatively with 3 days of hospitalization (Table 3).

Diagnostic Yield for Malignancy

The diagnostic yield of each method and the triple approach for malignancy are shown in Table 4. Of the 75 lesions evaluated by EUS–FNA with on-site cytopathology, 50 were classified correctly as malignant (sensitivity 70.4 %; 95 % CI 59.8–81.0), and the sensitivity of cytology using the Papanicolaou stain was 80.3 % (95 % CI 71.0–89.5), and of histology using IHC was 78.9 % (95 % CI 69.4–88.4). The sensitivity of the triple approach was significantly higher than that of cytology using Papanicolaou stain alone (95.8 vs. 80.3 %, $p = 0.004$). The diagnostic accuracies of on-site cytology, cytology using Papanicolaou stain, and histology using IHC were 70.7 % (95 % CI 60.4–81.0), 80.0 % (95 % CI 71.0–89.1), and 80.0 % (95 % CI 71.0–89.1), respectively. Combining these three methods improved the diagnostic accuracy to 94.7 % (95 % CI 89.6–99.8), which was significantly

Table 5 Diagnostic result comparisons between histology and cytology for malignant lesions (n = 71)

Histology with IHC [n, (%)]	Cytology with Papanicolaou stain		Total
	Positive	Negative	
Positive	50 (70.4)	6 (8.5)	56 (78.9)
Negative	12 (16.9)	3 (4.2)	15 (21.1)
Total	62 (87.3)	9 (12.7)	

greater than that observed for cytology with the Papanicolaou stain alone ($p = 0.007$). In comparing the diagnostic result for confirmed malignant lesions between cytology with Papanicolaou and histology with IHC, cytology revealed that 12 (16.9 %) of 71 malignant lesions were not diagnosed by histology with IHC, and the histology revealed that six (8.5 %) malignant lesions were not diagnosed by cytology (Table 5). Therefore, a total of 18 (25.4 %) patients were diagnosed with malignancy by a complementarity between cytological and histologic analyses of EUS–FNA specimens.

Discussion

In the present study, we used a triple approach for diagnosis of solid pancreatic masses and intra-abdominal lymph nodes. This approach used a single specimen from a needle pass that was divided into thirds for simultaneous on-site cytology using Diff-Quick staining, cytology using Papanicolaou staining, and histology with IHC. The diagnostic accuracy of this triple approach for EUS–FNA was 94.7 % (95 % CI 89.6–99.8) using a median of 2.0 needle pass, which was significantly higher compared with cytology using Papanicolaou staining alone (94.7 vs. 80.0 %; $p = 0.007$).

Over the past two decades, EUS–FNA has become an indispensible method for tissue acquisition. In previous studies, the accuracy of EUS–FNA for the diagnosis of pancreatic masses and lymph nodes has been analyzed extensively, although the overall accuracy of this procedure varied from 71 to 98 % [1–6, 16]. Recently, several studies have been performed to overcome limitations and optimize

Table 4 Diagnostic yields of each method compared with a triple approach for malignancy (n = 75)

% (95 % CI)	On-site cytology	Cytology using Papanicolaou stain	Histology with IHC	Triple approach	p value*
Sensitivity	70.4 (59.8–81.0)	80.3 (71.0–89.5)	78.9 (69.4–88.4)	95.8 (91.1–100)	0.004
Specificity	100 (51.0–100)	100 (51.0–100)	100 (51.0–100)	100 (51.0–100)	1.000
Accuracy	70.7 (60.4–81.0)	80.0 (71.0–89.1)	80.0 (71.0–89.1)	94.7 (89.6–99.8)	0.007

CI confidence interval, *IHC* immunohistochemical stain

* Statistical comparison between cytology and a triple approach

the diagnostic yield of the EUS–FNA procedure. These studies identified several factors that determined the technical outcomes and the diagnostic yields of the FNA procedure, including the use of suction during tissue acquisition, location and nature of the lesion, presence of an on-site cytopathologist, the combination of histologic and cytologic analyses, and the experience of the endosonographer [7, 13, 17–19].

The importance of on-site cytopathological examination on the diagnostic yield of EUS–FNA has been reported in several studies [8, 17, 20]. Klapman et al. [17] compared the diagnostic yield of EUS–FNA from two different hospitals, with and without on-site cytopathology. The diagnostic yield from the hospital with on-site cytopathology was twice that of the hospital without on-site cytopathology. Additionally, the presence of an attending on-site cytopathologist during the procedure was cost-effective. According to the report from Iglesias-Garcia et al. [8], on-site cytopathological evaluation improved the diagnostic yield of EUS–FNA for the cytological diagnosis of solid pancreatic masses and was associated with a significantly lower number of needle pass. In cases of inadequate samples, the on-site cytopathologist was able to provide information guiding the puncture area, which can impact the number of passes needed to obtain an appropriate sample.

Another factor affecting the diagnostic accuracy of EUS–FNA was the combination of histologic and cytologic analyses, since cytology alone is insufficient for verification of cellular arrangement and tissue architecture. Compared with cytology, histologic analysis of a tissue seems to have several advantages, such as better distinction between well-differentiated adenocarcinoma and chronic pancreatitis, an appropriate cellular subtyping and architectural analysis for the diagnosis of tumors (i.e., lymphoma, metastatic tumor, stromal tumor, NET), as well as the possibility of using disease-specific IHC stains. And cytological analysis alone might be limited to the diagnosis of lymph node and NET because they tend to more contamination of blood [2, 10–12]. Haba et al. [20] evaluated the diagnostic accuracy of EUS–FNA for 996 solid pancreatic lesions and reported that both cytological and cell-block preparations improved accuracy. In another study of 192 pancreatic masses, adequate tissue and sensitivity for histology were 86.4 and 60 % and were 92.7 and 68.1 % for subsequent cytology, respectively, which together were significantly more accurate than cytology alone (87.5 vs. 77.6 %, $p = 0.0155$) [21]. In our study, cytology and histology were complementary for diagnosis of pancreatic malignancy in 18 of 71 patients (25.4 %). Based on these results, a device for EUS-guided fine needle biopsy (FNB) with Procore reverse bevel technology (Wilson-Cook Medical, Winston-Salem, NC, USA) was developed

recently to obtain simultaneously tissue for cytology and sufficient core tissue for histology [22].

The diagnostic accuracy of the triple approach was 94.7 %, and the mean number of needle pass for diagnosis was 2.0 in our study. This was a relatively high level of diagnostic accuracy and a low number of needle pass compared with previous studies. Although insufficient specimens may be possible due to dividing up the tissues for each procedure, the triple approach increased the diagnostic accuracy of EUS–FNA based on previous studies and used standardized EUS–FNA methods. Our study determined that cytology identified 12 (16.9 %) of 71 malignant lesions that were not diagnosed by histology using IHC and that histology identified six (8.5 %) malignant lesions that were not diagnosed by cytology in patients with malignant lesions. In addition, the diagnostic accuracies using on-site cytology, cytology with Papanicolaou stain, and histology with IHC were 70.7, 80.0, and 80.0 %, respectively, and the diagnostic accuracy of the triple approach improved to 94.7 %. Therefore, in our study, combining cytological and histologic findings was more adaptable than cytology or histology alone for diagnostic accuracy of EUS–FNA. In addition, we used a 25-G needle exclusively for the transduodenal approach and a 22-G needle for the transgastric approach based on a previous publication [7]. Even though the technical performance or diagnostic yield between the 22-G and 25-G needle was not statistically different in our study, previous randomized controlled studies have reported better performance using the 25-G needle for the transduodenal approach [23–25]. Moreover, we sampled multiple areas within a mass lesion using a fanning method. This fanning method samples multiple areas, which may improve diagnostic accuracy, because the center of a cancerous mass is considered to be more necrotic than the periphery [26].

The standardization of EUS–FNA is very important, since it is a very sensitive procedure for determining tissue diagnoses in patients with suspected GI malignancies and periluminal lesions [27, 28]. In recently published reports, an algorithm designed to improve the technical outcome and optimize resource use for FNA procedures was proposed, based on previous studies and Phase I and II clinical studies [14]. In our study, we performed EUS–FNA using similar methods based on this algorithm to demonstrate acceptable diagnostic accuracy. However, technical performance and diagnostic yield need to be improved, and further studies are thus needed to establish an optimal algorithm.

This study has certain limitations. The major limitation is the lack of a control group. There was no direct comparison of the individual methods, because this study was designed to be a descriptive study demonstrating the feasibility and diagnostic yield of EUS–FNA using a triple approach for pancreatic solid lesions and intra-abdominal

lymph nodes in a single arm. Furthermore, only a small number of patients had a rare pancreatic tumor, which included NET in one (1.7 %) and metastatic tumors in four (6.8 %) of 59 pancreatic masses. The subgroup analysis after classification of a rare pancreatic tumor and pancreatic adenocarcinoma is more precise, because the diagnoses for rare pancreatic tumors are usually more difficult. However, since there were only a small number of rare pancreatic tumors, we could not perform a subgroup analysis. Finally, in the present study, samples obtained with EUS–FNA were divided into three groups of slides for on-site cytology using Diff-Quick stain, cytology using Papanicolau stain, and histology using IHC. Therefore, the volume of samples of each approach in three methods seems to be less than single approach alone, and diagnostic accuracy in each approach could be relatively low in our results. However, after confirmation of quality (diagnosis for malignancy and adequacies) of every each pass using on-site cytology, we decided whether terminated procedure or performed additional needle pass. Because, if the positive for malignancy or adequate cellularity was confirmed by on-site analysis, there was high probability to diagnose malignancy using cytology and histology of same pass. In other words, for increasing diagnostic accuracy of EUS–FNA, we focused quality of each pass than amount of specimen for cytologic or histologic analysis.

In conclusion, a triple approach for diagnosis of pancreatic solid masses and intra-abdominal lymph nodes shows comparable diagnostic accuracy of EUS–FNA using a low number of needle pass. Since the use of EUS–FNA for tissue diagnosis in GI and certain non-GI malignancies is more generalized, standardized methods of EUS–FNA are required, and further studies are needed to establish an optimal algorithm.

Conflict of interest None.

References

1. Savides TJ, Donohue M, Hunt G, et al. EUS-guided FNA diagnostic yield of malignancy in solid pancreatic masses: a benchmark for quality performance measurement. *Gastrointest Endosc.* 2007;66:277–282.
2. Wiersema MJ, Vilmann P, Giovannini M, et al. Endosonography-guided fine-needle aspiration biopsy: diagnostic accuracy and complication assessment. *Gastroenterology.* 1997;112:1087–1095.
3. Gress FG, Hawes RH, Savides TJ, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy using linear array and radial scanning endosonography. *Gastrointest Endosc.* 1997;45:243–250.
4. Harewood GC, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. *Am J Gastroenterol.* 2002;97:1386–1391.
5. Mitsuhashi T, Ghafari S, Chang CY, et al. Endoscopic ultrasound-guided fine needle aspiration of the pancreas: cytomorphological evaluation with emphasis on adequacy assessment, diagnostic criteria and contamination from the gastrointestinal tract. *Cytopathology.* 2006;17:34–41.
6. Chhieng DC, Jhala D, Jhala N, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy: a study of 103 cases. *Cancer.* 2002;96:232–239.
7. Sakamoto H, Kitano M, Komaki T, et al. Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. *J Gastroenterol Hepatol.* 2009;24:384–390.
8. Iglesias-Garcia J, Dominguez-Munoz JE, Abdulkader I, et al. Influence of on-site cytopathology evaluation on the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration (EUS–FNA) of solid pancreatic masses. *Am J Gastroenterol.* 2011;106:1705–1710.
9. Gleeson FC, Kipp BR, Caudill JL, et al. False positive endoscopic ultrasound fine needle aspiration cytology: incidence and risk factors. *Gut.* 2010;59:586–593.
10. Ribeiro A, Vazquez-Sequeiros E, Wiersema LM, et al. EUS-guided fine-needle aspiration combined with flow cytometry and immunocytochemistry in the diagnosis of lymphoma. *Gastrointest Endosc.* 2001;53:485–491.
11. Erickson RA, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. *Gastrointest Endosc.* 2000;51:184–190.
12. Mesa H, Stelow EB, Stanley MW, et al. Diagnosis of nonprimary pancreatic neoplasms by endoscopic ultrasound-guided fine-needle aspiration. *Diagn Cytopathol.* 2004;31:313–318.
13. Moller K, Papanikolaou IS, Toermer T, et al. EUS-guided FNA of solid pancreatic masses: high yield of 2 passes with combined histologic-cytologic analysis. *Gastrointest Endosc.* 2009;70:60–69.
14. Bang JY, Ramesh J, Trevino J, et al. Objective assessment of an algorithmic approach to EUS-guided FNA and interventions. *Gastrointest Endosc.* 2013;77:739–744.
15. Lin F, Staerkel G. Cytologic criteria for well differentiated adenocarcinoma of the pancreas in fine-needle aspiration biopsy specimens. *Cancer.* 2003;99:44–50.
16. Eloubeidi MA, Jhala D, Chhieng DC, et al. Yield of endoscopic ultrasound-guided fine-needle aspiration biopsy in patients with suspected pancreatic carcinoma. *Cancer.* 2003;99:285–292.
17. Klapman JB, Logrono R, Dye CE, et al. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol.* 2003;98:1289–1294.
18. Mertz H, Gautam S. The learning curve for EUS-guided FNA of pancreatic cancer. *Gastrointest Endosc.* 2004;59:33–37.
19. Lee JK, Choi JH, Lee KH, et al. A prospective, comparative trial to optimize sampling techniques in EUS-guided FNA of solid pancreatic masses. *Gastrointest Endosc.* 2013;77:745–751.
20. Haba S, Yamao K, Bhatia V, et al. Diagnostic ability and factors affecting accuracy of endoscopic ultrasound-guided fine needle aspiration for pancreatic solid lesions: Japanese large single center experience. *J Gastroenterol.* 2013;48:973–981.
21. Noda Y, Fujita N, Kobayashi G, et al. Diagnostic efficacy of the cell block method in comparison with smear cytology of tissue samples obtained by endoscopic ultrasound-guided fine-needle aspiration. *J Gastroenterol.* 2010;45:868–875.
22. Iglesias-Garcia J, Poley JW, Larghi A, et al. Feasibility and yield of a new EUS histology needle: results from a multicenter, pooled, cohort study. *Gastrointest Endosc.* 2011;73:1189–1196.

23. Fabbri C, Polifemo AM, Luigiano C, et al. Endoscopic ultrasound-guided fine needle aspiration with 22- and 25-gauge needles in solid pancreatic masses: a prospective comparative study with randomisation of needle sequence. *Dig Liver Dis*. 2011;43:647–652.

24. Camellini L, Carlinfante G, Azzolini F, et al. A randomized clinical trial comparing 22G and 25G needles in endoscopic ultrasound-guided fine-needle aspiration of solid lesions. *Endoscopy*. 2011;43:709–715.

25. Siddiqui UD, Rossi F, Rosenthal LS, et al. EUS-guided FNA of solid pancreatic masses: a prospective, randomized trial comparing 22-gauge and 25-gauge needles. *Gastrointest Endosc*. 2009;70:1093–1097.

26. Polkowski M, Larghi A, Weynand B, et al. Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline. *Endoscopy*. 2012;44:190–206.

27. Othman MO, Wallace MB. The role of endoscopic ultrasonography in the diagnosis and management of pancreatic cancer. *Gastroenterol Clin North Am*. 2012;41:179–188.

28. Gill KR, Ghabril MS, Jamil LH, et al. Endosonographic features predictive of malignancy in mediastinal lymph nodes in patients with lung cancer. *Gastrointest Endosc*. 2010;72:265–271.