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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Diagnostic ability and factors affecting accuracy of endoscopic ultrasound-guided fine needle aspiration for pancreatic solid lesions: Japanese large single center experience

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Abstract
Background Several studies have investigated the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) for pancreatic lesions, but they have included only limited patient populations. This study aimed to clarify the diagnostic accuracy of EUS-FNA in a large number of pancreatic lesions, and to describe the factors that influence it.
Methods From March 1997 to May 2010, 944 consecutive patients who had undergone EUS-FNA for pancreatic solid lesions were evaluated retrospectively. Factors affecting EUS-FNA accuracy were then analyzed.
Results A total of 996 solid pancreatic lesions were sampled by EUS-FNA. The overall sampling adequacy and diagnostic accuracy of these lesions were 99.3 % (989/996) and 91.8 % (918/996), respectively. The sensitivity and specificity for differentiating malignant from benign lesions were 91.5 % (793/867) and 97.7 % (126/129), respectively. The diagnostic performance was significantly higher when both cytological and cell-block examinations were carried out than with only cytological examination. In multivariate analysis, final diagnosis, location of lesion, lesion size, availability of on-site cytopathological evaluation, and experience of EUS-FNA procedure were independent factors affecting the accuracy of EUS-FNA. On-site cytopathological evaluation and lesion size were found to be the most weighted factors affecting diagnostic accuracy.
Conclusions EUS-FNA for pancreatic solid lesions yielded a high accuracy and low complication rate. Both cytological and cell-block preparations and on-site cytopathological evaluation contributed to improve the accuracy. The diagnostic ability of EUS-FNA was less for smaller lesions, and repeated procedures may be needed if malignancy is suspected.

Keywords Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) · Pancreatic solid lesion · Accuracy · Cell-block examination · On-site evaluation

Abbreviations

AC	Adenocarcinoma
EUS	Endoscopic ultrasound
EUS-FNA	Endoscopic ultrasound-guided fine needle aspiration

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IHC	Immunohistochemical
NET	Neuroendocrine tumor
SPN	Solid pseudopapillary neoplasm
FP	Focal pancreatitis
AIP	Autoimmune pancreatitis
SCN	Serous cystic neoplasm

Introduction

Pancreatic solid lesions comprise a variety of benign and malignant neoplasms and non-neoplastic lesions. The majority of these pancreatic tumors are invasive ductal adenocarcinomas (ACs), which require surgical resection or chemotherapy. However, the therapeutic strategies and prognoses of pancreatic tumors differ greatly depending on the pathological subtype [1, 2]. Endoscopic ultrasound (EUS) is the most accurate method for the detection of pancreatic lesions [3, 4]. However, accuracy of morphologic appearance of pancreatic lesions on EUS for the tumor subtype is limited [5, 6]. EUS-guided fine needle aspiration (EUS-FNA) has accuracy rates of 65–96 % for cyto-histopathological diagnosis of pancreatic lesions [7]. One of the factors that may affect the diagnostic accuracy of EUS-FNA is the size of lesions. EUS-FNA for small lesions of the pancreas is technically difficult [8, 9]. Although several studies have reported the yield of EUS-FNA for pancreatic lesions in relation to their size [9–14], all such studies have been based on limited patient populations. Accurate diagnosis of pancreatic cancer while it remains small is of paramount importance for this rapidly progressive malignancy. The main aim of this study was to define the diagnostic accuracy of EUS-FNA for pancreatic lesions and to identify factors affecting the accuracy of EUS-FNA in a large single center.

Methods

Between March 1997 and May 2010, 1089 patients with pancreatic solid lesions were evaluated at the Aichi Cancer Center Hospital, Nagoya, Japan. Among these patients, EUS-FNA of pancreatic solid lesions was attempted in 954 (87.9 %) of cases. Most of the remaining other 135 patients (12.4 %; 135/1089) in whom EUS-FNA was not performed were seen in earlier days of the study period, and alternative methods (pancreatic juice cytology, brushing cytology of pancreatic duct, biliary biopsy, brushing cytology of bile duct and biopsy from metastatic lesion) for confirming the diagnosis had been utilized. In recent years, all patients with suspected pancreatic solid lesions undergo EUS-FNA to obtain pathological diagnoses subsequent to radiological evaluation, regardless of whether the lesions are suspected to be benign or malignant, and whether the lesions are planned for resection or not. Ten of 954 patients (10 procedures) were excluded from statistical analyses because their clinical courses after the procedures could not be followed. Finally, we retrospectively evaluated 944 patients who had undergone EUS-FNA for pancreatic solid lesions. Informed consent was obtained from all patients before the procedure. Collection of data for this study was approved by the institutional review board at our institution.

EUS-FNA procedures were performed with the patient under conscious sedation using 35 mg of intravenous pethidine hydrochloride (Mitsubishi Tanabe Pharma, Osaka, Japan) and 5–10 mg of intravenous midazolam (Astellas, Tokyo, Japan). EUS-FNA was performed using a GF-UC30P, GF-UC240P-AL5 or GF-UCT240-AL5 convex array echoendoscope (Olympus Medical Systems, Tokyo, Japan) connected to an ultrasound scanning system (SSD-5500, Prosound SSD α-10; Hitachi Aloka Medical, Tokyo Japan). Different types of needles (19-, 22-, or 25-gauge Echo Tip Ultra; Cook Medical, IN, USA or NA-200H-8022; Olympus Medical Systems, Tokyo, Japan) were employed. The type and size of needle were chosen at the discretion of the endosonographer. After spraying the aspirated material onto glass slides, one slide was fixed by air-drying, stained with modified Giemsa stain (Diff-Quik; Kokusai Shiyaku, Kobe, Japan) and reviewed immediately (on-site cytopathological evaluation) by the cytopathologist or cytotechnologist to ensure specimen adequacy. Another slide was fixed by immediate immersion in 95 % alcohol and then stained with Papanicolaou stain. Additional material was obtained from each lesion unless on-site evaluation confirmed the presence of malignant cells or a sufficient number of representative cells from the lesion. Subsequently the remaining material, as well as the specimen obtained by one more pass, was submitted for cell-block preparation. The cell-block material was processed by fixation in 10 % neutral-buffered formalin solution, and then embedded in paraffin to be handled as a routine tissue block. Thin sections from paraffin-embedded cell blocks were cut and stained with hematoxylin and eosin.

Cytological diagnoses were interpreted as “insufficient”, “no atypia” (normal pancreatic tissue), “atypical” (including regenerative atypia by inflammation change), “suspicious”, or “malignant”. The cell-block materials were evaluated by hematoxylin-eosin staining and specific immunohistochemical (IHC) stains, if indicated. Cell-blocks were evaluated to both distinguish malignant from benign tissue, and also for a histological subtype of the lesion. Thus, cell-block diagnoses were assigned as

"insufficient", "no atypia" (normal pancreatic tissue), "atypical" (including regenerative atypia by inflammation change), "suspicious" for neoplasm, and the determined histological diagnoses of each neoplasms, such as "adenocarcinoma", "neuroendocrine tumor" and so on.

Pathological features of any surgically resected specimen and the clinical follow-up determined the final diagnosis. Pancreatic ACs, pancreatic neuroendocrine tumors (NETs), metastatic tumors from extra-pancreatic malignant tumors, pancreatic carcinomas other than AC, and solid-pseudopapillary neoplasms (SPNs) were considered malignant lesions. Lesions such as focal pancreatitis (FP), autoimmune pancreatitis (AIP) and other non-neoplastic lesions were considered as benign, in the absence of malignant findings on cytological and cell-block examinations and a lack of progression for at least 1 year during the follow-up period.

In this study, the sampling adequacy rate was defined by the proportion of lesions where adequate material for cytopathological diagnoses could be obtained. The accuracy of EUS-FNA diagnosis was defined as follows. In malignant lesions, EUS-FNA diagnoses were considered "accurate" when cytological and/or cell-block diagnoses of EUS-FNA matched the final diagnosis not only in distinguishing between malignant and benign lesions, but also in identifying the histological subtype of the tumor. In addition, when cytological and/or cell-block diagnoses were reported as "suspicious", we included them as accurate diagnoses for malignant lesions. When cytological and cell-block diagnoses were reported as "atypical", we included these as inaccurate diagnoses for malignant lesions. In benign lesions, EUS-FNA diagnoses were considered "accurate" when malignant findings were absent in both cytological and cell-block examinations. When cytological or cell-block diagnoses were reported as "suspicious" or "atypical", we also included them as accurate diagnoses. Furthermore, diagnostic sensitivity and specificity in differentiating between malignant and benign lesions were also evaluated. The diagnostic yield of EUS-FNA was then stratified according to lesion sizes of $\leq$10 mm, 10–20 mm and >20 mm. Lesion size was measured based on the EUS image. Finally, factors affecting the accuracy of EUS-FNA were analyzed using uni- and multivariate analyses. Variables employed for uni- and multivariate analyses were final diagnosis of the lesion (AC, NET, Metastatic tumor, carcinoma other than AC, SPN, FP, AIP and other benign lesion), location of the lesion (pancreatic head, body/tail), size of the lesion ($\leq$10 mm, 10–20 mm and >20 mm), presence or absence of on-site cytopathological evaluation, period when EUS-FNA procedure was performed (period I: March 1997–2002; annual number of EUS-FNA procedures for pancreatic lesion was under 50, period II: 2003–2006; annual number was over 50 and under 100, and period III: 2007–May 2010; annual number was over 100), and number of needle passes (once/twice and 3–7 times).

Statistical analysis was undertaken using the chi-square test, McNemar test and Mann–Whitney test in univariate analyses and logistic regression analysis in multivariate analysis. Values of $P < 0.05$ were regarded as statistically significant. All statistical analyses were undertaken using StatMate IV software (ATMS, Tokyo, Japan).

Results

Among the 944 patients with pancreatic solid lesions, 6 patients had two lesions each. Another 49 patients had undergone two or more EUS-FNA procedures. Consequently, the total number of EUS-FNA procedures for pancreatic solid lesions were 1009. Among them, 13 procedures in 13 patients failed because of failure to visualize the lesion in 6 patients, and inability to find a safe puncture tract in 7 patients. In 1 of the former patients, and 4 of the latter patients, repeated procedures succeeded on subsequent days. However, repeated procedures were not performed in the remaining 8 patients. Finally, 996 EUS-FNA procedures for 936 patients were successfully performed, with a success rate of 98.7 % (996/1009). The mean follow up of these patients were 16.3 months (range; 0.5–17.0 months).

On-site cytopathological evaluation was started from May 1998 in our institute. On-site examinations were therefore performed for 968 of the 996 lesions.

Table 1 Patients, lesions and characteristics of EUS-FNA procedures

Sex (patients)	
Male/female	593/351
Age (year)	
Median (range)	63 (20–86)
Location of lesion (patients)	
Head/body/tail	415/396/133
Number of lesion (patients)	
1 lesion/2 lesions	938/6
Number of EUS-FNA procedures performed (patients)	
1/2/3/4/5	895/43/3/2/1
EUS-FNA procedures (patients)	
Success/failure	936/8
Lesion size (mm)	
Median (range)	32 (3.4–130)
Number of needle passes	
Median (range)	2 (1–7)
On-site evaluation (patients)	
Performed/not performed	968/28

Characteristics of patients, lesions and EUS-FNA procedures are shown in Table 1.

The final diagnoses for the 996 lesions with successfully performed EUS-FNA are shown in Table 2. Of them, 867 lesions were found to be malignant, and the remaining 129 lesions were benign. Primary lesions for metastatic tumors in the pancreas were renal cancer (6 lesions), lung cancer (5 lesions), malignant lymphoma (3 lesions), bile duct cancer (2 lesions), rhabdomyosarcoma, orbital cancer, ovarian cancer, leiomyosarcoma, malignant melanoma, breast cancer (1 lesion each), and primary unknown cancers (2 lesions). Pancreatic carcinomas other than AC included adenosquamous carcinoma (10 lesions), acinar cell carcinoma (6 lesions) and anaplastic carcinoma (3 lesions). Final diagnoses were confirmed from surgically resected specimens for 143 lesions, including 92 ACs, 32 NETs, 7 metastatic tumors, 5 pancreatic carcinomas other than AC, 3 SPNs, 1 AIP and 3 other benign tumors (2 serous cystic neoplasms (SCNs) and 1 lymphoepithelial cyst). Diagnoses for the remaining 853 lesions were confirmed from the clinical courses.

The overall median lesion diameter was 32 mm (range, 3.4–130 mm). Forty lesions were ≤10 mm in diameter, and included 9 ACs (22.5 %; 9/40) and 16 NETs (40.0 %; 16/40). One hundred twenty one lesions were 10–20 mm in diameter, and included 71 ACs (58.7 %; 71/121) and 17 NETs (14.0 %; 17/121). The remaining 835 lesions were >20 mm in diameter, and included 683 ACs (81.8 %; 683/835) and 23 NETs (2.8 %; 23/835). The median number of needle passages was 2 (range, 1–7) (Tables 1, 2).

Table 2 Final diagnoses of pancreatic lesions performed EUS-FNA

	≤10 mm	10–20 mm	>20 mm	Total
Malignant lesion				
AC	9	71	683	763
NET	16	17	23	56
Metastatic tumor	1	8	15	24
Carcinoma other than AC	0	2	17	19
SPN	0	2	3	5
Benign lesion				
Focal chronic pancreatitis	11	16	46	73
Focal autoimmune pancreatitis	0	2	39	41
Other benign tumor	3	3	9	15
Total	40	121	835	996

AC adenocarcinoma, *NET* neuroendocrine tumor, *SPN* solid-pseudopapillary neoplasm

Sampling adequacy and diagnostic accuracy are shown in Table 3. Cytological examinations were not performed for 13 lesions because the cell-block examination alone was considered sufficient by the attending physicians of each patient. Cell-block examinations were not performed for 37 lesions because no materials remained after preparation for cytological examination. The overall sampling adequacy rate using the combination of cytological and cell-block examinations was 99.3 % (989/996). Sampling adequacy increased significantly when both cytological and cell-block examinations were performed, compared to cytological examination alone (Table 3).

Diagnostic accuracies with cytological and cell-block examination were 86.1 % (846/983) and 75.0 % (717/959), respectively. Fifty-nine of the 137 (43.1 %) lesions that could not be diagnosed by cytology were correctly diagnosed by cell-block examination. On the other hand, 168 of 242 (69.4 %) lesions undiagnosed from cell-block examinations alone could be correctly diagnosed by cytological examination. An accurate pathological diagnosis was reached in a total of 914 lesions, with a diagnostic accuracy of the combination of cytological and cell-block examination reaching 91.8 % (914/996), which was significantly higher than that of cytological examination alone (Table 3).

Diagnostic accuracies of cytological, cell-block, and their combination according to the tumor type are shown in Table 4. Accuracies were higher with cytological examination than with cell-block examination for AC and metastatic tumor. However, in NET or carcinoma other than AC, cell-block examination was more useful for obtaining accurate diagnosis. Of the 32 NETs and 11 carcinomas other than AC that were undiagnosed with cytological examination alone, 19 NETs (59.4 %; 19/32) and 10 carcinomas other than AC (90.9 %; 10/11) were diagnosed correctly with cell-block examination (Table 4). Particularly in these lesions, IHC staining was a necessary adjunct for establishing the diagnosis of 18 NETs and 6 carcinomas other than AC.

EUS-FNA performance for differentiating between malignant and benign lesions is shown in Table 5. For

Table 3 Sampling adequacy and accuracy of EUS-FNA

	Cytology $N = 983$	Cell-block $N = 959$	Combination $N = 996$	P value *
Sampling adequacy rate	99.1 % (974/983)	91.0 % (873/959)	99.3 % (989/996)	<0.01
Accuracy	86.1 % (846/983)	75.0 % (717/959)	91.8 % (914/996)	<0.01

* McNemar test; the differences comparing between cytology and combination

Table 4 Diagnostic accuracy in each final diagnoses (malignant lesion)

	Cytology	Cell-block	Combination	*P* value *	Undiagnosed lesion with cytology	Accuracy of cell-block in cytologically undiagnosed lesion
Total	84.7 % (726/857)	86.1 % (725/842)	90.9 % (788/867)	<0.01	137	42.3 % (58/137)
Final diagnosis						
AC	89.3 % (674/755)	74.5 % (552/741)	92.7 % (707/763)	<0.01	81	59.3 % (48/81)
NET	42.9 % (24/56)	71.7 (38/53)	76.8 % (43/56)	<0.01	32	59.4 % (19/32)
Metastatic tumor	77.3 % (17/22)	66.7 % (16/24)	75.0 % (18/24)	0.50	5	20.0 % (1/5)
Carcinoma	42.1 % (8/19)	84.2 % (16/19)	89.5 % (17/19)	0.04	11	90.9 % (10/11)
Other than AC						
SPN	60.0 % (3/5)	60.0 % (3/5)	60.0 % (3/5)	1	2	50.0 % (1/2)

AC adenocarcinoma, *NET* neuroendocrine tumor, *SPN* solid-pseudopapillary neoplasm

* McNemar test; the differences comparing between cytology and combination

Table 5 Diagnostic performance to differentiate malignant and benign lesions

	Cytology	Cell-block	Combination	*P* value**
Sensitivity	88.0 % (754/857)	74.9 % (631/842)	91.5 % (793/867)	<0.01
Specificity	95.2 % (120/126)	78.6 % (92/117)	97.7 % (126/129)	<0.01
Positive predictive value[a]	100 % (754/754)	99.8 % (631/632)	99.9 % (793/794)	
Negative predictive value[a]	54.5 % (120/220)	38.2 % (92/241)	64.6 % (126/195)	

[a] Excluding lesions whose EUS-FNA result were "insufficient"

** McNemar test; the differences comparing between cytology and combination

calculation of the sensitivity and specificity rates, lesions where EUS-FNA samples were not adequate also included. However, for calculating the positive predictive values (PPV) and negative predictive values (NPV), the lesions where EUS-FNA could not provide adequate samples were excluded. In malignant lesions, EUS-FNA could not reveal malignancy with cytological and cell-block examination (false-negative), in 100 of 857 (11.7 %) lesions and 149 of 842 (17.5 %) lesions, respectively. With a combination of both examinations, false-negative results were seen in 69 lesions. Including 5 lesions where adequate samples could not been obtained, sensitivity with combination reached 91.5 % (793/867). On the other hand, 1 lesion finally diagnosed as SCN by resected specimen was falsely diagnosed as NET (false-positive) with cell-block examination including positive IHC staining (CD56). No other benign lesion was diagnosed as malignant by EUS-FNA. Including the 2 lesions sampled inadequately, specificity with the combination of cytological and cell-block examination was 97.7 % (126/129). Sensitivity and specificity of either cytological or cell-block examinations alone were unsatisfactory due to the insufficient results seen in several lesions. However, with the combination of the two examinations, sensitivity and specificity increased significantly when both cytological and cell-block examinations were performed, compared with cytological examination alone. PPV and NPV were 99.9 % (793/794), and 64.6 % (126/195), respectively.

Sampling adequacy rates, diagnostic accuracies, sensitivities, specificities and numbers of needle passages stratified according to tumor diameter are shown in Table 6. The sampling adequacy rate was unrelated to the tumor size. However, the diagnostic accuracy and sensitivity for distinguishing malignant from benign lesions were significantly better for larger lesions than for smaller lesions. The numbers of needle passes also differed significantly between tumor sizes.

To clarify factors affecting the diagnostic accuracy of EUS-FNA, uni- and multivariate analyses were conducted (Table 7). Compared with AC, diagnostic accuracies were

Table 6 Diagnostic yield of EUS-FNA categorized by size of lesions

	≤10 mm	10–20 mm	>20 mm	*P* value*	*P* value**
Sampling adequacy rate	97.5 % (39/40)	98.3 % (119/121)	99.5 % (831/835)	0.67	0.96
Accuracy	82.5 % (33/40)	83.5 % (101/121)	93.4 % (780/835)	0.03	<0.01
Sensitivity	73.1 % (19/26)	81.0 % (81/100)	93.5 % (693/741)	<0.01	<0.01
Specificity	100 % (14/14)	100 % (21/21)	96.8 % (91/94)	0.74	0.68
Number of needle passes median, (range)	2, (1–4)	2, (1–4)	2, (1–7)	<0.01	<0.01

* "≤10 mm" vs. ">10 mm" Chi-square test

** "≤20 mm" vs. ">20 mm" Chi-square test

Table 7 Uni- and multivariate analysis of factors affecting the accuracy of EUS-FNA

Variable		Number of lesion	Accuracy (%)	Univariate analysis	Multivariate analysis	Odds ratio
Final diagnosis	AC	763	92.7	1	0.04	1.17
	NET	56	76.8	0.03		
	Metastatic tumor	24	75.0	<0.01		
	Carcinoma other than AC	19	89.5	0.20		
	SPN	5	60.0	0.06		
	FP	73	100	0.03		
	AIP	41	95.1	0.55		
	Other benign lesion	15	93.3	0.92		
Location	Body/tail	554	93.7	1	0.03	1.72
	Head	442	89.4	0.01		
Lesion size	>20 mm	835	93.4	1	<0.01	2.77
	10–20 mm	121	83.5	<0.01		
	≤10 mm	40	82.5	<0.01		
On-site evaluation	Performed	968	92.6	1	<0.01	4.97
	Not performed	28	64.3	<0.01		
Period[a]	III: 2007–2010	473	94.1	1	0.02	1.52
	II: 2003–2006	347	90.8	0.07		
	I: 1997–2002	176	87.5	<0.01		
Number of needle passes	1–2	642	92.5	1	0.09	0.66
	3–7	354	90.4	0.24		

AC adenocarcinoma, *NET* neuroendocrine tumor, *SPN* solid-pseudopapillary neoplasm, *FP* focal pancreatitis, *AIP* autoimmune pancreatitis

[a] Period I: EUS-FNA procedures are <50 times in each year. Period II: EUS-FNA procedures are 50–100 times in each year. Period III: EUS-FNA procedures are >100 times in each year

significantly lower for NET and metastatic tumor, and significantly higher for FP on univariate analysis. Lesions located in the pancreatic body or tail could be diagnosed with higher accuracy than lesions located in the pancreatic head. The accuracy of EUS-FNA was lower when on-site cytopathological evaluation was not available. The accuracy of EUS-FNA performed in latest period was significantly higher than in earliest period. And the accuracy was relatively higher when EUS-FNA procedure concluded in less number of needle passes. In multivariate analysis, final diagnosis ($P = 0.04$; odds ratio (OR) = 1.17), location ($P = 0.03$; OR = 1.72), lesion size ($P < 0.01$; OR = 2.77), presence of on-site cytopathological evaluation ($P < 0.01$; OR = 4.97) and period ($P = 0.02$; OR = 1.52) were found to be significant independent factors.

Eight patients (0.9 %; 8/936) in this study experienced complications following EUS-FNA. Six patients had gastrointestinal hemorrhage, defined as a decrease in peripheral blood hemoglobin >2 g/dL and/or necessity for endoscopic treatment. Portal vein thrombosis occurred in 1 patient due to acute pancreatitis [15], and pseudoaneurysm formation occurred in the splenic artery of another patient [16]. There were no patients with peritoneal dissemination or needle tract seeding which could be ascribed to the

EUS-FNA procedures. No procedure-related deaths were encountered in this study.

Discussion

In the present study, EUS-FNA for solid pancreatic lesions had a high diagnostic accuracy of 91.8 % in a large population treated at a single center. This study has presented the largest data set of consecutive EUS-FNA procedures for histologically and clinically confirmed pancreatic solid tumors to date. The present data set is also the largest to clarify the diagnostic ability of EUS-FNA for tiny pancreatic lesions ≤10 mm. We found that the diagnostic accuracy increased significantly when both cytological and cell-block examinations were performed, compared with cytological examination alone. However, the diagnostic accuracy was significantly lower for lesions ≤20 mm in diameter than for larger lesions. Presence of on-site cytopathological evaluation was also found to positively affect the diagnostic accuracy of EUS-FNA, as has been previously described [17]. In this study, multiple factors such as final diagnosis, location of lesion, lesion size, presence or absence of on-site cytopathological evaluation, and experience of EUS-FNA procedure were represented to affect the accuracy. Especially, on-site cytopathological evaluation and lesion size were revealed to be more weighted factors.

Previous studies which have described the diagnostic yields of EUS-FNA for pancreatic tumors, have reported a sensitivity of 54–95 %, specificity of 71–100 %, and overall accuracy rates of 65–96 % [7]. Although the diagnostic accuracy of EUS-FNA is high in most of the studies, the quality of data from these studies had varied a great deal. Some studies have confined their results only to pancreatic ACs or NETs [18–20]. Definitions of accurate diagnosis have also differed between many studies, with some studies including suspicious and/or atypical cytology as positive results [17, 21, 22], while other studies have assigned these as negative findings [9, 10, 23–25]. Furthermore, some studies have reported the yield of EUS-FNA for distinguishing malignant from benign pancreatic lesions [23, 26, 27], while others have only differentiated between neoplastic and non-neoplastic lesions [28]. In our series, the diagnostic yield of EUS-FNA was calculated in different ways. Diagnostic performance of EUS-FNA was defined not only for the presence of malignancy, but also for defining the pathological subtype of the tumor. With these criteria, the results can reveal very useful information from the clinical perspective.

Most of the previous studies have only used cytological examinations for analyses [9, 17–19, 23, 25, 27]. Combining both cytological and cell-block examinations in the present study achieved significantly better results in terms of sampling adequacy, diagnostic accuracy, sensitivity and specificity. We feel that cytology and cell-block analysis are complementary techniques. A few previous studies [24, 29] have reported incremental yield with the combination of the both of those methods (Table 8). Furthermore, establishing a cytological diagnosis from a tiny tissue specimen obtained by EUS-FNA is difficult for some pancreatic solid lesions such as NET or acinar cell carcinoma. Disease-specific IHC staining of cell-block specimens is a very useful method that helped to achieve an accurate diagnosis for 61 lesions in the present series. In 29 of these 61 lesions, IHC examinations were necessary because diagnoses from cytological examination were inaccurate.

In the present study, many type of tumors such as NET (5.6 %; 56/996), metastatic tumor (2.4 %; 24/996), carcinoma other than AC (1.9 %; 19/996) and SPN (0.5 %; 5/996) were included. In these rare pancreatic tumors, 24 NETs, 17 metastatic tumors, 14 carcinomas other than AC, and 2 SPNs were finally diagnosed by non-surgical methods. It has been commonly accepted that the diagnoses for these rare pancreatic tumors are difficult, and therefore the reliability of final diagnoses for these lesions becomes controversial. The proportion of these rare pancreatic tumors ranges from 3.5 to 17.8 % in the published literatures [9, 13, 17, 20, 22, 28, 30–32]. Our data include comparable proportion of these lesions. Furthermore, when we compare the lesions finally diagnosed by resection and

Table 8 Articles describing the diagnostic yield of EUS-FNA divided by cytology, cell-block, and combination of both

Author	No. of lesions	Accuracy			Sensitivity			Specificity		
		Cytology	Cell-block	Combination of both	Cytology	Cell-block	Combination of both	Cytology	Cell-block	Combination of both
Kopelman et al. [29]	102	81	78	88	73	63	84	94	100	94
Noda et al. [24]	33	61	79	94	–	–	–	–	–	–
Present study	996	86	75	92	88	75	92	95	79	98

the lesions finally diagnosed from clinical information, diagnostic accuracies for each final diagnoses were similar ($P = 0.36$ for NET, $P = 0.96$ for metastatic tumor, $P = 0.06$ for carcinoma other than AC and $P = 0.30$ for SPN). Hence we believe that our final diagnoses for these rare tumors were reliable for evaluation of EUS-FNA accuracy.

A small number of reports have compared the diagnostic accuracy of EUS-FNA according to lesion size. Agarwal et al. [10] reported that the diagnostic accuracy of EUS-FNA was lower for suspected pancreatic cancer <20 mm in diameter than for lesions ≥21 mm. In contrast, Uehara et al. [9] showed that diagnostic accuracies were equally good for small lesions <10 mm in diameter. These reports were based on data from smaller populations than the present study. In our series, the diagnostic accuracy of smaller lesions was significantly lower with both thresholds of 10 mm and 20 mm. Multivariate analysis identified lesion size as one of the independent factors affecting diagnostic accuracy. EUS-FNA for pancreatic lesions <10 mm is thought to be technically challenging not only in targeting the lesion, but also in obtaining an adequate specimen. Although no data are available to clarify the influence of operator experience specifically for small lesions, one multicenter study showed improvement of operator technique with experience was one factor enhancing the overall accuracy of EUS-FNA [33]. Data from our series accumulated from over a decade showed that both success rate and diagnostic accuracy of EUS-FNA increased each year with increasing experience. Furthermore, as Clary et al. [34] reported, the majority of cytohistological interpretive errors represented undercalls (the original diagnosis was less severe than the review diagnosis) when only a few malignant cells were present on the aspirate. When interpreting the findings for tiny specimens obtained by EUS-FNA, diagnosing whether the low numbers of atypical cells are associated with inflammation or malignancy is difficult. Indeed, 47 of 129 benign lesions in our series were diagnosed as showing "atypical" cytohistology on EUS-FNA specimens. Increasing experience thus improves the results of not only the endoscopist, but also cytopathologist.

Only a small number of investigations have reported the influence of different factors on the accuracy of EUS-FNA. Hwang et al. [12] reported that lesion size was marginally significant ($P = 0.08$) on multivariate analysis. A multivariate analysis by Rocca et al. [25] showed that lesion sizes >20 mm and benign solid lesions were independently associated variables. Another report from Turner et al. [20] reported that the presence of on-site cytopathological evaluation was the only factor significantly influencing diagnosis. All these reports were based on a much smaller number of cases or lesions, up to 560, and the present series appears to represent the largest undertaken to date.

There are some limitations in the present study, because of its retrospective design. The presence or absence of on-site cytopathological evaluation was determined by the period when EUS-FNA was performed. Furthermore, as FNA needle sizes used were described only in the records of 699 lesions (70.2 %; 699/996), its effect on the diagnostic yield could not be analyzed. The diagnostic accuracies with 19-gauge, 22-gauge and 25-gauge needles were 100 % (6/6), 86.3 % (571/662) and 95.4 % (63/67), respectively, and there was no significant difference in accuracies with these three type of needle. One previous report showed significant difference of accuracy between 19-gauge and 22-gauge needles [7]. A prospective comparative study [35] showed that the accuracy of EUS-FNA categorized by needle size varies according to location of lesion, and other retrospective study showed no significance of needle size [9]. Hence, the influence of needle size on EUS-FNA results appears likely to be limited.

In conclusion, we evaluated the diagnostic yield of EUS-FNA for pancreatic masses in the largest subject population to date. Pancreatic masses were diagnosed by EUS-FNA with high accuracy and a low complication rate. Both cytological and cell-block examinations were complementary for improving the accuracy. However, lesions ≤20 mm were difficult to diagnose correctly. On-site cytopathological evaluation was a significantly important factor affecting the accuracy of EUS-FNA. Although the diagnostic yield of EUS-FNA for pancreatic lesions is high, a variety of complementary methods should be tried when accurate diagnosis of a lesion seems difficult to obtain.

Conflict of interest The authors declare that they have no conflict of interest.

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