TIMELY REVIEW

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Assessment of preoperative pancreatic biopsy, cytological/histological review of cell-block-specimens obtained by endoscopic ultrasound-guided fine-needle aspiration: Laboratory-based study

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Abstract

Background: Pancreatic cancer is among the most lethal cancers worldwide due to the limited availability of techniques for early detection of signs and symptoms. Reportedly, it is the fourth-leading cause of cancer-related mortality among Japanese adults. With the advent of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) for diagnosing pancreatic cancer, the rate of the cytological and histological diagnoses of cell-block-specimens has significantly increased in Japan.

Methods: The cytological specimens of 165 patients with pancreatic lesions obtained using EUS-FNA between January 2010 and July 2016 at the Kyorin University Hospital were investigated. The clinical course of 153 patients was assessed from their clinical records, which included information on their imaging diagnosis, laboratory data, final clinical diagnosis and treatment; moreover, the accuracy of the cytological/histological examination and clinical diagnosis at our hospital were analysed.

Results: The number of cells in cell-block-specimens was too small to estimate data. However, cytological specimens were sufficient to observe the findings of suspected malignancy such as necrosis. Biopsy was deemed necessary for diagnosis using both histological and cytological specimens.

Conclusion: EUS-FNA can be used not only to diagnose benign or malignant types of pancreatic cancers but also to assess the sensitivity of molecular target drugs and chemotherapy methods. Therefore, both histological and cytological diagnoses are required to enhance diagnostic precision both in our hospital and at other institutions.

KEYWORDS
cytological/histological cell-block-specimens diagnosis, EUS-FNA, pancreatic biopsy, pancreatic cancer, specimen

1 | INTRODUCTION

Due to the limited range of available diagnostic techniques for detecting the early signs and symptoms of pancreatic cancer, it has become the most lethal cancer worldwide and the fourth-leading cause of cancer-related mortality in Japanese adults.

Endoscopic ultrasound (EUS) is the most effective method for the management of pancreatic cancer; it leads to a higher diagnostic yield than ultrasonography, computed tomography, magnetic resonance imaging and polyethylene terephthalate for the detection of early pancreatic tumours.[1,2] Endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA), as reported by Vilmann et al,[3] is often used for the qualitative preoperative diagnosis of tumours and for the assessment of medical treatment strategies. Recently, in addition to the increased use of EUS-FNA for the diagnosis of pancreatic lesions, the rate of cytological and histological examinations has also significantly increased in Japan. In the present study, we investigated samples obtained using EUS-FNA over 7 years in our hospital. We analysed the accuracy of cytological, histological and clinical diagnoses of cell-block-specimens to enhance diagnostic precision. A comparative examination of biopsy, cytological diagnosis, histological diagnosis and clinical diagnosis revealed that cytological diagnosis had a higher sensitivity for the detection of pancreatic cancer and other related malignancies than the other diagnostic methods.

2 | MATERIALS AND METHODS

Specimens from 165 patients with pancreatic lesions obtained using EUS-FNA between January 2010 and July 2016 at the Kyorin University Hospital were examined. The mean patient age was 68.7 (range, 34-86) years. In total, 86 males and 79 females were recruited in this case study. Both cytological and histological examinations were conducted on the samples collected using EUS-FNA.

EUS-FNA was performed using 22-G processing needles with stylets. After tissue sampling, the needles were washed once with laboratory saline, and the resulting small tissue samples were fixed in bottles containing 15% formalin solution. We examined the small tissue samples as cell-block-specimens for histological diagnosis. For cytological examination, the needles were washed, and the samples obtained were transferred onto glass slides and preserved with 95% ethanol. The slides were submitted to the pathology laboratory for cytological examination using fixed smear and Diff–Quik staining techniques. Rapid on-site evaluation was performed on specific specimens as needed. Formalin-fixed samples submitted for histological diagnosis were processed in an automated tissue processor for approximately one night and were penetrated with paraffin liquid via de-ethanol and de-xylene staining. Subsequently, using machine processing, ethanol was replaced with paraffin, and the solution was then hand-stirred, subjected to pressure and further treated. Small tissue samples were wrapped with organic solvent-resistant sponges in a cassette as individual paraffin blocks.

Pancreatic cancer was diagnosed when it was suspected from clinical data, laboratory imaging findings, clinical diagnoses at our hospital and cytology/histology biopsy diagnoses of carcinoma and/or suspected carcinoma.

Based on clinician reports, the cytological and histological cell-block-specimens results from all 165 patients were classified into the following five categories: 'no malignancy', 'atypical cells', 'suspected carcinoma', 'carcinoma' and 'other malignancy'. The cytological and histological categories of each patient were subsequently compared. Furthermore, the clinical course of the 153 patients was investigated from their clinical records, which included information on their imaging diagnosis, laboratory data, final clinical diagnosis and treatment. Patients who underwent surgery or chemotherapy as treatment were considered to have 'pancreatic cancer', whereas patients who were followed up due to a clinical diagnosis of no malignancy were considered to have a 'benign lesion'. Four patients who were diagnosed with malignant lymphomas were excluded. The cytological and histological reports and clinical diagnoses of these patients were compared to analyse the diagnostic accuracy of cytology (Table 2). The results from the cytological, histological and clinical diagnoses were analysed to identify the clinical course and generate a comparison chart using the results from the cytological diagnoses.

3 | ETHICS

This study was performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Kyorin University School of Medicine (permission ID number 1190). Informed consent was obtained from patients in the form of a patient opt-out on the website.

4 | RESULTS

Initially, cytological diagnoses were compared with the results from biopsy. For 93 of 161 patients (58%), the histological and cytological diagnoses were in complete accordance. Furthermore, carcinomas and suspected carcinomas were diagnosed through cytological and histological cell-block-specimens diagnoses in 81 patients (50%). Table 1 presents a comparison of the cytological and histological diagnoses.

Based on the results of the clinical diagnoses and a comparison with the cytological/histological diagnoses, 153 patients were followed up to determine their clinical course, of which 5 were deemed to have atypical lesions. One patient was diagnosed with adenocarcinoma by cytology; however, after the diagnosis, this patient was confirmed by operation to have an atypical lesion (Figure 4). Of the remaining 148 patients, malignancy was diagnosed in 138 patients, whereas malignancy was not established in the remaining 10 patients (Table 2). The 10 patients are currently being followed up. Of the 138 patients diagnosed with pancreatic cancer, 31 were confirmed by operation to have a pathological diagnosis of adenocarcinoma. Ascites were detected in 5 of 138 patients, and the cytological diagnosis of these patients was adenocarcinoma. Four of them were confirmed by liver biopsy, and the histological diagnosis was adenocarcinoma. Other

TABLE 1 Comparison of cytological and histological cell-block-specimens diagnoses in 165 patients

Histology \ cytology	No malignancy	Atypical cells	Carcinoma suspected	Carcinoma	s/o malignancy without Ca.	Malignancy without Ca.
No malignancy	15	1				
Atypical cells	11	26	1	4	0	2
Carcinoma suspected	2	10	4	9		
Carcinoma	1	9	20	48		
s/o malignancy without Ca.					1	
Malignancy without Ca.						1

Note: No malignancy: No atypical cells and suspicion of benign lesion. Atypical cells: Atypical cells, no suspicion of malignancy and intraductal papillary mucinous neoplasia. Carcinoma suspected: Few cancer cells; suspicion of adenocarcinoma and neuroendocrine tumour. Carcinoma: A lot of cancer cells. s/o malignancy without Ca.: Few malignant cells without carcinoma; suspicion of lymphoma. Malignancy without Ca.: A lot of malignant cells, suspicion of lymphoma.

TABLE 2 Comparison of clinical course and cytological/histological diagnoses in 153 patients

Clinical		Cytology		Histology	
*Carcinoma	138	Carcinoma	101	Carcinoma	90
				Atypical	9
				No malignancy	2
		Atypical	32	Malignancy	5
				Atypical	19
				No malignancy	8
		No malignancy	5	No malignancy	5
*Atypical	5	Atypical	3	Atypical	3
		s/o carcinoma	1★	Atypical	1
		No malignancy	1	No malignancy	1
*No malignancy	10	No malignancy	10	No malignancy	9
		Atypical	0	Atypical	1
Total	153				

Note: Clinical *Carcinoma: Detection of irregular and large-size mass, suspicion of malignancy in ultrasound (US) and confirmed by surgery and detection of body fluid and lymph node enlargement. *Atypical: only irregular mass in US (★ case is only irregular mass). *No malignancy: no mass/small mass; suspicion of no malignancy and/or benign lesion in US.

than the cases mentioned above, the imaging findings were confirmed.

Of the 138 patients, cytology revealed that 101 had malignancy, 32 had atypical cells and 5 had no malignancy. Of the five cases, one was discovered to have metastasis to the kidney, whereas for the remaining four, the malignancy was clear, and chemotherapy was given. Of the 101 patients identified with a malignancy, histological biopsy diagnoses confirmed malignancy in 90 patients, atypical cells in 9 patients and no malignancy in 2 patients (Table 2). The example patients indicated in Table 2 are presented in Figures 1–5.

5 | DISCUSSION

Pancreatic tumours are defined as adenocarcinomas, which are primarily external secretion tumours that commonly develop from the pancreatic duct.[4] The cellular images of pancreatic cancer do not vary greatly from those of adenocarcinomas in other organs.

The histological cell-block-specimens of pancreatic tissue yield a small volume of tissue from the harvested material, resulting in a small yield of cells to derive any significant data. Nevertheless, the cytology samples obtained from our laboratory were enough to detect background necrosis, which is suggestive of malignancy. A diagnostic approach for the evaluation of pancreatic cancer (malignancy) was established based on biopsy findings; however, our results demonstrate that cytology was more effective in inferring malignant tumours. These findings emphasise the need for the sampling of both histology and cytology specimens during diagnosis. Thus, the significant information obtained from cytology suggests that it is the fundamental diagnostic approach for the assessment of pancreatic cancer. Although malignancy can be established using biopsy analysis, cytological diagnosis (using FNA) is required for the identification of the variants of malignancy, highlighting the different sampling possibilities within tissue specimens. Although the same specimen was subjected to both cytological and histological biopsy diagnoses, the correlation of both tests was not significantly high. We considered that in some cases, cytological/histological examination findings and the unevenness of reported contents resulted in this insufficient correlation.

Klapman et al examined the presence or absence of quick cytological diagnoses and the diagnostic ability of EUS-FNA and observed that the sampling rate significantly decreased when an on-site cytological diagnosis was not promptly performed.[5]

Owing to its expense, on-site cytological diagnosis is not conducted at our hospital,[6] and the distance between the endoscopy room and pathology laboratory in our hospital may discourage the presence of an endoscopist. However, regulating the number of punctures is necessary for assessing tumour cells.[7,8] Smear technology and Diff–Quik staining enable physicians and endoscopists to determine whether a tumour
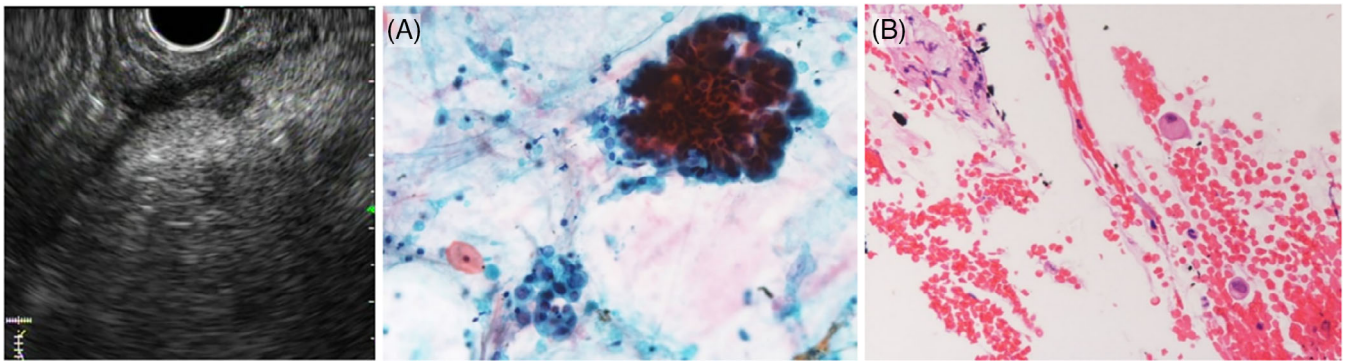
FIGURE 1 Cytology: Adenocarcinoma; Histology: No malignancy. Endoscopic ultrasound of a 76-year-old woman. The ultrasound shows a low echoic lesion of 26 mm in the pancreatic body. For cytological analysis, (A), necrotic material was obtained in the background, and clearly atypical cells, as suspected, demonstrated adenocarcinoma. In the histological analysis, (B), only red blood cells and histiocytes were observed in the background, and almost none of the epithelial cells were observed [Color figure can be viewed at wileyonlinelibrary.com]
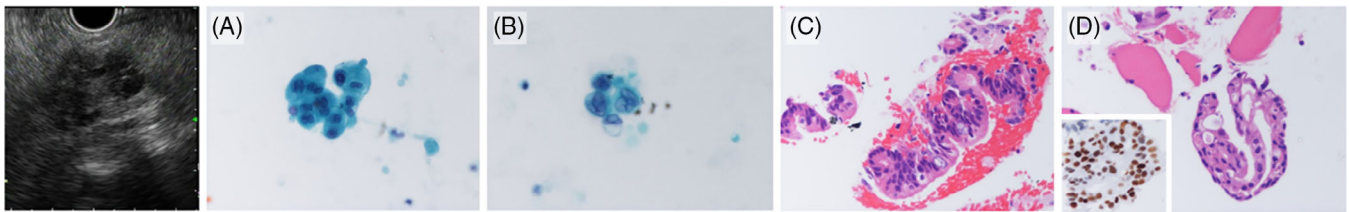

FIGURE 2 Cytology: Atypical; histology: No malignancy. Endoscopic ultrasound of a 69-year-old woman. Expansion of the pancreatic duct and 30 mm-sized low echoic lesion. A cluster of atypical columnar cells showed slightly moderate, large denaturation in the cytological diagnosis, (A) and (B), and biopsy showed no malignancy, (C). The duct indicating the variant was found in the re-examined biopsy sample, (D). P53 was positive (in a small part of (D)), confirming Adenocarcinoma [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Cytology: No malignancy; histology: Atypical. Endoscopic ultrasound of a 67-year-old woman. The ultrasound showed a simple cystic mass with a heterogeneous interior. Numerous red blood cells were clustered together, and cytological diagnosis was performed with only a few epithelial cells (cytology, (A)). The duct of the tissue samples showed some sequence irregularity and a slight variant form, but it was difficult to assess whether the sample was benign or malignant (histology, (B)). An infectious pancreatic cyst and drainage was clinically performed with subsequent follow-up [Color figure can be viewed at wileyonlinelibrary.com]

sample should be subjected to further testing. Generally, seven or eight cytotechnologists and pathologists are engaged in routine cytological diagnoses in our laboratory.

In the present study, formalin-fixed samples submitted for histological diagnosis were subjected to rigorous processing (as described in Section 2). However, this rigorous procedure is more likely to diminish the number of cells if the initial sample is small. To avoid this, the sample was wrapped with organic solvent-resistant sponges in a cassette as individual paraffin blocks. Small liquid components, such as necrotic materials, can be eluted by ethanol or xylene to consequently disappear during processing. The remaining components also often penetrate the exclusive sponge and are therefore not detected by encapsulation.
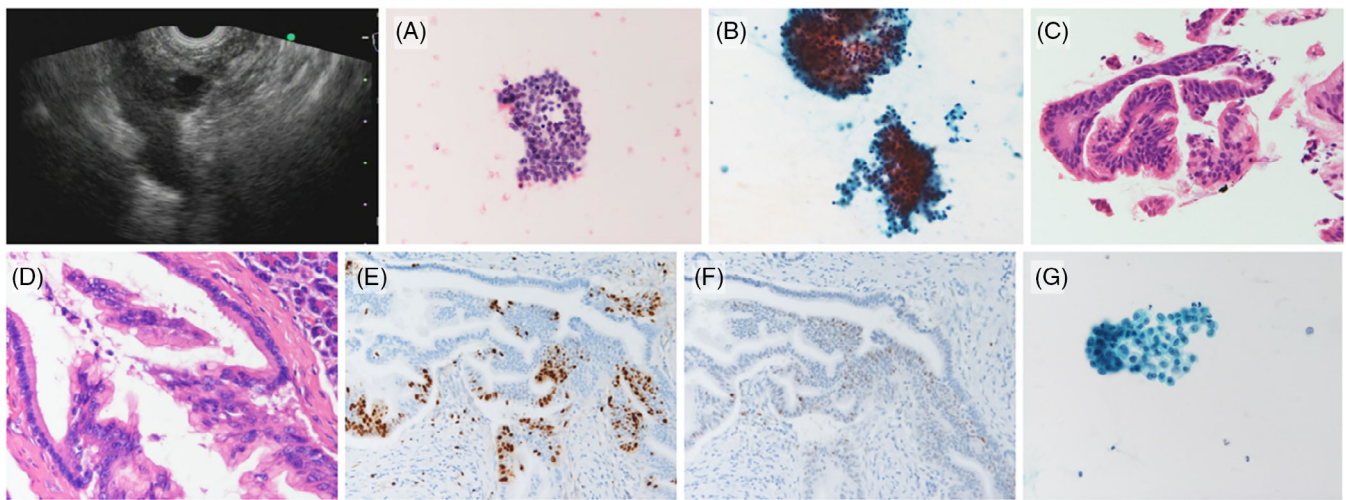
FIGURE 4 Cytology: Suspected adenocarcinoma; histology: Atypical. Endoscopic ultrasound of a 60-year-old man (★case from Table 2). Cystic lesions in the pancreatic body were noted with tail transition and heterogeneous interior. Testing of the mass shadow was repeated three times. Both cytology and histology identified a variant, but a suspected Class IV adenocarcinoma was reported by cytological diagnosis once (photograph, right (B)). Cytology (A) and (B) and histology ((C) biopsy and (D) surgical specimen). Segmental resection of the pancreas and duodenum was performed, C and D; an atypical lesion (PanIN-1 or two equivalents) was subsequently diagnosed and viewed using staining for Ki-67, (E), and P53, (F) from surgical specimen. A cluster of cells was observed in a part according to a Class IV specimen and a diagnosed specimen. The nucleolus is outstanding, but the cell's border is evident; the N/C ratio is small, similar to a repair cell-like change ((G) re-examination specimen) [Color figure can be viewed at wileyonlinelibrary.com]
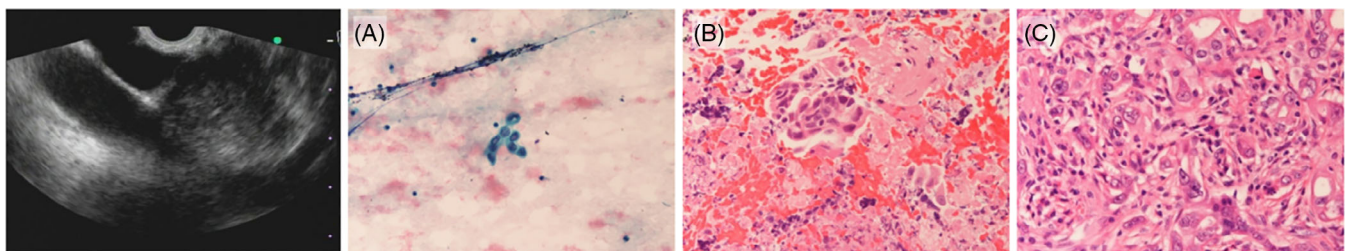

FIGURE 5 Cytology: Atypical; histology: Adenocarcinoma. Endoscopic ultrasound of a 72-year-old man. The ultrasound showed an irregular mass (20 mm) on the head of the pancreas with a generally uniform interior. The cytological specimens mostly comprised red blood cells and almost no epithelium with very few variant acinar cells (cytology (A)). The duct in which cancer was strongly suspected was identified in the biopsy (histology (B)). Subsequently, surgery was performed, and poorly differentiated Adenocarcinoma was confirmed (surgical (C)) [Color figure can be viewed at wileyonlinelibrary.com]

In this study, during cytological evaluation, smears were directly added to the slide glass and fixed. In fluid samples, such as those used in this study, the smear spins down and stains the ethanol-fixed slide glass with stain solution, resulting in a specimen that is suitable for microscopic examination. Staining improves cell visibility; moreover, if necrotic samples, viscous liquid, cyst samples and inflammatory cells are preserved, an increase in the number of epithelial cells facilitates the diagnosis of tumour cells.

The influence of anagenesis on the cells obtained and frequent testing must be considered during cytology and histology. Furthermore, inflammatory changes and fibrosis affect both cytological and histological biopsy diagnoses in specimens showing evidence of chronic pancreatitis or autoimmune pancreatitis.[9]

A majority of pancreatic tumours are adenocarcinomas. Therefore, immunohistochemical analysis is necessary for the detection of neuroendocrine tumours, acinar cell carcinomas and solid papillary pseudotumours, similar to that required for the detection of pancreatic cancer.[10] In the future, EUS-FNA should be used to confirm the treatment course. In addition, tissue specimens and cell-block specimens have been estimated to have a greater sensitivity to molecular targeted drugs than for cytological/histological diagnosis. The histological diagnosis of necrotic samples and small tissue samples is challenging; therefore, cytological diagnosis is often a more suitable option based on Papanicolaou staining and standard criteria.

In their study on atypical lesions, Evan et al identified pancreatic lymphomas in 5% of their samples, benign lesions in 11% and pancreatic ductal carcinomas in 21%.[11] Similar to the cytological diagnoses of tumours of the breast and thyroid gland and based on the Bethesda system, the cytological and histological diagnosis of pancreatic specimens should be performed while receiving constructive feedback with the possibility of re-examination.[10,12]

Although immunostaining, which is enabled by a cytological diagnosis and executed via decolouration and transcription, can be used to study target cells, there is a limited range of available staining antibodies; furthermore, this procedure is time consuming and requires sophisticated techniques. Recently, liquid-based cytology (LBC) has become the standard technique for use in cytological analyses; however, this method requires a separate examination of cell sap while it is preserved. Additionally, the LBC reagent used for this type of analysis increases the occurrence of cellular contractions and the presence of cyst contents and artefacts such as those following mucolysis.[13-15] In cytological diagnosis, factors such as background mucus and cyst contents are important for determining the nature of lesions.

The increased use of pancreatic EUS-FNA has facilitated the improvement in biopsy using ultrasound. Nonetheless, further studies are warranted to improve the use of endoscopy. EUS-FNA can be used not only to diagnose benign or malignant pancreatic cancers but also to assess the sensitivity of molecular target drugs and chemotherapy. Therefore, both histological biopsy and cytological diagnoses are required to enhance diagnostic precision in our hospital and other institutions. Additionally, clinicians, pathologists, cytotechnologists and endoscopic engineers should cooperate in institutions to enhance diagnostic accuracy.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

N.M., M.M. and M.T. conceived and designed the experiments. N.M., M.M. and M.T. performed the experiments. N.M. and M.M. analysed the data. Finally, N.M. wrote the article.

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