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Original Article

Diagnostic efficacy of different pathologic methods for assessing tissue obtained by endoscopic ultrasound-guided fine needle aspiration: a prospective study

Jialiang Huang[1*], Yulin Liang[3*], Longjiang Xu[2], Duanmin Hu[1]

Departments of [1]Gastroenterology, [2]Pathology, Second Affiliated Hospital of Soochow University, Suzhou City, Jiangsu Province, China; [3]Department of Gastroenterology, Suzhou BenQ Medical Center, Suzhou City, Jiangsu Province, China. [*]Co-first authors.

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Abstract: Aims: The best method for processing specimens by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has not been standardized and varies considerably between medical centers. The purpose of this study is to explore whether a combination of histologic and cytologic methods can increase the diagnostic efficacy of EUS-FNA in solid lesions around the digestive tract. Methods: We recruited 52 patients (65 cases total) with solid lesions around the digestive tract who underwent EUS-FNA as performed by the same endoscopic physician from December 2016 to January 2018. All the EUS-FNA specimens were processed by conventional smear cytology (CS), liquid-based cytology (LBC), cell block (CB), and histopathology. All the pathologic results were tracked to investigate the diagnostic value of the methods. Results: Fifty-three malignant lesions and 12 benign lesions were analyzed. The diagnostic accuracy levels of the CS, LBC, CB, and histopathology were 96.9%, 89.2%, 91.9%, and 48.1%, respectively. CS had a higher diagnostic accuracy than CB ($P < 0.05$) and LBC ($P < 0.05$). The cytologic methods had a significantly higher diagnostic accuracy than histopathology ($P < 0.05$). The combined diagnostic accuracy of all the methods was 100%. The diagnostic sensitivities of the CS, LBC, CB and histopathology were 96.2%, 86.8%, 90.4%, and 37.2%, respectively, and the diagnostic specificity of each of the four methods was 100%. Conclusions: Different pathological methods can compensate for one another, substantially improving the overall positive detection rate of EUS-FNA. Combining cytology and histology can contribute additional diagnostic efficacy to EUS-FNA in solid lesions around the digestive tract.

Keywords: Endoscopic ultrasound-guided fine needle aspiration, smear cytology, liquid-based cytology, cell block, histopathology

Introduction

With the application and popularity of type-B ultrasound, CT, and MRI, the detection rate of solid lesions around the digestive tract is increasing. These lesions are very common in the clinic and include enlarged lymph nodes, pancreatic space-occupying lesions, retroperitoneal space-occupying lesions, and some solid tumours of the abdominal cavity. Doctors must obtain pathologic evidence to devise treatment plans and determine a prognosis. In the 1990s, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was implemented in the clinic, and it replaced many invasive and risky diagnostic procedures, such as mediastinoscopy, laparoscopy, and laparotomy or thoracotomy [1, 2]. Currently, EUS-FNA is the preferred method for diagnosing solid lesions around the digestive tract.

Although EUS-FNA is an effective procedure, controversy remains regarding the suitable needle size, slow-pull and fanning techniques, necessity of rapid on-site cytological evaluation (ROSE), and specimen processing methods [2, 3]. In recent years, scholars have reached a consensus on some of these issues, but the best method to process the limited specimens has not been standardized and varies considerably between medical centers [4, 5]. Using proper pathological methods is crucial to the

diagnostic accuracy of EUS-FNA, and previous studies reported a diagnostic accuracy ranging from 60-90% for conventional smear cytology (CS) and liquid-based cytology (LBC) [6, 7]. In recent years, the specimen processing method has been continuously improved to increase the diagnostic accuracy of EUS-FNA to the greatest extent possible. To date, in addition to CS and LBC, cell block (CB) and histopathology have been increasingly widely used for pathologic diagnosis by EUS-FNA [8]. Since cytology and histology have their respective advantages in the field of pathologic diagnosis, the purpose of our study is to explore whether the combination of the two approaches can provide additional diagnostic efficacy in EUS-FNA.

The study included 52 patients (65 cases total) with solid lesions around the digestive tract. The EUS-FNA specimens from all the patients were processed by CS, LBC, CB, and histopathology. By analyzing the diagnostic results of the four pathologic methods, we were able to calculate the diagnostic yields of these methods and assess whether the combination of histologic and cytologic methods could increase the diagnostic accuracy of EUS-FNA for solid lesions around the digestive tract.

Materials and methods

Patients

From December 2016 to January 2018, 52 patients with solid lesions around the digestive tract were treated at our hospital. The study protocol was approved by the Clinical Research Ethics Committee of Second Affiliated Hospital of Soochow University. Informed consent was obtained from all patients, and all authors had access to the study data and reviewed and approved the final manuscript. Patients undergoing EUS-FNA in this study were included based on the following inclusion criteria: Imaging findings (CT/MRI/PET-CT) that suggested space-occupying lesions of an unknown nature in the abdominal cavity or enlarged abdominal or mediastinal lymph nodes.

Patients with the following conditions were excluded: (1) patients with severe heart, lung, liver or other diseases who were unable to tolerate EUS-FNA or had serious coagulation dysfunction; and (2) patients with an inability to complete the follow-up.

EUS-FNA procedure

The EUS-FNA procedures were performed in all the patients by a physician experienced in endoscopy using the protocol described below with the same types of instruments. A linear-array echoendoscope (EG-530; Fuji Film Ltd., Tokyo, Japan) was used for EUS. FNA was performed with a 22-gauge needle in all cases (Echo Tip; Cook Medical, Winston-Salem, NC, USA).

Under EUS guidance, the endoscopist chose the most suitable path and performed FNA. Lastly, an air-filled syringe was used to spray the specimens onto the glass slides. The specimens were considered satisfactory if the presence of non-hemorrhagic small tissue filaments was observed with the naked eye or the target cells were observed by ROSE [9].

For each site, at least 3 needle passes were performed (**Figure 1**). On the first needle pass, parts of the specimens were smeared for ROSE and hematoxylin-eosin (H&E) staining. Visible tissue strips were then selected from the remaining specimens and placed in bottle A with a fixation fluid (4% neutral formaldehyde). On the second needle pass, all the specimens were placed in bottle B with cell preservation solution, and selected visible tissue strips (by naked eye examination) were moved from bottle B to bottle A. The remaining specimens in bottle B were then sent for LBC. All the specimens obtained from the third needle pass were placed in bottle A. The specimens in bottle A were evenly divided into two plates and were diagnosed using CB and histopathology. The decision about whether a fourth needle pass was needed was based on the judgement of the on-site pathologist. In our study, there were 10 cases in which the specimens in bottle A were not sufficient for CB and histopathology at the same time, and the pathologists prioritized CB because it had a higher diagnostic rate than histopathology when specimens were limited [10]. In addition, 3 case specimens were insufficient so that neither CB nor histopathology could be performed, and these specimens were processed only by CS and LBC in the end.

The vital signs of the patients were monitored after EUS-FNA to evaluate complications such as infection, perforation, and bleeding.

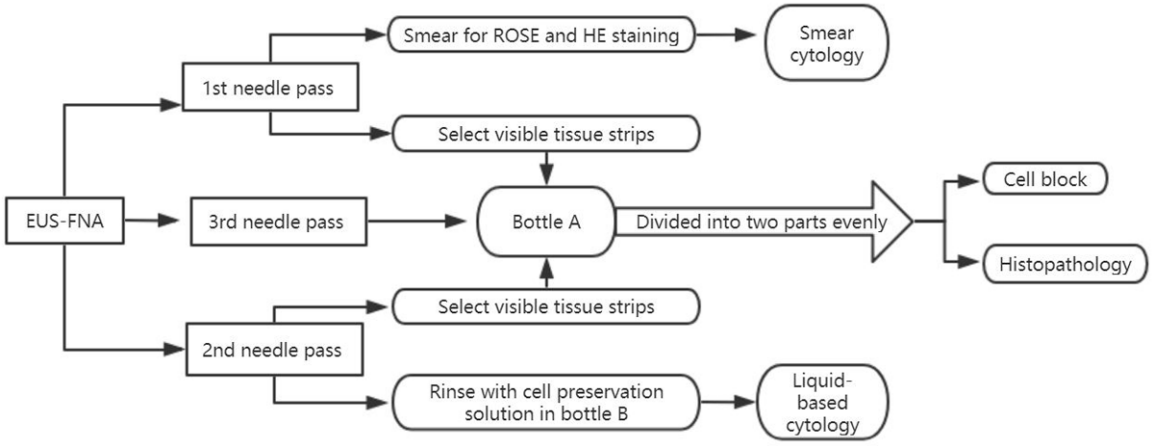

Figure 1. The EUS process.

Specimen processing methods

All the specimens were processed sequentially using CS, LBC, CB, and histopathology when sufficient. The four pathologic methods were independently diagnosed by four different pathologists. When the diagnoses of the same specimen by different methods were not consistent, the positive diagnosis was considered superior to the negative diagnosis. In addition, if the positive diagnoses were inconsistent, the final diagnosis would be obtained following a discussion among four pathologists.

Conventional smear cytology

The first FNA specimen was smeared onto a glass slide, air-dried, and stained with Giemsa for ROSE by a pathologist. The remaining specimens were placed in a fixation fluid (95% alcohol) and sent to the Department of Pathology for routine H&E staining [8].

Liquid-based cytology

Bottle B was centrifuged, and the liquid supernatant was discarded; then, slides were created by an automatic smear and dye machine (Becton, Dickinson and Company). All the specimens were stained for a Papanicolaou test [8].

Cell block and immunohistochemistry

One part of the specimen from bottle A was randomly selected and placed in a centrifuge tube; the specimen was centrifuged and the liquid supernatant was discarded. Then, a 4% neutral buffered formaldehyde solution was added to the centrifuge tube and mixed. The tube was centrifuged and the liquid supernatant was discarded again. The visible specimen was removed with a cuspidal stick and wrapped in filter paper. Following specimen dehydration, embedding and slicing, the pathologist stained them with H&E and IHC [10].

Histopathology

Another portion of the specimens from bottle A was sent to the Department of Pathology. Following dehydration, embedding and slicing, the specimens were stained with H&E.

During this study, two pathologists were invited to diagnose the specimens independently using the four pathologic methods. If their diagnoses were inconsistent, a third pathologist was consulted.

Benign and malignant definitions

The pathologic results of malignancy or suspicion of malignancy were categorized as malignant, while atypical or benign cells were categorized as benign.

The histopathology results of surgically excised tissues was considered the gold standard. In non-resected cases, a tentative diagnosis was determined based on the pathological results obtained from EUS-FNA, imaging diagnosis, and clinical manifestations. Patients without evidence of malignancy were followed for at least 12 months; if clinical manifestations, such as emaciation, jaundice, or abdominal

pain, were not observed, then benign diseases were considered.

Statistical analysis

SPSS 19.0 software was used for the statistical analysis. The accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and Youden index for the CS, LBC, CB and histopathology were estimated and compared using the McNemar chi-square test. $P < 0.05$ was considered significant.

Results

Baseline characteristics of the patients

Fifty-two patients with solid lesions around the digestive tract were enrolled in the study, including 34 males and 18 females, with an average age of 65.3 years. Among these individuals, two sites were punctured during the first EUS-FNA in 11 patients, and a second EUS-FNA was performed in 2 patients for re-examination or further diagnosis, all of which were recorded as 2 cases. Hence, 65 punctures were analyzed. Among the 65 punctures, 28 cases of pancreatic lesions, 23 cases of lymph nodes, and 14 cases of abdominal and pelvic space-occupying lesions were identified. The space-occupying lesions included 7 liver lesions, 3 pelvic cavity lesions, 2 bile duct lesions, 1 duodenal lesion, and 1 gallbladder lesion.

Final diagnosis

Based on the pathology results of the specimen, the imaging diagnosis, the clinical manifestation, the pathology of the surgically resected tissue and the follow-up condition, 53 lesions were diagnosed as malignant, and 12 lesions were ultimately diagnosed as benign. Among the 12 benign cases, 1 case was retroperitoneal tuberculosis, and 11 cases were unexplained lymphadenectasis. No special findings were observed in 9 of these lymphadenectasis cases. After 12 months of follow-up, the lesions did not progress and the patients had no other clinical symptoms; thus, they were conclusively diagnosed as benign lymph node hyperplasia. The remaining 2 patients presented with enlarged abdominal lymph nodes, but pancreatic cancer was diagnosed according to the postoperative pathology results or pathology results of other puncture sites. Although the specimens were satisfactory during the puncture process, no malignant cells were observed in the pathology samples of the lymph nodes. Finally, the selected lymph node from the puncture process was judged to contain no malignant tumor cells.

Comparing the diagnostic performance of the four pathologic methods

The diagnostic results for the four pathologic methods are compared in **Tables 1** and **2**. The diagnostic accuracies of the CS, LBC, CB, and histopathology were 96.9% (63/65), 89.2% (58/65), 91.9% (57/62), and 48.1% (25/52), respectively. The combined diagnostic accuracy of the four methods was 100% (65/65). The diagnostic accuracy rate of CS was higher than that of CB and LBC ($P < 0.05$). The diagnostic accuracy of the three cytology methods was significantly greater than that of the histopathology method ($P < 0.05$). The diagnostic sensitivities of CS, LBC, CB and histopathology were 96.2% (51/53), 86.8% (46/53), 90.4% (47/52), and 37.2% (25/43), respectively. The diagnostic specificity of the four methods was 100%. The following figures show four cases diagnosed using EUS-FNA that were ultimately confirmed using the surgically excised tissue from the present study (**Figures 2**-**5**).

In this study, no bleeding, perforation, infection or other complications occurred among the 65 cases of puncture.

Discussion

The primary purpose of EUS-FNA is to obtain tissues for pathological examination. However, a consensus on the best method for processing the aspirated specimens has not been reached because different methods exert different effects on the diagnosis [5, 8]. Currently, many researchers report on comparisons of different cytologic methods in EUS-FNA samples, but few studies have determined whether the diagnostic rate of EUS-FNA can be further improved using multiple cytologic methods combined with histology. This article is the first to combine the results for the CS, LBC, CB and histopathology and shows that the combination of cytology and histology provides additional diagnostic efficacy to EUS-FNA for solid lesions around the digestive tract.

Table 1. Pathologic diagnosis and accuracy of EUS-FNA

Final diagnosis	Case	Diagnostic accuracy			
		Conventional smear cytology	Liquid-based cytology	Cell block	Histopathology
Malignant					
Pancreas (N=28)					
Adenocarcinoma	23	22/23	21/23	20/23	3/17
Neuroendocrine tumor	2	2/2	2/2	2/2	1/1
Solid pseudopapillary tumor	2	2/2	2/2	2/2	1/2
Metastatic carcinoma	1	0/1	0/1	1/1	1/1
Lymph node (N=11)					
Metastatic carcinoma	9	9/9	7/9	9/9	3/8
Lymphoma	2	2/2	1/2	2/2	0/2
Abdominal space-occupying lesion (N=14)					
Neuroendocrine tumor	2	2/2	2/2	2/2	1/2
Metastatic/primary carcinoma	12	12/12	11/12	9/11	6/11
Total malignant cases	53	96.2% (51/53)	86.8% (46/53)	90.4% (47/52)	37.2% (16/43)
Benign					
Lymph node (N=12)					
Tuberculosis	1	1/1	1/1	0/0	0/0
No special discovery	11	11/11	11/11	10/10	9/9
Total benign cases	12	100% (12/12)	100% (12/12)	100% (10/10)	100% (9/9)
Total	65	96.9% (63/65)	89.2% (58/65)	91.9% (57/62)	48.1% (25/52)
		100% (65/65)			

Table 2. Comparison of the four pathologic methods

Index	Smear cytology	Liquid-based cytology	Cell block	Histopathology
Sensitivity	96.2% (51/53)	86.8% (46/53)	90.4% (47/52)	37.2% (16/43)
Specificity	100% (12/12)	100% (12/12)	100% (10/10)	100% (9/9)
Positive predictive value	100% (51/51)	100% (46/46)	100% (47/47)	100% (25/25)
Negative predictive value	85.7% (12/14)	63.2% (12/19)	66.7% (10/15)	25% (9/36)
Youden index	0.962	0.868	0.904	0.372

CS has been widely used to analyze specimens collected using EUS-FNA, and CS is easy to prepare, inexpensive, fast and causes no cellular trauma [11, 12]. However, the false-negative rate of CS for malignant diseases is high [13]. According to Arcidiacono et al. [14], approximately 30% of patients with a negative biopsy may have a malignant pancreatic tumour. Three primary explanations for this phenomenon have been proposed. First, part of the positive cells will be abandoned during the smearing process. Second, blood cells, digestive tract mucus, and other impurities on the slide will hinder the observation of the cells. Third, the process of making slices by hand may lead to cell swelling, dissolution and destruction. Fortunately, the endoscopists in our hospital had access to ROSE. ROSE can allow further sampling at the time of the initial procedure if the specimen is deemed to be inadequate, thus improving the efficacy and safety of EUS-FNA. Iglesias-Garcia et al. [15] similarly reported that ROSE may increase the diagnostic yield of EUS-FNA by 10%-30% in hospitals with diagnostic accuracy rates < 90%. In this study, the diagnostic accuracy, sensitivity, specificity, and positive and negative predictive values of CS were 96.9%, 96.2%, 100%, 100% and 85.7%, respectively. These results indicate that CS can prevent unnecessary surgeries because of its high sensitivity and negative predictive value. Notably, ROSE increased the accuracy of CS to 96.2% in our study, which was considered similar to studies by other researchers [16, 17].

Although ROSE has many advantages, it cannot be performed in most hospitals because it requires a long time and greater manpower [18,

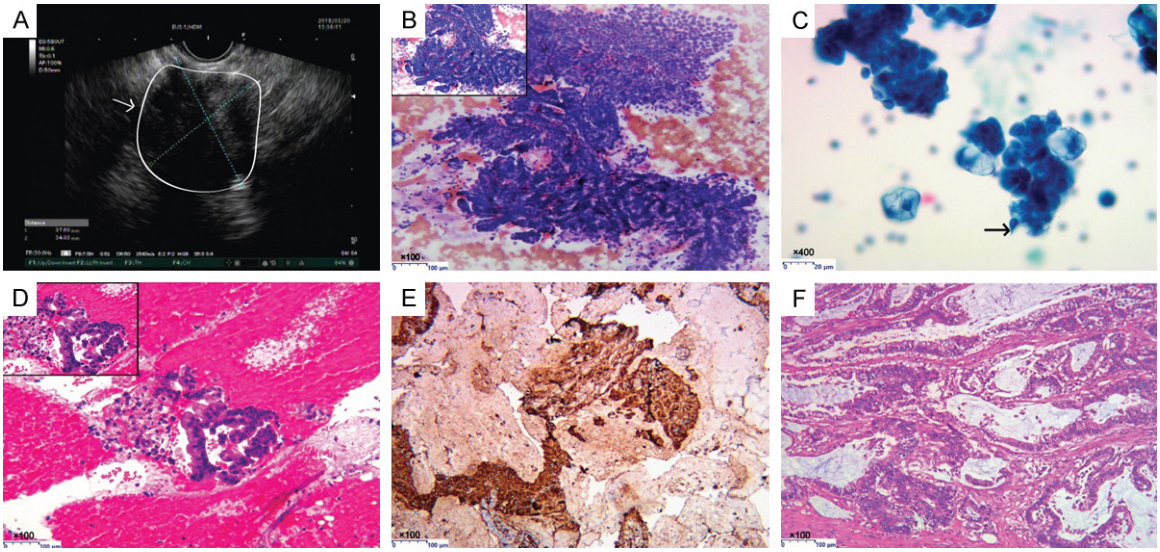

Figure 2. Pancreatic adenocarcinoma. A. EUS: A hypoechoic mass approximately 37*34 mm in size is located in the pancreatic neck and wraps around the truncus coeliacus and small blood vessels. B. Smear cytology preparation shows overlapping cells that are crowded in a nested pattern, and adenoid structures are observed in the center of the cell cluster (H&E staining, ×100); inset (H&E stain, ×200). C. The LBC preparation shows tumor cells in a papillary arrangement. The 'three-dimensional' structure of the cell cluster is clear, the sizes of the nuclei vary, and the chromatin is unevenly distributed. The cell indicated by the arrow shows a protruding nucleolus (Papanicolaou stain, ×400). D. CB preparation showing mild nuclear atypia, a disordered arrangement, and abnormal polarity. These tumor cells are arranged in a glandular pattern (H&E stain, ×100); inset (H&E stain, ×400). E. The tumor cells were strongly positive for CA19-9 immunostaining (×100). F. Postoperative pathology of the pancreas: Infiltrating growth of tumor cells, fibrous collagen hyperplasia around the tumor and mucous secretion (H&E stain, ×100).

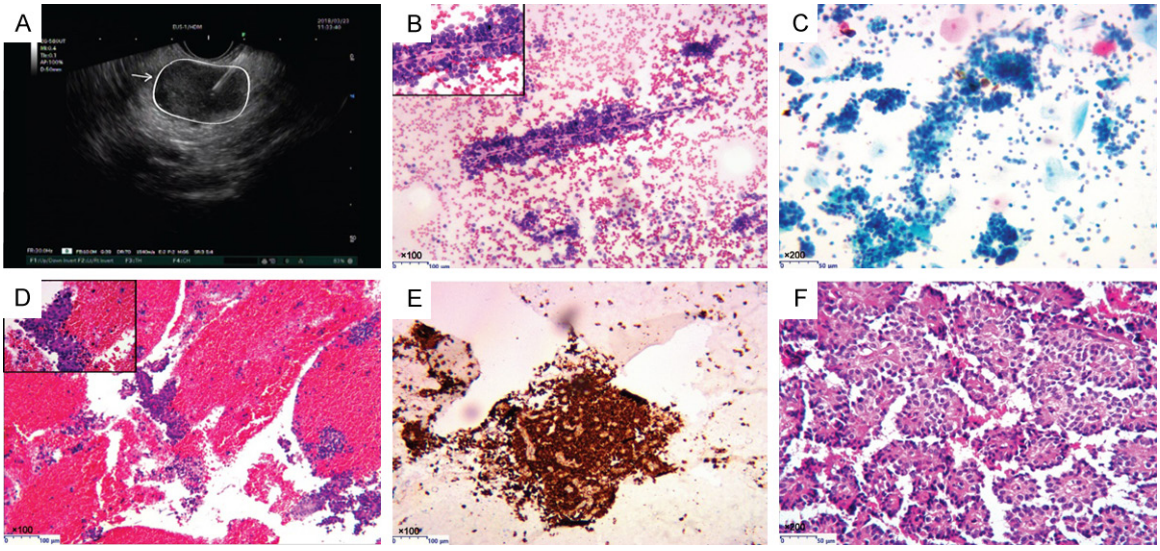

Figure 3. Pancreatic solid pseudopapillary tumor. A. EUS: Solid lesion located in the pancreatic body was approximately 18*25 mm in size with no blood flow signal, and the lesion was clearly delineated from the surrounding pancreas. B. Smear cytology preparation showing the tumor cells growing around the axis of the blood vessel in the blood background. The morphology of the tumor cells is small and consistent (H&E stain, ×100); inset (×400). C. LBC preparation showing the growth of tumor cell clusters around blood vessels. Some tumor cells are scattered after shedding from the peripheral blood vessels (Papanicolaou stain, ×200). D. CB preparation showing typical branched papillary structures and tumor cells growing around the vascular axis (H&E staining, ×100); inset (H&E stain, ×400). E. The cytoplasm and nucleus of the tumor cells were positive for β-catenin immunostain (×100). F. Postoperative pathology of the pancreas: Multiple blood vessel cross-sections with thickened walls suggest transparent degeneration. The surface of the blood vessel adheres loosely to tumor cells of the same size and shape. These cells are slightly to moderately atypical and present as papillary groups (H&E stain, ×200).

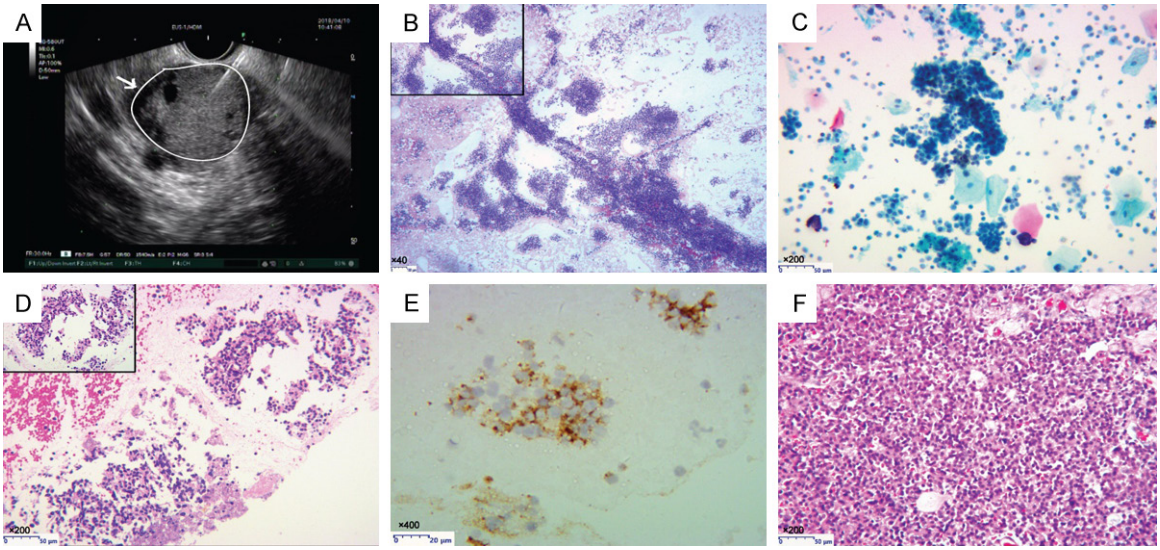

Figure 4. Pancreatic neuroendocrine tumor. A. EUS: Round solid space-occupying lesion at the tail of the pancreas with a clear boundary and a size of 25*26 mm. B. The smear cytology preparation shows abundant tumor cells. The trailing phenomenon is observed because the specimens are pulled by hand smears (H&E stain, ×40); inset (H&E stain, ×100). C. LBC preparation showing abundant cells loosely arranged in clusters. The tumor cells are slightly to moderately atypical with branched papillary structures (Papanicolaou stain, ×200). D. The CB preparation shows that the tumor cells are arranged in a sieve-like pattern with a scattered distribution, and a few cells are arranged in an acinar pattern. The cells are small and morphologically identical, with slight atypia (H&E stain, ×200); inset (×400). E. The cytoplasm of tumor cells was positive for CgA after immunostaining (×400). F. Postoperative pathology of the pancreas: The tumor cells are small and morphologically identical, with slight atypia, and abundant capillaries are interspersed between the cells. The cytoplasm of the tumor cells is abundant and eosinophilic (H&E stain, ×200).

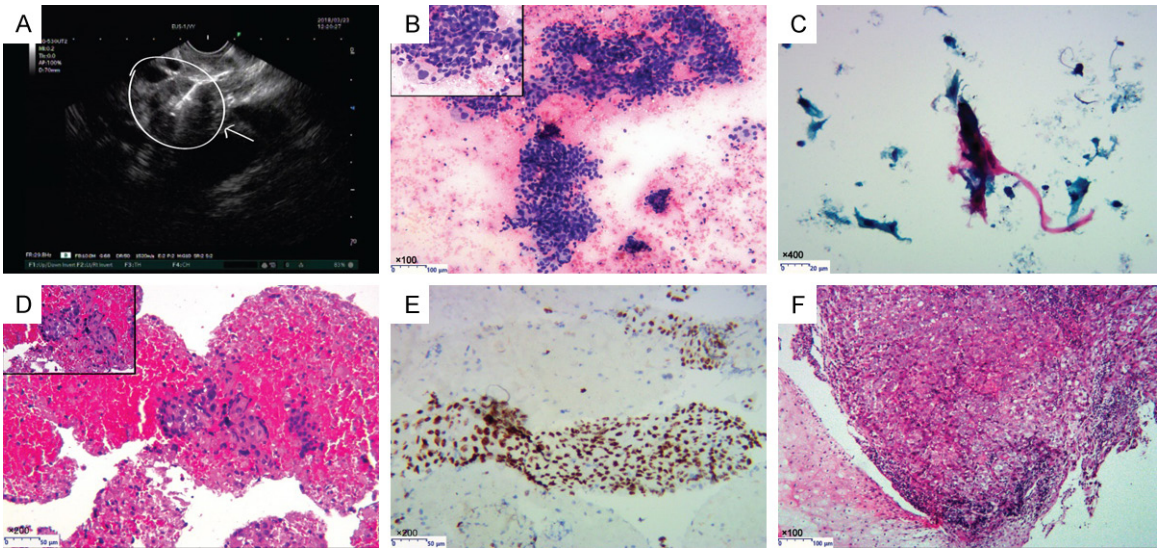

Figure 5. Esophageal squamous cell carcinoma with lymph node metastasis. A. EUS shows that the retroperitoneal lymph nodes are swollen, fused, and surround the truncus coeliacus and branched blood vessels. B. The smear cytology preparation shows the patchy distribution of tumor cells. Obvious atypia and mitotic activity are detected. A small number of lymphocytes is observed in the background (H&E stain, ×100); inset (×400). C. The LBC preparation shows obvious atypia of the tumor cells, deep staining of the nuclei and keratinized tumor cells (Papanicolaou stain, ×400). D. The CB preparation shows obvious atypia in tumor cells with a patchy distribution, and the karyoplasmic ratio of the cells is elevated (H&E stain, ×200); inset (×400). E. The nuclei of tumor cells were positive for p40 immunostaining (×200). F. Postoperative pathology of the esophagus shows poorly differentiated squamous cell carcinoma. The tumor cells display a patchy distribution, and normal squamous epithelial cells are observed on the left (H&E stain, ×100).

19]. For small hospitals without ROSE, a reasonable alternative is the combination of various pathologic examinations. Therefore, the next choice is LBC. LBC is part of exfoliative cytology. This approach is intended to place specimens that are difficult to handle using the traditional method into an intermediate liquid to remove the interfering components that affect the diagnosis, such as blood and mucus. Initially, the diagnostic value of cervical cytology using LBC for uterine cervical cancer was established worldwide [20, 21]. Currently, LBC has been applied for the effusion of the serous cavity, puncture cytology, and other processes. The diagnostic accuracy, sensitivity and specificity of LBC in this study were 89.2%, 86.8% and 100%, respectively. Compared with CS, the greatest advantage of LBC is that it significantly increases the specimen adequacy by reducing the number of inadequate diagnoses due to ambiguities caused by inflammation, blood contamination, and poor fixation. Consequently, LBC serves as a good complement to cytology, particularly when the aspirates are substantially affected by blood contamination due to a large number of needle passes [8, 22, 23]. Unfortunately, LBC changes the morphology and arrangement of the cells in the specimens, causing malignant cells to disperse and vanish into the carcinomatous background. Furthermore, with LBC, the nature and source of borderline lesions and suspected lesions are difficult to determine. Therefore, other techniques are needed to analyse these specimens.

CB combined with IHC can substantially compensate for this defect and not only distinguish the benign or malignant nature of the lesion but also help to detect non-morphologic markers that distinguish the specific type of tumours and guide the next clinical treatment [24]. In patients with suspected neuroendocrine tumors, the metastasis of unknown primary, mesenchymal neoplasms, lymphoproliferative disorders, or any other unusual cases, IHC plays an important role in determining the histologic type and source of the tumor [13, 25]. For example, many similarities in morphology and immunophenotypes have been identified between pancreatic solid pseudopapillary tumors and neuroendocrine tumors involved in the present study, but the nuclei of solid pseudopapillary tumor cells show almost 100% positive β-catenin immunostaining (**Figure 3E**). This finding is the most important distinguishing feature between these diseases. Due to these advantages, this combination has attracted the attention of many researchers. As shown in the study by Noda et al. [26], CB combined with IHC improves the final diagnostic efficacy of EUS-FNA. According to Qin et al. [27], CB combined with IHC for EUS-FNA specimens leads to a higher diagnostic efficacy for pancreatic lesions than CS and LBC. In our study, the diagnostic accuracy, sensitivity and specificity of CB combined with IHC were 91.9%, 90.4% and 100%, respectively. Three cases could not be successfully diagnosed. The reasons determined by a retrospective analysis of these failures were as follows: the low number of specimens, and the mixing of the specimens with a large amount of blood or necrotic tissue. We should increase the number of needle passes and change the puncture angle to improve this method.

Histology is used less frequently than cytology in assessing EUS-FNA specimens because of the difficulty in obtaining high-quality and high-volume tissue. With the development of medical equipment, Giovannini et al. [28] reported that satisfactory histological specimens were obtained using 22-gauge EUS histology needles. In the present study, EUS-FNA was also performed with a 22-gauge needle, but 13 lesions could not be analysed using histopathology because the quality or volume of tissues was unsatisfactory. The final diagnostic accuracy, sensitivity, and specificity of histopathology were 48.1%, 37.2%, and 100%, respectively. The diagnostic accuracy and sensitivity were significantly lower than the other three cytologic methods, which was closely related to the puncture procedure. First, some tumor cells are hidden in fragments of specimens, and the tissue strip sent for examination is actually bleeding and necrotic tissue. Second, the specimens are easily broken and thus positive cells remain in the cell preservation solution. Therefore, performing a pathologic diagnosis with EUS-FNA primarily depends cytological methods, and histopathology can be a supplemental method when the amount of tissue obtained from the puncture is sufficient.

Although EUS-FNA specimens should be analyzed initially using cytologic methods, histopathology potentially plays an important role. In

the present study, one pancreatic space-occupying lesion did not show a sufficiently high number of cells in CS and LBC. Although cell atypia was observed, this finding was not sufficient to diagnose the malignant tumor. Nevertheless, CB and histology clearly suggested squamous cell carcinoma, and this patient was ultimately diagnosed with metastatic pancreatic carcinoma.

In this study, we combined four pathologic methods to evaluate the diagnostic yield of EUS-FNA. The final diagnostic yield was as high as 100%, which is higher than the yield for any pathologic method alone. This result confirmed our hypothesis that the combination of cytology and histology contributed additional diagnostic efficacy to EUS-FNA in the solid lesions around the digestive tract. We analyzed the false-negative cases identified using each pathologic method and found that the different pathological methods compensate for one another, substantially improving the overall positive detection rate. The success rate and the diagnostic efficiency of the cytologic method in EUS-FNA specimens were significantly higher than those of the histology. CB combined with IHC can help clinicians distinguish the tissue origin and classify the diagnosis of the lesion.

A weakness of this study was that 77% of the patients lacked surgical pathology samples because the malignant lesions developed distant metastases, and thus there was not the opportunity for surgery. However, we propose that the patients we recruited may be very similar to the patients encountered in routine clinical practice.

In conclusion, EUS-FNA is a minimally invasive, safe, and effective method for diagnosing solid lesions around the digestive tract. The combination of CS, LBC, CB, and histopathology improves the diagnostic accuracy of EUS-FNA for solid lesions in this area. We recommend further multi-center studies to validate the clinical value of different pathologic methods for tissue specimens obtained using EUS-FNA, including but not limited to solid lesions around the digestive tract.

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Disclosure of conflict of interest

None.

Address correspondence to: Longjiang Xu, Department of Pathology, Second Affiliated Hospital of Soochow University, 1055 Sanxiang Road, Suzhou City, Jiangsu Province, China. E-mail: dbyjr0627@163.com; Duanmin Hu, Department of Gastroenterology, Second Affiliated Hospital of Soochow University, 1055 Sanxiang Road, Suzhou City, Jiangsu Province, China. E-mail: huduanmin2020@163.com

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