

## Role of the preoperative usefulness of the pathological diagnosis of pancreatic diseases

Kazuya Matsumoto, Yohei Takeda, Takumi Onoyama, Soichiro Kawata, Hiroki Kurumi, Masaru Ueki, Norimasa Miura, Hajime Isomoto

Kazuya Matsumoto, Yohei Takeda, Takumi Onoyama, Soichiro Kawata, Hiroki Kurumi, Hajime Isomoto, Department of Gastroenterology, Tottori University Hospital, Yonago 683-8504, Japan

Masaru Ueki, Department of Next Generation Advanced Medical Promotion Center, Tottori University Hospital, Yonago 683-8504, Japan

Norimasa Miura, Division of Pharmacotherapeutics, Department of Pathophysiological and Therapeutic Science, Faculty of Medicine, Tottori University, Yonago 683-8504, Japan

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**Correspondence to:** Kazuya Matsumoto, MD, PhD, Department of Gastroenterology, Tottori University Hospital, 86 Nishi-cho, Yonago 683-8504, Japan. [matsumot@med.tottori-u.ac.jp](mailto:matsumot@med.tottori-u.ac.jp)  
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### Abstract

Pancreatic cancer is the fifth leading cause of cancer death and has the lowest survival rate of any solid cancer. Endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) is currently capable of providing a cytopathological diagnosis of pancreatic malignancies with a higher diagnostic power, with a sensitivity and specificity of 85%-89% and 98%-99%, compared to pancreatic juice cytology (PJC), whose sensitivity and specificity are only 33.3%-93% and 83.3%-100%. However, EUS-FNA is not effective in the cases of carcinoma *in situ* and minimally invasive carcinoma because both are undetectable by endoscopic ultrasonography, although PJC is able to detect them. As for the frequency of complications such as post endoscopic retrograde cholangiopancreatography pancreatitis, EUS-FNA is safer than PJC. To diagnose pancreatic cancer appropriately, it is necessary for us to master both procedures so that we can select the best methods of sampling tissues while considering the patient's safety and condition.

**Key words:** Endoscopic ultrasound-guided fine-needle aspiration biopsy; Cytology; Pathology; Pancreatic juice cytology; Pancreatic cancer

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**Core tip:** In the era of cyto-pathological diagnosis of pancreatic cancer, endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) and pancreatic juice cytology (PJC) represent the most promising procedures for diagnosing pancreatic malignancies. However, there haven't been any reports that compared the utilities and faults of these procedures. In this review we have

highlighted the current role of EUS-FNA and PJC in the diagnosis process for pancreatic malignancies.

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## INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) currently ranks fifth when it comes to death involving cancer. It also, when it comes to solid cancers, has the lowest survival rate<sup>[1,2]</sup>. The current survival rate for patients with PDAC after 5 years with the condition is less than 3.5%<sup>[3,4]</sup>. An early diagnosis is crucial to improve the prognosis. However, for a number of reasons, including the inaccessibility of the pancreas and the highly malignant property of the disease, an early diagnosis is still difficult to obtain, despite the constant improvements in diagnostic imaging. Furthermore, it is especially difficult to distinguish between a PDAC and a pancreatic inflammatory lesion, which includes chronic pancreatitis (CP), or a benign stricture of the main pancreatic duct (MPD), and between intra-ductal papillary mucinous carcinoma IPMC and intra-ductal papillary mucinous neoplasm (IPMN). Being able to differentiate PDAC from other conditions is important, because not only are the treatments for each of these conditions different, but the prognosis for CP and other rare tumors is better than that for PDAC. A cyto-pathological diagnosis is desirable before beginning therapy in cases in which a qualitative diagnosis for the pancreatic mass by various imaging studies is not possible. In fact, 5%-10% underwent pancreatoduodenectomy based on a diagnosis that was made before surgery. However, after performing surgery of the primary pancreatic or periampullary malignancy, it is later proven histopathologically to be CP, a benign fibrous common bile duct stricture or so on<sup>[5-7]</sup>. After performing endoscopic ultrasound-guided fine-needle aspiration biopsy (EUS-FNA) for a pancreatic mass, the frequency of a PDAC does not reach 80%<sup>[8,9]</sup>, which is very important because the treatment strategy for a resection case and an unresectable case are different between PDAC and pancreatic neuroendocrine tumor cases<sup>[10-14]</sup>.

There are many diagnostic procedures in cytopathological treatment including, abdominal ultrasound guided fine-needle aspiration biopsy, computed tomography guided fine-needle aspiration biopsy, EUS-FNA, pancreatic juice cytology (PJC), and Endoscopic pancreatography guided biopsy. I will give an outline mainly on EUS-FNA and PJC in this review.

## PROCEDURE OF AN EUS-FNA

Vilmann *et al.*<sup>[15]</sup> was the first person to describe the EUS-FNA of a pancreatic mass in 1992. These days, EUS-FNA is the preferred method to sample pancreatic mass lesions, replacing for the most part other methods because EUS-FNA is considered the best diagnostic modality for pancreatic masses with a higher accuracy than that of biopsies under CT or US guidance.

There is a door knocking method and a fanning method in EUS-FNA. The door knocking method is a nice procedure that is useful in obtaining a specimen from a mass, especially one with fibrotic tissue, and, as for the fanning method, the utility is proved by RCT<sup>[16]</sup>.

FNA needles, which are available in sizes from 19 to 25 gauge (G), are available commercially. A recent meta-analysis suggests that a 22-G and a 25-G needle have a similar specificity rate after being used with 1292 patients being diagnosed with pancreatic malignancies<sup>[17]</sup>. The same study showed that the 25-G needle did appear to have a higher sensitivity when compared to the 22-G needle. Another study found that 25-G needles seemed to be more advantageous over the 22-G needles when it comes to the adequacy of passes. No difference in accuracy, number of passes or complications was found<sup>[18]</sup>. However, 25-G needles should be considered first in cases in which one must sample from the pancreatic head or uncinated process lesions, as in some studies it has appeared that the 25-G needle has a reduced chance of experiencing technical failures over 22-G needles in such situations<sup>[19,20]</sup>. 19-G needles, on the other hand, are not often used in the duodenum because of their natural rigidity. However, recently, a more flexible needle has been made of nitinol to improve its ability to function well (Flex 19, Boston Scientific, Natick, MA). An initial study using these new and improved needles included 38 patients. Thirty-two of the 38 patients had pancreatic head/uncinate lesions. The use of the needles provided adequate samples for cytological analysis in all 32 patients. There were no reported technical failures or procedure related complications<sup>[21]</sup>. Ramesh *et al.*<sup>[22]</sup> reported that there is no significant difference in the performance of flexible 19-G and 25-G needles although the procurement of histological core tissue with the flexible 19-G needles was significantly higher (88% vs 44%,  $P < 0.001$ ).

As for aspiration, there is a report that compared non-aspiration, aspiration of 10 mL, the aspiration of the slow pull method, and 10-20 mL, but a constant opinion was not obtained from the sampling rate about accuracy<sup>[23-27]</sup>.

EUS-FNA accuracy is also impacted by the skill level and whether or not a cytopathologist is available<sup>[28-30]</sup>. It has recently been shown, in a meta-analysis that covered 34 studies, that rapid on-site evaluation had a significant determinant on the accuracy of EUS-FNA when it comes to the diagnosis of pancreatic masses<sup>[28]</sup>. Two studies have evaluated the optimal number of EUS-

FNA passes<sup>[29,31]</sup> to be 5-7 passes for pancreatic masses in order to get the best diagnostic yield. For situations in which rapid pathology interpretation is not possible, this information may prove to be useful.

It is considered that the white specimens in EUS-FNA samples include histological evidence, and, as for the red specimen, it is thought to be the blood component. When inspected by a 19-G needle, a histologic core was found to be present in white specimens 78.9% of the time, and in red specimens 9.3% of the time<sup>[32]</sup>. It is reported in multiple meta-analysis that ROSE is useful in solving the problem mentioned above<sup>[28,33]</sup>.

Whereas, a meta-analysis suggests 25-G needles have a higher sensitivity rate than 22-G needles when it comes to diagnosing pancreatic malignancy<sup>[17]</sup>, it is expected in the future that EUS-FNA by using a 25-G needle will become more mainstream because of the ease of its puncture. At that time, reexamination re-examination may be required if it is necessary to perform immunohistochemical staining after performing ROSE, due to the smaller sample size meaning a decreased chance of there being a histologic core in the sample. Furthermore, there is a fundamental problem in that globally, there are not enough pathologists capable of performing ROSE.

We developed the target sample check illuminator (TSCI) to be a device that would solve the above problem<sup>[34]</sup>. The mean number of needle punctures was 2.4 (range, 1-5), and the agreement rate between TSCI and histopathology in 142 samples was 93.7% (133/142). No differences in detection capacity were observed in cancerous or non-cancerous lesions. When presence of the target specimen was confirmed by TSCI, 91.4% (53/58) of the patients were able to finish the tests, and the mean number of needle punctures was 1.2 (67/58).

## DIAGNOSTIC POWER OF EUS-FNA

Two recent studies reported a sensitivity of 85% and 89% based on cytology for the diagnosis of malignancy. The specificity for the same was found to be 98% and 99% respectively<sup>[28,35]</sup>.

It is useful in the improvement of the diagnostic ability of EUS-FNA to use a genetic analysis from EUS-FNA samples. Recent meta-analysis reported that combining K-ras mutation analysis with routine cytology moderately improves the ability of EUS-FNA to differentially diagnose between PDAC and pancreatic inflammatory masses. In a total of eight studies, with 696 cases of PDAC and 138 cases of pancreatic inflammatory masses, the pooled sensitivity, specificity, positive likely ratio and negative likely ratio of K-ras mutation analysis combined with cytopathology for diagnosis of PDAC vs pancreatic inflammatory masses were 90%, 95%, 13.45, and 0.13, respectively. Especially, among total 123 patients whose EUS-FNA results were inconclusive or negative, fifty-nine

had K-ras mutations and were finally diagnosed with PDAC (48%, 59/123)<sup>[36]</sup>. In addition, there are several possible means of processing aspirated samples obtained by EUS-FNA for molecular and other ancillary tests<sup>[37]</sup>.

## COMPLICATION WITH EUS-FNA

Complications from EUS-FNA include pain, bleeding, fever, and infection. Rare complications such as, acute portal vein thrombosis<sup>[38]</sup>, peritoneal seeding of tumor cells<sup>[39]</sup>, and ruptured pseudoaneurysm of the splenic artery<sup>[40]</sup> have also been reported. A recent systematic review by Wang *et al.*<sup>[41]</sup>, who identified 51 articles with a total of 10941 patients, reported that the mortality rate attributable to EUS-FNA-specific morbidity was 0.02% (2/10941) and that out of 8246 patients with pancreatic lesions only 60 (0.82%) patients reported any complications. About 36/8246 patients had pancreatitis. Of those patients, 75% of the cases were mild. Out of the total number of patients, one of them with severe pancreatitis died. The total rates of pain, bleeding, fever and infection were 0.38%, 0.10%, 0.08% and 0.02% respectively. Two point two percent of patients were reported to have peritoneal seeding of tumor cells after receiving EUS-FNA. However, it seems to be lower than that caused by CT-guided FNA (16.3%)<sup>[42]</sup>. There was no increase in the risk of peritoneal carcinomatosis in pancreatic masses to be found<sup>[43]</sup>. Beane *et al.*<sup>[44]</sup> found there to be no difference in the survival rate of patients with PDAC who underwent EUS-FNA than with those who did not. Not only was there no difference, but a recent study that looked at the risk of gastric/peritoneal recurrence in cases were EUS-FNA was performed found EUS-FNA was not associated with increased needle track seeding<sup>[45]</sup>. Furthermore, preoperative EUS-FNA was evaluated in 498 patients, and it was found that it had no negative effect on the survival rate of patients with resected pancreatic cancer<sup>[46]</sup>.

## LIMITATION FOR EUS-FNA

Even though EUS-FNA has an excellent accuracy and a low incidence of major complications, it does have several limitations. We cannot perform EUS-FNA when we cannot detect a tumor in EUS. Actually, we cannot identify the carcinoma *in situ* (CIS) in EUS<sup>[47]</sup>. Secondly, even though EUS-FNA has a very high sensitivity rate, when it comes to pancreatic tumors, its negative predictive value is only 55%-65%<sup>[35,48]</sup>. As such, EUS-FNA does not allow us to rule out the possibility of a malignancy. Third, if the patient has CP the diagnostic accuracy of EUS-FNA decreases<sup>[49,50]</sup>. It might also hinder cytological interpretation of pancreatic FNA, thus giving EUS-FNA a decreased sensitivity<sup>[51]</sup>. Fourth, EUS-FNA for pancreatic cancer has a false-positive rate of 1.1%, usually in patients with CP<sup>[52]</sup>. Fifth, we may not be able to perform EUS-FNA when we cannot

discontinue the use of an antithrombotic drug.

## PROCEDURE, DIAGNOSTIC POWER, AND COMPLICATION OF PJC

McCune *et al.*<sup>[53]</sup> developed the ERCP process in 1968. As for the sampling of the pancreas lesion, Endo was the first person to perform a collection of pancreatic juice under the ERP<sup>[54]</sup>. The process of PJC is used in all of the following procedures: Brushing cytology, cytodiagnosis with pancreatic duct lavage fluid (PDLF), cytodiagnosis by using endoscopic nasopancreatic drainage (ENPD), and cytodiagnosis by using secretin. Now I will present the methods, diagnosis results, and complications of each procedure.

### Brushing cytology

A cytopathological diagnosis by using brushing cytology is easier than conventional aspiration cytology because it can collect fresh cells.

However, the sensitivity (33.3%-65.8%) and the accuracy (46.7%-76.4%) are not so good because it is difficult to perform and collect enough cells<sup>[55,56]</sup>. Recently, scraping cytology with a guide-wire yielded 71.4%-93% sensitivity, 100% specificity, 100% positive predictive value, 75%-84.4% negative predictive value, and 88.8%-94% accuracy<sup>[8,57]</sup>.

However, this diagnosis rate is shown to improve by mastering the procedure<sup>[56]</sup>.

When we diagnose a CIS by PJC for a pancreatic duct stenosis case, when we are unable to see the pancreatic mass in imaging studies, and resected it, it is usually the case that there is no cancer at the site of stenosis in the MPD. The stenosis is caused by inflammation due to a CIS, which was derived from a branch duct. For this reason, the diagnostic power of brushing cytology is uncertain. As for the complications rate of brush cytology, it has been reported that acute pancreatitis is a possible complication with a rate of 4.2%-33.3%<sup>[8,55-57]</sup>.

### ENPD method

The ENPD method places 5 or 6-French ENPD tubes in the patient for up to 2-3 d<sup>[58,59]</sup>. Iiboshi *et al.*<sup>[58]</sup> diagnosed 15 CIS using this method. Sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of the ENPD method for pancreatic cancer were 80%-100%, 83.3%-100%, 93.3%-100%, 71%-100%, and 87%-95%, respectively, revealing significantly higher sensitivity than the conventional method ( $P = 0.0001$ )<sup>[58]</sup>. As for the complications of the ENPD method, post endoscopic pancreatitis (PEP) has a rate of 7.5%. In particular, the incidence rate of PEP of ENPD method for BD-IPMN is higher than the conventional method<sup>[60]</sup>.

### Cytodiagnosis by using secretin

Due to the fact that secretin stimulates pancreatic exocrine function, we are able to obtain more pancreatic

juice when secretin is present than without it. Finally, we can obtain pancreatic epithelial cells by using secretin. Administration of secretin was performed conventionally before collecting pancreatic juice for cytodiagnosis<sup>[54,60]</sup>. Secretin may be required in cases in which a sufficient amount of material was not able to be obtained by conventional methods or it may be needed to aspirate mucous fluid in intraductal papillary mucinous neoplasm<sup>[57]</sup>. Nakaizumi reported that the sensitivity for PDAC was 76% in PJC by using secretin<sup>[61]</sup>. As for the complications of secretin, at the top of the attached document, it shows a rate of 1.9% for nausea, 0.7% for flushing, and 0.5% for stomachache and vomiting<sup>[62]</sup>. We have not experienced any adverse events with the secretin administration. Also, we confirmed that the quantity of pancreatic juice significantly increases even though the secretin load in diluted form is 1/32.

### Cytodiagnosis with pancreatic duct lavage fluid

Imamura's process requires us to inject a saline from injection lumen, before aspirating it by the negative pressure from a guide-wire lumen with a different syringe at the same time by using double or triple-lumen cannule after brushing cytology in ERP. The sensitivity of pancreatic cancer diagnosis by this procedure is 83%, and pancreatitis was not a side-effect due to PDLF<sup>[63]</sup>. We choose to do PJC by using secretin if a catheter is able to pass through the narrow segment of the MPD, and PDLF if the catheter cannot pass.

If secretin is used in cases where a catheter is unable to pass the stenosis of the MPD, the pancreatic ductal pressure in the caudad area past the stenosis increases, and this causes pancreatitis.

## GENETIC ANALYSIS WITH PANCREATIC JUICE

It is useful in the improvement of the diagnostic ability of PJC to use a genetic and molecular analysis from PJC samples in cases in which a small quantity of specimen was obtained from PJC and the adjuvant diagnosis of the cytodiagnosis is negative. In a diagnosis for pancreatic cancer, sensitivity improves by adding the *K-ras* mutation analysis with routine cytology<sup>[64]</sup>. There are some reports about the utilities of telomerase activity<sup>[65]</sup>, DNA methylation<sup>[66]</sup>, Smad4<sup>[67]</sup>, and KL-6<sup>[68,69]</sup> measurement in pancreatic juice.

## LIMITATION OF PJC

First, the accuracy of PJC is generally only around 40%-70%<sup>[55,56]</sup> except in some institutions<sup>[8,57,58]</sup>. Second, we cannot diagnose pancreatic neuroendocrine tumor, solid pseudopapillary neoplasm, or pancreatic acinar cell carcinoma, because they are not connected to the MPD. Third, it is hard to perform immunostaining because it is difficult to obtain a specimen as compared with EUS-FNA. Fourth, around 4.2%-33.3% of complications such as PEP



can occur after PJC<sup>[8,55-57,60]</sup>, but it is reported that the risk decreases for PEP with diclofenac administration. Elmunzer *et al.*<sup>[70]</sup> reported that post-ERCP pancreatitis developed in 27 of 295 patients (9.2%) in the indomethacin group and in 52 of 307 patients (16.9%) in the placebo group ( $P = 0.005$ ). Moderate-to-severe pancreatitis developed in 13 patients (4.4%) in the indomethacin group and in 27 patients (8.8%) in the placebo group ( $P = 0.03$ ).

## USE OF EUS-FNA AND PJC

Generally, EUS-FNA is better in diagnostic ability and adverse events than PJC. Therefore, if we can perform EUS-FNA, we should choose EUS-FNA, and it is desirable to only choose PJC in the following cases: (1) we can not detect a mass in EUS; (2) it is difficult to perform EUS-FNA when avoiding blood vessels and the MPD; (3) it is difficult to stop use of antithrombotic medicine; and (4) there aren't any institutions capable of performing EUS-FNA in the neighborhood.

Furthermore, there are some reports that the diagnostic accuracy of EUS-FNA and/or PJC was significantly higher than that of EUS-FNA or PJC alone<sup>[8,71]</sup>.

In conclusion, although there are some complications such as acute pancreatitis and dissemination, if the frequency of complications and the physical burden of surgery for patients are taken into consideration, it is perhaps better to obtain tissue before treatment begins. Since there are various methods of sampling tissue, it is important to choose the procedure while considering the patient's condition and safety.

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