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

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

Matsumoto K, Takeda Y, Onoyama T, Kawata S, Kurumi H, Ueki M, Miura N, Isomoto H. Role of the preoperative usefulness of the pathological diagnosis of pancreatic diseases. World J Gastrointest Oncol 2016; 8(9): 656-662 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i9/656.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i9.656

PROCEDURE OF AN EUS-FNA

Vilmann et al\(^{15}\) 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\(^{16}\).

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\(^{17}\). 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\(^{18}\). 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\(^{19,20}\). 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/uncinated 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\(^{21}\). Ramesh et al\(^{22}\) 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\(^{23-27}\).

EUS-FNA accuracy is also impacted by the skill level and whether or not a cytopathologist is available\(^{28-30}\). 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\(^{30}\). Two studies have evaluated the optimal number of EUS-
FNA passes\textsuperscript{[28,31]} 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\textsuperscript{[32]}. It is reported in multiple meta-analysis that ROSE is useful in solving the problem mentioned above\textsuperscript{[28,31]}.

Whereas, a meta-analysis suggests 25-G needles have a higher sensitivity rate than 22-G needles when it comes to diagnosing pancreatic malignancy\textsuperscript{[17]}, 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\textsuperscript{[34]}. 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\textsuperscript{[28,33]}. It is useful in the improvement of the diagnostic ability of EUS-FNA to use a genetic analysis from EUS-FNA results were inconclusive or negative, fifty-nine had K-ras mutations and were finally diagnosed with PDAC (48%, 59/123)\textsuperscript{[46]}. In addition, there are several possible means of processing aspirated samples obtained by EUS-FNA for molecular and other ancillary tests\textsuperscript{[47]}. Complications from EUS-FNA include pain, bleeding, fever, and infection. Rare complications such as, acute portal vein thrombosis\textsuperscript{[38]}, peritoneal seeding of tumor cells\textsuperscript{[39]}, and ruptured pseudoaneurysm of the splenic artery\textsuperscript{[40]} have also been reported. A recent systematic review by Wang et al\textsuperscript{[41]}, 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%)\textsuperscript{[42]}. There was no increase in the risk of peritoneal carcinomatosis in pancreatic masses to be found\textsuperscript{[43]}. Beane et al\textsuperscript{[44]} 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\textsuperscript{[45]}. 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\textsuperscript{[46]}

**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\textsuperscript{[47]}. Secondly, even though EUS-FNA has a very high sensitivity rate, when in comes to pancreatic tumors, its negative predictive value is only 55%–65%\textsuperscript{[35,48]}. 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\textsuperscript{[49,50]}. It might also hinder cytological interpretation of pancreatic FNA, thus giving EUS-FNA a decreased sensitivity\textsuperscript{[51]}. Fourth, EUS-FNA for pancreatic cancer has a false-positive rate of 1.1%, usually in patients with CP\textsuperscript{[52]}. 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.*\(^{53}\) 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\(^{54}\). 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\(^{55,56}\). 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\(^{6,57}\).

However, this diagnosis rate is shown to improve by mastering the procedure\(^{56}\).

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%\(^{8,55-57}\).

**ENPD method**

The ENPD method places 5 or 6-French ENPD tubes in the patient for up to 2-3\(^{58,59}\). Iiboshi *et al.*\(^{58}\) 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 \((P = 0.0001)\)^{56}. As for the complications of the ENPD method, 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\(^{60}\).

**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\(^{54,60}\). 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 mucus fluid in intraductal papillary mucinous neoplasms\(^{57}\). Nakaizumi reported that the sensitivity for PDAC was 76% in PJC by using secretin\(^{61}\). 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\(^{62}\). 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\(^{63}\). 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 stenos 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\(^{64}\). There are some reports about the utilities of telomerase activity\(^{65}\), DNA methylation\(^{66}\), Smad4\(^{67}\), and KL-6\(^{68,69}\) measurement in pancreatic juice.

**LIMITATION OF PJC**

First, the accuracy of PJC is generally only around 40%-70%\(^{55,56}\) except in some institutions\(^{5,57,58}\). 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
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 cannot 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[6,7,11].

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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