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New strategy for evaluating pancreatic tissue specimens from endoscopic ultrasound-guided fine needle aspiration and surgery

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Abstract

Background and Aim: Preoperative histological evaluation of pancreatic neoplasms is important for guiding the resection strategy and preventing postoperative adverse events. However, conventional endoscopic methods have technical limitations that reduce the accuracy of the histopathological examination. Probe electrospray ionization mass spectrometry (PESI-MS) may be a useful technique for rapidly evaluating small specimens.
Methods: This single-center prospective study included patients with pancreatic neoplasms between October 2018 and December 2019. Pancreatic ductal adenocarcinoma (PDAC) specimens were obtained via endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) and non-neoplastic tissue was obtained via surgery. Specimens were subjected to PESI-MS and the mass spectra were analyzed using partial least squares regression-discriminant analysis.
Results: The study included 40 patients with 20 nonneoplastic specimens and 19 PDAC specimens (1 case of neuroendocrine carcinoma was omitted). All nonneoplastic specimens were sufficient for PESI-MS analysis, although only 7 of 19 PDAC specimens were sufficient for PESI-MS analysis because of poor sample quality or insufficient quantity (<1 mg). Among the 27 analyzed cases, the mass spectra clearly differentiated between the PDAC and nonneoplastic specimens.
Conclusions: This study revealed that PESI-MS could differentiate between PDAC and nonneoplastic specimens, even in cases where EUS-FNA produced very small specimens.

Introduction

Early diagnosis of pancreatic cancer is difficult and almost 80% of cases are diagnosed at stage IV disease. Thus, the prognosis of pancreatic cancer is very poor, with a 5-year overall survival rate of only 2.7%.[1] However, the 5-year overall survival rates improve to 50.0% if the tumor is detected at a diameter of 10–20 mm and 80.4% if the tumor is detected at a diameter of <10 mm.[1] Therefore, given the challenges or even impossibility of surgical intervention for advanced pancreatic cancer, it is critical to identify pancreatic tumors at an early stage. However, early diagnosis is often extremely difficult because computed tomography and magnetic resonance imaging cannot accurately detect or guide the differential diagnosis of pancreatic lesions.

Another challenge is the differentiation between pancreatic cancer and other pancreatic diseases, such as cystic tumors, autoimmune pancreatitis, and neuroendocrine neoplasms. Thus, a cytohistological evaluation is strongly recommended for diagnosing pancreatic cancer, which typically involves endoscopic techniques, such as endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). However, these techniques do not always provide a sufficient specimen quantity and quality to support an accurate diagnosis. Furthermore, there may be questions regarding whether the specimen was collected at the precise target location. Rapid intraoperative evaluations of the surgical margin are also extremely important to ensure complete resection and prevent recurrence. Unfortunately, intraoperative techniques, such as frozen sections, are usually time-consuming and provide lower-quality specimens than paraffin-embedded preparations. Thus, alternative methods are needed to rapidly and accurately diagnose pancreatic cancer, and it would be very useful if these methods were compatible with small specimens.

Probe electrospray ionization mass spectrometry (PESI-MS) is a derivative of the electrospray ionization (ESI) technique, but provides advantages in terms of ease of handling, compatibility with small samples, and rapid analytical procedures. Previous studies have indicated that PESI-MS was useful for diagnosing clinical specimens from human neoplasms, such as hepatocellular carcinoma and renal cell carcinoma.[2,3] Moreover, a recent study revealed that using PESI-MS to evaluate serum from patients with pancreatic cancer provided greater specificity and sensitivity, relative to CA19-9 testing.[4] Therefore, this technique might be useful for rapid on-site diagnosis of pancreatic cancer using EUS-FNA or surgical specimens. This study aimed to evaluate whether PESI-MS could differentiate between pancreatic ductal adenocarcinoma (PDAC) and nonneoplastic tissues, and whether the PESI-MS results were comparable to the findings from a traditional histopathological examination.

Methods

Study design. This is a prospective, pilot study. The patients aged older than 20 years who underwent EUS-FNA or surgery for pancreatic neoplasms at a university hospital between October 2018 and December 2019 were included after obtaining informed consent. The study procedures complied with the 2008 revision of the Declaration of Helsinki. The study protocol was approved by the institutional review board (20130330) and all patients provided their informed consent. Data were collected regarding age, sex, tumor size, tumor location, and histological findings for PDAC and normal specimens. The study procedures are summarized in Figure 1.

EUS-FNA procedure. The EUS-FNA procedure was performed to diagnose pancreatic lesions using a convex-type endoscope (GF-UCT260; Olympus Medical Systems, Tokyo, Japan) under conscious sedation with benzodiazepine (0.1 mg of flunitrazepam or 2.5 mg of midazolam) and pethidine (35 mg). Puncture routes were determined based on the tumor location, with duodenal puncture used for pancreatic head tumors and gastric puncture used for pancreatic body and tail tumors. All procedures were performed using a 22-G needle (Acquire; Boston Scientific, Natick, MA, USA, or EZ shot3; Olympus Medical Systems) and approximately 30 strokes using the suction or slow-pull techniques. The maximum number of puncture attempts was 5, which was based on the total amount of obtained tissues. The collected specimens were subjected to general pathological examinations and only the remaining tissue was used for the present study. The remaining tissue was temporarily placed in saline solution, washed using phosphate-buffered saline (PBS), and then stored at −30°C.

Collection of surgical tissue. A specimen of normal tissue was also collected from patients who underwent surgery for pancreatic neoplasms. After resection of the pancreatic neoplasm, a specimen of normal tissue (approximately 5 mm × 5 mm × 5 mm) was obtained from the edge of the resected specimen. The normal tissue was then temporarily placed in saline, washed using PBS, and stored at −30°C.

PESI-MS procedure. The samples were prepared for PESI-MS as previously described.[2] Defrosted tissues were rinsed by PBS and gently wiped with BEMCOT paper (AsahiKASEI,
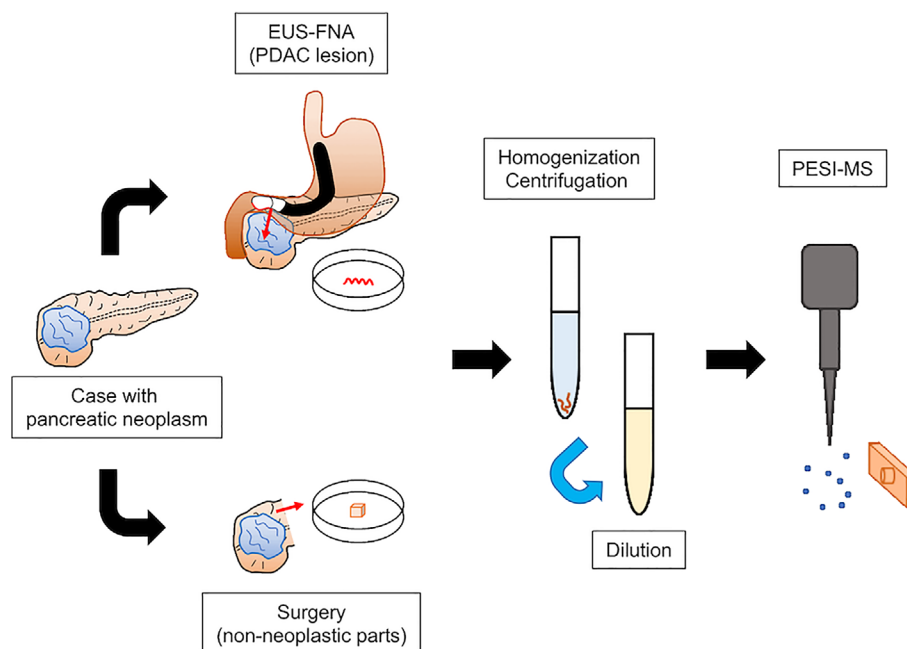

Figure 1 Study procedures. Samples of pancreatic ductal adenocarcinoma (PDAC) were obtained via endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) and samples of nonneoplastic tissue were obtained via surgery. The specimens were homogenized, centrifuged, and diluted before being subjected to probe electrospray ionization mass spectrometry (PESI-MS).

Tokyo, Japan) to remove the superfluous PBS. The tissues were then weighed with UniBloc analytical balance AUW-D (Shimadzu Corp., Kyoto, Japan) to trim them to approximately 4 mg for further analysis. Twenty-five μL of 50% ethanol (extraction solvent) were added to the sample per mg and manually homogenized using a pestle (Argos Technologies, Vernon Hills, IL, USA) in a 0.6-mL disposable plastic microtube (Watson, Tokyo, Japan). Defrosted tissues were rinsed using PBS and gently wiped using BEMCOT paper (AsahiKASEI). The tissues were then weighed and an approximately 4-mg sample was removed for analysis. The sample was added to 100 μL of 50% ethanol (extraction solvent) in a 0.5-mL disposable plastic tube and then manually homogenized using a pestle (Argos Technologies). The homogenized sample was centrifuged at $15\,000 \times g$ for 5 min and then the supernatant was removed and diluted four-fold with the extraction solvent. Nine microliters of the resulting solution were placed in a bespoke sample plate (Shimadzu Corp.,). In cases where only very small samples were available (e.g. approximately 1 mg), the volumes were adjusted proportionally. The PESI-MS analysis was performed using a triple quadrupole mass spectrometer (DPiMS-8060; Shimadzu Corp.), which has a PESI ion source that replaces the ESI module of the original LCMS-8060 spectrometer. The mass spectra were acquired for nonneoplastic and PDAC specimens in the positive ion mode, and all subsequent analyses were performed as previously described previously.[5–9]

Statistical analysis. Continuous variables were compared using the nonparametric test and categorical variables were compared using Fisher's exact test. Differences were considered statistically significant at two-sided *P* values of <0.05. These analyses were performed using JMP software (version 15.0 for Mac; SAS Institute, Tokyo, Japan). The spectra obtained via PESI-MS were subjected to partial least square regression-discriminant analysis (PLS-DA) using MetaboAnalyst software (Xia Lab, McGill University).

Results

Patients, specimens, and clinical characteristics. The study enrolled 40 patients with pancreatic neoplasms (Fig. 2). Nonneoplastic specimens were available for 20 patients who underwent surgical treatment and diagnostic specimens from EUS-FNA were obtained from 19 PDAC lesions and 1 neuroendocrine carcinoma (which was omitted from the analysis). All nonneoplastic specimens were evaluable using PESI-MS, although 12 of 19 PDAC specimens could not be analyzed because the total specimen size was <1 mg.

The patients' clinical characteristics are summarized in Table 1. The median ages were 73.5 years (range: 40–85 years) in the nonneoplastic group and 66 years (range: 44–81 years) in the PDAC group (*P* = 0.37). There were no significant intergroup differences in terms of sex (*P* = 0.15) or tumor location (*P* = 0.98). The median tumor sizes of treated or diagnosed pancreatic neoplasm were 28.5 mm (range: 8–74 mm) in the nonneoplastic group and 27 mm (range: 21–68 mm) in the PDAC group (*P* = 0.63).

Minimum EUS-FNA sample size required for PESI-MS analysis. Next, we compared the sample weight of specimens that were and were not evaluable by PESI-MS (Fig. 3). The average sample weights were 14.265 mg (range: 9.59–21.9 mg)
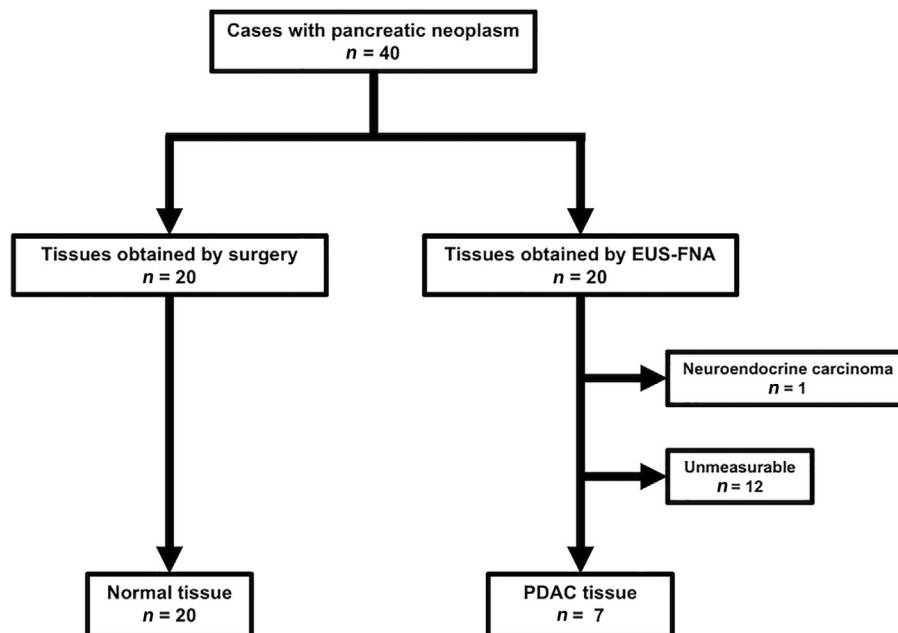

Figure 2 Study flowchart. The study included 20 patients who underwent surgery and 20 patients who underwent endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) for pancreatic neoplasms. A case of neuroendocrine carcinoma was omitted and probe electrospray ionization mass spectrometry (PESI-MS) results were available for 7 of 19 EUS-FNA specimens.

Table 1 Patient and tumor characteristics

	Nonneoplastic specimens ($n = 20$)	PDAC ($n = 7$)	P value
Age in years, median [range]	73.5 [40–85]	66 [44–81]	0.37
Sex			0.15
Male	12	2	
Female	8	5	
Location			0.98
Head	4	2	
Body	9	2	
Tail	7	3	
Tumor size in mm, median [range]	28.5 [8–74]	27 [21–68]	0.63

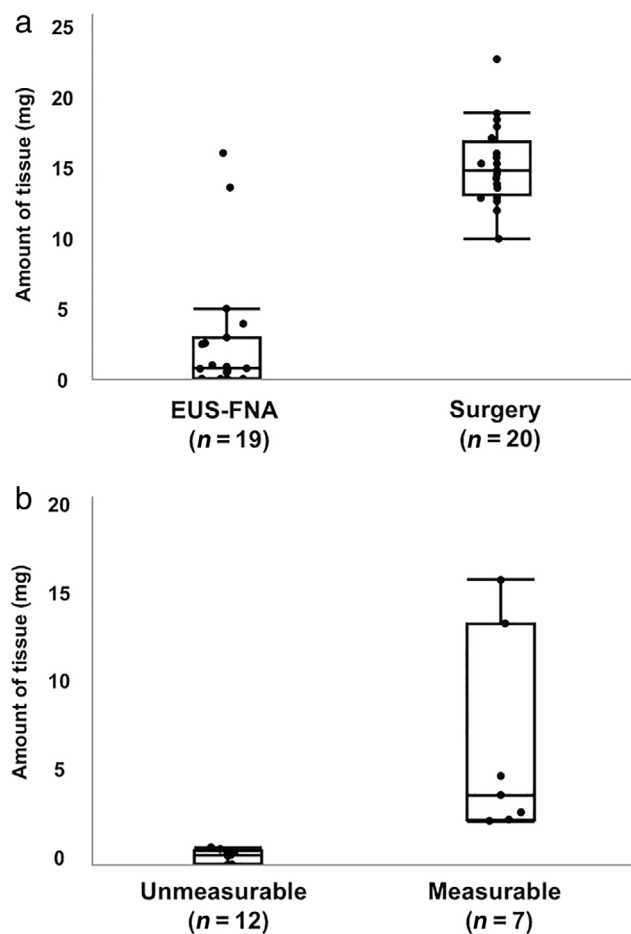PDAC, pancreatic ductal adenocarcinoma.

Figure 3 Tissue amounts for probe electrospray ionization mass spectrometry. (a) Surgery provided a significantly larger amount of tissue, relative to endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) ($P < 0.001$). (b) A significantly larger amount of tissue was obtained for cases in which probe electrospray ionization mass spectrometry was possible ($P < 0.001$).
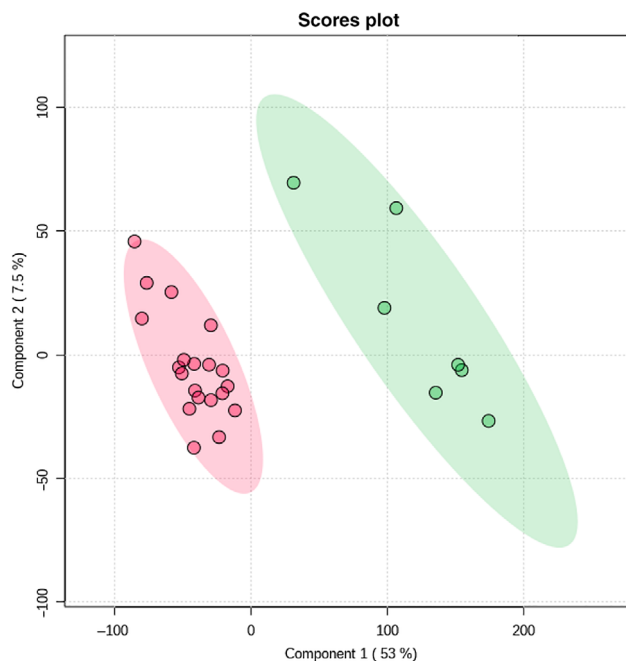

Figure 4 Partial least squares regression-discriminant analysis of spectrums from normal tissue and pancreatic ductal adenocarcinoma. Probe electrospray ionization mass spectrometry was performed for normal and pancreatic ductal adenocarcinoma (PDAC) tissues. The spectrums were analyzed using partial least squares regression-discriminant analysis, which revealed a clear differentiation between the nonneoplastic and PDAC tissues. (○), non-neoplasm; (○), PDAC.

from the surgery group and 0.72 mg (range: 0–15.47 mg) from the EUS-FNA group ($P < 0.001$, Fig. 3a). Most surgical specimens were initially cut using a diameter of approximately 1 cm, which provided sufficient tissue for the PESI-MS analysis, while the specimens in the EUS-FNA group were very limited. The mean sample weight from cases that were evaluable using PESI-MS was 3.77 mg (range: 2.37–15.47 mg), which was significantly heavier than the mean weight from cases that were not evaluable using PESI-MS (mean: 0.475 mg, range: 0–0.93 mg; $P < 0.001$) (Fig. 3b).

The obtained mass spectra are shown in the supplementary files. Relative to nonneoplastic tissue (Figure S1, Supporting information), the PDAC tissues exhibited a cluster of peaks at approximately *m/z* 800–900 and several minor peaks at *m/z* 400 (Figure S2). These results agree with previously reported results.[2,6]

Using PLS-DA to differentiate between PDAC and nonneoplastic tissues.

The acquired spectra were processed as previously reported to evaluate whether they could differentiate between PDAC and nonneoplastic specimens.[4,6] The data were analyzed using PLS-DA, which clearly differentiated between the PDAC and nonneoplastic specimens (Fig. 4). Although only a subset of the EUS-FNA cases were evaluable using PESI-MS (*vs* all cases in the surgery group), the agreement between the EUS-FNA and pathological diagnoses was 100%, which suggests that evaluating EUS-

FNA specimens using PESI-MS is a potentially valuable technique in the clinical setting.

Discussion

This study evaluated a mass spectrometry-based system for diagnosing pancreatic neoplasms, which revealed that the system could easily and rapidly differentiate between neoplastic and nonneoplastic tissues without laborious preparation steps. Thus, we suspect that a PESI-MS-based diagnostic system would be extremely useful for intraoperative assessment of surgical margins or in cases where EUS-FNA re-puncture is required to confirm that the specimen was retrieved from the intended target.

Pathological evaluation of specimens obtained via ERCP or EUS-FNA is often required to diagnose pancreatic cancer, although these techniques do not always provide sufficient specimens for the evaluation. In cases of pancreatic cancer, EUS-FNA has a sensitivity of 85–86.8% and specificity of 95.8–98%, although EUS-FNA fails to identify the pancreatic tumor in 5–15% of cases.[10,11] Moreover, pathological evaluation of EUS-FNA specimens can be limited by a shortage of well-trained pathologists, which may necessitate exploratory laparotomy or laparoscopic resection if malignancy cannot be ruled out.

Our PESI-MS-based strategy can minimize the need for exploratory surgery by allowing a clinical evaluation of even small specimens, such as those obtained via EUS-FNA. Nevertheless, the PESI-MS was only possible for 7 of 19 cases that involved EUS-FNA, which was typically related to collected tissue amounts of <1 mg. In some cases where PESI-MS was impossible, a conventional pathological diagnosis was also impossible due to low amount of tissue such as insufficient puncture of the tumor. However, in such situation, combining our PESI-MS-based method with EUS-FNA might help guide the decision regarding whether to perform additional punctures to reach a definitive diagnosis. It is also worth noting that all surgical specimens were evaluable using PESI-MS, which suggests that it might be a useful technique for intraoperatively evaluating the completeness of tumor resection.

The advantages of our strategy lie in its rapidity, ease of sample handling, and versatility. For example, a diagnosis can be achieved based on a very small specimen (>1 mg/test) without any complicated pretreatment. Furthermore, it only takes approximately 2 min to complete the mass spectrometry analysis, judge the results, and determine whether cancer is present. These advantages highlight the potential for combining endoscopic specimen collection with PESI-MS to provide a rapid on-site diagnostic platform, and we have used this system to differentiate renal cell carcinoma and breast cancer from nonneoplastic specimens.[3,6] In addition, this system can be used to identify PDAC using serum samples, with sensitivity and specificity values of >90% that are even superior to the conventional CA19-9 marker.[4] Thus, we believe that this system has value for diagnosing pancreatic cancer, given the difficulty of collecting a sufficient diagnostic specimen without surgical intervention. This system may also be useful for clinically diagnosing other cancers, including pancreatic, craniofacial, and hepatic cancers.[4,5,12] Finally, it is possible that our system might be useful for predicting outcomes and treatment responses, if the findings can be combined with data regarding pathological findings, TMN staging, chemotherapy outcomes, and prognosis.

This study has several limitations. First, the sample size was too small to construct a database for machine learning. Furthermore, a large proportion of the EUS-FNA cases were not evaluable using PESI-MS and mass spectra could not be generated for those cases. Second, this system still relies on non-automated processes, and further studies are needed to develop a strategy that standardizes and automates sample preparation, PESI-MS analysis, data processing, data transfer, and data interpretation. If these processes can be developed, we believe that our strategy can be readily and effectively implemented in the clinical setting.

In conclusion, a PESI-MS-based strategy could distinguish between PDAC and nonneoplastic tissues, even when using small specimens. This new diagnostic strategy may be useful for preoperative diagnosis of pancreatic lesions and for intraoperative evaluation of surgical margins.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1. The probe electrospray ionization mass spectrometry spectra for non-neoplastic tissues. The spectra for each non-neoplastic tissue are shown with intensity on the Y-axis and *m/z* values on the X-axis.

Figure S2. The probe electrospray ionization mass spectrometry spectra for pancreatic ductal adenocarcinoma tissues. The spectra for each pancreatic ductal adenocarcinoma (PDAC) tissue are shown with intensity on the Y-axis and *m/z* values on the X-axis.