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SurePath® liquid-based cytology in molecular diagnostics of pancreatic ductal adenocarcinoma: a case report and literature review

Relevance: *Pancreatic ductal adenocarcinoma (PDAC) has an aggressively malig-nant nature, and its prognosis remains extremely poor in Kazakhstan as well as worldwide. Currently, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) techniques are widely used as routine clinical practice. However, this tech-nique is not enough to diagnose PDAC for several reasons.*

Purpose of the research: *to evaluate the possibility to use KRAS mutations in SurePath® liquid-based cytology (SP-LBC) specimens obtained by EUS-FNA for PDAC diagnostics.*

Results: *PDAC was diagnosed using SP-LBC slide that contained a few suspicious malignant cells. The presence of a KRAS mutation (G12D) was confirmed by DNA extraction and PCR using SP-LBC and histological samples.*

Conclusion: *KRAS mutation in SP-LBC specimen obtained by EUS-FNA can ac-curately identify PDAC. SP-LBC is a useful technique for collecting high-quality cellular samples for genetic analysis as well as conducting an exploratory evaluation of appropriate molecular diagnostics for PDAC. However, there is a need for more data on the use of SP-LBC in PDAC diagnostics.*

Keywords: *Pancreatic ductal adenocarcinoma; EUS-FNA cytology; KRAS muta-tion; SurePath® based LBC.*

Introduction. Pancreatic ductal adenocarcinoma (PDAC) has an aggressively malignant nature, and its prognosis remains extremely poor in Kazakhstan as well as worldwide [1]. Cytological evaluation by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is used to define an optimal therapeutic strategy [2, 3]. EUS-FNA has replaced conventional diagnostic approaches in many countries. However, in some situations, these approaches do not allow to diagnose PDAC, i.e., when cellular samples have a limited number of cell populations [4].

A semi-automated technique of Liquid-Based Cytology (LBC) has recently gained popularity as a method of collecting and processing both gynecologic and non-gynecologic cellular specimens [5-8]. Although the cost of material (LBC slides) and the preparation time for LBC are bigger than those required for conventional smears, LBC has several advantages like rapid and proper fixation and fewer cases of air-drying of artifacts [9]. Two LBC systems, ThinPrep® based LBC (TP-LBC) and SurePath® based LBC (SP-LBC), are currently used worldwide. These systems have gradually replaced conventional smears as the primary test method in both gynecological and non-gynecological screening programs [10]. LBC preparations are increasingly being used in non-gynecological cytology due to their high cell-recovery rates. Several authors have examined pancreatic cytology using SP-LBC. TP-LBC uses filtration, while SP-LBC uses density gradient centrifugation (cell enrichment) with a sampling device and collection vials for the preparation of the final slide. Therefore SP-LBC provides much higher diagnostic accuracy for cervical glandular neoplasms than TP-LBC [11]. Moreover, residual SP-LBC samples can be used for further immunocytochemistry examination and molecular analysis [12, 13].

In the presented study, we examined Kirsten-ras (*KRAS*) mutations in SP-LBC specimens and checked whether they could be used for PDAC diagnosis.

Materials and methods.

Case information. A 50-year-old man with back pain was referred to the Kurume University Hospital for examination. Abdominal CT has shown a pancreatic tail tumor – a mass 52 32 mm with low-to-high density – and liver metastases (Figure 1A). Serum tumor markers such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were increased (CA19-9 – 2590 U/mL, CEA – 7.0 ng/mL). Endoscopic ultrasonography has revealed a 22-mm mass in the pancreatic tail (Figure 1B). The clinical symptoms, imaging and lab results have shown a suspected case of pancreatic head cancer cT3 cN1 cM1, stage IV. On the basis of SP-LBC treatment of EUS-FNA cellular specimen, the patient was indeterminately diagnosed with PDAC.

SP-LBC protocol. The specimens were stored in vials filled with a preservative solution (CytoRich® Red Preservative, Becton Dickinson). Each vial was centrifuged at 1,500 rpm for 5 min on the day of sampling, and CytoRich® Red was decanted. Then, the vials were centrifuged again with distilled water. After decanting the vials, 0.5 ml of fresh distilled water was added, and the sediment was stirred. A 0.5-ml sample was then dispensed into the settling chamber, and preserved cells were allowed to rest on the slide for at least 10 min. Approximately 1 ml of 95% ethanol was added to the settling chamber. The slide rack was inverted, and excess sample was discarded. The settling chamber was carefully removed, and the LBC slides were immediately fixed in 95% ethanol. After overnight fixation, the slides were finally stained by conventional Papanicolaou staining.

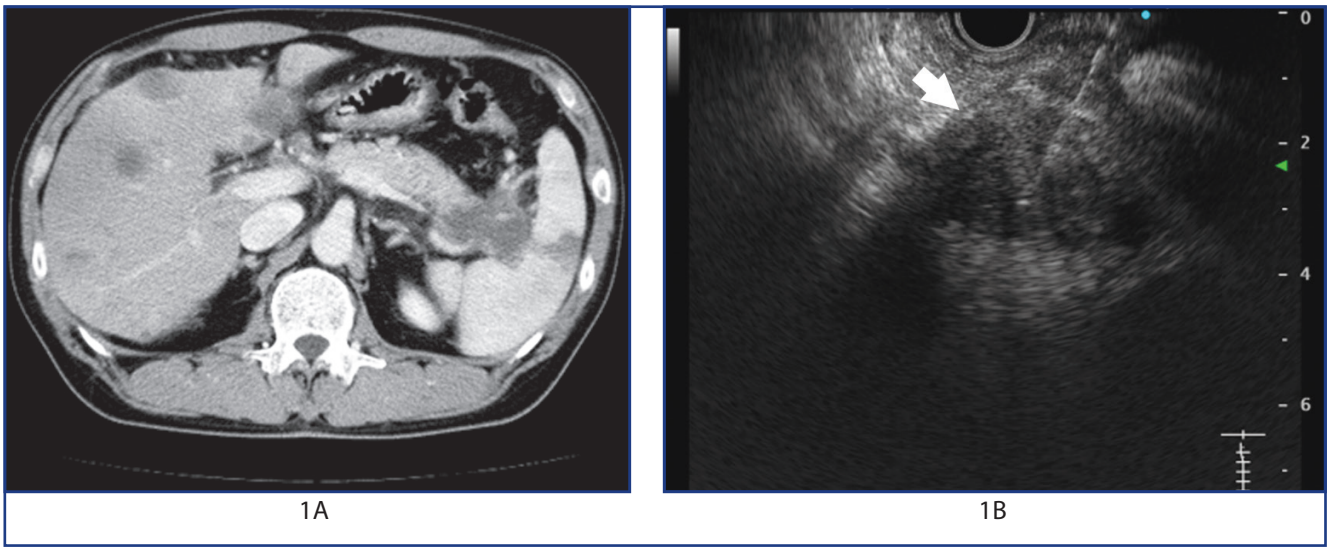

Figure 1 - Imaging of a pancreatic tumor
(A) Pancreatic tail tumor (52 × 32 mm) and liver metastasis detected during abdominal CT. (B) Endoscopic ultrasonography shows a 22-mm mass in the pancreatic tail.

DNA extraction and KRAS mutation analysis. The covered glass of the SP-LBC slide was removed for genetic analysis. Genomic DNA was purified using the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Subsequently, mutations in the *KRAS* codons G12D, G12V, G12C, G12R, G12S, G12A, and G13D in the *KRAS* wild-type gene were examined using fluorescence resonance energy transfer-based preferential homoduplex formation assay (F-PHFA; Riken Genesis Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions.

Tissue specimens were routinely fixed in 10% buffered neutral formalin and embedded in paraffin. Paraffin-embedded tissues were sliced into thin sections of 8-μm thickness, and 2-3 sections were used for DNA extraction using QIAamp DNA FFPE Tissue kit, according to the manufacturer's instructions. *KRAS* mutation analysis was next performed using the same F-PHFA assay, which was used for LBC specimens.

Results.

Cytological and histological findings of pancreatic cancer. Cytological diagnosis by EUS-FNA was "indeterminate"; however, histopathological examination of the tumors proved it to be tubular adenocarcinoma (Figure 2). SP-LBC treated slides from the pancreatic tail tumor revealed several clusters and isolated cells with a necrotic background. Cytological analysis of tumor cells showed small and bland nuclei with abundant cytoplasm. Some of the clusters were composed of cells with enlarged nuclei and prominent nucleoli, and the malignant cells were suspected to be adenocarcinoma cells (Figure 3). Finally, the cytological diagnosis was "indeterminate" for the tumor cells presented on the SP-LBC slide.

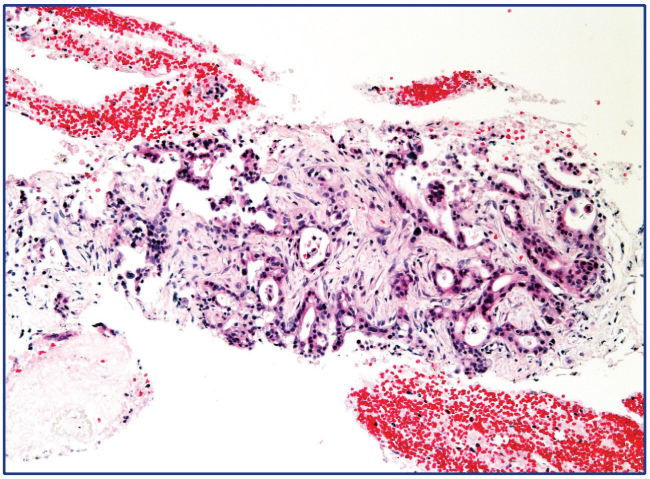

Figure 2 - Histological findings of pancreatic cancer
Well-defined gland formation in a tissue sample from pancreatic tumor adenocarcinoma. HE, 100×.

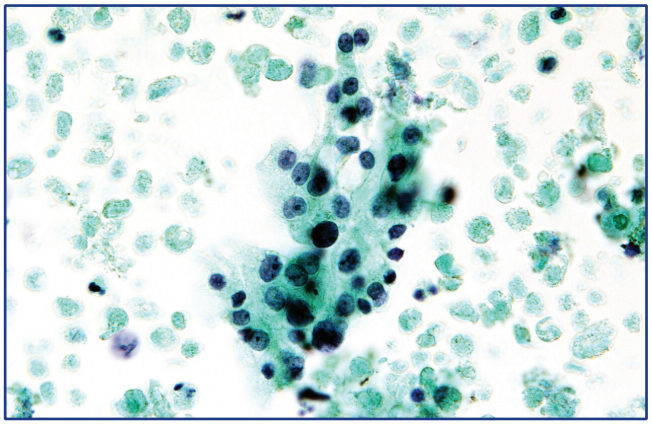

Figure 3 - Cytological findings of tumor cells on SP-LBC slide
Tumor clusters with slightly overlapping or unevenly spaced nuclei. Tumor cells show large and irregular nuclei with large, prominent nucleoli. Papanicolaou, 400×.

Molecular diagnosis for SP-LBC sample. We used a EUS-FNA cellular specimen on the basis of which a patient was indeterminately diagnosed with PDAC. The cytological sample obtained from the SP-LBC slide was subjected to DNA extraction and PCR reactions, and have confirmed the *KRAS* mutation (G12S). *KRAS* mutation was also examined using histological tissue sample for double check, and we also confirmed the *KRAS* mutation (G12S).

Discussion. In the presented study, *KRAS* mutation in an SP-LBC specimen obtained by the EUS-FNA was used to accurately identify PDAC. A literature review of the PubMed database (2010–2018) was performed to identify several major genes involved in the development and progression of neoplastic diseases identified by LBC. These articles are summarized in Table 1. Recently, many molecular diagnostic studies using TP-LBC and SP-LBC were made concerning breast [14], uterine cervix [15], lung [16], thyroid gland [17], and urinary bladder [18] cancers. In gynecology, the improvement of sample adequacy using TP-LBC was attributed to the ability of that technique to remove obscuring elements from cervical specimens. In addition, LBC has also facilitated molecular testing for human papillomavirus (HPV) from the same cervical cytology specimens. Molecular diagnostics using LBC had higher sensitivity compared to cytology. It also allowed identifying *BRAF*, *EGFR*, and *KRAS* mutations in LBC samples. Among them, *EGFR* status was an important factor for molecular targeted therapy of lung cancers; thus, molecular diagnostics using LBC has facilitated the choice of therapeutic strategy in several diseases. However, few studies have been made on cytological diagnostics of PDAC using LBC [12]. Moreover, no one reported the use of DNA extracted from LBC samples for the examination of PDAC.

Table 1 – Results of the literature review of the PubMed data base (2010–2018)

No.	Author	Year	Country	Organ	LBC	Target gene	Reference No.
1	Nishimura R*	2016	Japan	Breast	Thin-Prep	*HER2*	14
2	Tewari P	2018	Ireland	Uterine cervix	Thin-Prep	*Human papillomavirus*	15
3	Malapelle U	2012	Italy	Lung	Thin Prep	*EGFR/ KRAS*	16
5	Rossi ED	2013	Italy	Thyroid gland	Thin Prep	*BRAF (V600E)*	17
6	Mian C*	2010	Italy	Urinary bladder	Thin-Prep	Chromosomes 3, 7 and 17 Loss of 9p21 locus	18
4	Sekita-Hatakeyama Y	2018	Japan	Pancreas	SurePath	*KRAS*	12

* Molecular diagnostics was made using FISH method

Interestingly, in the presented study an indeterminate diagnostic SP-LBC sample has been used to confirm the *KRAS* status in PDAC patients. Therefore, the results of this study suggest that DNA extracted from an LBC sample may be used for molecular diagnostics of PDAC. However, it cannot be used to make a conclusion about the malignancy of the tumor.

Previous studies have shown that PDAC is associated with several genetic abnormalities involving the *KRAS*, *TP53*, *CDKN2A*, and *SMAD4/DPC4* genes [19, 20]. Among all of them, *KRAS* is considered to be an important molecular diagnostic target for PDAC, as this mutation is observed in 90% of all PDAC cases [21]. EUS-FNA cytology techniques may not always yield adequate quantities of tissue specimens for diagnosis, and a negative EUS-FNA result is not definitive evidence of a benign tumor. Therefore, *KRAS* mutation analysis by SP-LBC, as explained in this report, may be used to improve the diagnostic accuracy of PDAC.

Specific advantages of LBC include rapid and proper fixation, a reduced incidence of air-drying of artifacts, and reduced or no background effects [22]. Therefore, it might be easy to perform molecular analysis using LBC sample such sample being more clear and more informative, without necrotic or blood contamination. Several reports describe the conduct of *EGFR*, *ALK*, and *KRAS* analysis using LBC specimens of lesions in other organs, especially the lungs [16, 23]. Previous studies have already shown that laser capture microdissection makes direct sequencing highly sensitive even on LBC preparations with only a few Papanicolaou-stained cells [16]. However, there are no reports of *KRAS* analysis using Papanicolaou-stained cells in SP-LBC slides of PDAC. Combining *KRAS* molecular diagnostics with cytological findings allows achieving high diagnostic accuracy for PDAC in case the tissue sample from EUS-FNA is inadequate.

The present study, however, has some limitations. The detection of *KRAS* mutation in Papanicolaou-stained cells in SP-LBC slides can be difficult in case of insufficient quantity of cells or the slide inadequacy. Thus, cytological studies have to be made first to ensure an adequate number of malignant cells in the SP-LBC slide.

Conclusions. *KRAS* mutation in an SP-LBC specimen obtained by the EUS-FNA can accurately identify PDAC. SP-LBC is a useful technique for collecting high-quality cellular samples for genetic analysis as well as conducting an exploratory evaluation of appropriate molecular diagnostics for PDAC. However, there is a need for more data on PDAC diagnostics using SP-LBC.

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