The Use of K-ras Gene Mutation Analysis, Mucins Expression Profile and MicroRNA-Based Test to Improve Endoscopic Ultrasound-Guided Fine Needle Aspiration Diagnosis of Pancreatic Cancer

1.0 Background:

Every year, over 200 thousand people die worldwide due to pancreatic cancer with the highest incidence and mortality rates being in the developed countries (1). In Europe and United States, pancreatic cancer is the 6th and 4th cause of cancer death respectively (2, 3). Following the pancreatic cancer high fatality rates, its morality rates are almost equal to incidence rates. Due to lack of screening tests and few early indicators of the illness, pancreatic cancer is diagnosed late. According to loos (3) the prognosis for pancreatic cancer is poor and this has made the disease to have a 5 years survival rate which represents 6%. Treatment for various pancreatic cancers for the past few decades has not improved substantially and has insignificant effect on prolonging the patient's survival (4). Metastatic pancreatic ductal adenocarcinoma (PDAC)

according to recent genetic evolution studies takes up to 20 years to develop. As a results patients will lose their lives from metastatic cancer even if they are undergoing curative pancreatic resection. Due to lack of effective treatment for pancreatic cancer, many studies are suggesting the use of prevention measures in order to reduce pancreatic cancer mortality. Environmental factors according to the international variations play an important role in pancreatic cancer aetiology. For example, pancreatic cancer incidence has been reported mainly for those individuals who smoke tobacco (2). Epidemiological studies on pancreatic cancer over the past decades have been associated with various methodological issues regarding fatal disease. As a result, inconsistent results findings from the epidemiological studies have hindered out understanding of pancreatic cancer aetiology (2). However, data from numerous studies have related pancreatic cancer with various medical conditions including diabetes mellitus and pancreatitis. Other potential risk factors that increase the risk of having pancreatic cancer include occupational exposure of certain pesticides, physical inactivity and dietary factors such as increased sugar intake (1).

1.1 Classification of Pancreatic Cancer

The classification of pancreatic neoplasia is mainly based on predominant cell lineage and gross configuration within a neoplasm. The gross appearance of neoplasm is classified as either intraductal, cystic of solid. Currently, pancreatic cancer is classified into pancreatic endocrine and pancreatic and pancreatic exocrine. Pancreatic exocrine tumours such as ductal adenocarcinoma are highly malignant neoplasm that have very poor prognosis (5). Although cigarette smoking has been identified as the main causative factor for pancreatic carcinoma

hereditary pancreatitis has also been shown to be an aetiological factor that causes the onset of the type of cancer. Ductal adenocarcinoma is the most common exocrine cancer affecting the pancreas and accounts for approximate 90% of all pancreatic cancers (6). Approximately 80% of ductal adenocarcinoma cases manifest in patients who are above 60 years and it's rare among individuals who are above 40 years (6). Acinar cell carcinoma is a rare pancreatic carcinoma that is known to cause excessive pancreatic lipase production. Intraductal Papillary-Mucinous Neoplasm (IPMN) on the other hand is a cystic tumour which is known to originate from the main pancreatic duct appearing as finger-like projections. Although IPMN may be benign during diagnosis, it eventually progresses to malignancy and become a precursor for adenocarcinoma (7). A rare malignant cystic tumour is Mucinous Cystadenocarcinoma. However, due to excessive use of modern imaging, Mucinous Cystadenocarcinoma among other ductal pancreatic cysts are diagnosed. Pancreatic ductal adenocarcinoma in comparison to other types of pancreatic cancers is the major cause of cancer deaths with an estimated 43,920 individuals being diagnosed with the disease in United States in 2012 (7).

Pancreatic neuroendocrine tumours are relatively rare in comparison to ductal adenocarcinoma representing 3-4% of all pancreatic cancer (8). Currently, the incidence rate of pancreatic neuroendocrine tumour is less than 1%. However, the number of individuals diagnosed with this type of cancer is increasing with advancement of abdominal imaging (9). Some of the examples of pancreatic endocrine tumours include Gastrinomas (Zollinger-Ellison Syndrome), Glucagonomas, Insulinomas and Somatostatinomas VIPomas. Most of the endocrine pancreatic neoplasm are functional and secrete some hormonal products into the blood resulting into hormone-producing pancreatic tumour syndrome such as hypoglycaemia (10).

1.2 Pathological Typing and Staging

The stage of a pancreatic cancer is important as it facilitates the doctor to choose the treatment option and determine the patient outlook (11). The staging process of pancreatic cancer is based on the results from biopsies, endoscopies and imaging tests. The staging system that is commonly used to determine how far a cancer has spread is TNM system which provides 3 keys of information. The first information and which is represented by T describes the size of the main tumour and determines whether it has grown outside. N describes the spread of the tumour cells to the lymph nodes and M on the other hand provides information on how far the cancer has spread to different parts of the body. In stage IA of the pancreatic cancer, the tumour is still within the pancreases (T1) with no distant metastasis (M0) and no regional lymph node metastasis (N0). The tumour is less than 2 cm in longest dimension. In stage IB of the cancer, the tumour is limited only in the pancreas (T2) and has more than 2 cm in longest dimension. The stage has no regional node metastasis. In stage IIA of the cancer, the tumour extends beyond the pancreas (T3). However, the cancer cells do not involve the superior mesenteric artery. In stage IIB, the cancer extends to regional lymph nodes. In stage III, the tumour extends beyond the pancreas resulting into distant metastasis. In the final stage (IV), the tumour cells spread to other parts of the body and this is considered the final stage.

Table 1: Pathological staging of pancreatic cancer.

Stage	**Tumour***	**Nodal Status***	**Distant Metastases***	**Annotations**
IA	T1	N0	M0	Tumour limited to the pancreas, ≤ 2 cm in

				longest dimension
IB	T2	N0	M0	Tumour limited to the pancreas, ≥ 2 cm in longest dimension
IIA	T3	N0	M0	The tumour extends beyond the pancreas, but the tumour does not involve the major arteries or veins near the pancreas
IIB	T1,T2,T3	N1	M0	The cancer has spread to regional lymph nodes
III	T4	N0 or N1	M0	The tumour extends beyond the pancreas into major arteries or veins near the pancreas. A T4 tumour is unrespectable
IV	Any T	N0 or N1	M1	Cancer cells spread to different parts of the body, including distant lymph nodes. Distant spread of pancreatic cancer occurs mainly in the liver, peritoneum (lining of the abdominal cavity), and lungs.

***T describes the size and location of the primary tumour; N refers to regional lymph nodes; M refers to distant metastases (12).**

1.3 Current diagnostic methods

Pancreatic cancer is very difficult to detect as there is no specific signs and symptoms that may lead to suspect it. However, even if the pancreatic cancer has been suspected, it can be difficult

to detect it. Therefore, there are a variety of diagnostic methods that can be used to diagnose pancreatic cancer.

These methods include:

- **Blood tests:** Such as **CA 19-9** which is a tumour marker for pancreatic cancer. CA19-9 levels are more useful to evaluate the prognosis of pancreatic cancer. High levels of CA 19-9 in the blood designates the progression of the cancer, while stable or low levels of CA19-9 indicates the response of the tumour to the treatment. Blood glucose level can be tested to detect pancreatic cancer. Abnormally high levels of glucose in the blood may indicate diabetes which is a risk factor of developing pancreatic cancer. However, abnormally low levels of glucose have been found in the very rare neuroendocrine pancreatic cancer called glucagonoma.
- **Radiographic imaging** is widely used to manage patients with pancreatic cancer. There are different technologies that can be used to visualize pancreatic lesions. These techniques include: Computerized axial tomography (CAT) scanning, endoscopic ultrasound (EUS), magnetic resonance imaging (MRI) and positron emission tomography (PET).
- **Biopsy interpreted by a pathologist:** The biopsy remains the gold standard for diagnosing pancreatic cancer. There are a number of forms of biopsy, these forms include fine needle aspiration (FNA), tissue core biopsy, or by excisional biopsy. The FNA samples can be collected via the EUS endoscope, or in combination with a CAT or MRI imaging. The biopsy is used to detect tumours as well as to classify the tumour type.

- **Endoscopic ultrasound (EUS)** the endoscopic ultrasound (EUS) was initially developed as an evaluation tool of the pancreas (13). EUS is a conjugation of endoscopic and ultrasonography. EUS is used to detect small masses. However, it is usually coupled with a biopsy as EUS cannot identify the tumour with certainty. One of the main advantages of EUS is the ability to provide FNA to evaluate patients with solid pancreatic masses.

1.4 Molecular diagnosis

K-RAS (Kirsten Rat Sarcoma Viral Oncogene Homolog) is the gene that provides instructions for making K-RAS protein. This protein relay signals from outside the cells to the nucleus within the cells. This signal pathway instructs the cells to divide, mature, grow or take a specialised function.

KRAS oncogene is mutated in human malignancies including the lung ovarian and colon cancer. Mutations in KRAS within the pancreatic cancer are found in more than 90% of samples from different patients (14). According to several researches, the presence of KRAS gene mutations in tissue improves the accuracy of pancreatic cancer diagnosis. (14, 25, 26, 27).

Mucins

These are heavily glycosylated and high molecular weight glycoproteins. Mucins are known in protecting tissues epithelial surface and also might involve in the regeneration and differentiation of the epithelium, cellular signalling and cellular adhesion. They also involve in the promotion of invasive and metastatic capability of tumours (15). Therefore, mucins can be used as a diagnostic marker by detecting the rate of expression of mucins in different malignant tumours including pancreatic cancer (16).

1.5 MicroRNA-based test diagnosis

Diagnosis of pancreatic cancer and pancreatitis often represents a clinical dilemma (17, 18). Today, a commonly used diagnostic method of masses suspected to be malignant is endoscopic ultrasound guided fine needle aspiration (EUS FNA) (19). EUS FNA has a reported positive predictive value that approach to 100% and high specificity and sensitivity. However, its negative predictive value is significantly lower (70%) making the diagnostic method to have a 30% false negative results. This error results from such focal chronic pancreatitis that happens to mimic pancreatic cancer (20, 21). Mature microRNAs are regulatory RNA that control gene expression and linked to many human cancers. MicroRNAs are sensitive biomarkers that have potential to be used in molecular diagnostic tests of pancreatic cancer. MicroRNAs have a varying level of expression across different pathological condition such as inflammatory disease, autoimmune and cancer. Using various methods, MicroRNAs can be extracted from the tissues and quantified to identify a specific signature which is used for diagnostic purpose (22, 23).

1.6 Rationale

The purpose of this literature is to analyse and determine how the endoscopic ultrasonography with fine needle aspiration in research and hospitals have become a common diagnosis of

pancreatic cysts. The endoscopic ultrasonography has significantly improved the detection and pathologic analysis of pancreatic lesion (24). In combination with other molecular analysis such as K-RAS and microRNAs will further improve the clinical sensitivity of endoscopic ultrasonography. For this reason, it is clinically important to introduce more specific and sensitive molecular tests when analysing fine needle aspirate samples in order to increase the accuracy of the diagnostic procedure currently being used. Therefore, this literature aims at analysing the use of molecular diagnosis and MicroRNA-Based test with an aim of improving endoscopic ultrasound-guided fine needle aspirations diagnosis of pancreatic cancer.

2.0 Materials and Methods

Authors	**Ginesta M, et al (26)**	**Ogura T, et al (25)**	**Bournet B, et al (27)**
Year	**2012**	**2012**	**2015**
Country	**Spain**	**India & Japan**	**France**
Topic	**Genetic and Epigenetic Markers in the Evaluation of Pancreatic Masses.**	**Clinical impact of K-RAS mutation analysis in EUS-guided FNA specimens from pancreatic masses.**	**Endoscopic Ultrasound–guided Fine-Needle Aspiration Biopsy Coupled With a KRAS Mutation Assay Using Allelic Discrimination Improves the Diagnosis of Pancreatic Cancer.**
Aim of the study	**To evaluate hypermethylation status of some genes as a diagnostic tool in combination with K-RAS gene mutation.**	**To asses clinical outcome of K-RAS mutation analysis in EUS-FNA specimens from pancreatic masses**	**To improve the diagnosis of pancreatic cancer by the combination of EUS-FNA and K-RAS mutation analysis.**

Experimental design	N= 61 samples Malignant (Adenocarcinoma) n=43. Benign n=18 benign.	N= 394 samples. Pancreatic ductal adenocarcinoma n=307. Benign lesions n=47. Other types of tumours n=40.	N= 186 Samples. Pancreatic adenocarcinoma n= 104. Benign lesions n=60. Other pancreatic malignant n=22
Methods	Cytological examination: FNA samples. Analysis of K-RAS mutation: PCR. In addition, they have analysed the methylation status of HRH2, EN1, SPARC, CDH13 and APC using melting curve analysis after DNA bisulphite treatment.	Cytological examination: FNA samples. Analysis of K-RAS mutation: Based on either paraffin-embedded sections of the cell blocks or fresh specimens. PCR coupled with direct sequencing.	Cytological examination: FNA samples. Analysis of K-RAS mutations: TaqMan MGB allelic discrimination

Authors	**Horn A, et al (28)**	**Wang Y, et al (16)**	**Brand et al. (29)**
Year	2013	2007	2014
Country	U.S.A	China	U.S.A
Topic	Immunocytochemistry for MUC4 and MUC16 Is a Useful Adjunct in the Diagnosis of Pancreatic Adenocarcinoma on Fine-Needle Aspiration Cytology.	Diagnostic value of mucins (MUC1, MUC2 and MUC5AC) expression profile in Endoscopic ultrasound-guided fine-needle aspiration specimens of the pancreas.	A MicroRNA-Based Test Improves Endoscopic Ultrasound–Guided Cytologic Diagnosis of Pancreatic Cancer.
Aim of the study	To Investigate the utility of MUC4, MUC16 and NGAL in evaluating pancreatic FNA samples.	To evaluate mucins expression profile in EUS-FNA samples in order to improve the diagnosis of pancreatic cancer and mucinous neoplasm.	To justify that "a MicroRNA test improves EUS-guided cytological diagnosis of pancreatic cancer.
Experimental design	N= 16 samples Malignant (carcinoma) n=11 Atypical/Suspicion n=5	N= 56 samples. Pancreatic cancer n=40. Benign lesions n=10. Chronic pancreatitis n=6.	N= 228 Samples of pancreatic lesions.

Methods	• **Cytological examination** • **Immunohistochemistry**	• **Cytological examination** • **Immunohistochemistry**	• **Cytological examination** • **Reverse-transcription and relative quantitative polymerase chain reaction**

- Ogura T, et al (25) was purposed to investigate the "Clinical impact of K-ras mutation in EUS-guided FNA specimens from pancreatic masses." The research involved the collection of material (pancreatic fluid) or specimen from 394 patients. Among these patients, "307, 47, and 40 had pancreatic ductal adenocarcinomas, inflammatory lesions, and other tumours respectively." The EUS-FNA was conducted using a connection of a curved, one-dimensional, uniform-radiation echo-endoscope with ultrasound (US) device and a 22-gauge needle at a frequency of 7.5MHz. Three individual portions were made from the material obtained to allow for cytological assessment, preparing a cell-block and K-ras mutation analysis. Evaluation, performed by cytologists, used "Diff-Quick staining" to diagnose the presence of cancer cells. Cell-block analysis was done using the aspirated specimens, which had been treated with a solution with a formalin-water ratio of 0.1, centrifuged, and fixed in paraffin. Finally, the prepared sections were observed (using hematoxylin and eosin) for the diagnosis. The K-ras mutation analysis (gene-analysis) used a fresh aspirated material or the cell-block paraffin-fixed material. A "reverse transcriptase polymerase chain reaction" correlated

with methods of determining nucleotides arrangement were used to analysis the total RNA from the freshly aspirated material. Further analysis of the cell-block section using a highly sensitive Cycleave PCR assay was performed when negative outcomes of the fresh portion analysis were obtained.

- The study by Bournet et al (27) intended to investigate the added value of incorporating K-ras mutation analysis in EUS-FNA in the diagnosis of pancreatic cancer. Pancreatic tissues were obtained from 186 patients with suggested or suspected pancreatic masses. Like the study by Ogura T, et al (25), the EUS-FNA was performed by a convex uniform-array echo-endoscope fitted to a US machine and a regular or EUS-Procore 22 gauge needle, depending on sufficiency. The pancreatic aspirates (tissues) were fixed in formalin while in the needle and examined for cancer cells, after which the needle was removed, sterilized, and discarded. The cellular content was frozen for DNA extraction. Similar to the previous study (25), the K-ras mutation analysis was performed using a highly sensitive method. The procedure started with centrifuging the sample and extracting DNA from the solid matter using a “QIAamp DNA micro-kit”. Categorization of nucleotides was done using an N-spectrophotometer (27). K-ras mutations were identified by conducting custom-based dual probes mutation detection assay, which were ran on the “ABI Prism 7300 and Roche LC480II detection systems”. A ten-milliliter volume of sample plus 20 Nanogram of DNA and 1X of the assay were involved in the

polymerase chain reaction. Additionally, a two-step PCR was assess the “cycling conditions”.

- The study by Ginesta et al. (26) focused on how K-ras mutation detection together with methylation markers can be utilized in the diagnosis of pancreatic cancer. The methodology used in this study is similar to the studies above (25, 27). Sixty-one patients with suspected pancreatic epithelial cancer participated in the study. Forty-three participants had pancreatic cancer and eighteen with pancreatitis after a fine-needle aspiration was performed (26). The study participants were 39 men and 22 women aged between 32 and 85 years. The aspirated material obtained using previous methods (25, 27) were separated thrice. The first portion was assessed fresh after staining. The second portion prepared a cell-block for cytological diagnosis by embedding it with a solution of 1:1 ethanol-water ratio, centrifuging, and fixed in paraffin (26). The third portion was used for DNA analysis for K-ras mutation. After amplifying a polymerase chain reaction, the detection of K-ras mutation, at codon 12, was performed using the “primer-mediated Restriction Fragment Length Polymorphism” technique. The most prevalent mutation was assessed using a discrimination assay. The method is highly sensitive with an average of 3.5% mutant-allele detection.

- The study by Horn A, et al (28) investigated the utility of MUC4, MUC16 and NGAL in evaluating pancreatic FNA samples. During the period of 10 years at Nebraska Medical Centre, a computer based research for all pancreatic fine-needle aspirations was performed. From this rundown of all cases, just those that fulfilled the following criteria were chosen for the study. Those are Paraffin carrying cell block was produced using the aspiration substance, and cases analysed as atypical/suspicious that had ensuing surgical/clinical analytic confirmation. Hematoxylin-eosin–stained slides from cell blocks were the cases that were ultimately chosen for survey to focus the vicinity or deficiency of diagnostic substance. However, around 65 studies had been reviewed that were atypical/suspicious of nature, furthermore, amongst those cases 16 (25%) fulfilled our criteria. Within those 16 cases, 11 were the cases that were positive for carcinoma and 5 were found to be negative. The other 48 cases chosen as positive and negative controls, which had satisfactory cell material, 31 (65%) were certain for adenocarcinoma, and 17 (35%) were negative for carcinoma on cytomorphologic evaluation and confirmed by resulting biopsy or clinical follow-up. However, sections without staining were immune-stained for MUC4, MUC16 and NGAL. The immunohistochemical staining was evaluated by using an H-score (range 0-3). Those samples were scored by two pathologists and differences were fixed by concurrent inspection. Later on, Graph-Pad Prism 5.0 was used to conduct the analysis of characteristic curve by comparing pancreatic FNA specimens with samples containing adenocarcinoma and benign cells.

- The study by Wang Y, et al (16) focused on the evaluation of mucins expression profile in EUS-FNA samples in order to improve the diagnosis of pancreatic cancer and mucinous neoplasm. The inclusion criteria of this research was the lesions on pancreas that were demonstrated with the help of CT and MRI scan. A data of 104 patients with pancreatic occupying lesion was collected from October 2005 – December 2006. A Needle of 19-22 gauge was used to acquire histological as well as cytological models. Pathological tissues were chosen to evaluate immunohistochemical reactivity of mucins antibodies. On location assessment of all EUS-FNA was given by a cytopathologist and a preparatory analytic impression was rendered for every situation. The final diagnoses of the specimen was done by pathologists. Contrasted FNA histological analysis and the highest quality level that is the final histological determination acquired at surgery or post-mortem examination and subsequent results. 56 patients constituted our study 32 of whom were males and 24 were females with a median age of 59.1 years (range, 33–79 years). The follow-up time was 11 months. **Immunohistochemical staining of Mucins:** After the process of de-paraffinization of each tumour in xylene and rehydration in ethanol, 0.4 µ of the tumour part was dipped in 0.01 mol/l citric acid buffer solution (pH 6.0) at 95C for approximately 12 mins to retrieve the antigens. To block the enzyme endogenous peroxidase, again the tumour part was dipped in 3% H_2O_2 at room temperature for 10 minutes. Later on, the tumour section was cleansed with phosphate buffered saline (PBS) and dipped again in 1% cow serum at room temperature for 20 minutes, so as to decrease the nonspecific staining. All arrangement included positive and negative controls. The classification was according to

immunostaining: negative or positive, negative consisted of less than 5% cells whereas the positive consisted of more than 5% cells.

- The study by Brand et al. (29) intended to justify that “a MicroRNA test improves EUS-guided cytological diagnosis of pancreatic cancer”. It involved the collection of specimens (pancreatic tissues) from eight institutions. The study involved 228 participants who were ≥21 years old, had to have no pancreatic tumours or cancer in the past were recruited. Molecular testing of the MicroRNA was conducted using a “reverse-transcription and relative quantitative polymerase chain reaction” for all the collected specimens. A discrimination analysis was performed to on ninety-five Formalin-fixed, paraffin-embedded tissues to distinguish pancreatic ductal adenocarcinoma and chronic pancreatitis. Validation of the predictive value of pancreatic ductal adenocarcinoma was done by applying an optimal MicroRNA classifier to the fresh fine-needle aspirations. Reports from cytopathologists at the eight study locations were used for cytological diagnosis. The results of the diagnosis were grouped as either “indeterminate” or “non-malignant” and based on surgery, disease evaluation, and follow-up. The indeterminate group comprised of the suspected and atypical cells while the non-malignant were non-diagnostic, adenocarcinoma, and benign. Statistical analysis was analysed using binary statistics for molecular testing. Fisher exact test for qualitative variables, regression for non-categorical data and multivariate analyses.

TABLE 1: Performances of Cytopathology, K-RAS mutation Analysis and Both Tests from Three Articles to Evaluate the Diagnosis of Pancreatic Cancer Versus Benign Solid Pancreatic Lesion Using EUS–guided Fine-Needle Aspiration.

Malignant vs. Benign Lesions	Type of analysis	Sensitivity%	Specificity%	PPV%	NPV%	Accuracy%
Bournet B, et al (27).	Cytopathology alone	77%	100%	100%	67%	84%
	KRAS analysis alone	59%	99%	99%	53%	71%
	Combination of cytopathology and KRAS analysis	91%	99%	99%	88%	94%
Ogura T, et al (25)	Cytopathology alone	87%	100%	100%	54%	89%
	KRAS analysis alone	87%	100%	100%	54%	89%
	Combination of cytopathology and KRAS analysis	93%	100%	100%	68%	94%
Ginesta M, et al (26).	Cytopathology alone	75.7%	100%	100%	59.1%	82%
	KRAS analysis alone	76%	100%	100%	64.3%	83.6%
	Combination of cytopathology and KRAS analysis	86%	100%	100%	75%	90.2%

NPV, negative predictive value; PPV, positive predictive value.

Pancreatic lesions	Number of cases	Positive rate of MUC1 expression	Positive rate of MUC2 expression	Positive rate of MUC5AC expression
Pancreatic cancer	40	77.5% (31/40)	10.0% (4/40)	80.0% (32/40)
Pancreatic benign lesion	16	25% (4/16)	31.3% (5/16)	43.8% (7/16)
Pancreatic mucinous neoplasms	18	66.7% (12/18)	38.9% (7/18)	88.9% (16/18)
Pancreatic no	38	60.5% (23/38)	38.9% (7/18)	57.9% (22/38)

mucinous neoplasms				

TABLE 2: Prevalence of Mucin Expression in EUS-FNA Specimens from Pancreatic Cancer and Pancreatic Benign Lesions/ Pancreatic Mucinous and Non-Mucinous (16).

Diagnosis	Test	Case number	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
Pancreatic cancer	Cytology analysis	56	65	93.8	73.2	96.3	51.7
	Cytology analysis + MUC1(+)	56	85	100	89.3	100	72.7
	Cytology analysis + MUC5AC (+)	56	90	93.8	91.1	97.3	78.9
Pancreatic mucinous neoplasms	Cytology analysis	56	38.9	60.5	51.8	31.8	67.6
	Cytology analysis + MUC1(+)	56	77.8	97.4	91.1	93.3	90.2
	Cytology analysis + MUC5AC (+)	56	100	71.1	80.4	62.1	100

TABLE 3: Performances of Cytopathology Alone and Plus MUC1/MUC5AC to Assess the Diagnosis of Pancreatic Cancer and Pancreatic Mucinous Neoplasm (16).

Positive (PPV) and negative (NPV) predictive value of diagnosis.

TABLE 4: Sensitivity and Specificity for Adenocarcinoma for MUC4 and MUC16, in Malignant Cases and in Atypical/Suspicious Cases (28).

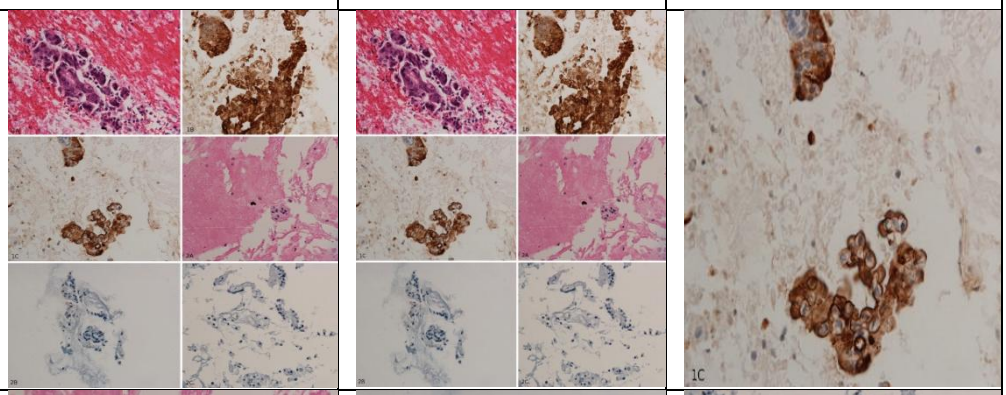
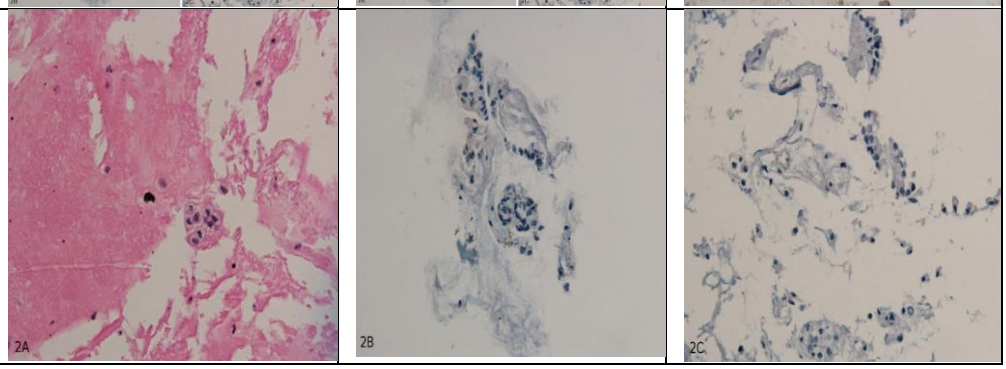
Diagnosis	Test	Sensitivity	Specificity
Malignant	MUC4	74	100
	MUC16	62.9	100
Atypical/Suspicious	MUC4	63.6	100
	MUC16	66.7	100
Diagnosed on cytology	**H&E (A)** **X400**	**anti-MUC4 (B)** **x400**	**anti-MUC16 (C)** **x400**
Pancreatic ductal adenocarcinoma. Figure 1			
Benign pancreatic ductal epithelium. Figure 2			

Figure 1: Pancreatic ductal adenocarcinoma, diagnosed on cytology: A, hematoxylin-eosin; B, anti-MUC4; and C, anti-MUC16 (X400) (28).

Figure 2: Benign pancreatic ductal epithelium: A, hematoxylin-eosin; B, anti-MUC4; and C, anti-MUC16 (X400) (28).

TABLE 5: Performance of Cytology and/or Molecular Testing in EUS-FNA Specimens

Brand R, et al (29)	Specimens number	Cytology alone	Molecular alone	Combined molecular cytology
Malignant detection rate (95% CI), %	184	78.8 (72.2–84.5)	82.6 (76.3–87.8)	90.8 (85.6–94.5)
Benign detection rate (95% CI), %	26	69.2 (48.2–85.7)	96.1 (80.4–99.9)	96.1 (80.4–99.9)

CI, confidence interval.

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