University of Szeged

Albert Szent-Györgyi Medical School

Doctoral School of Theoretical Medicine

DIAGNOSTIC INTERPRETATION AND RISK OF MALIGNANCY OF ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION CYTOLOGY IN SOLID PANCREATIC LESIONS

Ph.D. Thesis

Candidate: Béla Vasas, MD

Department of Pathology

Albert Szent-Györgyi Clinical Center

University of Szeged

Supervisors:

Zoltán Gábor Szepes, MD, habil., Ph.D.

Renáta Bor, MD, Ph.D.

Gastroenterology Center, Department of Internal Medicine

University of Szeged

Szeged

2024

List of full papers related to the subject of the thesis:

I.   **Vasas B**, Fábián A, Bősze Zs, Hamar S, Kaizer L, Tóth T, Bacsur P, Resál T, Bálint A, Farkas K, Molnár T, Szepes Z and Bor R. **Comparison of risk of malignancy and predictive value of diagnostic categories defined by Papanicolaou Society of Cytopathology System and WHO Reporting System for Pancreaticobiliary Cytopathology in solid pancreatic lesions**. *Therap Adv Gastroenterol.* 2024; Publication: 35142208. Subject area and category: Scopus - Gastroenterology SJR indicator: Q1. Impact factor: 3.9 *

II.   Bor R[#], **Vasas B[#]**, Fábián A, Szűcs M, Bősze Zs, Bálint A, Rutka M, Farkas K, Tóth T, Resál T, Bacsur P, Molnár T, Szepes Z. **Risk Factors and Interpretation of Inconclusive Endoscopic Ultrasound-Guided Fine Needle Aspiration Cytology in the Diagnosis of Solid Pancreatic Lesions**. *Diagnostics (Basel).* 2023; 13(17): 2841. Publication: 34129891. Subject area and category: Scopus - Clinical Biochemistry SJR indicator: Q2. Impact factor: 3.0 ([#]Co-first authors)

1. INTRODUCTION

Solid pancreatic lesions include both neoplastic (benign, premalignant, malignant) and non-neoplastic diseases. While most are pancreatic ductal adenocarcinomas (PDACs), others like neuroendocrine tumors (NETs), solid pseudopapillary neoplasms (SPNs), and metastases also occur. Non-neoplastic masses, such as chronic pancreatitis and autoimmune pancreatitis, can mimic cancer, complicating diagnosis. Endoscopic ultrasound-guided tissue acquisition (EUS-TA) via fine-needle aspiration (FNA) and biopsy (FNB) is crucial for identifying these lesions and distinguishing benign from malignant conditions. It has high sensitivity, specificity, accuracy and safety, facilitating treatment decisions, including staging and resectability. The European Society of Gastrointestinal Endoscopy (ESGE) recommends EUS-guided FNA as the first-line technique for suspected solid pancreatic lesions. However, challenges remain due to inconclusive results, such as low cellularity of smears or the presence of atypical cells of undetermined significance, which complicate definitive diagnoses and delay treatment. In

parallel with this, the increasing number of small tissue samples has made it difficult for pathologists to provide accurate and consistent interpretations. This underscores the need to minimize the rate of inconclusive samples and improve communication between pathologists and the multidisciplinary team managing pancreatic cancer, especially in the cases of uncertain or ambiguous sampling results.

This thesis presents two retrospective clinical studies. The first study investigates the frequency and risk factors of inconclusive EUS-FNA results, along with their clinical outcomes related to the risk of malignancy (ROM). The second study compares two standardized cytopathology reporting

PSC system			WHO system	
Category		Specific lesions	Category	
I.	Nondiagnostic			Inadequate /insufficient/ nondiagnostic	I.
II.	Negative (for malignancy)	Non-neoplastic only	Non-neoplastic and neoplastic (SCA)	Benign/Negative (for malignancy)	II.
III.	Atypical			Atypical	III.
IV.	Neoplastic				
IVa.	Neoplastic: benign	SCA	Low-grade MCN, Low-grade IPMN, Low-grade PanIN, BilIN	Pancreatobiliary neoplasm – low-risk/grade (PaN-low)	IV.
IVb.	Neoplastic: other	IPMN, MCN, PanNET, SPN, IOPN, ITPN, PanIN, BilIN	High-grade MCN High-grade IPMN IOPN, ITPN High-grade PanIN, BilIN	Pancreatobiliary neoplasm − high-risk/grade (PaN-high)	V.
V.	Suspicious (for malignancy)			Suspicious (for malignancy)	VI.
VI.	Positive (for malignancy)	PDAC, Acinar cell carcinoma, PanNEC PBL	PDAC, Acinar cell carcinoma, PanNET, PanNEC, SPN, PBL	Malignant	VII.

Table 1. Comparison of the PSC and WHO reporting systems: Lesions in red represent changes in tumor classification from the PSC system to the WHO system.

systems: the Papanicolaou Society of Cytopathology (PSC) System and the new World Health Organization (WHO) Reporting System. Both frameworks help pathologists consistently categorize lesions and provide uniform diagnostic information. The WHO system refines tumor categorization,

aligning with the PSC system but reorganizing certain neoplasms based on ROM (*Table 1*). This study evaluates and compares the predictive value and ROM of the WHO and PSC systems in diagnosing solid pancreatic lesions.

2. AIMS

2.1. Assessment of the clinical significance of inconclusive EUS-FNA cytology in the diagnosis of solid pancreatic lesions

2.1.1. Determination of the frequency and predictors of inconclusive cytological findings of the first pancreatic EUS-FNA sampling

2.1.2. Determination of the outcome of disease in patients with inconclusive cytology results

2.1.3. Identification of clinical factors influencing the ROM of EUS-FNA sampling

2.2. Comparison of clinical value of diagnostic categories defined by PSC and WHO reporting system for pancreaticobiliary cytopathology in solid pancreatic lesions

2.2.1. Comparison of ROM of diagnostic categories defined by PSC system and WHO system in solid pancreatic lesions

2.2.2. Comparison of predictive values of diagnostic categories defined by PSC system and WHO system in solid pancreatic lesions

3. PATIENTS AND METHODS

3.1. Patient enrollment, determination of subgroups and description of endpoints

3.1.1. Assessment of the clinical significance of inconclusive EUS-FNA cytology in the diagnosis of solid pancreatic lesions

This retrospective, single-center cohort study was conducted at a Hungarian tertiary-level gastroenterology center in collaboration with the pathology department. It included all patients who underwent EUS-FNA sampling for solid pancreatic lesions between January 2014 and December 2021. Patients were divided into two subgroups: conclusive and inconclusive cytology,

based on the diagnostic value of the EUS-FNA samples. Inconclusive results were defined using the PSC system, specifically cases classified as "nondiagnostic" (I) or "atypical" (III), and selected cases from the "negative for malignancy" (II) category when malignancy was clinically suspected. All other PSC categories were classified as conclusive cytology subgroups, including the "suspicious for malignancy" (V) category, due to its high ROM in an appropriate clinical setting.

The study aimed to identify predictors of inconclusive results, considering patient-related factors (age, gender, lesion location, size, and benign vs. malignant diagnosis) and procedure-related factors (investigator, needle size, number of punctures, biliary stent placement, diagnosis based on EUS image). The ROM was determined based on the final diagnosis obtained through follow-up procedures, including repeated biopsies, surgical intervention, autopsy, or the clinical course of the disease. ROM was calculated by dividing the number of malignant cases by the total number of cases in each category. False-positive and false-negative cases were identified by comparing cytological findings with the final diagnosis. Inconclusive "nondiagnostic" (I) and "atypical" (III) categories were considered true-negative for benign final diagnoses and false-negative for malignant ones.

3.1.2. *Comparison of the clinical value of diagnostic categories defined by PSC system and WHO reporting system in cytopathology for solid pancreatic lesions*

This retrospective cohort study at the University of Szeged, Hungary, included all patients who underwent EUS-FNA for solid pancreatic lesions from January 2014 to December 2021. Exclusion criteria were cystic pancreatic lesions, extrapancreatic lesions, and patient refusal for data use. Each cytological finding was compared with follow-up pathological or clinical data to determine the absolute ROM for each category. Absolute ROM was calculated based on the proportion of malignant diagnoses, supported by

histology, clinical evidence (e.g., weight loss, tumor marker rise), or radiologic evidence of malignancy. Lack of clinical or radiologic evidence, or no disease progression during follow-up, indicated a benign lesion. The relative ROM was calculated as the ratio of each category's absolute ROM to that of the "negative for malignancy" category.

The diagnostic predictive value of cytological categories was assessed using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Nondiagnostic categories were excluded. False-positive cases were benign lesions misdiagnosed as malignant, and false-negative cases were malignant lesions incorrectly identified as benign. The challenging interpretation of the "atypical" category was analyzed using three methods: classifying as negative, positive, or excluding from evaluation as inconclusive.

3.2.   EUS-FNA procedure and pathological evaluation

EUS-FNA samplings were performed by two experienced endoscopists using various needle sizes (19G, 22G, 25G) and techniques (standard suction, slow-pull) depending on the lesion characteristics. Samples were processed into alcohol-fixed smears, formalin-fixed, paraffin-embedded (FFPE) cell blocks, and cytospins. Pathological diagnosis was based on direct smears, cytospins, and FFPE cell blocks, stained with hematoxylin-eosin (HE). Immunohistochemistry was performed on most FFPE tissues and selected smears. PSC categories were assigned prospectively, with retrospective reclassification into the WHO system.

3.3.   Ethics approval

The study was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged, Hungary (approval number: 182/2015 SZTE).

3.4. Statistical analysis

Statistical analysis was conducted using R version 3.6.0 and SPSS version 28; p values of less than 0.05 were considered significant. Descriptive statistics were presented as means, medians, and ranges. Categorical variables were reported as event rates and frequencies, and continuous variables as means with standard deviation. Logistic regression, Pearson Chi-squared, and Fisher's exact tests were used to identify factors influencing inconclusive cytology and ROM. Fisher's exact test also assessed the significance of ROM differences across categories.

4. RESULTS

4.1. Assessment of the clinical significance of inconclusive EUS-FNA cytology in diagnosing solid pancreatic lesions

In this study, 473 patients with solid pancreatic lesions underwent 521 EUS-FNA procedures, with multiple samplings in some cases. The first sampling outcome was analyzed for each patient. The clinical characteristics of patients and EUS-FNAs are summarized in Table 2. Cytological analysis confirmed a neoplastic cause in 340 cases (71.88%), categorized as "malignant," "suspicious for malignancy," or "neoplastic: other." Only 33 samples (6.98%) were classified as "negative for malignancy." After a mean follow-up of 13.77 months, 392 cases (82.88%) were found to be malignant. The final diagnosis was histologically confirmed in 185 cases (39.11%), while in the remaining 288 patients (60.89%), diagnosis was based on clinical follow-up. The sensitivity, specificity, and diagnostic accuracy of initial EUS-FNA sampling were 85.43%, 100.00%, and 87.74%, respectively, improving to 89.92%, 100.00%, and 91.54% after repeated sampling. Complications occurred in five cases, including duodenal perforation, bleeding, pancreatitis, and amylase elevation. Diagnostic errors included misclassifying two PDACs as NETs and two cases of chronic pancreatitis as malignancies; one PDAC was initially reported as low-grade IPMN due to presumable peritumoral sampling.

CHARACTERISTICS OF PATIENTS		CHARACTERISTICS OF EUS-FNAs	
Male/female	229/244	Examiners A/B:	348/125
Age (year)	66.63±11.81 (18-95; median: 68)	Mean number of puncture per examination	3.44±1.07
Mean size of lesion (mm)	33.83±14.18	Number of puncture per examination	
Size of lesion		≤ 2 punctures	90 (53.93%)
≤20 mm	76 (16.07%)	3-4 punctures	311 (14.78%)
20-40 mm	257 (54.33%)	> 4 punctures	72 (19.19%)
≥ 40 mm	140 (29.60%)		
		Mean number of smear pairs per examination	2.11±1.01
Location of lesion			
head	255 (53.91%)	**Sampling technique**	
uncinate process	67 (14.16%)	only slow-pull (SP)	73 (15.43%)
body	90 (19.03%)	only standard suction (SS)	46 (9.73%)
tail	60 (12.68%)	both SP and SS	354 (74.84%)
diffuse	1 (0.21%)		
Histology of lesion		**Size of EUS needle**	
Ductal adenocarcinoma	352 (74.42%)	19G	33 (6.98%)
Primary bile duct carcinoma	2 (0.42%)	22G	395 (83.51%)
Solid pseudopapillary npl.	3 (0.63%)	25G	45 (9.51%)
Well-differentiated NET	15 (3.17%)		
Neuroendocrine carcinoma	3 (0.63%)	**Biliary stent**	129 (27.27%)
Low-grade IPMN	1 (0.21%)	**Type of lesion based on EUS image**	
High grade IPMN (clinical suspicion of malignancy)	2 (0.42%)	benign	54 (11.42%)
Myxofibrosarcoma	1 (0.21%)	malignant	419 (88.58%)
Hematolymphoid tumor	2 (0.42%)		
Metastatic carcinoma	15 (3.17%)	**Cytological finding based on PSC system**	
Ancient schwannoma	1 (0.21%)		
Serous cystadenoma	1 (0.21%)	"nondiagnostic"	72 (15.22%)
Intrapancreatic spleen	1 (0.21%)	"benign"	33 (6.97%)
Acute necrosing pancreatitis	12 (2.54%)	"atypical"	28 (5.92%)
Autoimmune pancreatitis	4 (0.85%)	"neoplastic: other"	19 (4.02%)
Chronic pancreatitis	31 (6.55%)	"suspicious for malignancy"	31 (6.55%)
Histologically unverified focal lesion disappeared during follow-up	27 (5.71%)	"malignant"	290 (61.31%)

Table 2. *Clinical characteristics of patients and EUS-FNA examinations.*

4.1.1. *Frequency and predictors of inconclusive cytological findings*

The first EUS-FNA sampling yielded inconclusive results in 108 cases (22.83%), with minimal variation over time. Significant increases in the odds of inconclusive cytological findings were observed for lesions with a benign final diagnosis (OR 11.20; 95% CI 6.56–19.54, p<0.001) as well as with the use of 25G FNA needles (OR 2.12; 95% CI 1.09–4.01, p=0.023) compared to 22G needles. Furthermore, the use of a single EUS-FNA technique compared

to the combined use of slow-pull and standard suction techniques (OR 1.70; 95% CI 1.06–2.70, p=0.027) and less than three punctures per procedure led to an elevation in the risk of inconclusive cytology (OR 2.49; 95% CI 1.49–4.14, p<0.001). Risk reduction in inconclusive cytology findings was observed in lesions between 2–4 cm (OR 0.40; 95% CI 0.23–0.68, p=0.001) and >4 cm (OR 0.16; 95% CI 0.08–0.31, p<0.001) compared to lesions ≤2 cm. EUS-FNAs that yielded both direct smears and FFPE were not associated with a reduction in the rate of inconclusive cytology compared to samplings that resulted in direct smears only (26.32% vs. 22.53%, p=0.594). Number of smears per puncture, and the presence of a biliary stent had no significant impact on the rate of inconclusive findings. Pancreatic tail lesions had a remarkably low rate of inconclusive cases (6.67%).

Multivariate analysis identified four predictors of inconclusive findings: localization in the pancreatic tail (OR 0.13 CI 95% [0.03–0.42], p=0.002), lesion size >4 cm (OR 0.24 CI 95% [0.10–0.54], p = 0.001), malignant EUS morphology (OR 0.11 CI 95% [0.02–0.38], p=0.002), all associated with decreased risk, and benign lesion origin (OR 56.97 CI 95% [17.40–272.78], p<0.001) (increased risk).

4.1.2.   Outcomes of patients with inconclusive cytology results

By the end of follow-up, 52.78% of cases with inconclusive cytology were found to be malignant, based on histopathological examination or clinical course. Some patients showed benign disease, including acute, chronic and autoimmune pancreatitis, and in others, lesions disappeared during follow-up. Thirteen patients did not undergo re-biopsy due to poor prognosis or refusal of treatment.

4.1.3.   Clinical Factors Influencing the ROM of EUS-FNA sampling

The overall ROM for EUS-FNA was 83.51%. The ROM was 88.11% for females and 78.60% for males (p=0.006), and older patients had a higher ROM (67.4±10.9 years vs. 62.4±15.1 years, p=0.001). Lesions <2 cm were

more likely benign, while larger lesions had a higher ROM (p<0.001). Elevated CA19-9 and CEA levels correlated with malignancy (p<0.001). In the inconclusive subgroup, malignancy was confirmed in 11.11% of cases classified as "negative for malignancy" based on EUS imaging. When EUS suggested malignancy, the ROM was 70.00%, and when EUS indicated benign lesions, the ROM was 3.57%. ROM increased when predictors such as malignant EUS morphology and larger lesion size were present together, particularly in the "atypical" (III) category, where ROM reached 94.74% for lesions >2 cm. In contrast, the ROM for small lesions with normal CA19-9 levels and benign EUS morphology was significantly lower.

4.2. Comparison of clinical value of diagnostic categories defined by PSC system and WHO reporting system in solid pancreatic lesions

This 8-year study analyzed 473 patients with solid pancreatic lesions who underwent EUS-FNA biopsy, resulting in 521 specimens. The male-to-female ratio was 229:244, with a mean age of 66.61 years. Most lesions were in the pancreatic head and uncinate process region (68.71%), with a mean diameter of 33.63 mm. Final diagnoses revealed 95 cases of benign disease (18.43%) and 426 cases of malignancy (81.76%).

Histological data were available for 205 cases, while clinical follow-up data covered 316 cases. The histologic specimens included biopsies from different modalities, repeat EUS-FNA samples, surgical excision specimens, and autopsy samples. In 60 cases, EUS-FNA samples were nondiagnostic or atypical, with 145 cases successfully diagnosed, and 5 cases involved diagnostic errors.

The PSC and WHO classification systems overlapped in several diagnostic categories, including nondiagnostic, negative for malignancy, atypical, and suspicious for malignancy. In 20 cases classified under the PSC IVb category (neoplastic: other), most were reclassified to the WHO VII category (positive

for malignancy). There were no cases in the PaN-low (WHO IV) category. All malignant cases under PSC VI were shifted to WHO VII.

4.2.1. *Comparison of ROM of diagnostic categories defined by PSC system and WHO reporting system in solid pancreatic lesions*

In 40 of 83 "nondiagnostic" (PSC I and WHO I) cases, neoplastic lesions were later confirmed, including 34 ductal adenocarcinomas and other malignancies. Benign conditions like chronic pancreatitis were common in undiagnosed

Diagnostic category defined by PSC system	Absolute ROM (%)	Relative ROM (%)	*p* value (Compared to negative for malignancy)
I – Nondiagnostic	48.19	21.23	< 0.0001
II – Negative for malignancy	2.27	-	-
III – Atypical	78.13	34.42	< 0.0001
IVa – Neoplastic: benign	-	-	-
IVb – Neoplastic: other	100.00	44.05	< 0.0001
V – Suspicious for malignancy	100.00	44.05	< 0.0001
VI – Malignant	99.34	43.76	< 0.0001
Diagnostic category defined by WHO system	**Absolute ROM (%)**	**Relative ROM (%)**	***p* value (Compared to negative for malignancy)**
I – Nondiagnostic	48.19	21.23	< 0.0001
II – Negative for malignancy	2.27	-	-
III – Atypical	78.13	34.42	< 0.0001
IV – PaN-low	-	-	-
V – PaN-high	100.00	44.05	< 0.0001
VI – Suspicious for malignancy	100.00	44.05	< 0.0001
VII – Positive (for malignancy)	99.38	43.78	< 0.0001

Table 3. *Absolute and relative ROM of cytological categories.*

cases. The absolute and relative ROMs for this category were 48.19% and 21.23%, significantly higher than for "negative for malignancy" (PSC II and WHO II). In the "atypical" (PSC III and WHO III) category, the ROMs were 78.13% and 34.42%, with low cellularity and blood contamination contributing to indeterminate diagnoses. Despite heterogeneity, the "neoplastic: other" (PSC IVb) category had 100% absolute ROM, similar to "suspicious for malignancy" (PSC V and WHO VI), which confirmed malignancy in all cases. The "malignant" (PSC VI and WHO VII) categories

had ROMs of approximately 99%, with two false positives due to misdiagnosed chronic pancreatitis *(Table 3)*.

4.2.2.   Comparison of predictive values of diagnostic categories defined by PSC system and WHO system in solid pancreatic lesion

Diagnostic categories considered as positive for malignancy		Sensitivity	Specificity	PPV	NPV	Validity
PSC and WHO	"Neoplastic: other"/"PaN-High" and "Suspicious for malignancy" and "Malignant" ("Atypical" considered positive for malignancy)	99.74%	82.69%	97.72%	97.73%	97.72%
	"Neoplastic: other"/"PaN-High" and "Suspicious for malignancy" and "Malignant" ("Atypical" considered as negative for malignancy)	93.26%	96.15%	99.45%	65.79%	93.61%
	"Neoplastic: other"/"PaN-High" and "Suspicious for malignancy" and "Malignant" (Excluded: "atypical" as an inconclusive category)	99.72%	95.56%	99.45%	97.73%	99.26%

Table 4. *Predictive value of cytological categories.*

Excluding nondiagnostic and atypical categories, the sensitivity, specificity, NPV, PPV, and validity of the PSC and WHO systems were identical at 99.72%, 95.56%, 97.73%, 99.45%, and 99.26%, respectively. Including the atypical category as malignant reduced specificity, while considering it benign reduced sensitivity, NPV, and validity *(Table 4)*.

5.   DISCUSSION

5.1.   Assessment of the clinical significance of inconclusive EUS-FNA cytology in the diagnosis of solid pancreatic lesions

5.1.1.   Frequency and predictors of inconclusive cytology in initial EUS-FNA sampling

Our study's diagnostic accuracy aligns with international findings, with pooled sensitivity at 84-89% and specificity at 96-99%. However, the negative predictive value (NPV) remains low (~50%). Larger needle diameters reduced inconclusive findings, contradicting some studies. Advanced needles like Franseen and fork-tip FNB needles show higher diagnostic accuracy,

especially for immunostaining, outperforming conventional needles in both pancreatic and nonpancreatic lesions.

The technique used also impacts outcomes. Combining suction techniques and multiple punctures per session minimized inconclusive results, with the fanning technique proving effective. Recent studies question the 2017 EUS-FNA guidelines recommending the use of 10 mL standard suction, suggesting that modified wet-suction techniques offer higher sample adequacy with less blood contamination. The optimal number of punctures remains unclear, with recommendations varying based on lesion size and presence of rapid on-site cytopathology (ROSE). Lesion size significantly affects outcomes; smaller lesions (<2 cm) are more prone to inconclusive results, as our study found. However, in large lesions, the risk of necrotic areas increases, making cells from these areas unsuitable for diagnosis. Tumor stiffness and fibrosis can also reduce sampling effectiveness, often requiring stronger suction. One study found that fibrosis negatively impacted EUS-guided tissue acquisition. In our study, obtaining both direct smears and FFPE samples did not yield more conclusive results, as many FFPE samples were histologically unevaluable. Macroscopic on-site evaluation (MOSE) could improve FFPE sample adequacy when ROSE is unavailable.

5.1.2.   Clinical predictors of malignancy in inconclusive cytology

We found strong correlations between PSC categories, EUS morphology, and ROM. For example, the ROM was 75% in the "atypical" category but only 3.03% in the "negative for malignancy" category. The "nondiagnostic" category did not correlate with ROM, indicating its limited utility in guiding further diagnostics. These results mirror international data, highlighting the variability in ROM across PSC categories.

5.2. Comparison of clinical value of diagnostic categories defined by PSC system and WHO reporting system in solid pancreatic lesions

5.2.1. Comparison of ROM of diagnostic categories defined by PSC system and WHO system in solid pancreatic lesions

The ROM values across PSC and WHO categories show significant variability, except in the "suspicious for malignancy" and "malignant" categories, where high ROM is consistently observed. The shift from PSC to WHO led to reclassification, mainly affecting categories IVa and IVb, but the impact on ROM was minimal in our study cohort. The ROM for cystic versus solid lesions differs notably, influenced by lesion morphology and interobserver variability among cytologists.

5.2.2. Comparison of predictive values of diagnostic categories defined by PSC system and WHO reporting system in solid pancreatic lesions

The "atypical" category (PSC III and WHO III) poses challenges in differentiating between benign and malignant conditions, often delaying treatment and increasing costs. Categorizing this group as positive for malignancy reduces specificity and increases false positives, while classifying it as negative reduces NPV and increases false negatives. The highest validity is achieved when excluding this category, confirming its inconclusiveness. Reducing the proportion of "atypical" diagnoses may improve diagnostic accuracy, and institutions should monitor and limit this category's use. The proportion of inconclusive results, including "nondiagnostic" and "atypical" categories, is influenced by lesion characteristics and EUS-FNA technique. Variation among cytopathologists in using indeterminate categories highlights the need for standardized criteria and training to improve diagnostic consistency. No current guidelines define the "atypical" rate as a quality indicator.

5.2.3.   Strengths and limitations of the studies

The study's strengths include a large, uniform cohort of solid pancreatic lesions, careful consideration of ROM differences between solid and cystic lesions, and collaboration between experienced cytologists using a standardized classification system to reduce variability. However, limitations include its single-center retrospective design, reliance on follow-up rather than histological confirmation, incomplete clinical data, and the absence of MOSE and ROSE, which may have affected sample adequacy and generalizability.

6.   CONCLUSION

Our first retrospective cohort study found that the rate of inconclusive EUS-FNA findings in solid pancreatic lesions can be reduced by using larger diameter needles (22G and 19G) and combining SP and SS techniques in a single procedure. Performing three or four punctures per procedure showed the highest clinical effectiveness without ROSE; fewer than two punctures increased inconclusive cases, while more than four did not improve efficiency. EUS morphology correlated closely with ROM, emphasizing the importance of the endoscopist's expertise and thorough examination. We recommend patient follow-up if EUS morphology suggests a benign lesion and cytology is "negative for malignancy" (PII). However, repeated sampling is needed if malignancy is suspected or in cases classified as "nondiagnostic" (PI) or "atypical" (PIII).

Our second study confirmed that the WHO system aligns with the PSC system in ROM and category predictive values for diagnosing solid pancreatic lesions. Reclassifying malignant lesions from the PSC IVb to the WHO VII category improved interdisciplinary communication and reduced misinterpretation of pathological findings.

Two practical recommendations emerged: First, the low ROM rate (2.27%) in the "negative for malignancy" category in our study reflects strict adherence

to the WHO system's diagnostic criteria. Therefore, cautious use of this category is recommended, considering the risk of false negatives that may arise from sampling errors. Second, nearly 80% of "atypical" cases are linked to malignancy but may delay diagnosis due to their inconclusive nature. To mitigate this, reducing the proportion of "atypical" cases through specialized pathologist training or multi-pathologist evaluations is advised.

7.   AKNOWLEDGEMENTS