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

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Ph.D. Thesis

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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)

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Number of publications in SJR *Q2* Journal: 5

LIST OF ABBREVIATIONS

AGA − American Gastroenterological Association

BilIN − biliary intraepithelial neoplasia

CA19-9 - carbohydrate antigen 19-9

CEA – carcinoembryonic antigen

CgA – chromogranin A

CI – confidence interval

EUS − endoscopic ultrasound

EUS-FNA − endoscopic ultrasound-guided fine-needle aspiration

EUS-FNB − endoscopic ultrasound-guided fine-needle biopsy

EUS-TA − endoscopic ultrasound-guided tissue acquisition

ESGE − European Society of Gastrointestinal Endoscopy

FFPE − formalin-fixed, paraffin-embedded

GNAS − guanine nucleotide binding protein alpha subunit

IOPN − intraductal oncocytic papillary neoplasm

IPAS − intrapancreatic accessory spleen

IPMN − intraductal papillary mucinous neoplasm

ITPN − intraductal tubulopapillary neoplasm

KRAS − Kirsten rat sarcoma virus oncogene homologue

MCN − mucinous cystic neoplasm

MOSE − macroscopic on-site evaluation

NEC − neuroendocrine carcinoma

NET − neuroendocrine tumor

NPV − negative predictive value

PBL − pancreaticobiliary lymphoma

PDAC − pancreatic ductal adenocarcinoma

PaN-high − pancreatic neoplasm: high-risk/grade

PaN-low − pancreatic neoplasm: low-risk/grade

PanIN − pancreatic intraepithelial neoplasia

PanNEC − pancreatic neuroendocrine carcinoma

PanNET − pancreatic neuroendocrine tumor

PPV − positive predictive value

PSC − Papanicolaou Society of Cytopathology

ROM − risk of malignancy

ROSE − rapid on-site evaluation

SCA − serous cystadenoma

SPN − solid pseudopapillary neoplasm

SS − standard suction technique

SP − slow-pull technique

WHO − World Health Organization

1. SUMMARY

1.2. Background

Solid pancreatic lesions represent a diverse group of benign and malignant diseases for which minimally invasive endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has become the leading diagnostic modality over the past decade. While this technique has high sensitivity and specificity, inconclusive cytological results remain a significant clinical challenge, often causing delays in treatment. To support the interdisciplinary interpretation of cytological findings in the management of pancreatic cancer, two reporting systems have been established: the Papanicolaou Society of Cytopathology (PSC) system and the World Health Organization (WHO) system, the latter of which aims to correct some aspects of the PSC system. Therefore, the aim of this thesis was to assess the frequency and examination- and patient-related risk factors of inconclusive cytological findings and to compare the PSC system and WHO system in terms of predictive value and risk of malignancy (ROM).

1.2. Methods

All consecutive patients with solid pancreatic lesions who underwent EUS-FNA sampling in University of Szeged from 2014 to 2021 were retrospectively enrolled. In assessing risk factors and interpreting inconclusive cytology findings, the “atypical” and “non-diagnostic” categories of the PSC system were considered inconclusive. The “negative for malignancy” category was also considered inconclusive if malignancy was suspected clinically. When comparing the two cytological reporting systems, the predictive value and ROM of cytological findings were determined with comparison to histologic outcome and/or clinical follow-up.

1.3. Results

A total of 473 first EUS-FNA samples were included, of which 108 cases (22.83%) were inconclusive. 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 25 G FNA needles (OR 2.12; 95% CI 1.09–4.01, $p=0.023$) compared to 22 G 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.

A total of 521 EUS-FNAs were performed with a malignancy rate of 81.76%. In both classification systems, the absolute ROM of “non-diagnostic”, “negative for malignancy”, “atypical”, “suspicious for malignancy” and “malignant” categories were 48.2%, 2.3%, 78.1%, 100.0% and 99.4%, respectively. Despite the heterogeneous nature of “neoplastic: other” category of PSC system, the absolute ROM for solid lesions was 100%. PaN-high category including only 2 endosonographically solid cases of high-grade IPMNs showed 100% ROM. There were no differences between PSC and WHO systems in sensitivity, specificity, negative and positive predictive value: excluding the “atypical” category, these were 99.7%, 95.6%, 97.7%, 99.5%, respectively. “Atypical” category considered as benign resulted in higher decrease in validity and negative predictive value, compared to “atypical” considered as true malignant (93.6% vs. 97.7%, and 65.8% vs. 97.7%).

1.4. Conclusions

The more than two punctures per EUS-FNA sampling with larger-diameter needle (19G or 22G) using the slow-pull and standard suction techniques in combination may decrease the probability of inconclusive cytological findings.

The WHO system was identical to the PSC system in terms of ROM and predictive values of categories for diagnosing solid pancreatic lesions. However, the reclassification of malignant lesions from the “neoplastic: other” (PSC IVb) category to the “positive for malignancy” (WHO VII) category not only harmonizes the systems but also enhances interdisciplinary communication, reducing the likelihood of misinterpreting pathological findings.

2. INTRODUCTION

Solid pancreatic lesions are a group of heterogeneous disease entities, that can be generally classified as either neoplastic (benign, premalignant and malignant) or non-neoplastic.[1] Most solid neoplasms are ductal adenocarcinomas and their subtypes, however, neuroendocrine tumors (NETs), solid pseudopapillary neoplasms (SPNs) and other rare primary and metastatic tumors can also show pancreatic involvement.[2] Non-neoplastic solid masses, like acute and chronic pancreatitis, autoimmune pancreatitis and intrapancreatic accessory spleen (IPAS) may mimic invasive cancer, therefore the diagnosis may be difficult and requires a multidisciplinary approach, including clinical, laboratory, imaging, cyto- and histopathologic and other ancillary studies.[3–5] Endoscopic ultrasound-guided tissue acquisition (EUS-TA) by fine-needle aspiration (FNA) and fine-needle biopsy (FNB) has become a key modality in the identification of solid pancreatic lesions and in the distinction of their benign and malignant origin with a high sensitivity, specificity, accuracy and safety, even in small lesions;[6,7] furthermore, it facilitates the therapeutic decision-making in terms of the precise staging and determination of resectability. The guideline of the European Society of Gastrointestinal Endoscopy (ESGE) recommends EUS-guided fine needle aspiration as a first-line sampling technique for suspected solid pancreatic neoplasms.[8,9]

Focusing on the side of sampling technique, numerous clinical trials and meta-analyses have demonstrated the efficacy and safety of this procedure. However, inconclusive cytological results (such as low cellularity or the presence of atypical cells of undetermined significance due to technical problems, bloodiness or other artifacts of the smears) still remain a major challenge in daily practice, as they do not allow for a definitive differential diagnosis between benign or malignant conditions.[10–12] In parallel with this, the growing number of cytological or minimal tissue samples have greatly challenged pathologists to provide accurate and reproducible diagnoses or interpretations. This raises two key issues for EUS-FNA sampling and pathological evaluation: how to reduce the proportion of inconclusive samples and how the pathologists can clearly communicate their findings to the multidisciplinary team involved in the management of pancreatic cancer, especially in the cases of uncertain or ambiguous sampling results. The two clinical studies in this thesis attempted to answer these questions.

In cases of inconclusive EUS-FNA samplings, re-evaluation of the pathology slides and surgery may be considered in addition to repeated sampling. However, these findings often lead to delays in treatment and increases the burden on patients and medical costs due to repeated interventions.[8,13] Therefore, the ideal solution to this problem would be to minimize the

proportion of EUS-FNA samples with inconclusive results as much as possible. To address this, the first retrospective study in this thesis focused on the determination of the frequency of these findings and assessing the examination- and patient-related risk factors associated with them. Furthermore, it examined the clinical outcomes of patients after EUS-FNA sampling of solid pancreatic lesions, particularly in relation to the risk of malignancy.

The second retrospective clinical study presented in this thesis focuses on the specificities of the interpretation of pathological findings by comparing the two currently accepted classification systems. We are witnessing a steady evolution of standardized reporting systems of pancreatic cytology: the Papanicolaou Society of Cytopathology (PSC) system guidelines and the atlas published in 2014 and 2015 classified the cytological diagnoses of the solid and cystic pancreaticobiliary lesions into the "nondiagnostic" (PSC I), "negative for malignancy" (PSC II), "atypical" (PSC III); "neoplastic: benign" (PSC IVa), "neoplastic: other" (PSC IVb), "suspicious for malignancy" (PSC V) and "malignant" (PSC VI) categories.[14,15] It also provided a guide to the definitions, terminology, diagnostic criteria, corresponding risk of malignancy (ROM), and a suggested therapeutic algorithm of each of the categories, strongly emphasizing the incorporation of radiological, biochemical, immunocytochemical and molecular based findings into the final cytopathology report. The newly published World Health Organization Reporting System for Pancreaticobiliary Cytopathology (WHO system) has been updated and refined with the PSC system, predominantly by reorganizing the heterogeneous tumors from neoplastic (IV) category into both established and newly created categories. *(Table 1)* The benign neoplasms (predominantly serous cystadenomas, SCAs) have been transferred from the "neoplastic: benign" to the "negative for malignancy" (WHO II) category, which also includes non-neoplastic lesions such as acute, chronic and autoimmune pancreatitis, pseudocyst, various benign cysts, and IPAS. Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) with low-grade dysplasia are shifted from PSC "neoplastic: other" to a newly formed "pancreatic neoplasm: low-risk/grade" category (PaN-low, WHO IV), however, the same entities with high-grade dysplasia now belong to the "pancreatic neoplasm: high-risk/grade" category (PaN-high, WHO V). The need for clear subdivision of the precursor intraductal/cystic neoplasms based on the severity of epithelial atypia (low-grade vs. high-grade) is strongly supported by some prospective studies, which indicated an increased ROM of 90-95.2% with high-grade atypia, and a ROM of 4.3-19% with low-grade atypia, respectively.[16,17] All low-grade malignancies (well-differentiated NETs and SPNs) previously classified into "neoplastic: other" of PSC system,

are now included in the "positive for malignancy" (WHO VII) category. With these modifications, the WHO system has seven interpretation categories: "insufficient/inadequate/nondiagnostic" (WHO I); "negative for malignancy" (WHO II); "atypical" (WHO III); "PaN-Low" (WHO IV); "PaN-High" (WHO V); "suspicious for malignancy" (WHO VI) and "positive for malignancy" (WHO VII).[18] In the current literature, only few recent studies are available that provide information on the ROM of each category that can be well translated into the new WHO system.[17,19,20] Therefore, the objective of our retrospective single-center study was to evaluate and compare the predictive value and ROM associated with the cytological categories of the WHO reporting system, contrasting them with the previously widely used PSC system in the diagnosis of solid pancreatic lesions.

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.[21]

(SCA – serous cystadenoma; MCN – mucinous cystic neoplasm; IPMN – intraductal papillary mucinous neoplasm; PanIN – pancreatic intraepithelial neoplasia; BilIN – biliary intraepithelial neoplasia; IOPN – intraductal oncocytic papillary neoplasm; ITPN – intraductal tubulopapillary neoplasm; PanNET – pancreatic neuroendocrine tumor; PanNEC – neuroendocrine carcinoma; PBL – pancreaticobiliary lymphoma; PDAC – pancreatic ductal adenocarcinoma)

3. AIMS

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

3.1.1. *Determination of the frequency and predictors of inconclusive cytological finding of the first pancreatic EUS-FNA sampling*

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

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

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

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

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

4. PATIENTS AND METHODS

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

4.1.1. 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 referral gastroenterology center in cooperation with the pathology department. All consecutive patients were enrolled between January 2014 and December 2021 who underwent EUS-FNA sampling for solid pancreatic lesions. The patients were divided into two subgroups based on the diagnostic value of the obtained EUS-FNA samples: subgroups of conclusive and inconclusive cytology. We considered cytological results inconclusive if they did not help establish a definitive diagnosis or reliably differentiate between the benign and malignant origins of the lesion. To objectively define these cases, we used the PSC system, which facilitates the interpretation of findings by providing information on the evaluability of the sample and the certainty of a malignant diagnosis.[22] All cytology cases classified as "nondiagnostic" (I) or "atypical" (III) in the PSC system were included in the group of inconclusive cases, regardless of the nature (benign or malignant) of the solid pancreatic lesion suggested by the EUS image. Furthermore, selected cases from the "negative for malignancy" (II) category were included in this group if malignancy was suspected based on the EUS image, due to the need for further diagnostic steps to validate the diagnosis. The "neoplastic: benign" (IVa), "neoplastic: other" (IVb), and "malignant" (VI) categories of the PSC system were classified as conclusive cytology subgroups, along with the "suspicious for malignancy" (V) category, due to its high risk of malignancy (ROM) in an appropriate clinical setting.[16,17]

The aims of the study were determined in relation to patients regardless of the number of EUS-FNA samplings performed during the study period. In assessing the predictors for the inconclusive cytological results, only the characteristics of the first EUS-FNA sample of the patient were evaluated to avoid possible bias due to the repetition of cases/factors. The predictors were identified by assessing the effect of patient-related (age, gender, location, and size of lesion, benign, or malignant final diagnosis) and procedure-related factors (investigator, size of needle, number of punctures per procedure, biliary stent placement prior sampling, diagnosis based on EUS image) on the two outcomes.

The ROM was determined based on the final diagnosis given at the end of patients' follow-ups, which was made in one of the following modalities: (1) conclusive repeated biopsy finding which could be obtained by repeated EUS-FNA, ultrasound-guided trans-abdominal biopsy,

endoscopic biopsy of tumor invading the upper gastrointestinal tract, etc., (2) surgical intervention (macroscopic morphology and/or histological examination); (3) autopsy finding; and (4) clinical course of the disease (in malignant cases - tumor progression or metastasis formation; in cases of inflammation - regression by imaging modalities or response to treatment, etc.). The clinical course of the disease was assessed after a follow-up of at least one month, except for patients who died from tumor-related causes within one month due to rapid cancer progression (although no autopsy was performed). The ROM was defined by the number of malignant cases divided by total number of cases within each category of clinical predictors. The efficacy data of EUS-FNA examinations were determined by the comparing the cytological findings with the final diagnosis. False-positive cases were defined as benign lesions which were incorrectly diagnosed as malignant by cytology. Similarly, false-negative cases were those in which a malignant neoplasm was incorrectly diagnosed as benign by cytology. Inconclusive "nondiagnostic" (I) and "atypical" (III) categories were considered to indicate the absence of malignancy. Therefore, these were classified as true-negative when the final diagnosis was benign and as false-negative when the diagnosis was malignant.

4.1.2. *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 enrolled all consecutive patients who underwent EUS-FNA sampling for solid pancreatic lesions at the University of Szeged, Hungary, from January 2014 to December 2021. The exclusion criteria were: (1) entirely or predominantly cystic pancreatic lesions confirmed during EUS examination; (2) EUS-FNA sampling of extrapancreatic lesions; (3) refusal of the patient to allow the use of clinical data for scientific purposes.

Each cytological finding was compared with pathological findings and/or clinical data obtained during follow-up to determine the absolute ROM. This was expressed as the absolute proportion of cases with a malignant final diagnosis within each category. To support a malignant diagnosis, histological samples obtained by other modalities (repeated biopsy, surgical specimen, autopsy) or, in their absence, the clinical evidence (weight loss, signs of local progression on endoscopy, gastric outlet or duodenal obstruction, rising tumor marker values) and radiologic evidence of neoplasm (disease progression, metastasis formation) were used. In calculating the ROM, malignant histologic follow-up findings included primary and metastatic malignancies such as carcinomas, NETs, NECs, SPNs, sarcomas, melanomas, and hematolymphoid tumors. Additionally, IPMNs with high-grade dysplasia were included, even in the absence of obvious invasion, due to their high risk of malignant transformation.[23,24]

Absence of clinical and/or radiologic evidence of disease, or lack of disease progression during the follow-up period, was considered indicative of a benign lesion. The relative ROM was determined as the ratio of absolute ROM of each diagnostic category to the absolute ROM of the "negative for malignancy" category (PSC II and WHO II).

The diagnostic predictive value of cytological categories was determined based on the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The "nondiagnostic" categories were excluded from the analysis because these cases were not suitable for pathological evaluation. A cytological finding was considered a false-positive if a benign neoplasm or a non-neoplastic lesion (e.g., chronic pancreatitis) was incorrectly diagnosed as malignant. Cases were regarded as false-negative if they were classified as non-neoplastic, benign neoplastic, or low-grade precursor neoplastic in either the PSC or WHO system, but the definitive diagnosis at the end of follow-up was malignant. The interpretation of "atypical" category (PSC III and WHO III) regarding neoplastic origin remains challenging. Therefore, three different assessment methods for this category were used in the analysis: (1) classifying as negative for malignancy; (2) classifying as positive for malignancy; (3) exclusion from the evaluation as diagnostically inconclusive cases.

4.2. EUS-FNA Procedure and Pathological Evaluation

EUS-FNA samplings were performed by two experienced endoscopists using linear echoendoscope (Olympus GF-UCT 140 or 160; Olympus Optical, Tokyo, Japan) and 19G, 22G, and 25G FNA needles (Echotip Ultra; Cook Ireland Ltd., Limerick, Ireland; EZ Shot 2 and 3, Olympus Optical, Tokyo, Japan). The punctures were performed using 5 or 10 mL continuous standard suction (SS) and/or slow-pull (SP) techniques with the same needle during approximately 7–10 back-and-forth movements performed in a fanning manner under continuous ultrasound control. The number of punctures, the suction force, and the size of the needle were not uniform, and depended on the endoscopist's preference and the characteristics of the lesion due to the retrospective nature of the study. The samples were used to prepare alcohol fixed direct smears, formalin-fixed paraffin-embedded (FFPE) cell blocks or small tissue fragments, and cytospins from needle rinsing fluid. The material obtained from the needle was spread onto slides with the reinsertion of the stylet, from which the grossly visible coherent pieces of tissue were removed and placed in a tube filled with 10% buffered formalin. This was done without macroscopic on-site evaluation (MOSE), and the tissue was processed according to the protocol for biopsy samples. Direct smears were made from the remaining specimen and fixed in 96% methanol for at least 10 minutes. The residual aspirated tissue was flushed out

from the needle to a native sampling tube and processed as cytospin preparations and/or cell blocks. Samples were prepared by EUS nurses or gastroenterologists assisting the endosonographer. No rapid on-site evaluation (ROSE) was done. The FFPE tissues, cytospin preparations and all direct smears were stained with hematoxylin-eosin (HE). Immunohistochemical testing was performed in most FFPE tissues and in selected cases of smears with high cellularity. The pathological diagnosis was based on the assessment of direct smears, cytospins, and FFPE cell blocks, which together were considered as a single EUS-FNA sample. No additional molecular studies for *KRAS* or *GNAS* mutations were performed. The smears were assessed by at least one of the three experienced cytopathologists involved in the study. In questionable cases, diagnoses were made based on the consensus of two pathologists. Throughout the whole study period, the PSC system was routinely used to classify pancreaticobiliary cytopathology findings and facilitate interdisciplinary communication. Consequently, PSC categories were assigned prospectively, while reclassification of cytological results into the WHO system was done retrospectively in 2022.

4.3. Ethics Approval and Consent to Participate

The methodology of both studies approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged, Hungary (ethics approval number: 182/2015 SZTE). All the included patients have signed an informed consent form for the scientific use of their medical data. The studies were carried out in accordance with the Declaration of Helsinki. The reporting of this study conforms to the STROBE statement.[25]

4.4. Statistical analysis

Statistical analysis was performed with R statistical software version 3.6.0 (R Foundation) and with SPSS software version 28 (SPSS Inc., Chicago, IL, USA); p values of less than 0.05 were considered significant. Descriptive statistics were expressed as means and medians with ranges. Categorical variables were reported as event rates and relative frequencies, and continuous variables as means with standard deviation and medians with ranges. Logistic regression model, Pearson Chi-squared, and Fisher's exact tests were applied to identify the clinical factors that can modify the incidence of inconclusive cytology and that can influence the ROM of pancreatic lesions. Fisher's exact test was also used to assess the statistical significance of the difference between the ROM values for each category and the "negative for malignancy" category (PSC II and WHO II).

5. RESULTS

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

A total of 473 patients with solid pancreatic lesions were enrolled and underwent 521 EUS-FNA examinations during the study period: in forty-four cases two samplings and in two cases three samplings were performed. For each patient, we assessed the outcome data from the first sampling. *(Figure 1)* Based on the EUS image, the endoscopist presumed the lesion to be malignant in 419 cases (88.58%) and benign in 55 cases (11.63%).

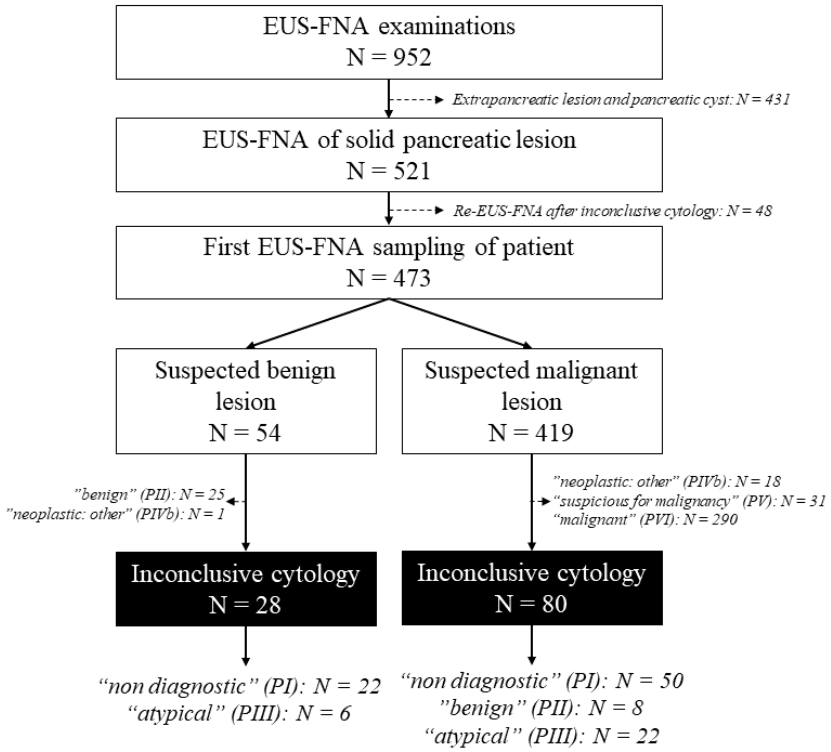

Figure 1. Patient enrollment in the study.

Most lesions were localized to the pancreatic head and uncinate process (n = 322, 68.08%) with a mean diameter of 33.83±14.18 mm (range 5–90 mm, median 30 mm). *(Table 2)* Cytological examination confirmed a definite neoplastic etiology in 340 cases (71.88%), including those classified as "malignant" (VI), "suspicious for malignancy" (V), and "neoplastic: other" (IVb) categories. Only 33 samples (6.98%) were classified as "negative for malignancy" (II) in the PSC system. There were no cases classified as "neoplastic: benign" (IVa) in the study cohort. In contrast, at the end of the mean follow-up of 13.77 months (range 0.1–106.4 months, median 5.67 months), the rate of neoplastic lesions was lower, at 83.51%. Of these, 392 cases (82.88%) were malignant, and 3 cases (0.63%) were benign neoplasms.

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		**Sampling technique**	
head	255 (53.91%)	only slow-pull (SP)	73 (15.43%)
uncinate process	67 (14.16%)	only standard suction (SS)	46 (9.73%)
body	90 (19.03%)	both SP and SS	354 (74.84%)
tail	60 (12.68%)		
diffuse	1 (0.21%)	**Size of EUS needle**	
Histology of lesion		19G	33 (6.98%)
Ductal adenocarcinoma	352 (74.42%)	22G	395 (83.51%)
Primary bile duct carcinoma	2 (0.42%)	25G	45 (9.51%)
Solid pseudopapillary npl.	3 (0.63%)		
Well-differentiated NET	15 (3.17%)	**Biliary stent**	129 (27.27%)
Neuroendocrine carcinoma	3 (0.63%)	**Type of lesion based on EUS image**	
Low-grade IPMN	1 (0.21%)	benign	54 (11.42%)
High grade IPMN (clinical suspicion of malignancy)	2 (0.42%)	malignant	419 (88.58%)
Myxofibrosarcoma	1 (0.21%)		
Hematolymphoid tumor	2 (0.42%)	**Cytological finding based on PSC system**	
Metastatic carcinoma	15 (3.17%)	"nondiagnostic"	72 (15.22%)
Ancient schwannoma	1 (0.21%)	"benign"	33 (6.97%)
Serous cystadenoma	1 (0.21%)	"atypical"	28 (5.92%)
Intrapancreatic spleen	1 (0.21%)	"neoplastic: other"	19 (4.02%)
Acute necrosing pancreatitis	12 (2.54%)	"suspicious for malignancy"	31 (6.55%)
Autoimmune pancreatitis	4 (0.85%)	"malignant"	290 (61.31%)
Chronic pancreatitis	31 (6.55%)		
Histologically unverified focal lesion disappeared during follow-up	27 (5.71%)		

Table 2. Clinical characteristics of patients and EUS-FNA examinations (n = 473).

The final diagnosis was validated histologically in 185 cases (39.11%), while in 288 patients (60.89%) the diagnosis was confirmed by the clinical course of the disease with a mean follow-up period of 10.54 months (range 0.1–106.4 months, median 2.0 months). The histologic specimens included 45 small biopsy samples with EUS-FNA or other modalities (24.32%), 107 surgical excision or resection specimens (57.84%), and 33 autopsy samples (17.84%). The sensitivity, specificity, and diagnostic accuracy of patients' first EUS-FNA sampling were

85.43%, 100.00%, and 87.74%, respectively, which increased to 89.92%, 100.00%, and 91.54% by repeated EUS-FNA of nondiagnostic cases. *(Figure 2)*

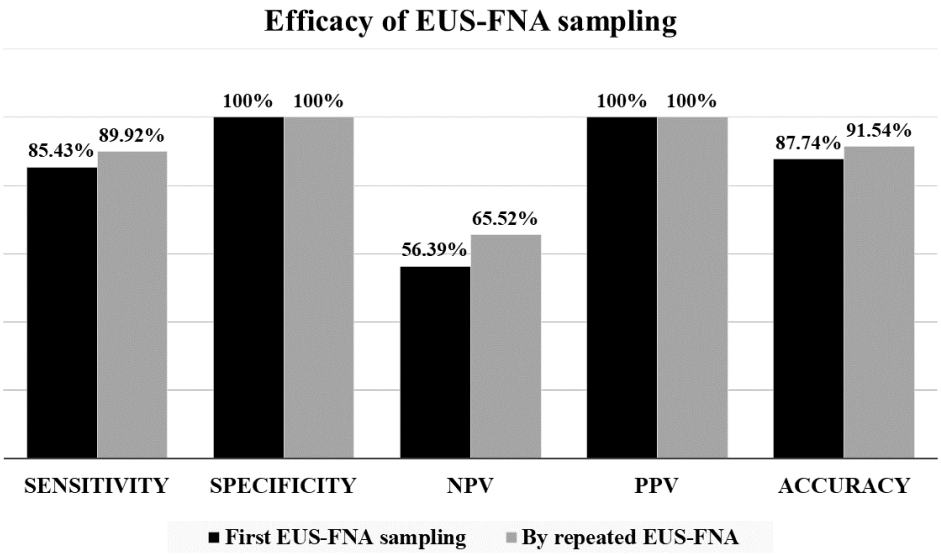

Figure 2. Efficacy of the EUS-FNA sampling of solid pancreatic lesions.

In 36 out of 46 cases, repeated EUS-FNA sampling was sufficient to establish the diagnosis. EUS-FNA sampling-related complications were recorded in five cases, which included one case of iatrogenic duodenal perforation, one case of gastrointestinal bleeding, one case of acute pancreatitis, and two cases of asymptomatic amylase elevation. Errors in the cytological diagnosis were identified in five cases. Two ductal adenocarcinomas were incorrectly diagnosed as NET, while in two cases, severe reactive abnormalities accompanying chronic pancreatitis complicated by acute inflammation were falsely interpreted as malignancies. Furthermore, one adenocarcinoma was initially reported as low-grade IPMN due to presumable peritumoral sampling.

5.1.1. Frequency and predictors of inconclusive cytological findings

The first EUS-FNA sampling of patients provided inconclusive results in 108 cases (22.83%), but there was no substantial fluctuation in the proportion of these cases over the study period. This rate varied between 16.67% and 25.58% over the years. Two examiners performed EUS-FNA sampling at our institute, and there was no significant difference between them in sampling efficacy or the proportion of inconclusive cases. *(Table 3)* Inconclusive samples were obtained more frequently for lesions ≤ 2 cm (43.42%) compared to lesions between 2–4 cm (23.35%, $p=0.001$) and ≥ 4 cm (10.71%, $p<0.001$). The use of the 19 G needle proved to be the most advantageous, but the difference compared to 22 G needles was not statistically significant

(OR 0.35, 95% CI [0.08–1.01], p=0.088). In contrast, the use of 25 G needles was associated with substantially higher odds of inconclusive findings (OR 2.12, 95% CI [1.09–4.01], p=0.023). The combined use of SP and SS techniques within a single EUS-FNA intervention reduced the proportion (20.34%) and risk (OR 1.70, 95% CI [1.06–2.70], p=0.027) of inconclusive cytology findings compared to the use of a single technique (30.25%). When comparing each technique using the combined method, a significant difference was detectable only in the case of SP (31.51%; OR 1.80, 95% CI [1.02–3.12], p=0.038). The use of 3 to 4 punctures per examination seems to be the most advantageous. Increasing the number of punctures did not reduce the risk of inconclusive findings. However, fewer than 3 punctures elevated the risk of inconclusive results (OR 2.49, 95% CI [1.49–4.14], p<0.001). The mean number of smears obtained per puncture had no influence on the rate of inconclusive results. Furthermore, EUS-FNAs that resulted in both direct smears and FFPE were not associated with a reduction in the rate of inconclusive cytology compared to samplings resulting in direct smears only (26.32% vs. 22.53%, p=0.594). The presence of a biliary stent did not increase the risk of inconclusive results (OR 1.08, 95% CI [0.67–1.71], p=0.748). The rate of successful EUS-FNA sampling was higher after metal stent implantation (absence of stent: 77.58%, plastic stent: 74.11%, metal stent: 83.87%), but the difference was not statistically significant (p=0.420).

The inconclusive results showed the strongest correlation with benign origin of the lesion determined by the end of follow-up, where their rate was 65.38% compared to 14.43% as seen in malignant cases (OR 11.20 CI 95% [6.56–19.54], p<0.001). This may also be due to the high rate of non-evaluable, particularly bloody, or cell-poor smears (“nondiagnostic” I) obtained when sampling benign lesions, significantly more often than in malignant lesions (47.44% vs. 8.86%, p<0.0001). Further reason for this may be that the smears with intact acinar cells or mild inflammatory abnormalities (“benign” II) may raise the possibility of peritumoral sampling if cross-sectional imaging and/or EUS images suggest suspicion of malignancy. When examining the effect of localization on the diagnostic value of sampling, we found that abnormalities in the pancreatic tail were associated with a remarkably low rate of inconclusive cases (6.67%) compared to other localizations (head 27.06%, uncinate process 25.37%, and body 20.00%, respectively).

Multivariate analysis confirmed the influence of four predictors on inconclusive findings: pancreas tail localization (OR 0.13 CI 95% [0.03–0.42], p=0.002), lesion size greater than 4 cm (OR 0.24 CI 95% [0.10–0.54], p = 0.001), and malignant EUS morphology (OR 0.11 CI 95%

[0.02–0.38], p=0.002) were associated with a decrease in risk, whereas the benign origin of the lesion (OR 56.97 CI 95% [17.40–272.78], p<0.001) led to an increase in risk.

	Conclusive N = 365	Inconclusive N = 108	Odds ratio (95% CI)	p value
Examiner				
ExA	274 (78.74%)	74 (21.26%)		
ExB	91 (72.80%)	34 (27.20%)	1.38 (0.86-2.20)	0.176
Location of lesion				
Head	188 (73.73%)	69 (27.06%)		
Uncinate process	50 (74.63%)	17 (25.37%)	0.92 (0.48-1.67)	0.781
Body	72 (80.00%)	18 (20.00%)	0.67 (0.37-1.19)	0.187
Tail	56 (93.33%)	4 (6.67%)	0.19 (0.06-0.49)	0.002
Size of lesion				
≤ 20 mm	43 (56.58%)	33 (43.42%)		
20 – 40 mm	197 (76.65%)	60 (23.35%)	0.40 (0.23-0.68)	0.001
≥ 40 mm	125 (89.29%)	15 (10.71%)	0.16 (0.08-0.31)	<0.001
Size of needle				
19G	30 (90.91%)	3 (9.09%)	0.35 (0.08-1.01)	0.088
22G	307 (77.72%)	88 (22.28%)		
25G	28 (62.22%)	17 (37.78%)	2.12 (1.09-4.01)	0.023
Sampling technique				
Both SP and SS	50 (68.49%)	72 (20.34%)		
SP or SS alone	33 (71.74%)	36 (30.25%)	1.70 (1.06-2.70)	0.027
Slow-pull (SP)	83 (69.75%)	23 (31.51%)	1.80 (1.02-3.12)	0.038
Standard suction (SS)	282 (79.66%)	13 (28.26%)	1.54 (0.75-3.02)	0.219
Number of punctures				
≤ 2 punctures	56 (62.22%)	34 (37.78%)	2.49 (1.49-4.14)	< 0.001
3-4 punctures	250 (80.39%)	61 (19.61%)		
> 4 punctures	59 (81.94%)	13 (18.06%)	0.90 (0.45-1.71)	0.763
Type of sample				
Only direct smears	28 (73.68%)	10 (26.32%)	1.23 (0.55-2.54)	0.594
Direct smears and FFPE	337 (77.47%)	98 (22.53%)		
Origin of lesion				
Benign	27 (34.62%)	51 (65.38%)	11.20 (6.56-19.54)	<0.001
Malignant	338 (85.57%)	57 (14.43%)		
EUS morphology				
Malignant	339 (80.91%)	80 (19.09%)		
Benign	26 (48.15%)	28 (51.85%)	4.56 (2.54-8.25)	<0.001
Presence of biliary stent				
Absence	256 (77.58%)	74 (22.42%)		
Presence	109 (76.22%)	34 (23.78%)	1.08 (0.67-1.71)	0.748
Plastic stent	83 (74.11%)	29 (25.89%)	1.21 (0.73-1.97)	0.453
Metal stent	26 (83.87%)	5 (16.13%)	0.67 (0.22-1.66)	0.720

Table 3. Predictors of inconclusive cytological findings (univariable analysis).

5.1.2. Outcomes of patients with inconclusive cytology results

By the end of the follow-up period, 57 cases (52.78%) in the inconclusive subgroup were found to be malignant. The final diagnosis was based on histopathological examination in 57 cases (52.78%) - including repeated EUS-FNA (n=7), transabdominal US-guided biopsy (n=19), surgical samples (n=24), and autopsy (n=7). In the remaining 51 cases (47.22%), the diagnosis was determined by the clinical course of the disease during a mean follow-up of 20.50 months (range 0.23–106.4 months, median 8.92 months). In 25 of these patients, the endosonographic image suggested benign disease, and no lesion was detected during the follow-up EUS examination which required repeated sampling. These histologically unidentified benign lesions were chronic pancreatitis (n=12), acute necrotizing pancreatitis (n=3), and autoimmune pancreatitis (n=1). Additionally, in nine cases, the lesion disappeared as observed through cross-sectional imaging and/or EUS during follow-up. In 13 of the 26 cases where the endosonographic morphology was suspicious for malignancy, benign disease was presumed based on cross-sectional imaging and/or repeated EUS examination results. This included cases where the focal lesion disappeared during follow-up (n=7), chronic pancreatitis (n=3), acute necrotizing pancreatitis (n=2), and a pseudocyst (n=1). Re-biopsy was waived for 13 patients with rapidly progressing underlying disease and deteriorating general condition due to lack of clinical relevance, as they were either no longer suitable for oncological treatment or had refused it.

5.1.3. Clinical factors influencing the ROM of EUS-FNA sampling

In the study cohort, the overall ROM of EUS-FNA was 83.51%, regardless of the EUS diagnosis that warranted the FNA sampling. The ROM was 88.11% for females and 78.60% for males (p=0.006). For the age groups, the risk was 73.08% for those below 60 years, 86.87% for those between 60 and 75 years, and 85.45% for those over 75 years. The mean age of patients with a malignant final diagnosis was significantly higher compared to patients with a benign diagnosis (67.4±10.9 years vs. 62.4±15.1 years, p=0.001). Lesion size had a significant correlation with ROM (p<0.001), as abnormalities smaller than 2 cm were more often benign (39.47%) compared to lesions between 2–4 cm (13.62%) and those larger than 4 cm (9.29%). *(Table 4)* Elevated CA19-9 (>27 U/mL) and CEA (>4.7 ng/mL) values above normal were also found more frequently in malignant cases (89.55% and 91.18%, p<0.001). The “nondiagnostic” (I) category showed no difference in the proportion of benign and malignant lesions at the end of follow-up (48.61% vs. 51.39%), whereas the “atypical” (III) category had a high ROM of 75.00%. The inconclusive subgroup comprised only those cytological specimens classified as

"negative for malignancy" (II), where malignancy was suspected based on EUS imaging. However, by the end of follow-up, malignancy was confirmed in only 11.11% of these cases. These values were even more pronounced when the entire study population was evaluated: the ROM for the "negative for malignancy" categories was 3.03%. Within the inconclusive subgroup, only one case judged to be benign using the EUS image had a final diagnosis of benign (ROM 3.57%), whereas the ROM for the EUS image suggestive of malignancy was 70.00%. These values were also slightly more explicit when we evaluated the entire population: ROM in benign EUS morphology was 3.70% compared to 93.79% for the ROM seen in malignant EUS images ($p<0.001$).

All cases N = 521	**Risk of malignancy**	**Odds ratio (95% CI)**	**p value**
Gender			
Female	88.11%	2.02 (1.23-3.36)	0.006
Male	78.60%		
Size of lesion			
≤ 20 mm	60.53%		
20 – 40 mm	86.38%	4.14 (2.31-7.42)	<0.001
≥ 40 mm	90.71%	6.37 (3.12-13.65)	<0.001
PSC category			
"nondiagnostic" (PI)	48.61%		
"benign" (PII)	3.03%	0.03 (0.00-0.17)	0.001
"atypical" (PIII)	75.00%	3.17 (1.25-8.90)	0.002
EUS morphology			
benign	3.70%	0.03 (0.00-0.01)	<0.001
malignant	93.79%		
Tumor markers			
CA 19-9 (n=270)		5.75 (2.99-11.22)	<0.001
CA19-9 elevation	89.55%		
CA19-9 normal	59.42%		
CEA (n=236)		3.94 (1.83-9.11)	0.001
CEA elevation	91.18%		
CEA normal	72.39%		

Table 4. Risk of malignancy (ROM) in patients with solid pancreatic lesion (univariable analysis). (*Limitation: data on CA19-9 and CEA were only available in 57.08% and 49.89% of patients, respectively).

In the subgroup of inconclusive cytological findings, the coexistence of identified predictors further increases the ROM. Specifically, in the "atypical" (III) category, lesions with malignant EUS morphology larger than 2 cm had a ROM of 94.74%. In contrast, in the "nondiagnostic" (I) and "negative for malignancy" (II) categories, the ROM for lesions smaller than 2 cm was

only 25.93%. This ROM decreased further to 0.00% for lesions with CA19-9 levels in the normal range and for those with benign EUS morphology.

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

A total of 473 patients with solid pancreatic lesions had undergone EUS-FNA biopsy with 521 specimens during the 8-year study period. The male-female ratio was 229:244. Mean age at time of sampling was 66.61±11.81years (range 18-95; median: 68). Lesions were located most frequently in the pancreatic head and uncinate process region (68.71%) and their mean diameter was 33.63±14.02 mm. In 482 cases, EUS-FNA sampling obtained both FFPE tissues and cytological smears, while in 38 cases only cytological specimens and in one case only FFPE tissue were forwarded for histological examination. The final diagnosis after an average follow-up of 13.77 months (range 0.1-106.4 months, median 5.67 months) was benign disease in 95 cases (18.43%) and malignant in 426 cases (81.76%). *(Figure 3)*

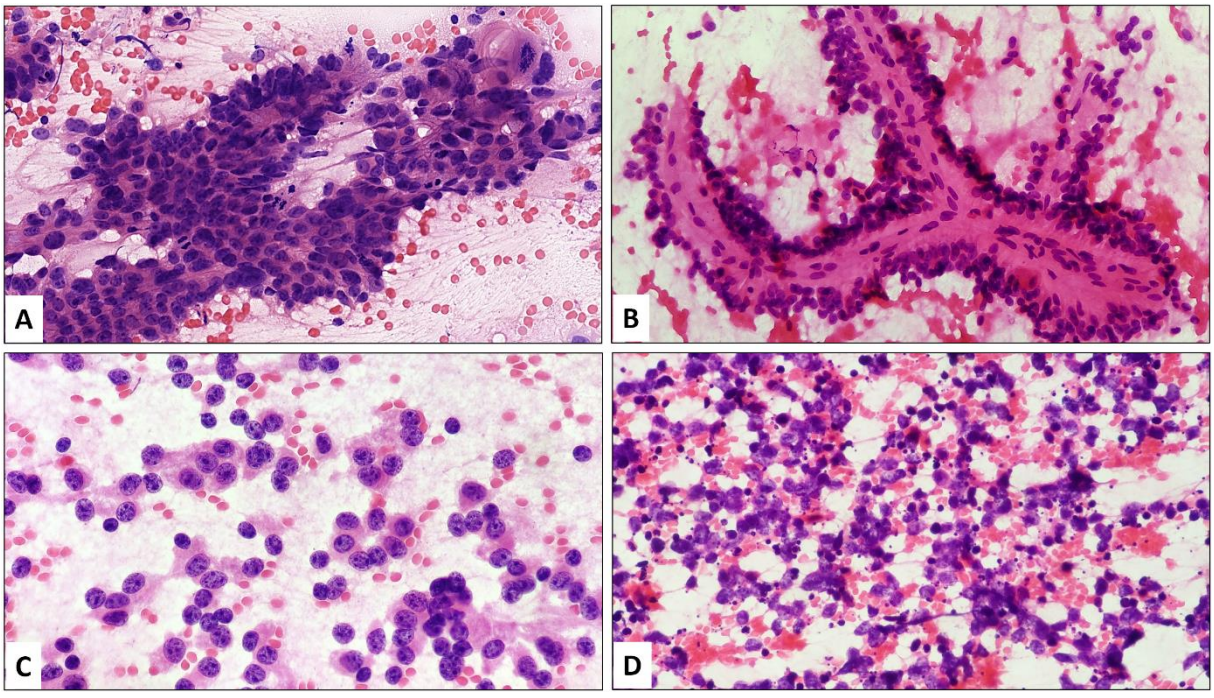

Figure 3. Samples representative of the most common pancreatic malignancies, including ductal adenocarcinoma (A), solid pseudopapillary neoplasm (B), neuroendocrine tumor (C), and metastatic small cell neuroendocrine carcinoma (D). Direct smears, H&E staining, 400x.

The histological classification and/or final clinical diagnosis of pancreatic lesions are shown in *Table 5*. Follow-up histologic reports were available for 205 cases (39.35%), and clinical follow-up data were used for 316 cases (60.65%). The histologic specimens included 40 small biopsy samples with other modalities, like transabdominal core needle biopsies and endoscopic

biopsies from tumors involving the stomach or duodenum (19.51%), 11 repeated EUS-FNA cell block samples (5.37%), 121 surgical excision or resection specimens (59.02%), and 33 autopsy samples (16.10%). In 60 patients with follow-up histology reports, the EUS-FNA sample was not suitable for diagnosis and was classified as "nondiagnostic" (PSC I and WHO I) or "atypical" (PSC III and WHO III). In 145 patients, the diagnosis was successfully established based on the EUS-FNA samples, which was confirmed by repeat histological examination in 140 cases.

Malignant (N = 426)		**Benign (N = 95)**	
Diagnosis	**N (%)**	**Diagnosis**	**N (%)**
Primary pancreatic cancer	**405 (95.07)**	**Benign pancreatic tumor**	**3 (3.16)**
- Ductal adenocarcinoma	375 (88.03)	- Low grade IPMN	1 (1.05)
- High-grade IPMN with the clinical suspicion of malignancy	2 (0.47)	- Microcystic serous cystadenoma	1 (1.05)
- Signet ring cell carcinoma	1 (0.23)	- Schwannoma	1 (1.05)
- Anaplastic carcinoma	4 (0.94)	**Non-neoplastic pancreatic lesion**	**59 (62.11)**
- SPN	3 (0.70)	- Acute necrotizing pancreatitis	13 (13.68)
- NEC	3 (0.70)	- Pseudocyst	2 (2.11)
- NET (well-differentiated)	16 (3.76)	- Autoimmune pancreatitis	5 (5.26)
- Myxofibrosarcoma	1 (0.23)	- Chronic pancreatitis	38 (40.00)
Bile duct carcinoma	**3 (0.70)**	- Intrapancreatic accessory spleen	1 (1.05)
Metastatic neoplasm	**16 (3.76)**	**Histologically unverified focal lesion disappeared during follow-up**	**33 (34.74)**
- Squamous cell carcinoma	4 (0.94)		
- Clear cell renal cell carcinoma	4 (0.94)		
- Small cell lung cancer	3 (0.70)		
- Lung adenocarcinoma	1 (0.23)		
- Colorectal carcinoma	1 (0.23)		
- Breast cancer	1 (0.23)		
- Malignant melanoma	1 (0.23)		
- Uterine leiomyosarcoma	1 (0.23)		
Hematolymphoid tumor	**2 (0.47)**		

Table 5. Histological classification and/or final clinical diagnosis of pancreatic lesions (SPN – solid pseudopapillary neoplasm; NEC – neuroendocrine carcinoma; NET – neuroendocrine tumor; IPMN – intraductal papillary mucinous neoplasm)

In 5 patients, diagnostic mistakes of histopathological assessment were revealed during follow-up by repeated sampling. Two cases of ductal adenocarcinoma were misdiagnosed as NETs due to misinterpretation of the initial technical difficulty to assess immunohistochemistry. In other two cases, severe reactive abnormalities accompanying chronic pancreatitis complicated by acute inflammation were mimicking the morphology of ductal adenocarcinoma (false positive). In one case, peritumoral EUS-FNA sampling was presumed in the background of histological

underestimation of the lesion, which was reported initially as chronic pancreatitis (false negative); the definitive diagnosis of ductal adenocarcinoma was confirmed by repeated transabdominal ultrasound guided biopsy within one month.

The PSC and WHO classification systems show a complete overlap in the definition of "nondiagnostic" (PSC I and WHO I), "negative for malignancy" (PSC II and WHO II), "atypical" (PSC III and WHO III) and "suspicious for malignancy" (PSC V and WHO VI) categories. Our study cohort had no cases in the "neoplastic: benign" (PSC IVa) category but included 20 cases in "neoplastic: other" (PSC IVb) category. Of these 20 cases, 3 SPN and 15 well-differentiated NETs were reclassified to "positive for malignancy" (WHO VII) category. There were no cases in "PaN-low" (WHO IV) category in our cohort, as this category, by definition, includes intraductal and/or cystic neoplasms with low-grade atypia. Despite the predominantly solid endosonographic features, the cytopathologist strongly suggested the diagnosis of high-grade IPMN of 2 cases, therefore these were transferred to "PaN-high" (WHO V) category. All components of the "malignant" (PSC VI) category were also shifted to the "positive for malignancy" (WHO VII) category.

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

In 40 of the 83 cases in the "nondiagnostic" (PSC I and WHO I) category, neoplastic lesions were confirmed at the end of follow-up. These included ductal adenocarcinoma in 34 cases, primary bile duct carcinoma in 2 cases, well-differentiated NET in 2 cases, and metastatic clear cell renal cell carcinoma in 2 cases. The unidentified benign lesions were most commonly chronic pancreatitis (n=19) and acute necrotizing pancreatitis (n=4), with one case each of autoimmune pancreatitis, pseudocyst, microcystic serous cystadenoma, and schwannoma. *(Table 6)* In addition, in 16 cases, disappearance of the lesion was noted by cross-sectional imaging and/or EUS during follow-up. Corresponding of these, the absolute and relative ROM of "nondiagnostic" (PSC I and WHO I) category were 48.19% and 21.23%, which is significantly higher compared to the "negative for malignancy" (PSC II and WHO II) category ($p<0.0001$). *(Table 7)*

Within the "negative for malignancy" (PSC II and WHO II) category, ductal adenocarcinoma was demonstrated during the follow-up of patients in one case, giving an absolute ROM rate of 2.27%. The clinical and endosonographic picture of this case suggested autoimmune pancreatitis, the repeated transabdominal ultrasound guided biopsy confirmed the final diagnosis within one month.

Diagnostic category defined by PSC system	Definitive diagnosis by the end of follow-up period		Total, N (%)
	benign, N (%)	malignant, N (%)	
I – Nondiagnostic	43 (51.81%)	40 (48.19%)	83 (15.93%)
II – Negative for malignancy	43 (97.73%)	1 (2.27%)	44 (8.45%)
III – Atypical	7 (21.88%)	25 (78.13%)	32 (6.14%)
IVa – Neoplastic: benign	-	-	-
IVb – Neoplastic: other	0 (0.00%)	20 (100%)	20 (3.84%)
V – Suspicious for malignancy	0 (0.00%)	37 (100%)	37 (7.10%)
VI – Malignant	2 (0.66%)	303 (99.34%)	305 (58.54%)
Total	95 (18.43%)	426 (81.76%)	521 (100%)
Diagnostic category defined by WHO system	**Definitive diagnosis by the end of follow-up period**		**Total, N (%)**
	benign, N (%)	**malignant, N (%)**	
I - Nondiagnostic	43 (51.81%)	40 (48.19%)	83 (15.93%)
II – Negative for malignancy	43 (97.73%)	1 (2.27%)	44 (8.45%)
III – Atypical	7 (21.88%)	25 (78.13%)	32 (6.14%)
IV – PaN-low	0 (0.00%)	0 (0.00%)	0 (0.00%)
V – PaN-high	0 (0.00%)	2 (100.00%)	2 (0.38%)
VI – Suspicious for malignancy	0 (0.00%)	37 (100%)	37 (7.10%)
VII – Positive (for malignancy)	2 (0.62%)	321 (99.38%)	323 (62.00%)
Total	95 (18.43%)	426 (81.76%)	521 (100%)

Table 6. Distribution of cytological categories proposed by WHO System and by PSC in the study cohort and their correlation with the definitive diagnosis of patients

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 7. Absolute and relative ROM of cytological categories proposed by WHO system and by PSC system for reporting pancreaticobiliary cytopathology

The absolute and relative ROM of “atypical” (PSC III and WHO III) category was 78.13% and 34.42%, respectively, which is significantly higher compared to the “negative for malignancy” (PSC II and WHO II) category ($p<0.0001$). The reason of indeterminate diagnosis was the markedly low cellularity of the smears and FFPE tissues in all cases, which was aggravated by a disturbing degree of blood contamination in 13 cases, pronounced inflammatory cells infiltration in 8 cases and significant contamination with upper gastrointestinal epithelial cells in 1 case. In “atypical” (PSC III and WHO III) category, the final diagnosis was based on histopathological examination in 62.50% of cases (repeated EUS-FNA n=5, transabdominal US-guided biopsy n=8, surgical sample n=4, autopsy n=3). The clinical course of the disease during follow-up was used to determine the final diagnosis in 12 cases. In 5 of these patients, the endosonographic image was suggestive for benign disease and no lesion was detected by follow-up EUS examination requiring repeat sampling. The re-biopsy of 7 patients with rapidly progressive underlying disease and deteriorating general condition was waived due to a lack of clinical relevance, as they were either no longer suitable for oncological treatment or had refused it. The definitive diagnosis at the end of follow-up was malignant in 25 cases (ductal adenocarcinoma n=22, primary bile duct carcinoma n=1, metastatic small cell lung cancer n=1, hematolymphoid tumor n=1) and benign in 7 cases (chronic pancreatitis n=3, focal lesion disappeared during follow-up n=2, acute necrotizing pancreatitis n=1, low-grade IPMN n=1).

Despite the heterogeneous nature of “neoplastic: other” (PSC IVb) category of PSC system, the absolute and relative ROM for solid lesions were 100% and 44.05%. This category included 14 well-differentiated NETs, 2 high-grade IPMNs, 3 SPNs and 1 ductal adenocarcinoma. It is important to note that one ductal adenocarcinoma was misdiagnosed by cytology as NET and therefore included in this category, however this did not affect the ROM. The 2 high grade IPMN cases of “PaN-high” (WHO V) category were considered malignant due to high-risk of malignant transformation with 100% absolute and 44.05% relative ROM. In these cases, the malignant nature of these tumors is supported only by clinical and radiological progressive disease course, histological verification of invasion was impeded by patients’ refusal of curative surgery.

All the cases in the “suspicious for malignancy” (PSC V and WHO VI) category also had a definitive diagnosis of malignancy (ductal adenocarcinoma n=35, signet ring cell carcinoma n=1, metastatic lung adenocarcinoma n=1). In the “malignant” (PSC VI and WHO VII) category, 2 ductal adenocarcinoma diagnoses were overestimated, being chronic pancreatitis with acute inflammation (false positive). The absolute and relative ROMs were 99.34% and

43.76% for “malignant” (PSC VI) and 99.38% and 43.78% for “positive for malignancy” (WHO VII) categories, respectively, due to the different case numbers.

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

Excluding “nondiagnostic” (PSC I and WHO I) and inconclusive “atypical” (PSC III and WHO III) categories, the sensitivity, specificity, NPV, PPV and validity of the cytopathological evaluation using the PSC and WHO systems were identical (99.72%, 95.56%, 97.73%, 99.45% and 99.26%). *(Table 8)* No substantial improvement in the sensitivity of pathological assessment could be achieved by including the “atypical” category in the analysis. By considering the “atypical” category as malignant, a substantial reduction in specificity was seen in both the PSC and the WHO system (from 95.56% to 82.69%). When the “atypical” category was considered as benign, a comparable reduction in sensitivity (from 99.72% to 93.26%), NPV (from 97.73% to 65.79%) and validity (from 99.26% to 93.61%) was observed.

	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 8. Predictive value of cytological categories proposed by WHO system and by PSC system based on the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

6. DISCUSSION

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

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

The diagnostic accuracy achieved in our study is consistent with the results published in international studies and high-quality meta-analyses, with a pooled sensitivity of 84–89% and a specificity of 96–99%.[6,26,27] Despite the convincing data, the NPV of EUS-FNA for suspected pancreatic tumors is considered low, and in our study, it was also barely above 50%; furthermore, inconclusive (atypical cells, suspicious for malignancy), negative for malignancy, or nondiagnostic results do not allow for the definitive diagnosis of benign conditions. We found that larger needle diameters were associated with a decrease in the rate of inconclusive cytological findings. This contradicts the results of a meta-analysis published in 2019, in which no significant difference was observed between the 22G and 25G needles used during EUS-FNA in the diagnosis of solid pancreatic lesions based on randomized trials.[28] Although most retrospective cohort studies have shown no difference in the efficacy of conventional 19G, 22G, and 25G needles, recent clinical trials have reported that conventional needles are inferior to newer types of FNA and FNB needles. The recently developed Franseen needles have shown superior diagnostic accuracy in EUS-guided sampling compared to conventional needles, especially in patients requiring immunostaining.[29] The novel fork-tip FNB needles were also found to be superior to FNA needles in terms of the proportion graded as a straightforward diagnosis (69% vs. 51%) and median pathology viewing time (188 vs. 332 s; $p<0.001$).[30] These two FNB needle types achieved the highest degree of cellularity in a single biopsy, with a diagnostic accuracy greater than 90%.[31] Furthermore, the FNB needles also outperformed FNA needles in the sampling of pancreatic and nonpancreatic lesions in terms of diagnostic accuracy (87% vs. 80%, $p=0.02$) and tissue core rate (80% vs. 62%, $p=0.002$).[32]

In addition to the type of needle applied, the technique of EUS-FNA sampling may also influence the outcome of the sampling. In our study, the combined use of the SP and SS techniques in a single examination, along with 3–4 punctures per sampling, resulted in the lowest proportion of inconclusive cytology findings. It should be noted that the fanning technique was used in all EUS-FNA cases, regardless of suction force, as previous studies have demonstrated its superiority over the standard approach.[33] A guideline on the technical aspects of the EUS-FNA was published in 2017 and has not been updated yet.[34] It

recommends the use of 10 mL standard suction for the EUS-guided sampling of solid masses with 25G or 22G FNA needles, however, the results of recently published prospective and retrospective clinical trials have questioned this practice.[35–37] A meta-analysis published in 2023, which compared the efficacy of SP, dry-suction, modified wet-suction, and no-suction techniques, found that the modified wet-suction technique provided the highest rate of sample adequacy; furthermore, dry suction was associated with significantly higher rates of blood contamination as compared with the SP technique (OR 1.44, 95% CI [1.15–1.80]).[38]

The number of punctures required to achieve the optimal diagnostic accuracy of EUS-FNA is still unclear.[39] The ESGE guideline recommends performing three to four needle punctures with an FNA needle or two to three punctures with an FNB needle when ROSE is unavailable.[34] In contrast, the white paper of the AGA also considers the size of the lesion when making recommendations based on clinical trials: in the absence of ROSE, optimally, four punctures should be performed to achieve the highest diagnostic accuracy in pancreatic solid lesions >2 cm in size and at least six punctures in lesions <2 cm.[40,41] Studies published in recent years have reported varying conclusions regarding the effect of pancreatic tumor size on the diagnostic yield of EUS-FNA. Uehara et al. highlighted that EUS-FNA was accurate in the evaluation of suspected pancreatic malignancies regardless of the size and location of lesion.[42] However, in another study, this accuracy was only achievable when ROSE was available.[43] Similar to several other studies, we have also verified that the size of the lesion influences the outcomes of EUS-FNA.[41,44,45] In our study, the rate of inconclusive cytological results was significantly increased for lesions smaller than 2 cm. The lower rate of inconclusive results in larger lesions may be explained by the fact that they are easier to identify and less frequently sampled peritumorally. Another important reason may be the high ROM of these lesions. Our study also pointed out that the most important predictor of inconclusive cytological findings is a benign final diagnosis. However, it should also be considered that in cases of large lesions, the risk of necrotic areas within the tumor is higher, as cells obtained from these areas are unsuitable for establishing a diagnosis. Furthermore, sampling particularly vascularized areas could also be disadvantageous due to massive blood contamination obscuring tumor cells on the smears. The stiffness of the tumor and the degree of fibrosis can also affect the effectiveness of sampling, as it is assumed that aspirating cells from hard, fibrotic cancers requires a technique using greater suction power.[46] Both pancreatic carcinomas and chronic pancreatitis are typically hard lesions due to prominent fibrotic stromal reactions. A retrospective study performed by Togliani et al. found that the adequacy of EUS-guided tissue

acquisition was negatively affected by the presence of fibrosis (OR 8.37 CI 95% [2.33–30.0]), and by the location of the lesion in the head/uncinate process (OR 0.37 CI 95% [0.14–0.99]).[47] However, the higher prevalence and grading of tissue fibrosis in lesions located in the head or uncinate process appear to negatively impact sample adequacy. In our study, there was no clear correlation between tumor location and the rate of inconclusive cytology results. The lesions in the pancreatic tail were associated with a significantly lower rate of inconclusive findings (6.67%) compared to other location; however, it is questionable whether this factor can be considered a true predictor of inconclusive cases, since this localization was the least frequent in the study population (n=60) and this group had a low rate of benign lesions (13.33%), lesions smaller than 2 cm (6.67%), and needle diameter of 25G (1.67%).

In our study, the proportion of conclusive results was not higher in EUS-FNA examinations where both direct smears and FFPE samples were obtained, compared to those where only direct smears were obtained. This may also be explained by the fact that in a large proportion of cases, the FFPE sample was not histologically evaluable and appeared only as a blood coagulum. Therefore, the proportion of conclusive cytology findings for FFPE samples was only slightly above 70%. The use of MOSE could be an alternative solution for the assessment of the adequacy of specimens if ROSE is unavailable, potentially enhancing the diagnostic yield of FFPE samples.[48,49] However, during the study period at our institute, MOSE was not implemented. A prospective pilot study by Iwashita et al. determined that the ideal cut-off value for the length of the macroscopically visible core on MOSE, indicating the presence of a histologic core specimen, is 4 mm. This achieved a sensitivity of 93.1% and specificity of 72.0%.[50] One of the most significant advantages of FFPE samples, as opposed to direct smears, is that they provide tissue architectural information in addition to cytomorphology. Moreover, they are compatible with a wide range of molecular and immunohistochemical techniques. Immunohistochemistry often proves essential for the differential diagnosis and prognostic evaluation of tumors, including pancreatic metastases and neuroendocrine tumors.[51,52]

6.1.2. *The outcome of disease in patients with inconclusive cytology results and the clinical factors influencing ROM*

We found a very strong correlation between PSC categories, EUS morphological diagnosis, and the ROM. The final diagnosis was 75.00% malignant in the “atypical” (III) category and 3.03% in the “negative for malignancy” (II) category, while the ROM for benign and malignant EUS morphological diagnoses was 3.70% and 93.79%, respectively. This may be explained by the

fact that a benign diagnosis is made with the utmost caution by both the gastroenterologist and the pathologist. The pathologists classified cytological findings as “negative for malignancy” (II) in very select cases: (i.) when there was a complete absence of cellular atypia and the EUS findings were negative for a neoplastic process, and (ii) when cytologic features were characteristic of specific non-neoplastic lesions, such as autoimmune pancreatitis or ectopic spleen. The situation was similar for a diagnosis established based on the EUS image by gastroenterologists. The “nondiagnostic” (PI) cytological findings, however, did not provide any guidance on the choice of further diagnostic steps to be taken and were not related to the ROM. These results are in accordance with international data.[53] The systematic review of eight studies by Nikas et al. showed that the ROM of PSC categories varied widely: the ROM of the “non diagnostic” (I), “negative for malignancy” (PII), and “atypical” (PIII) categories were in the ranges of 8–50%, 0–40%, and 28–100%, respectively.[54] The largest meta-analysis, which included 3566 patients from 23 studies, separately assessed the outcomes of atypical cytological findings from EUS-FNA in solid pancreatic masses and found that the ROM of this category was 58% (95% CI 47%–69%).[55] The presence of a mass and absence of a history of pancreatitis were significant predictors for pancreatic malignancy in cases of the cytological diagnosis of “atypical cells”, while the absence of a mass in the EUS images or history of chronic pancreatitis was more likely to be associated with a benign lesion.[56] The authors are in agreement that institutions (both cytopathologists and endoscopists) should monitor and keep their “atypical” cytology rates low, but there is no consensus recommendation or guideline that defines atypical cytology rate as a quality indicator or determines its minimum standard value. In our institute, the rate of “atypical” (III) PSC category was low, with 5.92%.

6.1.3. Advantages and limitations of the study

The advantage of our study is that it was carried out in close collaboration with experienced cytologists in the department of pathology and the PSC classification system for solid pancreatic tumors was routinely applied during the study period to facilitate interdisciplinary communication. EUS-FNA sampling was performed by one of the two endosonographers and the cytological samples were assessed by at least one of the three experienced pathologists, and in challenging cases by two of them. The small number and similar level of expertise of doctors involved in the evaluation allowed for the elimination of interobserver variability.

The greatest limitation was its single-center, retrospective cohort nature, which resulted in restricted availability of clinical data on patients’ symptoms (e.g., abdominal pain, jaundice, weight loss), and tumor marker findings (e.g., CEA, CA19-9, CgA). The gastroenterological

evaluation of pancreatic lesions and EUS-FNA samplings were performed at our institute as a tertiary-level referral medical center, however, the patients' follow-up was frequently performed in primary- or secondary-level medical institutions, which limited the availability of these data. Additionally, confirmatory cytological and/or histological sampling was performed in only a small number of patients, so the definitive diagnosis was determined mainly based on the behavior of the disease during follow-up. The absence of MOSE and ROSE might have affected the initial diagnostic accuracy and the rate of inconclusive results.

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

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

The clinical significance of the "atypical" (PSC III and WHO III) category is notable as it does not permit a definitive differential diagnosis between benign and malignant conditions. This often results in delays in treatment, increasing the burden of patients and raising medical costs due to repeated interventions. Interpreting this category poses challenges in establishing the predictive value of cytological categories. If categorized as positive for malignancy, specificity decreases (82.69%) and the number of false positives (n=9) rises. Conversely, if classified as negative for malignancy, the NPV decreases (65.79%) and the number of false negatives (n=26) increases. The classification system demonstrates its highest validity (99.26%) when the atypical category is excluded from the analysis, consequently, our results confirmed the inconclusive nature of this category and that it does not contribute to the diagnostic process. Our findings align with studies assessing the predictive value of both the PSC system and the WHO system.[17,19,20,53] Reducing the proportion of cytological findings in the "atypical" (PSC III and WHO III) category may be the ideal solution for these problems, therefore institutions (both cytopathologists and endoscopists) should monitor and keep the rate of this low. In terms of sampling, the proportion of inconclusive cytological findings including the "nondiagnostic" (PSC I and WHO I) and "atypical" (PSC III and WHO III) categories is influenced by the characteristics of the lesion (uncinate process location, less than 2 cm in size, presence of necrotic areas, increased vascularization, benign etiology) and the technical aspects of EUS-FNA (smaller needle diameter, suction technique, use of contrast enhanced EUS or EUS elastography, technique of smear preparation, obtaining FFPE samples, ROSE, MOSE).[42–44,46,57] Previous studies have shown a significant variation among cytopathologists in the use of indeterminate diagnostic categories.[58,59] The enhancement of

cytological diagnostic criteria, standardization of specimen quality evaluation, and training for cytopathologists have the potential to enhance agreement among cytopathologists, which would lead to increased repeatability of cytological diagnosis and reduction of inconclusive and false negative cases.[60] Unfortunately, there is currently no guideline that defines the "atypical" cytology rate as a quality indicator or specifies its minimum standard value.[61]

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

Several prospective and retrospective studies have assessed the ROM values of the standardized categories of the PSC system, showing significant variation in most categories. Exceptions to this trend are the "suspicious for malignancy" (PSC V) and "malignant" (PSC VI) categories, where consistently high ROM has been reported. In the systematic review including eight studies conducted by Nikas et al. before shifting into the new WHO system, the absolute ROM values for PSC I, II, III, IVb, V, and VI categories were 8-50%, 0-40%, 28-100%, 0-34%, 82-100%, and 97-100%, respectively.[54] The common categories between the PSC and WHO reporting systems, which have not undergone substantial changes in relation to solid pancreatic lesions, are "nondiagnostic" (PSC I and WHO I), "negative for malignancy" (PSC II and WHO II), "atypical" (PSC III and WHO III) and "suspicious for malignancy" (PSC V and WHO VI), so there should be no difference in the value of ROM between the reporting systems. The difference in the risk stratification of the two reporting systems could only be the result of the reclassification of the PSC IVa and PSC IVb categories. This reclassification resulted in the establishment of PaN-low (WHO IV) and PaN-high (WHO V) categories. Additionally, all malignant tumors, including low-grade ones, were transferred to the "positive for malignancy" (WHO VII) category. In our study cohort, only 3.84% of cases needed to be reclassified due to the transition to the WHO system, which did not result in significant changes in the relative and absolute ROM values of the categories, which were as follows: PSC IVb 100% and 44,05%, PSC VI 99,34% and 43,76%, "PaN-high" (WHO V) 100% and 44,05%, WHO VII 99,38% and 43,78%, respectively. A retrospective study carried out by Lui et al. evaluated the absolute ROM of 2562 EUS-FNA samples in seven standardized categories of the WHO system and found that it was 50%, 29%, 70%, 15%, 100%, 99% and 100% for solid pancreas lesions.[62] Simultaneously, it was emphasized that the absolute ROM value is significantly influenced by whether the lesion exhibits cystic or solid morphology. In their study cohort, the absolute ROM for cystic lesions exhibited notable distinctions from those of solid lesions, with values of 7%, 0%, 19%, 13%, 38%, 78%, and 100% in WHO I-VII categories. Remarkably, in this study, the

ROM value for the “negative for malignancy” (WHO II) category is very high, exceeds the value observed in the majority of other studies.[20,21,54] The observed differences may primarily stem from a notable level of interobserver variability among cytologists in the assessment of EUS-FNA samples.[63] In our cohort, the absolute ROM in this category was only 2.27%. The explanation for this is that pathologists categorized cytological findings as “negative for malignancy” (WHO II) in very selective cases, specifically when the cytologic features were characteristic for certain non-neoplastic lesions (such as chronic pancreatitis, autoimmune pancreatitis, ectopic spleen) and/or when the complete absence of cellular atypia correlated with EUS findings indicating no malignant involvement.

6.2.3. *Strengths and limitations of the study*

A significant strength of our study is the relatively large number of included cases and the creation of a clinically uniform cohort by including only solid pancreatic lesions. Solid and cystic pancreatic lesions exhibit substantial differences in incidence, malignant potential, clinical behavior, diagnostic and therapeutic protocols, as well as prognosis. Furthermore, previous studies have highlighted that the standardized categories of the PSC and WHO systems show significant variations in risk of malignancy (ROM) values for solid versus cystic lesions.

The most important limitation of our study is its single-center retrospective design. Additionally, it is important to note that the final diagnosis relied on clinical follow-up data in more than 60% of cases, with histological diagnosis confirmed by follow-up histologic reports in only 39.35% of cases. The study did not utilize MOSE and ROSE, which might have affected the adequacy of cytological samples and their subsequent diagnostic yield.

7. CONCLUSIONS

Our first retrospective cohort study found that the rate of inconclusive EUS-FNA findings in the sampling of solid pancreatic lesions can be successfully reduced by using larger diameter needles (22G and 19G) and by the combined use of SP and SS techniques within a single intervention. In addition, three or four punctures per procedure were associated with the highest clinical effectiveness without the use of ROSE: fewer than two punctures increased the proportion of inconclusive cases, whereas more than four punctures did not improve the sampling efficiency.

The EUS morphology of lesions showed the closest correlation with ROM, therefore, the endoscopist's proficiency, the thoroughness of the examination, and the adequate evaluation of lesions (description, image documentation) are of critical importance in the interpretation of inconclusive cases. Based on this, our recommendation is that in the case of EUS morphological signs suggestive for a benign lesion and "negative for malignancy" (PII) cytological findings, a follow-up with the patient may be sufficient; in contrast, repeated sampling is required if malignancy is suspected based on EUS morphology or in the cases of "nondiagnostic" (PI) and "atypical" (PIII) cytological categories.

Our second retrospective cohort study confirmed that the WHO system was identical to the PSC system in terms of ROM and predictive values of categories for diagnosing solid pancreatic lesions. However, the reclassification of malignant lesions from the "neoplastic: other" (PSC IVb) category to the "positive for malignancy" (WHO VII) category not only harmonizes the systems but also enhances interdisciplinary communication, reducing the likelihood of misinterpreting pathological findings.

The large number of cases included in our study allowed us to draw two practical recommendations on the ROM interpretation of categories:

- The relatively low rate of absolute ROM of the "negative for malignancy" category (PSC II and WHO II) in our study (2.27%) was attributed to the strict adherence to the definition determined by WHO system (specific diagnosis of non-neoplastic or benign neoplastic lesion can be made, or when cytology reveals solely normal pancreatic cells without any evident mass lesion on ultrasound). Therefore, we recommend a judicious application of the "negative for malignancy" category due to the potential for false negatives, which may be caused by sampling error.
- Our study also confirmed that specimens categorized as "atypical" (PSC III and WHO III) are associated with malignancy in almost 80% of cases but may lead to delay in diagnosis

due to their inconclusive nature. Therefore, the proportion in this category should be reduced, which could be facilitated by specific training of pathologists or by the evaluation of questionable cases by multiple pathologists.

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10. FIGURES AND TABLES

Figure 1. Patient enrollment in the study.

Figure 2. Efficacy of the EUS-FNA sampling of solid pancreatic lesions.

Figure 3. Samples representative of the most common pancreatic malignancies, including ductal adenocarcinoma (A), solid pseudopapillary neoplasm (B), neuroendocrine tumor (C), and metastatic small cell neuroendocrine carcinoma (D). Direct smears, H&E staining, 400x.

Table 1. Comparison of the PSC and WHO reporting system: lesions in red represent changes the classification of tumors in the WHO system compared to the PSC system. (SCA − serous cystadenoma; MCN − mucinous cystic neoplasm; IPMN − intraductal papillary mucinous neoplasm; PanIn − pancreatic intraepithelial neoplasm; BilIn − biliary intraepithelial neoplasm; IOPN − intraductal oncocytic papillary neoplasm; ITPN − intraductal tubulopapillary neoplasm; PanNET − pancreatic neuroendocrine tumor; PanNEC − neuroendocrine carcinoma; PBL − pancreaticobiliary lymphoma; PDCA − pancreatic ductal adenocarcinoma)

Table 2. Clinical characteristics of patients and EUS-FNA examinations (n=473).

Table 3. Predictors of inconclusive cytological findings (univariable analysis).

Table 4. Risk of malignancy (ROM) in patients with solid pancreatic lesion (univariable analysis). (*Limitation: data on CA19-9 and CEA were only available in 57.08% and 49.89% of patients, respectively).

Table 5. Histological classification and/or final clinical diagnosis of pancreatic lesions (SPN – solid pseudopapillary neoplasm; NEC – neuroendocrine carcinoma; NET – neuroendocrine tumor; IPMN – intraductal papillary mucinous neoplasm).

Table 6. Distribution of cytological categories proposed by WHO system and by PSC in the study cohort and their correlation with the definitive diagnosis of patients.

Table 7. Absolute and relative ROM of cytological categories proposed by WHO system and by PSC system for reporting pancreaticobiliary cytopathology.

Table 8. Predictive value of cytological categories proposed by WHO system and by PSC system based on the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).