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Clinical Impact of Molecular Biomarkers in the Management of Patients with Pancreatic Cysts

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Abstract

Background: Pancreatic cysts are common and often discovered incidentally. Accurate differentiation is essential to guide management, as some cysts may progress to malignancy. Common pancreatic cyst includes intraductal papillary mucinous neoplasms, pseudocysts, neuroendocrine tumors, serous cystadenoma, and mucinous cystic neoplasms. Current diagnostic approaches, including magnetic resonance imaging, endoscopic ultrasound, biochemical assays, and cytopathology, have an accuracy of approximately 70% in distinguishing benign from precancerous lesions. Molecular analysis of intracystic fluid, particularly KRAS and GNAS mutations, increases the diagnostic accuracy by up to 90%. These mutations are indicative of mucinous cystic lesions; however the efficacy of next-generation sequencing in routine clinical practice remains poorly studied.

Methods: This retrospective study included 85 patients who underwent EUS-guided fine-needle aspiration for pancreatic cysts at a single center between 2014 and 2021. Clinical data, EUS features, cytopathological results, and molecular testing (KRAS/GNAS mutations) were analyzed.

Results: Among the patients (mean age 63, 39% male), 73% of cysts were discovered incidentally. The most common type of cyst was intraductal papillary mucinous neoplasm (58%). KRAS and/or GNAS mutations were present in 16 patients with mucinous lesions, including 12% of the cases without elevated carcinoembryonic antigen. KRAS/GNAS mutations had 94.4% specificity but 23.7% sensitivity for mucinous differentiation. Surgery revealed high-grade dysplasia or cancer in 11 of the 25 operated cases.

Conclusions: This study found that molecular analysis enhances the classification of pancreatic cysts but does not improve the detection of malignancy. Further research is needed to characterize the role of molecular biomarkers in pancreatic cyst management.

Keywords: Pancreatic cysts; Mucinous cysts; Non-mucinous cysts; Molecular analysis

Introduction

Pancreatic cysts (PCs) are typically discovered incidentally following the increased use of abdominal imaging. The reported prevalence of PCs in patients aged over 40 years is 2.6% and up to 49.1% using computed tomography and magnetic resonance imaging (MRI), respectively [1-3]. Prevalence increases with age and is estimated to be between 10% and 15% at age 75 and more than 20% after 80 years [4]. The malignant potential of PCs depends on the etiology of the lesion. The American Gastroenterological

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Association's technical review of incidental PCs estimated an annual incident risk of malignancy of 0.25% with a prevalent malignant risk of 0.25% at the time the cyst was identified [5]. PCs can be classified into two categories: neoplastic (mucinous and non-mucinous) and non-neoplastic cysts (e.g., pseudocysts). The most frequently encountered benign PCs include serous cystadenoma (SCA), mucinous cystic neoplasm (MCN), and intraductal papillary mucinous neoplasm (IPMN). Mucinous PCs have the potential to progress to invasive pancreatic ductal adenocarcinoma (PDAC), whereas non-mucinous and non-neoplastic PCs have no risk of malignancy. Neoplastic cysts such as SCAs and pseudocysts do not progress to malignancy and require conservative follow-up. Nevertheless, low-risk mucinous cysts, namely IPMNs and MCNs with low-grade dysplasia (LGD), require surveillance because of their possible progression to malignancy. Surgery is indicated for high-risk mucinous/malignant lesions, including premalignant cysts such as IPMNs and MCNs with high-grade dysplasia (HGD), and malignant cysts (PDACs, or IPMNs and MCNs with invasive carcinoma). Table 1 summarizes the epidemiology and characterization of different types of PCs. According to the Sendai guidelines first published in 2006 by the International Association of Pancreatology [6] and subsequently revised in 2012 (Fukuoka guidelines) [7] and then in 2017 (Sendai guidelines) [8], surgical resection is recommended for main duct-type IPMNs (MD-IPMNs), mixed main and branch duct-type IPMNs, and MCNs. Depending on clinical symptoms and imaging features, branch duct-type IPMN (BD-IPMN) is managed by surgery or surveillance. Using the Sendai guidelines, high sensitivity (90-100%) but low specificity (21-31%) and low positive predictive value were reported for detecting advanced neoplasia in mucinous cysts. The updated 2012 Fukukoa guidelines modified the surgical resection criteria for BD-IPMN by removing the cut-off cyst size and subsequently including obstructive jaundice as an objective clinical finding for resection. Furthermore, endoscopic ultrasound (EUS) was recommended for cyst ≥3 cm and for mucinous PCs with "worrisome" features. These "worrisome" features include the presence of a clinically obvious form of pancreatitis and imaging features such as cyst size ≥3 cm, thickened/enhancing cystic wall, main duct size 5-9 mm, non-enhancing mural nodule, abrupt change in pancreatic duct caliber with distal atrophy, or lymphadenopathy.

However, most observers pointed out that both the Sendai and Fukukoa guidelines are subject to a low positive predictive value. Moreover, a major drawback of these guidelines is that they are concerned only with IPMNs and MCNs. For the first time, the 2018 European guidelines [9] introduced the idea that MCN <4 cm with no mural nodules or symptoms could benefit from surveillance instead of surgical resection because of the lower risk of progression to PDAC. To better characterize PCs, the first step consists of cross-sectional imaging (pancreatic MRI) to highlight the "worrisome" features in addition to the morphological features of the lesion and the connection or involvement of the cyst with the main or branch pancreatic duct. Subsequently, EUS allows for high-resolution imaging of PCs and the main pancreatic duct, although morphological features alone are still poor predictors of the PC type and advanced neoplasia. The use of EUS is enhanced when coupled with fine-needle aspiration (FNA), cytopathological evaluation, and carcinoembryonic antigen (CEA) and amylase measurements. The reported sensitivity of cytopathology according to PC type and malignancy varies widely from 25% to 88%. Indeed, elevated CEA levels are considered one of the most accurate markers for diagnosing mucinous PC. However, different cut-off values have been proposed with different levels of sensitivity ranging from 70% to 80% and specificity from 79% to 84%. Recent advances in molecular technologies have allowed investigators to detect variations in DNA, RNA, protein content, and small molecules collected from limited amounts of fluid or tissue. The cellular content of PC fluid is generally suboptimal, although it can be analyzed for alterations in DNA, miRNA, protein, and metabolites. This has opened new avenues for biomarker exploration. In a large cohort of 113 patients with PC fluid analysis extracted from surgical specimens of IPMNs, Wu et al. performed next-generation sequencing (NGS) and found GNAS mutations in 66% of cases, KRAS mutations in 81%, and both mutations in 51% [10]. Further assessment of MCNs and SCAs demonstrated the lack of GNAS mutations in both types of PC. In a study of PC fluid extracted using EUS-FNA, KRAS and GNAS mutations were reported in 68% and 39% of IPMNs, respectively, with a mutation in either gene in 83% of IPMNs [11]. Overall, in the preoperative setting, the sensitivity and specificity were 70% and 98% for KRAS mutations in IPMNs and 36 and 100% for GNAS mutations, respectively. The combination of both genes for IPMNs had sensitivity of 84% and specificity of 98%.

Few studies have assessed the clinical impact of DNA profiling on PCs. In addition to enhancing sensitivity, NGS allows for the simultaneous evaluation of multiple genetic alterations in PC fluid. Using a broad panel of genes, including KRAS, GNAS, VHL, TP53, CDKN2A, and SMAD4, Jones et al. showed that molecular studies changed the clinical diagnosis in 12% of the cases [12]. KRAS and/or GNAS mutations were observed in 48% of PCs without elevated CEA levels. Similarly, Singhi et al. [13] analyzed PC fluid from 225 patients for genetic alterations using a highly sensitive NGS panel comprising KRAS, GNAS, TP53, PIK3CA, and PTEN. The sensitivity and specificity for the diagnosis of mucinous PC were 96% and 100%, respectively. Using this information, the authors proposed an algorithmic approach for PC evaluation and management; that integrates PC fluid DNA testing. Based on this retrospective study, Singhi et al.

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reported that this algorithmic approach could detect advanced neoplasia within mucinous PC with 100% sensitivity, 90% specificity, 79% positive predictive value, and 100% negative predictive value [13]. The prevalence of pancreatic cancer or HGD in PCs accounts for up to 42% of resected lesions. Currently, the only curative approach to PDAC is surgical resection with an overall survival of 25% at 5 years compared to 10% when non-operable [14]. Operative risks are also non-negligible, and the mortality rate for pancreatic resection in the context of PCs is estimated to be between 1% and 7% with a morbidity rate of up to 64%. Therefore, it is important to select patients who may require surgery, especially because the incidence of PC lesions increases with age. This study aimed to evaluate the clinical impact of molecular analysis in the management of patients with PCs.

Material and Methods

We retrospectively evaluated 85 patients with PCs who underwent EUS-FNA at Geneva University Hospital, Switzerland, between November 2014 and September 2021. The cystic fluid was subsequently subjected to molecular analysis. Ethical approval was obtained from the Swissethics Committee, which granted authorization for the reuse of personal health data in the absence of explicit patient consent. Imaging impressions were obtained by reviewing MRI, CT, EUS, and radiology reports for all patients. Cysts are broadly classified into non-mucinous and mucinous categories. EUS was performed under general anesthesia with orotracheal intubation in all patients. EUS assessment was conducted using the Olympus EUME3 device, and both transgastric and transduodenal approaches were used to evaluate the pancreatic parenchyma and ducts. Once Doppler confirmed the absence of vascular interposition, a needle was inserted under EUS guidance. The needle was then advanced through the gastrointestinal wall into the cyst. After proper positioning, cyst fluid was aspirated and sent for analyses. A single dose of the prophylactic antibiotic (ciprofloxacin) was administered to all patients. In cases where the cyst fluid could not be completely aspirated, patients received antibiotics (ciprofloxacin and metronidazole) for 3 days to prevent infection.

The aspirated cyst fluid was delivered to the Department of Diagnostics for cytological, biochemical, and molecular analyses. An aliquot of ≥0.3 mL of fresh homogenized cyst fluid was used for molecular analysis. The remaining cyst fluid was centrifuged to prepare a cytospin for cytologic analysis, and the supernatant cyst fluid was analyzed for CEA and amylase levels. CEA levels were recorded, and the imaging diagnosis was further classified based on CEA levels less than or greater to 192 ng/mL. Cyst fluid cytology was classified as either with or without HGD (high-grade dysplasia or invasive carcinoma). NGS was performed on a sample of 0.3 mL DNA extracted from native fluid using either an Illumina sequencer with the NGS100v1 custom panel (Agilent SureSelect), which covers approximately 500 hotspot mutations on 100 genes linked to various hematological and pathological tumors, or an Ion Torrent Proton sequencer with the Ion Ampliseq Cancer Hotspot v2 custom panel, which covers approximately 2,800 mutations in 50 genes linked to different cancers.

In the case of high fluid viscosity or insufficient sample volume preventing NGS analysis, targeted sequencing using the Sanger method was performed on KRAS (exons 2, 3, and 4) and GNAS (exons 8 and 9), where most of the changes occured. For these two genes, if the comparison of the sequence of the PCR product with a “wild type” sequence showed 100% homology, this indicated an absence of mutations. However, this analysis is not as sensitive as NGS analysis, and a negative result does not necessarily indicate the absence of mutations, especially if the sample’s tumor cellularity is <30-40% (15). Data are expressed as percentages for categorical variables and mean +/- standard deviation for quantitative variables. The sensitivity and specificity of CEA level, expert advice, cytology, and their combination in addition to KRAS and/or GNAS mutational status in PC fluid were evaluated for mucinous cysts. The 95% confidence intervals were calculated using the Copper-Pearson exact method. The final diagnoses of PCs were based on surgical specimens or, in the non-surgical cohort, on the combination of PC fluid CEA, cytology, and outcome after prolonged follow-up (2 years). A sub-analysis was performed on patients with a definitive diagnosis (via surgical pathology, EUS-FNA cytology, imaging, or >2-year follow-up) to evaluate the discriminative power of molecular analysis in differentiating mucinous from non-mucinous cysts. Statistical analyses were performed using the R software. Because not all tests were performed for each patient, the number of observations for each test was variable.

Results

A total of 85 patients were included between November 2014 and September 2021. The demographic and clinical characteristics are detailed in Table 2. In our cohort, 52 patients were female (61.2%), with a mean age of 63.1 ± 15 years. Most PCs were unique (76.5%) or unilobed (63.5%). Regarding the distribution of cysts, 31.8% were located in the head of the pancreas, 7% in the isthmic region, 24.7% in the body, and 11.8% in the tail. One pseudocyst was in the retrograstric region. The median diameter of the PCs was 23 mm (IQR 17-28.50), with 23.5% being >3 cm. Most cysts were detected incidentally on radiological imaging (72.9%). However, 27.1% of the patients had clinical symptoms such as, abdominal pain or discomfort (65.2%) or pancreatitis (26.1%). Of note, among the patients diagnosed with IMPN, two had a history of colon cancer and five had diabetes. Overall, 45.9% of the patients presented with at least one

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worrisome feature during EUS assessment. Worrisome features were based on the Sendai 2017 classification: cyst size >3 cm, enhanced mural nodule, thickened/enhanced cyst walls, main pancreatic duct size >5-9 mm, abrupt change in pancreatic duct caliber, or presence of lymphadenopathy. After EUS-FNA, CEA levels were obtained in 61.4% of patients, and cytology was conclusive in 95.3% of cases. CEA levels were not determined in 32/85 (37.65%) PCs because of technical issues associated with the high viscosity of the fluid or insufficient sample volume. CEA was >192 kU/L in 19 cases (22.25%), and both CEA and amylase increased concomitantly in nine patients (10.6%). The glucose levels were not assessed. Among the lesions, 63 (74.12%) were classified as mucinous because they met at least one of the criteria, and 15 (17.6%) as non-mucinous. The technical success rate was 100%. One case of pneumoperitoneum was reported as a complication of a pseudocyst drainage.

Table 1: Epidemiology and characterization of pancreatic cyst types

Characteristics	MCN	IPMN	SCA	Pseudocyst
Sex (% female)	>95%	55%	70%	<25%
Age (decade)	5th to 7th	5th to 7th	5th to 7th	4th to 5th
Incidental detection	50%	>90% if small	50%	rare
Localization	Body and tail	Head (70%)	Variable	Variable
Calcification	Rare, peripheral	No	30-40%, central	No
Typical imaging characteristics	Orange	Grape	Honeycomb	Variable
Multifocal	No	Yes	No	Common
Connection or involvement with MPD	Rare	Yes	No	Common
MPD	Normal or deviated	Normal or dilated	Normal or deviated	Normal or irregularly dilated

IPMN: intraductal papillary mucinous neoplasm; MCN: mucinous cystic neoplasm; MPD: main pancreatic duct; SCA: serous cystadenoma

Table 3 summarizes the different cysts types. The main type of PCs was IPMN in 57.6% of the cases, followed by serous cystadenoma (14.1%) and MCN (10.6%). In this series, eight patients had IPMN with HGD, three had mucinous malignant lesions (two PDAC and one invasive carcinoma), and two had probable mucinous malignant lesions (inconclusive cytology, palliative care). Other non-mucinous lesions included one lymphoepithelial cyst, one adrenal adenoma, one para-pancreatic celiac neurofibroma, and one simple mucinous cyst. Non-specific cysts were identified in patients with an inconclusive assessment (EUS, chemical fluid, wild-type KRAS/GNAS mutations) who were unfit to pursue the investigations and who would not have

Table 2: Demographic and clinical characteristics of the 85 patients with pancreatic cysts

Female sex, n (%)		52 (61.1%)
Mean age at EUS-FNA, years (±SD)		63.1 (± 15)
Alcohol consumption, n (%)		7 (8.2%)
Tobacco consumption, n (%)		6 (7.0%)
Diabetes, n (%)		10 (11.8%)
Clinical symptoms prior to EUS imaging	None	62 (72.9%)
	Abdominal discomfort/pain	15 (65.2%)
	Pancreatitis	6 (26.1%)
	Jaundice	3 (13%)
	Weight loss	4 (17.4%)
Cyst location, n (%)	Head	27 (31.8%)
	Isthmic	6 (7.0%)
	Body	21 (24.7%)
	Tail	10 (11.8%)
	Retrogastric	1 (1.2%)
	Multiple cyst locations	17 (20%)
	Dilatation of MPD	3 (3.5%)
Median cyst size, mm (IQR)		23 (17-28.50)
Cyst size >3 cm, n (%)		20 (23.5%)
Cyst with nodule/mass, n (%)		8 (9.4%)
Unique cyst, n (%)		54 (63.5%)
EUS sign of chronic pancreatitis		5 (5.9%)
EUS imaging, n (%)	No worrisome features	46 (54.1%)
	≥1 worrisome features	39 (45.9%)
PC fluid CEA, n (%)	CEA <192 ng/mL	33 (38.8%)
	CEA ≥192 ng/mL	19 (22.3%)
	No available results	32 (38.6%)
PC fluid amylase, n (%)	Amylase<350 U/L	20 (23.5%)
	Amylase≥350 U/L	20 (23.5%)
	No available results	45 (52.9%)
PC fluid CEA ≥192 ng/mL and amylase ≥350 U/L		9 (10.6%)
Cytology	No diagnosis	13 (4.7%)
	Negative for HGD	66 (77.6%)
	Positive for HGD or PDAC	6 (7.0%)
Treatment decision	Surgery	25 (29.4%)
	Surgery, refused by patient	3 (3.5%)
	Endoscopic drainage	1 (1.2%)
	Palliation	2 (2.3%)
Mortality		7 (8.2%)
Complication due to EUS-guided drainage, n (%)		1 (1.2%)
Post-operative complications (n=25), n (%)		2 (8.0%)

CEA: carcinoembryonic antigen; EUS: endoscopic ultrasound; FNA: fine-needle aspiration; HGD: high-grade dysplasia; IPMN: intraductal papillary mucinous neoplasm; IQR: interquartile range; MCN: mucinous cystic neoplasm; MPD: main pancreatic duct; PC: pancreatic cyst; PDAC: pancreatic ductal adenocarcinoma; SCA: serous cystadenoma

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benefited from surgical resection if a malignant lesion was suspected. These two patients are currently alive. Complete NGS analysis was performed in 17 patients (Table 4). Among them, ten patients had mucinous lesions, and one had a non-mucinous lesion. Overall, KRAS and GNAS mutations were found in six cysts. Single KRAS mutations were found in seven cysts and single GNAS mutations in four cysts. KRAS and/or GNAS mutations were observed in 11.7% of the PCs without elevated CEA levels.

Of the 85 patients included in the study, 25 underwent surgery for suspected mucinous lesions, although only 11 had HGD (eight) or cancer (three). Two other patients had advanced unresectable malignancies but without KRAS and/or GNAS mutations. Table 5 shows the distribution of KRAS/GNAS mutations among 85 patients. Mutations in KRAS and/or GNAS were found in 6/11 (54.5%) lesions classified as malignant and in 3/14 (21.4%) non-malignant lesions that were operated on (Table 5). Sequencing of KRAS and GNAS mutations was performed using the Sanger methods (PCR) in 77.6% of cases, while 5.9% of cases were performed using NGS with the 100-gene panel (Illumina), and 16.5% of cases were performed using NGS with the 50-gene panel (Ion Torrent Proton). One ABL1 mutation was found in one patient, but the pathogenicity of the mutation was unknown. No other mutations were found in any of the analyzed genes. Of the 25 patients who underwent surgery, both KRAS and GNAS mutations were found in four cysts, only the KRAS mutation in three cysts, and only the GNAS mutation in two cysts. No mutations were found in the 8/25 patients operated for mucinous lesions, five of which were malignant lesions. The two patients who died from advanced unresectable probable PDAC had neither KRAS nor GNAS mutations.

Table 3: Types of pancreatic cysts

Mucinous	Intraductal papillary mucinous neoplasm	49 (57.6%)
	Mucinous cystic neoplasm	9 (10.6%)
	Malignant lesions	5 (5.9%)
Non-mucinous	Serous cystadenoma	12 (14.1%)
	Pseudocyst	4 (4.7%)
	Other	4 (4.7%)
Non-specific		2 (2.3%)

Table 6 summarizes the characteristics of the 25 resected cysts. The final histopathologic description of the 25 patients with an indication for surgical resection was three malignant cysts (two PDAC and one IPMN-IC), eight IPMN with HGD, seven mucinous lesions with LGD (four IPMN-LGD, two MCN-LGD, and one mucinous simple cyst), four benign cysts (SCA), and, three other pathologies (schwannoma, adrenal adenoma, and pseudocyst). Two patients had postoperative complications, with one case of hemoperitoneum due to

Table 4: SevTable 1enteen cases with KRAS/and or GNAS mutations

Cytology	Sex	Age	CEA	Amylase	KRAS	GNAS	Histology
Operated patients							
IPMN-HGD	F	71	High	Low	Positive	Negative	IPMN-HGD
IPMN-LGD (MPD)	M	50.2	NA	NA	Positive	Positive	IPMN-LGD (MPD)
Inconclusive	F	78.8	High	Low	Positive	Positive	IPMN-LGD
IPMN-LGD (MPD)	M	75	NA	NA	Negative	Positive	IPMN-HGD (MPD)
IPMN-HGD (MPD)	M	72.6	High	NA	Positive	Positive	IPMN-HGD (MPD)
Adenocarcinoma	M	76	High	High	Positive	Positive	IPMN-HGD (MPD)
IPMN-LGD (MPD)	F	78.5	High	Low	Positive	Negative	Invasive carcinoma
Mucinous neoplasia	M	46.4	Low	Low	Positive	Negative	SCA
IPMN-LGD (MPD)	M	68.9	High	Low	Negative	Positive	IPMN-HGD (MPD)
Non-operated patients							
IPMN-LGD	M	64	Low	Low	Positive	Positive	
IPMN-LGD	F	63.9	NA	NA	Positive	Negative	
IPMN-LGD	M	58.5	NA	NA	Positive	Positive	
IPMN-LGD	M	54.5	Low	NA	Positive	Negative	
MCN	M	71.6	NA	NA	Negative	Positive	
IPMN-LGD	F	68.8	High	Low	Positive	Negative	
IPMN-LGD	F	86.3	Low	NA	Positive	Negative	
IPMN-LGD	F	61.5	Low	High	Negative	Positive	

HGD: high-grade dysplasia; IPMN: intraductal papillary mucinous neoplasm; LGD: low-grade dysplasia; MCN: mucinous cystic neoplasm; MPD: main pancreatic duct; NA: not available; SCA: serous cystadenoma

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Table 5: Distribution of KRAS/and or GNAS mutations among the 85 patients

Diagnosis	KRAS and/or GNAS mutation	Wild type
Non-mucinous lesions(n=20)	1	19
Mucinous lesions (low-grade dysplasia) (n=50)	10	40
Mucinous lesions (high-grade dysplasia) (n=8)	5	3
Cancer (n=5)	1	4
Non-specific (n=2)	0	2

Table 6: Characteristics of the 25 resected cysts

c	CEA	Amylase	KRAS	GNAS	Histology
Absence of mucus, pseudocyst	High	High	Negative	Negative	MCN
IPMN-HGD	NA	NA	Negative	Negative	IPMN-HGD
IPMN-HGD	High	Low	Positive	Negative	IPMN-HGD
IPMN-HGD	NA	NA	Negative	Negative	IPMN-HGD
Mucinous neoplasia	NA	NA	Negative	Negative	SCA
IPMN-LGD (MPD)	NA	NA	Positive	Positive	IPMN-LGD (MPD)
Mucinous neoplasia	High	High	Negative	Negative	MCN
IPMN-HGD	High	Low	Negative	Negative	IPMN-HGD
Mucinous neoplasia	Low	NA	Negative	Negative	PDAC
Inconclusive	High	Low	Positive	Positive	IPMN-LGD
IPMN-LGD (MPD)	High	High	Negative	Negative	IPMN-LGD
Mucinous neoplasia	NA	NA	Negative	Negative	SCA
Mucinous neoplasia	NA	NA	Negative	Negative	SCA
IPMN-LGD (MPD)	NA	NA	Negative	Positive	IPMN-HGD (MPD)
IPMN-HGD (MPD)	High	NA	Positive	Positive	IPMN-HGD (MPD)
Mucinous neoplasia	NA	NA	Negative	Negative	Schwannoma
Mucinous neoplasia	Low	NA	Negative	Negative	Simple mucinous cyst
Mucinous neoplasia	High	Low	Negative	Negative	PDAC
IPMN-LGD (MPD)	NA	NA	Negative	Negative	IPMN-LGD (MPD)
Adenocarcinoma	High	High	Positive	Positive	IPMN-HGD (MPD)
Inconclusive	Low	Low	Negative	Negative	Adrenal adenoma
IPMN-LGD (MPD)	High	Low	Positive	Negative	Invasive carcinoma
Mucinous neoplasia	Low	Low	Positive	Negative	SCA
Mucinous neoplasia	Low	Low	Negative	Negative	Pseudocysts
IPMN-LGD (MPD)	High	Low	Negative	Positive	IPMN-HGD (MPD)

HGD: high-grade dysplasia; IPMN: intraductal papillary mucinous neoplasm; LGD: low-grade dysplasia; MCN: mucinous cystic neoplasm; MPD: main pancreatic duct; NA: not available; SCA: serous cystadenoma

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Table 7: Diagnostic performance of the different tests used to diagnose mucinous lesions

Variable	Sensitivity (95% CI)	Specificity (95% CI)	n (number of observations)
CEA	0.571 (0.340 to 0.782)	0.846 (0.546 to 0.981)	34
Expert advice	0.973 (0.858 to 0.999)	0.353 (0.142 to 0.617)	54
Cytology	0.939 (0.798 to 0.993)	0.467 (0.213 to 0.734)	48
Combination of CEA, expert advice and cytology.	1.000 (0.903 to 1.000)	0.133 (0.017 to 0.405)	51
KRAS	0.184 (0.077 to 0.343)	0.944 (0.727 to 0.999)	56
GNAS	0.158 (0.060 to 0.313)	1.000 (0.815 to 1.000)	56
KRAS or/and GNAS	0.237 (0.114 to 0.402)	0.944 (0.727 to 0.999)	56

CEA: carcinoembryonic antigen; CI: confidence interval

Table 8: Diagnostic performance of KRAS or/and GNAS according to the sequencing method

Variable		Sensitivity (95% CI)	Specificity (95% CI)	n (number of observations)
Sanger method	KRAS	0.156 (0.053 to 0.328)	1.000 (0.735 to 1.000)	44
	GNAS	0.125 (0.035 to 0.290)	1.000 (0.735 to 1.000)	44
	KRAS or/and GNAS	0.188 (0.072 to 0.364)	1.000 (0.735 to 1.000)	44
NGS method	KRAS	0.333 (0.043 to 0.777)	0.833 (0.359 to 0.996)	12
	GNAS	0.333 (0.043 to 0.777)	1.000 (0.541 to 1.000)	12
	KRAS or/and GNAS	0.500 (0.118 to 0.882)	0.833 (0.359 to 0.996)	12

CI: confidence interval

arterial bleeding in the pancreatic tissue section, and one case of stump necrosis. Both the patients were successfully treated surgically. To understand the discriminant power of molecular analysis between the two categories of cystic lesions (i.e., non-mucinous vs mucinous), we performed a sub-analysis of patients with a definitive diagnosis based on either a surgical pathological specimen or diagnostic EUS-FNA cytology, imaging features, and prolonged follow-up (>2 years). Table 7 shows the performance of the different tests, performed alone and in combination, to diagnose mucinous cysts. CEA had the highest sensitivity of 57.1% (CI 34–78%) and specificity of 84.6% (CI 54.6–98.1%). Molecular analysis performed less well with a low sensitivity of 23.7% (CI 11.4–40.2%) but a high specificity of 94.4% (CI 72.7–99.9%). Expert advice based on EUS imaging, MRI, and/or computed tomography had a sensitivity of 97.3% (CI 85.8–99.9%) with a specificity of 35.3% (CI 14.2–61.7%). The combined use of expert advice, CEA, and cytology had a sensitivity of 100% (CI 90.3–100%) and specificity of 13.3% (CI 1.7–40.5%).

Regarding the mutations, KRAS had 18.4% sensitivity (CI 7.7–34.3%) and 94.4% specificity (CI 72.7–99.9%), while GNAS had 15.8% sensitivity (CI 6–31.3%) and 100% specificity (CI 81.5–100%) for mucinous cyst diagnosis. When combined with KRAS and/or GNAS mutations, they had 23.7% sensitivity (CI 11.4–40.2%) and 94.4% specificity (CI 72.7–99.9%). When sequencing methods used to detect KRAS and/or GNAS were separated (Sanger methods or NGS), molecular mutations detected using NGS showed better diagnostic performance in our population (Table 8). During the follow-up, seven patients (8.23%) died. Five deaths were related to pancreatic disease, and two were unrelated (one case of acute mesenteric ischemia and one case of septic shock due to dialysis catheter infection). Median follow-up time after EUS assessment was 1.48 years (IQR 0.33-3.94).

Discussion

With the increased use of medical imaging in the general population and the aging of the population, the detection of PCs is expected to significantly increase over time. Their management continues to be a challenge. Therefore, it is crucial to accurately identify cystic lesions with that have the potential to progress to malignancy. Current guidelines recommend surgical resection for high-risk mucinous cysts (cysts with HGD or invasive carcinoma), whereas lesions with LGD should undergo surveillance. Non-mucinous lesions did not require follow-up. In our series, intracystic CEA was

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measured in 34 patients, and cytology was informative in 48 cases. CEA levels may help to detect mucinous lesions with a sensitivity of 57.1% and specificity of 84.6%, while cytology had a better sensitivity (93.9%) but lower specificity (46.7%). When combining CEA, expert advice, and cytology, the sensitivity was good (100%), but the specificity decreased to 13.3%. In this cohort of patients, we observed that molecular biomarkers (KRAS and/or GNAS mutations) play a limited but potentially informative role in the clinical management of PCs. Our study is in line with earlier publications reporting that KRAS mutations were highly specific for identifying mucinous lesions with a specificity of 94.4% [10,15]. However, they showed a low sensitivity (18.4%), thus overlooking many patients with mucinous lesions. One of our patients presented with mucinous cells on cytology and KRAS mutation, but the lesion was later identified as SCA after resection. Non-mucinous cysts such as SCA or cystic acinar transformations of the pancreas are generally described in the literature as KRAS and GNAS wild types. One explanation could be that the cytology obtained during EUS-FNA revealed pancreatic intraepithelial neoplasia cells in resected pancreatic lesions carrying this mutation. Furthermore, three other patients with no KRAS or GNAS mutations but cytological findings of mucinous cells underwent surgery, which revealed SCA. In all these cases, the mucinous cells identified in the cytology likely resulted from contamination of the digestive tract. These observations highlight the importance of interpreting the molecular results with caution, especially in the context of cytological and clinical findings.

Molecular analysis was also performed in two patients, as the lesions could not be classified as mucinous or non-mucinous. One patient had KRAS and GNAS mutations and was diagnosed with IPMN without HGD, while no mutations were found in the other cyst, which was an adrenal adenoma. Molecular analysis was carried out in one patient with suspected adenocarcinoma in cytology with mutations in KRAS and GNAS, although no malignant cells were identified in the resected pancreas (but IPMN-HGD). Of the three resected malignant lesions, two were pancreatic ductal adenocarcinomas that developed on IPMN with HGD, and one was invasive carcinoma. Of note, the latter patient was suspected to have IPMN with LGD on cytology. However, surgical resection was performed because of MPD involvement. Of the two cases of PDAC, both were wild type; only one of these patients harbored high levels of CEA, while the other with IC had only KRAS mutations and high levels of CEA. Mutations in KRAS and/or GNAS were found in 6/11 (54.5%) lesions classified as malignant and in 3/14 (21.4%) of the non-malignant lesions that were underwent surgery.

The limitations of Sanger sequencing compared to NGS in PC evaluation are its lower sensitivity due to its ability to detect low-frequency mutations and overlook mutations present in a small fraction of cells within a heterogeneous cyst. In contrast, NGS has high sensitivity because it can simultaneously detect multiple mutations, even at low variant allele frequencies. Furthermore, the lower prevalence of KRAS mutations found in the present study may be related to the cyst types (mostly non-surgical), low PC fluid volume, and predominant use of Sanger sequencing compared to NGS (77.7% or 66/85 vs 22.3% or 19/85), which has lower sensitivity than the latter [15]. Indeed, the sensitivity for detecting KRAS/GNAS using NGS was previously reported to be approximately 50%, which is in line with our findings [16]. A causative link between diabetes mellitus and chronic pancreatitis has been observed in patients with IPMN. In our cohort, only five patients with IPMN had diabetes at the time of PC detection (out of a total of 10 patients with diabetes). In the literature, 10-45% of individuals with IPMN are diabetic [3]. Most patients in this study cohort (70.6%) did not undergo surgery given the absence of high-risk imaging features and ancillary tests to identify high-risk cysts. Clinical decisions based on the EUS-FNA findings suggested surgical resection in 25 patients (29.4%). Nevertheless, three patients rejected the proposal of surgical resection despite being highly recommended; two of these patients were oriented to palliative care, and one had endoscopic drainage. Fifty patients with low-risk cysts were referred for follow-up evaluation. Patients with PCs without worrisome features were followed-up by MRI every 6 months during the first year and then once a year. In the case of the appearance of worrisome features or cyst growth ≥5mm every 2 years, patients were referred to our center.

This study has several limitations. First, only 25/85 patients underwent surgery, thus limiting the number of available surgical specimens. Second, the median follow-up duration was short (1.48 years). Third, chemical analyses (CEA and amylase in PC fluid) were not available for every patient. In summary, our results suggest that routine use of KRAS and/or GNAS mutations in the analysis of PC lesions may not be essential for every patient. Imaging studies, chemical analyses (CEA levels), and cytology performed well in distinguishing non-mucinous lesions from mucinous lesions, demonstrating higher sensitivity in our cohort. However, while the contribution of molecular diagnostics in daily practice appeared limited in this study, it is important to consider that the techniques used such as Sanger sequencing may have affected these outcomes. We acknowledge that newer technologies such as NGS mutational analysis could improve these results due to their greater sensitivity in detecting lesions like IPMN and MCN. Preliminary trends suggest that NGS may offer better test performance than Sanger sequencing, although these findings should be interpreted cautiously, given the overlapping confidence intervals between these two methods. Therefore, the sequential use of mutational analysis may still be

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recommended in specific cases, such as diagnosing mucinous cysts in younger patients after non-diagnostic cytology and CEA levels <192 ng/mL, where a false-negative result could otherwise stop the necessary follow-up.

Advances in biomarker research to distinguish between PC types are ongoing, with promising developments in metabolite-based markers such as glucose. Elevated glucose levels have demonstrated a sensitivity of 94% and specificity of 64% for differentiating mucinous from non-mucinous PCs, showing a diagnostic accuracy comparable to that of CEA [17]. Although neither glucose nor CEA can reliably identify the presence of advanced neoplasia, glucose is a cost-effective alternative for the identifying mucinous PCs. Importantly, glucose testing could be more accessible and less invasive than CEA measurement. However, the potential role of molecular analyses in patients with low glucose levels remains unclear and requires further investigation. The recently published Kyoto Guidelines on Pancreatic Cyst Management include recommendations to analyze a broader range of molecular markers. Among them, TP53, SMAD4, CDKN2A, and PIK3CA are used for identifying high-grade dysplasia and invasive carcinoma, as well as VHL mutations for distinguishing serous cystic neoplasms [18]. While our study primarily focused on KRAS and GNAS mutations due to their established diagnostic role, future research should integrate this broader molecular panel to enhance diagnostic accuracy. These additional markers could improve the detection of high-risk cysts and refine surveillance strategies. The long-term management of patients with molecular-negative pancreatic cysts remains an area of ongoing investigation. Current surveillance strategies typically rely on imaging modalities, such as MRI and EUS at regular intervals, and in cases of doubt sooner, at the discretion of the multidisciplinary board. In the case of molecular-negative cysts, monitoring should be based on the cyst size, growth rate, and clinical presentation. The Kyoto Guidelines highlight the utility of integrating metabolic markers, such as glucose levels into surveillance protocols. Incorporating these findings into follow-up strategies could provide a more adaptive approach.

Conclusions

The accurate assessment of PCs in the preoperative setting remains challenging. While various guidelines provide a suboptimal strategy for managing PCs, the clinical approach to patients with PCs should be individualized based on their clinical status, presence of comorbidities, stratification of risk for developing malignancy, and personal preference. Our aim was to accurately identify predictive signs of malignancy to allow for early surgery and improve long-term survival while sparing patients with LGD cysts and the morbidity and mortality associated with pancreatic surgery.

Although DNA-based biomarkers have made tremendous progress in recent years and are available in some specialized centers, their clinical utility remains a subject of ongoing debate. The lack of robust prospective data makes it challenging to determine the optimal role of DNA testing for the evaluation of PC lesions. In our study, molecular analysis did not significantly enhance the classification of PCs as either mucinous or non-mucinous. However, these findings highlight the need for further research to better understand the potential contributions of molecular testing, particularly in specific clinical contexts or with the use of advanced sequencing technologies.

Conflicts of interest and source of funding

The authors declare that they have no conflicts of interest and that no external funding was received for this study.

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