

SEMMELWEIS EGYETEM
DOKTORI ISKOLA

Ph.D. értekezések

3489.

GYIMESI GYÖRGY

**Viscerális megbetegedések intervenciós, sebészeti kezelése és a hasüregi
szervek transzplantációja**
című program

Programvezető: Dr. Szijártó Attila, egyetemi tanár

Témavezető: Dr. Hritz István, egyetemi tanár

Biomarkers and genetic markers in the diagnosis of cystic lesions of the pancreas

PhD thesis

György Gyimesi M.D.

Semmelweis University Doctoral College
Surgical Medicine Division



Supervisor:

István Hritz, MD, Ph.D

Official reviewers:

Antal Tivadar Dezsőfi-Gottl MD, PhD
Tibor Gyökeres MD, Ph.D

Head of the Complex Examination Committee: Béla Molnár MD, DSc

Members of the Complex Examination Committee:

Pál Kaposi Novák MD, Ph.D
Ádám Domonkos Tárnoki MD, Ph.D

Budapest
2026

TABLE OF CONTENTS

LIST OF ABBREVIATIONS.....	4
1. INTRODUCTION.....	6
1.1. Definition.....	6
1.2. Prevalence.....	6
1.3. Classification.....	7
1.4. The main types of pancreatic cysts.....	8
1.4.1. Pseudocysts.....	8
1.4.2. Serous cystic neoplasm (SCN)/Serous cystadenoma (SCA).....	11
1.4.3. Mucinous cystic neoplasm (MCN)/Mucinous cystadenoma (MCA).....	12
1.4.4. Intraductal Papillary Mucinous Neoplasm (IPMN).....	14
1.4.4.1. Main-Duct IPMN.....	15
1.4.4.2. Branch-Duct IPMN.....	16
1.4.4.3. Mixed-Type IPMN.....	17
1.4.5. Solid Pseudopapillary Neoplasm (SPN).....	18
1.4.6. Cystic Pancreatic Neuroendocrine Tumor (cNET).....	19
1.4.7. Cystic Pancreatic Ductal Adenocarcinoma.....	20
1.5. Methods in the diagnosis of Pancreatic Cystic Lesions.....	21
1.5.1. Diagnostic Approach to Pancreatic Cystic Lesions.....	21
1.5.2. Role of Endoscopic Ultrasound in the Evaluation of Pancreatic Cystic Lesions.....	22
1.5.3. Endoscopic Ultrasound-Guided Fine-Needle Aspiration (EUS-FNA).....	23
1.5.4. Macroscopic Evaluation of Cyst Fluid.....	23
1.5.5. Cyst Fluid Viscosity and the “String Sign”.....	23
1.5.6. Fluid Cytology.....	24
1.5.7. Cyst Fluid Amylase.....	24
1.5.8. Cyst Fluid Carcinoembryonic Antigen (CEA).....	24
1.5.9. Other Tumor Markers.....	25
1.5.10. Cyst Fluid Glucose.....	25

1.5.11. Mutational analysis.....	25
1.5.11.1.VHL (von Hippel–Lindau) Gene Mutations.....	26
1.5.11.2. KRAS Mutations.....	26
1.5.11.3. GNAS Mutations.....	26
1.5.11.4. Other Genetic Markers.....	27
1.6. Additional Diagnostic Techniques for Pancreatic Cystic Lesions.....	27
1.6.1. EUS-Guided Through-the-Needle Biopsy (EUS-TTNB).....	27
1.6.2. Endoscopic Ultrasound-Guided Needle-Based Confocal Laser Endomicroscopy (EUS-nCLE).....	28
2. OBJECTIVES.....	29
2.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts.....	29
2.2.Clinical benefits of KRAS and GNAS mutation analysis in addition to morphology and conventional cyst fluid testing for the characterization of pancreatic cysts.....	29
3. METHODS.....	30
3.1.Study design.....	30
3.2.Inclusion and exclusion criteria.....	30
3.3.Endoscopic ultrasound (EUS).....	31
3.4.CT and MRI morphology.....	32
3.5.Conventional cyst fluid analysis.....	32
3.5.1. String sign.....	32
3.5.2. Cytology.....	32
3.5.3. CEA and Glucose.....	33
3.5.4. Amylase.....	33
3.6. KRAS/GNAS mutational analysis.....	33
3.7. Follow-up.....	33
3.8. Statistical analysis.....	34
3.9. Language editing.....	34
4. RESULTS.....	35

4.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts.....	35
4.2. Clinical benefits of KRAS and GNAS mutation analysis in addition to morphology and conventional cyst fluid testing for the characterization of pancreatic cysts.....	38
5. DISCUSSION.....	43
5.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts.....	43
5.2. Clinical benefits of KRAS and GNAS mutation analysis in addition to morphology and conventional cyst fluid testing for the characterization of pancreatic cysts.....	46
6. CONCLUSIONS.....	50
6.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts.....	50
6.2. Clinical benefits of KRAS and GNAS mutation analysis in addition to morphology and conventional cyst fluid testing for the characterization of pancreatic cysts.....	50
7. SUMMARY.....	52
8. REFERENCES.....	53
9. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS.....	67
10. ACKNOWLEDGEMENTS.....	69

LIST OF ABBREVIATIONS

ACG	American College of Gastroenterology
AGA	American Gastroenterological Association
ASGE	American Society for Gastrointestinal Endoscopy
BD-IPMN	Branch duct intraductal papillary mucinous neoplasm
CA19-9	Carbohydrate antigen 19-9
CEA	Carcinoembryonic antigen
CH-EUS	Contrast-harmonic endoscopic ultrasound
cNET	Cystic neuroendocrine tumor
CT	Computed tomography
DM	Diabetes mellitus
ESGE	European Society of Gastrointestinal Endoscopy
EUS	Endoscopic ultrasound
EUS-FNA	Endoscopic ultrasound-guided fine-needle aspiration
EUS-nCLE	Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy
EUS-TTNB	Endoscopic ultrasound-guided through-the-needle biopsy
HbA1c	Hemoglobin A1c
IPMN	Intraductal papillary mucinous neoplasm
IQR	Interquartile range
KRAS	Kirsten rat sarcoma viral oncogene homolog
GNAS	Guanine nucleotide-binding protein alpha subunit
MCN	Mucinous cystic neoplasm
MCA	Mucinous cystadenoma
MD-IPMN	Main duct intraductal papillary mucinous neoplasm
MDCT	Multidetector computed tomography
MPD	Main pancreatic duct

MRI	Magnetic resonance imaging
MRCP	Magnetic resonance cholangiopancreatography
MT-IPMN	Mixed-type intraductal papillary mucinous neoplasm
NGS	Next-generation sequencing
NPV	Negative predictive value
PCF	Pancreatic cyst fluid
PCL	Pancreatic cystic lesion
PPV	Positive predictive value
SCA	Serous cystadenoma
SCN	Serous cystic neoplasm
SD	Standard deviation
SPN	Solid pseudopapillary neoplasm
VHL	Von Hippel–Lindau
WON	Walled-off necrosis
χ^2	Chi-square test

1. INTRODUCTION

1.1. Definition

Pancreatic cystic lesions (PCLs) are defined as circumscribed, fluid-containing structures arising in the pancreatic parenchyma, encompassing a heterogeneous group of entities with distinct underlying mechanisms of development and variable biological behavior.

1.2. Prevalence

Pancreatic cystic lesions (PCLs) were initially regarded as rare entities, as reflected in early reports from 1934. [1] In the following decades, however, accumulating pathological and clinical observations demonstrated that these lesions are considerably more common than previously assumed. [2] Data derived from autopsy series indicate that pancreatic cysts are far more prevalent than previously assumed, with reported frequencies ranging between 33.2% and almost 50% [3,4]. These findings suggest that a large proportion of pancreatic cystic lesions remain clinically silent throughout life. In parallel with the increasing availability and routine use of abdominal imaging techniques — most notably cross-sectional modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) — the rate of incidentally detected pancreatic cysts has risen substantially. Importantly, prevalence estimates in asymptomatic populations show marked variability depending on the imaging modality employed. In a large population-based cohort, transabdominal ultrasound detected pancreatic cysts in 3.5% of individuals [5].

Detection rates are significantly higher for cysts larger than 10 mm than for smaller lesions, and cysts located outside the pancreatic tail are more frequently recognized. [6] In asymptomatic populations examined by CT, the prevalence ranges between 2.1% and 2.7%. [7,8] It has been demonstrated that the most sensitive non-invasive method for identifying pancreatic cystic lesions is magnetic resonance imaging in conjunction with magnetic resonance cholangiopancreatography (MRI/MRCP). Zerboni et al. used MRCP to report a prevalence of 24.8% among asymptomatic persons in a comprehensive meta-analysis. [8] With the application of more advanced MRI hardware and software, even higher detection rates have been described, with incidental pancreatic cysts identified in

up to 56.3% of examined subjects. [9] A strong association has been demonstrated between increasing cyst prevalence, larger cyst diameter, and advancing age. [10]. The prevalence of pancreatic cysts rose from 2.4% in patients under 40 to 8.7% in those between 60 and 69 and 37.7% in those over 80 in an MRI-based study involving 616 participants. [11] Prevalence variations by geography have also been documented. According to Zerboni et al.'s meta-analysis, Western populations have much higher MRI/MRCP-based detection rates (United States: 12.6%; Europe: 7–9%) than Asian cohorts (3.1%). [8]

1.3. Classification

Pancreatic cystic lesions are currently divided into neoplastic and non-neoplastic categories, representing a clinically and pathologically diverse group of entities. Prior theories were mostly limited to serous and mucinous cystic lesions, however since the 1980s, major advances in cross-sectional imaging and other diagnostic modalities have markedly increased the detection of pancreatic cysts and stimulated extensive research into their pathogenesis, morphology, clinical behavior, and management. As a result, several classification systems have been proposed, and pancreatic cystic lesions are now broadly separated according to histological criteria into neoplastic and non-neoplastic entities, and then further separated into epithelial and non-epithelial cysts. [12–14]

Pancreatic pseudocysts, congenital cysts, lymphoepithelial cysts, and retention cysts are the non-neoplastic cysts that are most clinically significant. Intraductal papillary mucinous neoplasms (IPMN), mucinous cystic neoplasms (MCN), serous cystic neoplasms (SCN), cystic neuroendocrine tumors (cNET), solid pseudopapillary neoplasms (SPN), and ductal adenocarcinomas with cystic degeneration are the most significant entities among neoplastic cystic lesions. Table 1 summarizes a histology-based classification of pancreatic cystic lesions.

Table 1. *Histology based classification of the most relevant PCLs*

Main category	Entities
Epithelial – neoplastic	Serous cystic neoplasm (SCN)/Serous cystadenoma (SCA); mucinous cystic neoplasm (MCN); Intraductal papillary mucinous neoplasm (IPMN); ductal adenocarcinoma with cystic degeneration; cystic neuroendocrine tumor (cNET); solid pseudopapillary neoplasm (SPN); acinar cell cystadenoma; cystic teratoma; cystic hamartoma; epidermoid cyst in intrapancreatic accessory spleen; cystic pancreatoblastoma and cystic metastatic epithelial neoplasm
Epithelial – non-neoplastic	retention cyst; congenital cysts (solitary idiopathic cyst, polycystic kidney disease, von Hippel–Lindau syndrome, cystic fibrosis); lymphoepithelial cyst; periampullary duodenal wall cyst; enteric duplication cyst; mucinous non-neoplastic cyst and endometrial cyst
Non-epithelial – non neoplastic	Pseudocyst and parasitic cyst
Non-epithelial – neoplastic	Sarcoma and Lymphangioma

Four primary morphological patterns of pancreatic cystic lesions can be distinguished based on cross-sectional imaging features [14]: 1. macrocystic lesions with cysts often larger than 1-2 cm (oligocystic or multicystic); 2. unilocular cysts without solid components, septations, or calcifications; 3. cystic tumors having solid components; and 4. microcystic tumors, which have cysts that are usually less than 1 cm in size.

Pseudocysts, congenital cysts, and IPMNs account for the majority of pancreatic cystic lesions in terms of overall frequency. Mucinous and serous cystadenomas follow, while solid tumors with cystic degeneration make up a small group of uncommon lesions. [12–14]

1.4. The main types of pancreatic cysts

1.4.1. Pseudocyst

Pancreatic pseudocysts constitute the predominant variety of pancreatic cystic lesions. They typically arise as a complication of acute or chronic pancreatitis and, less commonly,

as a result of forceful or penetrating abdominal trauma or iatrogenic damage. [15,16] Pseudocyst development is more prevalent during chronic pancreatitis than acute pancreatitis and is more common in alcohol-related cases compared to non-alcohol-related pancreatitis. [15] Pseudocysts are encased by a distinct wall consisting of fibrous and inflammatory tissue and, unlike actual cysts, do not possess an epithelial lining. They generally communicate with the pancreatic ductal system either directly or indirectly via the pancreatic parenchyma. Their genesis is ascribed to the disruption of the pancreatic duct due to elevated intraductal pressure stemming from ductal stenosis, calculi, or protein plugs, or from pancreatic necrosis subsequent to an episode of acute pancreatitis. [15, 17, 18] A fluid collection developing after acute pancreatitis is classified as an acute pseudocyst only if it persists for over 4–6 weeks and is surrounded by a wall of fibrous or granulation tissue. [15]

Clinical history and presentation are essential for differentiating pseudocysts from cystic neoplasms. The clinical development may vary from an asymptomatic state to an acute abdomen resulting from severe consequences. Transabdominal ultrasonography is typically the primary imaging technique employed. On ultrasound, pseudocysts generally manifest as well-defined, round or oval, anechoic lesions exhibiting distal acoustic enhancement and possessing a smooth wall. In the first stage, they may exhibit internal echoes of varied intensity due to necrotic debris, a phenomenon more prevalent in pseudocysts resulting from acute necrotizing pancreatitis than in those linked to chronic pancreatitis. In most cases, this debris gradually resolves. Increased internal complexity may also be observed in the presence of intracystic hemorrhage or superinfection. [15]

In the acute setting, computed tomography (CT) is often preferred because bowel gas may reduce the sensitivity of ultrasound. CT provides detailed information on surrounding structures and may reveal associated findings such as calcifications or dilation of the pancreatic or the biliary ductal system. Magnetic resonance imaging and magnetic resonance cholangiopancreatography (MRI/MRCP) allow accurate assessment of cyst morphology, internal contents (including debris), and ductal communication, exhibiting great sensitivity for identifying small cystic lesions. [19,20]



Figure 1. *Axial reconstructions of the portal venous phase CT scans demonstrating a pseudocyst in the pancreatic tail. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)*

Endoscopic ultrasonography (EUS) is pivotal in the diagnostic assessment of pancreatic cysts, facilitating high-resolution morphological characterization and enabling cyst fluid sampling using fine-needle aspiration (FNA). The aspirated fluid is generally examined for cytology, carcinoembryonic antigen (CEA), glucose, and amylase levels. Pseudocysts, devoid of an epithelial lining, do not include epithelial cells in cytological samples. The fluid exhibits low viscosity and may seem black, turbid, or hemorrhagic, containing inflammatory cells like macrophages and histiocytes. Amylase levels are generally increased, and a measurement below 250 U/mL effectively rules out a pseudocyst. [21]. Pseudocysts are defined by low CEA levels (<192 ng/mL) and high glucose concentrations (>50 mg/dL or 2.8 mmol/L). The majority of pseudocysts resolve with supportive medical treatment; however, a subset may develop complications. Acute complications include hemorrhage (mostly resulting from splenic artery erosion and pseudoaneurysm formation), infection, and rupture. Chronic problems include gastric outlet obstruction, biliary obstruction, and thrombosis of the splenic or portal vein. [15] Hemorrhage is linked to significant morbidity and mortality, most commonly resulting

from the erosion of a major vessel close to the cyst. Interventional radiology is essential for identifying the source of bleeding and endovascular embolization. [15,22]

The clinical consequences of pseudocyst rupture depend on its site. Rupture into the gastrointestinal tract may be asymptomatic or may present with melena or hematemesis requiring urgent intervention, whereas rupture into the peritoneal cavity may lead to peritonitis and, occasionally, hemorrhagic shock, usually necessitating emergency surgery. [15]. Although infected pseudocysts may initially be managed conservatively, most patients ultimately require intervention, preferably by endoscopic drainage. Additional indications for drainage include persistent abdominal pain, gastric or duodenal obstruction, and biliary obstruction. Endoscopic techniques have become the preferred therapeutic approach in these settings. [15,23]

1.4.2. Serous cystic neoplasm (SCN)/Serous cystadenoma (SCA)

Serous cystic neoplasm and serous cystadenoma refer to the same entity, they comprise roughly 16% of all pancreatic cystic lesions. Approximately 65–70% of cases occur in women, usually between the fifth to seventh decades of life, with the majority of lesions originating in the body or tail of the pancreas. About 60% of patients are asymptomatic upon diagnosis. [24, 25] Four morphological patterns of SCNs are identified: microcystic, macrocystic (oligocystic), mixed, and solid. The microcystic variant, distinguished by numerous small cysts typically measuring less than 2 cm and divided by thin septa, accounts for around 50% of cases. A central fibrous scar with calcification may be observed in up to 30% of microcystic SCNs, resulting in the characteristic "sunburst" appearance. On CT, a circumvascular sign may be observed, reflecting the hypervascular nature of the lesion. [25] A honeycomb pattern may also be seen, corresponding to numerous tiny cysts. Macrocystic SCAs consist of a limited number of cysts over 2 cm, delineated by thin septations, and represent roughly 30% of instances. These lesions do not have a central scar but frequently exhibit external lobulation similar to the microcystic variant. Because of their appearance, macrocystic SCAs may be difficult to distinguish from other cystic pancreatic neoplasms. The solid variant presents as a hypervascular mass with or without small cystic components. [24–26]

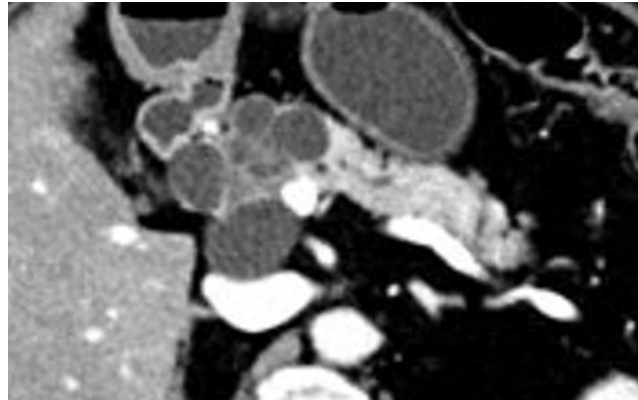


Figure 2. *Axial reconstructions of the portal venous phase CT scans of a serous cystic neoplasm in the pancreatic head. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)*

CT, MRI, and EUS can demonstrate the characteristic morphological features, such as the honeycomb pattern or clusters of microcysts. Lesions with a microcystic architecture and central scar can be confidently identified as SCAs and usually do not necessitate further diagnostic procedures. In contrast, lesions with atypical morphology, including microcystic SCNs lacking a central scar, macrocystic, or solid variants, should undergo EUS-guided FNA when feasible. [25]

The cyst fluid obtained from serous cystadenomas exhibits low viscosity, and cytological analysis shows bland, glycogen-rich cuboidal epithelial cells. Intracystic CEA levels are low (<192 ng/mL), and elevated glucose concentrations are characteristic of these lesions (> 50 mg/dL or 2.8 mmol/L). [21] According to the American College of Gastroenterology Guideline, asymptomatic SCNs do not require routine follow-up. [19]. European guidelines recommend imaging control after one year and reserve surgical resection for symptomatic lesions causing obstruction of the common bile duct, stomach, duodenum, or portal vein [27].

1.4.3. Mucinous cystic neoplasm (MCN)/Mucinous cystadenoma (MCA)

Mucinous cystic neoplasms, formerly called as mucinous cystadenomas, are usually solitary cysts that affect almost exclusively women, particularly those who are young or

middle-aged. In over 70% of instances, they are found in the body or tail of the pancreas. [28]

Histologically, MCNs are characterized by the presence of ovarian-type stroma, a thick cyst wall, and the absence of communication with the pancreatic ductal system. Although uncommon, peripheral “eggshell” calcifications represent a characteristic imaging feature. [2,21]

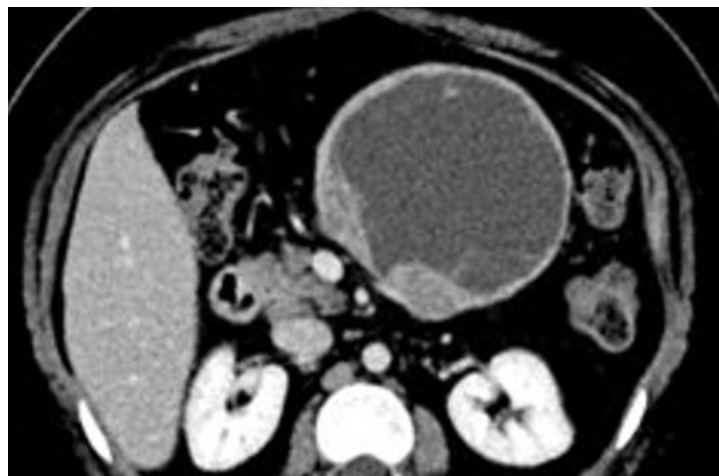


Figure 3. Axial reconstructions of the portal venous phase CT scans of a large mucinous cystic neoplasm of the pancreas. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)

The cyst fluid is highly viscous and contains mucin and columnar epithelial cells on cytological examination. However, the presence of columnar cells is not specific, as they may also be found in intraductal papillary mucinous neoplasms (IPMNs). [21] Amylase and lipase levels in the cyst fluid are usually low, whereas CEA concentrations are elevated (>192 ng/mL) and glucose levels are decreased (<50 mg/dL or 2.8 mmol/L). [21,28]

In a large systematic review of resected MCNs, malignancy was observed in 0–34% of cases; however, among lesions smaller than 4 cm, invasive carcinoma was identified in only 0.03%. [29] Surgical resection is recommended for MCNs measuring ≥ 40 mm, for symptomatic lesions, and for those exhibiting high-risk features such as mural nodules.

In patients with cysts between 30 and 40 mm, additional factors including age, comorbidities, operative risk, and patient preference should be considered.

For MCNs smaller than 40 mm without mural nodules or symptoms, lifelong surveillance is advised as long as the patient remains fit for surgery. Follow-up with MRI, EUS, or a combination of both is recommended every six months during the first year and annually thereafter if no changes are observed. In cysts smaller than 30 mm, a definitive diagnosis of MCN may be difficult, and differentiation from other cystic lesions can be challenging. Therefore, surveillance strategies similar to those applied for small IPMNs are recommended for MCNs and IPMNs measuring less than 3 cm [27].

1.4.4. Intraductal Papillary Mucinous Neoplasm (IPMN)

Intraductal papillary mucinous neoplasms (IPMNs) were originally identified in 1982 in patients initially diagnosed of pancreatic adenocarcinoma, who later demonstrated a more favorable clinical prognosis. Throughout time, other synonyms have been employed, such as mucinous ductal ectasia, mucin-producing carcinoma, intraductal mucin-producing tumor, intraductal papillary hyperplasia, intraductal papillary tumor, and intraductal papillary neoplasm. [28,30]

IPMNs are defined by segmental or diffuse dilation of the pancreatic ductal system, which is lined by mucin-producing epithelial cells. They may arise throughout the pancreas, with a marked predominance in the pancreatic head (80–90%). [21,28] According to ductal involvement, IPMNs are classified into main-duct IPMN (MD-IPMN), branch-duct IPMN (BD-IPMN), and mixed-type IPMN (MT-IPMN). This distinction is of major clinical relevance, as it impacts the risk of malignant transformation and therapeutic strategy. [28]

In surgically resected series, the reported prevalence of high-grade dysplasia or invasive carcinoma is approximately 31% (15–48%) for BD-IPMNs and 62% (36–100%) for MD-IPMNs; the corresponding rates of invasive carcinoma are 19% (6–38%) and 43% (11–81%), respectively. [31]. When considering the large proportion of indolent, unresected BD-IPMNs, the true overall risk of malignancy in BD-IPMN is substantially lower, confirming that MD-IPMN carries a significantly higher malignant potential. Mixed-type IPMNs exhibit a risk of malignant progression comparable to that of MD-IPMN.

Histopathological studies differentiating microscopic and macroscopic main duct involvement demonstrated markedly different rates of high-grade dysplasia or carcinoma (17% vs. 70%). [27,32]. However, the biological behavior of MT-IPMN remains partly unclear, as radiological classification may underestimate microscopic main duct involvement, while some BD-IPMNs show pathological main duct extension without significant ductal dilatation. [33]

Histologically, IPMNs are subdivided into gastric, intestinal, and pancreatobiliary epithelial subtypes. These subtypes are associated with distinct prognostic profiles: gastric-type lesions are predominantly low-grade BD-IPMNs, which have the most favorable prognosis, followed by intestinal-type IPMNs. In contrast, pancreatobiliary-type IPMNs provide the greatest risk of high-grade dysplasia and invasive cancer. [33]

1.4.4.1. Main-Duct IPMN

MD-IPMNs are defined by segmental or diffuse dilation of the main pancreatic duct exceeding 5 mm. A patulous ampullary orifice exhibiting mucin extrusion, commonly referred as „fish-mouth papilla” and/or mural nodules may be observed. Although many patients are asymptomatic, others may present with abdominal or back pain related to pancreatitis, jaundice, or nonspecific gastrointestinal symptoms. Laboratory evaluation includes liver function tests, measurement of pancreatic enzyme levels, and tumor markers (CEA and CA19-9). Elevated tumor marker levels, and/or new-onset or worsening diabetes mellitus, assessed by HbA1c, may indicate malignant transformation.

Contrast-enhanced MRI/MRCP and multidetector CT represent first-line imaging modalities. Endoscopic ultrasound (EUS) is valuable for detecting mural nodules and other features suggestive of high-grade dysplasia or invasive carcinoma. [33]. EUS-guided fine-needle aspiration may demonstrate highly viscous fluid with mucin-producing columnar or cuboidal epithelial cells, occasionally with atypia or malignant features. Typically, intracystic CEA is elevated (>192 ng/mL) and glucose is low (< 50 mg/dL or 2.8 mmol/L). [21,28]

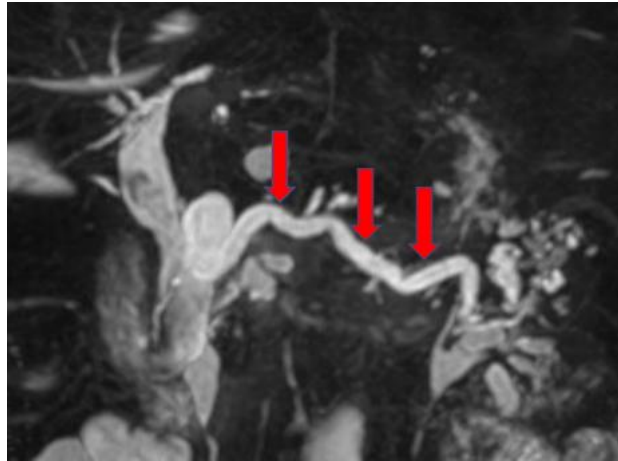


Figure 4. MRI with maximum intensity projection (MIP) reconstruction demonstrating a main-duct IPMN. Arrows indicate the dilated main pancreatic duct. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)

Surgical resection is indicated in the presence of so-called high-risk stigmata:

- main pancreatic duct diameter ≥ 10 mm,
- obstructive jaundice associated with a cystic lesion in the pancreatic head,
- enhancing mural nodule ≥ 5 mm or solid component,
- positive or suspicious cytology for high-grade dysplasia or carcinoma.

Relative “worrisome features” include duct diameter 5–9 mm, abrupt duct caliber change with distal parenchymal atrophy, acute pancreatitis, elevated CA19-9, recent onset or worsening diabetes, lymphadenopathy, and recurrent pancreatitis. Operative decisions must integrate oncologic risk with patient comorbidity, life expectancy, and preference. [33]

1.4.4.2. Branch-Duct IPMN

BD-IPMNs arise from the side branches and appear as clusters of small cysts communicating with the ductal system, often producing a grape-like configuration. They typically occur in the sixth to seventh decade and are most frequently localized in the pancreatic head or uncinate process; multifocal disease is present in 20–40% of cases. [21,33] Imaging characteristics and cyst fluid profiles resemble those of MD-IPMN.

Differential diagnoses include mucinous cystic neoplasms and ductal adenocarcinoma with cystic degeneration.

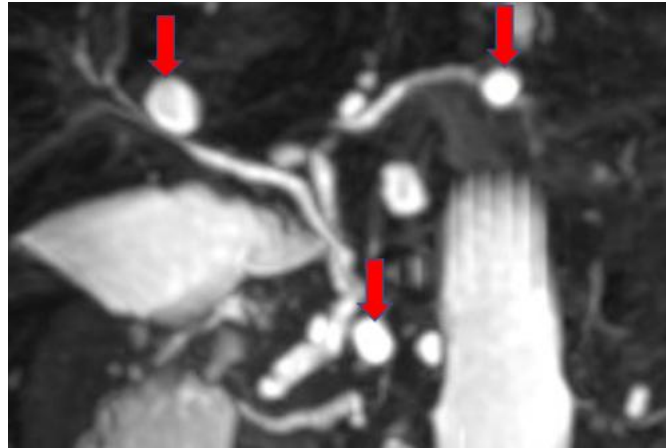


Figure 5. MRCP demonstrating multiple branch-duct IPMNs. Arrows indicate the cystic lesions. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)

High-risk stigmata prompting surgical consideration are identical to those for MD-IPMN. Worrisome features specific to BD-IPMN include cyst diameter ≥ 30 mm, mural nodules > 5 mm, thickened or enhancing cyst walls, growth ≥ 2.5 mm/year, acute pancreatitis, elevated CA19-9, recent-onset diabetes, and lymphadenopathy. In the absence of these factors, surveillance is recommended according to cyst size. The Kyoto guidelines propose MRI/MRCP, MD-CT, and/or EUS at 6- to 18-month intervals, combined with laboratory follow-up including tumor markers and HbA1c. The patient's age, comorbidities, life expectancy, and risk of concomitant pancreatic ductal adenocarcinoma should all be taken into consideration while determining the length of surveillance. [33]

1.4.4.3. Mixed-Type IPMN

Mixed-type IPMNs show both main duct dilation and cystic side-branch involvement. Given their malignant potential, which approximates that of MD-IPMN, they should be managed according to the same therapeutic principles [27].

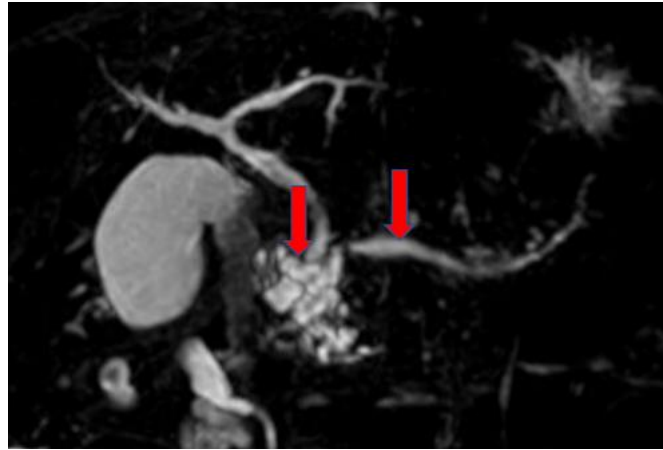


Figure 6. MRCP demonstrating a mixed-type IPMN. Arrows indicate the grape-like cluster of small cysts and the dilated main pancreatic duct. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)

1.4.5. Solid Pseudopapillary Neoplasm (SPN)

Solid pseudopapillary neoplasms, previously termed papillary cystic tumor, solid pseudopapillary tumor, or Frantz–Gruber tumor, primarily affect young women, comprising around 90% of cases [28]. They usually present between the second and fourth decades of life and are predominantly located in the pancreatic body or tail. Symptoms are typically nonspecific; jaundice and pancreatitis are uncommon. SPNs are characteristically solitary, well-defined, and often large. Imaging by CT or MRI reveals an encapsulated lesion with mixed solid and cystic components caused by hemorrhagic degeneration; peripheral calcifications may be present, similar to MCNs. [21,28]. EUS-guided biopsy is reserved for diagnostically uncertain cases. [34] Cytology demonstrates characteristic papillary structures with fibrovascular cores and uniform tumor cells with eosinophilic or foamy cytoplasm. Immunohistochemistry typically shows positivity for vimentin, α 1-antitrypsin, neuron-specific enolase, and progesterone receptor. [35,36]. Cyst fluid shows high viscosity with low amylase, lipase, and CEA levels. [28] Although the overall malignant potential is relatively low (approximately 5–15%), complete surgical resection is suggested for all patients due to the potential of local invasion and metastasis. [21,27,28,37]

1.4.6. Cystic Pancreatic Neuroendocrine Tumor (cNET)

Cystic pancreatic neuroendocrine tumors (cNETs) originate from endocrine cells of the pancreas and represent cystic variants or degenerative forms of pancreatic neuroendocrine tumors (pNETs). [2,38] They can develop in any region of the pancreas and are typically solitary, unless associated with hereditary syndromes such as multiple endocrine neoplasia type 1 (MEN1; pituitary adenoma, parathyroid hyperplasia, pancreatic neuroendocrine tumors) or von Hippel–Lindau disease. Cystic pNETs, in contrast to their solid counterparts, are often larger at diagnosis, more frequently symptomatic, and more often non-functioning, suggesting that they may constitute a biologically distinct subgroup. [39]

Radiologically, cNETs appear as well-demarcated lesions that usually do not communicate with the main pancreatic duct and often demonstrate thick, contrast-enhancing walls or intralesional solid components, while the adjacent pancreatic parenchyma and ductal system are usually preserved. [21] More than 80% of these tumors express somatostatin receptors, allowing detection by positron emission tomography using radiolabeled somatostatin analogues such as octreotide or DOTATATE. [2]

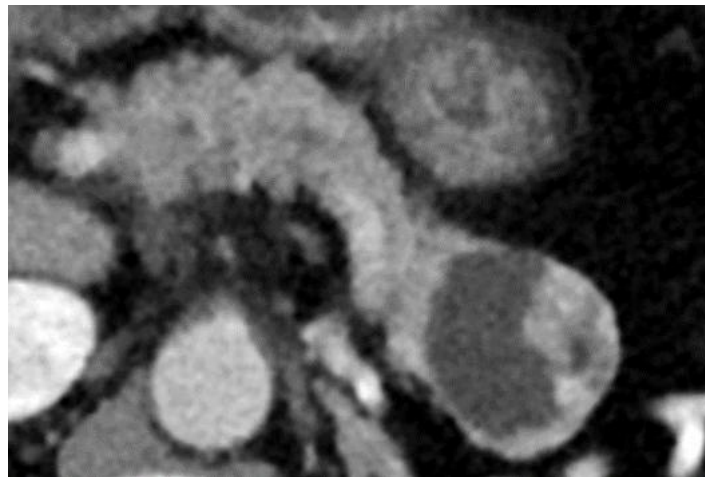


Figure 7. Axial reconstructions of the portal venous phase CT of a cystic neuroendocrine tumor in the pancreatic tail. (Courtesy of Dr. Laura Tamás-Hangody and the Medical Imaging Center, Semmelweis University)

Endoscopic ultrasound with fine-needle aspiration (EUS-FNA) can assist in diagnosis. The aspirated fluid generally has low viscosity and may be hemorrhagic; intracystic amylase and lipase levels are variable, whereas carcinoembryonic antigen (CEA) concentrations are usually low. [21] Cytologically, the tumors consist of uniform, medium-sized polygonal cells with finely granular amphophilic to eosinophilic cytoplasm. [38] Immunohistochemical staining is positive for chromogranin A, synaptophysin, CD56, CDX2, and neuron-specific enolase. [21]

Adverse prognostic factors include the presence of symptoms, tumor size ≥ 20 mm, high histological grade, Ki-67 proliferation index $\geq 3\%$, and lymphovascular invasion. [40] Owing to the limited number of studies specifically addressing cystic variants, therapeutic strategies largely follow those established for solid pancreatic neuroendocrine tumors. Surgical resection is the sole potentially curative option for localized disease and is recommended for all functional tumors irrespective of size, for non-functional tumors larger than 2 cm, and for symptomatic lesions. Active surveillance may be considered in selected patients with small (< 2 cm), non-functional, asymptomatic tumors, particularly in the presence of significant comorbidity or high operative risk. In advanced or unresectable disease, or in patients unfit for surgery, systemic treatment options include somatostatin analogues, targeted therapies (such as everolimus or sunitinib), cytotoxic chemotherapy, and peptide receptor radionuclide therapy (PRRT). For hormonally active, locally advanced or metastatic disease, or in patients unfit for surgery, ablative palliative treatment may be considered. [41]

1.4.7. Cystic Pancreatic Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) may occasionally present with cystic features. In a large histopathological series, approximately 8% of PDACs demonstrated a cystic component. [42] Four principal morphological patterns have been described: lesions with large glandular structures; tumors showing cystic degeneration; associated with retention cysts; or accompanied by pseudocyst formation. This morphological heterogeneity reflects distinct underlying pathogenic mechanisms. [42]

Macroscopically, cystic PDACs may range from lesions dominated by a single large cavity to tumors containing multiple small cystic spaces producing a honeycomb-like morphology. Imaging reveals irregular, thick-walled cystic lesions with enhancing solid

components. EUS-guided fine-needle aspiration, directed preferentially at the solid areas, is useful for confirming the diagnosis. [21] Tumor staging and therapeutic management follow the same principles as for solid pancreatic ductal adenocarcinoma, including surgical resection when feasible and multimodal oncological treatment according to disease stage.

1. 5. Methods in the diagnosis of Pancreatic Cystic Lesions

1.5.1. Diagnostic Approach to Pancreatic Cystic Lesions

The main aim of the diagnostic assessment of pancreatic cystic lesions is to stratify cysts into benign entities without malignant potential and lesions carrying low, intermediate, or high risk for advanced neoplasia. [2] The diagnostic workup should begin with a comprehensive medical history, with particular attention to previous episodes of acute or chronic pancreatitis and to a family history of pancreatic cancer or hereditary cancer syndromes linked to an elevated risk of pancreatic malignancy. [43] Clinical assessment should encompass the evaluation of symptoms, although most pancreatic cysts are detected incidentally and remain asymptomatic.

Obstructive jaundice represents a high-risk clinical feature. Pancreatitis caused by ductal obstruction or mucin hypersecretion and cyst-related abdominal pain are considered intermediate-risk features, although a causal relationship is often difficult to establish. [2]

Laboratory investigations should include serum amylase and lipase, liver transaminases and cholestatic parameters, as well as tumor marker carbohydrate antigen 19-9 (CA19-9) and glycated hemoglobin (HbA1c). Elevated CA19-9 levels and new-onset or worsening diabetes mellitus are associated with an increased risk of advanced neoplasia. [44,45] As the next diagnostic step, high-resolution magnetic resonance imaging of the pancreas with magnetic resonance cholangiopancreatography (MRI/MRCP) at 1.5 or 3 Tesla is recommended [43]. MRI demonstrates an accuracy of approximately 55–76% for distinguishing benign from malignant cysts, although its accuracy for defining the specific cyst subtype is lower (40–50%). MRI is particularly valuable for detecting communication with the main pancreatic duct and is therefore superior in the diagnosis of branch-duct IPMN. [19,46] If MRI is contraindicated, contrast-enhanced multidetector computed tomography using a dedicated pancreatic protocol should be performed.

Imaging findings guide subsequent management decisions, including the need for endoscopic ultrasound with or without fine-needle aspiration, surgical resection, or radiological surveillance. [43]

When cyst characterization remains uncertain after cross-sectional imaging or when IPMN-associated risk features cannot be reliably excluded, endoscopic ultrasound (EUS), optionally combined with contrast enhancement or fine-needle aspiration, is recommended. [47,48] In addition to cytological or histological examination—which is frequently constrained by low cellularity—measurement of cyst fluid biomarker levels and analysis molecular alterations may aid in cyst type differentiation. [47]

1.5.2. Role of Endoscopic Ultrasound in the Evaluation of Pancreatic Cystic Lesions

B-mode EUS is recommended as a complementary modality to cross-sectional imaging. Although EUS alone is imperfect for precise cyst typing and is subject to interobserver variability, its high spatial resolution enables superior visualization of key morphological features. [27,50] Systematic documentation should include the number, location, and size of cysts; their relationship to the pancreas; and detailed assessment of wall thickness, mural nodules or associated solid components, macrocystic or microcystic architecture, septations, and the presence of intraluminal mucus or debris. [21].

A major advantage of EUS is its sensitivity for detecting small mural nodules. Differentiation between true mural nodules and intraluminal mucin globules may be challenging. Mucin appears as a round, hyperechoic structure with a hypoechoic center and may be shown to be mobile by patient repositioning or by displacement during EUS-guided needle puncture. In equivocal cases, contrast-enhanced EUS (CE-EUS) can be employed. After intravenous administration of microbubble contrast agents, vascularized tissue demonstrates enhancement, whereas avascular mucin does not, enabling differentiation between neoplastic nodules and mucus. [21]. Septations can likewise be better characterized using contrast enhancement.

EUS also allows accurate assessment of ductal communication, with diagnostic performance comparable to MRI/MRCP. [51] Endoscopic inspection of the major papilla during the same procedure may reveal a characteristic “fish-mouth” appearance indicative

of mucin extrusion. Limitations of EUS include its invasive nature, the requirement for sedation, and notable interobserver variability.

1.5.3. Endoscopic Ultrasound-Guided Fine-Needle Aspiration (EUS-FNA)

EUS-FNA may yield supplementary diagnostic insights for cyst characterization and the identification of high-grade dysplasia or invasive cancer. Needle selection is guided by cyst size, location, and operator preference. Potential adverse events include bleeding, infection, pancreatitis, and, rarely, needle-tract seeding. To minimize complications, a single puncture is recommended, and complete aspiration of the targeted cyst compartment should be attempted. According to the 2025 American College of Gastroenterology (ACG) and the American Society for Gastrointestinal Endoscopy (ASGE) quality indicators, routine antibiotic prophylaxis for EUS-guided aspiration of pancreatic cysts is not routinely recommended, in contrast to earlier practice, as current evidence does not demonstrate a significant reduction in infectious complications. [21,52]

1.5.4. Macroscopic Evaluation of Cyst Fluid

Gross inspection of aspirated cyst fluid may provide limited diagnostic clues. Fluid from pseudocysts is often brownish, hemorrhagic, or purulent due to inflammatory debris and neutrophils, whereas mucinous cysts are characterized by clear, highly viscous fluid that may be difficult to aspirate. [21]

1.5.5. Cyst Fluid Viscosity and the “String Sign”

Cyst fluid viscosity can be evaluated using the so-called “string sign,” which reflects the ability of mucin-rich fluid to form an elongated filament when stretched between two surfaces. After EUS-FNA, a droplet of fluid is placed between gloved fingers or observed dripping from the needle. A positive string sign—classically defined as a filament ≥ 1 cm in length persisting for a minimum of 1 second—is highly specific for mucinous cysts. [21,53] A shorter filament length of ≥ 3 mm lasting ≥ 1 second has also been proposed as indicative of mucinous etiology. [54] However, the sensitivity of the method is moderate and interobserver variability remains substantial. [55]

1.5.6. Fluid Cytology

Cyst fluid cytology demonstrates high specificity but only moderate sensitivity for the detection of malignant pancreatic cystic lesions, with reported values of approximately 96% and 62%, respectively. [56] This discrepancy reflects the inherent difficulty of cytological interpretation, particularly in specimens with low cellularity. Several factors may further reduce diagnostic sensitivity. Sampling error is common in both microcystic and mucinous lesions, in which epithelial atypia may be focal, resulting in false-negative findings. In addition, contamination with blood or with benign epithelial cells from the gastric or duodenal mucosa may complicate cytological evaluation. [21]

When the volume of aspirated fluid is insufficient for conventional cytology, sampling of the cyst wall can be performed by repeated to-and-fro movements of the needle through the collapsed cyst cavity. [57] The presence of glycogen-rich cuboidal cells supports a diagnosis of serous cystadenoma, whereas identification of mucin-producing epithelial cells suggests a mucinous cystic lesion. Nevertheless, false-negative results are frequent, and mucin-secreting cells may also be encountered in the normal pancreatic ductal epithelium. [28]. In addition to cellular analysis, assessment for extracellular mucin has been shown to be highly specific for mucinous cysts. [58,59]

1.5.7. Cyst Fluid Amylase

Measurement of cyst fluid amylase requires approximately 0.5 mL of aspirate and is particularly useful when a pancreatic pseudocyst is considered in the differential diagnosis. Because pseudocysts typically communicate with the pancreatic ductal system, they almost invariably exhibit markedly elevated amylase concentrations. An amylase level below 250 U/mL effectively excludes a pseudocyst. [21,60] Although intraductal papillary mucinous neoplasms usually show elevated amylase levels owing to ductal communication, some mucinous cystic neoplasms may also display intermediate or high amylase activity, thereby limiting the specificity of this marker. [60,61]

1.5.8. Cyst Fluid Carcinoembryonic Antigen (CEA)

Determination of carcinoembryonic antigen (CEA) in cyst fluid generally requires 0.25–1 mL of aspirate. It should be noted that CEA assays are standardized for serum rather than cyst fluid, and inter-assay variability complicates direct comparison between studies.

Elevated CEA concentrations are commonly observed in mucin-producing cysts, whereas low levels are characteristic of pseudocysts and serous cystadenomas. Diagnostic performance depends on the applied threshold. In clinical practice, a cutoff value of 192 ng/mL is most widely used. [21] A large meta-analysis reported, at this threshold, a pooled sensitivity of 60.4% and a pooled specificity of 88.6% for distinguishing mucinous from non-mucinous cysts. [62]

Use of extreme cutoff values—either very high (>800 ng/mL) or very low (<5 ng/mL)—increases specificity to more than 95% for mucinous (IPMN or MCN) and non-mucinous cysts, respectively, but reduces sensitivity to approximately 50%. [19,63] Importantly, CEA levels do not reliably correlate with the presence of high-grade dysplasia or invasive carcinoma, and therefore cyst fluid CEA is not suitable for differentiating benign from malignant pancreatic cystic lesions. [64]

1.5.9. Other Tumor Markers

Several additional cyst fluid protein biomarkers, including CA 72-4, CA 125, CA 19-9, and CA 15-3, have been investigated for the characterization of pancreatic cystic lesions. However, their diagnostic performance has been shown to be inferior to that of CEA, and therefore they are not recommended for routine clinical use. [19,63]

1.5.10. Cyst Fluid Glucose

In recent years, intracystic glucose has emerged as a valuable biomarker for distinguishing mucinous from non-mucinous pancreatic cystic lesions. Mucinous cysts exhibit reduced glucose concentrations, usually below 50 mg/dL or 2.8 mmol/L, reflecting increased metabolic activity and glucose consumption by the neoplastic epithelium. A meta-analysis reported a pooled sensitivity of 90% and a specificity of 82% for intracystic glucose in identifying mucinous pancreatic cysts. [65] Glucose measurement requires only a minimal volume of cyst fluid (<0.002 mL), is inexpensive, and can be performed rapidly using point-of-care glucometers, allowing on-site assessment during EUS-FNA. [66]

1.5.11. Mutational Analysis

The extraction and analysis of DNA from cyst fluid have substantially expanded the diagnostic armamentarium for pancreatic cystic lesions. Specific somatic mutations are

associated with distinct cystic neoplasms and can assist in cyst classification, particularly when imaging, cytology, and biochemical markers are inconclusive or when only limited fluid volumes (<0.5 mL) are available. [2,67] Certain genetic alterations are primarily useful for identifying mucinous differentiation, whereas others are more closely associated with high-grade dysplasia or invasive carcinoma.

1.5.11.1. VHL (von Hippel–Lindau) Gene Mutations

Both sporadic and von Hippel–Lindau syndrome–associated serous cystadenomas frequently harbor inactivating mutations or loss of heterozygosity of the VHL gene. A large meta-analysis by Pflüger et al. demonstrated a pooled sensitivity of 56% and a specificity of 99% for VHL alterations in diagnosing SCAs, the most frequent benign pancreatic cystic neoplasm. [68] The detection of a VHL mutation provides strong evidence for a serous etiology and may justify discontinuation of radiological surveillance with high diagnostic confidence. [64]

1.5.11.2. KRAS Mutations

The RAS oncogene family, comprising KRAS, HRAS, and NRAS, includes the most commonly mutated oncogenes in human malignancies. KRAS (Kirsten rat sarcoma viral oncogene) is the predominant isoform involved in pancreatic tumorigenesis, with activating mutations most frequently affecting codons 12, 13, and 61, leading to constitutive signaling through proliferative and anti-apoptotic pathways. KRAS mutations are characteristic of several epithelial malignancies, including cholangiocarcinoma, colorectal, bronchial and pancreatic adenocarcinoma. [69] In pancreatic cyst fluid, KRAS mutation analysis shows high specificity (99%) but moderate sensitivity (61%) for the identification of mucinous cysts. However, its performance in distinguishing high-grade dysplasia or invasive carcinoma from low-grade lesions is limited (pooled sensitivity 73%, specificity 35%). [64]

1.5.11.3. GNAS Mutations

The GNAS (Guanine nucleotide-binding protein alpha subunit) gene encodes the stimulatory G-protein α -subunit (G α s). Activating somatic mutations lead to increased cyclic AMP signaling and enhanced cellular proliferation. Such mutations are well recognized in endocrine neoplasms, fibrous dysplasia, and McCune–Albright syndrome.

[70]. In pancreatic cyst fluid, GNAS mutations are highly specific for mucinous cysts (pooled specificity 100%) but demonstrate modest sensitivity (44%). Their potential to predict high-grade dysplasia or malignancy is limited (sensitivity 49%, specificity 51%). [64]

1.5.11.4. Other Genetic Markers

Additional mutations, including alterations in CDKN2A, PIK3CA, SMAD4, and TP53, exhibit low sensitivity but very high specificity for the detection of mucinous differentiation, high-grade dysplasia, or invasive carcinoma. Reported sensitivities for mucinous cysts are 6%, 11%, 4%, and 16%, respectively, with corresponding specificities of 99%, 100%, 98%, and 99%. For the identification of advanced neoplasia, sensitivities range from 9% to 42% with specificities between 95% and 98%. [64]

1.6. Additional Diagnostic Techniques for Pancreatic Cystic Lesions

1.6.1. EUS-Guided Through-the-Needle Biopsy (EUS-TTNB)

EUS-TTNB employs a microforceps introduced through a 19-gauge FNA needle, enabling histological sampling of the cyst wall, septa, or mural nodules. Currently available devices have jaw opening widths of 3.2 mm and 4.3 mm. The principal indication is the evaluation of morphologically indeterminate cystic lesions in which imaging, EUS-FNA cytology, and cyst fluid analysis fail to establish a definitive diagnosis, particularly in unilocular or oligocystic lesions such as SCAs, MCNs, IPMNs, and cystic neuroendocrine tumors. A further indication is risk stratification of IPMNs by assessing the grade of dysplasia or the presence of invasive carcinoma. [71]

EUS-TTNB specimens are also suitable for DNA sequencing, allowing integrated histological and molecular characterization. [71,72] In a network meta-analysis, Li et al. demonstrated superior diagnostic performance of EUS-TTNB compared with other modalities, with pooled sensitivity and specificity of 87% and 83% for mucinous cysts, and 97% and 95% for malignancy, respectively. [73] Contraindications include cysts smaller than 15 mm and clinical situations in which the results would not alter management. IPMNs require careful selection due to a higher complication rate. A large multicenter retrospective study reported an overall adverse event rate of 11.5%, including intracystic hemorrhage, acute pancreatitis, infection, and abdominal pain; three fatal

outcomes among 506 patients were documented (two from pancreatitis and one from sepsis). [74]

1.6.2. Endoscopic Ultrasound-Guided Needle-Based Confocal Laser Endomicroscopy (EUS-nCLE)

Needle-based confocal laser endomicroscopy enables real-time in vivo microscopic imaging of cyst epithelium. A 0.85-mm diameter miniprobe (Cellvizio AQ-Flex) is introduced through a standard 19-gauge FNA needle, providing a field of view of 325 μm at a resolution of 3.5 μm and an imaging depth of 40–70 μm . Following cyst puncture, the probe is positioned against the cyst wall, and 2.5 mL of 10% fluorescein is administered intravenously to permit contrast imaging. Video sequences are recorded for up to 10 minutes, after which cyst fluid is obtained for biochemical, cytological, and molecular analyses. Antibiotic prophylaxis is routinely administered prior to the procedure. [75,76]

Distinct nCLE patterns correspond to specific cyst types. A superficial vascular network is characteristic of serous cystadenomas; papillary projections or thick epithelial bands indicate mucinous cysts such as IPMN or MCN; and pseudocysts show an avascular, paucicellular fibrous wall appearing as a dark background. Reported diagnostic performance includes a sensitivity of 87% and specificity of 91% for mucinous cysts, and 81% and 98% for malignancy, respectively. [73] Limitations of the technique include high cost, limited availability, and a substantial learning curve. Complications comprise acute pancreatitis, intracystic bleeding, infection, and fluorescein-related adverse reactions, with pancreatitis being the most frequent (incidence 1.3%–12%). [76]

2. OBJECTIVES

2.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts

The objectives of this study were:

- To determine and compare the sensitivity, specificity, positive predictive value, and negative predictive value of intracystic CEA and glucose levels in distinguishing mucinous from non-mucinous pancreatic cystic lesions.
- To evaluate the clinical utility of intracystic glucose measurement as a biomarker for the identification of mucinous pancreatic cysts.

2.2. Clinical benefits of KRAS and GNAS mutation analysis in addition to morphology and conventional cyst fluid testing for the characterization of pancreatic cysts

The objectives of this study were:

- To assess the sensitivity, specificity, positive predictive value, and negative predictive value of intracystic KRAS and GNAS mutational analysis for the identification of mucinous pancreatic cystic lesions.
- To compare the diagnostic performance of genetic analysis with conventional diagnostic parameters, including cyst morphology, cyst fluid viscosity, and intracystic CEA and glucose measurement
- To assess the usefulness of KRAS and GNAS testing in clinical practice

3. METHODS

3.1. Study design

Two prospective observational studies investigating the diagnostic value of cyst fluid biomarkers and molecular testing in pancreatic cystic lesions (PCLs) were conducted at the Department of Surgery, Transplantation and Gastroenterology, Semmelweis University, Budapest. The first study evaluated the diagnostic efficacy of intracystic carcinoembryonic antigen (CEA) and glucose in differentiating mucinous and non-mucinous pancreatic cysts and was conducted between September 2020 and December 2023. The second study evaluated the clinical utility of KRAS and GNAS mutational analysis in addition to cyst morphology and conventional cyst fluid testing and was performed between April 2022 and July 2025.

Both studies were approved by the Ethics Committee of Semmelweis University (approval number: 1121-1/2020/EKU). Written informed consent was obtained from all participants prior to endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). Clinical data, imaging reports, laboratory results, cytology, pathology, molecular analyses, and surgical records were collected in a prospective manner and documented systematically.

3.2. Inclusion and exclusion criteria

Adult patients (>18 years) with pancreatic cystic lesions of uncertain etiology were eligible for inclusion if EUS-FNA and cyst fluid analysis were expected to influence clinical management, particularly in differentiating mucinous from non-mucinous cysts or in identifying advanced neoplasia.

In the biomarker study, only cysts in which both intracystic CEA and glucose measurements were successfully obtained were included.

In the molecular study, patients were enrolled if cyst fluid analysis, including KRAS/GNAS testing, was considered clinically relevant for risk stratification or therapeutic decision-making.

Exclusion criteria in both studies comprised contraindications to endoscopy or FNA, cysts with unequivocal radiological features of invasive malignancy, and cases in which a consensus final diagnosis could not be established by expert review.

Final cyst diagnoses were determined retrospectively by three experienced gastroenterologists using all available clinical, imaging, biochemical, cytological, and (when available) histopathological data. In the first study, assessors were blinded to glucose results; in the second study, they were blinded to KRAS/GNAS findings. This composite clinical diagnosis served as the reference standard in cases without histology or definitive cytology.

3.3. Endoscopic ultrasound (EUS)

All EUS examinations were performed by two expert endosonographers with extensive experience in pancreatic imaging, using a UCT-180 linear echoendoscope connected to an EU-ME2 Premium ultrasound processor (Olympus GmbH, Hamburg, Germany). Only cysts measuring at least 15 mm were sampled in the molecular study, whereas no minimum size threshold was applied in the biomarker study.

During EUS, the following parameters were systematically recorded:

- cyst location, size, and number
- cyst contour and lobularity
- cyst wall thickness
- presence of septations, mural nodules, or associated solid components
- main pancreatic duct diameter
- ductal communication
- presence of regional lymphadenopathy

Fine-needle aspiration was performed using 19-, 22-, or 25-gauge EchoTip Ultra needles (Cook Medical, USA). In a limited number of cases, alternative 22-gauge needles (Micro-Tech Areus or Trident, China) were used. Aspiration was carried out with negative pressure using a 10-mL syringe. No procedure-related complications were observed in either cohort.

3.4. CT and MRI morphology

All patients underwent contrast-enhanced multidetector computed tomography (MDCT) and/or magnetic resonance imaging with magnetic resonance cholangiopancreatography (MRI/MRCP) prior to EUS-FNA. The same morphological criteria used during EUS evaluation were applied to cross-sectional imaging for cyst characterization. Imaging studies were independently reviewed by experienced radiologists and pancreatologists. In cases suspected of premalignant or malignant disease, findings were additionally discussed in a multidisciplinary pancreatic tumor board.

3.5. Conventional cyst fluid analysis

3.5.1. String sign

Cyst fluid viscosity was assessed using the string sign test. A drop of the obtained cyst fluid was placed between the thumb and index finger, and the fingers were slowly separated while measuring the maximal length of the fluid strand before rupture. [21,53]

In the first study, the test was considered positive if the strand reached at least 3–4 mm and persisted for ≥ 1 second.

In the second study, a stricter criterion was applied, defining positivity as a strand length of ≥ 5 mm lasting for ≥ 1 second.

3.5.2. Cytology

Cytological evaluation was performed at the Department of Pathology, Forensic and Insurance Medicine, Semmelweis University, Budapest. Air-dried smears stained with hematoxylin–eosin and Papanicolaou technique were used.

Samples were assessed for:

- malignant cells or cellular atypia
- mucin-producing epithelial cells
- glycogen-rich cells
- inflammatory cells
- extracellular mucin

3.5.3. CEA and Glucose

Cyst fluid samples were immediately transported to the clinical laboratory and processed within four hours. CEA concentrations were measured by electrochemiluminescence using enzyme-labeled sandwich immunoassays, while glucose levels were determined spectrophotometrically using Hexokinase.

In both studies, the standard cut-off values were applied:

- CEA: >192 ng/mL to indicate mucinous cysts [77]
- Glucose: <50 mg/dL (2.8 mmol/L) to indicate mucinous cysts [78]

At least 1 mL of fluid was required for combined biochemical testing in the molecular study.

3.5.4. Amylase

Intracystic amylase levels were measured when sufficient fluid volume was available. An amylase concentration below 250 U/L was used to exclude pancreatic pseudocysts.

3.6. KRAS/GNAS mutational analysis

Molecular testing was performed at the Department of Pathology, Forensic and Insurance Medicine, Semmelweis University, Budapest, results were interpreted by molecular pathologists blinded to clinical, imaging, cytological, and biochemical data. A minimum of 0.5 mL of cyst fluid was used for analysis. DNA was extracted using the HighPur PCR Template Kit (Roche). Exon 2 of the KRAS gene (NM_004985.5) and exon 8 of the GNAS gene (ENST00000371085.8) were amplified with intronic sequence-specific primers. Bidirectional Sanger sequencing was conducted using the BigDye® Terminator v1.1 Cycle Sequencing Kit, followed by purification with the BigDye® XTerminator™ kit and analysis on a SeqStudio Genetic Analyzer (Thermo Fisher Scientific). During the initial phase, only KRAS testing was performed; subsequently, combined KRAS and GNAS analysis was implemented.

3.7. Follow-up

In the biomarker study, the mean follow-up duration was 921 days.

In the molecular study, patients were followed until July 2025, with a median follow-up of 252 days (range: 80–751 days).

Follow-up data included clinical status, imaging findings, surgical outcomes when applicable, and disease progression.

3.8. Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics version 25, GraphPad Prism (version 10.6.1), and Microsoft Excel (Microsoft 365). Normality of continuous variables was evaluated using the Anderson–Darling, D’Agostino–Pearson, Shapiro–Wilk, and Kolmogorov–Smirnov tests. Comparisons between groups were performed using the independent two-sample Student’s t-test when data followed a normal distribution. In cases where normality assumptions were not met, the Mann–Whitney U test was applied. Categorical variables were analyzed using the chi-square (χ^2) test or, when expected frequencies were below five, Fisher’s exact test was used instead. Continuous variables were presented as mean $\pm$ standard deviation or median with range/interquartile range, depending on distribution characteristics. All statistical tests were two-tailed, and p value below 0.05 was interpreted as statistically significant. [79,80]

3.9. Language editing

Language editing was performed with the assistance of an AI-based language tool (ChatGPT, OpenAI, San Francisco, CA, USA).

4. RESULTS

4.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts

Pancreatic cyst fluid samples were obtained from 97 patients and analyzed for cytology, amylase activity, CEA, and glucose concentrations. Six cases were excluded from the final analysis: four due to clear evidence of cyst infection based on cytological and/or microbiological findings, and two because of marked blood contamination detected by macroscopic inspection, as these conditions may lead to falsely altered glucose and CEA measurements. Consequently, 91 patients were included in the final cohort.

Among these patients, 28 (30.8%) were male and 63 (69.2%) were female. Patients with mucinous cystic lesions were significantly older than those with non-mucinous cysts (median age, 72.4 years [IQR, 65.95–76.58] vs. 56.4 years [IQR, 42.6–71.2], respectively; $p < 0.0001$). The median cyst diameter measured by endoscopic ultrasonography was 28 mm in the mucinous group and 38 mm in the non-mucinous group, showing a trend toward larger lesions in the latter group ($p = 0.054$).

Regarding morphology, 43 cysts were unilocular and 48 were multilocular. Anatomical distribution revealed 28 cysts in the uncinate process or pancreatic head, 59 in the pancreatic body, and 4 in the tail. Detailed demographic and morphological characteristics are summarized in Table 2.

Table 2. *Population demographics and cyst characteristics*

Parameter	Mucinous	Non-mucinous	p-value
Male, n (%)	18 (35)	10 (26)	
Female, n (%)	34 (65)	29 (74)	
Age, median (IQR)	72.4 (65.95-76.58)	56.4 (42.6-71.2)	<0.0001
Cyst characteristics	Mucinous	Non-mucinous	p-value
Localization of the largest cyst, n, (%)			
Uncinate process	3 (6)	0(0)	
Head	12(23)	13(33)	
Genu of pancreas	8(15)	8(21)	
Body	16 (30)	14 (36)	
Body-tail border	11 (21)	2 (5)	
Tail	2 (4)	2 (5)	
Largest cyst diameter (mm)	28 (24–32)	38 (25–41)	0.054
Unilocular, n (%)	24 (46)	19 (49)	
Multilocular, n (%)	28 (54)	20 (51)	

Definitive diagnosis was established by postoperative histopathology in 13 patients (14.3%) and by conclusive cytological examination of cyst fluid in 5 cases (5.5%). For the remaining patients, the reference diagnosis was based on integrated clinical follow-up, cross-sectional and EUS morphology, cytological features, and string sign testing. Final classification was determined by consensus among three experienced pancreatologists. Reference standards are presented in Table 3.

Table 3. *Reference standards*

	Mucinous	Non-mucinous
Diagnosis based on	Number of cysts (%)	Number of cysts (%)
Follow up, Imaging	37 (71)	36 (92)
Postoperative histopathology	12 (23)	1 (3)
EUS guided FNA – definitive cytopathology	3 (6)	2 (5)

Overall, 52 cysts were classified as mucinous, including 33 intraductal papillary mucinous neoplasms (IPMN), 12 mucinous cystic neoplasms (MCN), and 7 pancreatic adenocarcinomas. The remaining 39 lesions were non-mucinous: 21 pseudocysts, 16

serous cystic neoplasms (SCN), one neuroendocrine tumor, and one lymphoepithelial cyst. Diagnostic categories are presented in Table 4.

Table 4. *Diagnostic categories*

Diagnosis	Mucinous n (%)	Non-mucinous n (%)
IPMN	33 (63.5)	—
MCN	12 (23)	—
Adenocarcinoma	7 (13.5)	—
Serous cystic neoplasm	—	16 (41)
Pseudocyst	—	21 (54)
Neuroendocrine tumor	—	1 (2.5)
Lymphoepithelial cyst	—	1 (2.5)

Median intracystic CEA concentration was 449.5 ng/mL (IQR: 58.23–1785) in mucinous cysts and 3 ng/mL (IQR: 3–16.3) in non-mucinous cysts ($p < 0.0001$). Conversely, median glucose levels were markedly lower in mucinous lesions than in non-mucinous cysts (8.1 mg/dL [IQR, 1.8–11.71] vs. 100.9 mg/dL [IQR, 55.86–115.3], respectively; $p < 0.0001$). Biomarker distributions are shown in Table 5.

Table 5. *Biomarker distributions*

Parameter	Mucinous	Non-mucinous	p-value
CEA (cut-off: 192 ng/mL)			
Median (IQR), ng/mL	449.5 (58.23-1785)	3 (3-16.3)	<0.0001
Elevated / non-elevated, n	36 / 16	0 / 39	
Glucose (cut-off: 50 mg/dL)			
Median (IQR), mg/dL	8.1 (1.8-11.71)	100.9 (55.86-115.3)	<0.0001
Elevated / non-elevated, n	2 / 50	31 / 8	
CEA or glucose positive	52 / 0	8 / 31	
Amylase (cut-off: 250 IU/L)			
Median (IQR), IU/L	2445 (1011–8825)	10360 (173–52800)	
Elevated / non-elevated, n	40 / 12	23 / 16	

Using a cut-off value of 192 ng/mL, elevated CEA levels were observed in 36 of 52 mucinous cysts, corresponding to a sensitivity of 69.2% for identifying mucinous lesions. In contrast, intracystic glucose concentrations below 50 mg/dL were detected in 50 of 52 mucinous cysts, yielding a sensitivity of 96.2%.

CEA demonstrated perfect specificity (100%), whereas glucose showed a specificity of 79.5%. When combining both biomarkers (CEA >192 ng/mL and/or glucose <50 mg/dL), diagnostic sensitivity increased to 100%, although specificity decreased compared with CEA alone (79.5% vs. 100%).

Diagnostic performance metrics, including positive and negative predictive values, are shown in Table 6. [79]

Table 6. *Diagnostic performance*

Parameter	CEA	Glucose	CEA + Glucose
Sensitivity	69.2%	96.2%	100%
Specificity	100%	79.5%	79.5%
PPV	100%	86.2%	86.7%
NPV	70.9%	93.9 %	100%

4.2. Clinical benefits of KRAS/GNAS gene mutation analysis in addition to morphology and conventional cyst fluid testing in differentiating pancreatic cysts

The study cohort comprised 47 patients (15 men and 32 women) with a broad spectrum of pancreatic cystic lesions. Thirty-three lesions (70.2%) were classified as mucinous, including 24 intraductal papillary mucinous neoplasms (IPMNs), five mucinous cystic neoplasms (MCNs), and four pancreatic ductal adenocarcinomas with cystic morphology. Fourteen cysts (29.8%) were non-mucinous, consisting of five pseudocysts or walled-off necroses (WONs), eight serous cystic neoplasms (SCNs), and one cystic neuroendocrine tumor (cNET). Twenty-six cysts were unilocular and twenty-one were multilocular. The median diameter of the largest cyst was 35.5 mm (IQR 25.0–48.5). Baseline demographic and morphological characteristics are summarized in Table 7.

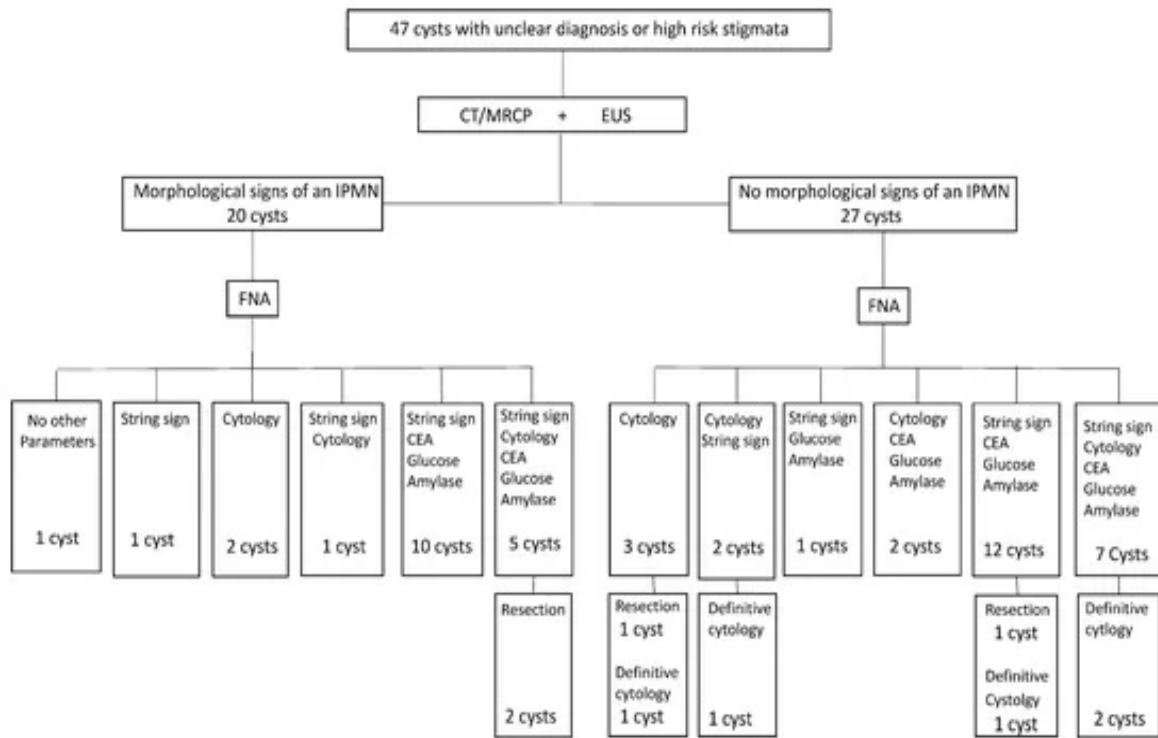
Table 7. Patient and cyst characteristics

Parameter	All (n=47)	Mucinous (n=33)	Non-mucinous (n=14)	p-value
Gender – Male, n (%)	15 (31.9%)	13 (39.4%)	2 (14.3%)	0.17
Gender – Female, n (%)	32 (68.1%)	20 (60.6%)	12 (85.7%)	
Age, mean ($\pm$ SD) (years)	65.8 ($\pm$ 14.8)	68.7 ($\pm$ 14.7)	58.9 ($\pm$ 13.1)	0.04
Localization – Uncinate process	1 (2.1%)	1 (3.0%)	0 (0.0%)	
Localization – Head	20 (42.6%)	13 (39.4%)	7 (50.0%)	
Localization – Genu	7 (14.9%)	4 (12.1%)	3 (21.4%)	
Localization – Body	14 (29.8%)	11 (33.3%)	3 (21.4%)	
Localization – Body–tail border	2 (4.3%)	2 (6.1%)	0 (0.0%)	
Localization – Tail	3 (6.4%)	2 (6.1%)	1 (7.1%)	
Largest cyst diameter, median (IQR), mm	35.5 (25.0–48.5)	32.0 (25.0–41.0)	47.5 (24.0–67.0)	0.11
Unilocular cyst, n (%)	26 (55.3%)	18 (54.5%)	8 (57.1%)	
Multilocular cyst, n (%)	21 (44.7%)	15 (45.5%)	6 (42.9%)	

Histopathological confirmation was available in four patients (8.5%), revealing one pancreatic intraepithelial neoplasia (panIN) with high-grade dysplasia, one IPMN with low-grade dysplasia, one IPMN with high-grade dysplasia, and one MCN. Definitive cytological diagnosis was established in five additional cases (10.6%), including four adenocarcinomas and one cNET, all of which were unresectable or surgery was declined.

The remaining 38 cysts (80.9%) were classified on the basis of a composite reference standard incorporating cross-sectional imaging, EUS morphology, cytology, string sign, and intracystic CEA and glucose levels (Figure 8).

Figure 8. Diagnostic strategy



Material for cytological evaluation was obtained in 45 patients (95.7%), with sample quality rated as satisfactory in 48.8%, suboptimal in 37.7%, and unsatisfactory in 13.3%. Intracystic CEA and glucose concentrations were available in 36 (76.6%) and 37 (78.7%) patients, respectively. In eight cases, biomarker measurement was intentionally omitted because imaging strongly suggested IPMN or cystic adenocarcinoma, and in three cases CEA assessment was technically unsuccessful.

KRAS mutational analysis was successfully performed in all 47 patients, whereas GNAS testing was available in 28 cases. KRAS mutations were detected in 14 of 33 mucinous cysts, corresponding to a sensitivity of 42.4% (95% CI: 27.2–59.2), while only one non-mucinous lesion harbored a KRAS mutation, yielding a specificity of 92.9% (95% CI: 68.5–99.6). Activating GNAS mutations were identified in 10 of 23 mucinous cysts tested (sensitivity 43.5 %, 95% CI: 25.6–63.2), and in none of the non-mucinous lesions, resulting in a specificity of 100% (95% CI: 56.6–100). Detailed results of the mutational analyses are presented in Table 8, and the corresponding diagnostic performance indices are summarized in Table 9.

Table 8. Results

Cyst Type	All	Mucinous	Non-mucinous
K-ras			
wild	32 (68.1%)	19 (57.6%)	13 (92.9%)
mutant	15 (31.9%)	14 (42.4%)	1 (7.1%)
GNAS			
wild	18 (38.3%)	13 (39.4%)	5 (35.7%)
mutant	10 (21.3%)	10 (30.3%)	0 (0.0%)
not analyzed	19 (40.4%)	10 (30.3%)	9 (64.3%)

Table 9. Results

K-Ras	value	(95% CI)
Sensitivity	0.4242	0.2724 to 0.5919
Specificity	0.9286	0.6853 to 0.9963
Positive Predictive Value	0.9333	0.7018 to 0.9966
Negative Predictive Value	0.4063	0.2552 to 0.5774
GNAS	value	(95% CI)
Sensitivity	0.4348	0.2563 to 0.6319
Specificity	1.0	0.5655 to 1.000
Positive Predictive Value	1.0	0.7225 to 1.000
Negative Predictive Value	0.2778	0.1250 to 0.5087

Among the six mucinous lesions with histologically or cytologically confirmed high-grade dysplasia or invasive carcinoma, KRAS mutations were present in five cases (83.3%), whereas GNAS mutations were detected in two of the five patients in whom GNAS analysis was available (40%). No KRAS or GNAS alterations were identified in the single cystic neuroendocrine tumor.

In terms of clinical impact, molecular testing influenced patient management in some cases. In one patient, conventional diagnostic modalities suggested a serous cystic neoplasm; however, the detection of a KRAS mutation raised the suspicion of a mucinous etiology, leading to intensified surveillance and consideration of repeat EUS-FNA in the event of morphological progression. Furthermore, in two additional patients in whom CEA measurement was unsuccessful, the presence of oncogenic mutations supported the diagnosis of mucinous cysts.

In these cases, molecular results complemented, rather than replaced other diagnostic features such as cyst morphology, string sign, and cytology, illustrating the added value of KRAS/GNAS analysis as part of a multimodal diagnostic approach.[80]

5. DISCUSSION

5.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts

The increasing use of cross-sectional abdominal imaging has led to a rising detection rate of incidentally identified pancreatic cystic lesions, many of which are asymptomatic. Given the heterogeneous malignant potential of these entities, accurate risk stratification and identification of lesions requiring surveillance or surgical intervention are of major clinical importance. At present, a definitive diagnosis can usually be established only by postoperative histopathology or by cytological confirmation of adenocarcinoma or neuroendocrine tumor from cyst fluid. However, no single diagnostic modality, nor any combination of currently available techniques, provides fully reliable discrimination across all cyst subtypes.

Current international guidelines recommend categorizing pancreatic cystic lesions into mucinous and non-mucinous groups, as mucinous lesions are considered premalignant and therefore warrant long-term surveillance or resection depending on risk features. [19,27,33] Routine clinical evaluation relies on patient history (including previous pancreatitis), cross-sectional imaging (CT and MRI/MRCP), and endoscopic ultrasound with fine-needle aspiration (EUS-FNA) and cyst fluid analysis. In cases of uncertain etiology, MRI/MRCP is considered the preferred imaging technique; nevertheless, its diagnostic accuracy for establishing a specific cyst type ranges widely from 20% to 86%, while accuracy for differentiating benign from malignant lesions is only 56%–76% according to systematic review data. [81]

A major benefit of EUS is the ability to acquire cyst fluid for biochemical and cytological analysis. When imaging findings are inconclusive and results are expected to influence management, guidelines recommend cytology, amylase, and CEA measurement. [19,27,33] Amylase levels are primarily useful for excluding pseudocysts when values are below 250 U/L. Cytology demonstrates high specificity (approximately 89%) but limited sensitivity (around 46%) for the detection of mucinous cysts. [73] Meta-analyses evaluating cyst fluid biomarkers have reported sensitivities of 56%–67% and specificities of 80%–96% for CEA at the conventional cut-off value of 192 ng/mL, whereas glucose

at a cut-off of 50 mg/dL shows markedly higher sensitivity (around 91%) with specificity between 75% and 86%. [65,66,82]

The optimal diagnostic threshold for intracystic glucose remains controversial. Zamir et al. proposed a cut-off of 87 mg/dL (4.8 mmol/L), achieving a sensitivity of 90.9% and a specificity of 83.3% in a cohort of 101 samples. [83] In contrast, Smith et al. reported favorable diagnostic performance using a substantially lower threshold of 25 mg/dL (1.4 mmol/L), with sensitivity of 88.1% and specificity of 91.2% in 93 cysts. [84]. In the present study, we applied the widely adopted cut-off value of 50 mg/dL (2.8 mmol/L).

Intracystic glucose measurement was first highlighted by Park et al. in 2013 as a promising biomarker for identifying mucinous cystic lesions. [85] Despite accumulating evidence over more than a decade, this method has not been incorporated as diagnostic standard into previous international guidelines. In contrast, the 2024 Kyoto Guideline acknowledges glucose as a useful marker [33], and more recently, the European Society of Gastrointestinal Endoscopy (EGSE), in a 2025 technical review suggests the use of intracystic glucose level measurement over CEA for the diagnosis of mucinous cysts. [86] Compared with CEA, glucose measurement offers several methodological advantages: while CEA assays are validated primarily for serum and demonstrate inter-laboratory variability in cyst fluid, glucose determination is standardized across multiple biological fluids and shows excellent reproducibility. [87]. Moreover, glucose analysis requires a substantially smaller sample volume (approximately 50 μ L versus ≥ 200 μ L for CEA). [65] The availability of point-of-care glucometers further enables immediate on-site testing, facilitating rapid clinical decision-making during EUS procedures. [66]

In this prospective single-center study, we directly compared the diagnostic performance of CEA and glucose for identifying mucinous pancreatic cystic lesions. CEA demonstrated a sensitivity of 69.2% and a specificity of 100%, whereas glucose exhibited markedly higher sensitivity (96.2%) but lower specificity (79.5%). Combining the two biomarkers (CEA >192 ng/mL or glucose <50 mg/dL) increased sensitivity to 100%, although specificity was reduced compared with CEA alone (79.5% vs. 100%). These findings are consistent with previous reports highlighting the superior sensitivity of intracystic glucose for the detection of mucinous cysts.

From a pathophysiological perspective, reduced glucose concentrations in mucinous cyst fluid are thought to reflect increased metabolic consumption by mucin-producing epithelial cells. [88] Infectious processes can similarly lower glucose levels due to bacterial utilization [89]; therefore, cysts with microbiological or cytological evidence of infection were excluded from the present analysis to avoid confounding.

The prospective design and standardized assessment represent strengths of this study. The single-center setting may also be advantageous for investigations involving operator-dependent procedures such as EUS, as it limits interobserver variability. All examinations were performed by two experienced endosonographers, with additional independent evaluation by a third gastroenterologist.

Nevertheless, several limitations must be acknowledged. Histological confirmation was available in only 14.3% of cases, and definitive cytological diagnosis of adenocarcinoma or neuroendocrine tumor in 5.5%. The majority of lesions were classified based on imaging, cyst fluid characteristics, and clinical follow-up, which may reduce diagnostic certainty. However, population-based data indicate that the prevalence of malignancy at initial detection is approximately 0.25%, with an annual conversion rate to invasive cancer of 0.24%, and only about 15% of surgically resected cysts prove malignant. [90] Thus, the limited availability of histology reflects real-world clinical practice, where most cysts are managed conservatively and cytology rarely yields definitive results. A further limitation is the relatively short mean follow-up period of 921 days, which may be insufficient to capture late malignant transformation.

When compared with emerging diagnostic approaches, such as cyst fluid mutational analysis or advanced “through-the-needle” techniques, intracystic glucose measurement may appear less sophisticated. Genetic testing for KRAS and GNAS mutations can identify mucinous cysts with high specificity, and European guidelines suggest considering such analysis when diagnostic uncertainty is likely to influence management. [27] However, despite modest sample volume requirements (0.2–0.5 mL), DNA yield may be inadequate in some cases. [89,90] In addition, mutational analysis remains costly and requires specialized infrastructure and expertise. Confocal laser endomicroscopy and through-the-needle biopsy demonstrate superior diagnostic accuracy [73] but are

associated with higher costs, increased complication rates, and a significant learning curve, limiting their routine application.

In summary, our findings support intracystic glucose as a simple, inexpensive, and highly sensitive biomarker for identifying mucinous pancreatic cystic lesions. Diagnostic performance can be further enhanced when combined with CEA measurement. Despite growing evidence, glucose remains underutilized in daily practice. Large-scale, prospective, multicenter studies are warranted to confirm its clinical utility and to support its inclusion in future international guidelines.

5.2. Clinical benefits of KRAS/GNAS gene mutation analysis in addition to morphology and conventional cyst fluid testing in differentiating pancreatic cysts

The differential diagnosis of pancreatic cystic lesions (PCLs) remains a major clinical challenge and is likely to continue to be so in the foreseeable future. Certain cyst types, particularly intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs), carry a relevant risk of malignant transformation, whereas others may already represent invasive carcinoma at the time of detection. In contrast, the majority of incidentally discovered, asymptomatic pancreatic cysts are benign, and the overall risk of malignant progression is low. [90] Underestimation of malignant potential may result in delayed diagnosis of pancreatic cancer, while overestimation may lead to unnecessary surveillance, substantial healthcare costs, and avoidable morbidity and mortality associated with unwarranted surgical resection. Accordingly, the diagnostic work-up of PCLs focuses first on differentiating mucinous from non-mucinous lesions and, subsequently, on identifying features associated with high-grade dysplasia or malignancy.

In current clinical practice, cyst characterization relies on a multimodal strategy including cross-sectional imaging (CT, MRI/MRCP), endoscopic ultrasound (EUS) morphology, assessment of the string sign, and analysis of cyst fluid cytology and carcinoembryonic antigen (CEA) levels. In recent years, molecular analysis of pancreatic cyst fluid has emerged as a promising adjunctive diagnostic modality. DNA obtained from cyst fluid allows detection of specific somatic mutations that may assist in defining cyst lineage and, potentially, neoplastic grade. For example, von Hippel–Lindau (VHL) mutations are highly specific for serous cystadenomas, although their sensitivity is limited to

approximately 50%. [64] Regarding mucinous lesions, combined testing of KRAS and GNAS mutations has shown encouraging diagnostic accuracy. A meta-analysis by McCarty et al. including 785 lesions reported a sensitivity of 75% and a specificity of 99% for detecting mucinous cysts using combined KRAS/GNAS mutational analysis. [82] Similar results were obtained in a network meta-analysis by Li et al., which demonstrated a sensitivity of 77% and a specificity of 93% when histopathology was used as the reference standard. [73] Furthermore, accumulating evidence indicates that GNAS mutations are highly specific for IPMNs. [93–95]

Recommendations regarding the clinical use of molecular testing have evolved over time. The revised Fukuoka Guidelines in 2017 considered cyst fluid molecular analysis investigational and suggested its use only in specialized centers. [31] The 2018 American Gastroenterological Association guideline described molecular diagnostics as an emerging research field with uncertain clinical utility [19]. In contrast, the European evidence-based guidelines published in the same year already recommended considering genetic testing for KRAS and GNAS mutations in cases with indeterminate diagnosis when the result could influence clinical management (evidence level 2C, strong agreement). [27] Most recently, the 2024 Kyoto consensus guidelines on IPMN management have further strengthened the role of cyst fluid genetic markers, endorsing their use for cyst type classification and for identifying high-grade dysplasia or invasive carcinoma. [33]

In the present prospective study, we assessed the clinical utility of KRAS and GNAS mutation analysis in the differentiation of pancreatic cystic lesions. The sensitivity of KRAS and GNAS for identifying mucinous cysts was 42.4% and 43.5%, respectively, while specificity reached 92.9% for KRAS and 100% for GNAS. Several limitations must be acknowledged. The cohort size was modest, and only a minority of lesions had a definitive diagnosis based on surgical histology (8.5%) or conclusive cytology for adenocarcinoma or neuroendocrine tumor (10.6%). Initial classification relied on cross-sectional imaging and EUS, particularly on the assessment of ductal communication, assuming the high diagnostic accuracy of MRCP and EUS for this purpose. [51] Final diagnosis was refined using cyst fluid viscosity, cytology, CEA, and glucose levels, which together were considered a composite reference standard. While this approach reflects everyday clinical practice and includes cysts with diverse size, morphology, and etiology,

it inevitably carries the risk of misclassification, especially when one or more parameters are unavailable or inconclusive. In our cohort, cytology was absent or non-diagnostic in 17.6% of cases, and CEA and glucose measurements were unsuccessful or not performed in 23.4% and 21.3%, respectively. Furthermore, KRAS analysis was initially performed alone, with combined KRAS/GNAS testing introduced only later. The relatively low sensitivity observed in our study compared with previous reports [73,82] may in part be explained by the use of Sanger sequencing rather than next-generation sequencing (NGS), which has a lower limit of mutation detection. [91]

Despite these limitations, molecular testing influenced clinical management in selected cases. In one patient, conventional assessment suggested a non-mucinous lesion, yet detection of a KRAS mutation—given the high specificity of this alteration for mucinous neoplasia—prompted reclassification and closer surveillance with the option of repeat EUS-FNA. In two additional patients, CEA measurement was not feasible, whereas genetic testing supported a mucinous diagnosis. As molecular analysis requires only a small volume of cyst fluid and is less affected by high viscosity, it may provide valuable complementary information when standard biochemical assays are inconclusive. For this reason, storage of frozen cyst fluid for potential future genetic analysis appears justified in selected cases.

The clinical value of KRAS/GNAS testing remains debated. Several studies have reported limited incremental benefit over conventional diagnostics. Mack et al. found KRAS/GNAS mutations in only 12% of cysts with low CEA levels and concluded that molecular testing had restricted impact on management [96]. Similarly, Faias et al. observed clinically relevant reclassification in only two of 52 cases following KRAS/GNAS analysis. [97] Herranz Pérez et al. analyzed cyst fluid samples from 36 pancreatic cysts, including cytology, CEA, glucose, and a panel of genetic markers. Among the six lesions initially classified as non-mucinous based on conventional diagnostic criteria, KRAS and GNAS mutations were detected in two cases. As a consequence, these two cysts were reclassified as mucinous neoplasms and the follow-up strategy was modified accordingly. Thus, similarly to our findings, molecular analysis resulted in a change in clinical classification in only a limited number of cases, indicating a modest incremental diagnostic yield over standard morphological and biochemical assessment. [98] More favorable results have been published using NGS-based

techniques. Schmitz et al. demonstrated that KRAS/GNAS analysis by deep targeted NGS was superior to both cytology and CEA in identifying mucinous cysts [99], and Rahal et al., in a large single-center cohort of 933 patients, showed that the addition of genetic testing significantly improved the sensitivity of CEA for differentiating mucinous from non-mucinous lesions when histopathology served as the reference standard. [100] Discrepancies among studies likely reflect differences in sequencing methodology (Sanger vs. NGS or deep-targeted NGS) and in the applied reference standards.

Our study is, to our knowledge, the first to compare KRAS/GNAS mutation analysis with an extended conventional diagnostic panel that also included the string sign and intracystic glucose measurement, in addition to morphology, cytology, and CEA. Given that the combination of glucose and CEA has been shown highly sensitive in identifying mucinous PCLs, the incremental diagnostic yield of KRAS/GNAS testing in this comprehensive setting appeared limited, with lower sensitivity than that of conventional multimodal assessment. Nevertheless, the very high specificity of these mutations may be particularly valuable in selected indeterminate cases and when standard biomarkers cannot be reliably measured.

KRAS mutations were identified in 83% and GNAS mutations in 40% of cysts with histologically or cytologically confirmed high-grade dysplasia or malignancy in our cohort. Although these findings are in line with previously published data, the number of cysts with high-grade dysplasia or invasive carcinoma in our cohort was limited, furthermore, KRAS and GNAS mutations per se are not suitable for distinguishing high-grade dysplasia or invasive malignancy. Finally, while GNAS mutations are considered highly specific for IPMNs, this feature did not lead to diagnostic reclassification in our series; however, it may provide important supportive evidence when other diagnostic modalities yield inconclusive results.

6. CONCLUSIONS

6.1. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in the differentiation of mucinous and non-mucinous pancreatic cysts

- Measurement of intracystic glucose demonstrated a sensitivity of 96.2% and a specificity of 79.5% for the identification of mucinous pancreatic cystic lesions at a cut-off level of 50 mg/dL (2.8 mmol/L).
- Compared with intracystic CEA (cut-off >192 ng/mL), glucose exhibited superior sensitivity but inferior specificity for the detection of mucinous pancreatic cystic lesions.
- The highest diagnostic sensitivity was achieved by combining elevated intracystic CEA with reduced glucose levels. The specificity of the combined approach was higher than that of glucose alone but remained lower than that of CEA alone.
- A limitation of the study is that final cyst classification was based predominantly on cross-sectional imaging, EUS morphology, cyst fluid viscosity, cytology, and clinical follow-up, with only a minority of cases confirmed by definitive cytology (adenocarcinoma or neuroendocrine tumor) or postoperative histology. Consequently, a degree of diagnostic uncertainty cannot be excluded in clinically classified cases.

New findings:

- Intracystic glucose measurement proved to be an easily accessible, inexpensive, and clinically useful biomarker in routine practice with an outstanding sensitivity in identifying mucinous cysts. Its main limitation is suboptimal specificity, which can be mitigated by combined interpretation with other parameters such as CEA and the string sign.

6.2. Clinical benefits of KRAS/GNAS gene mutation analysis in addition to morphology and conventional cyst fluid testing in differentiating pancreatic cysts

- In our cohort, KRAS mutation analysis showed a sensitivity of 42.4% and a specificity of 92.9% for identifying mucinous cystic lesions, while GNAS analysis

exhibited a sensitivity of 43.5% and a specificity of 100%. Overall, the diagnostic sensitivity of genetic testing was inferior to that of conventional cyst fluid analysis.

New findings:

- Owing to their high specificity, the detection of KRAS and/or GNAS mutations may provide supportive evidence for a mucinous etiology in diagnostically inconclusive cases.
- KRAS/GNAS testing requires only a small volume of cyst fluid and can be performed even in highly viscous samples, representing a practical advantage in situations where CEA or glucose measurements are technically unsuccessful.

7. SUMMARY

Over the past decade, an increasing number of studies have consistently demonstrated that cyst fluid glucose represents a reliable, inexpensive, and widely accessible biomarker with superior sensitivity compared with the traditionally used carcinoembryonic antigen (CEA). The results of the first part of this work confirm these observations by showing that intracystic glucose measurement provides markedly higher sensitivity for the identification of mucinous pancreatic cysts than CEA, while CEA retains higher specificity. The combined application of the two markers further enhanced diagnostic sensitivity, albeit at the expense of some loss in specificity. Although advanced endoscopic techniques such as confocal laser endomicroscopy and through-the-needle biopsy offer higher overall diagnostic accuracy, their limited availability, higher cost, and increased procedural risk restrict their use to specialized centers and selected cases. Given its favorable analytical characteristics, minimal sample volume requirement, low cost, and potential for point-of-care testing, intracystic glucose determination represents a practical adjunct to conventional cyst fluid analysis, especially in centers without access to advanced endoscopic or molecular diagnostic modalities.

In the second part of this work, the clinical contribution of KRAS and GNAS mutation analysis to the diagnostic work-up of pancreatic cystic lesions was evaluated in the context of conventional morphological and cyst fluid-based assessment. Although the sensitivity of these genetic alterations for identifying mucinous cysts was lower than that of the combined standard diagnostic approach, their very high specificity indicates that the presence of KRAS and/or GNAS mutations can provide strong supportive evidence for a mucinous origin in diagnostically uncertain cases. This may be of particular relevance when imaging, cytology, string sign, and biochemical markers yield discordant or equivocal results. From a practical perspective, molecular testing also offers technical advantages, as it can be performed from small-volume samples and is less influenced by high cyst fluid viscosity. However, the application of molecular testing is limited by its relatively high cost, the need for specialized laboratory infrastructure and expertise. Consequently, it should be considered a supplementary tool rather than a replacement for standard cyst fluid-based assessment strategies.

8. REFERENCES

1. Hawes CH. Pancreatic Cyst. *N Engl J Med* 1934;211:714-716
2. Gonda TA, Cahen DL, Farrell JJ. Pancreatic Cysts. *N Engl J Med*. 2024 Sep 5;391(9):832-843.
3. Matsuda Y, Kimura W, Matsukawa M, Aida J, Takubo K, Ishiwata T, Furukawa T, Mino-Kenudson M, Arai T. Association Between Pancreatic Cystic Lesions and High-grade Intraepithelial Neoplasia and Aging: An Autopsy Study. *Pancreas*. 2019 Sep;48(8):1079-1085.
4. Kimura W, Nagai H, Kuroda A, Muto T, Esaki Y. Analysis of small cystic lesions of the pancreas. *Int J Pancreatol*. 1995 Dec;18(3):197-206.
5. Soroida Y, Sato M, Hikita H, Hagiwara S, Sato M, Gotoh H, Kato S, Iwai T, Yamazaki T, Yatomi Y, Sasano T, Ikeda H. Pancreatic cysts in general population on ultrasonography: Prevalence and development of risk score. *J Gastroenterol*. 2016 Dec;51(12):1133-1140.
6. Yu MH, Kim JH, Kang HJ, Choi SY, Park YS, Lee ES, Park HJ. Transabdominal Ultrasound for Follow-Up of Incidentally Detected Low-Risk Pancreatic Cysts: A Prospective Multicenter Study. *AJR Am J Roentgenol*. 2021 Jun;216(6):1521-1529.
7. Chang YR, Park JK, Jang JY, Kwon W, Yoon JH, Kim SW. Incidental pancreatic cystic neoplasms in an asymptomatic healthy population of 21,745 individuals: Large-scale, single-center cohort study. *Medicine (Baltimore)*. 2016 Dec;95(51):e5535.
8. Zerboni G, Signoretti M, Crippa S, Falconi M, Arcidiacono PG, Capurso G. Systematic review and meta-analysis: Prevalence of incidentally detected pancreatic cystic lesions in asymptomatic individuals. *Pancreatol*. 2019 Jan;19(1):2-9.

9. Moris M, Bridges MD, Pooley RA, Raimondo M, Woodward TA, Stauffer JA, Asbun HJ, Wallace MB. Association Between Advances in High-Resolution Cross-Section Imaging Technologies and Increase in Prevalence of Pancreatic Cysts From 2005 to 2014. *Clin Gastroenterol Hepatol*. 2016 Apr;14(4):585-593.e3.
10. Kromrey ML, Bülow R, Hübner J, Paperlein C, Lerch MM, Ittermann T, Völzke H, Mayerle J, Kühn JP. Prospective study on the incidence, prevalence and 5-year pancreatic-related mortality of pancreatic cysts in a population-based study. *Gut*. 2018 Jan;67(1):138-145.
11. Lee KS, Sekhar A, Rofsky NM, Pedrosa I. Prevalence of incidental pancreatic cysts in the adult population on MR imaging. *Am J Gastroenterol*. 2010 Sep;105(9):2079-84.
12. Gupta A, Chennatt JJ, Mandal C, Gupta J, Krishnasamy S, Bose B, Solanki P, H S, Singh SK, Gupta S. Approach to Cystic Lesions of the Pancreas: Review of Literature. *Cureus*. 2023 Mar 28;15(3):e36827.
13. Klöppel G, Kosmahl M. Cystic lesions and neoplasms of the pancreas. The features are becoming clearer. *Pancreatology*. 2001;1(6):648-55.
14. Kloth C, Haggenmüller B, Beck A, Wagner M, Kornmann M, Steinacker JP, Steinacker-Stanescu N, Voge D, Beer M, Juchems MS, Schmidt SA. Diagnostic, Structured Classification and Therapeutic Approach in Cystic Pancreatic Lesions: Systematic Findings with Regard to the European Guidelines. *Diagnostics (Basel)*. 2023 Jan 26;13(3):454.
15. Habashi S, Draganov PV. Pancreatic pseudocyst. *World J Gastroenterol*. 2009 Jan 7;15(1):38-47.
16. Walt AJ, Bouwman DL, Weaver DW, Sachs RJ. The impact of technology on the management of pancreatic pseudocyst. Fifth annual Samuel Jason Mixter Lecture. *Arch Surg*. 1990 Jun;125(6):759-63.
17. Sanfey H, Aguilar M, Jones RS. Pseudocysts of the pancreas, a review of 97 cases. *Am Surg*. 1994 Sep;60(9):661-8.

18. Gumaste VV, Pitchumoni CS. Pancreatic pseudocyst. *Gastroenterologist*. 1996 Mar;4(1):33-43.
19. Elta GH, Enestvedt BK, Sauer BG, Lennon AM. ACG Clinical Guideline: Diagnosis and Management of Pancreatic Cysts. *Am J Gastroenterol*. 2018 Apr;113(4):464-479.
20. Kim YH, Saini S, Sahani D, Hahn PF, Mueller PR, Auh YH. Imaging diagnosis of cystic pancreatic lesions: pseudocyst versus nonpseudocyst. *Radiographics*. 2005 May-Jun;25(3):671-85.
21. Hawes R, Fockens P, Varadarajulu S. *Endosonography*. Philadelphia: Elsevier; 2019. Fourth edition. p185-200.
22. Kudaravalli P, Garg N, Pendela VS, Gambhir HS. Hemorrhagic pancreatic pseudocyst: A rare complication. *Am J Emerg Med*. 2021 May;43:243-244.
23. Agalianos C, Passas I, Sideris I, Davides D, Dervenis C. Review of management options for pancreatic pseudocysts. *Transl Gastroenterol Hepatol*. 2018 Mar 21;3:18.
24. Jais B, Rebours V, Malleo G, Salvia R, Fontana M, Maggino L, Bassi C, Manfredi R, Moran R, Lennon AM, Zaheer A, Wolfgang C, Hruban R, Marchegiani G, Fernández Del Castillo C, Brugge W, Ha Y, Kim MH, Oh D, Hirai I, Kimura W, Jang JY, Kim SW, Jung W, Kang H, Song SY, Kang CM, Lee WJ, Crippa S, Falconi M, Gomatos I, Neoptolemos J, Milanetto AC, Sperti C, Ricci C, Casadei R, Bissolati M, Balzano G, Frigerio I, Girelli R, Delhaye M, Bernier B, Wang H, Jang KT, Song DH, Huggett MT, Oppong KW, Pererva L, Kopchak KV, Del Chiaro M, Segersvard R, Lee LS, Conwell D, Osvaldt A, Campos V, Aguero Garcete G, Napoleon B, Matsumoto I, Shinzeki M, Bolado F, Fernandez JM, Keane MG, Pereira SP, Acuna IA, Vaquero EC, Angiolini MR, Zerbi A, Tang J, Leong RW, Faccineto A, Morana G, Petrone MC, Arcidiacono PG, Moon JH, Choi HJ, Gill RS, Pavey D, Ouaiissi M, Sastre B, Spandre M, De Angelis CG, Rios-Vives MA, Concepcion-Martin M, Ikeura T, Okazaki K, Frulloni L, Messina O, Lévy P. Serous cystic neoplasm of the pancreas: a multinational study of 2622 patients under the auspices of the International Association of Pancreatology and

- European Pancreatic Club (European Study Group on Cystic Tumors of the Pancreas). *Gut*. 2016 Feb;65(2):305-12.
25. Ning K, Salamone A, Manos L, Lafaro KJ, Afghani E. Serous Cystadenoma: A Review on Diagnosis and Management. *J Clin Med*. 2023 Nov 25;12(23):7306.
 26. Chu LC, Singhi AD, Haroun RR, Hruban RH, Fishman EK. The many faces of pancreatic serous cystadenoma: Radiologic and pathologic correlation. *Diagn Interv Imaging*. 2017 Mar;98(3):191-202.
 27. European Study Group on Cystic Tumours of the Pancreas. European evidence-based guidelines on pancreatic cystic neoplasms. *Gut*. 2018 May;67(5):789-804.
 28. Dietrich FC. Endoscopic ultrasound. An intraductory manual and atlas. Stuttgart-New York: Thieme; 2011. Second edition. p337-352.
 29. Nilsson LN, Keane MG, Shamali A, Millastre Bocos J, Marijinissen van Zanten M, Antila A, Verdejo Gil C, Del Chiaro M, Laukkarinen J. Nature and management of pancreatic mucinous cystic neoplasm (MCN): A systematic review of the literature. *Pancreatology*. 2016 Nov-Dec;16(6):1028-1036.
 30. Farrell JJ, Brugge WR. Intraductal papillary mucinous tumor of the pancreas. *Gastrointest Endosc*. 2002 May;55(6):701-14.
 31. Tanaka M, Fernández-Del Castillo C, Kamisawa T, Jang JY, Levy P, Ohtsuka T, Salvia R, Shimizu Y, Tada M, Wolfgang CL. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. *Pancreatology*. 2017 Sep-Oct;17(5):738-753.
 32. Sahora K, Fernández-del Castillo C, Dong F, Marchegiani G, Thayer SP, Ferrone CR, Sahani DV, Brugge WR, Warshaw AL, Lillemoe KD, Mino-Kenudson M. Not all mixed-type intraductal papillary mucinous neoplasms behave like main-duct lesions: implications of minimal involvement of the main pancreatic duct. *Surgery*. 2014 Sep;156(3):611-21.
 33. Ohtsuka T, Fernandez-Del Castillo C, Furukawa T, Hijioka S, Jang JY, Lennon AM, Miyasaka Y, Ohno E, Salvia R, Wolfgang CL, Wood LD. International

- evidence-based Kyoto guidelines for the management of intraductal papillary mucinous neoplasm of the pancreas. *Pancreatology*. 2024 Mar;24(2):255-270.
34. Pontrelli A, Di Meo G, Prete FP, De Simone B, Testini M. Management and outcomes of solid pseudopapillary neoplasm of the pancreas: a systematic review. *Clin Exp Med*. 2025 Oct 25;25(1):318.
 35. Master SS, Savides TJ. Diagnosis of solid-pseudopapillary neoplasm of the pancreas by EUS-guided FNA. *Gastrointest Endosc*. 2003 Jun;57(7):965-8.
 36. Bardales RH, Centeno B, Mallery JS, Lai R, Pochapin M, Guiter G, Stanley MW. Endoscopic ultrasound-guided fine-needle aspiration cytology diagnosis of solid-pseudopapillary tumor of the pancreas: a rare neoplasm of elusive origin but characteristic cytomorphologic features. *Am J Clin Pathol*. 2004 May;121(5):654-62.
 37. Kang CM, Kim KS, Choi JS, Kim H, Lee WJ, Kim BR. Solid pseudopapillary tumor of the pancreas suggesting malignant potential. *Pancreas*. 2006 Apr;32(3):276-80.
 38. Xiao SY, Ye Z. Pancreatic cystic tumors: an update. *Journal of Pancreatology* 1(1):p 2-18, December 2018/JP9.
 39. Bordeianou L, Vagefi PA, Sahani D, Deshpande V, Rakhlin E, Warshaw AL, Fernández-del Castillo C. Cystic pancreatic endocrine neoplasms: a distinct tumor type? *J Am Coll Surg*. 2008 Jun;206(6):1154-8.
 40. Rindi G, Mete O, Uccella S, Basturk O, La Rosa S, Brosens LAA, Ezzat S, de Herder WW, Klimstra DS, Papotti M, Asa SL. Overview of the 2022 WHO Classification of Neuroendocrine Neoplasms. *Endocr Pathol*. 2022 Mar;33(1):115-154.
 41. Ma ZY, Gong YF, Zhuang HK, Zhou ZX, Huang SZ, Zou YP, Huang BW, Sun ZH, Zhang CZ, Tang YQ, Hou BH. Pancreatic neuroendocrine tumors: A review of serum biomarkers, staging, and management. *World J Gastroenterol*. 2020 May 21;26(19):2305-2322.

42. Kosmahl M, Pauser U, Anlauf M, Klöppel G. Pancreatic ductal adenocarcinomas with cystic features: neither rare nor uniform. *Mod Pathol.* 2005 Sep;18(9):1157-64.
43. Lee LS. Updates in diagnosis and management of pancreatic cysts. *World J Gastroenterol.* 2021 Sep 14;27(34):5700-5714.
44. Levink IJM, Jaarsma SC, Koopmann BDM, van Riet PA, Overbeek KA, Meziani J, Sprij MLJA, Casadei R, Ingaldi C, Polkowski M, Engels MML, van der Waaij LA, Carrara S, Pando E, Vornhülz M, Honkoop P, Schoon EJ, Laukkarinen J, Bergmann JF, Rossi G, van Vilsteren FGI, van Berkel AM, Tabone T, Schwartz MP, Tan ACITL, van Hooft JE, Quispel R, van Soest E, Czako L, Bruno MJ, Cahen DL; PACYFIC-registry work group. The additive value of CA19.9 monitoring in a pancreatic cyst surveillance program. *United European Gastroenterol J.* 2023 Sep;11(7):601-611.
45. Schweber AB, Agarunov E, Brooks C, Hur C, Gonda TA. New-Onset Diabetes Is a Potential Marker for the Malignant Transformation of Pancreatic Cysts: A Real-World Population Cohort Study. *Pancreas.* 2022 Oct 1;51(9):1186-1193.
46. Jones MJ, Buchanan AS, Neal CP, Dennison AR, Metcalfe MS, Garcea G. Imaging of indeterminate pancreatic cystic lesions: a systematic review. *Pancreatology.* 2013 Jul-Aug;13(4):436-42.
47. Schmitz D, Stenzinger A, Grützmann R, Esposito I, Raatschen HJ, Seufferlein TTW. The Differential Diagnosis and Management of Pancreatic Cystic Neoplasms. *Dtsch Arztebl Int.* 2025 Oct 31;122(22):617-622.
48. Kitano M, Yoshida T, Itonaga M, Tamura T, Hatamaru K, Yamashita Y. Impact of endoscopic ultrasonography on diagnosis of pancreatic cancer. *J Gastroenterol.* 2019 Jan;54(1):19-32.
49. Guilabert L, Nikolic S, de-Madaria E, Vanella G, Capurso G, Tacelli M, Maida M, Vladut C, Knoph CS, Quintini D, Rancatore G, Infantino G, Tarantino I, Rizzo GEM. Endoscopic ultrasound for pancreatic cystic lesions: a narrative review. *BMJ Open Gastroenterol.* 2025 Sep 25;12(1):e001893.

50. Ahmad NA, Kochman ML, Brensinger C, Brugge WR, Faigel DO, Gress FG, Kimmey MB, Nickl NJ, Savides TJ, Wallace MB, Wiersema MJ, Ginsberg GG. Interobserver agreement among endosonographers for the diagnosis of neoplastic versus non-neoplastic pancreatic cystic lesions. *Gastrointest Endosc.* 2003 Jul;58(1):59-64.
51. Kim YC, Choi JY, Chung YE, Bang S, Kim MJ, Park MS, Kim KW. Comparison of MRI and endoscopic ultrasound in the characterization of pancreatic cystic lesions. *AJR Am J Roentgenol.* 2010 Oct;195(4):947-52.
52. Mishra G, Lennon AM, Pausawasdi N, Shami VM, Sharaiha RZ, Elmunzer BJ. Quality Indicators for EUS. *Am J Gastroenterol.* 2025 May 1;120(5):973-992.
53. Bick BL, Enders FT, Levy MJ, Zhang L, Henry MR, Abu Dayyeh BK, Chari ST, Clain JE, Farnell MB, Gleeson FC, Kendrick ML, Pearson RK, Petersen BT, Rajan E, Vege SS, Topazian M. The string sign for diagnosis of mucinous pancreatic cysts. *Endoscopy.* 2015 Jul;47(7):626-31.
54. Oh SH, Lee JK, Lee KT, Lee KH, Woo YS, Noh DH. The Combination of Cyst Fluid Carcinoembryonic Antigen, Cytology and Viscosity Increases the Diagnostic Accuracy of Mucinous Pancreatic Cysts. *Gut Liver.* 2017 Mar 15;11(2):283-289.
55. Hakim S, Coronel E, González GMN, Ge PS, Chari ST, Thosani N, Ramireddy S, Badillo R, DaVee T, Catalano MF, Sealock RJ, Parupudi S, Hernandez LV, Joshi V, Irisawa A, Rana S, Lakhtakia S, Vilmann P, Saftoiu A, Sun S, Giovannini M, Katz MH, Kim MP, Bhutani MS. An international study of interobserver variability of "string sign" of pancreatic cysts among experienced endosonographers. *Endosc Ultrasound.* 2021 Jan-Feb;10(1):39-50.
56. Ding C, Yang JF, Wang X, Zhou YF, Gu Y, Liu Q, Shen HZ, Zhang XF. Diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration-based cytology for distinguishing malignant and benign pancreatic cystic lesions: A systematic review and meta-analysis. *PLoS One.* 2025 Feb 20;20(2):e0314825.
57. Hong SK, Loren DE, Rogart JN, Siddiqui AA, Sendekci JA, Bibbo M, Coben RM, Meckes DP, Kowalski TE. Targeted cyst wall puncture and aspiration during EUS-

- FNA increases the diagnostic yield of premalignant and malignant pancreatic cysts. *Gastrointest Endosc.* 2012 Apr;75(4):775-82.
58. Walsh RM, Henderson JM, Vogt DP, Baker ME, O'malley CM Jr, Herts B, Zuccaro G Jr, Vargo JJ, Dumot JA, Conwell DL, Biscotti CV, Brown N. Prospective preoperative determination of mucinous pancreatic cystic neoplasms. *Surgery.* 2002 Oct;132(4):628-33; discussion 633-4.
 59. Shami VM, Sundaram V, Stelow EB, Conaway M, Moskaluk CA, White GE, Adams RB, Yeaton P, Kahaleh M. The level of carcinoembryonic antigen and the presence of mucin as predictors of cystic pancreatic mucinous neoplasia. *Pancreas.* 2007 May;34(4):466-9.
 60. Park WG, Mascarenhas R, Palaez-Luna M, Smyrk TC, O'Kane D, Clain JE, Levy MJ, Pearson RK, Petersen BT, Topazian MD, Vege SS, Chari ST. Diagnostic performance of cyst fluid carcinoembryonic antigen and amylase in histologically confirmed pancreatic cysts. *Pancreas.* 2011 Jan;40(1):42-5.
 61. Oh HC, Kang H, Brugge WR. Cyst fluid amylase and CEA levels in the differential diagnosis of pancreatic cysts: a single-center experience with histologically proven cysts. *Dig Dis Sci.* 2014 Dec;59(12):3111-6.
 62. Khan I, Baig M, Bandepalle T, Puli SR. Utility of Cyst Fluid Carcinoembryonic Antigen in Differentiating Mucinous and Non-mucinous Pancreatic Cysts: An Updated Meta-Analysis. *Dig Dis Sci.* 2022 Sep;67(9):4541-4548.
 63. van der Waaij LA, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc.* 2005 Sep;62(3):383-9.
 64. Pflüger MJ, Jamouss KT, Afghani E, Lim SJ, Rodriguez Franco S, Mayo H, Spann M, Wang H, Singhi A, Lennon AM, Wood LD. Predictive ability of pancreatic cyst fluid biomarkers: A systematic review and meta-analysis. *Pancreatology.* 2023 Nov;23(7):868-877.
 65. Faias S, Cravo M, Chaves P, Pereira L. Comparative analysis of glucose and carcinoembryonic antigen in the diagnosis of pancreatic mucinous cysts: a

- systematic review and meta-analysis. *Gastrointest Endosc.* 2021 Aug;94(2):235-247.
66. Guzmán-Calderón E, Md BM, Casellas JA, Aparicio JR. Intracystic Glucose Levels Appear Useful for Diagnosis of Pancreatic Cystic Lesions: A Systematic Review and Meta-Analysis. *Dig Dis Sci.* 2022 Jun;67(6):2562-2570.
 67. Singh H, McGrath K, Singhi AD. Novel Biomarkers for Pancreatic Cysts. *Dig Dis Sci.* 2017 Jul;62(7):1796-1807.
 68. Vortmeyer AO, Lubensky IA, Fogt F, Linehan WM, Khettry U, Zhuang Z. Allelic deletion and mutation of the von Hippel-Lindau (VHL) tumor suppressor gene in pancreatic microcystic adenomas. *Am J Pathol.* 1997 Oct;151(4):951-6.
 69. Uprety D, Adjei AA. KRAS: From undruggable to a druggable Cancer Target. *Cancer Treat Rev.* 2020 Sep;89:102070.
 70. Weinstein LS, Liu J, Sakamoto A, Xie T, Chen M. Minireview: GNAS: normal and abnormal functions. *Endocrinology.* 2004 Dec;145(12):5459-64.
 71. Vilas-Boas F, Ribeiro T, Macedo G, Dhar J, Samanta J, Sina S, Manfrin E, Facciorusso A, Conti Bellocchi MC, De Pretis N, Frulloni L, Crinò SF. Endoscopic Ultrasound-Guided Through-the-Needle Biopsy: A Narrative Review of the Technique and Its Emerging Role in Pancreatic Cyst Diagnosis. *Diagnostics (Basel).* 2024 Jul 23;14(15):1587.
 72. Rift CV, Melchior LC, Kovacevic B, Klausen P, Toxværd A, Grossjohann H, Karstensen JG, Brink L, Hassan H, Kalaitzakis E, Storkholm J, Scheie D, Hansen CP, Lund EL, Vilmann P, Hasselby JP. Targeted next-generation sequencing of EUS-guided through-the-needle-biopsy sampling from pancreatic cystic lesions. *Gastrointest Endosc.* 2023 Jan;97(1):50-58.e4.
 73. Li SY, Wang ZJ, Pan CY, Wu C, Li ZS, Jin ZD, Wang KX. Comparative Performance of Endoscopic Ultrasound-Based Techniques in Patients With Pancreatic Cystic Lesions: A Network Meta-Analysis. *Am J Gastroenterol.* 2023 Feb 1;118(2):243-255.

74. Facciorusso A, Kovacevic B, Yang D, Vilas-Boas F, Martínez-Moreno B, Stigliano S, Rizzatti G, Sacco M, Arevalo-Mora M, Villarreal-Sanchez L, Conti Bellocchi MC, Bernardoni L, Gabbrielli A, Barresi L, Gkolfakis P, Robles-Medrandá C, De Angelis C, Larghi A, Di Matteo FM, Aparicio JR, Macedo G, Draganov PV, Vilmann P, Pecchia L, Repici A, Crinò SF. Predictors of adverse events after endoscopic ultrasound-guided through-the-needle biopsy of pancreatic cysts: a recursive partitioning analysis. *Endoscopy*. 2022 Dec;54(12):1158-1168.
75. Wu CCH, Lim SJM, Tan DMY. Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy for pancreatic cystic lesions: current status and future prospects. *Clin Endosc*. 2024 Jul;57(4):434-445.
76. Chin YK, Wu CCH, Tan DMY. The Role of Needle-Based Confocal Laser Endomicroscopy in the Evaluation of Pancreatic Cystic Lesions: A Systematic Review. *Clin Endosc*. 2021 Jan;54(1):38-47.
77. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology*. 2004 May;126(5):1330-6.
78. Zikos T, Pham K, Bowen R, Chen AM, Banerjee S, Friedland S, Dua MM, Norton JA, Poultides GA, Visser BC, Park WG. Cyst Fluid Glucose is Rapidly Feasible and Accurate in Diagnosing Mucinous Pancreatic Cysts. *Am J Gastroenterol*. 2015 Jun;110(6):909-14.
79. Gyimesi G, Keczer B, Rein P, Horváth M, Szűcs Á, Marjai T, Szijártó A, Hritz I. Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in differentiation of mucinous and non-mucinous pancreatic cysts. *Pathol Oncol Res*. 2024 Oct 10;30:1611881.
80. Gyimesi G, Keczer B, Rein P, Horváth M, Gellért B, Marjai T, Tóth E, Szűcs Á, Szijártó A, Barbai T, Székely E, Hritz I. Clinical Benefits of KRAS/GNAS Gene Mutation Analysis in Addition to Morphology and Conventional Cyst Fluid Testing in Differentiating Pancreatic Cysts. *J Clin Med*. 2025 Dec 7;14(24):8671.

81. Mohamed E, Jackson R, Halloran CM, Ghaneh P. Role of Radiological Imaging in the Diagnosis and Characterization of Pancreatic Cystic Lesions: A Systematic Review. *Pancreas*. 2018 Oct;47(9):1055-1064.
82. McCarty TR, Garg R, Rustagi T. Pancreatic cyst fluid glucose in differentiating mucinous from nonmucinous pancreatic cysts: a systematic review and meta-analysis. *Gastrointest Endosc*. 2021 Oct;94(4):698-712.e6.
83. Zamir E, Zelnik Yovel D, Scapa E, Shnell M, Bar N, Bar Yishay I, Ziv-Baran T, Younis F, Phillips A, Lubezky N, Shibolet O, Ben-Ami Shor D. Pancreatic cyst fluid glucose: a rapid on-site diagnostic test for mucinous cysts. *Therap Adv Gastroenterol*. 2022 Nov 4;15:17562848221133581.
84. Smith ZL, Satyavada S, Simons-Linares R, Mok SRS, Martinez Moreno B, Aparicio JR, Chahal P. Intracystic Glucose and Carcinoembryonic Antigen in Differentiating Histologically Confirmed Pancreatic Mucinous Neoplastic Cysts. *Am J Gastroenterol*. 2022 Mar 1;117(3):478-485.
85. Park WG, Wu M, Bowen R, Zheng M, Fitch WL, Pai RK, Wodziak D, Visser BC, Poultides GA, Norton JA, Banerjee S, Chen AM, Friedland S, Scott BA, Pasricha PJ, Lowe AW, Peltz G. Metabolomic-derived novel cyst fluid biomarkers for pancreatic cysts: glucose and kynurenine. *Gastrointest Endosc*. 2013 Aug;78(2):295-302.e2.
86. Facciorusso A, Arvanitakis M, Crinò SF, Fabbri C, Fornelli A, Leeds J, Archibugi L, Carrara S, Dhar J, Gkolfakis P, Haugk B, Iglesias Garcia J, Napoleon B, Papanikolaou IS, Seicean A, Stassen PMC, Vilman P, Tham TC, Fuccio L. Endoscopic ultrasound-guided tissue sampling: European Society of Gastrointestinal Endoscopy (ESGE) Technical and Technology Review. *Endoscopy*. 2025 Apr;57(4):390-418.
87. Boot C. A review of pancreatic cyst fluid analysis in the differential diagnosis of pancreatic cyst lesions. *Ann Clin Biochem*. 2014 Mar;51(Pt 2):151-66.
88. Rossi G, Petrone MC, Tacelli M, Zaccari P, Crippa S, Belfiori G, Aleotti F, Locatelli M, Piemonti L, Doglioni C, Falconi M, Capurso G, Arcidiacono PG.

- Glucose and lactate levels are lower in EUS-aspirated cyst fluid of mucinous vs non-mucinous pancreatic cystic lesions. *Dig Liver Dis.* 2024 May;56(5):836-840.
89. Ribaldone DG, Bruno M, Gaia S, Cantamessa A, Bragoni A, Caropreso P, Sacco M, Fagoonee S, Saracco GM, De Angelis C. Differential diagnosis of pancreatic cysts: A prospective study on the role of intra-cystic glucose concentration. *Dig Liver Dis.* 2020 Sep;52(9):1026-1032.
 90. Scheiman JM, Hwang JH, Moayyedi P. American gastroenterological association technical review on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. *Gastroenterology.* 2015 Apr;148(4):824-48.e22.
 91. Singhi AD, McGrath K, Brand RE, Khalid A, Zeh HJ, Chennat JS, Fasanella KE, Papachristou GI, Slivka A, Bartlett DL, Dasyam AK, Hogg M, Lee KK, Marsh JW, Monaco SE, Ohori NP, Pingpank JF, Tsung A, Zureikat AH, Wald AI, Nikiforova MN. Preoperative next-generation sequencing of pancreatic cyst fluid is highly accurate in cyst classification and detection of advanced neoplasia. *Gut.* 2018 Dec;67(12):2131-2141.
 92. Nikiforova MN, Khalid A, Fasanella KE, McGrath KM, Brand RE, Chennat JS, Slivka A, Zeh HJ, Zureikat AH, Krasinskas AM, Ohori NP, Schoedel KE, Navina S, Mantha GS, Pai RK, Singhi AD. Integration of KRAS testing in the diagnosis of pancreatic cystic lesions: a clinical experience of 618 pancreatic cysts. *Mod Pathol.* 2013 Nov;26(11):1478-87.
 93. Singhi AD, Nikiforova MN, Fasanella KE, McGrath KM, Pai RK, Ohori NP, Bartholow TL, Brand RE, Chennat JS, Lu X, Papachristou GI, Slivka A, Zeh HJ, Zureikat AH, Lee KK, Tsung A, Mantha GS, Khalid A. Preoperative GNAS and KRAS testing in the diagnosis of pancreatic mucinous cysts. *Clin Cancer Res.* 2014 Aug 15;20(16):4381-9.
 94. Springer S, Wang Y, Dal Molin M, Masica DL, Jiao Y, Kinde I, Blackford A, Raman SP, Wolfgang CL, Tomita T, Niknafs N, Douville C, Ptak J, Dobbyn L, Allen PJ, Klimstra DS, Schattner MA, Schmidt CM, Yip-Schneider M, Cummings OW, Brand RE, Zeh HJ, Singhi AD, Scarpa A, Salvia R, Malleo G, Zamboni G, Falconi M, Jang JY, Kim SW, Kwon W, Hong SM, Song KB, Kim SC, Swan N,

- Murphy J, Geoghegan J, Brugge W, Fernandez-Del Castillo C, Mino-Kenudson M, Schulick R, Edil BH, Adsay V, Paulino J, van Hooft J, Yachida S, Nara S, Hiraoka N, Yamao K, Hijioka S, van der Merwe S, Goggins M, Canto MI, Ahuja N, Hirose K, Makary M, Weiss MJ, Cameron J, Pittman M, Eshleman JR, Diaz LA Jr, Papadopoulos N, Kinzler KW, Karchin R, Hruban RH, Vogelstein B, Lennon AM. A combination of molecular markers and clinical features improve the classification of pancreatic cysts. *Gastroenterology*. 2015 Nov;149(6):1501-10.
95. Lee JH, Kim Y, Choi JW, Kim YS. KRAS, GNAS, and RNF43 mutations in intraductal papillary mucinous neoplasm of the pancreas: a meta-analysis. *Springerplus*. 2016 Jul 26;5(1):1172.
96. Mack S, Bichard P, Frossard J-L. Clinical Impact of Molecular Biomarkers in the Management of Patients with Pancreatic Cysts. *Journal of Surgery and Research*. 8 (2025): 442-451.
97. Faías S, Duarte M, Albuquerque C, da Silva JP, Fonseca R, Roque R, Dias Pereira A, Chaves P, Cravo M. Clinical Impact of KRAS and GNAS Analysis Added to CEA and Cytology in Pancreatic Cystic Fluid Obtained by EUS-FNA. *Dig Dis Sci*. 2018 Sep;63(9):2351-2361.
98. Herranz Pérez R, de la Morena López F, Majano Rodríguez PL, Molina Jiménez F, Vega Piris L, Santander Vaquero C. Molecular analysis of pancreatic cystic neoplasm in routine clinical practice. *World J Gastrointest Endosc*. 2021 Feb 16;13(2):56-71.
99. Schmitz D, Kazdal D, Allgäuer M, Trunk M, Vornhusen S, Nahm AM, Doll M, Weingärtner S, Endris V, Penzel R, Kirchner M, Brandt R, Neumann O, Sültmann H, Budczies J, Kienle P, Magdeburg R, Hetjens S, Schirmacher P, Bergmann F, Rudi J, Stenzinger A, Volckmar AL. KRAS/GNAS-testing by highly sensitive deep targeted next generation sequencing improves the endoscopic ultrasound-guided workup of suspected mucinous neoplasms of the pancreas. *Genes Chromosomes Cancer*. 2021 Jul;60(7):489-497.

100. Rahal MA, DeWitt JM, Patel H, Schmidt CM, Ceppa EP, Simpson RE, Sherman S, Al-Haddad M. Serial EUS-Guided FNA for the Surveillance of Pancreatic Cysts: A Study of Long-Term Performance of Tumor Markers. *Dig Dis Sci*. 2022 Nov;67(11):5248-5255.

9. BIBLIOGRAPHY OF THE CANDIDATE’S PUBLICATIONS

Publications forming the basis of the thesis

G. Gyimesi, B. Keczer, P. Rein, M. Horváth, Á. Szűcs, T. Marjai, A. Szijártó, and I. Hritz, “Diagnostic performance of intracystic carcinoembryonic antigen (CEA) versus glucose in differentiation of mucinous and non-mucinous pancreatic cysts,” *PATHOLOGY AND ONCOLOGY RESEARCH*, vol. 30, 2024.

IF: 2.3 [Q2] (Medicine)

G. Gyimesi, B. Keczer, P. Rein, M. Horváth, B. Gellért, T. Marjai, E. Tóth, Á. Szűcs, A. Szijártó, T. Barbai, E. Székely, and I. Hritz, “Clinical Benefits of KRAS/GNAS Gene Mutation Analysis in Addition to Morphology and Conventional Cyst Fluid Testing in Differentiating Pancreatic Cysts,” *JOURNAL OF CLINICAL MEDICINE*, vol. 14, no. 24, 2025.

IF: 2.9 [Q1] (Medicine)

Additional publications form the candidate

A. Fábíán, R. Bor, E. Szabó, V. Kardos, A. Bálint, K. Farkas, Á. Milassin, M. Rutka, K. Szántó, T. Molnár, M. Szűcs, K. Lőrinczy, Á. Orbán-Szilágyi, T. Gyökeres, **G. Gyimesi**, A. Szepes, V. Kovács, I. Rácz, and Z. Szepes, “Endoscopic treatment of gastric antral vascular ectasia in real-life settings,” *JOURNAL OF DIGESTIVE DISEASES*, vol. 22, no. 1, pp. 23–30, 2021.

IF: 3.366 [Q2] (Gastroenterology)

G. Gyimesi, F. Widmer, and M. C. Sulz, “Acute pancreatitis caused by gastric balloon: a case report,” *AME CASE REPORTS*, vol. 8, 2024.

IF: 0.7

G. Gyimesi, S. Kormann, M. Mueller, D. Mueller, and M. C. Sulz, “Gastric banding adjustment catheter dislodgement and perforation into the colon: case report,” *AME CASE REPORTS*, vol 9, 2025.

IF: 0.7

G. Gyimesi, “Uralhatatlan nyelőcsővarix-vérzés kezelése öntáguló fémstenttel,” *CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE*, vol. 7, no. 3, pp. 145–148, 2021.

G. Gyimesi, “Az endoluminális vákuumterápia alkalmazása nyelőcsőperforáció és varratelégtelenség esetén: irodalmi áttekintés,” *CENTRAL EUROPEAN JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY / GASZTROENTEROLÓGIAI ÉS HEPATOLÓGIAI SZEMLE*, vol. 6, no. 4, pp. 192–197, 2020.

G. Gyimesi, “Protonpumpa-gátló kezelés az indikációk és a mellékhatások tükrében: kinek, mennyit és meddig?,” *HÁZIORVOS TOVÁBBKÉPZŐ SZEMLE*, vol. 23, no. 6, pp. 354–356, 2018.

10. ACKNOWLEDGEMENTS

In connection with the completion of my doctoral work, I wish to express my deepest gratitude to my supervisor, **Dr. István Hritz**, for granting me the opportunity to conduct scientific research within the Division of Interventional Gastroenterology at the Department of Surgery, Transplantation and Gastroenterology of Semmelweis University. I am sincerely thankful for his continuous support, professional guidance, and invaluable advice, as well as for his assistance in the preparation of the related publications and this dissertation. I am equally grateful for his outstanding clinical work, particularly for performing the endoscopic ultrasound examinations and sampling procedures at the highest professional standard.

I would also like to extend my sincere appreciation to **Professor Attila Szijártó** for supporting my research activities within the institute.

My gratitude further goes to **Dr. Miklós Horváth**, who played a significant role in the performance of the endoscopic ultrasound examinations and sampling procedures.

I am indebted to **Dr. Bánk Keczer** for his valuable assistance in data collection and statistical analysis.

I would like to express my appreciation to **Dr. Tamás Barbai**, **Dr. Eszter Székely**, and the staff of the Department of Pathology, Forensic and Insurance Medicine at Semmelweis University for performing the cytological and genetic analyses and for their support in data interpretation.

I also wish to convey my heartfelt thanks to my former and current colleagues and mentors—**Dr. Attila Szepes**, **Dr. Zsolt Dubravcsik**, **Dr. Andreas Heer** and **Dr. Michael Sulz**—who introduced me to the field of interventional endoscopy and provided continued guidance and encouragement throughout my professional development.

Finally, I would like to express my gratitude to my family, friends, and current colleagues for their unconditional support and patience, and for providing a stable and encouraging background throughout the years of research and during the preparation of this dissertation.

I dedicate this dissertation to the memory of my late father, whose inspiration led me to choose the medical profession.