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Risk stratification in pancreatic cystic lesions: towards a precision medicine-based approach

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TITLE

Risk stratification in pancreatic cystic lesions: towards a precision medicine-based approach

TÍTULO

Estratificação de risco em lesões císticas do pâncreas: rumo a uma abordagem baseada em medicina de precisão

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LIST OF PUBLICATIONS

This thesis includes papers published in several peer-reviewed journals.

The list of publications is hereby presented:

1. Vilas-Boas F, Macedo G. **Pancreatic Cystic Lesions: New Endoscopic Trends in Diagnosis.** J Clin Gastroenterol. 2018 Jan;52(1):13-19.
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2. Vilas-Boas F, Macedo G. **Management Guidelines for Pancreatic Cystic Lesions: Should we Adopt or Adapt the Current Roadmaps?** J Gastrointestin Liver Dis. 2019;28(4):495-501.
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3. Vilas-Boas F, Costa-Moreira P, Moutinho-Ribeiro P, Martins D, Lopes J, Macedo G. **Through-the-needle biopsy sampling may allow preoperative intraductal papillary mucinous neoplasia subtyping.** Gastrointest Endosc. 2020;92(1):235-6.
   DOI: 10.1016/j.gie.2020.02.037. PubMed PMID: 32586560

4. Vilas-Boas F, Ribeiro T, Costa-Moreira P, Barroca H, Lopes J, Martins D, Moutinho-Ribeiro P, Macedo G. **Endoscopic Ultrasound Through-The-Needle Biopsy of Pancreatic Cysts: Toward Procedure Standardization.** Dig Dis. 2022 Aug 15. Online ahead of print.
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5. Vilas-Boas F, Ribeiro T, Afonso J, Cardoso H, Lopes S, Moutinho-Ribeiro P, Ferreira J, Mascarenhas-Saraiva M, Macedo G. **Deep Learning for Automatic Differentiation of Mucinous versus Non-Mucinous Pancreatic Cystic Lesions: A Pilot Study.** Diagnostics. 2022 Aug 24;12(9):2041.
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During the period of implementation of the research projects included in this thesis, the author actively participated in parallel studies that influenced the conduct of his own work.

Works related to this dissertation in which the author was involved:

1. Facciorusso A, Kovacevic B, Yang D, Vilas-Boas F, Martinez-Moreno B, Stigliano S, et al. **Predictors of adverse events after endoscopic ultrasound-guided through-the-needle biopsy of pancreatic cysts: a recursive partitioning analysis.** Endoscopy. 2022;54(12):1158-1168.
   DOI: 10.1055/a-1831-5385. PubMed PMID: 35451041

2. Saraiva MM, Ribeiro T, Ferreira JPS, Vilas-Boas F, Afonso J, Santos AL, et al. **Artificial intelligence for automatic diagnosis of biliary stricture malignancy status in single-operator cholangioscopy: a pilot study.** Gastrointest Endosc. 2022;95(2):339-48.
   DOI: 10.1016/j.gie.2021.08.027. PubMed PMID: 34508767

3. Morais R, Vilas-Boas F, Santos-Antunes J, Pereira P, Macedo G. **Single-Operator Pancreatoscopy for Diagnosis, Evaluation, and Staging of Mixed-Type Intraductal Papillary Mucinous Neoplasm.** GE Port J Gastroenterol. 2020;27(5):368-71.
   DOI: 10.1159/000505273. PubMed PMID: 32999911

LIST OF ABBREVIATIONS

AI	Artificial intelligence
ACG	American College of Gastroenterology
AE	Adverse event
AGA	American Gastroenterological Association
ANN	Artificial neural network
ASGE	American Society of Gastrointestinal Endoscopy
AUROC	Area under the receiver operator curve
BD-IPMN	Branch-duct intraductal papillary mucinous neoplasia
CA 19-9	Carbohydrate antigen 19-9
CDX2	Caudal type homeobox 2
CEA	Carcinoembryonic antigen
CLE	Confocal laser endomicroscopy
CNN	Convolutional neural network
CT	Computed tomography
DL	Deep learning
DNA	Deoxyribonucleic acid
DSS	Disease specific survival
ER	Estrogen receptors
EUS	Endoscopic ultrasound
EUS-FNA	Endoscopic ultrasound-guided fine needle aspiration
EUS-FNB	Endoscopic ultrasound-guided fine-needle biopsy
EUS-TTNB	Endoscopic ultrasound-guided through-the-needle biopsy
FNA	Fine needle aspiration
FNB	Fine needle biopsy
GI	Gastrointestinal
HGD	High-grade dysplasia
HRS	High-risk stigmata
IAP	International Association of Pancreatology
ICG	International consensus guidelines
IOPN	Intraductal oncocytic papillary neoplasm
IPMC	Intraductal papillary mucinous carcinoma
IPMN	Intraductal papillary mucinous neoplasm
LGD	Low-grade dysplasia
MCN	Mucinous cystic neoplasm
MD-IPMN	Main-duct intraductal papillary mucinous neoplasia
MEN-1	Multiple endocrine neoplasia type 1
ML	Machine learning
MPD	Main pancreatic duct

MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
MT-IPMN	Mixed-type intraductal papillary mucinous neoplasia
MUC	Mucin
NGS	Next-generation sequencing
NSAID	Non-steroid anti-inflammatory drug
PanIN	Pancreatic intraepithelial neoplasia
PB	Pancreatobiliary
PC	Pseudocyst
PD	Pancreaticoduodenectomy
Pca	Pancreatic cancer
PCL	Pancreatic cystic lesion
PCN	Pancreatic cystic neoplasm
PDAC	Pancreatic ductal adenocarcinoma
PNET	Pancreatic neuroendocrine tumor
PR	Progesterone receptors
RMN	Ressonância magnética nuclear
SCA	Serous cystadenoma
SPN	Solid pseudopapillary neoplasm
TC	Tomografia computorizada
TTNB	Through-the-needle biopsy
VHL	Von Hippel-Lindau
WF	Worrisome features
WHO	World Health Organization

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ACKNOWLEDGEMENTS

This project emerged as a challenge that first resulted from my pancreatology and endoscopic ultrasound fellowship at University Hospital of Santiago de Compostela. Since then, my interest in endoscopic ultrasound and the continuous diagnostic dilemmas imposed by pancreatic cystic lesions, motivated the development of a research project aimed at establishing a more individualized approach of the patients.

This project is, above all, the result of a teamwork and depended on São João Hospital's enormous clinical and research role and the strong work from the Gastroenterology Department team that I have joined in 2010.

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CONTENTS

OUTLINE OF THE THESIS

In the Summary a brief description of this thesis is presented, focusing on the published scientific papers.

In Chapter 1, the rationale for choosing the topic of pancreatic cystic lesions (PCLs) is explained.

Chapter 2 (Introduction and Literature Review) highlights the frequency and spectrum of PCLs, the importance of mucinous lesions as precursors of pancreatic cancer (Pca) and the role of endoscopic ultrasound (EUS) in their management. We discuss the increasingly important role of new tools in the endoscopic armamentarium for cyst diagnosis and risk stratification and point to the development of artificial intelligence (AI) tools to reduce misdiagnosis and interobserver variability in visual classification of EUS images.

In Chapter 3, the aims of the thesis are described.

In Chapter 4, the full texts of the papers that build the core of the thesis are presented.

In Chapter 5, an integrated discussion of the articles is presented supporting the major conclusions of the thesis and the implications for clinical practice.

Finally, in Chapter 6, the possibilities for future research are highlighted.

SUMMARY

Pancreatic cysts represent an increasingly prevalent group of lesions with distinct prognosis, heterogeneous morphology and often indistinct radiological features. The overall risk of malignancy is very low and almost exclusively found in mucinous lesions. The diagnosis of mucinous lesions represents an opportunity for the early detection and prevention of a small but relevant subset of pancreatic malignancy.

Current clinical algorithms are imperfect assigning the most appropriate patient management because they are based on recommendations with low grades of evidence. In fact, preoperative characterization and risk stratification of pancreatic cystic lesions (PCLs) is still challenging even if it is based on current standard of care that includes clinical history, imaging and endoscopic ultrasound (EUS).

Most guidelines show high sensitivity but low specificity, which results in surgical overtreatment that is associated with significant morbidity and non-negligible mortality that could be mitigated if we were able to improve patient risk stratification. Moreover, the costs of long-term PCLs follow-up and the anxiety it generates for the patients should be carefully considered.

Intraductal papillary mucinous neoplasms (IPMNs) represent most incidental PCLs and comprise a heterogeneous group of tumors with variable grades of dysplasia and different subtypes that determine the risk of malignancy, recurrence and overall survival. The possibility to preoperatively establish IPMN dysplasia grade and phenotype may have a significant impact on management.

EUS was shown to have an important role in the evaluation of PCLs, because of its higher diagnostic yield and accuracy over computed tomography (CT) and magnetic resonance imaging (MRI) for cyst type prediction and malignancy risk determination. Moreover, EUS-guided fine needle aspiration (EUS-FNA) allows for cyst fluid analysis that may overcome the limitations of morphology alone. Nevertheless, fluid scant cellularity, frequently insufficient cyst fluid volume, carcinoembryonic antigen (CEA) levels overlap and lack of correlation between CEA and dysplasia grade are important limitations. In fact, for branch-duct IPMNs (BD-IPMNs), the determination of high-risk

lesions continues to rely mostly on morphological criteria, although morphology alone is known to have low accuracy and only moderate interobserver agreement, even among expert endosonographers.

New EUS-based techniques expanded the endoscopic armamentarium for PCLs evaluation and have been shown to impact clinical management.

Endoscopic ultrasound-guided through-the-needle biopsy (EUS-TTNB) using a dedicated microforceps (Moray® - STERIS, Mentor, OH, USA), was found to be superior to EUS-FNA cytology and cyst fluid analysis by several systematic reviews but there is high heterogeneity among the included studies, that suggests the need for procedure standardization. Moreover, safety concerns have been raised because of the recently reported high rate of adverse events (AEs).

The convergence of precision-based Medicine and artificial intelligence (AI) is revolutionizing disease management. AI is particularly helpful in endoscopy as it has demonstrated outstanding performance for image analysis, possibly reducing misdiagnosis and interobserver variability in visual classification.

A convolutional neural network (CNN) is a form of deep learning (DL) exploiting hierarchical relations in data and has recently been shown to outperform clinicians in identifying several pathologies based on imaging.

Few EUS-based AI models have been described and its use for pancreatic disease evaluation is scarce, especially regarding PCLs management.

With this thesis we evaluated the use of EUS-TTNB for the diagnosis and risk stratification of PCLs. We evaluated its diagnostic performance and its impact on clinical management and explored several technique variations that could impact procedure success and minimize AEs.

We studied and performed EUS-TTNB in a group of 40 patients with PCLs. For mucinous cyst diagnosis, through-the-needle biopsy (TTNB) revealed higher sensitivity (75.9% vs 58.6%) and accuracy (80.9% vs 67.5%) than EUS-FNA, with similar specificity and with no significant AEs. Moreover, for most patients, TTNB allowed the

preoperative diagnosis of specific cyst type, showed higher value for mucinous cyst identification and in 1 out of 5 cases, had impact on clinical management. Regarding IPMNs, subtype definition was possible for most lesions (63%), which may impact decision-making. We reported that forceps preloading is mandatory for cysts smaller than 20 mm, two specimens may be enough to guarantee histologic adequacy and the samples can be processed as cell-block to minimize tissue loss.

We were also able to develop and train an EUS-based CNN for automatic identification of mucinous PCLs using 5505 EUS images (3725 from mucinous PCLs and 1780 from non-mucinous PCLs). The CNN revealed an excellent performance with 98.5% accuracy and image processing speed of 139 frames per second. This fact predicts the possibility for future algorithm real-time implementation and its incorporation in the management framework of PCLs.

In conclusion, the identification and risk stratification of mucinous lesions is a crucial step to optimize patient management. The use of AI models can help physicians classify EUS images and mitigate the low interobserver agreement previously described for EUS cyst morphology. If these results can be validated and tested in multicenter studies using larger datasets, they may have a significant impact reducing the number of fine needle aspiration (FNA) procedures and ultimately contributing to minimize inappropriate surgical resections and follow-up procedures.

Regarding EUS-TTNB, it has significant clinical impact in the case of morphologically indeterminate cysts, when cyst type identification is essential to define management. In the case of mucinous lesions, it allows for histological grade definition but for presumed BD-IPMNs, careful patient selection and the use of the correct technique are fundamental to minimize the risk of AEs.

RESUMO

As lesões císticas do pâncreas representam um grupo de lesões cuja deteção incidental é muito frequente e que são caracterizadas por uma história natural, morfologia e características imagiológicas muito variáveis. No geral, o risco de malignidade é muito baixo e exclusivo das lesões mucinosas. Desta forma, o diagnóstico de lesões mucinosas, representa uma oportunidade para a deteção precoce e prevenção de um pequeno, mas relevante grupo de doentes em risco de cancro do pâncreas.

Os atuais algoritmos nos quais baseamos o seguimento e tratamento destes doentes encontram fundamento em recomendações com baixo grau de evidência. A caracterização pré-operatória e a estratificação de risco das lesões císticas do pâncreas constituem um desafio, mesmo quando baseadas no atual conjunto de modalidades diagnósticas constituído pela história clínica, os exames de imagem seccional e a ecoendoscopia. As recomendações internacionais revelam elevada sensibilidade, mas reduzida especificidade que resulta num elevado número de doentes com lesões benignas ou mucinosas com displasia de baixo grau, submetidos a cirurgia pancreática que tem significativa morbilidade e mortalidade. Para além disso, os custos associados à vigilância e a ansiedade que a mesma gera nos doentes, deverá ser tida em consideração.

Os IPMNs representam a maioria das lesões císticas do pâncreas detetadas incidentalmente e constituem um grupo heterogéneo de lesões com graus variáveis de displasia e diferentes subtipos que determinam o risco de malignidade e o prognóstico. A possibilidade de, no pré-operatório, definir o fenótipo e o grau de displasia dos IPMNs, poderá ter um impacto significativo na abordagem destas lesões.

A ecoendoscopia já demonstrou ter um papel relevante na avaliação das lesões císticas do pâncreas, devido à elevada rentabilidade e acuidade diagnósticas quando comparada com a tomografia computorizada (TC) e a ressonância magnética nuclear (RMN) no que respeita à determinação do tipo de quisto e ao risco de malignidade. Para além disso, a ecoendoscopia possibilita a realização de punção aspirativa com

obtenção de líquido para estudo citológico e análise bioquímica que poderão ajudar a reduzir as limitações impostas pela simples avaliação morfológica. Contudo, o volume limitado de líquido, a reduzida celularidade das amostras e a sobreposição dos valores de CEA para o diagnóstico diferencial, para além da ausência de correlação destes valores com o grau de displasia, constituem importantes limitações desta técnica. No caso dos IPMNs, a definição de lesões de alto risco continua a basear-se sobretudo em critérios morfológicos, contudo a morfologia isoladamente é caracterizada por acuidade e concordância inter-observador reduzidas, mesmo entre ecoendoscopistas experientes.

Novas técnicas baseadas na ecoendoscopia resultaram numa recente expansão das opções para avaliação das lesões císticas do pâncreas e demonstraram potencial impacto clínico. A biópsia intra-quística utilizando a pinça Moray® demonstrou em várias revisões sistemáticas ter maior rentabilidade diagnóstica que a simples punção aspirativa com análise do líquido do quisto, contudo a elevada heterogeneidade entre os estudos incluídos sugere a necessidade de uniformização técnica do procedimento. Para além disso, foram recentemente reportadas preocupações com a elevada taxa de complicações associada a esta técnica.

A convergência entre a medicina de precisão e a inteligência artificial tem atualmente um papel relevante na abordagem de múltiplas patologias. A inteligência artificial revelou uma utilidade particularmente relevante na endoscopia, devido à elevada performance para a análise de imagens, contribuindo para a redução das limitações impostas pela variabilidade inter-observador na classificação de imagens.

As redes neurais convolucionais constituem uma forma de *deep learning* que explora as relações hierárquicas entre os dados e que recentemente demonstraram superar a avaliação dos especialistas na identificação de patologias com base na interpretação de imagens. Até à data, foram descritos um número reduzido de modelos de inteligência artificial baseados na ecoendoscopia e o seu uso para avaliação de doenças pancreáticas é escasso, sobretudo no caso das lesões císticas do pâncreas.

Com esta tese, avaliamos o uso da biópsia intra-quística durante a ecoendoscopia para o diagnóstico e estratificação de risco nas lesões císticas do pâncreas. Avaliamos

o seu papel no diagnóstico, o impacto clínico e as diferentes variações da técnica que poderão alterar o seu sucesso e minimizar os eventos adversos. Realizamos biópsia intra-quística num grupo de 40 doentes com lesões císticas pâncreas. Na nossa coorte, a biópsia intra-quística revelou maior sensibilidade (75,9% vs 58,6%) e acuidade (80,9% vs 67,5%) que a punção aspirativa para o diagnóstico de lesões mucinosas, com especificidade semelhante e sem eventos adversos significativos. Para além disso, para a maioria dos doentes, a biópsia permitiu o diagnóstico histológico específico, revelou maior capacidade para diagnóstico de lesões mucinosas e para 1 em cada 5 casos, teve impacto clínico que se traduziu numa mudança da estratégia na abordagem dos doentes. Relativamente aos IPMNs, a definição do subtipo histológico foi possível para a maioria das lesões (63%), o que poderá ter impacto na abordagem dos doentes. Concluímos que o pré-carregamento da pinça na agulha de punção é mandatório para quistos menores que 20 mm, duas amostras poderão ser suficientes para garantir um diagnóstico histológico e as amostras podem ser processadas como citobloco para minimizar perdas de material.

Também fomos capazes de desenvolver e treinar uma rede neural convolucional baseada na ecoendoscopia para identificação automática de lesões císticas mucinosas utilizando 5505 imagens de ecoendoscopia (3725 imagens de lesões mucinosas e 1780 de lesões não mucinosas). A rede neural convolucional revelou um desempenho excelente com precisão de 98.5% e velocidade de processamento de imagem de 139 *frames* por segundo. Este facto permite prever a possibilidade de futura incorporação em tempo real do algoritmo nas plataformas de ecografia.

Em conclusão, a identificação e a estratificação de risco de lesões mucinosas é fundamental para definir a abordagem dos doentes. A utilização de algoritmos de inteligência artificial poderá ajudar os clínicos a classificar as imagens de ecoendoscopia e mitigar a já descrita baixa concordância inter-observador na avaliação destas imagens. Se estes resultados forem confirmados em estudos subsequentes, idealmente multicêntricos e utilizando *datasets* mais robustos, poderão contribuir para minimizar o número de cirurgias pancreáticas e exames de vigilância inadequados.

CHAPTER 1
RATIONALE

Pancreatic cancer arises most frequently from pancreatic intraepithelial neoplasia (PanIN), the classic pre-neoplastic lesion, but can also develop from larger precursor lesions namely, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) [1]. One of the reasons for the dismal prognosis of Pca is the inability to detect it at an early stage, before lesions become locally advanced or metastatic [2].

Mainly because of the widespread use of cross-sectional imaging, PCLs are discovered with increasing frequency [3, 4]. A recently published systematic review that included 17 studies (48860 patients), revealed a pooled rate of incidentally detected PCLs of 8% (range 0.2-46%) and 4.3% pooled prevalence of mucinous cysts, representing around 60% of all incidentally diagnosed PCLs [5]. In patients over the age of 70, the prevalence of PCLs can be as high as 10% [6].

Patients with PCLs have an increased risk of pancreatic malignancy compared with the general population. Using the data of a large cohort of veterans, Munigala et al. reported an overall 19.64 (95% CI, 12.12-31.82; p <0.0001) times higher risk of Pca in these patients compared with the rest of the individuals in the database [7]. Especially for this reason, the identification of PCLs may generate significant anxiety for both patients and clinicians [8, 9].

PCLs are broadly classified as non-neoplastic (mostly pseudocysts) or neoplastic. Recent series reported that more than 50% are neoplastic lesions and constitute 10-15% of all pancreatic neoplasms [10]. Neoplastic PCLs are differentiated in mucinous and non-mucinous lesions, and the type of epithelial lining determines the risk of malignancy. Malignancy occurs virtually only in those with mucinous structure, namely IPMN and MCN. Identification of precancerous mucin-producing cysts offers the potential for the early detection and prevention of an important subset of Pca [2].

PCLs present a unique diagnostic dilemma and are an increasing source of referral to gastroenterologists and surgeons and a major driver of resource utilization [9]. When dealing with these patients, physicians must account for the cost of repeat imaging and the morbidity and mortality associated with pancreatic surgery [9]. In fact, most incidental pancreatic cysts will do no harm to the patients, especially in the case of

the elderly population with significant comorbidities [11-13]. The challenge is to find and act on the few lesions that are pre-malignant or the ones that already harbor malignancy, but there is a lack of good evidence on prospective large cohorts to support this decision [2]. Considering the morbidity of pancreatic surgery and the costs of long-term PCLs follow-up, a correct identification of benign cystic lesions is also crucial in clinical practice and there is evidence that cysts with benign behavior are those in which there is lower diagnostic accuracy with current modalities. Imaging characteristics of MCNs and serous cystadenomas (SCAs) frequently overlap and, for example, in a study published De Pretis et al., all the cases of presumed MCN ultimately turned out to be benign lesions. Moreover, a significant proportion of pseudocysts (PCs) and simple cysts were preoperatively considered MCNs [14]. In fact, currently available clinical tools are imperfect assigning the most appropriate strategies for patients with pancreatic cysts. From surgical series, we learned that up to 25% of patients who have resection are finally diagnosed with cysts with no malignant potential and almost 80% of mucinous cysts are ultimately found not to have advanced histology (high-grade dysplasia or cancer) [2, 15, 16].

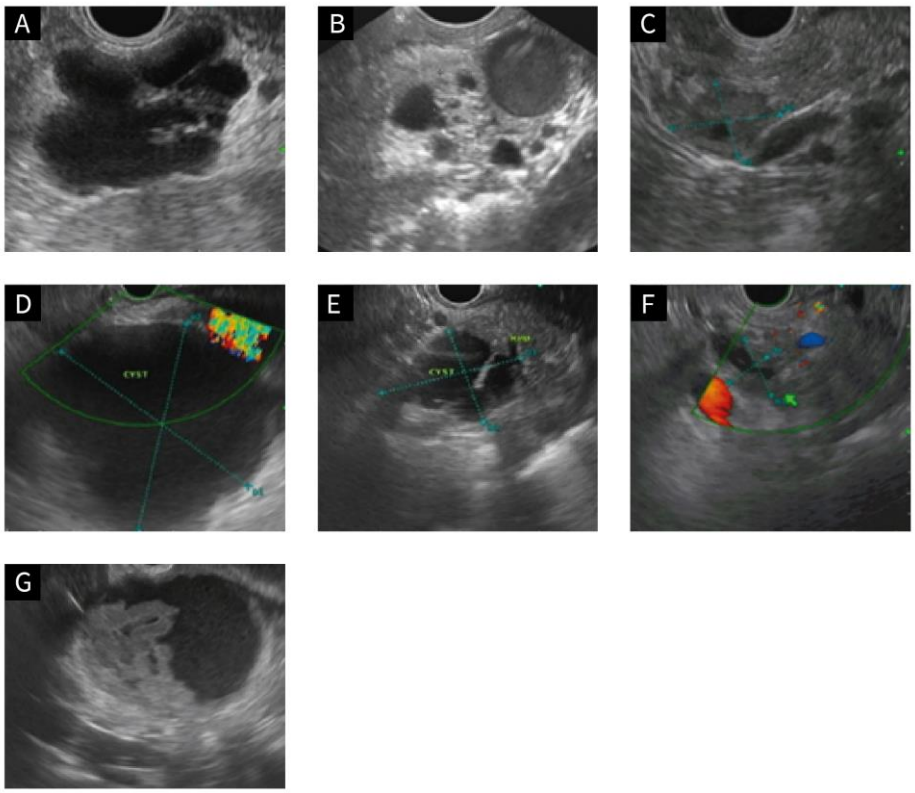
The preoperative characterization of pancreatic cysts in clinical practice is still difficult [14]. There are many challenges associated with achieving an accurate diagnosis and, arguably more importantly, identifying reliable and reproducible methods to stratify the risk of cancer for these patients. This fact turns clinical decision-making difficult [9].

CHAPTER 2
INTRODUCTION AND LITERATURE REVIEW

2.1 SPECTRUM OF CYST TYPES

When considering pancreatic cysts, there is a wide variety of entities that include non-neoplastic and neoplastic lesions. These later lesions encompass a spectrum of benign, malignant and borderline neoplasms that are either primarily cystic or result from cystic degeneration of solid tumors [17]. Figure 1 shows the EUS features of different types of PCLs.

Figure 1. EUS features of different types of pancreatic cystic lesions.



A: Mucinous cystic neoplasia: lobulated cyst with incomplete septa and no connection with the main pancreatic duct located in the pancreatic tail; B: Serous cystadenoma: pancreatic head well-circumscribed microcystic lesion ("honeycomb" pattern), with a macrocystic area. The fibrous portion of the lesion is hyperechoic and the cystic portion is anechoic; C: Cystic neuroendocrine tumor: small hypoechoic lesion with regular borders and a small cystic component in the body of the pancreas; D: Pseudocyst: large, round, doppler negative anechoic lesion with smooth wall in a patient with history of acute pancreatitis; E: BD-IPMN: bilocular septated cyst with main pancreatic duct connection in the head of the pancreas; F: MD-IPMN: main pancreatic duct dilation with irregular wall thickening; G: Solid pseudopapillary neoplasia: mixed solid and cystic well demarcated pancreatic tail lesion in a young female patient.

Author's own images

PCLs are characterized by heterogeneous morphology with often absent defining radiological features and frequently constitute a diagnostic challenge [18].

Pancreatic cystic neoplasms (PCNs) like SCAs have a benign clinical course whereas IPMNs and MCNs represent precursor lesions to invasive adenocarcinoma. Moreover, solid pseudopapillary neoplasms (SPNs) are regarded as low-grade malignant neoplasms, but a small subset of these lesions may show metastatic behavior [19]. Table 1 describes the features of the most common PCLs.

Table 1. Features of common pancreatic cystic lesions.
Adapted from Vilas-Boas F. et al. J Gastrointestin Liver Dis 2019 [20].

	SCA	MCN	IPMN	SPN	PC
Age (years)	60-80	30-50	60-80	20-30	Variable
Gender	Female > Male	Nearly all female	Male > Female	Female	Male = Female
Location	Any	Body/tail	Head	Body/tail	Any
Malignant potential	Very rare	Moderate/high	MD, MT – High; BD – Low	Low	None
Communication with main duct	No	No	Yes	No	Variable
Cytology	Cuboidal cells	Columnar cells	Columnar cells	Fibrovascular stroma	Cyst contents

BD: Branch-duct; IPMN: Intraductal papillary mucionous neoplasia; MCN: Mucinous cystic neoplasm; MD: Main duct; MT: Mixed type; PC: Pseudocyst; SCA: Serous cystadenomas; SPN: Solid pseudopapillary neoplasm.

2.1.1 NON-NEOPLASTIC CYSTS

Non-neoplastic cysts are benign lesions that can be further classified as either non-epithelial or epithelial [21]. PCs are the most frequent and correspond to non-epithelial cysts. Non-neoplastic epithelial pancreatic cysts are rare, benign lesions which include retention, squamoid, lymphoepithelial and enterogenous cysts [22].

2.1.1.1 PSEUDOCYSTS

PCs correspond to collections of debris, inflammatory cells and blood, secondary to acute or chronic pancreatitis or pancreatic trauma. They are usually diagnosed 4

weeks from the onset of acute non-necrotizing pancreatitis, are surrounded by a fibrous wall not lined by epithelium and contain essentially no solid material [23, 24]. On CT, PCs show as solitary, unilocular, low-attenuation cysts with accompanying signs or history of acute or chronic pancreatitis [17].

The critical issue for their management is sometimes the differentiation of PCs from the far more common neoplastic lesions [9].

If the diagnosis is unclear, EUS with FNA usually reveals a dark, string-sign negative fluid, with high amylase and low CEA levels. Cytologic analysis shows histiocytes and inflammatory cells [24].

Most PCs resolve spontaneously, possibly because ductal disruption that leads to extravasation seals spontaneously, resulting in the fluid reabsorption [25]. When symptomatic, PCs present with abdominal pain, jaundice, early satiety or weight loss which correlate with local mass effect [25].

PCs drainage is indicated in the presence of symptoms, complications (hemorrhage or infection) or increasing size. The options include percutaneous, endoscopic or surgical techniques. Currently the general view is that the management should be determined by available local expertise [25].

2.1.2 NEOPLASTIC CYSTS

Neoplastic cysts are divided in mucinous and non-mucinous. Mucin-producing neoplasms have malignant potential and are classified by the World Health Organization (WHO) as two distinct entities: MCNs and IPMNs. The group of non-mucinous lesions includes SCA, SPN and pancreatic neuroendocrine tumors (PNETs) with cystic features [26].

2.1.2.1 SEROUS CYSTADENOMA

SCAs of the pancreas are rare, slow-growing, benign tumors that comprise 1-2% of all pancreatic neoplasms and 10-16% of PCLs [27, 28]. A retrospective, multicenter study

that included over 2500 SCAs reveals that these lesions are more common in the elderly and show female predominance [27].

Nowadays, the majority of SCAs are diagnosed incidentally but when symptomatic, abdominal pain and a palpable abdominal mass are the most common clinical findings. The presence of symptoms is significantly related to cyst size, but no clear cut-off could yet be defined [27]. The tumor cells are presumed to be of intercalated duct cells or of centroacinar cell lineage. Histologically, SCAs are characterized by glycogen-rich small cuboidal epithelial cells (Periodic Acid Schiff-positive) with round nuclei, and a prominent epithelium-associated microvascular meshwork [29]. The degree of dysplasia categorizes the cysts into SCA or serous cystadenocarcinoma, the latter being its malignant counterpart. The risk of serous cystadenocarcinoma is very low (0.1%) and there is very limited data about this tumor even though it is considered a distinct tumor category by the WHO, which requires the presence of distant metastasis for its diagnosis [27, 29]. Specific mortality related to SCA is nearly zero and cases reported as malignant do not fulfill the WHO criteria so there is controversy around the existence of malignant SCAs [29, 30].

On CT and MRI, SCAs are generally single microcystic honeycomb-like lesions with no communication with the main pancreatic duct (MPD). In up to 30% of cases, SCAs may show a central stellate scar and calcification, which can help in the differential diagnosis [31]. The presence of multiple SCAs should suggest Von Hippel-Lindau (VHL) syndrome. In fact, VHL gene mutation is known to play an important role in the pathogenesis of SCAs [29]. Four morphological variants of SCA are described. Microcystic SCA is characterized by a cluster of multiple tiny cysts filled with clear serous fluid, separated by fibrous septa in a honeycomb-like appearance, which constitutes the classical pattern and the majority of the lesions [27-29]. The macrocystic/oligocystic variant reveals fewer (typically less than 10) and larger (>1 cm) cystic spaces and is especially difficult to distinguish from MCN. In a mixed-type lesion we can find features of both micro and oligocystic types and lastly, the solid variant, which is rare (~2% of cases), lacks any grossly visible cystic spaces, resulting in the creation of a well-demarcated nodule on macroscopic exam and is usually

preoperatively confused with PNETs [27, 29]. The variants of SCAs do not differ significantly from the more common microcystic variant except for the difficult differential diagnosis with other neoplasms on imaging [29].

The cytological diagnosis of SCA using EUS-FNA is challenging because of the know cell cohesiveness and adhesion to the tissue but a cyst fluid CEA level <5 ng/mL is highly suggestive of SCA [29]. SCAs have an excellent prognosis and do not require follow-up, unless the diagnosis is uncertain [30]. Surgical resection is curative (no recurrences are described) but should only be considered for symptomatic cases.

2.1.2.2 SOLID PSEUDOPAPILLARY NEOPLASM

SPN or Frantz tumor is a rare neoplasia, first reported in 1959 and accounts for 3-5% of PCLs and 1-2% of exocrine pancreatic neoplasms [32].

It occurs most commonly in young women in their 30s and its origin has not yet been clarified. In fact, the term solid pseudopapillary neoplasm is a descriptive designation, leaving the histogenesis open, since its phenotype does not correlate to pancreatic, epithelial, neuroendocrine or histiocytic lineage [33, 34].

SPNs usually present as a single, well-circumscribed, solid-cystic lesion most commonly in the body and tail of the pancreas. In fact, the presence of necrosis and hemorrhage is frequent, especially in the case of larger lesions. Nowadays, the incidental finding on cross-sectional imaging is the most common form of presentation but some patients report undefined upper abdominal pain due to tumor compression [35]. On CT, SPNs present as heterogenous masses with a solid portion and similar enhancement to pancreatic parenchyma in arterial and venous phases [35]. The mean size is 6-8 cm but the diameter may reach over 20 cm and rare cases of spontaneous rupture of the tumors have been described [34].

On EUS, SPNs are usually hypoechoic, solid appearing masses with varying degrees of cystic degeneration and when FNA is performed, the aspirated fluid is usually bloody and reveals low CEA levels.

Macroscopically, a clear demarcation of the tumor from the pancreatic tissue is found and microscopically two types of arrangements are observed: solid and papillary [34]. The WHO describes SPNs as “low-grade malignant neoplasms” [36] but up to 10 to 15% are reported as aggressive lesions showing local infiltration, vascular or perineural invasion and can metastasize to the liver and peritoneum [37]. We should acknowledge that there is a known inconsistent correlation between pathologic features of the tumor and the clinical course of the disease [34] but some studies suggest larger tumors (>5 cm), lymphovascular and perineural invasion and advanced nuclear grade are associated with malignant disease [37].

Surgical resection is the mainstay of treatment for SPNs in fit patients [35]. Parenchyma-preserving techniques such as enucleation or central pancreatectomy may be used in selective cases but are associated with increased incidence of pancreatic fistula [35, 38]. The need for lymph node dissection is debatable because lymph node metastasis are infrequent. In the presence of metastasis or unresectable SPNs, chemo and radiotherapy have been used with varying success [35]. SPNs are associated with excellent prognosis after complete resection with 10-year survival rate of 96%. Even in the case of aggressive disease, the 5- and 10-year survival rates are 71% and 66%, respectively [37]. European guidelines recommend life-long imaging surveillance, even in benign SPNs, as long as the patient is fit for surgery because if recurrence occurs, it should be aggressively resected [30].

2.1.2.3 PANCREATIC NEUROENDOCRINE TUMORS

PNETs account for less than 10% of all pancreatic neoplasms and are classified clinically in functional or non-functional according to whether they secrete hormones. Non-functional PNETs represent around 90% of all these lesions [17, 39, 40]. PNETs are typically solid lesions but can present as cystic neoplasms presumably as a result of tumor necrosis or hemorrhage, but this assumption is subject of discussion, as some studies suggest they may represent a distinct biological entity [40]. It is estimated that around 13 to 17% of all PNETs show some kind of cystic degeneration and should be considered in the differential diagnosis of cystic pancreatic tumors

[39]. Moreover, some studies found that cystic PNETs are more frequently associated with multiple endocrine neoplasia type 1 (MEN-1) and can show less aggressive behavior, usually presenting with lower pathologic grade and Ki-67 proliferation index [10]. In fact, a large single-center series of PNETs found less frequency of metastatic disease and lower pathologic stage at presentation for cystic PNETs when compared with the solid counterpart [40].

PNETs are usually diagnosed in the fourth to sixth decade of life with equal gender distribution and up to 60% of cases are asymptomatic on presentation [40, 41]. Cystic PNETs are more common in the tail and in contrast with solid PNET are usually larger and more often symptomatic [42].

Preoperative diagnosis is challenging, and these lesions are frequently misdiagnosed as IPMNs, MCNs or SPNs by CT or MRI studies. They typically show as a well-circumscribed cystic lesion with a peripheral rim enhancement. Calcifications are rare [24]. EUS minimizes the risk of misdiagnosis and frequently shows a cystic or mixed solid-cystic lesion with a thick wall. For EUS-FNA, a sensitivity as high as 89% is reported. Fluid CEA level is low, as is amylase, because PNETs do not communicate with MPD [24, 40]. Somatostatin receptor scintigraphy uses radiolabeled somatostatin analogs and relies on receptors expressed by PNETs. This modality has sensitivities ranging from 75 to 100% and is also useful to evaluate the presence of metastatic disease [10].

Histologically, cystic PNETs show polygonal, plasmacytoid looking cells with round or oval and slightly peripheral nuclei, “salt and pepper’ chromatin, and positive staining for chromogranin A and synaptophysin [40].

PNETs are usually managed by surgical resection but some authors consider that for pure cystic PNETs surveillance can be considered [43]. Concerning prognosis, Koh et al. found no significant difference in survival between solid and cystic NETs [42] and in a retrospective study, Cloyd et al. reported no metastasis, recurrence or death after resection of purely cystic PNETs [43].

2.1.2.4 MCNs

In the past, MCNs and IPMNs were frequently confused but nowadays it is clear that these are two separate entities with different biologic behavior and pathologic features, including prevalence of invasive cancer and recurrence after resection [44]. In fact, it was not until 1996 that the WHO classified IPMNs and MCNs as two distinct entities and not until 2000 that the presence of ovarian-like stroma was required for MCN diagnosis [44].

MCNs are frequently discovered incidentally and in a large surgical series, accounted for 16% of resected cysts [45]. They are single lesions, located mostly (>90%) in the body and tail of the pancreas and affect almost exclusively middle-aged women (peak incidence in the fifth decade of life) [4, 46]. Morphologically, MCNs are typically large macrocystic multilocular or unilocular lesions with thickened wall and no communication with the MPD [47]. In fact, there is not a definitive preoperative finding with which to identify them but recently the ability to perform EUS-guided intracystic biopsies allowed the unequivocal MCN diagnosis through the detection of ovarian-like stroma [48].

MCNs do not arise in the ductal system of the pancreas and are lined by mucin producing columnar epithelium surrounded by an ovarian-type stroma. The epithelium is associated with low to high-grade dysplasia or invasive cancer [4]. A systematic review published in 2016 reported malignancy rates that range from 0 to 34%. Patients with malignant MCNs are older and more frequently report abdominal pain and weight loss [47].

Ovarian-type stroma is composed of densely packed, spindle-shaped cells with round or elongated nuclei and sparse cytoplasm, which usually shows positive staining for estrogen receptors (ER) and progesterone receptors (PR). These receptors seem to drive tumor growth and explain the almost exclusive occurrence in women [47].

Like IPMNs, as mucin producing lesions, MCNs are characterized by high cyst fluid CEA and the presence of mucin on cytology.

There is debate about whether MCNs can be surveilled or if all should undergo surgical resection. In MCN, the risk of malignancy is low if the lesion is smaller than 4 cm and shows no mural nodule, solid component or eggshell calcification [47]. However because of the young age of most of the patients and the required decades of follow-up, surgical resection is usually recommended [4]. Because the great majority of MCNs is encountered in the pancreatic body or tail, the lesions are amenable to distal or middle pancreatectomy which are safe procedures in high-volume centers, and in the case of middle pancreatectomy, associated with less risk of exocrine and endocrine insufficiency [44, 49, 50].

The prognosis is excellent for patients who undergo resection of non-invasive MCNs, with 5-year survival rate of 100% [44, 47]. Moreover, complete resection is curative, with no risk of recurrence and in the absence of adenocarcinoma no follow-up is required. For patients with invasive cancer, the 5-year survival rate is around 60%, like the observed for IPMNs but much higher than that of ductal adenocarcinoma [4, 44].

2.1.2.5 IPMNs

IPMNs are grossly visible (by definition >5 mm) intraductal epithelial neoplasms of mucin-producing cells, first recognized by the WHO in 1996 [51]. IPMNs usually present in patients in their 60s or 70s, with similar distribution between men and women, although geographic differences in gender distribution were described [52]. IPMNs are most often found in the head/uncinate of the pancreas and represent the most frequent diagnosis in published series of surgically resected cysts [4, 45, 53].

Based on imaging and macroscopic features, these lesions are characterized as main-duct IPMN (MD-IPMN) and branch-duct IPMN (BD-IPMN). Some patients present with both main and branch duct components, which is referred to as a mixed-type (MT) IPMN [54].

MD-IPMN is distinguished by segmental or diffuse dilatation of the MPD of more than 5 mm, after exclusion of other causes of obstruction, while BD-IPMN presents as a

pancreatic cyst of more than 5 mm that communicates with the MPD. MT-IPMNs meet both these criteria [55].

BD-type has a better prognosis than the variant that involves the main duct, with a rate of malignancy of 25% (range 6-46%) for the former and 50% for the later (range 40-72%) [4].

Beyond MPD communication, another key characteristic of IPMN is its multifocality. Up to 40% of IPMNs are multiple and this finding is useful in the differential diagnosis [56].

Most patients with IPMNs are incidentally discovered during cross sectional imaging studies (CT or MRI). Radiologically, BD-IPMN manifests as a single cyst or a cluster of cysts without MPD dilation. MRCP usually allows the demonstration of cyst communication with the MPD [46].

When symptomatic, IPMNs usually present with abdominal pain, weight loss, jaundice, steatorrhea, diabetes or pancreatitis. In fact, pancreatitis was a sentinel symptom in 15% of cases of histologically confirmed IPMNs in a multicenter study from two important referral centers published in 2010 [57].

2.2 NATURAL HISTORY OF IPMNs

IPMNs comprise most incidental PCLs and pose a great challenge to clinicians because they are precursors of pancreatic ductal adenocarcinoma (PDAC) [58].

These tumors always exhibit at least low-grade dysplasia and should be considered premalignant in all clinical situations. However, the natural history regarding progression to cancer is not well characterized [9].

Our understanding of the natural history of IPMNs has evolved significantly in the last decade. Globally, the rate of malignancy development in IPMNs during follow-up is low. A recent large cohort study of BD-IPMN harboring no high-risk stigmata (HRS), found overall cumulative incidence rates of carcinoma of 3.5% at 10 years and 12% at 15 years after diagnosis [54].

This fact and the non-negligible risk of morbidity and mortality associated with pancreatic surgery, justify a shift from an aggressive to a more conservative approach when dealing with BD-IPMNs. However, the difficult balance between the risk of malignant progression and overtreatment, make patients with these neoplasms challenging to manage [59].

Patients with BD-IPMNs are often referred to surveillance programs, and surgical resection is recommended when features suggesting development of high-grade dysplasia or carcinoma are observed during the follow-up period [54, 56].

When initiating a surveillance program there are many aspects that should be considered and discussed with the patient. Moreover, the psychological burden of patients undergoing surveillance must be considered [8], although a recent international cohort study revealed low surveillance-related anxiety and depression scores in these patients [60].

International guidelines introduced two categories of clinical and morphological risk factors for malignancy: worrisome features (WF) and HRS. HRS warrants surgical resection in fit patients while in patients with WF, EUS with the possibility to perform FNA is recommended [11]. Limited data is available for patients with BD-IPMN

harboring HRS or WF undergoing non-surgical management. Recently, a retrospective multicenter study that included 281 elderly patients, found that the risk of IPMN-related death in the case of IPMNs with HRS is 40%, reinforcing that surgical resection should be offered to fit patients. For elderly patients with IPMN harboring WF, the 5-year disease specific survival (DSS) was 96%, which favors conservative management. In multivariate analysis only age and severe comorbidities were independent predictors of DSS in patients with WF [11]. In fact, scientific society guidelines drive recommendations based mostly on cyst features, but several other factors impact on patient outcomes and patient-oriented PCLs management, with stratification based on Charlson comorbidity index combined with cyst features is suggested by a recent cohort study from the USA [12].

Pancreatic carcinogenesis in patients with branch-duct IPMNs is driven by two major pathways: carcinoma derived from IPMN and *de novo* PDAC in the duct apart from the IPMN, differentiated by imaging and pathological examinations. This differentiation is often difficult and serial imaging data and genetic information, if available, is crucial [54]. In fact, IPMN represents a “field defect”, with the entire organ at risk of neoplasia, but recent findings suggest that both PDAC concomitant with IPMN and PDAC derived from IPMN may have more favorable biological behaviors or be diagnosed earlier than other PDAC [4, 61].

Intraductal papillary mucinous carcinoma (IPMC) is the term used to describe an IPMN lesion which progressed to invasive carcinoma. IPMC accounts for about 10% of resected Pca of ductal origin [51].

2.3 IPMN SUBTYPES AND THEIR CLINICAL IMPORTANCE

The term IPMN was first used in the 1990s, and by that time it was established as a special entity among the pancreatic neoplasms [62].

Microscopically, IPMNs result from the proliferation, in the MPD or branch ducts, of columnar mucin producing cells, usually in a papillary arrangement or rarely as flat lesions. The papillae range from microscopic folds of neoplastic epithelium to grossly visible finger-like projections [51].

Based on the highest degree of cytoarchitectural atypia in the epithelium, IPMNs are classified as having low-grade or high-grade dysplasia. Intermediate-grade category was abandoned and is now included in the low-grade group [51].

Based on morphological resemblance to the epithelium of other gastrointestinal organs and the expression of specific mucins, IPMNs were historically divided into gastric, intestinal, pancreatobiliary and oncocytic subtypes [51, 63]. Four types of mucins are used to classify the different histological subtypes, namely MUC1, MUC2, MUC5AC and MUC6 [51].

Recent data show that the natural history, risk of malignancy, as well as overall survival are tied to the epithelial subtype. In fact, IPMN subtypes appear to develop along different molecular pathways and have been associated with different rates of malignant transformation as well as different types of invasive carcinoma, making them an interesting feature for risk stratification [46, 63, 64].

Since 2019, the WHO classification of tumors of the digestive system considers intraductal oncocytic papillary neoplasms (IOPNs) a distinct entity because of distinguishable genomic and morphological features [36].

Gastric subtype constitutes the majority of IPMNs (50-60%), exhibiting a tall, columnar mucinous epithelium, indistinguishable from gastric mucosa. Immunohistochemistry shows staining for MUC5AC with or without MUC6 and absence of MUC1 and MUC2 expression [51]. These lesions have basally located nuclei, therefore tend to have low-

grade dysplasia and rarely exhibit malignant transformation. When invasive carcinoma occurs, it is usually of the tubular type, with a survival almost as poor as regular PDAC [46, 56, 65].

Intestinal type IPMN accounts for 20-30% of all lesions, is morphologically similar to colon villous adenomas and expresses MUC2, MUC5AC and CDX2. It typically involves the MPD and high-grade dysplasia (HGD) is found in about 50% of cases. Invasive carcinoma arising from intestinal type IPMN is commonly of the colloid type, which has a better prognosis than tubular adenocarcinoma [46, 51, 56, 65].

The pancreatobiliary (PB) type accounts for 10-15% of IPMN lesions and has a complex papillary configuration, with cuboidal cells harboring HGD. Some authors suggest that gastric and PB subtypes may represent the same lesion with different grades of dysplasia and even intestinal IPMNs might derive from gastric lesions [63]. PB subtype stains for MUC5AC, MUC1 and MUC6 but not for MUC2 or CDX2 [46, 51, 56, 65].

IOPNs are distinguished from IPMNs by the morphologic appearance of the epithelium lining the papillae, that is composed of large cells with granular eosinophilic cytoplasm. IOPNs do not have specific mucin expression and do not harbor the genetic alterations seen in IPMNs [66].

One important issue concerning IPMN subtyping is the fact that different subtypes can be mixed in the same lesion, supporting the theory of a common origin. Moreover, the high interobserver variability for the classification of IPMN further complicates subtyping [63].

Preoperative diagnosis of the histologic subtypes of IPMN may, in the future, have an important and relevant impact on the management strategy of non-invasive IPMNs [65].

2.4 SCIENTIFIC SOCIETIES GUIDELINES: CLINICAL MANAGEMENT

The current management of PCLs is largely driven by consensus guidelines [55] or evidence-based guidelines with low grades of evidence available for the majority of the recommendations [30, 67, 68]. The first major document was the 2006 International Consensus Guidelines (ICG) that resulted from a meeting of experts in Sendai, Japan, so they are also known as Sendai Guidelines. Since then, this document was updated twice, the last time in 2017 in Fukuoka, Japan (Revised Fukuoka guidelines) [55]. The European Study Group on Cystic Tumors of the Pancreas also published their recommendations in 2013 and a revised version in 2018 [30]. Two American scientific societies, the American Gastroenterological Association (AGA) and the American College of Gastroenterology (ACG) published another set of recommendations in 2015 and 2018, respectively [67, 68] In the case of AGA, the guideline was preceded by an extensive literature technical review [9].

Several imaging features were found to be predictive of the risk of malignancy and are used for clinical decision-making. Selecting only the studies with surgical specimen as gold standard, the AGA technical review found that the risk of malignancy was significantly increased in patients with cysts >30 mm (odds ratio [OR], 2.97; 95% confidence interval [CI], 1.82-4.85) and cysts with associated solid component (OR, 7.73; 95% CI, 3.38-17.67) but found no significant increased risk in those with dilated MPD (OR, 2.38; 95% CI, 0.71-8.0) [9]. However, several series including surgically resected IPMNs have reported a risk of HGD or cancer of 37-91% in the case of MPD diameter of 5-10 mm [30]. Concerning growth rate, the AGA technical review did not found a statistically significant effect on risk of cancer [9], but a multicenter study with imaging surveillance for 36 months detected a 20-fold higher risk of malignant progression in IPMN whose size increased >5 mm/year or had a total growth of 10 mm [69].

Taking this evidence into account, it is clear that clinical management cannot be based only on single predictive factors of malignancy [55]. Instead, clinicians should

make their decision, taking into account the presence of more than one risk factor, as shown in a recent multicenter study evaluating the effect of multiple risk factors on the likelihood of malignancy in IPMN [70, 71]. More importantly, Zelga et al. found that the risk of malignancy in IPMN correlated with the presence of WF and increased in a stepwise fashion with the number of these features. The risk was 22%, 34%, and 59% if one, two or 3 WF were present, respectively ($p = 0.001$) [71].

Several investigation groups have published nomograms that can be used in clinical practice including multiple risk factors to predict the presence of high-risk lesions [72, 73].

Guidelines on PCLs are broadly concordant in that patients with MD-IPMNs, MT-IPMNs and SPNs should be evaluated for surgery [20]. The same is true for BD-IPMNs with HRS defined by the International Association of Pancreatology (IAP) guidelines: obstructive jaundice in a patient with a cystic lesion of the head of the pancreas, enhancing solid nodule or definite solid nodule ≥5 mm, positive cytology for malignancy or MPD size ≥10 mm [55]. The HRS match the absolute indications for resection defined by the European Guidelines [30].

Moreover, IAP guidelines describe the WF on imaging that should trigger further work-up and close surveillance. They include cyst size ≥30 mm, enhancing mural nodule <5 mm, thickened enhanced cyst walls, MPD diameter of 5-9 mm, abrupt MPD caliber change with distal parenchymal atrophy, lymphadenopathy, elevated serum CA19-9 levels and cyst growth >5 mm/2 years. European guidelines consider WF as relative indications for resection but change the cutoffs for 40 mm in the case of cyst size and ≥5 mm/year for the growth rate. From the clinical standpoint, both guidelines also recommend evaluation for surgery in the case of acute pancreatitis (caused by the IPMN) and, in the case of European guidelines, the development of new-onset diabetes mellitus should also prompt surgical resection in fit patients [30, 55].

The decision, however, should always be individualized and depends not only on the risk of invasive carcinoma or HGD, but also on the patient's life expectancy, comorbidities and cyst location [11].

Especially in the case of BD-IPMNs, guidelines are discordant concerning indications for EUS, the surveillance intervals and the discontinuation of surveillance [30, 55, 68]. These topics were subject of extensive discussion in one of our review papers that constitute the starting point of the original research [20]. The topic of surveillance discontinuation is especially relevant and controversial. AGA guidelines suggest against continued surveillance of cysts with no change in characteristics after 5 years [68], but several studies including a recent large cohort study concluded that the malignancy risk in BD-IPMNs is maintained after the 5-year mark [54, 74, 75]. One exception may be cysts that remain 15 mm or smaller during surveillance for more than 5 years, which may be considered low-risk for progression to malignancy [75].

2.5 ROLE OF ENDOSCOPIC ULTRASOUND FOR PCLs MANAGEMENT

CT and MRI are the imaging modalities recommended by scientific societies guidelines for the initial assessment of PCLs [30, 68, 76]. MRCP is more sensitive than CT for establishing the presence of communication between the cyst and the ductal system while CT is superior for calcification detection. The accuracy and interobserver agreement of CT and MRI is suboptimal for cyst subtype definition and for the differentiation of malignant and pre-malignant cysts [14]. Moreover, there is increasing concern with repeated imaging, that is usual in patients with PCLs under surveillance, as 1.5-2.0% of cancers in the USA are thought to be related to radiation from CT scans [77].

EUS is part of a multimodality diagnostic evaluation of PCLs and was found by Khashab et al. to increase diagnostic yield and accuracy over CT and MRI in presurgical prediction of cyst type and to determine the presence of malignancy [78]. EUS is helpful to identify cysts, like PCs and SCAs, that do not require follow-up, when imaging is equivocal and is ideally suited for IPMN evaluation given its ability to detect MPD communication and its impact in the identification of true mural nodes versus mucus clogs and in the detection concomitant solid masses [79].

There are some discrepancies between different guidelines concerning the indications for EUS/EUS-FNA and it is often unclear when EUS should be performed [80]. The IAP guidelines recommend EUS in the presence of any WF, while the AGA guidelines require the presence of two or more high-risk features on cross-sectional imaging (dilated MPD, ≥3 cm cyst or non-enhancing solid component). The European study group suggests the performance of EUS when there are clinical or radiological features of concern and the ACG when cyst type is unclear or when EUS results are likely to alter management [30, 67, 68, 76]. Several predictive features have been described for risk stratification but there continues to be a high discrepancy between risk stratification using standard of care and pathological diagnosis. A recent retrospective study reviewing 338 patients with PCLs, found that EUS changed

management when CT showed a cyst >4 cm in size or >3 cm with solid component. Additionally, specific patient characteristics including male sex, age less than 50 years and smoking history were significantly associated with EUS change in management [80].

One main advantage of EUS is the possibility to aspirate cyst fluid for cytologic and biochemical analysis using FNA, that may overcome the limitations of morphology alone [78].

In clinical practice, cyst fluid analysis usually includes cytology and determination of CEA and amylase levels. EUS-FNA rarely results in diagnostic cytology because of fluid scant cellularity [81]. Cyst fluid CEA levels greater than 192 ng/mL have traditionally been used to predict the likelihood of mucinous lesion [26]. However, CEA levels do not correlate with the degree of dysplasia and there is considerable overlap between mucinous and non-mucinous cysts CEA levels, so only values that are either very low (non-mucinous) or very high (mucinous) are truly helpful [15, 46]. Recently, cyst fluid glucose levels have shown better performance than CEA for mucinous vs non-mucinous cyst differentiation [82].

Nevertheless, a solitary PCL may remain a diagnostic challenge after completion of these investigations. In fact, especially in the case of BD-IPMNs, we continue to rely mostly on morphological criteria for risk stratification [18].

2.5.1 NEW EUS-BASED DIAGNOSTIC TECHNIQUES

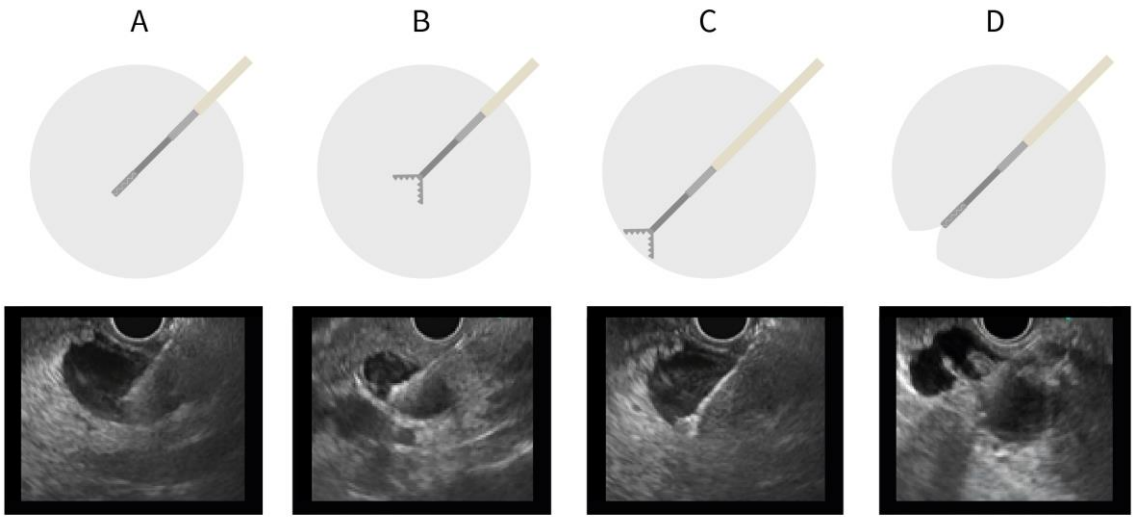
Endoscopic innovations based on EUS and new cyst fluid biomarkers have emerged in the last decade and have potential impact in the diagnostic workflow of PCLs [18, 83]. Molecular analysis of DNA-based biomarkers in cyst fluid and confocal laser endomicroscopy (CLE) have been described but its clinical application is hampered by technology availability and cost [83, 84]. EUS-TTNB of cystic lesions is emerging as an important tool in the endoscopic armamentarium for risk stratification in PCLs [83].

2.5.1.1 THROUGH-THE-NEEDLE BIOPSY

TTNB of PCLs was developed to overcome the low sensitivity of cyst fluid cytology during EUS-FNA [18, 85]. The technique was first described in 2010 by Aparicio et al. in a pilot study that showed the feasibility of cyst wall biopsy with real-time ultrasonographic control [85]. A dedicated forceps, designed to fit through a 19-gauge EUS needle, was latter developed. Moray® microforceps (STERIS, Mentor, OH, USA), allows sampling of cyst wall, septa or mural nodules for histologic analysis of epithelium and stroma [48, 86] (Figure 2).

EUS-TTNB may provide definite information regarding cyst type and the grade of dysplasia in the case of mucinous cysts. Moreover, for IPMN, the histologic features and mucin expression by immunohistochemistry can be important for risk stratification [65, 87]. Two recent meta-analyses [88, 89] confirmed the feasibility, high specimen adequacy and diagnostic yield of EUS-TTNB. Nonetheless, the procedure is not standardized, and safety concerns have been raised [87, 90]. In fact, an important multicenter study including 506 patients identified the predictors for adverse events after EUS-TTNB. On multivariate analysis, age, the number of passes, the inability to completely aspirate the cyst, and the diagnosis of IPMN were associated with higher risk of adverse events [91].

Figure 2. Diagram and corresponding EUS images showing EUS-TTNB procedure steps.



A: After cyst puncture, the microforceps is advanced through the needle; B and C: The forceps is opened inside the cyst and pushed against the wall; D: After forceps closure, it is pulled back creating a tenting effect of the tissue. Author's own images.

2.5.1.2 ARTIFICIAL INTELLIGENCE

Artificial intelligence (AI) involves computer programs that perform functions associated with human intelligence, such as learning and problem solving. Its application in Medicine has recently emerged as a breakthrough technology to identify lesions and differentiate images in various specialties, including in endoscopy to aid the physician in the interpretation of multimodality images [92-94]. In fact, several experts predict that almost every medical specialties will be using AI technology in the future [95].

AI and in particular its subfield of machine learning (ML), is ideal for deciphering patterns in large datasets and offers unique opportunities for advancing precision oncology [96].

ML involves the use mathematical models for capturing structure in data, in which a machine performs repeated iterations of models progressively improving performance of a specific task. After the optimization procedure on example data – training – the models can be used to make predictions about new, unseen data – validation and test sets [92, 93] (Figure 3).

Supervised ML refers to training with labeled data, for example “mucinous” or “non-mucinous”, to ultimately predict the labels of new data based on the model learnt from labelled examples. On the other hand, unsupervised learning concerns training with unlabeled data and aims only to find the underlaying structure, to predict what data points are similar. It can be helpful when a gold standard is not available, or the objective is to split the data into groups that share certain properties. Deep learning (DL) is a form of ML in which an artificial neural network (ANN) is used, exploiting hierarchical relations in the data [93]. ANN are ML models inspired by the brain’s neural connections. Each neuron is a computing unit, and all neurons are connected to each other to build a network. Signals travel from the first (input), to the last (output) layer, possibly after going through multiple hidden layers. Convolutional neural network (CNN), which consists of multilayers of ANN with step-by-step minimal processing, showed outstanding performance in image analysis [92, 94].

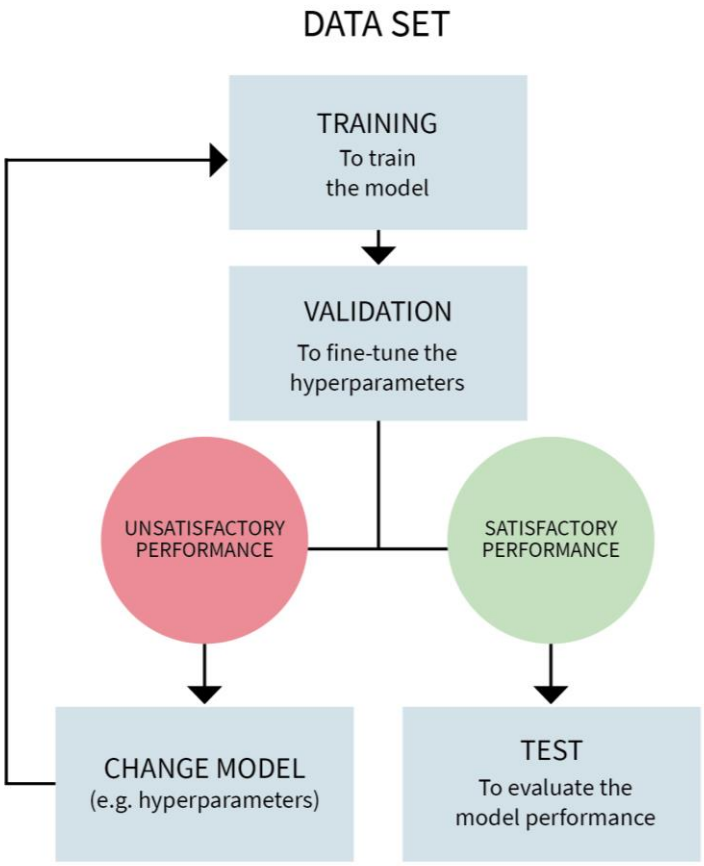
Training a CNN involves dividing the data into a training set, which helps to define the architecture of the network and to find out the various weights between the nodes, and then a test set (validation dataset) to assess the capability of the CNN to predict the desired output. During training, weights of interneuron are adjusted to optimize classification. The so called hyperparameters consist of optional settings defined by the investigators that affect the behavior of a model and are used to optimize its performance. The validation set should be large enough to find the right hyperparameters and prevent overfitting [92, 93].

Several mathematical ML models, built based on training datasets, are used to describe the relation between an input (e.g. image) and an output (e.g. label). The model should afterwards work for new data, not used for training, that allows generalization. If the model is too tightly fitted to the training data and does not generalize toward new data, this is called overfitting [93].

DL algorithms have recently been shown to outperform clinicians in identifying pathologies in images [97]. AI has multiple applications in Gastroenterology and seems particularly valuable in endoscopy. In fact, several assistance tools for lesion detection and characterization are already approved for clinical practice [92, 98]. Most studies focus on AI tools dedicated to improving diagnostic accuracy in the case of colorectal polyps or cancer and also on the diagnosis of premalignant lesions of the upper GI tract (esophagus and stomach), but some publications in the field of pancreatology, test for example, the ability of AI to aid in the detection of PDAC based on EUS [99].

In the case of PCLs, different types of cysts carry distinct risk of malignant transformation, therefore, it is imperative to correctly identify the cystic lesions and their characteristics to stratify their malignant potential. Given the limitations of current modalities based on imaging and cyst fluid analysis, novel diagnostic AI models are being tested to better risk-stratify PCLs [98].

Figure 3. Diagram showing the steps for building a machine learning model. Adapted from Van der Sommen et al. GUT 2020;69(11):2035-2045 [93].



2.6 PANCREATIC SURGERY

In the case of IPMNs, the goal of surveillance and management should be to identify the lesions that have the potential to progress to cancer and to resect them before an invasive component develops [100].

Pancreatic surgery, even in expert centers, is associated with significant morbidity and non-negligible mortality, especially in the case of pancreaticoduodenectomy (PD), that should be taken into account for decision-making in the management of PCLs [101]. In fact, several published series report morbidity rates over 30% and mortality up to 5% in patients undergoing PD [102, 103]. Although associated with a low mortality, distal pancreatectomy has an approximate 25% morbidity rate, which includes a 15% to 20% risk of diabetes [30]. The AGA technical review identified 77 studies that evaluated PCLs surgery-related morbidity and 49 studies that included information about surgical mortality and report an overall morbidity and mortality rate of 30% and 2.1%, respectively [9].

Regarding IPMNs, indications for resection have changed significantly over the last few decades, from resection of all lesions to resection of only those meeting specific criteria that were topic of ongoing refinement in recent international guidelines [104]. Current guidelines have a high sensitivity in terms of Pca prevention, but the low specificity has led to high rates of unnecessary surgical resection worldwide [105]. A recent multicenter American study that included 478 patients that underwent resection for IPMN, showed that only 23% of surgical specimens revealed high-grade dysplasia or invasive carcinoma [104]. This was also the case in an international multicenter study that included expert centers from 3 continents that showed that 63% of resected BD-IPMNs had only low-grade dysplasia [100]. Moreover, other studies reported that 25-64% of patients undergoing resection have PCLs with no malignant potential [2].

2.7 PRECISION MEDICINE

The concept of precision-based Medicine is an emerging approach for disease management. The basic principle is to create diagnostic, prognostic, and therapeutic strategies tailored to each patient [106].

For PCLs, we nowadays determine patients' management based on demographics, imaging and cyst fluid analysis but current guidelines are imperfect for risk stratification [18].

In contrast to the "one-size-fits-all" approach of traditional medicine, precision medicine provides health care adapted for individual patients [107]. In the case of precision diagnosis, emerging tools are used for improving and individualizing patients' risk stratification, follow-up and treatment decisions. EUS-based AI models and EUS-TTNB are two examples of those such tools.

Multiple AI algorithms were described for medical image analysis and endoscopic image interpretation, but very few were published reporting their use for EUS image analysis [108, 109]. However, some studies proved the possibility to include DL models for the evaluation of pancreatic lesions, including PCLs [98].

In the case of EUS-TTNB several studies proved its usefulness for pancreatic cyst subtype definition and in the case of mucinous lesions, for determination of dysplasia grade [87, 110, 111]. Moreover, for IPMNs, EUS-TTNB gives the possibility for histological subtyping using morphology and mucin expression, that can impact decision-making [90].

CHAPTER 3

AIMS

The **main aim** of this thesis was:

To determine the role of a new endoscopic technique – EUS-TTNB – in the diagnosis and risk stratification of PCLs and to develop and validate an EUS-based CNN for the differentiation of mucinous and non-mucinous PCLs, ultimately contributing for a precision-based medicine approach of these lesions and reducing the number of inappropriate resections and follow-up procedures.

The specific aims were:

1. To evaluate the use of EUS-TTNB for definition of cyst histotype.
2. To define the possibility to determine the grade of dysplasia in mucinous cysts and perform IPMN subtyping using EUS-TTNB samples.
3. To evaluate the clinical impact and safety of EUS-TTNB to ultimately improve patient selection.
4. To contribute to EUS-TTNB procedure standardization.
5. To explore the use of AI tools to mitigate EUS image misclassification.
6. To develop and to validate an EUS-CNN for the automatic identification of mucinous PCLs.

CHAPTER 4

RESULTS – PUBLICATIONS

4.1 REVIEW ARTICLES

PANCREATIC CYSTIC LESIONS: NEW ENDOSCOPIC TRENDS IN DIAGNOSIS

Filipe Vilas-Boas, Guilherme Macedo

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Pancreatic Cystic Lesions
New Endoscopic Trends in Diagnosis

Filipe Vilas-Boas, MD and Guilherme Macedo, PhD, FACG, FASGE, AGAF

Abstract: The diagnosis of incidental pancreatic cystic lesions is increasing in the general population because of the routine use of cross-sectional imaging modalities. Not all cysts are neoplastic, and the majority of cystic neoplasms have a low overall likelihood of progression to malignancy. Current management is based on imaging and cyst fluid analysis, but we are not able to diagnose the specific type of cyst and its malignant potential in a significant number of patients. Better diagnostic tools are required to avoid unnecessary surgical resections that carry an important risk of morbidity and mortality. Herein we review current evidence concerning the use of new endoscopic modalities for the evaluation of pancreatic cystic lesions. We focus our discussion on the new cyst fluid markers, and the advancements on modalities such as confocal endomicroscopy, contrast-enhanced endoscopic ultrasound, and the use of Spyglass. We also discuss the use of new devices to improve the cellular yield from cyst fluid and to obtain cyst-wall tissue, namely the cytology brush, the fine needle biopsy, and forceps for cyst-wall biopsy.

Key Words: pancreatic cystic lesions, endoscopic ultrasound, cyst fluid analysis, contrast-enhanced endoscopic ultrasound, confocal laser endomicroscopy, pancreatoscopy and cystoscopy

(*J Clin Gastroenterol* 2018;52:13–19)

The prevalence of pancreatic cysts is not known, but they are incidentally found in about 2.4% to 2.6% of patients undergoing cross-sectional imaging for reasons not related to the pancreas; this finding is strongly correlated with advancing age. In patients over the age of 70, the prevalence can be as high as 10%.[1,2]

Pancreatic cystic lesions (PCLs) present a unique diagnostic dilemma. The majority of incidental pancreatic cysts will do no harm to the patient. The challenge is to find and act on the few that are premalignant or the ones that already harbor malignancy, but there is a lack of good evidence for this decision on prospective large cohorts.

When considering pancreatic cysts, there is a wide variety of entities that include congenital, inflammatory, and neoplastic lesions. Among the most common, pseudocysts (PCs) and serous cystadenomas (SCAs) have a benign clinical course, whereas intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs) represent precursor lesions to invasive adenocarcinoma.[3] Table 1 summarizes key features of the main pancreatic cyst types.

The management of asymptomatic pancreatic cysts is largely driven by consensus guidelines with low grades of evidence available for the majority of recommendations.[4,5]

In the last decade, the evaluation and treatment of PCLs changed from an aggressive to a more conservative approach.

The current management of pancreatic cysts is based on clinical presentation, imaging, and cyst fluid analysis, but a solitary PCL may remain a diagnostic challenge after completion of all available investigations. Defining the best approach to managing incidental pancreatic cysts could potentially spare patients unnecessary testing, radiation, and surgery. This fact justifies that better diagnostic tools are required.

Herein we present a review of the recent advances with regard to the evaluation of PCLs that hopefully will allow better recognition of the type of lesion and risk for malignancy in the near future.

ENDOSCOPIC ULTRASOUND–FINE-NEEDLE ASPIRATION (EUS-FNA) AND CYST FLUID ANALYSIS

EUS is considered a valuable technique in the evaluation of pancreatic cysts because of superior spatial resolution.[6] It provides details of the morphologic characteristics of the lesions and allows sampling of both fluid and solid components. EUS also has a role in the follow-up and documentation of morphologic changes over time and in ascertaining resectability in case of malignancy.[7]

EUS-FNA with cyst fluid analysis will likely have little benefit as first test or for surveillance of asymptomatic solitary or multiple cysts <1 cm and in the presence of classic microcystic SCA. On the contrary, it will be useful in the differential diagnosis of macrocystic SCA versus mucinous cystadenoma, in the evaluation of cystic degeneration of a solid lesion, and in the presence of focal or diffuse main duct dilatation. Accuracy of EUS imaging alone in the differentiation of mucinous versus nonmucinous cysts is ~50%.

One of the limitations of EUS is the low interobserver agreement for the diagnosis of neoplastic versus non-neoplastic lesions, specific type, and EUS features of PCLs.[6] This issue is still valid for different observer groups considered as experts, semiexperts, or novices.[7]

Cyst fluid analysis is nowadays an important part of the evaluation of PCLs. It allows measurement of tumor markers and cytologic evaluation that may distinguish different types of cysts (serous vs. mucinous) and may allow grading the epithelium of mucinous cysts. The issue is that interpretation is limited by specimen cellularity, degeneration, and contamination with gastrointestinal epithelium.

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The authors declare that they have nothing to disclose.
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TABLE 1. Characteristics of Common Pancreatic Cystic Lesions

Characteristics	SCA	MCN	IPMN	SPN	PC
Age	60-80	30-50	60-80	20-30	Variable
Gender	Female > male	Nearly all female	Male > female	Female	Male = female
Location	Any	Body/tail	Head	Body/tail	Any
Malignant potential	Very rare	Moderate/high	MD, MT-high; BD-Low	Low	None
Communication with main duct	No	No	Yes	No	Variable
Cytology	Cuboidal cells	Columnar cells	Columnar cells	Fibrovascular stroma	Cyst contents

BD indicates branch duct; IPMN, intraductal papillary mucinous neoplasia; MCN, mucinous cystic neoplasm; MD, main duct; MT, mixed type; PC, Pseudocyst; SCA, serous cystadenomas; SPN, solid pseudopapillary neoplasm.

In the Cooperative Pancreatic Cyst study (CPC study) that involved 341 patients with PCLs, sensitivity of cyst fluid cytology for diagnosing mucinous cysts was only 34% because of the low number of cells found in cystic fluid.[8]

More recently, problems with cytology in EUS-guided cyst aspiration were delineated in a prospective, dual-center study in which, similarly to the CPC study, only 31% of the samples had adequate cellularity for analysis.[7]

The epithelial cells of MCNs harbor several proteins that can be found in the cyst fluid. Several studies show that the analysis of the fluid for carcinoembryonic antigen (CEA), carbohydrate-associated antigen (CA) 19-9, and CA 72-4 are useful to differentiate between mucinous and nonmucinous lesions (Table 2).

Mucinous cysts are lined by endoderm-derived columnar epithelium capable of secreting CEA. The addition of CEA analysis increased the diagnostic yield to ~80% in separating mucinous cysts from nonmucinous cysts in the CPC study.[8]

Cyst fluid CEA is a reliable marker for mucinous cystic lesions but is not a predictor of malignancy or dysplasia.[8,10]

In the CPC study, cyst fluid concentrations of CEA, CA 72-4, CA 125, CA 19-9, and CA 15-3 were measured.[8] The ROC curve area was greatest for CEA (0.79), followed closely by CA 72-4 (0.72). The cutoff value of 192 ng/mL for CEA provided the greatest accuracy (0.79) for differentiating between mucinous and nonmucinous cysts with moderate sensitivity (0.73) and specificity (0.84).[8]

Amylase levels in the cyst fluid can also aid in the differential diagnosis of PCLs. Van der Waaij et al[9] published a pooled analysis that looked at the ability of cyst fluid amylase, CA 19-9, and CEA to distinguish between SCA, MCN, and PC.

The authors included 12 studies. Amylase in the fluid was measured in 155 PCLs (32 SCA, 60 PC, 32 MCN, and 31 mucinous adenocarcinoma). A cutoff of 250 U/L for cyst fluid amylase was associated with a sensitivity of 44% and a specificity of 98% for the diagnosis of PC. The conclusion is that amylase <250 U/L virtually excludes the presence of a PC.[9]

EUS-FNA with cyst fluid analysis is the current standard practice, but, according to the International Consensus Guidelines published in 2012, EUS is operator dependent and the cytology of cyst fluid difficult to interpret.

NEW TRENDS: CYST FLUID ANALYSIS

Intracystic Markers

There is a parallel between histologic and molecular progression in mucinous cysts, involving the same molecular events seen in the development of pancreatic cancer, including *KRAS* mutation, *p53* overexpression, and loss of *p16* and *SMAD4*.[11]

Recently, the combination of clinical and molecular features was pointed in some studies to be more accurate for assessing cyst type and the need for resection.

The multicentric prospective Pancreatic Cyst DNA Analysis Study (PANDA study) investigated the additive value of molecular analysis to CEA for distinguishing mucinous and nonmucinous cysts and the role of cyst fluid DNA analysis for predicting malignancy.[11] The addition of *KRAS* to CEA levels >148 ng/mL was shown to raise the sensitivity for the diagnosis of mucinous cysts from around 70% to 84%, maintaining the specificity at around 67%. In this study, the presence of a *KRAS* mutation was 96% specific (odds ratio, 20.9) for detecting a mucinous lesion. In the absence of *KRAS* mutation, the cyst fluid CEA level remains significantly associated with mucinous cyst.[11]

For the detection of malignancy, analysis incorporating DNA concentration, number and sequence of mutations, and mutational amplitude were significantly associated with advanced lesions in a univariate analysis but not in the multivariate regression model. All malignant cysts with false-negative cytologic findings manifested at least 1 DNA analysis variable associated with malignancy.

The authors conclude that a combination of tests to include cytologic evaluation, CEA level, and a detailed DNA analysis can maximize the diagnostic yield of pancreatic cyst FNA.

TABLE 2. Accuracy of Different Cyst Fluid Markers in Differentiating Mucinous and Nonmucinous Pancreatic Cystic Lesions

Cyst Fluid Marker	*CEA (ng/mL)	*CA 15.3 (U/mL)	*CA 19.9 (U/mL)	*CA 72.4 (U/mL)	*CA 125 (U/mL)	†Amylase (U/L)
Sensitivity (%)	73	19	68	80	83	44
Specificity (%)	84	94	62	61	37	98
Accuracy (%)	79	57	66	72	60	65

Cutoff values: CEA: 192; CA 15.3: 121; CA 19.9: 2900; CA 72.4: 7; CA 125: 9; amylase: 250.
*Data from the Cooperative Pancreatic Cyst Study (Brugge et al[8]).
†Data from pooled analysis (Van der Waaij et al[9]).
CA indicates carbohydrate-associated antigen; CEA, carcinoembryonic antigen.

In the study published by Singhi et al,[12] dual *KRAS* and *GNAS* mutations showed 84% sensitivity and 98% specificity for IPMN diagnosis, but had no correlation with grade of dysplasia of the lesions.

More recently a multicentric retrospective study from the United States of America, Europe, and Japan used massive parallel DNA sequencing in 130 cyst fluid samples collected during surgery during 9 years.[13]

Cysts were considered as appropriately resected if they were found on histopathologic examination to be solid pseudopapillary neoplasia, MCNs, or IPMNs with high-grade dysplasia/invasive adenocarcinoma. The authors looked for mutations in 6 oncogenes (*BRAF*, *CTNNB1*, *GNAS*, *KRAS*, *NRAS*, and *PIK3CA*) and 5 tumor suppressor genes (*CDK2NA*, *RNF43*, *SMAD4*, *TP53*, and *VHL*), loss of heterozygosity (LOH) in the same 5 tumor suppressor genes, and the presence of aneuploidy.

The authors found at least 1 genetic alteration in 92% of the cyst fluid samples and described a mutational profile for each cyst type.

KRAS was the most commonly mutated gene in cyst fluid samples from MCNs and IPMNs. In the case of MCNs, these lesions were identified with 100% sensitivity and 75% specificity by the absence of *CTNNB1* or *GNAS* mutations, chromosome 3 LOH, or aneuploidy in chromosome 1q or 22q.

Globally, 86 (91%) of the IPMNs had a mutation in *KRAS* or *GNAS* and 45 (47%) had a mutation in both genes. *GNAS* mutations were not found in any other cyst type.

The presence of a mutation in *GNAS* and *RNF43*, LOH in chromosome 9, or aneuploidy in chromosome 1q or 8p had 76% sensitivity and 97% specificity for the diagnosis of IPMN.

The composite molecular markers (presence of a mutation in *SMAD4*, chromosome 17 LOH, the region containing *RNF43*) or aneuploidy in chromosome 5p, 8p, 13q, or 18q) correctly identified IPMNs with high-grade dysplasia or invasive carcinoma with a sensitivity of 75% and a specificity of 92%. The combination of both the clinical and molecular features increased the sensitivity to 89%, but the specificity was only 69%.[13]

Springer and colleagues propose an algorithm that uses a combination of molecular and clinical features to categorize the cysts on the basis of distinct mutational profiles. These profiles would potentially reduce the number of unnecessary surgeries by 91%.

Importantly, the study found consistency between the sample sets for 24 lesions for which cyst fluid was collected both during surgery and EUS-FNA.[13]

Cyst fluid cytokines are the other promising clinically relevant biomarkers that are worth mentioning. Cytokines are proteins that lead signaling cascades modulating immune responses.

Inflammation is associated with the pathogenesis of several malignancies. This is true, for example, in chronic pancreatitis wherein continued inflammation may lead to dysplasia and adenocarcinoma. Specific lymphocytes and the cytokines they produce may serve as markers of neoplasia.[14]

Lee and colleagues reported on the use of a multiplex bead-based protein assay for the evaluation of inflammatory proteins in pancreatic cyst fluid collected during EUS-FNA. They compared the inflammatory protein profile of 5 IPMNs and 5 inflammatory cysts. Granulocyte-macrophage colony-stimulating factor was shown to be of particular interest, as it appeared in all 5 inflammatory cyst fluid samples and in none of the BD-IPMN samples. In addition, 2 other proteins, eotaxin and hepatocyte growth factor, were detected with higher concentrations in inflammatory cysts compared with BD-IPMN cysts. The authors conclude that these proteins may serve as diagnostic biomarkers and provide insights into the malignant potential of pancreatic cystic neoplasms, but their results need validation with greater sample size studies.[15]

The study from Maker and colleagues evaluated the pancreatic cyst fluid aspirates from 40 resected IPMN specimens (19 high-risk lesions with high-grade dysplasia or invasive disease, and 21 low-risk lesions with low-grade or moderate-grade dysplasia). The authors showed that IL1β levels had a high sensitivity and specificity (odds ratio, 17) to distinguish low-risk lesions from high-risk lesions. In a validation set, IL1β maintained a high positive predictive value (PPV) for correctly identifying high-risk cysts. The authors conclude that IL1β levels correlated with the degree of cyst dysplasia and were highly predictive of high-risk lesions.[16]

NEW TRENDS: ENDOSCOPY

Contrast-enhanced EUS

Contrast-enhanced-EUS has been reported as an useful adjunct in the differential diagnosis of pancreatic solid tumors, but there is limited experience in its use for the study of PCLs.

A first study from Japan published in 2013 involving 17 IPMNs with mural nodules compared the findings of EUS with the surgical specimens and showed that the evaluation of vascularity by contrast-enhanced endoscopic sonography could be useful for distinguishing mural nodules from mucus clots.[17]

One year later, Hocke et al[18] showed that CE-EUS could be useful for differential diagnosis of PCs/dysontogenic cysts and cystic neoplasia.

The same conclusions were more recently presented in a paper from Bologna, Italy, that included 76 patients with pancreatic cysts.[19] CE-EUS allowed differentiation between PC (hypoenhancement) and PCLs (hyperenhancement) but could not differentiate between SCAs and MCNs (both with hyperenhancement). CE-EUS clearly showed malignant vegetations inside PCLs as solid components with hyperenhancement.

Cytology Brush (Echobrush)

To increase the sensitivity of EUS-FNA, other technical methods to improve the cellular yield and to obtain cyst-wall tissue have been recently studied.

Cytology brush using Ecobrush (Cook Endoscopy, Winston-Salem, NC) has been tested in several studies with conflicting results and a high rate of complications (8% to 20%), including 1 death.

The first pilot study was published in 2007 by Al-Haddad and colleagues and involved 10 patients, all of them but 1 having mucinous neoplasms. The authors reported a clear superiority of cytobrushing over EUS-FNA in terms of cellularity and detection of mucinous epithelium (90% vs. 20%).[20]

The same authors published the complete series in 2010 that included 37 patients and reached similar results, as intracellular mucin was detected more frequently with

Echobrush than with EUS-FNA (62% vs. 23%, $P = 0.001$). The overall complication rate was 19% (7 patients). There were 4 minor adverse events (2 abdominal pain, 2 self-limiting intracystic bleeds) and 3 patients with significant complications (2 acute pancreatitis, 1 major bleed).

In the same year, Sendino and colleagues published their results on the use of Ecobrush in 30 patients. Brushing was superior to the aspirated fluid for detecting diagnostic cells (73% vs. 36%, $P = 0.08$) and mucinous cells (50% vs. 18%, $P = 0.016$), but the technical failure rate was 27% (8 patients) in relation to difficulties in sampling lesions located in the head. Three patients had complications (10%), including 1 fatal subacute retroperitoneal hemorrhage in a patient under anticoagulation therapy that was stopped 2 days before the procedure.[21]

The other preliminary study was published by Bruno et al[22] and, among other lesions, included 12 pancreatic cysts, obtaining adequate material for cytologic analysis in 50% of the cases.

Fine-Needle Biopsy

A new EUS needle equipped with a side fenestration (EchoTip ProCore, Cook Endoscopy Inc., Limerick, Ireland) was developed to obtain histologic samples in different solid lesions.

A prospective study from 2 Italian centers published in 2014 with a mean follow-up of 11 ± 4.2 months included 58 patients with 60 PCLs that consecutively underwent EUS-FNA/biopsy.[23]

The overall sample adequacy for cytohistologic diagnosis was 65% with a high percentage of samples collected being adequate for histologic evaluation (46.1%). In PCLs with solid components, the cellular material was adequate for cytohistologic evaluation in 17/18 (94.4%) lesions.

The authors reported 2 procedure-related adverse events (1 self-limited bleeding and 1 patient who developed fever).

Pancreatoscopy and Cystoscopy (Spyglass)

The development of Spyglass (BostonScientific, Natick, MA, USA) allowed endoscopic exploration of biliary and pancreatic duct systems.

A series of case reports and smaller studies have reported that single-operator SpyGlass technology can be useful for evaluation of pancreatic duct lesions such as IPMN because it allows visualization of the pancreatic duct epithelium and the performance of directed biopsies

A study from Sweden including data from 41 patients showed that pancreatoscopy using SpyGlass provided additional diagnostic information in 39 of the 41 cases (95%). The authors concluded that pancreatoscopy affected clinical decisions in 76% of cases. Post-ERCP pancreatitis occurred in 7 patients (17%), 6 of them had normal or slightly dilated MPD.[24]

In 2014, Japanese authors published another study evaluating the use of SpyGlass in the assessment of possible IPMN with MPD dilation.[25] This retrospective study involved 17 patients (main duct-IPMN = 10; branch duct-IPMN = 2; mixed type-IPMN = 5).

SpyScope pancreatoscopy was attempted in 13 of the 17 patients. The target lesion could be observed in 12 patients and targeted biopsy had only 25% sensitivity for the detection of malignant IPMN. The authors explained the low sensitivity by citing the technical limitations of working in the small pancreatic duct and the fact that IPMN may include dysplasia varying from low grade to high grade in the same lesions.

In 3 patients, SpyGlass pancreatoscopy was useful for determining the excision line before surgery, avoiding total pancreatectomy, especially in patients with diffuse MPD dilation.

Symptomatic pancreatitis was not observed in any of the 12 patients who underwent pancreatoscopy.

The first report of SpyGlass use outside the biliary tree and main pancreatic duct was published in 2009.[26]

Antillon and collaborators[26] performed EUS to evaluate an 8 cm cystic lesion of the pancreas suspected to be a PC with internal debris or a CPN with mural nodules. After creating a cystogastrostomy opening, SpyGlass was introduced through the accessory channel of the echoendoscope and then into the cyst, allowing the inspection of the cyst wall and the performance of biopsies.

More recently Chai et al[27] from China published a retrospective observational study that examined the use of through-the-needle fiberoptic pancreatic cystoscopy with SpyGlass for the diagnosis of PCLs. The authors performed cystoscopy in 43 patients and correlated intracystic characteristics with pathology results (surgical, FNA pathology, and cyst fluid cytology results). There were no significant adverse events. They described the cystoscopic characteristics of each type of cystic lesion, summarizing the blood vessel distribution on the cystic wall, partition or ridge-like structure, and papilla-like structure. According to these authors, the tree-like branching pattern of blood vessel distribution may be an SCN-specific characteristic, and, for MCNs, intracystic papilla-like structure observed during cystoscopy may support its diagnosis. Intracystic partitions and the puncture holes in the partitions were observed more commonly in SCNs (69%) than in MCN (11%). Red papilla-like structure with a rich blood supply was found in 60% of the IPMNs. Ridge-like shape structures on the cystic wall surrounded by abundant blood vessels were more commonly found in MCNs (33%) than in SCNs (15%).

In the case of solid pseudopappilary neoplasms, cystoscopy was unable to show clear images because of small and blurred cystic cavities, and, in PCs, the authors describe the presence of protein-like substances or necrotic deposits.

The authors concluded that cystoscopy had great diagnostic value for PCLs and could serve as an important adjunct to EUS, but their results need validation from prospective studies.

Cyst-wall Biopsy

Cyst-wall biopsy may allow us to overcome the problems in EUS-guided cyst fluid aspiration as regards the limited cellularity of the samples.

In the previously cited pilot study from Aparicio et al,[28] the authors performed biopsies from the cyst wall using a 0.8 mm diameter forceps (Lumenis Surgical).

In both cystic lesions submitted for biopsies, the sample findings were consistent with MCN. The authors found that the forceps was clearly seen by EUS, and it is possible to control in real time its opening and closing and its contact with the cystic wall.

One of the patients developed severe pancreatitis 1 month later, but the authors claim this was unlikely to be related to the biopsy.

Recently Shakhatreh and colleagues from Virginia, USA, published his work on the use of a specifically developed forceps to use in cystic lesions. The Moray micro

forceps (US Endoscopy) was used to perform cyst-wall biopsies in 2 patients and yielded mucinous columnar epithelium in both samples.[29]

Confocal Laser Endomicroscopy (CLE)

CLE (Cellvizio; Mauna Kea Technologies, France) is a real-time laser-assisted microscopic imaging of tissue facilitating in vivo histopathology. IV fluorescein is used to stain vessels and delineate tissue structures and is the most commonly used contrast agent for CLE imaging.

In EUS-guided needle-based CLE (nCLE), a 19-G needle is loaded with the AQ-Flex miniprobe (diameter of 0.85 mm).

Konda et al[30] published the first human pilot study demonstrating the feasibility of EUS-guided nCLE for pancreatic lesions in 2011.

This study from 2 centers in the United States included 18 cases (16 cystic lesions and 2 solid masses). The authors reported that the procedure was technically feasible in 17 of 18 cases. Two patients developed pancreatitis that required hospitalization.

A second study, INSPECT,[31] included 66 patients from 8 reference centers in the United States and in Europe. This trial focused on the characterization of IPMN and correlated some identified nCLE structures (papillae) with histologic features (Fig. 1). In this study, nCLE revealed epithelial villous structures that were associated with neoplastic cystic lesions with a sensitivity of 59%, specificity of 100%, and PPV of 100%. The overall complication rate was 9%.

The single-center DETECT study[32] included 30 patients and assessed the feasibility, safety, and diagnostic yield of a combination of cystoscopy using Spyglass and nCLE in the diagnosis of CPLs. nCLE alone had a sensitivity of 80%, specificity of 100%, PPV of 100%, NPV of 80%, and accuracy of 89% for diagnosis of mucinous cysts. Sensitivity reached 100% with the combination of both Spyglass and nCLE imaging.

The most recently published results concerning CLE use in PCLs come from the CONTACT study.[33]

CONTACT is a multicenter study from France developed in 2 phases. CONTACT 1 evaluated the value of nCLE for the characterization of benign PCLs that could have a direct impact on patient management by avoiding unnecessary surgery. The authors included 31 patients and were able to identify an nCLE pattern called "superficial vascular network," which was a unique feature of SCA (Fig. 2).

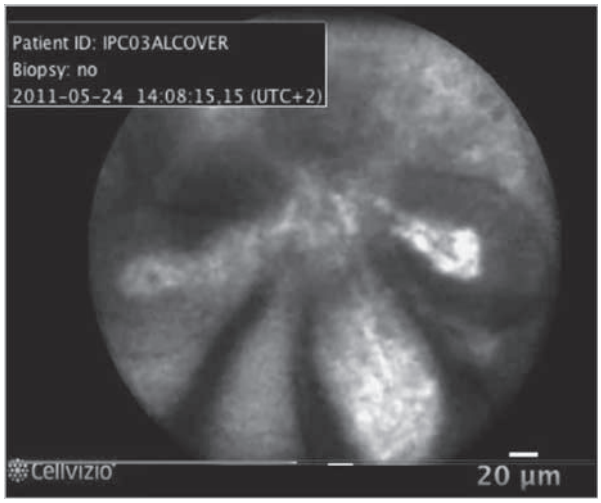

FIGURE 1. nCLE image showing papillary projections that characterize IPMN. Image reproduced with permission from Prof. Marc Giovannini, Head of the Endoscopy Unit, Paoli-Calmettes Institute, Marseille, France. Permission to reproduce must be obtained from the rightsholder.

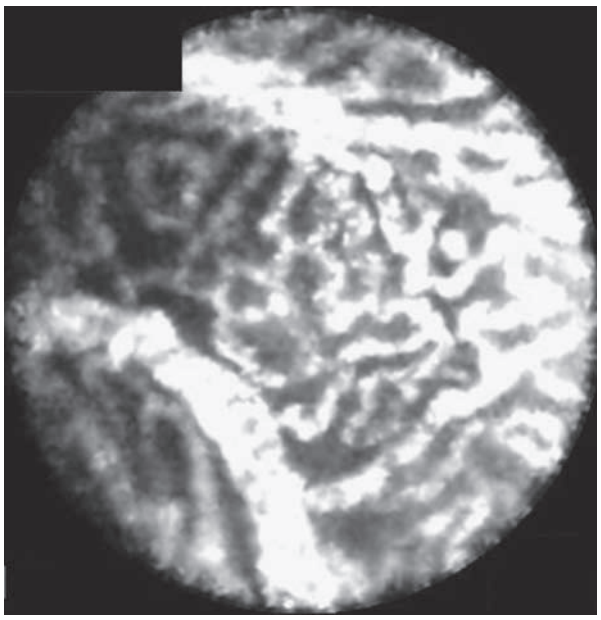

FIGURE 2. nCLE image revealing superficial vascular network of SCA. Image reproduced with permission from Prof. Marc Giovannini, Head of the Endoscopy Unit, Paoli-Calmettes Institute, Marseille, France. Permission to reproduce must be obtained from the rightsholder.

The same authors published a second paper in which they determined new nCLE criteria for the diagnosis of MCN and PC and carried out a first evaluation of the performance of these criteria as well as a second validation of the previously detailed criteria for SCA and IPMN.[34]

A recent meta-analysis of 2 studies[31,32] that applied nCLE for the diagnosis of PCLs produced a pooled sensitivity and specificity of 68% and 90%, respectively. The accuracy of nCLE for classifying PCLs on the basis of their malignant potential is low at 46%, and the interobserver agreement for identification of nCLE findings is slight ($\kappa = 0.13$).[35,36]

A larger prospective study— CONTACT 2 is currently underway and should confirm the set of criteria previously described for PCLs.

There are several limitations of nCLE. In BD-IPMN, the distribution of the papillary epithelium is patchy, and the limited intracystic mobility might restrict and prevent imaging the involved area of the cystic lesion. In the case of SCA, "superficial vascular network" was not observed in nearly one-third of the cases, and, in MCN, ovarian stroma has not been characterized by nCLE;

Combining all 3 trials (INSPECT, CONTACT, and DETECT) the rate of postprocedural pancreatitis was 4.3%.

CONCLUSIONS

Despite recent advances in imaging techniques and endoscopic procedures, the diagnosis of cystic pancreatic lesions remains a challenge.

A more conservative approach is nowadays adopted by most clinicians owing to the improved knowledge of the pathology and natural history of these neoplasms.

EUS is nowadays the most important tool for the evaluation of PCLs, as it combines high-resolution images with the possibility of FNA for cyst fluid analysis.

In recent years, several advances related to cyst fluid analysis and endoscopic techniques allow us to better risk stratify the patients and decide their best management.

In the field of cyst fluid analysis, the search for new molecular markers and the use of DNA sequencing techniques will provide distinct mutational profiles associated with each type of cyst, reducing the number of unnecessary surgeries. The search for biomarkers that identify high-risk lesions or the likelihood of progression, especially in the case of BD-IPMNs, must be our main goal and investigation should be encouraged.

EUS will continue to have a major role in the evaluation of PCLs. Preliminary work shows that CE-EUS is useful for the differential diagnosis of these lesions but more data are needed on the application of this technology to determine the nature of internal solid components within the cysts.

The collection of biopsy specimens during pancreatoscopy in IPMNs has low sensitivity, but this technique will probably have a role in the planning of surgical resection in some cases of multifocal lesions, especially in the case of main duct-IPMN with diffuse duct dilation.

Nowadays and in the future, tissue will continue to be the issue. Echobrush has a prohibitive complication rate that discourages its use. We also need more data on the usefulness of EUS biopsies carried out using a needle equipped with a side fenestration.

In the results from pilot studies performing cyst-wall biopsy with a trough, the needle forceps seems promising and will probably improve when we are able to target areas suspicious of advanced histology identified during cystoscopy.

The perspective of being able to perform in vivo histopathology in PCLs is overwhelming, but nCLE data need to be prospectively validated in clinical practice. The tide is still rising in the quest for the most accurate tools to elucidate diagnosis in PCLs, but we are on the right track.

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MANAGEMENT GUIDELINES FOR PANCREATIC CYSTIC LESIONS: SHOULD WE ADOPT OR ADAPT THE CURRENT ROADMAPS?

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REVIEW

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Management Guidelines for Pancreatic Cystic Lesions: Should we Adopt or Adapt the Current Roadmaps?

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ABSTRACT

Pancreatic cystic lesions are very prevalent, especially in elderly patients and are increasingly being diagnosed because of the massive use of cross sectional imaging. Our knowledge about the natural history of these lesions is limited, especially in the case of intraductal papillary mucinous neoplasms. This fact explains why scientific societies guidelines statements are based on evidence graded as very low quality and helps the understanding of some of the different guidelines recommendations. Several guidelines have been recently revised to incorporate the new evidence published in the literature with the aim to help clinicians make the best decisions. American Gastroenterological Association guidelines, a revision of the International Consensus Guidelines, the American College of Gastroenterology and the European Study Group guidelines are the most recent.
Herein we review the current guidelines on pancreatic cysts and focus our discussion on controversies and updates about the best imaging modalities, the indications for endoscopic ultrasound guided fine needle aspiration, cyst fluid analysis, indications for resection and surveillance strategies.

Key words: pancreatic cystic lesion – IPMN- guidelines – endoscopic ultrasound – cyst fluid analysis.

Abbreviations: ACG: American College of Gastroenterology; AGA: American Gastroenterological Association; BD: branch-duct; CT: computer tomography; CE-EUS: contrast-enhanced endoscopic ultrasound; CLE: confocal laser endomicroscopy; ESG: European Study Group; EUS: endoscopic ultrasound; EUS-FNA: endoscopic ultrasound guided fine needle aspiration; HGD: high-grade dysplasia; HRF: high-risk features; HRS: high-risk stigmata; ICG: International Consensus Guidelines; IPMN: intraductal papillary mucinous neoplasm; MCN: mucinous cystic neoplasia; MD: main duct; MRCP: Magnetic Resonance Cholangiopancreatography; NGS: next generation sequencing; PCL: pancreatic cystic lesions; SCA: serous cystadenoma; WF: worrisome features.

INTRODUCTION

The diagnosis of pancreatic cystic lesions (PCLs) is increasingly performed. Two recent studies reporting on the use of cross sectional imaging for health screening programs that included around 25,000 patients showed a global prevalence of PCLs around 2.5% [1, 2].

Patients with PCLs have an increased risk of pancreatic malignancy compared with the general population [3]. Using the data of a large cohort of veterans, Munigala et al. [3] reported an overall 19.64 (95% CI, 12.12-31.82; p <0.0001) times higher risk of pancreatic cancer in these patients compared with the rest of the patients in the database.

In PCLs, malignancy occurs virtually only in those with mucinous structure. Branch duct (BD)-intraductal papillary mucinous neoplasms (IPMNs) comprise the majority of incidental pancreatic cystic lesions [4] and pose a great challenge to clinicians because they are precursor lesions of pancreatic adenocarcinoma.

However, the rate of malignancy development in IPMNs during follow-up is low. A systematic review of 37 case series predominantly from Japan and Italy reported 112 invasive cancers in 3,980 patients during 14,830 patient-years of follow-up. The proportion of patients developing invasive neoplasia was 2.8% overall (95% CI, 1.8%–4.0%), 0.72% per year [5].

The development of trustworthy guidelines is a key priority for healthcare providers and is necessary to promote the best

care for patients [6]. Current guidelines on PCLs are too many and confusing for the clinicians.

This paper will address the main differences between the most recently published guidelines on PCLs, in an effort to assist clinicians in the management of these patients. We will focus on the controversies and updates regarding the recommended imaging modalities, the indications for endoscopic ultrasound guided fine needle aspiration (EUS-FNA), cyst fluid analysis, surgery and surveillance strategies.

CURRENT GUIDELINES

American Gastroenterological Association (AGA) guidelines [7], a revision of the International Consensus Guidelines (Fukuoka-ICG) [8], the American College of Gastroenterology (ACG) [9] and the European Study Group guidelines (ESG) [10] were recently published.

In medical science, guidelines should be evidence-based, developed as a group process using validated methods to achieve consensus after rigorous disclosure of conflicts of interest [6, 11]. The Grading of Recommendations Assessment, Development and Evaluation (short GRADE) is a transparent approach to grading quality (or certainty) of evidence and strength of recommendations and is now considered the standard in guideline development. Except for the revised ICG (consensus symposium), the other three more recent guidelines were developed using the GRADE framework. Guidelines on PCLs are broadly concordant in that patients with main duct (MD)-IPMNs, mixed-IPMNs and solid pseudopapillary neoplasia (SPN) should be evaluated for surgery. The same is true for BD-IPMNs with "high-risk stigmata" (HRS) defined by the ICG (enhancing solid nodule or definite solid nodule ≥5mm, positive cytology for malignancy or main pancreatic duct – MPD - dilation over 10 mm).

There is a consensus that the risk of malignancy in mucinous cystic neoplasia (MCN) is low if the lesion is smaller than 4 cm without a mural nodule. In the case of serous cystadenoma (SCA) there is no need for resection or surveillance, except if the lesion is causing symptoms.

On the other hand, especially in the case of BD-IPMNs, guidelines are discordant concerning the EUS indications, the impact of cyst size, the threshold for surgery, the surveillance intervals and the discontinuation of surveillance.

Several reasons might explain the disagreement of the different medical societies guidelines. The most obvious is our poor knowledge of PCLs natural history and the low quality of the evidence supporting the guidelines statements. In addition, the main focus used to be on cyst features rather than on the patient characteristics and ultimate prognosis.

Imaging Modalities

All current guidelines agree that magnetic resonance cholangiopancreatography (MRCP) is the best procedure to characterize the pancreatic cysts and ICG recommends its use in all patients with cysts ≥5mm in size. Pancreatic protocol computed tomography (CT) is the alternative modality suggested by the ICG, ACG and the ESG to characterize PCLs.

The reported accuracy in identifying the specific type of PCL is between 40% and 95% for MRCP and between 40% and 81% for CT [12]. MRCP is more sensitive to detect the communication between the PCL and the pancreatic duct, the presence of mural nodules or internal septa [12]. Multifocality pointing towards the IPMN diagnosis is better evaluated by MRCP. But the presence of calcifications is better diagnosed using CT.

Sainani et al. [13] retrospectively compared the performance of dedicated pancreatic protocols CT and MRCP for 38 pathologically confirmed PCLs ≤ 3cm in size. They found a better accuracy for MRCP to detect morphologic features of the cysts, but the differences were not statistically significant. More importantly, the accuracy of these two techniques for assessment of histological aggressiveness was similar (CT vs MRI, 75-78% vs 78-86%, respectively; $p > 0.05$) [13].

EUS-FNA

Endoscopic Ultrasound is considered useful in the evaluation of pancreatic cysts because of its superior spatial resolution when compared with cross sectional imaging studies [14]. Endoscopic ultrasound guided-FNA with cyst fluid analysis will likely have little benefit as a first test or for the surveillance of asymptomatic solitary or multiple cysts <1 cm and in the presence of classic microcystic SCA. On the contrary, it will be useful in the differential diagnosis of macrocystic SCA versus mucinous cystadenoma, in the evaluation of cystic degeneration of a solid lesion, in the diagnosis of main duct involvement in IPMNs and to confirm presence of solid component/mural nodule. Cysts that have clear indication for resection based on imaging or presence of symptoms do not need EUS.

Accuracy of EUS imaging alone in the differentiation of mucinous versus non-mucinous cysts is around 50% [15]. Because of this limitation, EUS-guided sampling in PCLs may help in the differential diagnosis and risk stratification. The recent technical guideline of the European Society of Gastrointestinal Endoscopy (ESGE) for EUS-guided sampling recommends emptying the cyst with a single pass in the case of lesions without a solid component. When the lesion contains a solid part, this component should be targeted because samples were shown to be more accurate for diagnosis than fluid aspirates [16].

Moreover, new diagnostic modalities for the evaluation of PCLs involving EUS, such as contrast-enhanced-EUS (CE-EUS) or confocal laser endomicroscopy (CLE) were recently developed and several papers report on their usefulness [15].

Contrast-enhanced-EUS is nowadays widely used as an adjunct for the differential diagnosis of pancreatic solid lesions. In the case of PCLs, Fusaroli et al. [17] demonstrated that CE-EUS allowed differentiation between pseudocysts (hypoenhancement) and cystic neoplasms (hyperenhancement) but could not differentiate between SCAs and MCNs (both with hyperenhancement). Also CE-EUS clearly showed malignant vegetations inside PCLs as solid components with hyperenhancement [17].

The revised ESG state that CE-EUS should be considered for the evaluation of mural nodules and septations [10]

Confocal laser endomicroscopy is a real-time laser-assisted microscopic imaging of tissue facilitating *in vivo* histopathology. Its first use in pancreatic lesions was described in 2011 [18]. Four important studies (INSPECT [18], DETECT

[19], CONTACT [20] and CONCYST-01 [21]) focused on the characterization of PCLs using CLE. A meta-analysis that included two studies that applied CLE for the diagnosis of PCLs produced a pooled sensitivity and specificity of 68% and 90%, respectively [22]. The accuracy of CLE for classifying PCLs on the basis of their malignant potential is low at 46%, and the inter-observer agreement for identification of CLE findings is slight (κ =0.13) [23]. This new technique will hopefully allow better recognition of the type of lesion and risk for malignancy in the near future, but at this time, its use is not recommended outside clinical trials.

The main differences between the guidelines concerning EUS are related to the indications (Table I). The ESG suggests EUS in the presence of clinical or radiological features of concern and the ACG guideline recommends EUS in the presence of any of the following: MD >5mm, cyst size ≥3 cm or change in MD caliber with upstream atrophy. According to the revised ICG, EUS is indicated in the presence of any of the so called "worrisome features" (WF) and AGA guidelines suggest its use only if there are two or more positive high-risk features (HRF) (dilated MD ≥5mm, cyst size ≥3 cm or non-enhancing solid component) on MRCP.

Table I. Indications for EUS-FNA

Guideline	EUS-FNA
ICG (Revised Fukuoka 2017)	*Worrisome features
AGA 2015	**≥2 high-risk features
European 2018	Clinical or radiological features of concern; Hyperenhancement on CE-EUS
ACG 2018	Cysts in which the diagnosis is unclear, and where the results are likely to alter management

ACG: American College of Gastroenterology; AGA: American Gastroenterological Association; ICG: International Consensus Guidelines; *Worrisome features: pancreatitis, cyst ≥3 cm, enhancing mural nodule < 5 mm, thickened/enhancing cyst walls, main duct size 5-9 mm, abrupt change in caliber of pancreatic duct with distal pancreatic atrophy, lymphadenopathy, increased serum CA19-9, cyst growth rate ≥ 5 mm/2 years; **AGA High-risk features: dilated MPD (≥5mm), ≥3 cm cyst or non-enhancing solid component

The previous ICG published in 2012 defined WF as presence of pancreatitis, cyst size ≥3 cm, thickened/enhancing cyst walls, MPD size 5-9 mm in diameter, abrupt change in the caliber of the pancreatic duct with distal pancreatic atrophy and lymphadenopathy [24]. In the revision of the ICG published in 2017 the authors added the presence of enhancing mural nodule <5 mm, increased serum CA19-9 and cyst growth rate ≥5 mm/2 years as WF [8].

The requirement of two or more positive HRF to perform EUS as recommended by the AGA guideline has been questioned. Kohli et al. [25] published a retrospective cohort study that included 210 patients who had EUS for PCLs characterization between 2004 and 2015. The requirement of ≥2 HRF, based on AGA practice guidelines, would have decreased the number of EUS procedures by 91%, but reduced the sensitivity for pancreatic malignancy to 50% [25].

Cyst Fluid Analysis

A CEA level of 192 ng/mL was found to be optimal by using receiver operating characteristic curves, with a 75% sensitivity and 84% specificity for differentiating between mucinous and non-mucin producing cysts, but CEA levels are inaccurate to differentiate benign versus malignant mucinous PCLs [26].

The combination of clinical and molecular features was recently pointed to be more accurate for assessing the cyst type and the need for resection.

Springer et al. [27] reported on the use of massive parallel DNA sequencing in 130 cyst fluid samples collected at the time of EUS or from resected surgical specimens in an interval of 9 years. The composite molecular markers (presence of a mutation in SMAD4, chromosome 17 LOH- region containing RNF43 - or aneuploidy in chromosome 5p, 8p, 13q, or 18q) correctly identified IPMNs with high-grade dysplasia or invasive carcinoma with a sensitivity of 75% and a specificity of 92%. The combination of both clinical and molecular features increased the sensitivity to 89%, but the specificity was only 69% [27].

Singhi et al. [28] prospectively evaluated the use of the next generation sequencing (NGS) using cyst fluid obtained during EUS-FNA (626 specimens from 595 patients). The assay targeted several genes known to be mutated and/or deleted in PCLs (KRAS, GNAS, NRAS, HRAS, BRAF, CTNNB1, TP53, PIK3CA, PTEN and AKT1). The authors found that mutations in KRAS and GNAS are highly sensitive and specific for IPMN, but not MCN. Moreover, detection of mutations/deletions in TP53, PIK3CA and/or PTEN were highly sensitive and specific for IPMNs with advanced neoplasia [28].

Besides the measurement of tumor markers and molecular studies, cyst fluid analysis allows cytologic evaluation that may distinguish different types of cysts (serous vs. mucinous) and may permit the grading of the epithelium of mucinous cysts [15]. But the cytological interpretation is limited by specimen cellularity, degeneration, and contamination with gastrointestinal epithelium. Concerning cytology, a meta-analysis published in 2013 revealed 42% sensitivity and 99% specificity for differentiating mucinous versus non-mucinous PCLs [29].

All guidelines on PCLs beside ICG recommend cyst fluid analysis with CEA level determination and cytology. The revision of ICG still considers EUS-FNA for cytology as investigational but mentions the added value of cyst fluid CEA levels. Most guidelines consider molecular analysis as investigational and not ready for clinical practice. The ACG guideline suggests that the use of molecular markers may be considered in the case of indeterminate diagnosis of cyst type when the results are likely to change the management (Table II).

In fact, molecular studies are nowadays still considered costly and may not add to the standard analysis.

Surgery

In a retrospective series from Massachusetts General Hospital involving 851 individuals undergoing resection of PCLs over 33 years, 60% of the lesions had a risk of harboring malignancy or progress to malignancy (about 40% IPMNs;

Table II. Cyst fluid analysis

Guideline	CEA	Biochemistry	Cytology	Molecular analysis
ICG (Revised Fukuoka 2017)	M	Amylase	Investigational	KRAS/GNAS Investigational
AGA 2015	R	(-)	R	Investigational
European 2018	R	Lipase	R	KRAS/GNAS (conditional)
ACG 2018	R	(-)	R	Not ready for clinical practice

ACG: American College of Gastroenterology; AGA: American Gastroenterological Association; ICG: International Consensus Guidelines; CEA: Carcinoembryonic antigen; GNAS: adenylate cyclase-stimulating G alpha protein; KRAS: Kirsten rat sarcoma viral oncogene homolog; M: mentioned; R: recommended; (-): not mentioned.

20% MCN; 3% SPN). This series also shows that the most incidentally found PCLs are IPMNs [30].

IPMNs encompass a spectrum of lesions from adenoma to invasive carcinoma, and are considered precancerous lesions [31].

The mean frequency of invasive carcinoma and high-grade dysplasia (HGD) in resected BD-IPMN is 31.1% and that of invasive cancer is 18.5% [8].

Pancreatic surgery carries a significant risk of morbidity, even in high-volume centers. Valsangkar et al. [30] found a 38% postoperative complication rate for PCLs resection. Crippa et al. [31] reported mortality rates of 1-3% and morbidity rates of 30-60% after surgical resection of IPMNs, depending on resection types.

Surgery in IPMNs is most valuable in the case of lesions harboring HGD or carcinoma in situ, so the focus should be to refine the diagnosis of HGD.

Several features in mucinous lesions are associated with an increased risk for HGD or cancer and are used to determine indications for resection. Table III shows the indications for surgery in PCLs according to society guidelines.

All guidelines (except AGA guidelines) recommend cyst resection in the presence of jaundice or acute pancreatitis or in the presence of positive cytology for malignancy.

Concerning the cyst size, the ICG and AGA moved away from size alone as criterion to indicate surgery because of its poor predictive value for invasive carcinoma and HGD, but the revised ESG consider lesion size ≥ 4 cm as a relative indication for resection. ACG recommends that for mucinous cysts ≥ 3 cm the pros and cons of surgery versus surveillance should be discussed.

Recent data showed that the growth rate may be more important than the cyst size itself. A large retrospective multicentric study from the USA and China [32] including 284 patients with BD-IPMNs without WF or HRS, showed that malignant BD-IPMNs (18.6 vs. 0.8 mm/year; p=0.05) grew at a faster rate compared to benign BD-IPMNs and that a growth rate ≥5mm/year had a hazard ratio of 19.5 (95% CI 2.4-157.8) for malignancy.

The presence of mural nodules was found to have the highest diagnostic odds ratio for malignancy in BD-IPMNs in a meta-analysis of 23 articles that included 1373 patients [33]. In fact, in all guidelines, mural nodules are an absolute indication for resection. The cut-off value size of mural nodules to identify high-risk lesions is set at 5mm by the revised ICG and the ESG. A mural nodule ≥5mm on EUS has a sensitivity of 73-85% and specificity of 71-100% for HGD or cancer [10].

Concerning MD, studies showed that the risk of advanced histology (HGD or cancer) was correlated with the duct size and with the presence of abrupt caliber change. In the previously cited meta-analysis [33], Kim et al. found the MD dilation to have a diagnostic odds ratio of 3.4 (95% CI 2.3-5.2), the highest after mural nodules. This finding justifies the inclusion of MD dilation by all the recent guidelines as an important feature for the management of IPMNs. An MD ≥10mm is considered an absolute indication for surgery by the ICG and the ESG, and a duct size of 5-9.9mm is considered a WF by ICG and is a relative indication for surgery as determined by the ESG.

Of note is the recent inclusion of high serum CA 19-9 as an important predictor of advanced histology in IPMNs. Concerning the indication for resection, the revision of ICG

Table III. Indications for surgery

Guideline	Symptoms	MPD	Mural nodule	Positive cytology	Size	Comments
ICG (Revised Fukuoka 2017)	+ jaundice	≥10mm	+ 5 mm cut-off	+	-*	HRS Consider life expectancy, comorbidities and location
AGA 2015	NA	dilated	+	+	-	and/or 2 features
European 2018	+ jaundice, acute pancreatitis	≥10 mm (5-9.9 mm relative indication)	+ 5mm cut-off	+	≥ 4cm (relative indication)	Growth rate ≥5mm/year new-onset DM high CA19.9
ACG 2018	+ jaundice, acute pancreatitis	≥5mm	+	+	≥3cm	Growth rate ≥3mm/year new-onset DM high CA19.9

ACG: American College of Gastroenterology; AGA: American Gastroenterological Association; CA 19.9: carbohydrate antigen 19.9; DM: Diabetes Mellitus; HRS: high-risk stigmata; ICG: International Consensus Guidelines; MPD: Main pancreatic duct; NA: not applicable; *cyst size alone is not an appropriate parameter to indicate surgery. Presence of more than one risk factor increases probability of HGD/inv carcinoma

included high serum CA 19-9 as a new WF and the ESG states high CA 19-9 as a relative indication for surgery.

All this discussion is focused on cyst features, but the trend is to adopt a more "patient"-centered strategy. This shift happens because of a better understanding of the natural history of PCLs, namely IPMNs.

Nowadays there is a need to personalize the decision and to focus on patient condition/status (comorbidities and expected survival) because factors besides cyst features have a significant impact on patient outcomes, and every multidisciplinary team taking care of these patients should be aware of this fact.

Two important studies brought new information concerning this problem. The first is a multicentric retrospective study from Italy and the USA that included 281 elderly patients with IPMNs (231 harboring WF and 50 HRS, as per ICG that undergone non-operative management with a median follow-up of 51 months) [34]. The disease-specific survival (DSS) of patients with IPMNs with WF was as high as 96%, highlighting noncancerous mortality of these elderly patients on long-term follow-up. In the case of patients with IPMNs harboring HRS, the authors found a 40% risk of IPMN-related death reinforcing that surgical resection should be offered to fit patients [34].

The second important report about competing risks for mortality in patients with PCLs was recently published by Kwok et al. [35]. The authors included 1800 patients with PCLs stratified based on Charlson comorbidity index. There were 402 deaths during a median follow-up of 5.7 years. Only 43 deaths were PCL-related, reinforcing that the association of patient-related factors and cyst features help guide the management.

The ESG now propose the management based on cyst features but also on the patient's life expectancy and comorbidities and cyst location. This stratification allows patients to be divided into two resection strategies: preemptive surgery in the case of the presence of relative indications for surgery, and cancer surgery in the case of absolute indication(s).

Surveillance

All guidelines support surveillance when patients are not submitted to surgery (Table IV).

The main controversy and perhaps the most striking feature introduced by the 2015 AGA guidelines is the possibility of stopping surveillance.

A retrospective multicentric study from Italy including 144 IPMNs with neither WF nor HRS reveals new-onset of WF and HRS in 26 patients (18%) after a median follow-up of 71 and 77.5 months, respectively. One out of six patients developed WF or HRS beyond 5 years of surveillance, so the authors conclude that persistent surveillance is required [36].

The possibility of stopping surveillance was evaluated by the group of Massachusetts General Hospital in a retrospective study of 577 patients with BD-IPMN, of whom 363 underwent surveillance over 5 years. Forty-five patients developed malignancy, 5 of them after 10 years of follow-up [37]. These results do not support AGA's recommendation for stopping surveillance.

The authors found, however, that cysts which remain smaller than 15 mm for more than 5 years might be considered low-risk. In this group of patients, the decision to stop surveillance may be adequate [37].

COMMENTS AND CONCLUSION

A solitary PCL may remain a diagnostic challenge after completion of all currently available investigations. The morphology and location of the cyst along with the presence of communication with MD and the patient characteristics, including age and gender are useful in the differential diagnosis of PCLs. In addition, a history of acute or chronic pancreatitis may point to the diagnosis of a pseudocyst (Table V). Currently, the CEA level in cyst fluid is the best modality to differentiate mucinous and non-mucin producing cysts but it is not a predictor of malignancy or dysplasia. In the future, if the usefulness of molecular studies is confirmed, analysis of key gene mutations may be part of clinical practice and aid in risk stratification.

To infer advanced histology/malignancy, the presence of mural nodules/solid component, dilation of MD and growth rate have the highest predictive value.

Several scientific societies issued guidelines for the management of PCLs. In the era of Evidence-based Medicine, clinicians must support their decisions on the best available data in the literature, but there are some controversies stated in the different guidelines that can lead to confusion. The clinicians frequently do not know which guideline they should follow. The controversies are justified by the fact that

Table IV. Surveillance (mucinous cysts)

Guideline	Follow-up indications	Stop surveillance
ICG (Revised Fukuoka 2017)	< 1cm – CT/MRI in 2-3 years 1-2 cm – CT/MRI yearly x 2 then lengthen as appropriate *2-3 cm – EUS in 3-6 months then lengthen as appropriate *>3 cm – MRI/EUS every 3-6 months up to 1year	Lifelong (until not fit for surgery)
AGA 2015	MRI after one year then MRI every 2 years	Stable appearance after 5 years
European 2018	EUS/MRI and CA 19-9 after six months then EUS/MRI and CA 19-9 yearly	Lifelong (until not fit for surgery) Intensification after 5 yrs?
ACG 2018	Cyst size guides surveillance (similar to ICG)	When not fit for surgery, assess utility in those >75 years

ACG: American College of Gastroenterology; AGA: American Gastroenterological Association; CA 19.9: carbohydrate antigen 19.9 ; CT: Computed tomography; EUS: Endoscopic Ultrasound; ICG: International Consensus Guidelines; MRI: Magnetic Resonance Imaging; *consider surgery in young fit patients with need for prolonged surveillance

Table V. Features of common pancreatic cystic lesions

	SCA	MCN	IPMN	SPN	PC
Age (years)	60-80	30-50	60-80	20-30	Variable
Gender	Female>Male	Nearly all female	Male>Female	Female	Male=Female
Location	Any	Body/tail	Head	Body/tail	Any
Malignant potential	Very rare	Moderate/high	MD, MT – high; BD - Low	Low	none
Communication with main duct	No	No	Yes	No	Variable
Cytology	Cuboidal cells	Columnar cells	Columnar cells	Fibrovascular stroma	Cyst contents

BD: branch-duct; IPMN: Intraductal papillary mucinous neoplasia; MCN: Mucinous cystic neoplasm; MD: main duct, MT – mixed type; PC: Pseudocyst; SCA: Serous cystadenomas; SPN: Solid pseudopapillary neoplasm.

data is limited, especially in regard to the natural history of BD-IPMN. All the evidence related to the management of pancreatic cysts is graded as very low quality. Furthermore, the guidelines reflect the motivations and are biased by the authors' background.

All guidelines agree on the importance of a detailed history and on the importance of MRCP for initial evaluation. EUS should be performed in the presence of any known WF. Regarding treatment and surveillance, there is consensus on the need for upfront multidisciplinary discussion and the need to include patients in the decision. Furthermore, surgery should be performed only at high-volume centers to ensure the best results with less morbidity.

The decision to observe versus to resect often remains individual. We must consider patient status, namely comorbidities and life expectancy, because recent data has evidenced that most patients will die from causes not related with a cyst.

The strict adherence to a particular guideline is probably not the best option; therefore, we should adapt a strategy aiming for a personalized approach.

Conflicts of interest: None to declare.

Authors' contribution: F.V.B. wrote the original draft of the manuscript. G.M. critically revised the manuscript and approved the final version.

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4.2 ORIGINAL RESEARCH ARTICLES

THROUGH-THE-NEEDLE BIOPSY SAMPLING MAY ALLOW PREOPERATIVE INTRADUCTAL PAPILLARY MUCINOUS NEOPLASIA SUBTYPING

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Through-the-needle biopsy sampling may allow preoperative intraductal papillary mucinous neoplasia subtyping

To the Editor:

We read with great interest the study by Crinò et al[1] regarding biopsy sampling of pancreatic cystic lesions (PCL). The investigators, besides establishing the number of samples needed to reach an adequate diagnosis using EUS-guided through-the-needle microforceps biopsy, make the first description that tries to standardize the diagnostic criteria for cystic lesions by use of through-the-needle biopsy (TTNB) specimens. To evaluate the diagnostic capabilities of TTNB sampling, the authors propose the assessment of 4 histologic criteria: (1) provide cyst-lining epithelium, (2) differentiate mucinous from nonmucinous cysts, (3) define the grade of dysplasia, and (4) provide a specific diagnosis of cyst histotype.

Intraductal papillary mucinous neoplasias (IPMNs) include a spectrum of diseases with both morphologic and immunohistochemical variations.[2] Several studies have found important differences in the potential for invasive progression, risk of recurrence, and overall prognosis between the different IPMN subtypes.[3]

After important multicenter studies had demonstrated its high diagnostic yield and clinical utility,[4-6] we started using TTNB sampling in our center for PCL characterization, especially in the case of suspected branch-duct (BD)-IPMN with worrisome features (Fukuoka consensus).[7] In our first 20 procedures, TTNB samples provided the diagnosis of cyst histotype in 17 cases, including 10 BD-IPMNs. On the basis of histologic features and mucin (MUC) expression (MUC1, MUC2, MUC5AC, and MUC6) in the TTNB samples, we were able to determine the predominant phenotype of IPMN in 8 cases (Figs. 1 and 2).

We suggest that, besides cyst epithelium characterization, cytoarchitectural atypia definition, and specific diagnosis of cyst histotype, IPMN immunophenotype classification should be included in the pathology report of TTNB samples. Also, we think that future studies evaluating the number of macroscopically visible specimens to be collected during EUS-TTNB should account for the importance of being able to determine IPMN (immuno) phenotype.

DISCLOSURE

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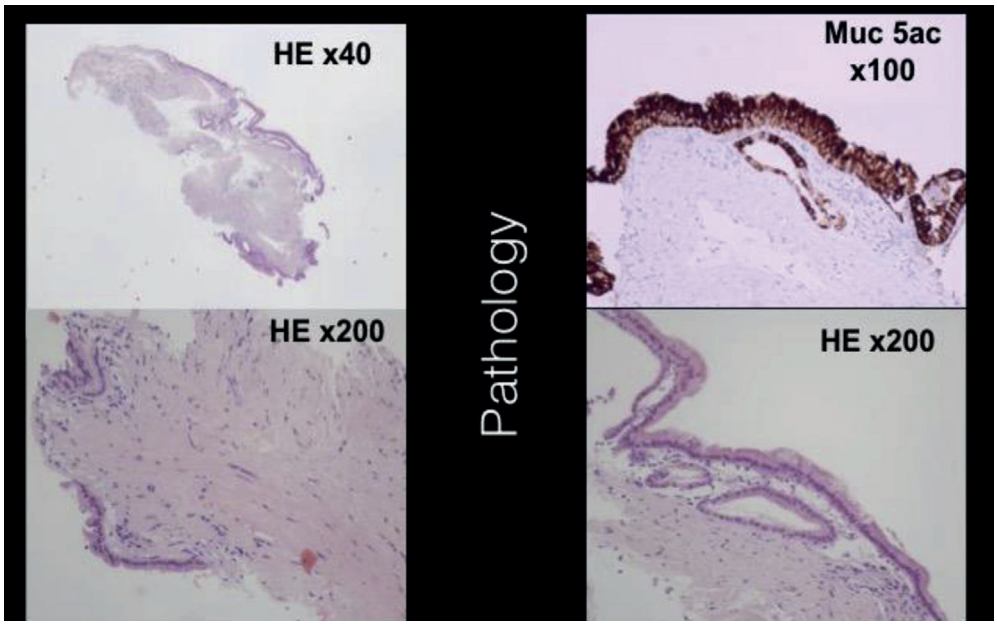

Figure 1. Mucinous epithelium, gastric type, low-grade dysplasia.

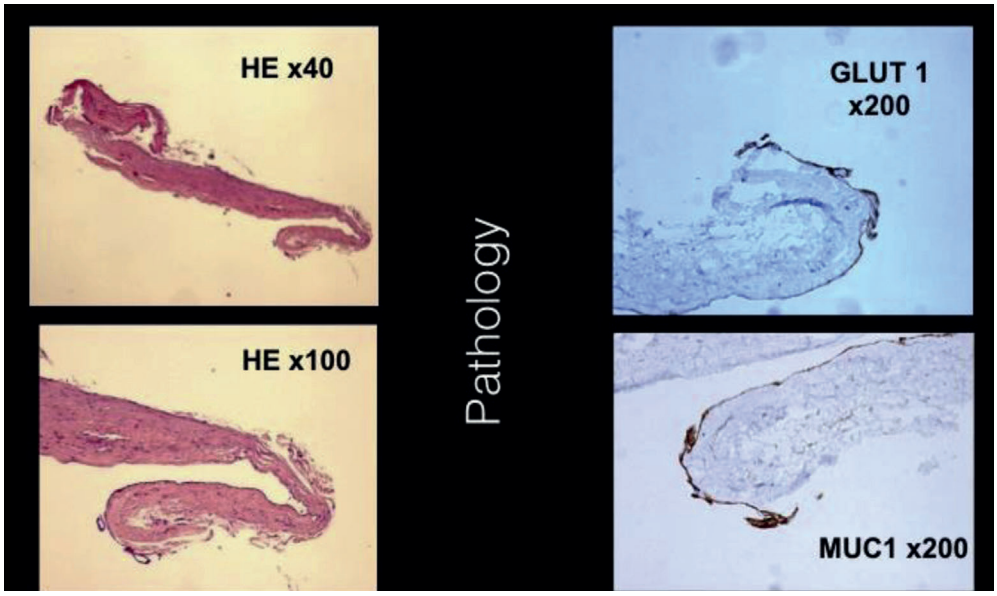

Figure 2. Mucinous epithelium, pancreatobiliary type, low-grade dysplasia.

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Response:

We thank Dr Vilas-Boas et al[1] for their comment on our article.[2] The authors raise an interesting point: the feasibility of intraductal papillary mucinous neoplasm (IPMN) subtyping on specimens collected with EUS-guided through-the-needle biopsy (TTNB).

TTNB is a very promising tool for the diagnosis of pancreatic cystic lesions and has been demonstrated to provide histologic specimens suitable for immunohistochemical staining in 80% to 87% of cases.[3-5] Moreover, the interobserver agreement among pathologists has been demonstrated to be substantial for cyst type definition,[6] further confirming the good quality of TTNB samples. Therefore, the feasibility of IPMN subtyping is an expected result, which was previously reported.[7]

This finding is relevant because intestinal and oncocytic subtypes give rise to colloidal and oncocytic carcinomas that are less aggressive than tubular adenocarcinoma, which frequently develops from gastric or pancreatobiliary subtypes.[8] Nevertheless, the gastric subtype is the most common and has the least likelihood of progression to invasive carcinoma.[8] In a preoperative setting, this information, added to clinical and imaging features, could affect the decision-making process.

However, before this information is implemented in standard practice for IPMN risk stratification, at least 2 points should be investigated. First is the reliability of IPMN subtyping and expression of mucins on TTNB samples compared with surgical pathology. Indeed, the intracystic variability of IPMN subtypes has been

ENDOSCOPIC ULTRASOUND THROUGH-THE-NEEDLE BIOPSY OF PANCREATIC CYSTS: TOWARD PROCEDURE STANDARDIZATION

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Endoscopic Ultrasound Through-The-Needle Biopsy of Pancreatic Cysts: Toward Procedure Standardization

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Keywords
Pancreatic cystic lesions · EUS-guided through-the-needle microforceps biopsy · Worrisome features

Abstract
Background and Aims: EUS-guided through-the-needle microforceps biopsy (EUS-TTNB) was introduced as a new diagnostic tool to establish pancreatic cyst histotype and help to better risk stratify the patients. The aim of this study was to describe the technical success, diagnostic yield, and adverse events of through-the-needle biopsy and discuss the technique variations, focusing on future procedure standardization. ***Methods:*** We performed a prospective single-center study including patients with presumed mucinous cysts harboring worrisome features or indeterminate cyst type on imaging, submitted to EUS-TTNB using Moray® microforceps between March 2018 and September 2021. Specimens were processed as a cell-block. ***Results:*** We included 40 patients. Technical success was 97.5%. The diagnostic yield was 72.5% for TTNB whereas for cyst fluid cytology/analysis it was 27.5%. Moreover, without TTNB 5 mucinous lesions would not have been diagnosed. TTNB had a sensitivity of 76% and a specificity of 91%, while FNA cytology had a sensitivity and specificity of 35% and 91%, respectively. Moreover for IPMN lesions, subtyping was possible in 63% of cases. TTNB resulted in change in clinical management in 20% of patients. We registered three adverse events: 2 self-limited intracystic bleeding and 1 patient with abdominal pain not associated with pancreatitis. ***Conclusion:*** TTNB proved superior to cyst fluid analysis and cytology for the definition of cyst histotype and mucinous cyst diagnosis with acceptable risk profile. Further studies should explore the best steps for procedure standardization.

Introduction

Patients with pancreatic cystic lesions (PCLs) have an increased risk of pancreatic malignancy compared with the general population [1, 2]. In PCLs, malignancy occurs virtually only in those mucinous in nature. Current guidelines recommend that the diagnostic framework for pre-

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sumed mucinous PCLs presenting with worrisome features (WF) should include endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) for cyst fluid analysis combining carcinoembryonic antigen (CEA) levels and cytology [2, 3].

The diagnosis of PCLs is difficult, and a significant discrepancy exists between presumed clinical or imaging diagnosis and the histologic analysis of surgical specimens [4]. Furthermore, cyst fluid cytology has low sensitivity (40%) and CEA levels do not correlate with the presence or risk of malignancy [2, 3, 5], thus leading to inappropriate pancreatic resections. Defining the exact diagnosis and accurately estimating the relevant risk of malignancy could spare patients unnecessary testing, radiation, and surgery [6].

Moray® microforceps (STERIS, Mentor, OH, USA) allows sampling of cyst wall, septa, or mural nodules for histologic analysis of epithelium and stroma [7, 8]. It is designed to fit through a 19-gauge EUS needle. EUS-guided through-the-needle microforceps biopsy (EUS-TTNB) may provide definite information regarding the cyst type and, for mucinous cysts, can define the grade of dysplasia. Histologic features and mucin expression by immunohistochemistry allow a proper establishment of the phenotype in the case of intraductal papillary mucinous neoplasm (IPMN) that can be important for risk stratification [9–11].

Several systematic reviews and meta-analysis have been published to evaluate the application of EUS-TTNB in PCLs. Two recent meta-analyses confirmed the feasibility, high specimen adequacy, and diagnostic yield of EUS-TTNB [12, 13]. However, the procedure is not standardized, the best technique has not been defined, and safety concerns in relation with higher rates of adverse events compared with standard EUS-FNA have been raised [9, 14].

Moreover, EUS-TTNB generated the need for technicians to handle and process very small specimens seldom evaluated before by pathologists. Therefore, specimen handling and processing needs to be standardized [15, 16].

In the current study, we report our experience using EUS-TTNB of PCLs, describing its technical success, diagnostic yield compared with FNA/cyst fluid analysis, change in clinical management, and rate of adverse events. We also report changes to the technique aimed to improve sample collection, handling, and processing. We discuss the possible different variations of the technique, during different steps, focusing on future procedure standardization.

Materials and Methods

Population and Study Design

We have performed a prospective, open-label, single-center study of consecutive patients undergoing EUS-TTNB between March 2018 and September 2021 for presumed mucinous cysts with WF, as defined by International Consensus Guidelines [2], or morphologically indeterminate cyst type over 15 mm with no connection to the pancreatic duct, after magnetic resonance cholangiopancreatography. Patients older than 18 years, fit for surgery, and able to provide written informed consent were included. Pregnancy, low platelet count (<50,000/mm^3), and coagulation disturbances (international normalized ratio >1.5 or hereditary diseases causing deficiencies in coagulation factors such as hemophilia or von Willebrand disease) hampering FNA were exclusion criteria. Anticoagulants and antiplatelet drugs were suspended before the procedure.

All data were prospectively collected and introduced into an electronic platform. Data related to patients' demographics and clinical features including age, gender, clinical presentation, history of pancreatitis, indication for EUS, smoking habits, family history of pancreatic disease, and follow-up visits were collected from individual electronic clinical records. Additionally, data regarding the cysts, namely, date of diagnosis, EUS features (location, size and morphology, wall thickness, and presence of mural nodules/solid components), and pathological findings were extensively recorded. Finally, data regarding definite diagnosis as well as total follow-up time were collected. Study protocol was approved by the Ethics Committee of São João University Hospital/Faculty of Medicine of the University of Porto (CE 33/2018).

EUS Procedures

Examinations were performed by two experienced endosonographers (FVB and PMR), each with more than 10 years of EUS practice and over 1,000 procedures. All procedures were performed using linear echoendoscopes Olympus® GF-UCT180 and Olympus® GF-UCT140 coupled with Olympus® EU-ME2 ultrasound processor. All procedures were conducted under anesthesiologist-directed sedation. Cystic lesions were punctured using a 19-gauge FNA needle (Expect™; Boston Scientific Corp., Marlborough, MA, USA) through the stomach for lesions located in the body or tail or through the duodenum for lesions in the head of the pancreas.

After cyst puncture, the stylet was retracted from the needle and the microforceps was advanced through the lumen of the needle. For lesions smaller than 20 mm in diameter, we preloaded the needle with the forceps before puncture. The forceps was opened inside the cyst and pushed against the wall. For most cases, the cyst wall was targeted. If a thick septum or a mural nodule was detected during the examinations, those structures were preferentially targeted. The assistant was asked to slowly close the forceps and then it was pulled back while watching for the tenting of the tissue (shown in Fig. 1). Only one bite per pass was performed. We aimed to collect a minimum of 4 macroscopically visible specimens from each sampled cyst.

After forceps removal, syringe suction was applied to aspirate cyst fluid. If sufficient volume was collected, the fluid was sent for CEA, amylase, and glucose quantification. Any additional fluid was sent for cytology. Finally, the needle was flushed with saline and the content was sent to the laboratory in phosphate buffered saline solution for cytological examination.

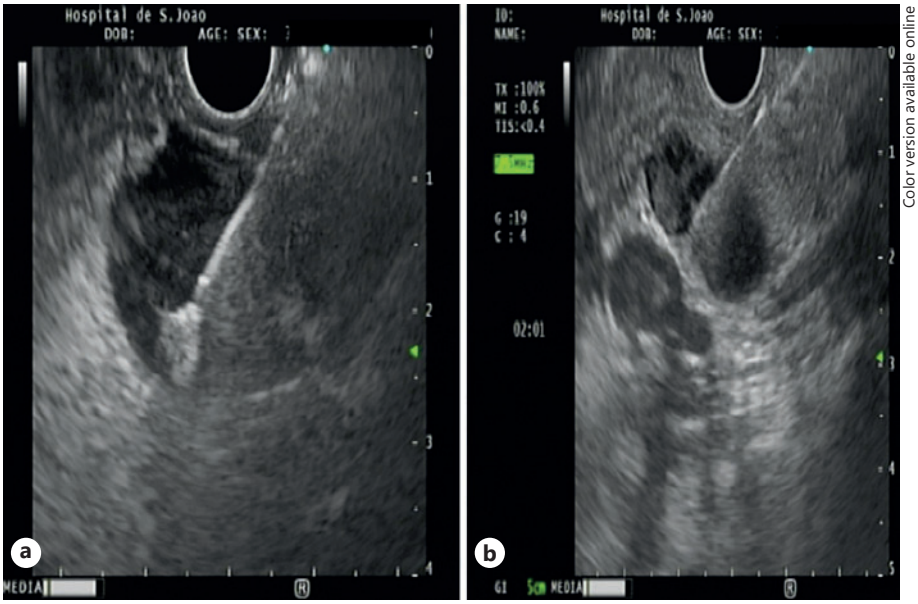

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Fig. 1. EUS-TTNB. Forceps opening inside the cyst (**a**). Tenting after forceps closure and pushing (**b**).

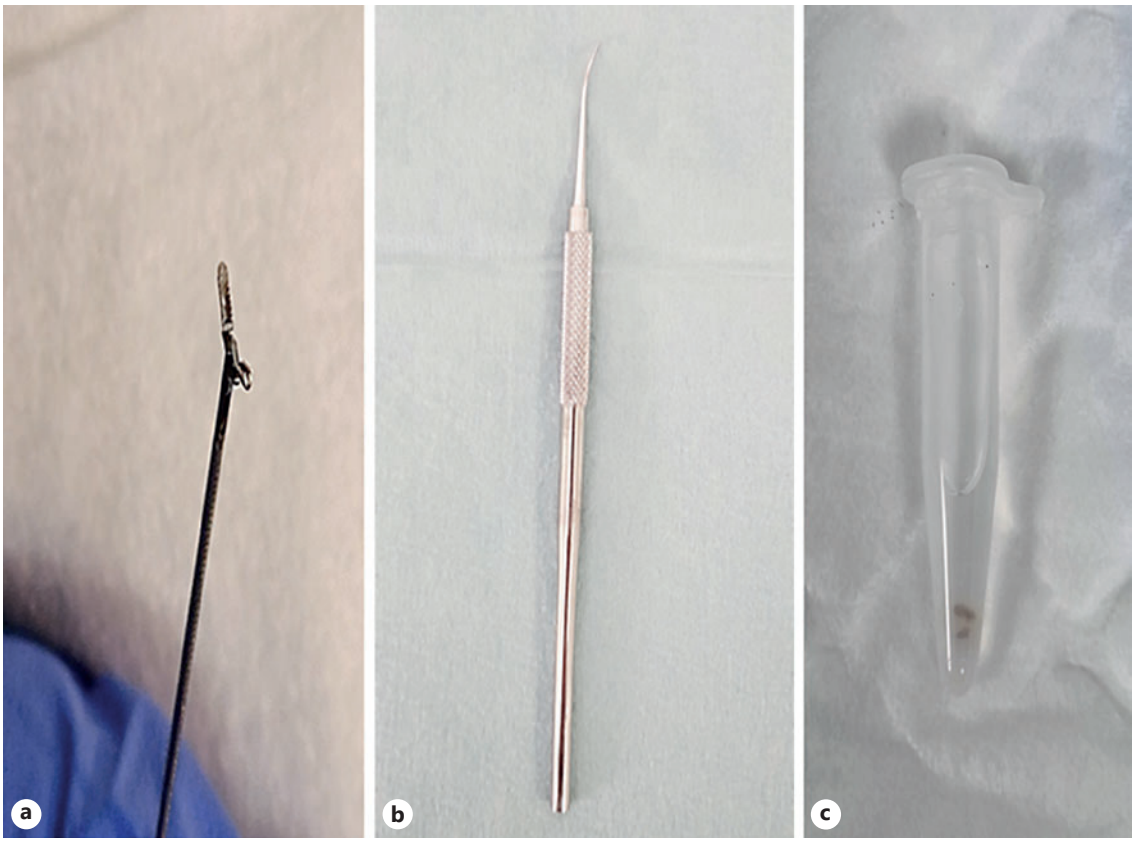

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Fig. 2. Moray® forceps with specimen (**a**), extraction pick (**b**), and Eppendorf container with two samples (**c**).

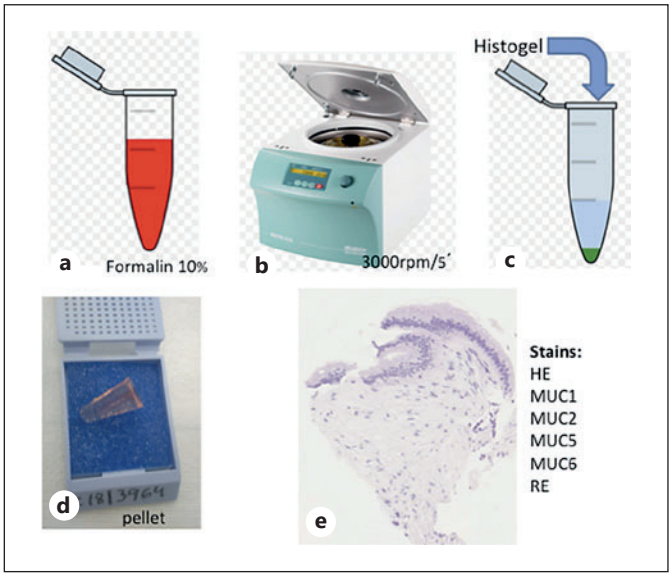

Fig. 3. Cell-block preparation. Step-by-step technique of gel-based cell-block method: **a** The specimen is placed in a 1.5-mL Eppendorf container with formalin. **b** The Eppendorf is centrifugated at 3,000 rpm for 5 min. **c** HistoGel (Thermo Scientific) is added to the tube and refrigerated for 5 min. **d** The obtained cell-block (gel with specimen tissue) is placed in a cassette for paraffin embedding and the paraffin block sectioned in 3-4 micron cuts for HE staining. **e** Additional cuts for immunohistochemical staining with a panel including MUC1, MUC2, MUC5AC, MUC6, CDX2 and estrogen receptors.

A single dose of intravenous ciprofloxacin 200 mg was administered during the procedure as prophylaxis. No continuing oral antibiotics were prescribed. After the procedure, patients were observed in the recovery room for at least 2 h before discharge, to screen for complications.

Specimen Handling

The specimen was removed from the forceps using the included extraction pick and placed in a 1.5-mL Eppendorf container with formalin as a preservative medium (shown in Fig. 2). We placed all the collected specimens together in a single container. At the pathology laboratory, the specimens were processed as one starting with centrifugation (3,000 rpms, rotor radius 17 cm) for 5 min.

The pellet with cells was then removed from the centrifuge and the supernatant was poured off taking care not to disrupt the sediment cells at the bottom. HistoGel (Thermo Scientific) was added to the tube and refrigerated for 5 min to solidify. The obtained cell-block (gel with specimen tissue) was placed in a cassette for paraffin embedding. The block was sectioned in 3-4 micron cuts and the sections were placed in glass slides for staining (shown in Fig. 3). If a morphological diagnosis of mucinous lesion was established, further sections on glass slides were prepared for immunohistochemical staining with a panel including MUC1, MUC2, MUC5AC, MUC6, CDX2, and ultimately estrogen receptors if a stromal component was identified (shown in Fig. 4).

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Table 1. Patient and cyst characteristics

Variable	Total
Age, median (IQR), years	67 (56–73)
Gender, *n* (%)	
Female	25 (62.5)
Male	15 (37.5)
Presentation, *n* (%)	
Incidental	29 (72.5)
Abdominal pain	6 (15.0)
Pancreatitis	4 (10.0)
Weight loss	1 (2.5)
Indication for EUS-FNA, *n* (%)	
Mucinous with WF	36 (90.0)
Indeterminate cyst type	4 (10.0)
Cyst location, *n* (%)	
Head	22 (55.0)
Body	7 (17.5)
Tail	11 (27.5)
Cyst size, median (IQR), mm	30.0 (15–75)
Cyst CEA levels, median (IQR), ng/mL*	63 (7.8–1,271.8)
Cyst glucose levels, median (IQR), mg/dL#	10 (10.0–47.5)
Cyst amylase levels, median (IQR), U/L§	566 (40.0–18,540.0)
Final diagnosis, *n* (%)	
IPMN	26 (65.0)
SCA	6 (15.0)
MCN	3 (7.5)
PC	3 (7.5)
SPN	2 (5.0)
Follow-up, median (IQR), months	12.0 (4.0–18.8)

EUS-FNA, endoscopic ultrasound fine needle aspiration; WF, worrisome features; CEA, carcinoembryonic antigen; IPMN, intraductal papillary mucinous neoplasm; SCA, serous cystadenoma; MCN, mucinous cystic neoplasm; SPN, solid pseudopapillary neoplasm; PC, pseudocyst; IQR, interquartile range. * Data available for 30 lesions. # Data available for 29 lesions. § Data available for 25 lesions.

Study Variables and Definitions

Technical success was defined as the successful puncture of the cyst and acquisition of at least one macroscopically visible specimen. The primary outcome was the diagnostic yield, defined as the proportion of cysts in which a histopathological diagnosis was attained out of the total number of EUS-TTNB procedures.

The final diagnosis of cyst type was based on surgical specimen for patients who underwent resection, on a conclusive TTNB histology result, or on morphological features at imaging plus cyst fluid cytology combined with CEA and glucose fluid levels (global evaluation). Cysts were considered mucinous if cytology revealed extracellular mucin or mucinous epithelial cells or, in their absence, CEA fluid levels >192 ng/mL and glucose levels <50 mg/dL. For the determination of the final diagnosis by global evaluation, the decision was made after multidisciplinary team discussion. Adverse events were defined and graded according to the American Society for Gastrointestinal Endoscopy (ASGE) terminology [17].

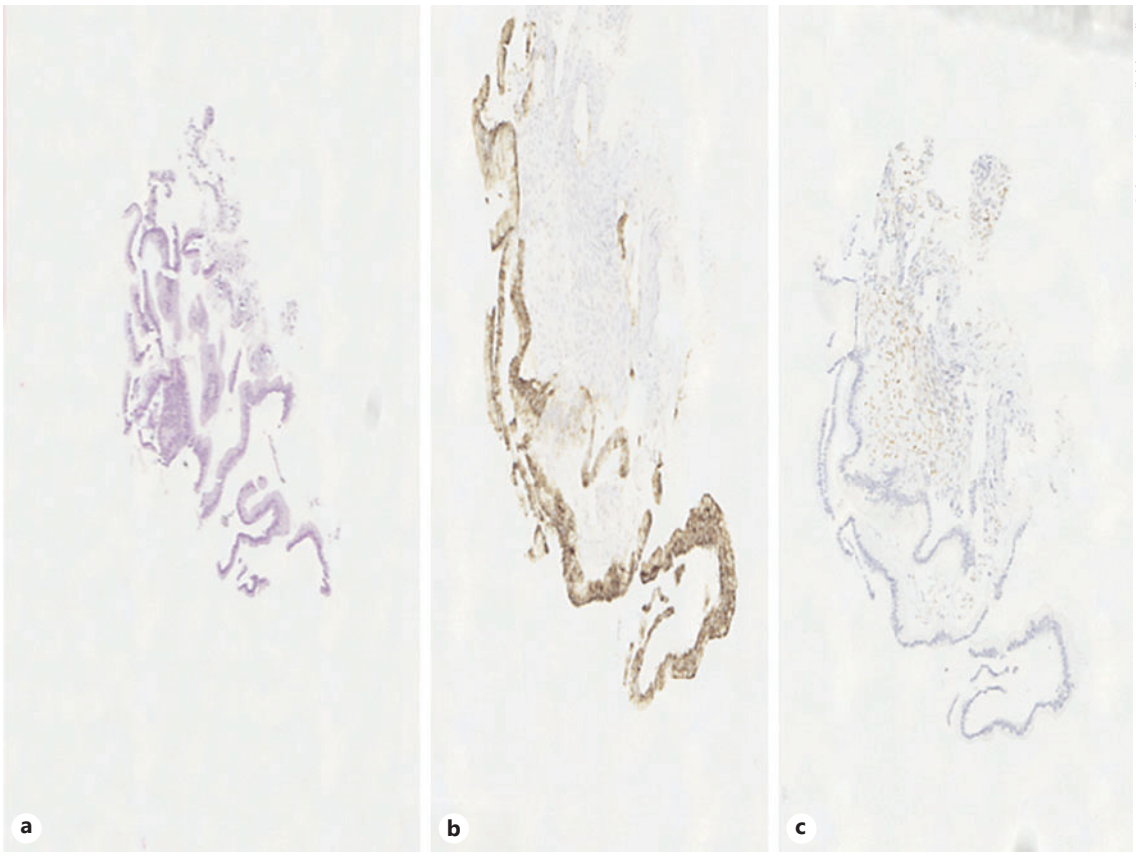

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Fig. 4. a HE, ×10. **b** MUC5, ×10. **c** Estrogen receptors, ×10. MCN lined by mucinous gastric-type epithelium, surrounded by characteristic dense ovarian-type stroma with expression of estrogen receptors.

Statistical Analysis

The diagnostic yield of EUS-TNNB constitutes the primary outcome and is expressed as percentage. Secondary outcome measures include technical success, sample adequacy, as well as the rate of adverse events and are expressed as percentages.

Categorical variables are expressed as frequency and percentages and were compared using the χ^2 test. Continuous variables are expressed as median and interquartile range. The performance metrics for the detection of mucinous lesions using cytology, cytology plus cyst fluid analysis, and TTNB were assessed by calculating the sensitivity, specificity, positive and negative predictive values, and overall accuracy. Statistical analysis was performed using SPSS Statistics version 22 (Armonk, NY, USA).

Results

Population and Cyst Characteristics

We included a total of 40 consecutive patients: 36 with presumed mucinous cysts with WF and 4 indeterminate cysts after imaging. Demographics and clinical features of the patients are shown in Table 1. Final diagnosis of cyst type was obtained by surgical specimens in 16 patients (40%), TTNB histology in 14 patients (35%), and global evaluation (cytology plus fluid CEA and glucose levels) in 10 cases (25%). Ultimately, there were 26 IPMNs, 6 serous cystadenomas, 3 mucinous cystic neoplasms, 2 solid pseudopapillary neoplasms, and 3 pseudocysts.

The median PCLs diameter was 30.0 mm (interquartile range 15–75 mm). Twenty-two cysts (55%) were localized in the head, 7 in the body, and 11 in the tail of the pancreas.

Cyst fluid CEA levels were determined for 30 lesions. In 10 patients, CEA levels were not available; in 7 cases because of insufficient cyst fluid volume; and in the remaining 3 lesions because of thick fluid that prevented the assay performance at the laboratory. In 5 of these 10 cases, the cyst fluid glucose levels were determined.

Technical Success and Diagnostic Performance of TTNB

Technical Success

Data regarding the technical and diagnostic performance of EUS-TTNB are summarized in Table 2. EUS-TTNB was

Table 2. Technical and diagnostic performance

Variable	Total
Technical success, *n* (%)	39 (97.5)
Complications, *n* (%)	3 (7.5)
Definite histologic characterization, *n* (%)	29 (72.5)
Type of lesion, *n* (%)	
Mucinous	23 (57.5)
Nonmucinous	6 (15.0)
Nonconclusive	11 (27.5)
IPMN subtype, *n* (%)	
Gastric	2 (10.5)
Pancreatobiliary	10 (52.6)
Nontypable	7 (36.8)
Method for final diagnosis, *n* (%)	
TTNB	14 (35.0)
FNA cytology	10 (25.0)
Surgical specimen	16 (40.0)
Sensitivity/specificity/accuracy for mucinous lesions (%)	
Cytology	34.5/90.9/50.0
Cytology + cyst fluid analysis	58.6/90.9/67.5
TTNB	75.9/90.9/80.9
Surgery, *n* (%)	
Yes	16 (40.0)
TTNB and surgical specimen agreement, *n* (%)	15 (93.8)

IPMN, intraductal papillary mucinous neoplasm; TTNB, through-the-needle microforceps biopsy.

Table 3. Individual data on the 16 patients who underwent surgery

	TTNB	Surgical specimen
Diagnosis		
BD-IPMN	12	12
MCN	2	2
PC	1	1
SPN	0	1
Inconclusive	1	0
Grade of dysplasia (data for 14 mucinous lesions)		
LGD	12	13
HGD	2	0
Malignant	0	1
IPMN subtype (data for 6 patients)		
Gastric	2	1
Pancreatobiliary	4	3
Intestinal	0	1
Mixed type	0	1

TTNB, through-the-needle biopsy; LGD, low-grade dysplasia; HGD, high-grade dysplasia; SPN, solid pseudopapillary neoplasia; PC, pseudocyst; MCN, mucinous cystic neoplasm.

successfully performed in 39 out of 40 cases (technical success 97.5%). The biopsies were not accomplished in 1 case because we were not able to pass the forceps through the needle, due to its angulation, during a transduodenal puncture. This case refers to a 29-mm multilocular cyst in the uncinate process. Because of the cyst size, as defined by the protocol, the forceps was not preloaded in the needle. FNA was performed via transgastric route in 23 (57.5%) cases and by transduodenal approach in 17 patients.

Diagnostic Yield

Concerning the primary outcome, TTNB samples were adequate for definition of cyst histotype in 29 cases out of 40 procedures (diagnostic yield 72.5%), whereas FNA cytology provided adequate samples in 11 patients (diagnostic yield of 27.5%). The sensitivity, specificity, and overall accuracy of TTNB for the diagnosis of mucinous lesions were 75.9%, 90.9%, and 80.9%, respectively. Oppositely, FNA cytology revealed lower performance, with 34.5% sensitivity, 90.9% specificity, and 50.0% accuracy (Table 2). The diagnosis of the specific cyst type by TTNB was possible in 29 patients; on the other hand, FNA cytology results allowed definite diagnosis in 5 patients.

Diagnostic Performance for Mucinous Lesions and Specific Cyst Type

TTNB diagnosed 23 mucinous cysts versus 11 diagnosed with fluid cytology and 13 using CEA fluid levels >192 ng/mL. Combining cytology results and CEA fluid levels, 18 mucinous cysts were diagnosed. Therefore, twelve mucinous cysts would not have been diagnosed if only cytology was available and, even when combined with fluid CEA levels, 5 mucinous cystic neoplasms would have been missed. However, in 3 cases of inconclusive TTNB result, a mucinous cyst was diagnosed based on cytology results. Moreover, while TTNB allowed the diagnosis of specific cyst type in 29 patients, based on FNA cytology results this was possible in only 5 patients.

For mucinous lesions, TTNB allowed the definition of dysplasia grade in all cysts. For IPMN, immunohistochemistry-based subtyping was possible for 12 (63%) out of a total of 19 lesions. There were 10 pancreatobiliary (shown in Fig. 5) and 2 gastric subtype IPMNs. The subtyping was not possible in 7 cases due to sample scarcity that precluded immunohistochemistry study.

Agreement between TTNB and Surgical Specimens

All but one TTNB diagnosis for specific cyst type were concordant with surgical specimen for the patients who had the cyst resected (15/16), whereas dysplasia grade was non-

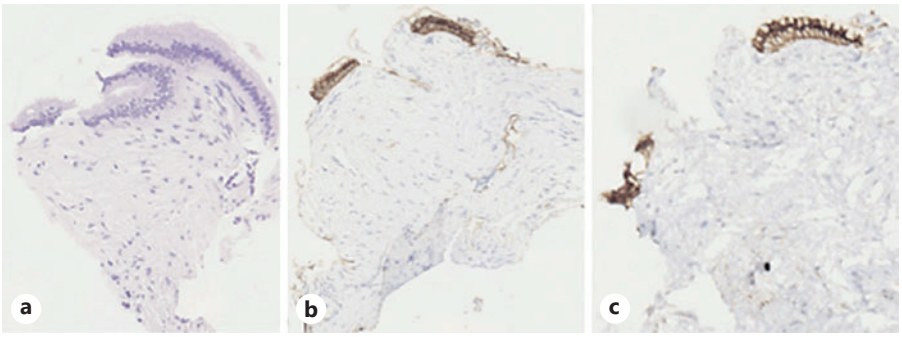

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Fig. 5. Pancreatobiliary-type IPMN with low-grade dysplasia. **a** Mucinous epithelium with low-grade dysplasia. **b** MUC1 staining. **c** MUC5 expression.

concordant in 3 cases; 1 patient had the diagnosis of low-grade dysplasia BD-IPMN in TTNB and was ultimately diagnosed with malignant BD-IPMN in the surgical specimen. In turn, 2 cases categorized with high-grade dysplasia BD-IPMN in the TTNB sample revealed to have only low-grade dysplasia BD-IPMN in the surgical specimen (Table 3).

The specific case of malignant BD-IPMN concerns a 33-mm cyst in the pancreatic body with no mural nodule or solid component that increased in size on serial assessments. EUS evaluation showed thickened cyst walls and confirmed the absence of mural nodules. TTNB histology and immunohistochemistry revealed pancreatobiliary IPMN subtype confirmed by the surgical specimen.

For TTNB IPMN subtyping, the concordance with surgical specimen subtype was 67% (4 in 6 patients). In the 2 nonconcordant cases, 1 patient with gastric subtype in TTNB was found to have a mixed (pancreatobiliary and gastric) type, and the other case classified as pancreatobiliary type in TTNB was ultimately defined as intestinal-type SB-IPMN in the surgical specimen (Table 3).

Impact on Clinical Management

Concerning the clinical impact of TTNB in our cohort, we registered a change in clinical management in 8 patients (20%). If TTNB had not been performed, 1 patient would not have been submitted to surgery (BD-IPMN misdiagnosed with malignant transformation); however, the performance of TTNB allowed the identification of 4 patients who would have been inadequately proposed for surgery. These patients were ultimately diagnosed with BD-IPMN with low-grade dysplasia ($n = 2$) and SCA ($n = 2$). Finally, 3 patients were successfully discharged from ongoing surveillance: two with SCA and one with pseudocyst.

Adverse Events

We registered 3 adverse events (7.5%) that concern 2 cases of intracystic bleeding (1 detected during the procedure and 1 delayed bleeding) and 1 case of post-procedure abdominal pain with no amylase or lipase elevation or evidence of pancreatitis on imaging studies. All adverse events were considered mild and resolved without any specific intervention. The patient with bleeding identified during the procedure was admitted to the hospital for surveillance and discharged after 24 h (overnight admission).

The patient who had delayed bleeding developed abdominal pain 3 days after the procedure and was observed in the emergency room. Abdominal ultrasound showed hypodense content in dependent position within the cystic lesion in the pancreatic body in relation with hemorrhage. Laboratory workup revealed normal complete blood count. She was observed in the emergency room for 2 h and discharged under analgesia with paracetamol.

Discussion

Accurate risk stratification is the most challenging step in the management of PCLs [18]. A precise determination of cyst type is key for estimating the risk of malignancy. Moreover, in the case of mucinous lesions the definition of histological grade is paramount for decision-making.

In our experience, TTNB of PCLs using microforceps was shown to have a high rate of technical success (97.5%) and a fair diagnostic yield (72.5%), with a low frequency of adverse events (7.5%). In the single case of technical failure, concerning an uncinate process cyst, the endoscope/needle set position did not allow the forceps passage. The use of new nitinol-based needles with enhanced flexibility may be a solution to overcome this difficulty, but this should be a subject for future studies [19, 20]. The results from our series are similar to the experience of other groups [7, 9, 21, 22], with several recent meta-analyses confirming the favorable diagnostic accuracy of TTNB and its superiority over FNA cytology [12, 13, 18].

Nevertheless, TTNB procedure is not yet standardized and several variations to the technique have been described. For example, some authors describe the microforceps can be preloaded in the needle [23], while others remove the stylet after puncturing the lesion and insert the forceps after that [24].

Our experience shows that preloading the needle with the microforceps is crucial in the case of lesions smaller than 20 mm. This step prevents the lesion collapse following the suction of cyst fluid into the needle lumen when the stylet is removed because approximately 1.1 mL of fluid is held inside a 19 G FNA needle and the volume of a 20-mm cyst is around 1 mL ($4/3 \times \pi \times r^3$ where r is the cyst radius) [25]. For the safe performance of the intracystic biopsies, enough space should be ensured to allow manipulation of the forceps within the cyst under EUS control. If the cyst is empty, some authors describe the injection of saline through the needle to expand it. However, this additional step may increase the risk of infection [26].

Another step for which different ways of proceeding have been described concerns the timing for cyst fluid aspiration. There are reports describing that the first step after puncturing the cyst is to aspirate a fluid sample for cyst fluid analysis while other authors describe the collection of the fluid sample at the end of the procedure, after performing microforceps biopsies, while draining the whole cyst [7, 9, 23, 24]. We performed cyst fluid aspiration after the biopsies. One important issue is to determine if the performance of biopsies prior to fluid aspiration will improve the cellularity of the samples. In theory, manipulation of the forceps in the cyst wall could result in cell flushing to the fluid, improving cellularity.

The number of passes and bites for each pass is also a matter of debate. Some authors propose 2–3 bites per pass while others perform only one bite [7, 24]. In this study, we performed a single bite per pass as we believe that the absence of a central spike in Moray® microforceps precludes the fixation of the first fragment, hindering the acquisition of two specimens per pass.

In our first protocol, we planned to collect 4 macroscopically visible specimens to improve sample adequacy. This number was suggested from studies of single-operator cholangioscopy-guided biopsies that use a similar microforceps [27].

More recently, Crino et al. [7] suggested that two macroscopically visible specimens could achieve 100% histological adequacy. Their results led us to change our protocol and aim to collect only 2 samples from each cyst, for patients included since June 2019. This procedural change contributed to an important reduction in examination time (because of fewer forceps passes) and theoretically could impact the rate of adverse events. When we compare our results concerning sample adequacy for histological analysis before and after protocol change, we did not observe a statistically significant difference (90.9% vs. 65.5%, $p = 0.233$). However, the small sample size should prevent the establishment of conclusions about this topic. Further studies are needed, especially concerning the need for more than 2 specimens in the case of IPMN subtyping.

Concerning specimen handling, most authors describe the processing of TTNB samples as a routine histology specimen (paraffin embedding, section, and staining) [7, 9, 16, 21]. However, in a multicenter study published in 2018, Barresi and coworkers [28] admit the preparation of a cell-block if the specimens were not clearly visible. In fact, Crino et al. [15] acknowledges that TTNB samples pose a challenge for pathologists as well and maximal care must be taken in their handling because such small specimens were seldom evaluated before by pathologists. As a complex diagnostic procedure, the goal should be to make the most of the collected samples [29].

After discussion with our pathologists and technicians, we assumed the option to process TTNB specimens as cytology samples and generate a cell-block. In this way, we guaranteed that no tissue was wasted and since its structure was intact, no information was lost. In addition, we were also able to perform immunohistochemistry studies.

TTNB specimens allow for the determination of the grade of dysplasia of mucinous lesions that can impact decision-making. However, our results (3 nonconcordant cases) add discussion to the known problem of the interobserver agreement of grade of dysplasia and invasion in IPMN already evaluated in previous studies [30]. We must also consider that the degree of epithelial atypia may vary within the cyst [31].

Several papers have reported important differences in the potential for invasive progression, recurrence risk, and overall prognosis between different IPMN subtypes. Determining which IPMN variant carries the higher risk for the development of invasive cancer is important for clinical management [9, 10, 23, 31]. In fact in a multicenter surgical series, Furukawa et al. [31] showed that IPMN morphological type was an independent predictor of patient prognosis, even in a subcohort of invasive cases. Gastric-type IPMN was associated with lower grade, absence of invasion, and fair survival. In contrast, pancreatobiliary type had higher histological grades and poor prognosis, but for the subset of noninvasive pancreatobi-

liary cases the survival is much better, which reveals the importance of preoperative diagnosis of this subtype [31]. This fact may ensure a personalized treatment or follow-up strategy for the individual patient [29], ultimately avoiding unnecessary surgeries. The feasibility of IPMN subclassification in TTNB samples was previously reported [9, 10, 23]. The results from our cohort confirm that evidence; however, the scarcity of specimen limited the performance of immunohistochemistry in some cases. Future studies should assess whether the acquisition of more than the two recommended samples can help increase the rate of IPMN subtyping. Furthermore, one issue with IPMN subtyping reliability in TTNB samples concerns the recognition of possible coexistence of different epithelial subtypes in the same lesion, which may lead to sampling errors [32].

Regarding adverse events, our results are in line with the rates reported in one recent meta-analysis [13]. We had no case of post-procedure pancreatitis nor infectious adverse event.

The use of antibiotic prophylaxis with a single IV shot is current practice despite the lack of conclusive evidence [3]. Two recently published studies add more solid evidence about the lack of additional benefit from this prophylaxis after EUS-FNA of cystic lesions, even when TTNB is performed [33, 34].

In a prospective study, Kovacevic et al. [9] reported a risk of adverse events of 10% and describe a protocol amendment adding measures to reduce the risk for acute pancreatitis (rectal NSAIDs and periprocedural IV fluids). Nevertheless, a case of death due to post-TTNB pancreatitis occurred despite these prophylactic measures [35]. We did not use any pancreatitis prophylaxis measures, but this issue should be explored in future studies. Moreover, the relation between adverse events occurrence and cyst type and the importance of main pancreatic duct communication should also be assessed. Our adverse events were registered in 3 patients diagnosed with IPMNs. Another unexplored issue is the possible association between adverse events and intracystic needle time.

An accurate selection of patients for TTNB is of extreme importance because of the risk of complications associated with the procedure [14]. One of the strengths of our study is that we have only included presumed mucinous cysts with WF or morphologically indeterminate cysts with no connection to the pancreatic duct.

Our study has some limitations. First, the small number of included patients makes it unreasonable to draw definite conclusions regarding the precise factors associated with the risk for adverse events. Moreover, the performance of a diagnostic test is best evaluated against a reference standard. The existence of surgical specimen as a gold standard for final diagnosis in a minority of cases may have resulted in biased accuracy estimates.

In conclusion, we showed that, when compared to the current standard of care (fluid analysis and cytology), TTNB proved to be superior in the establishment of a definitive diagnosis. TTNB is a better tool for detection of mucinous lesions and in the determination of degree of dysplasia, with an acceptable risk profile. In the case of IPMNs, it allowed subtyping in most cases, which may also prove useful for risk stratification and decision-making, toward a precision medicine-based approach.

Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee on human research of São João University Hospital/Faculty of Medicine of the University of Porto, approval number CE 33/2018. Written informed consent was obtained from all participants before inclusion in the protocol.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Filipe Vilas-Boas: procedure performance, data collection, and drafting the manuscript. Tiago Ribeiro and Pedro Costa-Moreira: data collection and revision of the manuscript. Helena Barroca, Joanne Lopes, and Diana Martins: pathological procedures performance and revision of the manuscript. Pedro Moutinho-Ribeiro: procedure performance and revision of the manuscript. Guilherme Macedo: revision of the manuscript. All authors approved final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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DEEP LEARNING FOR AUTOMATIC DIFFERENTIATION OF MUCINOUS VERSUS NON-MUCINOUS PANCREATIC CYSTIC LESIONS: A PILOT STUDY

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Deep Learning for Automatic Differentiation of Mucinous versus Non-Mucinous Pancreatic Cystic Lesions: A Pilot Study

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Abstract: Endoscopic ultrasound (EUS) morphology can aid in the discrimination between mucinous and non-mucinous pancreatic cystic lesions (PCLs) but has several limitations that can be overcome by artificial intelligence. We developed a convolutional neural network (CNN) algorithm for the automatic diagnosis of mucinous PCLs. Images retrieved from videos of EUS examinations for PCL characterization were used for the development, training, and validation of a CNN for mucinous cyst diagnosis. The performance of the CNN was measured calculating the area under the receiving operator characteristic curve (AUC), sensitivity, specificity, and positive and negative predictive values. A total of 5505 images from 28 pancreatic cysts were used (3725 from mucinous lesions and 1780 from non-mucinous cysts). The model had an overall accuracy of 98.5%, sensitivity of 98.3%, specificity of 98.9% and AUC of 1. The image processing speed of the CNN was 7.2 ms per frame. We developed a deep learning algorithm that differentiated mucinous and non-mucinous cysts with high accuracy. The present CNN may constitute an important tool to help risk stratify PCLs.

Keywords: pancreatic cystic lesions; mucinous cystic neoplasm; intraductal papillary mucinous neoplasm; endoscopic ultrasound; artificial intelligence

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1. Introduction

Pancreatic cystic lesions (PCLs) are very common. A recent systematic review including 17 studies found a pooled prevalence of 8% [1]. PCLs include a wide range of entities, namely congenital, inflammatory, and neoplastic lesions. Patients with PCLs have an increased risk of pancreatic malignancy compared with the general population [2]. However, malignancy occurs virtually only in those with PCLs of mucinous phenotype. Intraductal papillary mucinous neoplasm (IPMN) is the most common pancreatic cystic neoplasia, accounting for nearly half of pancreatic resections due to cystic lesions at a reference academic hospital in the USA [3].

The diagnosis of PCLs based on endoscopic ultrasound (EUS) has important limitations [4]. In fact, the range of accuracy in differentiating mucinous from non-mucinous lesions is 48–94% with a sensitivity of 36–91% and a specificity of 45–81% [4]. However, one of the main limitations of EUS is its low interobserver agreement for the diagnosis of neoplastic versus non-neoplastic lesions and the determination of the specific type of PCL. These concerns remain valid for a wide spectrum of endoscopists, with different degrees of expertise in EUS (experts, semi-experts, or novices) [5,6].

The application of artificial intelligence (AI) algorithms for the interpretation of medical imaging has been the focus of intense research across several areas [7,8]. The implementation of these automated systems for the automatic analysis of endoscopic images has provided promising results [9]. The ever-increasing computational power allows the analysis of large image datasets through deep learning algorithms. Convolutional neural networks (CNNs) are a type of multi-layer deep learning algorithm resembling the visual cortex, which is tailored for automatic image analysis [10].

To date, only a small number of studies reported the use of deep learning systems for the automatic interpretation of EUS images [11]. To optimize the diagnosis based on EUS morphology and mitigate the low interobserver agreement, we aimed to develop a CNN algorithm for the automatic diagnosis of mucinous PCLs using EUS images.

2. Materials and Methods

2.1. Patient Population and Study Design

We conducted a retrospective study using a prospectively maintained hospital database of patients submitted to EUS for PCL characterization. All patients whose EUS exam was recorded as a video file were included. All videos were recorded using the same EUS device. Images retrieved from these examinations were used for the development, training, and validation of a CNN-based model for the automatic identification of mucinous PCLs.

This study was approved by the ethics committee of São João University Hospital/Faculty of Medicine of the University of Porto (CE 41/2021) and was conducted respecting the Declaration of Helsinki. This study is of a non-interventional nature.

2.2. Data Collection

We retrieved the videos from 28 patients for high-quality EUS image analysis. These images comprised still frames acquired during the EUS procedure as well as images obtained through the decomposition of recorded videos into frames. The fragmentation of videos into still images was performed using the VLC media player (VideoLAN, Paris, France). The complete set of images was evaluated by an expert in EUS (FVB) with an experience of more than 1000 EUS exams. All non-relevant frames were excluded. A total of 5505 images were ultimately extracted. From this pool, 3725 depicted mucinous PCLs and 1780 showed non-mucinous PCLs.

Clinical and demographic data were obtained from the electronic clinical record of each patient. Any information deemed to potentially identify the subjects was omitted. Each patient was assigned a random number in order to guarantee effective data anonymization. A team with Data Protection Officer (DPO) certification confirmed the non-traceability of data and conformity with the general data protection regulation (GDPR).

2.3. Endoscopic Ultrasound Procedures and Definitions

All EUS procedures were performed under anesthesiologist-directed sedation using linear echoendoscopes (Olympus® GF-UCT180 and Olympus® GF-UC140) coupled with an Olympus® EU-ME2 ultrasound processor under anesthesiologist-directed sedation. Cyst type was determined based on surgical specimen, intracystic biopsy forceps samples (Moray® micro forceps, STERIS) or cyst fluid cytology combined with carcinoembryonic antigen (CEA) and glucose fluid levels. PCLs were considered mucinous if cytology revealed mucinous epithelial cells or, in their absence, CEA fluid levels >192 ng/mL and glucose levels <50 mg/dL. Patients with cystic neuroendocrine tumors and solid pseudopapillary neoplasms were excluded.

2.4. Development of the Convolutional Neural Network

A deep learning CNN was developed for the automatic identification and differentiation of mucinous and non-mucinous PCLs. In the former group, we included IPMNs and mucinous cystic neoplasms (MCN), while the latter included neoplastic (serous cystadenoma) and non-neoplastic (pseudocyst) lesions. From the collected pool of images

(*n* = 5505), 3725 depicted mucinous and 1780 showed non-mucinous lesions. This pool of images was divided for the constitution of training and validation datasets. The training dataset was composed of 80% of the extracted images (*n* = 4404). The remaining 20% was used as the validation dataset (*n* = 1101). The performance of the CNN was assessed using the validation dataset. A flowchart summarizing the study design is presented in Figure 1.

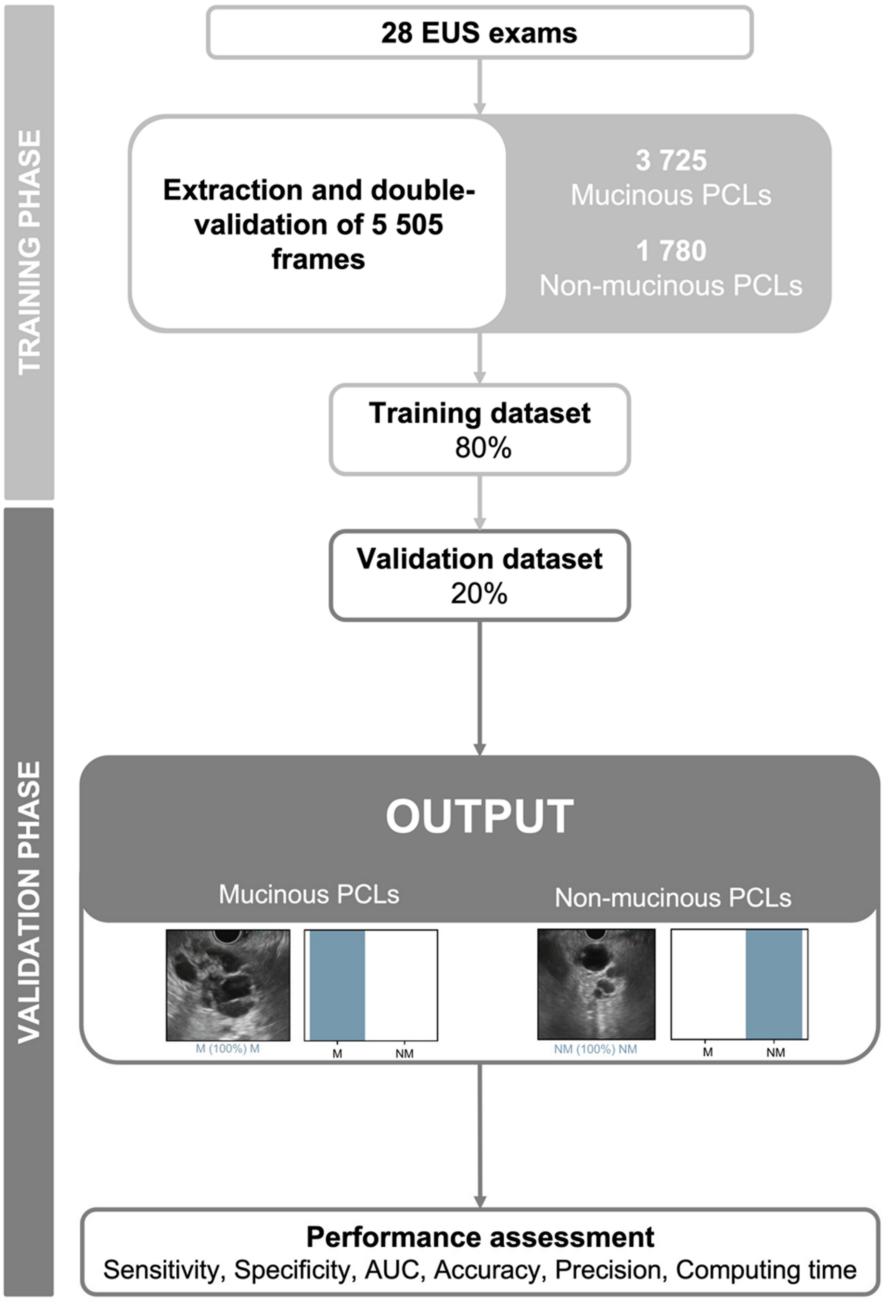

Figure 1. Study design for the construction of the convolutional neural network and subsequent evaluation of its performance. EUS—endoscopic ultrasound; PCLs—pancreatic cystic lesions; M—mucinous pancreatic cystic lesion; NM—non-mucinous pancreatic cystic lesion; AUC—area under the receiving operator curve.

The CNN was created using the *Xception* model with its weights trained on *ImageNet* (a large-scale image dataset aimed for use in development of object recognition software). To transfer this learning to our data, we kept the convolutional layers of the model. We removed the last fully connected layers and attached fully connected layers based on the

number of classes we used to classify our endoscopic images. We used two blocks, each having a fully connected layer followed by a dropout layer of 0.25 drop rate. Following these two blocks, we add a dense layer with a size defined as the number of categories to classify (three: normal pancreatic parenchyma, mucinous PCLs and non-mucinous PCLs). The learning rate of 0.00015, batch size of 32, and the number of epochs of 30 was set by trial and error. We used *Tensorflow* 2.3 and *Keras* libraries to prepare the data and run the model. The analyses were performed with a computer equipped with a 2.1 GHz Intel® Xeon® Gold 6130 processor (Intel, Santa Clara, CA, USA) and a double NVIDIA Quadro® RTX™ 4000 graphic processing unit (NVIDIA Corporate, Santa Clara, CA, USA).

2.5. Model Performance and Statistical Analysis

The primary outcome measures included sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), and the accuracy in differentiating mucinous and non-mucinous lesions. Moreover, we used receiver operating characteristic (ROC) curves analysis and area under the ROC curves (AUC) to measure the performance of our model in the distinction between categories. The classification provided by the CNN was compared to the definitive diagnosis (mucinous or non-mucinous cyst), the latter being considered the gold standard. For each image, the trained CNN calculated the probability for each category. A higher probability translated in a greater confidence in the CNN prediction. The category with the highest probability score was outputted as the CNN's predicted classification (Figure 2). Additionally, the image processing performance of the network was determined by calculating the time required for the CNN to provide output for all images in the validation image dataset. Clinical and demographic data are presented as median (interquartile range) or frequency (percent). Continuous data were compared using the Mann–Whitney U test. Differences in the distribution of categorical variables were assessed using the chi-square test. Statistical analysis was performed using Sci-Kit learn v0.22.2 [12].

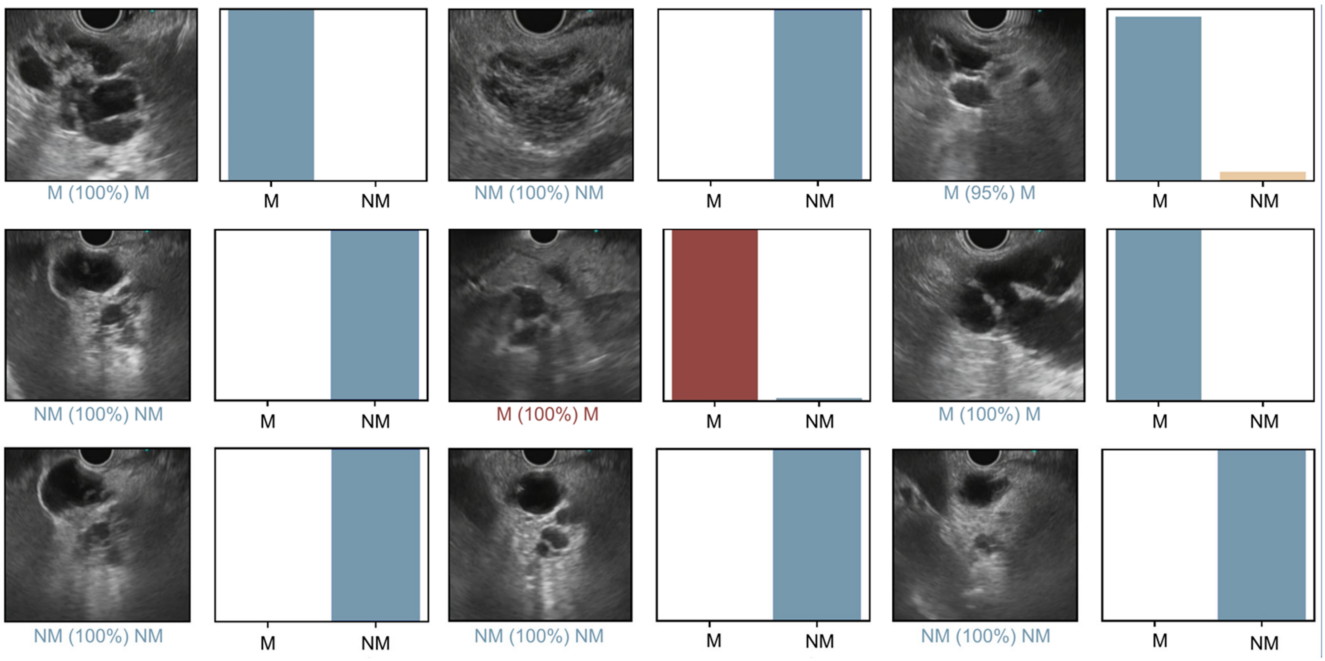

Figure 2. Output provided during the validation phase of the convolutional neural network. The bars represent the probability estimated by the algorithm. The finding with the highest probability was outputted as the predicted classification. A blue bar represents a correct prediction. Red bars represent an incorrect prediction. M—mucinous pancreatic cystic lesion; NM—non-mucinous pancreatic cystic lesion.

3. Results

3.1. Clinical and Demographic Data

A total of 28 videos from patients submitted to EUS for pancreatic cystic lesion characterization between November 2017 and August 2021 were used for image retrieval. From these patients, 16 were female (57%) and had a median age of 65 years (IQR 53–70). A total of 17 (61%) individuals had a final diagnosis of mucinous cysts, while 11 (39%) were ultimately diagnosed with a non-mucinous lesion. Surgical specimens were reported for eight lesions. Histology using intracystic biopsy forceps samples (Moray® micro forceps, STERIS) was available for five patients. The remaining cysts (n = 15) were considered mucinous based on fluid cyst analysis (cytology plus CEA and glucose levels). Concerning cyst histotype, we included 16 IPMNs, 1 mucinous cystic neoplasm (MCN), 8 SCA (five of which were of the macrocystic variant), and 3 pseudocysts (PC). The median follow-up time was 18 months (3–29). The characteristics of the patients and lesions including demographic data and lesion size and location are summarized in Table 1. Most lesions (86%) were incidentally found, 30% were located in the head and neck of the pancreas and the median size was 34.5 mm (19.3–44.8 mm). In this cohort, 14 patients underwent EUS for presumed mucinous lesions with worrisome features as per international consensus guidelines and 14 because of indeterminate cyst type after clinical and imaging integration (unilocular/oligocystic lesion without clear communication with the main pancreatic duct). Mucinous cysts were smaller in size compared to non-mucinous lesions, respectively, 26.0 mm (IQR 17.5–44.5) vs. 37.0 mm (IQR 26.0–46.0), although this difference was not statistically significant (p = 0.29). The location of the lesions had a similar distribution for mucinous and non-mucinous lesions (p = 0.90) and were more frequently found in the head and neck of the pancreas (47% and 55%, respectively). No adverse events were reported for the EUS procedures, nor for EUS-FNA (including through-the-needle biopsies).

Table 1. Baseline clinical and demographic data.

	Mucinous PCLs (n = 17)	**Non-Mucinous PCLs (n = 11)**	***p* Value**
Sex			0.57
Female, *n* (%)	10 (58.8)	6 (54.5)	
Age			0.64
Years, median (IQR)	64.0 (53.0–69.5)	65.0 (53.0–72.0)	
Presentation			0.22
Incidental, *n* (%)	13 (76.5)	11 (100.0)	
Abdominal pain, *n* (%)	2 (11.8)	-	
Pancreatitis, *n* (%)	2 (11.8)	-	
Indication for EUS			<0.01
Worrisome features, *n* (%)	13 (76.5)	1 (9.1)	
Indeterminate cyst type, *n* (%)	4 (23.5)	10 (90.9)	
Cyst location on EUS			0.90
Pancreatic head, *n* (%)	8 (47.1)	6 (54.5)	
Pancreatic body, *n* (%)	6 (35.3)	3 (27.3)	
Pancreatic tail, *n* (%)	3 (17.6)	2 (18.2)	
Cyst morphology			0.63
Unilocular, *n* (%)	6 (35.3)	4 (36.4)	
Multilocular, *n* (%)	11 (64.7)	7 (63.6)	
Cyst diameter			
mm, median (IQR)	26.0 (17.5–44.5)	37.0 (26.0–46.0)	0.29

Abbreviations: EUS—endoscopic ultrasound; PCLs—pancreatic cystic lesions; IQR—interquartile range.

Overall, a total of 5505 frames were extracted for the construction of the CNN: 3725 of mucinous cysts (IPMNs and MCN) and 1780 of non-mucinous lesions (SCA and PC). The accuracy of the algorithm increased as data were repeatedly input into the multi-layer architecture of the CNN (Figure 3).

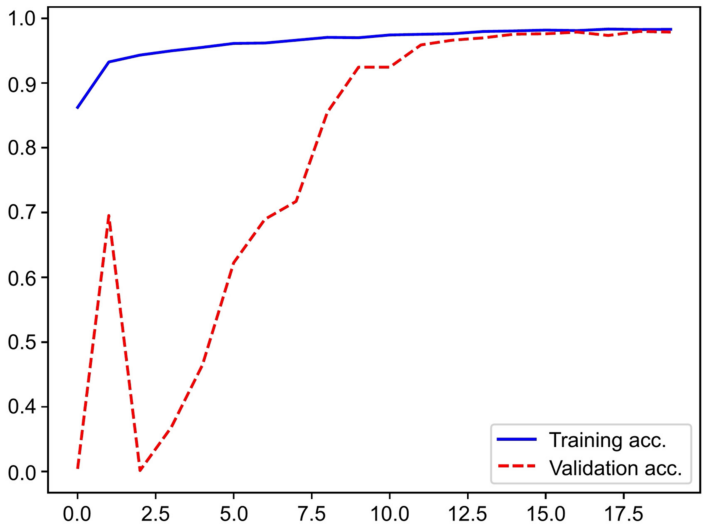

Figure 3. Evolution of the accuracy of the convolutional neural network during training and validation phases, as the training and validation datasets were repeatedly input in the neural network.

3.2. Performance of the Convolutional Neural Network

The full-size dataset was split for the constitution of training and validation datasets as follows: 80% of the retrieved images were used as a training dataset, and the remaining 20% were used as a validation dataset for evaluation of the CNN's performance. The confusion matrix between the trained CNN and final diagnosis is shown in Table 2. Overall, the algorithm had an accuracy of 98.5%. The sensitivity, specificity, PPV and NPV for the detection and differentiation of mucinous cysts versus normal or non-mucinous structures were, respectively, 98.3%, 98.9%, 99.5% and 96.4%. The AUC of the CNN for the discrimination of mucinous and non-mucinous cystic lesions was 1.00 (Figure 4).

Table 2. Confusion matrix of the automatic detection versus final diagnosis.

		Final Diagnosis	
		Mucinous	**Non-Mucinous**
CNN	Mucinous	743	9
	Non-mucinous	12	337

Abbreviations: CNN—convolutional neural network; Mucinous—mucinous pancreatic cystic lesions; Non-mucinous—non-mucinous pancreatic cystic lesions.

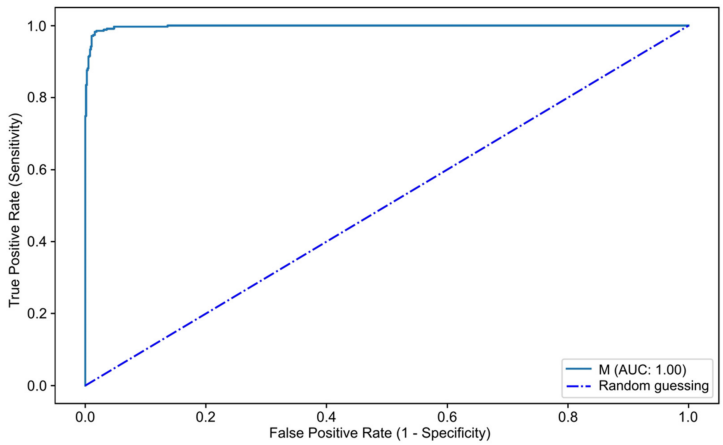

Figure 4. ROC analysis of the network's performance in the detection of mucinous pancreatic cystic lesions. AUC—area under the receiver operating characteristic curve. M—mucinous pancreatic cystic lesion.

3.3. Computational Performance of the CNN

The CNN completed the reading of the validation dataset in 6 seconds at a speed of 5.2 ms/frame. This translates into an approximated reading rate of 191 frames per second.

4. Discussion

The development of AI algorithms is a hot topic in medical literature. Several reports show promising results regarding gains in diagnostic accuracy, particularly for medical specialties highly dependent on imaging [9]. The application of machine learning (ML) in endoscopy has shown encouraging results [10].

In this proof-of-concept study, we have developed a CNN for the automatic identification of mucinous pancreatic cysts during EUS. The algorithm demonstrated an excellent discriminatory ability with 98.5% accuracy for the differentiation of mucinous cysts from non-mucinous lesions. This proof-of-concept study represents a pilot effort to minimize the limited interobserver agreement regarding the EUS characterization of pancreatic cysts. Evidence on the application of AI to EUS for the study of pancreatic lesions is limited. Particularly, studies focusing on the detection and differentiation between mucinous and non-mucinous pancreatic cystic lesions based on EUS images are scarce. To the authors' knowledge, only one study focusing on this subject has been previously published [13]. Nevertheless, the development of AI algorithms for the evaluation of pancreatic diseases is a subject of increasing interest [14–19].

The development of a deep learning model accurately predicting the phenotype of PCLs during EUS procedures may have a substantial impact on patient management. The main goals when approaching these lesions is defining their type (mucinous vs. non-mucinous) and, subsequently, attaining a definite histotype. The first step of this sequence is of particular relevance, as the malignant potential is virtually restricted to mucinous lesions. Therefore, we developed a deep learning algorithm for the automatic classification of PCLs as mucinous vs. non-mucinous. Nguon and coworkers implemented a CNN model to differentiate MCN and SCA using EUS images [13]. Their algorithm achieved an overall accuracy around 80%, which is in line with the classification performance of experienced endosonographers. The authors explained this suboptimal accuracy as the result of the inclusion of EUS images obtained using both radial and linear echoendoscopes as well as variations in the demarcation of single or multiple regions of interest (ROI), which included the cyst as well as surrounding tissue. This study differs from ours as we only included linear EUS images and our CNN model included complete images, without pre-selected ROI. Nevertheless, the main difference between the studies resides in the spectrum of included lesions, as our study focuses on group classification rather than differentiating between two different cyst types. Our model was built including EUS images from IPMNs in the mucinous group, in addition to MCN. IPMNs are the most frequent pancreatic cystic neoplasia and constitute a big challenge when it comes to correctly risk stratifying the malignant potential of each lesion. The work by Kuwahara et al. expands the reach of our study [15]. This group developed a deep learning algorithm to predict the malignancy potential of IPMNs using images from patients with malignant and non-malignant IPMNs. The authors used the output value of deep learning calculated after training as the predictive value of malignancy (AI value). The mean AI value of malignant lesions was higher than that of benign IPMNs. In this study, the CNN had a higher diagnostic performance than that of the endoscopists diagnosis and the predictive factors provided by scientific societies guidelines. Further studies on deep learning tools for application to EUS should expand the knowledge in this issue and address the challenge of automatic detection of cysts with advanced neoplasia, therefore minimizing the need for cyst puncture, ultimately preventing unnecessary surgeries.

The development of AI solutions for PCLs differentiation has expanded to other endoscopic tools complementary to conventional EUS. Confocal laser endomicroscopy (CLE) has been proven useful for differentiating various types of PCLs and more recently was shown to outperform international guidelines in the prediction of malignancy in IPMNs.

However, image interpretation is observer dependent, and CLE is not widely available [20]. Recently, Machicado et al. described the development of a CNN algorithm based on CLE images to risk stratify IPMNs [16]. They used CLE videos from 35 histopathologically confirmed IPMNs and developed two CNN algorithms whose accuracy was compared to International Consensus Guidelines and American Gastroenterology Association criteria for advanced lesion/surgical indication. The results showed the higher accuracy of the CNN algorithm compared with the guidelines.

We conducted a proof-of-concept study assessing the potential of deep learning tools for the differentiation of mucinous and non-mucinous PCLs. This study has several highlights. First, to our knowledge, it is the first study to provide a clinically useful tool for the differentiation of PCLs as mucinous or non-mucinous. The accurate differentiation between both entities allows a prompt estimate of malignant potential, which has significant impact in patient management and follow-up. Second, our model was demonstrated to be highly sensitive, specific, and accurate. Finally, our algorithm had a high image processing performance with an approximate reading rate of 139 frames per second. An adequate image processing performance is a key element for subsequent real-time implementation of this proof-of-concept CNN model.

This study has several limitations. First, a small number of patients were enrolled and, therefore, some cyst types were underrepresented. The inclusion of a large pool of frames extracted from full-length videos, in addition to the routine still frames included in the standard EUS report, with distinct resolution and viewing angles contributed to provide an adequate variability to our dataset. Second, we performed a single-center retrospective study. Subsequent robust multicenter prospective studies are required to assess the clinical significance of our results. Further development of this technology will require the inclusion of large numbers of patients. Additionally, the refinement of the algorithm will require the inclusion of other types of pancreatic cysts as this should be required before it reaches clinical practice, providing automatic differentiation between several classes. Third, our proof-of-concept algorithm was developed and assessed using a single EUS suite. Therefore, our results may not be generalizable to other EUS platforms. Finally, the absence of surgical specimens or histological samples as the gold standard for all the included cysts is a significant limitation for establishing a reliable and reproducible gold standard for the development of the automated algorithm. An automated predictive model can only be as good as the gold standard for defining the true classification. Furthermore, the future application of AI tools into real-life EUS practice will require going through a strict regulatory pathway. The Food and Drug Administration (FDA) has approved several AI/ML-based Software as medical device (SaMD) with locked algorithms and changes beyond original market authorization requiring FDA premarket review [21]. Additionally, the FDA accepts the evolving and changing nature of AI/ML-based SaMD, namely convolutional neural networks. This particular matter constitutes a change from the previous paradigm for medical device regulation, as it was not initially designed for adaptive deep learning models. Indeed, a new framework is being gradually developed to provide appropriate regulatory oversight.

Artificial intelligence is gradually changing the landscape in digestive health care. Indeed, accurate, faster, and tireless AI tools will disrupt clinical practice and play a key role in endoscopic ultrasound. The potential of deep learning algorithms to impact the care of patients with pancreatic disease is vast and may contribute to improving the prognosis of these patients. We believe this AI model constitutes a significant milestone in the phenotypic differentiation of PCLs. Indeed, this work highlights the technological feasibility of accurately achieving morphologic pattern identification of pleomorphic pancreatic lesions.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The extensive use of cross-sectional imaging has not only identified a plethora of PCNs, but it has also offered the opportunity to focus efforts on a selected population at risk for Pca, namely patients harboring mucin-producing pancreatic cysts (IPMNs and MCNs), in whom active surveillance could play an important role in improving survival [100].

Fortunately for most patients, the majority of cystic lesions will not impact their ultimate survival and decisions should be individualized based on patient comorbidities, cyst type, features and location [12, 13, 24]. This is especially relevant as an important series from an expert center reported that 25% of patients undergoing surgical resection have PCLs with no malignant potential and several surgical series report that 60-78% of resected BD-IPMN are ultimately found to harbor low-grade dysplasia [45, 100, 104]. The experience from our hospital is similar to that published by other centers: from 39 resected BD-IPMN, 28 (72%) specimens showed low-grade dysplasia. The surgical complication rate (Clavien-Dindo grade ≥3) was 44% and one patient died of complications related to surgery (unpublished data, presented at Semana Digestiva 2017). More recently, our group, reported on the use of glypican-1 for Pca screening in high-risk individuals and included 40 mucinous cystic lesions. Fifteen lesions (37.5%) were resected and 12 (80.0%) were found to be benign [112]. Therefore, there is a need to improve patient selection for surveillance and surgery and accurate preoperative assessment of PCLs is crucial for adequate patient management [91].

The current standard of care for PCLs diagnosis and risk stratification includes history/demographics, cross-sectional imaging and EUS with or without FNA (cytology and cyst fluid analysis). However, as we discussed in our review article, PCLs may remain indeterminate concerning subtype and malignancy risk after completion of these investigations [18].

CT and MRI are imperfect for cyst type determination and differentiation of malignant and pre-malignant cysts [14]. EUS-FNA allows for cyst fluid cytology and cyst fluid biochemical analysis, however cytology is hindered by the low cellularity of the samples and gastrointestinal contamination [113]. In fact in a meta-analysis of 16

studies including 1024 patients by Wang et al., cyst fluid cytology revealed 94% specificity but only 51% sensitivity [81]. Our results are in line with previously published data. In our cohort of 40 patients, cytology showed 34.5% sensitivity and 90.9% specificity [114].

Cyst fluid CEA levels >192 ng/mL are associated with the diagnosis of mucinous lesions. This cut-off was derived from the 2004 prospective, multicenter cooperative cyst study published by Brugge et al. and demonstrated a sensitivity and specificity of 75% and 84%, respectively [26]. In fact, a more recent meta-analysis by Thornton et al. including 18 studies with 1438 patients corroborated these results using the same cut-off [16].

Several limitations have been pointed to the clinical use of cyst fluid CEA: the overlap for CEA levels between mucinous and non-mucinous cysts, insufficient cyst fluid volume for CEA determination and lack of correlation between CEA levels and grade of dysplasia [15, 46, 81].

In our cohort of 40 patients, cyst fluid CEA levels were available for 30 lesions because in 7 cases, cyst fluid volume was insufficient for the quantification and in 3 lesions the viscosity of the fluid precluded the performance of the assay in the laboratory [114]. In 5 of these 10 cases, cyst fluid glucose levels were determined using point-of-care glucometer. With the cutoff of 192ng/mL, fluid CEA levels allowed the diagnosis of 13 mucinous cysts (in a total of 29 ultimately diagnosed as mucinous lesions) and when combined with cytology, 18 mucinous lesions were diagnosed [114].

In 2013, Park et al. reported for the first time the utility of cyst fluid glucose levels for differentiating mucinous and non-mucinous lesions [115]. Three recent metanalysis demonstrated that cyst fluid glucose levels perform better than CEA for cyst differentiation (mucinous vs non-mucinous) and its measurement is simple and requires only a very small fluid volume [82, 116, 117].

We retrospectively reviewed our EUS database and identified 78 patients (62% female) with PCLs submitted to EUS-FNA that had determination of pancreatic cystic fluid glucose levels, from October 2017 to December 2022. For mucinous cyst diagnosis, the

sensitivity using a glucose cut-off of 50 mg/dL was 93.2% and the specificity was 76.5% (AUROC 0.87). In the same cohort, intracystic CEA levels (cut-off 192 ng/mL) had a sensitivity and specificity of 55.5% and 87.5%, respectively (AUROC 0.81). The correlation between on-site and laboratory glucose levels was excellent (Pearson correlation 0.947) (unpublished data).

There are nowadays two relevant challenges that could potentially spare patients with PCLs unnecessary testing, radiation, and surgery [18]:

1) To differentiate mucin-producing cysts from the ones with no malignant potential, that do not require resection or follow-up;

2) To differentiate mucin-producing cysts with low-grade dysplasia from the ones that harbor high-grade dysplasia or early invasive cancer, who will benefit from surgery.

These points are especially important given the fact that pancreatic surgery is associated with significant morbidity and non-negligible mortality [102, 103].

As suggested in the recently published study by Lobo et al., the focus should be to detect a potentially preventable or curable malignancy but clinicians should be aware of the harm, including cost, morbidity, and mortality, that accompanies more aggressive surveillance or early intervention [118].

Paper 1 describes our experience using EUS-TTNB for diagnosis and risk stratification of PCLs and discusses several points concerning technique variations that can help improve its diagnostic yield and standardize the procedure.

Paper 2 describes the development of a deep learning algorithm for the automatic identification of mucinous PCLs using EUS images, with the aim to mitigate the low interobserver agreement previously described for cyst EUS morphology.

5.1 PAPER 1
EUS-TTNB for PCLs diagnosis and risk stratification

5.1.1 TTNB FOR CYST TYPE DEFINITION

Recent advances concerning the evaluation of PCLs, namely in the endoscopic field, are thought to allow better recognition of the type of lesion and determination of risk of malignancy, as we mentioned previously and discussed thoroughly in our review paper [18].

The performance of EUS-FNA for histological type determination and malignancy risk establishment is disappointing [91] and there is growing need to find accurate and affordable tests to improve diagnosis [84]. Cyst fluid DNA analysis using next-generation sequencing (NGS) obtained preoperatively by EUS-FNA or from resected surgical specimens, was shown to be useful for cyst classification and for prediction of malignancy in mucinous lesions [2, 119, 120]. NGS has several advantages over Sanger sequencing (requirement of smaller amounts of DNA, ability to assay multiple genes simultaneously and higher sensitivity)[119] but it is not available in most centers and its cost-efficiency is not validated to justify its use in current clinical practice [121].

EUS-TTNB using a microforceps has recently shown promising results with higher diagnostic performance in the evaluation of PCLs, compared with EUS-FNA, as shown in several systematic reviews [88, 89, 110]. Also important is the fact that interobserver agreement among pathologists was found to be substantial for cyst type definition in a previous study [122].

Our group found similar results to the three previously cited systematic reviews, with TTNB revealing 75.9% sensitivity, 90.9% specificity and 80.9% accuracy versus 58.6% sensitivity, 90.9% specificity and 67.5% accuracy for EUS-FNA in the diagnosis of mucinous lesions [114]. A systematic review published by Faias et al. that included 8 studies (203 PCLs with surgical pathology as reference standard for diagnosis)

revealed that EUS-TTNB had higher diagnostic yield than genetic analysis, being especially useful for identifying benign lesions, for which both surgery and surveillance are unnecessary. However genetic testing was found to have higher accuracy in the diagnosis of malignant and high-risk cysts [84]. In our cohort of 40 patients undergoing TTNB using Moray® microforceps we reported as primary outcome, the proportion of cysts in which a histopathological diagnosis was attained. The diagnostic yield of TTNB was 72.5% as samples were adequate for cyst type definition in 29 cases out of 40 procedures, whereas FNA cytology revealed a diagnostic yield of 27.5% [114]. Moreover, in our experience, the preoperative diagnosis of 12 mucinous cysts would not be possible if only cytology was available and the combination of cytology and CEA levels would have also resulted in the missing of five of these lesions [114]. This higher diagnostic yield for the identification of mucinous cysts has been previously shown in other published TTNB series [87, 111, 123].

The differentiation between different specific cyst types has important implications for management, as for example SCAs do not require surveillance or surgery, except in the presence of mass effect [113] and in the case of MCNs, there is the possibility to avoid immediate resection in specific circumstances [124]. Previous studies reported on the limitations of cyst fluid cytology for the identification of serous epithelial cells [125, 126]. For these lesions, the detection of VHL mutation is useful [119, 120] but is not readily available in most centers. In our cohort, six lesions were ultimately diagnosed as SCAs. Using TTNB alone, we were able to diagnose three SCAs and to determine the successful discharge from ongoing surveillance of two of these patients who would otherwise have continued testing [114].

In patients with MCNs less than 50 mm, without wall enhancement or mural nodules, the malignancy risk is negligible and initial surveillance is acceptable [124]. Based on clinical and imaging modalities, MCN diagnosis is often presumptive and there is a 20% risk of misdiagnosis [48, 124]. Moreover, the morphological distinction between MCN and IPMN is challenging as sometimes the connection between the cyst and the MPD is difficult to document. In fact, MCN diagnosis demands the histological

documentation of ovarian-type stroma beneath the epithelium, which is impossible with current standard of care tools because during EUS-FNA, subepithelial stroma is not sampled, so it is not possible to distinguish MCNs from IPMNs [48, 113].

EUS-TTNB was previously shown to allow the detection of ovarian-like stroma that allows unequivocal preoperative diagnosis of MCN [48]. In our study, Moray® microforceps specimens from three patients, revealed mucinous epithelium with subepithelial ovarian-type stroma. Two patients had resection and surgical specimens were concordant with the preoperative TTNB diagnosis [114].

Moreover, some studies have also shown the possibility for accurate SPN and cystic PNET preoperative diagnosis based on TTNB alone [111]. In our experience, TTNB was not able to provide the diagnosis in two lesions which were ultimately diagnosed as SPNs in surgical specimens. We speculate that the frequent presence of necrosis and hemorrhage in these tumors, may obscure the classical solid and papillary microscopic arrangement that characterizes SPNs in TTNB specimens, precluding its diagnosis. Because these lesions usually have an important solid component, we suggest that EUS-FNA or FNB directed to the solid part should be preferred, as previously reported [127].

One important limitation of the studies that reported on the use of TTNB is the small number of patients that underwent surgery [88]. The systematic review by Tacelli et al., based on five studies that reported on a subgroup of resected lesions, including 62 patients, found that the diagnostic concordance between EUS-TTNB and surgery was 87%. After exclusion of lesions without adequate TTNB samples for histological diagnosis, the concordance raised to 93% [89].

In our cohort, all but one TTNB diagnosis for specific cyst type were concordant with surgical specimen for the patients who had resection (15/16), which adds to the evidence that the concordance with surgical histology is very high for TTNB [114].

5.1.2 TTNB FOR IPMN RISK STRATIFICATION

BD-IPMNs comprise the majority of incidental PCLs and are considered a great challenge to clinicians because they are precursors of PDAC [20]. The likelihood of lesion progression and the timepoint of its occurrence in a specific patient is hard to determine [128].

The ultimate goal in the management of IPMNs, is to determine which neoplasms will eventually evolve into malignancy and to avoid the risks of unnecessary surgery as most patients will not develop invasive cancer [100]. In fact, a systematic review reported 112 invasive cancers in 3,980 patients during 14,830 patient-years of follow-up. The overall proportion of patients developing invasive neoplasia was 2.8% overall (95% CI, 1.8%-4.0%), 0.72% per year [9].

Currently, we lack accurate clinical, biological, and morphological risk factors for invasive IPMNs, so guidelines use a combination of features to predict HGD or cancer that determine the indication for resection [20]. Table 2 describes the indications for surgical resection according to the most recent scientific societies guidelines.

Table 2. Indications for surgery according to recent guidelines.
Adapted from Vilas-Boas F. et al. J Gastrointestin Liver Dis. 2019;28(4):495-501 [20].

Guideline	Symptoms	MPD	Mural nodule	Positive cytology	Size	Comments
ICG (Revised Fukuoka 2017)	jaundice	≥10 mm	+ 5 mm cut-off	+	-*	HRS Consider life expectancy, comorbidities and location
AGA 2015	NA	dilated	+	+	-	And/or 2 features
European 2018	jaundice, acute pancreatitis	≥10 mm (5-9.9 mm relative indication)	+ 5 mm cut-off	+	≥4 cm (relative indication)	Growth rate ≥5 mm/ year, new-onset DM, high CA 19-9
ACG 2018	jaundice, acute pancreatitis	≥5 mm	+	+	≥3 cm	Growth rate ≥3 mm/ year, new-onset DM, high CA 19-9

ACG: American College of Gastroenterology; AGA: American Gastroenterological Association; CA 19-9: carbohydrate antigen 19.9; DM: Diabetes mellitus; HRS: High-risk stigmata; ICG: International Consensus Guidelines; MPD: Main pancreatic duct; NA: not applicable.

*Cyst size alone is not an appropriate parameter to indicate surgery. Presence of more than one risk factor increases probability of HGD/invasive carcinoma.

Guidelines perform better identifying very high-risk and very low-risk lesions, but in clinical practice most patients reveal intermediate-risk features. Several surgical series reported rates of 0-26% of HGD and 0-37% of invasive cancer in resected BD-IPMNs, which resulted in the definition of guidelines with high sensitivity but low specificity in detecting malignancy and subsequent surgical overtreatment for IPMNs [100, 129]. A retrospective study published in 2017, found that the 2012 ICG and the 2015 AGA guidelines, were associated with unnecessary surgery in 54% and 12% of cases, respectively [130]. Moreover, a recent observational study of non-operated IPMNs reported a 5-year disease-specific survival rate of over 95% among patients with WF, that adds to the discussion that for elderly patients, even in the presence of WF, resection should be avoided [11]. Furthermore, Sahora et al. in a large cohort of 725 patients with IPMNs, identified a subgroup of frail patients (age adjusted Charlson comorbidity index ≥7), with high risk of non-IPMN-related mortality within a few years after diagnosis. These group of patients did not benefit from surveillance or resection [13].

Our results are in line with the data published by other centers. In a cohort of 54 surgically resected BD-IPMNs, 68% revealed low-grade dysplasia (unpublished data, presented at the World Congress of Gastroenterology 2019). Previously, we had already published the data of a similar cohort of 39 resected IPMNs where we compared the accuracy of IAP and AGA guidelines for the prediction of advanced IPMNs (HGD or invasive cancer). Both guidelines performed suboptimally with 82% sensitivity and 86% specificity for AGA guidelines and 100% sensitivity and 39% specificity for IAP guidelines [131].

Beyond guidelines, other tools like scores and nomograms aimed to establish a preoperative calculation of the risk of malignancy for an individual patient [72, 73, 128].

We had the opportunity to apply two nomograms [72, 73] to a group of 54 resected IPMNs at our institution and found a moderate accuracy for prediction of advanced histology (area under the receiver operating curve (AUROC) of 0.72 for both scores) (unpublished data, presented at the World Congress of Gastroenterology 2019).

The Shin score, published in 2010, comprises five variables (age ≥60 years, history of pancreatitis, Carbohydrate antigen 19-9 (CA 19-9)>37 IU/mL, presence of mural nodules and MPD diameter ≥6 mm) and was validated in an Asian cohort [132]. Recently Manuel-Vásquez et al. performed an external validation of the Shin score in a multicenter cohort of 567 patients from Europe with BD-IPMNs harboring WF. The authors confirmed a significant association of the score with the presence of malignancy and a high score (≥3) warrants resection as the risk of malignancy is high. However, lower scores are associated with smaller risk so better stratification tools are still needed [128].

These data add to the need for better tools to risk stratify patients with IPMNs, which has been the subject of intense research for several years.

To increase sensitivity of EUS-FNA, other EUS devices like the cytology brush (Echobrush®, Cook Medical) and EUS-FNB using a needle with side fenestration (EchoTip ProCore®, Cook Medical) have been studied [18]. The results have been disappointing both for the use of ProCore FNB needle, especially for cysts without solid component and for the Echobrush®, in this latter case because of high rates of technical failure and adverse events [133, 134].

The determination of the grade of dysplasia in mucinous lesions can impact decision-making. In addition to provide definite information regarding the cyst type and additional diagnostic yield for mucinous lesions over FNA, EUS-TTNB was also shown to allow the definition of dysplasia grade in cystic mucinous lesions with improved diagnosis of advanced neoplasia [87, 110, 111]. The systematic review published by Westerveld et al. that included four studies that provided information on histological grading of mucinous lesions, revealed that TTNB, in comparison with FNA was significantly more likely to match the histologic grade of surgical pathology specimens (OR 10.4; 95% CI: 2.3-14.1) [110].

In our cohort of 40 patients submitted to TTNB, the grading of dysplasia was possible for all 23 mucinous cysts. However in the case of 3 patients (in a total of 16) who had resection, the dysplasia grade established in the TTNB sample was non-concordant with the surgical specimen [114]. This finding adds to the discussion of the lack of

interobserver agreement for dysplasia grade in IPMN, reported in some studies with surgical specimens [135] but not in others using TTNB samples [87, 122]. Moreover, TTNB does not allow the sampling of the whole cyst, as it is limited by the point of entrance of the needle and we must also consider that the degree of epithelial atypia may vary within the lesion, resulting in inhomogeneous distribution of dysplasia. Furthermore, there is also the issue of cyst epithelium denudation pointed by some studies that explains the lack of lining epithelium in some TTNB samples [89, 136]. This problem is seen with greater extent in MCNs than in IPMNs [137].

5.1.3 TTNB FOR IPMN SUBTYPING

IPMNs are divided in three subtypes – gastric, intestinal and pancreatobiliary - based on morphologic features and specific mucin expression [51, 63]. IOPN is considered a distinct entity since 2019 [36]. Recent data show that the natural history, risk of malignancy, as well as overall survival in patients with IPMNs is related to the epithelial subtype, so preoperative phenotype definition can be important for risk stratification [65, 87, 138, 139]. The gastric subtype is the most common (~60%) and is rarely associated with high grade dysplasia. On the other hand, pancreatobiliary subtype corresponds to around 6% of cases and is more often associated with invasive carcinoma [64].

For the first time preoperatively, TTNB gives us the opportunity to define cyst histotype, because we obtain samples retaining the histologic architecture of the tissue [89]. This fact allows the performance of immunohistochemistry on the epithelium and stroma that is relevant for IPMN subtyping and MCN diagnosis [136]. In fact, in high surgical risk patients with IPMNs harboring WF, but without HRS, subtyping may be useful to support decision-making [90].

A first study published by Kovacevic et al. reported on the feasibility of IPMN subtyping using TTNB specimens [139]. In fact, according to two systematic reviews, in 80% to 87% of the cases, TTNB specimens are suitable for immunohistochemical staining, which is useful to complement morphologic evaluation [88, 89]. Commenting on a

paper published by Crinò et al. in 2019 [86], our group also reported an initial experience of using TTNB samples for morphologic and immunohistochemical subtyping of IPMN and discussed the relevance of this topic in a group of 10 IPMNs, diagnosed during our first 20 EUS-TTNB procedures. Based on morphology and MUC expression (MUC1, MUC2, MUC5AC, and MUC6), we were able to determine the phenotype of 8 lesions [140].

In 2021, Kovacevic et al. published a prospective single center study that included 101 patients that underwent EUS-TTNB. Fifty-seven IPMNs were diagnosed, and subtyping was successfully performed in all the cases. The authors, however, do not describe the concordance rate of IPMN subtyping in the group of 13 patients that underwent resection [87].

In our cohort of 40 patients that included 19 IPMNs, immunohistochemistry-based subtyping was possible for 12 lesions (63%). Subtyping was not possible in 7 cases because of insufficient samples that precluded immunohistochemistry studies [114].

5.1.4 TTNB IMPACT ON CLINICAL MANAGEMENT

As we previously discussed, EUS-TTNB is helpful for the elucidation of specific cyst type and has a significantly higher diagnostic yield for the identification of mucinous cysts than cytology or cyst fluid CEA levels. Moreover, the good concordance rate for histologic grade among resected mucinous lesions justifies the possibility of using TTNB results to improve preoperative risk stratification, especially in patients with no HRS, that can have a significant impact on clinical management [110].

Concerning the TTNB impact on management in our cohort of 40 patients and assuming a correct TTNB diagnosis, we found that 4 patients would have inadequately undergone resection based on standard of care, and 3 patients who would be kept on surveillance, were discharged. Conversely, 1 patient who had the diagnosis of LGD BD-IPMN in TTNB but despite that, latter had resection because of rapid growth rate, was ultimately diagnosed with malignant BD-IPMN in the surgical specimen [114]. This

underestimation of the real grade of dysplasia of the lesions is an important limitation that has been previously described [141].

In 2018, Mittal et al. published a retrospective single center experience on 27 patients undergoing EUS-TTNB and described that the biopsy results changed the diagnosis in 7 patients (26%) providing the diagnosis of 2 mucinous cysts, 4 SCAs and 1 NET, but do not describe how this impacted decision-making [142].

Chessman et al. reported on the impact on clinical management of EUS-TTNB and CLE compared to standard of care ("composite standard"). The authors concluded that TTNB led to an overall change in clinical management in 38.6% of cases, including an increase in discontinuation of surveillance, reduction in indication for follow-up imaging and endoscopic studies and referral for surgery in 2 of 28 patients that would have undergone further surveillance [143].

The first prospective study published on TTNB was the one by Yang et al. in 2018 that included 114 consecutive patients. Regarding patient management, the authors reported the diagnosis of HGD by TTNB alone in two patients with IPMNs with no HRS or WF that would not have been submitted to surgery [111].

In the more recent prospective study published in 2021 by Kovacevic et al., TTNB led to a change in clinical management in 11.9% of patients, allowing the diagnosis of SCA (especially the diagnostically challenging oligocystic variant) in 10 patients, leading to follow-up discontinuation [87]. The authors, however, did not specified in how many cases TTNB avoided inappropriate surgery for these benign lesions [90].

5.1.5 TTNB PROCEDURE STANDARDIZATION

Two recent systematic reviews on EUS-TTNB found a high heterogeneity among the included studies, that suggests that a standardization of the procedure is urgently required [88, 89]. Moreover, few studies have described the details of the technical steps during EUS-TTNB [89].

In fact, several technique variations have been described among the published studies, regarding for example, forceps preloading, timing for cyst fluid aspiration, number of passes and bites per pass, number of collected specimens and specimen handling [86, 87, 111, 123, 139, 144]. In the study by our group, we discuss the details of the technical variations during the different steps of the procedure, focusing on future standardization [114].

Safe performance of TTNB depends on the existence of space for forceps manipulation inside the cyst. The fluid content of the cyst will keep the lesion distended, so in our opinion, aspiration of the cyst for cytology and CEA levels should be performed after biopsy sampling, avoiding the need of saline injection for cyst re-expansion, that can add to the risk of infection. Concerning forceps preloading, we concluded that it is a crucial step in the case of cystic lesions smaller than 20 mm, to prevent cyst collapse after puncture with a 19G needle. Moreover, because of the absence of a central spike in the forceps, only a single bite per pass should be performed [114].

The previously cited systematic reviews describe that during EUS-TTNB, the mean number of passes performed in the included studies was 3 [88, 89]. The number of passes performed should be systematically described because it will determine the procedure-induced trauma and will also impact the procedure duration, which could affect the adverse event rate [89]. Also important, and not necessarily the same, is the number of visible collected samples necessary to reach histologic adequacy.

In an important study by Crinò et al., the authors make the first description that tries to standardize the diagnostic criteria for PCLs using TTNB specimens. The authors proposed the assessment of 4 histologic parameters (provide cyst-lining epithelium, differentiate mucinous from non-mucinous cysts, define dysplasia grade, and determine cyst histotype) to evaluate the adequacy of the samples and determined that the acquisition of two visible specimens reaches a histologic adequacy of 100% [86]. In a letter to the editor that we have published commenting on this study, we suggested that IPMN immunophenotype classification should also be included in the pathology report of TTNB samples and stressed that future studies should evaluate

the need for more than 2 visible specimens to allow IPMN subtyping [140]. However, the optimal number of collected specimens to improve diagnostic performance may also depend on specimen processing technique and on pathologist experience [114, 145] because as acknowledged by Crinò et al., TTNB samples pose a challenge for pathologists because of the small size of the specimens, seldom evaluated before [146].

Concerning specimen handling, after discussion with our pathologists and technicians, we decided to process TTNB specimens as we do for cytology samples and generate a cell-block, as previously described in other observational study [141]. We believe that cell-block preparation avoids sample loss, while preserving tissue architecture for histologic assessment and immunohistochemistry studies [114].

5.1.6 TTNB ADVERSE EVENTS

The enthusiasm related to the development of new endoscopic tools must be balanced with the possibility of AEs. This fact was well demonstrated during the evaluation of the cytology brush (Echobrush®, Cook Medical) that was compromised by the development of serious adverse events that led to the abandonment of the technique [18].

The first case reports and retrospective case series on EUS-TTNB reported no adverse events or only mild complications that resolved spontaneously with no need for specific treatment. However, a systematic review [89] described an 8.6% (ranging from 1-23%) overall rate of AEs. Moreover, the recent single-center prospective study by Kovacevic et al., reported a 10% adverse event rate, including one death related to post-TTNB pancreatitis. The impact of prophylactic measures has not been fully evaluated and the authors were not able to find predictive factors for adverse events [87].

Faciorusso et al., recently published a multicenter retrospective analysis of 506 patients who underwent TTNB and generated a prognostic model for post-TTNB AEs. We had the opportunity to participate is this study that reported an overall AE rate of

11.5% (58/506), including 15 (3%) moderate, 9 (1.8%) severe and 3 (0.6%) fatal (2 acute pancreatitis with multiorgan failure and 1 septic shock). In this study the most frequent adverse event was acute pancreatitis (5.7%) [91].

Systematic reviews reported that the most common adverse event of TTNB is intracystic bleeding (4%-6%) but it was almost always described as self-limiting, not requiring additional interventions [88, 89, 110]. This fact justifies that in the multicenter study by Faciorusso et al., these bleedings were considered incidents according to American Society of Gastrointestinal Endoscopy (ASGE) lexicon and were not included in the determination of AE rate.

In multivariate analysis, the diagnosis of IPMN, patient age over 64 years, the number of passes and the impossibility to completely aspirate the cyst, were identified as significant predictive factors of AEs [91].

Several prophylactic measures have been suggested to reduce the AE rate, namely periprocedural hydration with Ringer's lactate, rectal non-steroid anti-inflammatory drugs (NSAIDs) and antibiotics. Concerning antibiotic prophylaxis, despite recommended by current guidelines [147], two recent studies question its need, even when TTNB is performed [148, 149]. Even though, in the multicenter study by Faciorusso et al., antibiotic prophylaxis was used in 96.2% of patients and it might be useful in the subgroup of patients with cysts that cannot be completely aspirated [91]. In our cohort, all patients had a single 200 mg IV dose of ciprofloxacin as determined by the initial research protocol and no infection occurred.

Regarding acute pancreatitis, in the same multicenter study by Faciorusso et al., 27.7% of patients received prophylaxis using rectal NSAIDs. On univariate analysis, rectal NSAIDs were found to be protective for AEs but not in multivariate analysis [91]. We did not use any prophylactic measures against acute pancreatitis but acknowledge the need for further studies on this topic.

In our cohort, we registered no infectious adverse event or acute pancreatitis. Two patients had self-limited intracystic bleeding and one developed delayed post-procedure abdominal pain with no evidence of acute pancreatitis.

5.2 PAPER 2

EUS-AI algorithm for mucinous PCLs identification

5.2.1 ARTIFICIAL INTELLIGENCE IN ENDOSCOPY

Gastroenterology is substantially reliant on vast amounts of images and has become an important subject for the application of AI models [108]. AI has emerged as a technology to help physicians handle various types of images and is presumed soon to have significant effects on clinical practice. The central goal using AI is to use large datasets to recognize patterns, ultimately allowing the learned function to be applied to new data [108]. In the field of endoscopy, AI focused on image analysis for the recognition of specific anatomical locations during upper endoscopy, detection of GI neoplasms, classification between benign and malignant lesions or evaluation of endoscopy quality metrics such as bowel preparation score [94, 108]. Specific examples are the detection of early neoplastic lesions in Barrett's esophagus, diagnosis of Helicobacter pylori infection, gastric cancer detection during upper endoscopy and polyp detection/characterization during colonoscopy (white light or using chromoendoscopy), including in real-time analysis [92, 94, 108]. In capsule endoscopy, several studies from our group focused on automatic detection of vascular, inflammatory and protruding lesions and for cholangioscopy we developed a CNN for the automatic differentiation of indeterminate biliary strictures [150-153].

5.2.2 EUS-BASED ARTIFICIAL INTELLIGENCE MODELS FOR PANCREATIC DISEASES

Multiple studies have described the development of AI algorithms for medical image analysis; however, few have been published on its use for pancreatic disease evaluation or EUS image analysis. More importantly, the application of EUS-based DL models for the evaluation of pancreatic lesions, including PCLs, is scarce [109]. The

first study that described the use of an EUS-AI model for pancreatic lesion evaluation was published in 2001 and relates to a ML algorithm. Norton et al., developed a ML-based algorithm to differentiate focal mass-forming pancreatitis from Pca, using EUS images [154]. Some more recent studies have tested EUS-based DL models for the diagnosis of PDAC [155, 156], differentiation of chronic pancreatitis versus PDAC [157] and differentiation of autoimmune pancreatitis versus PDAC [109]. In this latter study, Marya et al., in an effort to overcome the limitations of current diagnostic modalities for differentiating autoimmune pancreatitis and Pca, developed an EUS-based algorithm, using a CNN model, that revealed an excellent performance.

Saftoiu et al., described the use of CNNs for the differential diagnosis of pancreatic solid tumors using EUS elastography [99] and contrast-enhanced EUS images [158]. In these studies, the authors used a multilayer perceptron model that relies on a numeric value as input data to the algorithm and not the images themselves which can impact diagnostic performance [159]. Until recently, given the diverse morphologies, it has been difficult to use AI for the assessment of PCLs.

5.2.3 EUS-BASED AI MODELS FOR THE STUDY OF PCLS

As previously discussed, despite the use of guideline-defined risk features and several diagnostic tools, the diagnosis of mucinous cysts and risk stratification of PCLs is still imperfect.

One special interest of AI application is to reduce misdiagnosis and interobserver variability in visual classification. In fact, computer-aided diagnosis has been proposed as a potential solution to standardization of endoscopic and radiological image interpretation, because it allows the processing of deeper layers of data, not discernable to humans [98, 108].

In the case of PCLs, the varied morphology adds to the difficulty to accurately distinguish different types of lesions, ranging from inflammatory to serous or

mucinous lesions. In recent years, AI has been applied to the classification and risk stratification of PCLs using cross-sectional imaging and EUS [98].

Radiomics, also known as quantitative imaging, uses feature extraction and pixel analysis from cross-sectional imaging studies to create radiological phenotypes. In the case of PCLs, CT and MRI-based radiomics have been described for cyst subtyping and malignancy prediction [98, 160]. In IPMNs, MRI radiomic models were proven superior to CT models in predicting malignant potential [161] and when combined with clinical features, show improved performance for the prediction of high-risk lesions [162].

Relevant limitations have been described concerning the use of EUS for the evaluation of PCLs [30]. EUS morphology alone revealed an accuracy of only 51% for the distinction of mucinous and non-mucinous cysts and the interobserver agreement is only fair to moderate, even among expert endosonographers [14, 163, 164].

The first study to describe the use of an EUS-CNN algorithm to differentiate between different types of PCLs was published in 2021 by Nguon et al. The model developed by these authors allowed the differentiation of MCN and SCA with an overall accuracy around 80% [165].

In our pilot study, we developed a CNN for the automatic identification of mucinous pancreatic cysts (MCNs and IPMNs) using EUS images that revealed an excellent performance with 98.5% accuracy [166] (Figure 4). Unlike the study by Nguon et al., our algorithm focused on group classification rather than on the differentiation between only two different types of cysts and included not only MCNs but also IPMNs [166]. This fact is clinically very relevant as IPMN comprises the most frequent cystic neoplasia and its diagnosis during EUS may be challenging in the event of a single lesion or if the connection with MPD cannot be established. Moreover, our algorithm revealed a high image processing capacity with a reading rate of 139 frames per second that is necessary for future real-time implementation.

Nguon and collaborators included 109 lesions (60 MCNs and 49 SCAs) but used only 1 to 4 EUS images from each patient (130 and 81 images from MCNs and SCAs,

respectively) that were expanded using data augmentation (zoom, rotation/flip, perspective). Moreover, EUS images with variations in the demarcation of single or multiple regions of interest were obtained using both linear and radial echoendoscopes [165].

An increasing number of studies describe the use of video analysis and its potential advantages over the use of still images for AI algorithm development. In fact, a video contains spatiotemporal information that is not available using still images [93].

For CNN algorithm development, training and validation, we used images obtained through video decomposition, allowing the extraction of 5505 images (3725 from mucinous PCLs and 1780 from non-mucinous PCLs). In our study we used a supervised learning in which an expert endosonographer classified data input (correspondence to mucinous or non-mucinous lesions) into specific subgroups and a CNN was trained and validated to predict a labelled output [166].

Concerning risk stratification, Kuwahara et al. developed a DL model using EUS images to predict malignancy in pathologically confirmed IPMNs and compared the algorithm performance with standard of care-based predictions [159]. One of the main limitations of our study was that we did not have a surgical specimen as diagnostic gold standard for all the included lesions. The study by Kuwahara was the first to test the application of AI for malignancy diagnosis in IPMN. The AI model revealed higher diagnostic performance (95.7% sensitivity, 96.2% specificity and 94% accuracy) than endoscopists (56% overall accuracy) and outperformed high-risk predictors described by scientific societies guidelines. In fact, in multivariate analysis, that included several putative risk factors of malignancy, only AI determined probability was identified as an independent risk factor for malignancy [159]. This year, another study described the use of DL with EUS images to predict histological grade in IPMNs [167]. Using the EUS images from 43 patients who underwent surgery, the authors trained a CNN that was able to distinguish low-grade IPMN from high-grade IPMN/early invasive carcinoma with very high accuracy (99.6%) when applied to the EUS images from a group of 27 patients used for testing/validation [167]. These two studies expand the

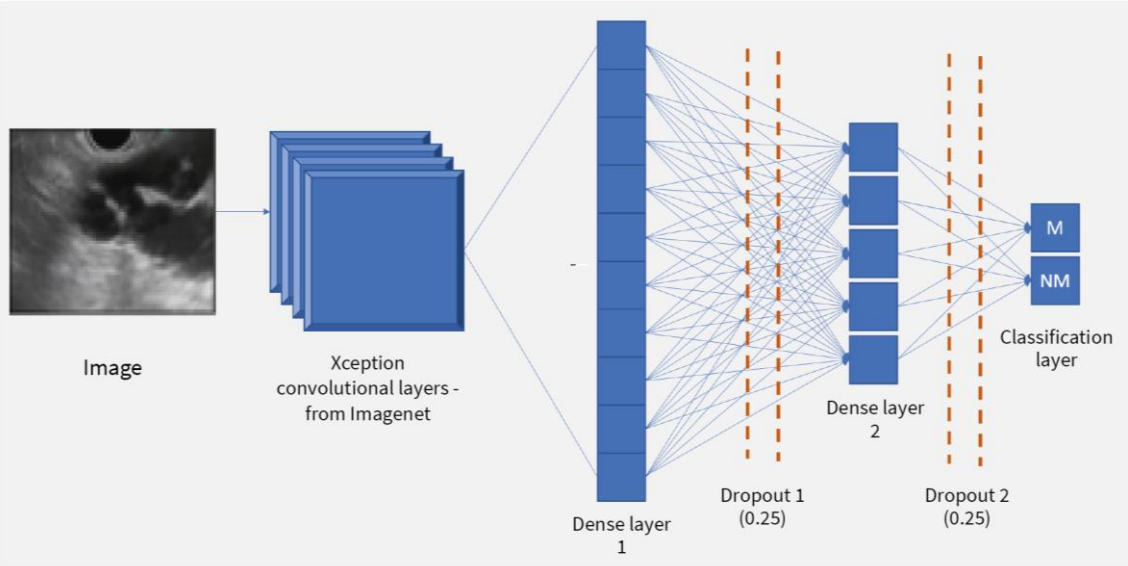
reach of our algorithm and together are a significant step forward towards the automatic detection of high-risk lesions, ultimately preventing unnecessary surgeries.

In another field, CLE is also recognized as an EUS-based complementary technique for PCLs evaluation. CLE is a real-time laser-assisted microscopic imaging of tissue facilitating *in vivo* histopathology. During EUS-CLE, a miniprobe is passed through a 19G needle but due to equipment costs and need to learn novel *in vivo* histopathological imaging, there is lack of widespread adoption of the technique [18, 98]. A recent systematic review that included 7 studies revealed a diagnostic accuracy measured by AUROC of 99% and low adverse event rate [168].

Machicado et al. developed a CNN algorithm using EUS-CLE images from 35 patients with histologically proven IPMNs. The model predicted the presence of HGD with 83.3% sensitivity, 88.2% specificity and 85.7% accuracy, which was superior to the prediction using the risk factors described by scientific guidelines [169].

Together, these studies prove the possibility to incorporate AI models in current management framework of PCLs, but the algorithms must be externally validated in prospective multicenter studies.

Figure 4. Overview of the architecture of the convolutional neural network. Each EUS image from the validation dataset was processed and ultimately classified by the algorithm in two distinct classes.



EUS – Endoscopic ultrasound; M – Mucinous pancreatic cystic lesion; NM – Non-mucinous pancreatic cystic lesion.

5.3. CONCLUSIONS

Because of the aging population and the increasingly frequent use of imaging studies, PCLs will continue to be a major referral indication to Gastroenterology consultation and an opportunity to identify the small but very relevant group of patients with Pca precursor lesions which will benefit from surgery or surveillance.

Currently, EUS morphology and EUS-FNA for cyst fluid biochemical and cytology analysis are imperfect for cyst type definition and risk stratification. Our research added more evidence to the previously published data concerning the relevant and relatively safe role of EUS-TTNB. TTNB, unlike for example CLE that depends on specific training, can be performed by any endosonographer with FNA skills. Also relevant is that, unlike molecular/genetic techniques, EUS-TTNB accessibility is easier and associated with lower costs.

Gastroenterology, and especially the field of endoscopy, relies on large amounts of data in the form of images, and has become one of the most important areas of application of AI. While for the detection and characterization of lesions in conventional endoscopy there are algorithms already in use in clinical practice, the application of AI in the analysis of EUS images and for PCLs classification, has been rarely described.

We demonstrated that:

- TTNB allows the definition of specific cyst type and has maximal clinical impact in the case of morphologically indeterminate cyst type with no communication with the MPD, when the exact identification of cyst nature is crucial to define management.
- For mucinous cysts, EUS-TTNB allows for dysplasia grade definition but correlation with surgical specimens is imperfect possibly because of inhomogeneous distribution of dysplasia along the cyst epithelium.

- EUS-TTNB samples allow for BD-IPMN subtyping based on morphology and MUC profile. Subtyping may have an impact on decision-making for high surgical risk patients with IPMNs harboring WF, but without HRS, because of the known differences in the potential for invasive progression and prognosis between different IPMN subtypes.
- Regarding the optimal EUS-TTNB technique, for cysts smaller than 20 mm, forceps preloading in the needle is essential to avoid cyst collapse and keep the space for forceps manipulation. Moreover, only one bite per pass should be performed because of the absence of a spike in the forceps that precludes the acquisition of two samples per pass.
- EUS-TTNB specimens may be processed as a cell-block that assures no loss of material due to the small size of the fragments, maintaining tissue structure.
- Two TTNB specimens may be enough for histological analysis as we did not observe significant differences between two and four specimens to ensure sample adequacy after protocol change during our study. This will reduce the risk of adverse events as we know it is linked to the number of passes, but must be balanced with possible limitations in the performance of IPMN subtyping
- A careful patient selection for EUS-TTNB is mandatory to reduce the risk of adverse events. We reported a 7.5% (3/40) adverse event rate, and if we exclude the two self-limited intracystic bleeding cases that some authors consider incidents rather than adverse events, this rate was much lower.
- AI may be applied successfully for the evaluation of EUS images to reduce misdiagnosis and standardize image interpretation.
- We developed a EUS-CNN that allowed automatic identification of mucinous pancreatic cyst with an excellent performance.
- The CNN revealed an image processing speed compatible with real-time implementation.

CHAPTER 6

FUTURE RESEARCH

The role of EUS-TTNB has been recently questioned, because of the risk of adverse events, especially in the case of presumed IPMNs and older patients. Given the availability of other modalities, namely CLE and molecular analysis, EUS-TTNB clinical impact should be evaluated in further, ideally larger, multicenter studies, with available surgical specimen correlation. In fact, the lack of surgical specimen for most of the enrolled patients is a problem of all studies evaluating the role of tissue sampling in PCLs.

Several questions remain without a definitive answer and should be explored in future investigations:

- It will be important to study the impact of preoperative IPMN subtyping for decision-making.
- Determine if TTNB performance prior to cyst fluid aspiration will improve cellularity of the sample, resulting in higher sensitivity for cytology, as manipulation of the forceps in the cyst wall could result in cell flushing to the fluid. This higher sensitivity could result in better diagnostic performance of EUS cyst puncture combining cytology and TTNB histology.
- Concerning adverse events, future research should explore the role of total procedure time and intracystic needle time in complication rate, as well as the mechanism(s) that results in higher risk of the technique in the case of IPMN lesions.
- The role of antibiotic prophylaxis as well as post-TTNB pancreatitis prevention using rectal NSAIDs or Ringer´s Lactate hyperhydration, should be further evaluated, ideally with specifically designed randomized controlled trials.

Regarding AI application in EUS evaluation of PCLs, several limitations of our pilot study should be addressed in future studies. The next step will be to expand our dataset to continue training and validation of the binary CNN, as the accuracy the AI algorithm will improve as the amount of data increases. This will be possible establishing collaborations with other centers, to share labeled datasets, ultimately putting forward a multicenter study that is already being designed – the “SmartCyst” study that will validate and test the algorithm using patient-split methodology.

Another future study will be to prospectively test the algorithm after integration in current EUS platforms that will allow real-time implementation. If we can significantly expand our dataset with EUS images from different types of cysts, the challenge will be to expand the mucinous vs non-mucinous automatic classification and develop a multi-class neural network with the ability to classify the specific cyst type.

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