From the Department of Clinical Science, Intervention and Technology (CLINTEC), Division of Surgery
Karolinska Institutet, Stockholm, Sweden

# IMPROVEMENT OF THE DIAGNOSIS AND MANAGEMENT OF CYSTIC TUMORS OF THE PANCREAS

Zeeshan Ateeb


Karolinska Institutet

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# Improvement of the Diagnosis and Management of Cystic Tumors of The Pancreas

Thesis For Doctoral Degree (Ph.D.)

By


## Zeeshan Ateeb


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*Principal Supervisor:*
**Professor Urban Arnelo**
Umeå University
Department of Surgical and Perioperative Sciences/Surgery
*and*
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Surgery

*Co-supervisor(s):*
**Professor Marco Del Chiaro**
University of Colorado - Anschutz Medical Campus
Department of Surgery
Division of Surgical Oncology

**Professor Margaret Sällberg Chen**
Karolinska Institutet
Department of Dental Medicine
Unit of Oral Diagnostics and Surgery

**Professor Matthias Löhr**
Karolinska Institutet
Department of Clinical Science, Intervention and Technology
Division of Surgery

**Stephan Haas, MD, PhD**
Karolinska Institutet
Department of Medicine Huddinge
Division of Gastroenterology and Rheumatology

*Opponent:*
**Professor Malin Sund**
University of Helsinki
Faculty of Medicine, Clinicum
Department of Surgery

*Examination Board:*
**Associate Professor Eva Szabo**
Örebro University
Department of Medical Sciences

**Professor Bengt Isaksson**
Uppsala University
Department of of Surgical Sciences
Division of Upper Abdominal Surgery

**Associate Professor Staffan Wahlin**
Karolinska Institutet
Department of Medicine Huddinge
Division of Gastroenterology and Rheumatology

*To my beloved **family** who has shown invaluable support, patience and trust on this journey.*

# POPULAR SCIENCE SUMMARY OF THE THESIS

Pancreatic cancer is around the tenth most common form of cancer. However, when it comes to the cancer-related cause of death, it is the fourth most common cause and is expected to become the second most common cause by 2030. The main reason for this is that pancreatic cancer is usually detected at a late stage when curative treatment is no longer possible. This, together with the aggressive nature of pancreatic cancer are reasons for the dismal prognosis. One strategy to reverse this negative trend is to improve and find new ways to diagnose pancreatic cancer or its precursors at a much earlier stage.

Pancreatic cysts occur in up to 50% of the population and consist of several different types of cysts. Some of these have the potential to progress to pancreatic cancer. The most common form of cyst with a malignant potential is the intraductal papillary mucinous neoplasm (IPMN). Of note, only 1-3% of these will develop into cancer. It is therefore important not only to correctly diagnose IPMN, but also to accurately identify those patients with IPMN that have a high risk for cancer. Current diagnostic methods have an accuracy of around 70-80% at most. This means that some patients with cysts are missed and develop pancreatic cancer and that some patients, on the other hand, undergo unnecessary, complicated and life-changing operations.

This thesis aimed to find new and improve existing diagnostic methods to diagnose cystic tumors of the pancreas.

In the clinical practice, imaging methods such as computed tomography and magnetic resonance imaging are used to characterize pancreatic cysts. One of the radiological markers used to find cyst with malignant potential is the width of the main pancreatic duct. It is known that a greater dilatation is associated with a high risk of cancer, but where this relationship (width of the main duct in millimeter) begins and the risk for cancer increases is still unclear. In **study I & II** we investigated this parameter more closely and correlated it to the histological (microscopic) findings. Patients who underwent surgery for IPMN between 2008 and 2017 were included and analyzed. The results showed that even a minor dilatation of more than 5 mm could imply a high risk of cancer. In addition to this parameter, it was also noted that elevated levels of the biomarker CA 19-9 were associated with cancer.

Until today, there are no good methods to analyze the fluid in the cysts. Those that exist have a low reliability and are therefore sparsely used. Analysis of metabolic products is an upcoming method with the potential to characterize and diagnose various tumors in more detail. The method, so-called metabolomics, has been applied to other types of tumors but has yet not been applied to pancreatic cysts to the same extent. The purpose of **studies III & IV** was to map the metabolic profile of the cyst types IPMN and SCN (a benign cyst) using metabolomics. Cyst fluid and blood were collected and analyzed from patients undergoing surgery for cystic tumors. The analyses in study III were able to identify several different metabolites in both cyst fluid and plasma. These could later be used in an analytical model to distinguish IPMN from SCN, i.e. the cyst type with the potential for cancer development from the benign cyst type.

These results were then validated in study IV in which we also found new metabolites. Moreover, the different cyst types could be distinguished with high reliability. In addition, we also found associations between specific metabolites and bacteria in the cyst fluid. The results from these studies show the potential of metabolomics and its application for pancreatic cystic tumors and plasma and could be implemented in clinical practice in the future.

Patients who undergo surgery for cystic tumors, where a part is removed, and a part is left, so-called partial resection, have a risk of recurrence of the tumor in the remaining part (remnant). However, the rate of recurrence and what strategy should be applied for these patients is still unclear and debated. The purpose of **study V** was thus to investigate the rate of recurrence in patients who underwent partial resection for IPMN. All patients who underwent partial resection for IPMN, between the years of 2008 and 2017 and with a follow-up time of at least two years were included. A total of 224 patients were included where the significant recurrence rate was 30.8%. As it is already known that cancer has a high recurrence rate, further analyses were performed in patients with pre-cancerous cysts (low-grade and high-grade dysplasia). The significant recurrence rate in these patients was 11.1%. The results show that even patients with pre-malignant histological findings have a significant risk of recurrence. This means that this category of patients should still be followed up regularly.

In summary, this thesis has provided more knowledge about new and existing diagnostic methods of pancreatic cystic tumors and has highlighted the importance of continued follow-up of already operated patients.

# POPULÄRVETENSKAPLIG SAMMANFATTNING

Cancer i bukspottskörteln, även kallad pankreas, är runt den tionde vanligaste förekommande formen av cancer. Däremot, när det gäller den cancerrelaterade dödsorsaken, innehar den plats fyra och beräknas bli den näst vanligaste orsaken tills år 2030. Den främsta anledningen till detta är att pankreascancer oftast upptäcks i ett sent skede när botande behandling inte längre är möjligt. Detta tillsammans med pankreascancerns aggressiva natur gör att prognosen är dyster. En strategi för att vända denna negativa trend är att försöka hitta pankreascancer eller dess förstadium i ett tidigt skede.

Pankreascystor, så kallade vätskefyllda tumörer i bukspottkörteln, förekommer upp till 50% i befolkningen och består av flera olika cysttyper. Några av dessa har en potential att utvecklas till pankreascancer. Den vanligaste formen av cystorna med potential till cancerutveckling är intraduktal papillär mucinös neoplasm (IPMN). Dock är det enbart 1-3% av dessa som kommer att utvecklas till cancer. Det gäller således att inte bara korrekt diagnosticera IPMN bland cystorna utan även hitta just de IPMN som har hög potential till cancerutveckling. Dagens diagnostiska metoder har en tillförlitlighet på som högst 70-80%. Detta gör att vissa patienter med cystor feltolkas och utvecklas till pankreascancer och att vissa patienter å andra sidan genomgår komplicerade operationer i onödan.

Denna avhandling syftade till att hitta nya och förbättra befintliga diagnostiska metoder för cystiska tumörer i pankreas.

I den kliniska vardagen används främst bildgivande metoder såsom skiktröntgen och magnetkamera för att karaktärisera cystorna. En av de radiologiska markörerna som används idag är vidden på huvudgången i pankreas. Det är känt att en större dilatation är associerad med hög risk för cancer, men var denna relation (vidden av huvudgången i millimeter) börjar och därmed risken för cancer ökar är ännu oklart. I **studie I & II** har vi närmare undersökt denna parameter och korrelerat det till de histologiska (mikroskopiska) fynden. Patienter som genomgått kirurgi för IPMN mellan 2008 och 2017 inkluderades och analyserades. Resultaten visade att även en mindre dilatation över 5 mm är kan innebära en hög risk för cancer. Utöver denna parameter noterades även att förhöjda nivåer av biomarkören CA 19-9 är associerade med cancer.

Än idag finns det inga väletablerade metoder för att analysera vätskan i cystorna. De metoder som finns har en låg tillförlitlighet och används därför sparsamt. Analys av ämnesomsättningsprodukter, s k metaboliter, är en kommande metod med potential att i mer detalj kunna karaktärisera och diagnosticera olika tumörer. Metoden, s.k. metabolomik, har kunnat appliceras på andra tumörer men har ännu inte utforskats på pankreascystor i samma utsträckning. Syftet med **studie III & IV** var att med hjälp av metabolomiken kunna kartlägga den metaboliska profilen av cysttyperna IPMN och SCN (en godartad cysta). Cystvätska och blod samlades in och analyserades från patienter som hade genomgått kirurgi för cystiska tumörer. Analyserna i studie III kunde identifiera flera olika metaboliter i både cystvätska och plasma. Dessa kunde senare användas i en analytisk modell för att urskilja IPMN från SCN dvs

den cysttypen med potential för cancerutveckling från den godartade cysttypen. Dessa resultat validerades därefter i studie IV där ytterligare metaboliter kunde identifieras. Även här kunde de olika cysttyperna gå att urskilja med hög tillförlitlighet. Utöver det sågs även en koppling mellan specifika metaboliter och bakterier i cystvätskan. Resultaten från dessa studier visar på potentialen av analysen av metaboliter i cystvätskan och skulle i framtiden kunna implementeras i den kliniska vardagen.

Patienter som genomgår kirurgi för cystiska tumörer, där en del av pankreas tas bort och en del lämnas kvar, så kallad partiell resektion, har en risk för återkomst av tumör i den kvarvarande delen. Hur stor denna risk är och vilken uppföljningsstrategi man bör använda för dessa patienter är dock ännu oklart och omdebatterat. Syftet med **studie V** var ett undersöka frekvensen av återfall hos patienter som genomgått en kirurgi för IPMN. Alla patienter som opererats för IPMN, där en del av bukspottkörteln tagits bort, mellan åren 2008 och 2017 och med en uppföljningstid på minst två år inkluderades. Totalt inkluderades 224 patienter där den signifikanta återfallsfrekvensen låg på 30,8%. Eftersom det redan är känt att pankreascancer har en hög återfallsfrekvens gjordes ytterligare analyser av patienter vars histologiska analyser visade att cystorna var förstadium till cancer (låggradig- och höggradig dysplasi). Den signifikanta återfallsfrekvensen för dessa patienter var 11,1%. Resultaten visar att även patienter med förstadium till cancer, innehar en icke försumbar risk för återfall. Detta innebär att även denna kategori av patienter bör följas upp noggrant och regelbundet.

Sammanfattningsvis har denna avhandling och dess ingående studier givit mer kunskap inom olika diagnostiska metoder av pankreascystor samt belyst vikten av fortsatt uppföljning av redan opererade patienter.

# ABSTRACT

Pancreatic cancer is on its course on becoming the second cause of cancer related mortality. Although improvements have been made in the treatment arsenal, still only a minority of patients are able to receive treatment with curative intention. This can be attributed to the fact that most of the pancreatic cancers are diagnosed at a later stage when curative therapy is no longer possible. Thus, efforts are being made to find pancreatic cancer and its precursor lesions at an earlier stage. Intraductal papillary mucinous neoplasms (IPMN) is the most common type of cystic lesion which has the potential for malignant transformation. Hence, an accurate and early diagnosis of this entity could contribute to reverse the dismal trend of pancreatic cancer.

**Study I & II**

***Aim:*** To identify and correlate risk factors for advanced histological findings in IPMN patients. ***Methods:*** These were retrospective studies in which patients undergoing resection for IPMN during 2008-2015 (study I) and 2004-2017 (study II) were included. Patients characteristics, radiological and histological data were prospectively collected. ***Results:*** One-hundred-fifty-two patients were included in study I and 796 patients in study II. In **study I**, main pancreatic duct (MPD) dilatation of 6-9.9 mm and >10 mm were associated with an increased risk of advanced IPMN histology, such as high grade dysplasia (HGD) and cancer, at odds of 2.92 (CI 1.38–6.20, $p$=0.005) and 2.65 (CI 1.12–6.25, $p$=0.02), respectively. In addition, jaundice and elevated levels of CA 19-9 were also associated with higher risk for HGD/cancer at odds of 15.36 (CI 1.94–121.22, $p$=0.009) and 4.15 (CI 1.90–9.05, $p$=0.0003), respectively. These associations remained significant at uni- and multivariable regression analysis. In **study II**, the results showed MPD-dilatation to be the lone significant variable associated with increased probability of HGD or IPMN-cancer at both uni- and multivariable analysis. MPD dilatation of 5-9.9 mm was associated with odds of 2.74 (CI 1.80–4.16) and 4.42 (CI 2.55–7.66) for HGD and IPMN-cancer respectively. MPD-dilatation over 10 mm was associated with greater odds of 6.57 (CI 3.94–10.98) and 15.07 (CI 8.21–27.65) for HGD and IPMN-cancer, respectively. A 5-7 mm diameter of the MPD was determined as the cut-off value to best discriminate between the lesions with low risk of malignancy to those with a high risk. ***Conclusions:*** Even a smaller dilatation of the MPD is associated with increased risk of HGD and IPMN-cancer. Dilatation of the MPD and elevated levels of CA 19-9 are important diagnostic markers of advanced histology, thereby facilitating proper selection of patients most suitable for surgery.

**Study III & IV**

***Aim:*** To define and validate the metabolic profile of patients with IPMN and serous cystic neoplasm (SCN) and to correlate the metabolite levels to histology and grade of dysplasia. ***Methods:*** Plasma and cyst fluid were prospectively collected from patients undergoing resection for IPMN and SCN. Targeted and untargeted analysis of metabolites and lipids species were performed and correlated to histology and clinical parameters. ***Results:*** From a cohort of 35 patients in study III and 57 patients in study IV several metabolites and lipids were identified in both cyst fluid and plasma. In **study III**, the metabolic profile showed significant

alterations in the lipid pathways. An integrated metabolomic and lipidomic analysis model was able to discriminate IPMN from SCN up to 100% accuracy. The results in **study IV** not only validated the results from study III but also found novel metabolites able to discriminate non-cancerous lesions (low-grade IPMN and SCN) from malignant (HGD and cancer). Furthermore, specific metabolites correlated to presence of bacteria sequences in the cyst fluid. ***Conclusion:*** Analysis of the metabolic profile in cyst fluid and plasma from IPMN patients has been able to discriminate IPMN from SCN with high accuracy and also to predict the degree of dysplasia within IPMN. This method has shown potential of clinical application which in turn could improve the diagnosis of pancreatic cystic lesions.

**Study V**

***Aim:*** To investigate the rate of new recurrence and progression of known IPMNs in the remnant pancreas after pancreatic surgery and to investigate if the grade of dysplasia at first histology affects the risk of recurrence. ***Methods:*** This was a retrospective study in which patients undergoing an elective partial pancreatic resection between 2008 and 2017 were included. Patients who underwent total pancreatectomy and/or had less than 2 years of follow-up were excluded. Patient characteristics and data of radiology, histology and recurrence was collected prospectively. Clinical significant recurrence was defined as findings resulting in a change in the management of the lesion. ***Results:*** Overall 224 patients were included in the study. The overall recurrence rate was 44.6% (100/224), whereas the clinical significant recurrence rate was 30.8% (69/224). Patients older than 65 years presented 4.4 odds (CI 1.5-13.1) of recurrence and patients with "known IPMN left in remnant" had 2.6 odds (CI 1.12-5.9) of recurrence. Patients with LGD and HGD at first histology without concomitant PDAC had a clinical significant recurrence rate of 11.1% (15/135). No differences regarding risk of recurrence could be found when comparing patients with LGD to HGD (HR 1.1 [CI 0.5-2.2]). ***Conclusion:*** Patients with LGD and HGD at first histology harbor a not negligible risk of future malignant transformation and should not be overlooked. The risk is further increased if the patient is older and/or have a previously known IPMN in the remnant.

The **overall conclusion** of the thesis is that the included studies have increased the knowledge on several aspects in the diagnosis and management of cystic tumors of the pancreas. A known radiological diagnostic marker, the dilatation of the main pancreatic duct, has been studied where the results show that even smaller dilatations over 5 mm may indicate malignancy. An upcoming field in metabolomics has been applied on pancreatic cystic neoplasms and has shown great potential as a future diagnostic method. Lastly, the post-operative management of IPMN patients has been studied where the results highlight the need for continued clinical surveillance due the risk of recurrence even after surgery.

# LIST OF SCIENTIFIC PAPERS

I. **Main pancreatic duct dilation greater than 6 mm is associated with an increased risk of high-grade dysplasia and cancer in IPMN patients.**
**Ateeb Z***, Valente R*, Pozzi-Mucelli RM, Malgerud L, Schlieper Y, Rangelova E, Fernandez-Moro C, Löhr JM, Arnelo U, Del Chiaro M. Langenbecks Arch Surg. 2019 Feb;404(1):31-37. doi: 10.1007/s00423-018-1740-8. Epub 2019 Jan 5. PMID: 30612152 **Equal contribution*

II. **Main Duct Dilatation Is the Best Predictor of High-grade Dysplasia or Invasion in Intraductal Papillary Mucinous Neoplasms of the Pancreas.**
Del Chiaro M*, Beckman R*, **Ateeb Z**, Orsini N, Rezaee N, Manos L, Valente R, Yuan C, Ding D, Margonis GA, Yin L, Cameron JL, Makary MA, Burkhart RA, Weiss MJ, He J, Arnelo U, Yu J, Wolfgang CL.
Ann Surg. 2020 Dec;272(6):1118-1124. doi: 0.1097/SLA.0000000000003174. PMID: 30672797 **Equal contribution*

III. **Integrated targeted metabolomic and lipidomic analysis: A novel approach to classifying early cystic precursors to invasive pancreatic cancer.**
Gaiser RA*, Pessia A***, Ateeb Z***, Davanian H, Fernández Moro C, Alkharaan H, Healy K, Ghazi S, Arnelo U, Valente R, Velagapudi V, Sällberg Chen M, Del Chiaro M.
Sci Rep. 2019 Jul 15;9(1):10208. doi: 10.1038/s41598-019-46634-6. PMID: 31308419; PMCID: PMC6629680 **Equal contribution*

IV. **Metabolic Characterization of Plasma and Cyst Fluid from Cystic Precursors to Pancreatic Cancer Patients Reveal Metabolic Signatures of Bacterial Infection.**
Morgell A*, Reisz JA*, **Ateeb Z**, Davanian H, Reinsbach SE, Halimi A, Gaiser R, Valente R, Arnelo U, Del Chiaro M, Chen MS, D'Alessandro A.
J Proteome Res. 2021 May 7;20(5):2725-2738. doi: 10.1021/acs.jproteome.1c00018. Epub 2021 Mar 15. PMID: 33720736
**Equal contribution*

V. **Recurrence and progress of IPMN in the remnant pancreas after partial resections**
**Ateeb Z**, Franco SR, Valente R, Ghorbani P, Sparrelid E, Gilg S, Del Chiaro M, Arnelo U.
*In manuscript*

**Scientific papers not included in the thesis:**

**Isolation of pancreatic microbiota from cystic precursors of pancreatic cancer with intracellular growth and DNA damaging properties**
Halimi A, Gabarrini G, Sobkowiak MJ, **Ateeb Z**, Davanian H, Gaiser RA, Arnelo U, Valente R, Wong AYW, Moro CF, Del Chiaro M, Özenci V, Chen MS. Gut Microbes. 2021 Jan-Dec;13(1):1983101. doi: 10.1080/19490976.2021.1983101. PMID: 34816784; PMCID: PMC8632270.

**Circulating and Salivary Antibodies to Fusobacterium nucleatum Are Associated With Cystic Pancreatic Neoplasm Malignancy.**
Alkharaan H, Lu L, Gabarrini G, Halimi A, **Ateeb Z**, Sobkowiak MJ, Davanian H, Fernández Moro C, Jansson L, Del Chiaro M, Özenci V, Sällberg Chen M. Front Immunol. 2020 Aug 28;11:2003. doi: 10.3389/fimmu.2020.02003. PMID: 32983143; PMCID: PMC7484485.

**Enrichment of oral microbiota in early cystic precursors to invasive pancreatic cancer.**
Gaiser RA, Halimi A, Alkharaan H, Lu L, Davanian H, Healy K, Hugerth LW, **Ateeb Z**, Valente R, Fernández Moro C, Del Chiaro M, Sällberg Chen M. Gut. 2019 Dec;68(12):2186-2194. doi: 10.1136/gutjnl-2018-317458. Epub 2019 Mar 14. PMID: 30872392; PMCID: PMC6872446.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| BD-IPMN | Branch-duct Intraductal Papillary Mucinous Neoplasm |
| CA 19-9 | Carbohydrate Antigen 19-9 |
| CEA | Carcinoembryonic Antigen |
| CI | Confidence Interval |
| DFS | Disease Free Survival |
| EUS-FNA | Endoscopic Ultrasound guided Fine Needle Aspiration |
| HG-IPMN | High Grade Intraductal Papillary Mucinous Neoplasm |
| HGD | High Grade Dysplasia |
| HR | Hazard Ratio |
| IPMN | Intraductal Papillary Mucinous Neoplasm |
| LG-IPMN | Low Grade Intraductal Papillary Mucinous Neoplasm |
| LGD | Low Grade Dysplasia |
| MCN | Mucinous Cystic Neoplasm |
| MD-IPMN | Main Duct Intraductal Papillary Mucinous Neoplasm |
| MGD | Moderate Grade Dysplasia |
| MT-IPMN | Mixed Type Intraductal Papillary Mucinous Neoplasm |
| OR | Odds Ratio |
| OS | Overall Survival |
| PC | Pancreatic Cancer |
| PCA | Principal Component Analysis |
| PCN | Pancreatic Cystic Neoplasm |
| PDAC | Pancreatic Ductal Adenocarcinoma |
| SCN | Serous Cystic Neoplasm |
| TAG | Triacylglycerol |
| TP | Total Pancreatectomy |
| WHO | World Health Organization |

# 1 INTRODUCTION

Pancreatic cancer (PC) is a rare but fatal disease and is currently the number four cause of cancer-related mortality in Western countries. With a 5-year survival rate of 7-10% (1-3) and a mortality rate being almost similar to the incidence rate of 80-90% (4), PC is considered to be one of the most lethal cancers (5). It is expected to become the number two cause of cancer-related death by the year of 2030 (6-8) and is for this reason considered to be a medical emergency (9).

In order to reduce the risk of mortality and morbidity, the strategy is to detect precancerous lesions at an early stage enabling curative therapy (6, 10). If PC is detected and treated early at stage 1 the 5-year survival rate could increase to 50-69% and if at stage 0, the 5-year survival rate could increase to as much as 80-85% (11, 12).

Pre-emptive surgery is a widely recognized form of prophylactic surgery where cystic or other precursor lesions are resected before they transform into malignancy. For instance, in esophageal surgery, endoscopic submucosal dissection (ESD) or ablation is performed for resection of high-grade dysplasia in patients with Barrett's esophagus. The corresponding pre-emptive surgery in pancreas could represent resection of the precursor lesion or even the whole pancreas (6). However, considering that only a small percentage of the precursor lesions in the pancreas progress to cancer (13), it is of utmost importance to have accurate diagnostic methods to prevent unnecessary and risky surgical procedures (14).

The two main precursor lesions for pancreatic ductal adenocarcinoma (PDAC) are pancreatic intraepithelial neoplasia (PanIN) and mucin producing cystic lesions of the pancreas (15). Although PanIN is the underlying cause of approximately 80% of PDAC it cannot be detected before malignant transformation and tumor formation the same way as cystic lesions in the pancreas can be detected at an earlier stage (16).

# 2 LITERATURE REVIEW

## 2.1 CYSTIC LESIONS

With the improvement of abdominal imaging modalities and increased preventive health-check-ups, pancreatic cystic neoplasms (PCN), are more commonly detected (7, 10). The reported prevalence of cystic lesions varies between studies and the used imaging modality. In studies in which the PCNs were detected by computed tomography (CT), the prevalence is 2.2-2.6% (10, 17, 18). When using magnetic resonance imaging (MRI) the rate increases to 20-49% (10, 17). In studies of post-mortem examinations, the prevalence is estimated to be up to 50% (7, 10). The prevalence increases with age and no difference has been noted between genders (19, 20).

Approximately 70% of cystic lesions are asymptomatic and the vast majority of PCNs, are benign (4, 17, 19). A systematic review and meta-analysis of 17 studies by Zerboni *et al.* showed an 8% incidental finding rate of cystic lesions of which 0.7% had radiological or clinical signs of malignancy (21). In contrast, a prospective study of 1077 patients showed no progress to cancer, however, a minimal progress was seen in half of the patients (20).

Pancreatic cystic neoplasms comprise a heterogeneous group of lesions of which a few harbor the potential to progress to malignancy (7, 22). The three main distinctive entities of pancreatic cystic tumors are: intraductal papillary mucinous neoplasia (IPMN), mucinous cystic neoplasm (MCN) and serous cystic neoplasm (SCN) (23). SCN is considered to be a benign lesion whereas the mucin producing cystic lesions, MCN and IPMN, harbor the risk of malignant transformation and can, in later stages, even metastasize (24). Hence, it is of great significance that these precursor lesions are correctly identified and characterized in order to manage the potential malignant lesions accordingly (17).

Until recently, the natural history of the pancreatic cysts was ill-defined. There is accumulating evidence that it takes over a decade to evolve progressively from a benign into a more aggressive malignant cyst (6). A retrospective analysis of 1815 patients with PCNs by Wu *et al.* showed a total cyst-related malignancy rate of 2.9% (25). Thirty-nine patients (2.1%) had a malignant cyst at diagnosis. Out of the surveillance cohort of 1735 patients, 14 (0.8%) developed a cyst related malignancy with an average duration of surveillance of 23.4 months and a total 3383 person-years of follow-up (25).

### 2.1.1 Pseudocyst

The most common cystic lesion in the pancreas is the pancreatitis-related pseudocyst, representing approximately 34% of all cystic lesions of the pancreas (17). The distinguishing factor is the recent history of pancreatitis prior to the discovery of the cyst. The cystic fluid is of low viscosity with low levels of CEA and high levels of amylase and lipase (26).

### 2.1.2 Serous Cystic Neoplasm

Serous cystic neoplasms (SCN) accounts for 1-2% of all pancreatic neoplasms and up to 33% of all cystic lesions (17). They are glycogen rich and arise from cuboidal epithelium (26). SCNs are generally benign lesions with a very small risk of under one percent for malignant transformation (17, 26).

SCNs are strongly related to an older age and the female gender with a ratio of 4:1 and is therefore nick-named the "grandmother lesion". The most common location is the head of pancreas where 40% of the SCNs are found. Importantly, they lack communication with the main pancreatic duct (MPD). There are three main appearances of SCNs. Seventy percent have a polycystic appearance and 20% have a honey-comb appearance. The oligocystic SCN stands for the remaining 10% and can be mistaken for the mucinous cystic neoplasm (MCN) which poses a diagnostic problem (17, 27). The etiology and origin are not well understood but there is a genetic disposition. Up to 15% of patients with von Hippel-Lindau syndrome (VHL) have SCNs (26).

In similarity to the pseudocysts, the cystic fluid is mucin free with low viscosity, but also has low levels of amylase, CEA and CA 19-9 (17, 26, 28). The only indication for surgical resection is the presence of clinical symptoms (28). Recurrence of SCNs after surgery is rare (26).

### 2.1.3 Mucinous Cystic Neoplasm

Mucinous cystic neoplasms are a form of mucin producing pancreatic cystic lesions that develops almost only in women and those who are mostly younger to middle-aged patients (22). It is therefore called "the mother lesion" and stands for 10% up to 45% of all PCNs (17, 26). In those rare cases where MCNs occur in men, the patients are usually older (17). The most common location of MCNs is in the tail in approximately 72% of the cases. The appearance is typically large, round or oval cysts without any connection to the pancreatic duct system. They can be both unilocular, which is most common, or multi-locular with septations (17, 26). The peripheral wall consists of a thick fibrous capsule which may calcify (17). The inner wall is constructed of columnar epithelium with underlying ovarian-like stroma. The epithelium can be stained for estrogen and progesterone receptor and beta-human chorionic gonadotropin (β-HCG) (26). This may explain why MCNs are mostly developed in women. The cystic fluid has a high viscosity high with high levels of CEA and low levels of pancreatic amylase (26).

MCN is a precursor lesion of PDAC with risk for malignant transformation ranging between 10% and 50%. Genetically, *KRAS*-mutation and the frequency of mutation is associated with extent of dysplasia and malignant transformation. Moreover, overexpression of *p53* is associated with invasive MCNs (29). In a study by Iacobuzio-Donahue CA *et al.* a loss of expression of the DPC4 protein was seen in 85% of invasive MCNs, however no benign MCNs were analyzed in the study (30).

Radiologic signs for malignancy are summarized in Table 1 (17). Using these features to distinguish malignant from benign MCNs, a sensitivity of 81% and a specificity of 83% has been calculated. The chance of malignancy is 95% if the cystic lesion has a thick surrounding wall, internal septations, and calcifications of the wall or septa. If two out of these three criteria are met, the chances of malignancy is 56% to 74% (17). The prognosis depends on the invasiveness of the lesion. If the malignant cyst is noninvasive, the prognosis is very good. Of note, the recurrence rate of MCN is low after surgical resection (17).

***Table 1*** *- Radiologic features correlated with malignant transformation of Mucinous Cystic Neoplasms, as reported by Burk et al. (17)*

| |
|---|
| Wall thickening/irregularity |
| Solid enhancing components |
| Mural nodularity |
| Papillary projections |
| Size ≥ 4 cm |
| Patients age ≥ 55 years |
| Peripheral eggshell calcifications |

### 2.1.4 Intraductal Papillary Mucinous Neoplasm

Intraductal papillary mucinous neoplasms (IPMN) was first reported by Ohashi *et al.* in 1982 and are as the name suggests mucin producing papillary-like neoplasms located in the duct system of the pancreas (17, 31, 32). Typically, the mucin is found within the cytoplasm of epithelial cells and within the acellular fluid matrix (26). When proliferation of these epithelial cells occurs, they form papillae and subsequently a thick mucus. These changes could create macroscopic masses and result in distal dilatation of the ducts which may be visible on imaging modalities (33, 34).

#### *2.1.4.1 Degree of Dysplasia*

The degree of changes or cellular atypia of the proliferative epithelial cells vary and the degree of atypia determines the aggressiveness (17). Low grade dysplasia is regarded as a benign condition with tall columnar cells with no or only minor changes of cellular atypia (17, 35). Moderate grade dysplasia has moderate cellular atypia with signs of hyperchromatic and enlarged nuclei (35). High-grade dysplasia are seen as pre-malignant as the following stage is invasiveness and malignancy (17). At this stage, the cellular and nuclear atypia is significant with loss of polarity and with enlarged and pleomorphic nuclei (35).

#### *2.1.4.2 Prevalence*

IPMN was recognized by the World Health Organization as a clinopathological entity in 1996 (26). The prevalence in the literature accounts the IPMN for 21% to 33% of all cystic lesions in the pancreas (17). However, as more knowledge is gained regarding these lesions along with improved imaging modalities, an increasing number of PCNs are classified as IPMN (33).

IPMN occur almost equally in men and women, with a slight predominance in men with 60% (10, 17, 33). Similar to the serous cystic neoplasms, they occur at an older age and are therefore

nicknamed as “the grandfather lesion”. The cystic fluid containing mucin is highly viscous with high levels of CEA and amylase (17, 26).

#### *2.1.4.3 Types of IPMN*

There are two major types of IPMN which differs in morphology and aggressiveness. The first is branch-duct IPMN, also called side-branch IPMN. As the name implies, these lesions occur in the side branches of the duct system and are usually asymptomatic (17). They stand for around 60% of the IPMN and usually occur in the head or uncinate process of the pancreas (17, 36).

The other type is the main duct-IPMN which involves the main duct and can lead to obstruction of the duct and pancreatitis (17). They are often segmental and affecting a small part of the main duct. The majority of MD-IPMNs are found in the pancreatic head in 58% and the pancreatic body in 23% of the cases. In 12%, the lesion is diffuse and may affect the whole main duct (17).

A third type, mixed-type IPMN involves both the main and branch ducts and is considered as a subtype of MD-IPMN (17).

#### *2.1.4.4 Risk of Progression and Malignancy*

In similarity to MCN, IPMN harbor a risk for malignant transformation (7). The exact risk is still unclear and the numbers vary in studies (33). In resected patients, MD-IPMN is associated with a malignancy rate of 4-80% (4). The malignancy rate for BD-IPMN is at 20% in resected patients.

Regarding the risk of progression, MD-IPMN is associated with a higher risk. MD-IPMN will progress into cancer in 57-92% of the patients and in 6-72% for patients with BD-IPMN (4, 35). Recently, several studies have shown that BD-IPMN, especially those that are small in size (<15 mm) and do not have any radiological risk factors at diagnosis, will rarely develop into cancer and may therefore be surveilled less frequently (37-40). However, surveillance is still recommended by the guidelines due to the risk of progression to cancer (41-43).

In the study by Han *et al.*, 0.9% of BD-IPMN patients transformed into malignancy in a 3-year period (38). However, IPMN is a slow proliferating disease which is supported by Pergolini *et al.* reporting that 8% of the patients followed-up for 10 years developed malignancy (44). Additionally, Del Chiaro *et al.* has shown that all IPMN will progress over time (45).

Considering the prognosis of resected IPMN, non-invasive IPMNs have a 5-year survival rate of 80-100% while invasive IPMNs have a 5-year rate of 40-60% (35). This supports and emphasizes that need to identify IPMN at an early stage where a resection at correct time could hinder the progression to adenocarcinoma (4, 46).

Radiological and tumor markers indicating malignancy or malignant transformation are described in Table 2 in terms of sensitivity, specificity and accuracy as reported in the systemic review and meta-analysis by Sultana et al. (47).

***Table 2*** *– Radiologic and tumor markers indicating malignancy in IPMN with respective sensitivity, specificity and accuracy, as reported by Sultana et al. (47).*

| Characteristic | Sensitivity (95% CI) | Specificity (95% CI) | Accuracy (95% CI) |
|---|---|---|---|
| Presence of risk features on CT/MRI | 0.809 (0.714–0.883) | 0.762 (0.654–0.851) | 0.856 (0.778–0.915) |
| Presence of risk features on PET | 0.968 (0.900–0.995) | 0.911 (0.815–0.998) | 0.985 (0.949–0.998) |
| Enhancing mural nodule | 0.690 (0.585–0.793) | 0.798 (0.722–0.862) | 0.819 (0.719–0.925) |
| Main ductal dilatation ≥ 10 mm | 0.614 (0.471–0.746) | 0.687 (0.564–0.799) | 0.702 (0.596–0.838) |
| Cyst Size ≥ 3 cm | 0.682 (0.575–0.789) | 0.574 (0.43–0.702) | 0.657 (0.575–0.766) |
| Cyst fluid elevated CEA levels | 0.636 (0.179–0.926) | 0.720 (0.48–0.894) | 0.843 (0.481–0.997) |
| Elevated serum CEA levels | 0.169 (0.074–0.321) | 0.933 (0.867–0.972) | 0.691 (0.375–0.996) |
| Elevated serum CA19-9 levels | 0.380 (0.156–0.634) | 0.903 (0.846–0.947) | 0.729 (0.651– 0.792) |
| Combinations | 0.743 (0.542–0.9) | 0.906 (0.782–0.963) | 0.907 (0.701–0.999) |

#### *2.1.4.5 Subtypes of IPMN*

There are four main morphological subtypes of IPMN depending on the type of papillae (48). Classification is based on immunohistochemistry against mucin proteins such as MUC1, MUC2, MUC5AC, MUC6 (48).

I. ***Gastric*** – The papillae are similar to thick-fingers or small tubules (48). They are positive for MUC5AC & MUC 6 (26, 48). The gastric subtypes is typically found in patients with BD-IPMN patients and is associated with low-grade dysplasia (26)
II. ***Intestinal*** – Villous papilla that are positive for MUC5AC & MUC2 (48). The intestinal subtype is associated with MD-IPMN (26, 49). A malignant transformation in intestinal type is of colloid-type adenocarcinoma and have a better prognosis (50).
III. ***Pancreatobiliary*** – This subtype has fern-like papillae positive for MUC1, MUC5AC & MUC6 (48). Can be found in both BD-or MD-IPMN (26). This subtype is associated with tubular type adenocarcinoma and have poor prognosis (50-52).
IV. ***Oncocytic*** – Tend to occur in younger population (53). The papillae is phylloid and is positive for MUC5AC & MUC 6 and perhaps also MUC1 & 2 (26). The prognosis is less favorable for this subtype (53).

#### *2.1.4.6 Recurrence of IPMN*

Follow-up and surveillance should continue even after resection of mucinous cystic lesions since there is a risk of recurrence or progression of remaining small lesions in the remnant (54). There are two possible underlying mechanisms. Firstly, a local recurrence of the initial cancer lesion and secondly, a metachronous occurrence of new primary tumor (55). Previous studies on IPMN recurrence have been relatively small in sample size and have reported a wide range of remnant progression rates between 8% and 50% (54, 56). Some of these previous studies have included patients with invasive IPMN which might underestimated the rate of recurrence due to early mortality of metastasis meaning the patients dies before a recurrence is found (54). Another issue with current published studies is the variety of definitions of progression and recurrence (54).

Al Efishat *et al.* found in their study comprising 319 patients, a cumulative recurrence rate of 10% at 2 years after resection and 26% at 5 years. They also found that patients with a lesion in the body and tail had a higher risk for progression compared to patients with a lesion in the pancreatic head and neck (HR 2.43; 95% CI [1.47-4.0], $p<0.001$) (54).

Hirono *et al.* performed a multicenter retrospective study of 1074 patients who underwent resection for IPMN. The results showed a recurrence rate of 14.4% at a median time of 24.0 months. The rate of extra pancreatic recurrence was 8.2%. A subgroup analysis of metachronous high risk lesions, which was defined as; symptoms, location of body/tail, MPD ≥10 mm and IPMC (IPMN with high-grade dysplasia), showed a rate of recurrence of 34.3% at 5 years. Also, those patients who underwent a second operation of these high-risk lesions, had a better overall survival compared to those who did not (57).

The International Consensus Guidelines (ICG) 2017 suggests that in the majority of patients, the recurrence and progression occur 5-10 years after surgery. Consequently, surveillance should be performed for as long as the patient is clinically fit to undergo surgery (50). The European expert consensus statement concur with the ICG and further suggests the interval of follow-ups to be based on the grade of dysplasia in the resected specimen (58). For patients with resected IPMN with low-grade dysplasia, the disease-free survival (DFS) is approximately 52 months. The DFS for patients with IPMN with high-grade dysplasia or invasive carcinoma is significantly shorter with 29 months (58).

## 2.2 DIAGNOSTIC METHODS

The currently available diagnostic methods have a low accuracy rate of around 60% to define the correct nature of the cystic lesion (59). The relatively low accuracy rate results in difficulties to determine a suitable management. New diagnostic approaches to improve the diagnostic accuracy such as molecular and genetic analysis of the cystic fluid are promising but are not yet implemented in clinical practice (60, 61).

### 2.2.1 Imaging Modalities

#### *2.2.1.1 Magnetic Resonance Imaging*

Improvement of the imaging modalities have resulted in an increased application of imaging methods to examine and follow changes in the pancreas. Magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) are established methods to examine the pancreas, pancreatic ducts and biliary tracts (62). MRI has a 91% specificity identifying IPMN and almost 100% sensitivity of identifying communication with the main pancreatic duct. It also has a 74-75% accuracy differentiating malignant from benign IPMN (17).

A new adaptation and addition to this modality is the secretin enhanced MRCP (s-MRCP) (10, 62). It improves detection of morphologic abnormalities of the pancreatic duct and may facilitate in distinguishing BD-IPMN from MD-IPMN (62, 63). Similarly, it can be used to distinguish MCN from BD-IPMN by identifying a potential communication with the duct system (26). However, it is currently only used in a few selected cases (10).

#### *2.2.1.2 Computed Tomography*

Computed tomography (CT) is one of the most utilized imaging modalities (23). When differentiating SCN from IPMN it has a 90% specificity and when identifying an IPMN it has an 86% specificity. The sensitivity for detection of septa is 74% and main duct communication 86%, respectively. Regarding the diagnostic accuracy of differentiating malignant from benign IPMN, the rate is 56–78% (17, 26). As an approach to expand the use of CT and to increase the diagnostic accuracy, cyst volumetry and elongation values have been tested but have shown no additional value (64).

#### *2.2.1.3 Endoscopic Ultrasound*

The main benefit of EUS is that it describes the cyst morphology well e.g. mural nodules. EUS has a 68% accuracy differentiating malignant from benign IPMN (17, 26). Contrast-enhanced EUS, in which contrast is administered intravenously, improves this modality and can differentiate nodules with advanced neoplasia from those with LGD (65-68). EUS can also be used to aspirate cells and fluid from the cyst using EUS-guided fine needle aspiration (EUS-FNA). It has shown to be a safe method, with a complication rate of 2-3% and is recommended by different guidelines (22). The risk for tumor seeding and peritoneal carcinomatosis has been shown by Yoon *et al.* to be negligible (69). However, the main issue with this method is that the number of cells aspirated for the cytological examination often remains too few for analysis (70, 71).

#### *2.2.1.4 Comparison of the Imaging Modalities*

Several studies report better specificity and diagnostic accuracy rates for MRI compared to the other imaging modalities. One reason may be because its better ability to identify communication with duct, mural nodules and multi-located cysts (19). Although the

differences may not be so high between the modalities, and the diagnostic accuracy is still low for both modalities (58), MRI is still preferred for following and evaluating cystic lesions in the pancreas (72). Both the International consensus guidelines and the European expert consensus statement recommend MRI for follow up (48, 58).

#### *2.2.1.5 Emerging Imaging Modalities*

***18-fluorodeoxyglucose positron emission tomography/computed tomography (18-FDG PET/CT)*** is a well-established imaging method for cancer (46). A few studies have investigated the ability of this method to distinguish malignant from benign neoplasms. A retrospective study by Yamashita *et al.* investigated the usefulness of 18-FDG PET/CT to differentiate malignant IPMN from benign in 39 patients. The results showed a sensitivity of 82% and a specificity of 71% (46).

Recently Lee *et al.*, reported in the their meta-analysis of 1018 patients from 17 studies that $^{18}$F-FDG-PET was the optimal modality to differentiate malignant from benign PCNs with an accuracy of 99.7% (73). However, this modality has not yet been recommended by neither the International nor the European guidelines yet.

***Needle-based confocal laser endoscopy (nCLE)*** is an emerging imaging modality which provides a live image at a microscopic level of the inner wall of the cystic lesion (74, 75). In a study by Palazzo *et al.* the addition of nCLE to EUS FNA when diagnosing a single large cystic lesion changed the therapeutic management for 28% of the patients. One of the consequences was that surveillance of benign SCA lesion dropped from 40% to 5% (74). The diagnostic yield was 85% which is significantly higher than cytology alone at around 30%. However, the sensitivity and specificity of this test remains unclear. Furthermore, this method may only be suitable for larger cysts.

### 2.2.2 Molecular Diagnostic Markers in Serum

#### *2.2.2.1 CA 19-9*

Carbohydrate antigen 19-9 (CA 19-9), also called cancer antigen 19-9, is a sialylated lacto-N-fucopentaose II related to the Lewis-a antigen, expressed in 90-95% of the population, and is adsorbed onto the surface of erythrocytes (76-78). It has been validated as a serum tumor marker for pancreatic cancer (79). CA 19-9 is produced by pancreatic and biliary ductal cells, gastric and colon epithelial cells and is normally present in the serum in low concentrations. There are several reasons for an increase of the CA 19-9 level ranging from benign gastrointestinal to malignant neoplasms (39).

The level of CA 19-9 correlates with presence of malignant IPMN. However, a normal value does not exclude malignancy since the sensitivity is between 79-100% and specificity around 82 % (33, 80) Also the positive predicted value (PPV) is quite poor currently at 74.0% PPV and at 81.7% diagnostic accuracy (58).

#### *2.2.2.2 Span-1*

Span1 is another antigen tumor marker which has a sensitivity to detect PDAC of 70-80%, however, it is not an established biomarker as CA 19-9 (81). In a study by Yamanaka *et al.*, elevated levels of Span-1 (>30U/ml) were found in patients with invasive IPMN and were associated with lymph node metastasis with an odds ratio of 7.32 (95% CI: 1.10 - 56) (81). Another finding in the study was a poorer survival for those patients with positive Span-1 (81).

In other studies, Span-1 has shown useful to monitor to detect recurrence after treatment with gemcitabine (82). Satake *et al.* showed that Span-1 has a higher sensitivity to detect PDAC than CA 19-9 but a lower specificity and diagnostic accuracy, meaning that no additional value was being added compared to analyzing/detecting CA 19-9 (83).

### 2.2.3 Molecular Diagnostic Markers in Cystic Fluid

#### *2.2.3.1 Cytology*

Cytological analysis can be obtained from pancreatic cystic lesions with the aid of EUS-FNA which is a well-established and safe procedure (10, 84, 85). Although findings of atypical cells have a high specificity of around 83%, this method is associated with low sensitivity and poor diagnostic accuracy below 50% (19, 26, 58, 86, 87). The reason being that this method relies on analysis of cells, however, the sampling of cells or epithelium is poor (10, 26). Estrada *et al.* showed in their study that there was no improvement in measuring diagnostic yield even when rapid on-site cytology (ROSE) was performed (84). Presence of mucin in cystic fluid cannot be accurately tested due to the risk of contamination from stomach or duodenum (84).

#### *2.2.3.2 Carcinoembryonic Antigen*

Another marker that can be analyzed in the cystic fluid is the glycoprotein carcinoembryonic antigen (CEA) (10). However, the results regarding the applicability of this test differs in studies. A study by Brugge *et al.* suggests that an elevated CEA (>192ng/ml) can accurately predict a mucinous lesion in 79% of the cases (88). Increasing the cut-off value will increase the specificity but at the same lower the sensitivity. The opposite will increase the difficulty in excluding mucinous cysts. CEA is considered to be able to distinguish between mucinous lesions and SCN (26, 89). This is also supported by the revised ICG 2017 (50). One study suggested that CEA of >30ng/ml obtained from pancreatic juice may be used to diagnose HGD and invasive carcinoma, however, other studies claim that the diagnostic accuracy is low and cannot be used to either confirm nor rule out malignant lesion (86).

#### *2.2.3.3 Glucose*

Analysis of intra-cystic levels of glucose has been reported by a few systematic reviews and meta-analyses as a possible marker to distinguish mucinous from non-mucinous cystic lesions (90, 91). In the meta-analysis reported by McCarty *et al.*, low glucose-levels (< 50 mg/dL) have been associated with mucinous cystic lesion with a pooled sensitivity of 91% (95% CI: 88-94), a specificity of 86 % (95% CI: 81-9) and an accuracy of 94% (95% CI: 91-96) (92). When low

levels of glucose was combined with elevated levels of CEA, the pooled sensitivity increased to 97% (95% CI: 90-99) and the pooled diagnostic accuracy was calculated to 97% (95% CI: 95-98).

#### *2.2.3.4 EUS-guided through the needle biopsy (EUS-TTNB)*

EUS-TTNB is a new concept of taking biopsies to be able to analyze the inner wall of the cystic lesion (93, 94). Barresi *et al.* performed a retrospective multicenter study where 56 samples of the cystic wall were taken with a novel micro-forceps through a 19-gauge EUS needle. This technique, EUS-TTNB, reached a diagnostic yield/sample adequacy of 83.9% (47/56; 95% CI, 72–92%). From 47 patients, cytology was also obtained out which 17 samples could be used for diagnosis, (36.2%; 95% CI, 23–51%) (95). Out of the 56 patients, 15 underwent surgical resection. EUS-TTNB reached a diagnostic accuracy of 91.7% in mucinous lesions (11/12 patients). When investigating the accuracy of severity of lesion (grade of dysplasia) it was estimated to 75% (9/12), when compared to histology (86).

#### *2.2.3.5 Genetic Analysis*

Molecular and genetic analysis of cystic fluid is becoming popular and is evolving (50, 72). Two of the mutations associated with adenocarcinoma are *KRAS* and *GNAS* (26, 58, 96). Detection of *KRAS*-mutation has in some studies shown to support the diagnosis of a mucin producing cystic lesion (50). However, it cannot be used to determine if the cyst is malignant. On the other hand, recent studies indicate that mutation of *GNAS* could aid in distinguishing a mucin producing cyst with malignant potential from those without (50). As analysis of cystic fluid with next generation sequencing (NGS) is developing (26), a study by Jones *et al.*, found mutations present in 60% of the cysts they studied. For detecting mucinous lesions with NGS, the specificity has been estimated to 75% and sensitivity to 86%.

Konings *et al.* compared two groups of patients with PCNs who have a higher risk of developing cancer (97). The first group included patients with mutation carriers of gene associated with a higher risk for pancreatic cancer (*CDKN2A, BRCA1, BRCA2,* or *TP53* gene, and individuals with Peutz-Jeghers or Lynch syndrome). The other group included patients with PCN with a family history of PDAC but without a mutation. The result showed the group with familial history had a higher prevalence of cysts over 10 mm of size (16% vs 5%, $p$=0.045). However, the group with mutation carriers had a higher rate of progression (16% vs 2%, $p$=0.050) (97).

A recommendation from the ICG is that molecular and cytological analysis ought to be performed only in centers with experienced endosonographers where adequate knowledge and skills are available to handle and interpret the fluid samples findings. A study by Springer *et al.* suggests that a combined analysis of molecular markers and clinical features could imply a sensitivity of 90-100% and a specificity of 92-98% (98). The European expert consensus statement recommend analysis of mutations only in those unclear cases where the result will have an impact on the management and treatment (58).

## 2.3 GUIDELINES

Due to limited knowledge, evidence and methods able to accurately diagnose and distinguish pre-malignant lesions, patients with cystic lesions are included in surveillance programs (7). However, different guidelines, of which there at least fifteen, are based on expert opinion rather than evidence, suggest different management strategies of these patients (7, 60, 72, 99). The implementation of these recommendations results in patients being followed-up for a long period of time with costly investigating methods (14).

### 2.3.1 International Guidelines

The first guidelines were published in 2006 after a meeting of the "International Association of Pancreatology" in Sendai, Japan (59). The revised version, the "Fukuoka Consensus guidelines" came six years later in 2012 (59). In regards of surveillance and indications for surgery, patients' symptoms, chemical parameters and radiological features were divided into two categories. Patients presenting with "high risk stigmata" were considered for up-front surgery *(Table 3a)*. Patients with "worrisome features" were suggested to undergo further examination with EUS and/or be follow-up with specified intervals according to the size of cystic lesion. (59)

The latest revised version of the International Consensus Guidelines was published in 2017 (50). No major changes were made in the guideline, however, a few radiological features and a chemical marker, Carbohydrate antigen 19-9 (CA 19-9) were added as "worrisome features" *(Table 3a)*. Another revision in regards of small cysts <1cm, was an initial close follow-up that could be prolonged if no there was no change between the follow-ups.

### 2.3.2 European Guidelines

The first European consensus guidelines were published in 2013 after a consensus meeting held in Stockholm, Sweden during the congress of United European Gastroenterology Week 2011 (7). Similar to the International guidelines, the European version had absolute and relative indications for surgery in regards of branch-duct IPMN.

The revised European evidence based guidelines were published in 2018 with several changes and revision of the absolute and relative indications for resection (58). The current definitions are summarized in Table 3b.

For IPMNs that do not meet criteria for surgery, the recommendations are a first follow-up after 6 months and thereafter annually. For patients with relative indication, a bi-annual follow-up is recommended (7, 58).

### 2.3.3 Comparison of Guidelines

Hasan *et al.* compared five guidelines: 1) 2015 American Gastroenterological Association AGA, 2) 2017 International Association of Pancreatology IAP, 3) American College of Gastroenterology ACG, 4) 2018 European Study Group and 5) American College of Radiology ACR. They concluded that the European guidelines was applicable for all patients with PCNs

whereas the other are more selective. The IAP guidelines only apply to patients with IPMN and the ACR to all incidental cysts. The AGA applies to all cysts but only for asymptomatic patients. The ACR is the only guidelines out of these five where the age of the patients matter (100).

A few studies have examined the performance of the guidelines, which is defined by its ability to identify lesions with high-grade dysplasia or invasiveness. The sensitivity and specificity vary for all guidelines. In case of the IAP, the sensitivity was 28-81% and specificity 34-88%. (100).

Another study by Lekkerkerker *et al.* compared the European guidelines, IAP and AGA and showed that surgery was justified in 53%, 54% and 59% respectively of the cases. The AGA would mean fewer resections with a risk of missing 12% of patients with HGD or cancer, whereas the European or the IAP would not have missed such a lesion in the cohort of the study (101). The study concluded that none of the analyzed guidelines had a sufficient diagnostic accuracy.

***Table 3*** *– Radiological and clinical parameters for assessment of pancreatic cystic lesions. a) 2017 International Consensus Guidelines (50) & b) 2018 European Evidence Based Guidelines (58)*

| **a) International Consensus Guidelines (2017)** | | **b) European Evidence Based Guidelines (2018)** | |
|---|---|---|---|
| ***High risk stigmata*** | ***Worrisome features*** | ***Absolute indications*** | ***Relative Indication*** |
| Obstructive jaundice in a patient with cystic lesion of the head of the pancreas | Pancreatitis | Positive cytology for malignant/high-grade dysplasia | Growth rate ≥5 mm/year |
| Enhancing mural nodule >5 mm | Cyst >3 cm | Jaundice (tumor related) | Increased serum CA 19-9 level (>37 U/mL in the absence of jaundice) |
| Main pancreatic duct >10 mm | Enhancing mural nodule <5 mm | Dysplasia or cancer | MPD diameter 5- 9.9 mm |
| | Thickened/enhancing cyst walls | Contrast-enhancing mural | Cyst diameter ≥40 mm |
| | Main duct size 5-9 mm | Nodule (≥5 mm) or solid mass | New-onset of diabetes mellitus |
| | Abrupt change in caliber of pancreatic duct with distal pancreatic atrophy | | Acute pancreatitis (caused by IPMN) |
| | Lymphadenopathy | | Contrast- enhancing mural nodules <5 mm |
| | Increased serum level of CA 19-9 | | |
| | Cyst growth rate >5 mm /2 years | | |

## 2.4 METABOLOMICS

### 2.4.1 Definition and Role of Metabolites

One of the established hallmarks of cancer is changes in metabolism, a process achieved by two main reasons (77, 102). The first is adaption to the microenvironment which is necessary for the cancer cells to grow in an environment with severe hypoxia and nutrition deprivation. The second reason is oncogenes of which *KRAS* mutations is seen in most pancreatic cancers >90% (77, 85).

A new area being explored in conjunction with genomics and proteomics is metabolomics (103, 104). This entails a non-biased qualitative and quantitative assessment of metabolites in a given biological sample (104, 105). Metabolites are essentially biochemical end-products of biological signaling and have shown to affect its environment and signaling mechanisms by, for instance, regulating epigenetic mechanisms, functioning as co-substrates and thereby regulating post-translational modifications and interacting with plasma proteins and affecting their transport capabilities (105).

### 2.4.2 Analytical Methodology of Metabolomics

The concept of metabolomics was proposed in 1999, however, the analytical method has only recently become feasible after development of the technology (103, 106). The process of metabolomics consists of four main steps (104, 106, 107):

I. **Sample collection** – Biological samples are collected from various sources e.g. plasma, tissue, urine and feces (104).
II. **Sample preparation** – This step includes the extraction of metabolites, compound pre-treatment and separation to finally produce a liquid mixture ready for analysis (106). Since the quality of the collected samples may vary, pre-treatment becomes a vital step for the success of the study (104).
III. **Chemical analysis** – The samples are then analyzed with nuclear magnetic resonance (NMR) or mass spectromtery (MS). NMR is less sensitive than MS, limiting its use if the samples have low levels of metabolites (107). However, unlike MS, it is not destructive, enabling for the samples to be recovered and remeasured multipe times (106). Different separation techniques can be coupled to MS such as gas chromotography-MS (GC-MS) and liquid chromatography- MS (LC-MS) (107).
IV. **Data analysis** – The large and often complex data from the chemical analysis is then processed, analyzed (including statisitcal analysis) and interpreted in computational tools (105). Recently, major developments and improvements in this field have made metabolomics as a diagnostic tool possible.

### 2.4.3 Types of Metabolomic Experiments

There are four main types of experimental designs in metabolomics which uses different analytical platforms (106).

I. **Targeted metabolomics** – This design is suitable for identifying and quantifying a small subset of compunds (50-500 compunds) making it ideal when testing for a hypothesis and detection of a known biomarker. Targeted metabolomics is frequently used in medical and clinical applications and requires NMR, LC-MS or GC-MS (105, 106).
II. **Untargeted metabolomics** – With this approach as many metabolites as possbible are characterized. Since it is more qualitative rather than quantitative, this often labor intensive design is ideal for generation of hypothesis and discovery of biomarkers (105, 106). The analytical platforms used are LC-MS, GC-MS, capillary electrophoresis-MS (CE-MS) and NMR. At times, a combination of the platorms are used, e.g. LC-MS for purifying and accumulating the compund and NMR for confirming its identity (106).
III. **Metabolic flux measurement** – This type, also called "Fluxomics", is a branch of targeted metabolomics which measures the reaction rates of the metabolites and the movement of isotopic labels through metabolic intermediates. Fluxomics require LC-MS or NMR to study the dynamics of cellular and physiological metabolism (106).
IV. **Metabolic imaging** – An emerging type invloving in vivo or vitro detection and visualization of metabolites in a specific tissue (105, 106). Using different metabolite imaging methods, such as NMR, magnetic resonance spectroscopy (MRS) and positron emission tomography (PET), a small number of metabolites (<20 metabolites) can be identified and semi-quantified. The primary application of metabolic imaging is the exploration and assessment of cell- tissue- and organ-specific metabolims but may also be suitable for pre-operative assesment (106, 108).

### 2.4.4 Utilities of metabolomics in cancer biomarker discovery

Metabolomics has proven to be a valuable tool in many fields, for instance, for discovery of new drugs and biomarkers and for monitoring of cancer (104, 109). In the field of cancer diagnostics, studies have shown a high sensitivity of metabolomics and metabolite alterations to discriminate tumor tissues from non-tumor tissues (103). A study by Budhu *et al.* showed that specific biochemical alterations can be used to distinguish different cancers, in this case liver, breast and pancreas cancer (103).

Metabolomics has been studied for diagnosing pancreatic cancer, however, very little has been studied on cystic tumors, especially IPMN cyst fluid. Park *et al.* analyzed for metabolites in 45 patients using mass spectrometry and compared mucinous cysts with non-mucinous cysts. Two metabolites, glucose and kynurenine, were found to have a high diagnostic accuracy for mucinous cysts with ROC-values of 0.92 and 0.94 respectively (110). It has later been shown that pancreatic cancer is characterized by enhanced glycolytic metabolism which could explain the significance of glucose as metabolite (111, 112).

# 3 RESEARCH AIMS

**Study I**

To correlate risk factors and the degree of dilatation of the main pancreatic duct to advanced findings at histological analysis.

**Study II**

To identify risk factors predictive of malignancy in patients with IPMN.

**Study III**

To define the metabolic and lipidomic profile of pancreatic cystic fluid and plasma in patients undergoing resection for IPMN and SCN.

**Study IV**

To validate metabolomics as a diagnostic method for differentiation between SCN and IPMN, and to distinguish the grade of tissue dysplasia among IPMN *(to validate the results from study III).*

To study the relation between IPMN associated plasma and cystic metabolites and the bacterial profile.

**Study V**

To investigate the recurrence and progress rate of known IPMNs in the remnant of patients who have undergone partial pancreatic resection.

To investigate if the grade of dysplasia at first histology affects the risk of recurrence.

# 4 MATERIALS AND METHODS

## 4.1 STUDY I

### 4.1.1 Study Design

This was a single-institution, retrospective study conducted at Karolinska University Hospital, a tertiary referral center. All consecutive patients undergoing resection for IPMN during 2008 to 2015 were included. Data of patient demographics, risk factors, radiological characteristics, clinical symptoms histological results were collected through the medical records system. For analysis of risk factors associated with advanced histology the patients were stratified as a) LGD & MGD and b) HGD & Invasive cancer. For the sub-analysis of the dilatation of the main-pancreatic duct (MPD) the groups were stratified as I) 0-5.9 mm, II) 6-9.9 mm III) ≥10 mm.

Ethical approval was obtained from the local Ethical Committee in Stockholm (Dnr 2015/1544–31/4).

### 4.1.2 Statistical Analysis

For categorical and continuous variables, Chi-square and Student's t-test were used, respectively. For the further analysis of different subclasses of MPD dilatation, Mantel-Haenszel test for trend was used as ordinal variables. Variables that were significant were further analyzed by uni- and multivariable logistic regression analysis. Probability score of <0.05 was considered statistically significant. All data analysis was performed on MedCalc (Mariakerke, Belgium).

## 4.2 STUDY II

### 4.2.1 Study Design

This was a retrospective cohort study including consecutive patients operated for IPMN at two tertiary referral centers, Karolinska University Hospital, Stockholm, Sweden and Johns Hopkins Hospital, Baltimore, MD, USA during the period of 2008-2017 and 2004-2017, respectively. The resected specimens were histologically confirmed.

A total of 901 patients were collected from the two centers *(Figure 1)*. Seventy-one patients had concomitant pancreatic cancer and for 34 patients the histology was not clear. They were thus excluded from the cohort leaving 796 patients for final analysis.

All patient data including demographic characteristics, symptoms, radiological features and histology were collected from the medical records.

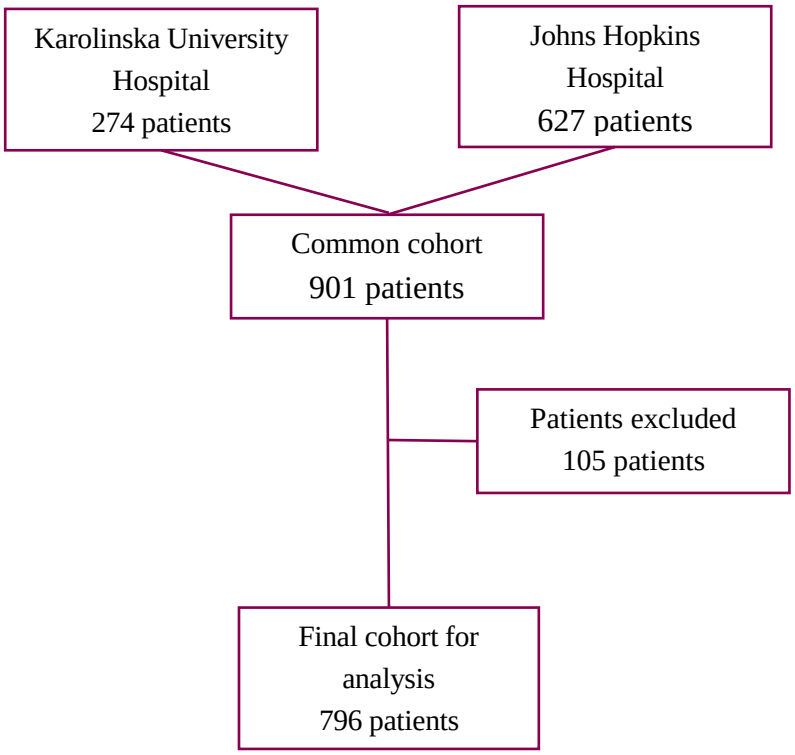


***Figure 1**- Flowchart of cohort selection of study II*

### 4.2.2 Statistical Analysis

The outcome was IPMN-histology which was categorized as low grade (LG) IPMN, high grade (HG) IPMN and invasive (Inv) IPMN. Moderate or intermediate grade IPMN were categorized as LG-IPMN. For the association between risk factors and above-mentioned three-way outcome, Pearson-Chi square test was used. All risk factors that met statistically significance, defined as $p<0.05$, were then analyzed in a multivariable model. Any missing data was dealt with a standard complete-case approach.

A multinominal logistic regression model (113) was used to calculate the probability of each outcome based on the dilatation of the MPD as quantitative risk factor.

For the third analysis, the object was to identify an interval of cut-off for the risk factor, MPD-dilatation, that best discriminates the LG-IPMN from advanced histology (HG-IPMN and Inv-IPMN). For this analysis HG-IPMN and Inv-IPMN was categorized together since these two groups are subject for intervention.

All data analysis was performed with Stata 15 (StataCorp) statistical software.

## 4.3 STUDY III AND IV

### 4.3.1 Study Population and Design

#### *4.3.1.1 Study III*

This was a prospective cohort study conducted at Karolinska University Hospital, a tertiary referral center. All patients over the age of 18, undergoing surgery for suspected PCN during February 2016 to January 2017 were included. Patients with histologically confirmed IPMN or SCN were selected for further analysis.

#### *4.3.1.2 Study IV*

In similarity to study III, patients undergoing resection for suspected PCN during 2016 to 2019 were eligible for inclusion. After histological verification, only patients with IPMN and SCN were selected for further analysis. Those patients who had already been included in Study III were excluded.

### 4.3.2 Sample Collection and Classification

The cystic fluid was collected from fresh resection specimen at the pathology lab within 20 minutes of resection. A macroscopic assessment was made by a pathologist with sub-specialization in upper GI-diseases. Fluid from cystic lesions was aspirated using a syringe with a needle, whereas fluid from the MPD was aspirated using a syringe without a needle *(Figure 2 & 3)*.

Venous whole blood was collected from the patients immediately prior to surgery and was processed within four hours. Plasma and the aspirated fluid were stored at -80°C.

The resected specimens were processed and microscopically examined per clinical routine. The findings were assigned to one of four groups: SCN, LGD-IPMN, HGD-IPMN or cancer.

### 4.3.3 Analysis

#### *4.3.3.1 Study III*

The metabolomics and lipodomics analyses were performed using liquid chromatography-mass spectrometry. A targeted (semi)quantative analysis of 100 metabolites and more than 1000 lipids was performed. The profiles of each cyst fluid and venous blood samples were then correlated with the histological diagnosis and clinical factors and were adjusted for confounders. Since patients with HGD and cancer are both subject for surgical resection, these groups were combined for the analysis. The statistical analyses were performed with R 3.5.1 and Stan 2.17.1 statistical software.

***Figure 2*** *– Cyst fluid aspiration in a resected pancreatic specimen.*

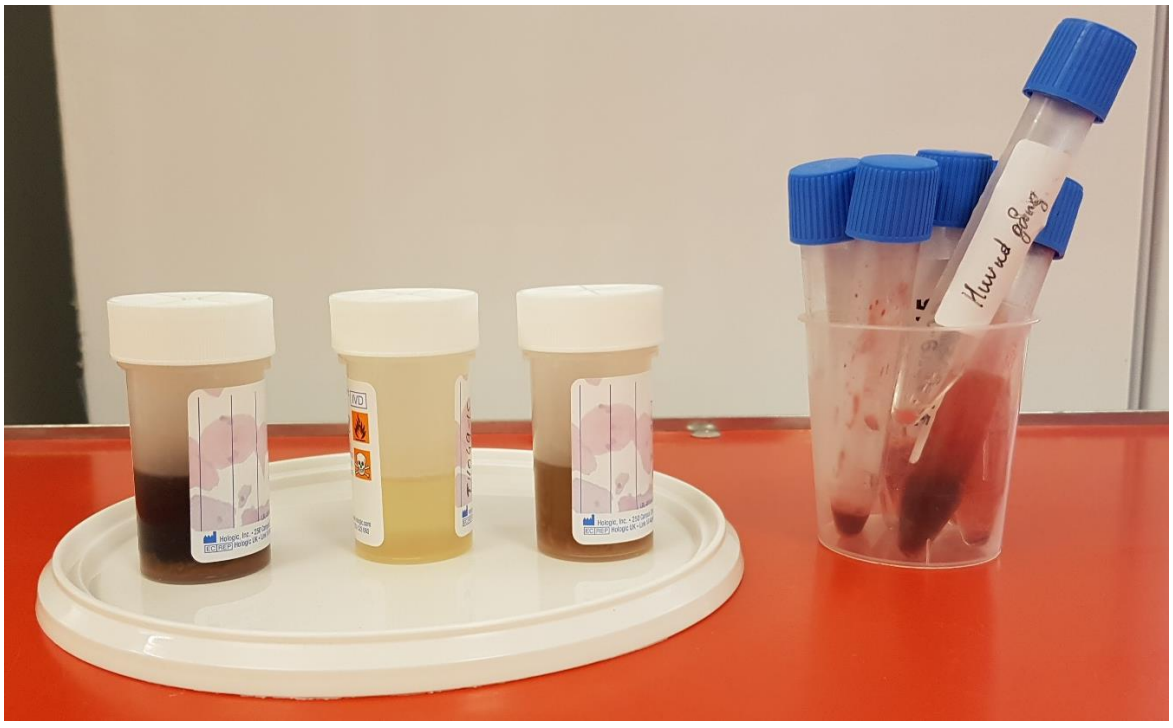

***Figure 3*** *- Fluid collected from cystic lesions and from main pancreatic duct*

#### *4.3.3.2 Study IV*

The samples were collected in same fashion as in Study III, however, for this study both targeted and untargeted metabolomic analyses were used. An additional analysis was performed to study the microbiome to study potential correlations between metabolites and the microbiome in the cyst samples. The microbiome was achieved by quantification and sequencing of 16S RNA gene by qPCR and sequencing of the V3-4 region of 16S gene on the Illumina platform.

### 4.3.4 Ethical Considerations

Both studies were approved by the local Ethical Committee in Stockholm (Dnr: 2015/1580-31/1. Written informed consent were collected from patients prior to surgery.

## 4.4 STUDY V

### 4.4.1 Study Population and Design

All patients who underwent surgery for IPMN between January 2008 and July 2017 were retrospectively included in the study with a minimal follow-up time of 2 years. Patients who underwent total pancreatectomy were excluded from further analysis *(Figure 4)*. Clinical parameters, radiological and histological characteristics and data of recurrence was collected in a prospective manner.

Ethical approval was obtained from the local Ethical Committee in Stockholm (Dnr: 2016/2542-31/1).

### 4.4.2 Classification and Definitions

All resected specimens were histologically verified by pathologist and graded as low-grade IPMN, high-grade IPMN and IPMN-cancer. All patients were discussed in multidisciplinary team conferences.

Recurrence within the pancreatic remnant was defined as local recurrence and any extra pancreatic recurrence was defined as systemic recurrence. Clinical significant recurrence was defined as findings that resulted in a change in the management of the lesion i.e. re-operation or palliative treatment. Progress of an existing lesion was defined as increase in size of $\geq 2$ mm and if the multidisciplinary team conference recognized it as a progress.

### 4.4.3 Statistical Analysis

The statistical analyses were performed using SPSS version 28.0 (IBM, Armonk, NY) and R version 4.1.1, R Project for Statistical Computing, Vienna, Austria. Univariable statistical comparisons between groups were performed using Pearson X2 Test or Mann-Whitney U. An alpha of 0.05 was deemed significant for all analyses.

For the multivariable analysis, significant predictors and clinically relevant risk factors (gender, diabetes mellitus, cyst diameter, diameter of main pancreatic duct and solid component/mural

nodule) were included. Different models were tested to define their independent association with recurrence of which the significant models were reported.

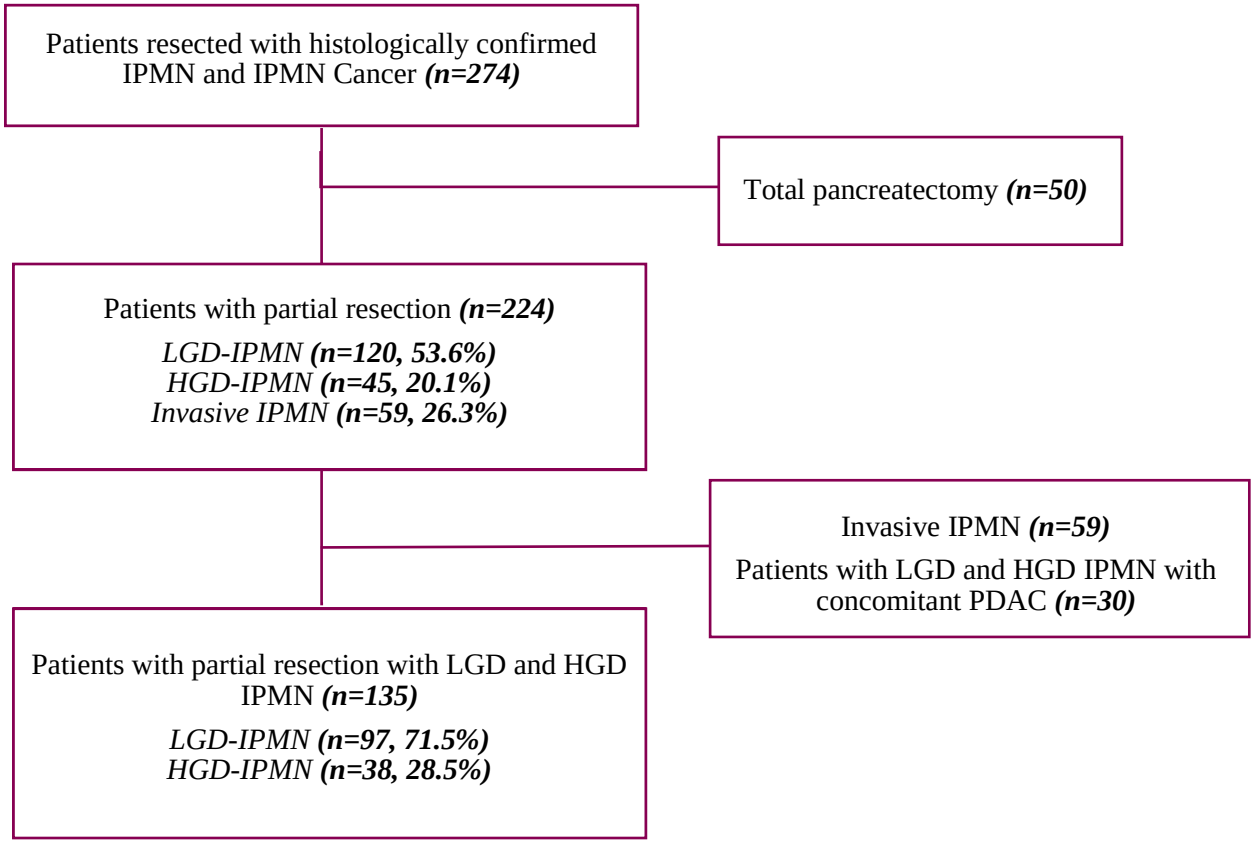


***Figure 4*** *- Flowchart of selection of patients of study V*

# 5 RESULTS

## 5.1 STUDY I

A total of 152 patients underwent resection for IPMN during the period of 2008 to 2015. The characteristics of the patients are shown in Table 4. The number of patients with LGD, HGD and cancer were 72, 29 and 51 respectively with almost half undergoing pancreaticoduodenectomy.

An analysis of risk factors was performed to investigate their prevalence in and association with advanced histology in the cohort. The results are shown in Table 5. Significant differences could be seen in patients with jaundice, elevated CA 19-9 and dilatation of the MPD.

Next, a uni- and multivariable logistic regression analysis was performed for the previously mentioned statistically significant risk factors which confirmed their association with advanced histology *(Table 6)*. MPD dilatation over 10 mm was not statistically significant at the sex and age-adjusted multivariable analysis, $p$=0.08, however, a trend could be seen.

In order to investigate if an increasing dilatation of the main pancreatic duct was associated with an increasing risk of advanced histology, Mantel-Haenszel test for trend was used, which confirmed this trend *(Table 7)*.

***Table 4*** *- Patient Characteristics*

| | |
|---|---|
| Patients | 152 |
| Male | 78 (51.3 %) |
| Age | 69.04 (67.76-70.31;  95% CI) |
| BMI | 25.72 (24.98-26.45;  95% CI) |
| Smokers | 56 (34.2 %) |
| Familial history of Pancreatic cancer | 4 (2.6 %) |
| Diabetes | 37 (24.3 %) |
| Symptoms | 49 (32.2 %) |
| Duct Mean Size (mm) | 7.61 (6.64-8.57; 95% CI) |
| Cyst Mean Size (mm) | 32.72 (28.84-36.59; 95% CI) |
| Multifocal | 69 (45.4 %) |
| Head | 63 (41.4 %) |
| Body-Tail | 56 (36.8 %) |
| Diffuse | 14 (9.1 %) |
| Uncinate | 19 (12.5 %) |
| Pancreaticoduodenectomy | 79 (52.0) |
| Distal Pancreatectomy | 48 (31.6) |
| Total Pancreatectomy | 19 (12.5 %) |

***Table 5*** *- Prevalence of high grade dysplasia (HGD)/cancer and low grade dysplasia (LGD) according to possible other risk factors.*

| Risk factor | HGD/cancer | LGD | p-value |
|---|---|---|---|
| **Cyst diameter ≥ 40 mm** | 22/80 (27.5%) | 25/72 (34.7%) | 0.33 |
| **Mural nodules** | 1 /80(1.2 %) | 1/72 (1.3%) | 0.94 |
| **Acute pancreatitis** | 4/80 (5.0%) | 8/72 (11.1 %) | 0.16 |
| **Diabetes** | 23/80 (28.7%) | 14/72 (19.4.%) | 0.18 |
| **Family History of PDAC** | 2/80 ( 2.5%) | 2/72 (2.7%) | 0.91 |
| **Multifocal** | 35/80 (43.7%) | 34/72 (47.2%) | 0.66 |
| **Uncinatus location** | 10/80 (12.5%) | 8/71 (11.2%) | 0.81 |
| **Head localization** | 37/80 (46.2%) | 25/71 (35.2%) | 0.17 |
| **Smoking** | 30/80 (37.5%) | 26/72 (36.1%) | 0.85 |
| **Jaundice** | 14/80 (17.50%) | 1/72 (1.30%) | **0.0009** |
| **CA 19-9 ≥ 37 kE/L** | 35/73 (47.9%) | 12/68 (17.6%) | **0.0001** |
| **MPD 1-5.9 mm** | 26/80 (32.50%) | 49/72 (68.05%) | **<0.0001** |
| **MPD 6-9.9 mm** | 32/80 (40.0%) | 14/72(19.44%) | **0.006** |
| **MPD ≥ 10 mm** | 22/80 (27.50%) | 9/72 (12.50%) | **0.02** |

***Table 6*** *- Sex- and age adjusted uni- and multivariable logistic regression analysis considering patients with a) MPD 6-9.9 mm and b) MPD ≥10 mm and jaundice and elevated CA 19-9 in respective groups.*

| | **Univariable** | **Multivariable** |
|---|---|---|
| ***a)*** | | |
| **MPD 6-9.9 mm** | OR 2.65, CI 1.12-6.25 ***(p=0.02)*** | OR 2.35, CI 0.88-6.23 *(p=0.08)* |
| **Jaundice** | OR 15.36, CI 1.94-121.22 ***(p=0.009)*** | OR 8.96, CI 1.06-75.52 ***(p=0.04)*** |
| **Elevated CA 19-9** | OR 4.15, CI 1.90-9.05 ***(p=0.0003)*** | OR 3.63, CI 1.61-8.19 ***(p=0.001)*** |
| ***b)*** | | |
| **MPD ≥10 mm** | OR 2.65, CI 1.12-6.25 ***(p=0.02)*** | OR 2.35, CI 0.88-6.23 *(p=0.08)* |
| **Jaundice** | OR 15.36, CI 1.94-121.22 ***(p=0.009)*** | OR 8.96, CI 1.06-75.52 ***(p=0.04)*** |
| **Elevated CA 19-9** | OR 4.15, CI 1.90-9.05 ***(p=0.0003)*** | OR 3.63, CI 1.61-8.19 ***(p=0.001)*** |

***Table 7*** *- Prevalence of high grade dysplasia (HGD)/cancer and low grade dysplasia (LGD) according to different classes of MPD-dilatation.*

| Risk Factor | HGD/Cancer | LGD | p-trend value |
|---|---|---|---|
| **MPD 1-5.9 mm** | 26/80 (32.50%) | 49/72 (68.05%) | **<0.0001** |
| **MPD 6-9.9 mm** | 32/80 (40.0%) | 14/72 (19.44%) | |
| **MPD ≥ 10 mm** | 22/80 (27.50%) | 9/72 (12.50%) | |

## 5.2 STUDY II

A total of 796 patients were included in the cohort for final analysis. Patient characteristics and the association of risk factors with HG-IPMN or Inv-IPMN are shown in Table 8. Almost 60% of the patients had LG-IPMN at final histology. Risk factors that were statistically proven to be associated with Inv-IPMN were male sex, age over 70, diabetes mellitus, jaundice, weight loss, elevated CA 19-9, unifocal lesion, pancreatic head location, solid component and if the lesion was classified as main duct (MD) or mixed type (MT) IPMN at pre-operative radiology. On the contrary, risk factors as familial history of pancreatic cancer and cyst size of ≥40 mm was associated to LG-IPMN. This analysis also suggested that increasing dilatation of MPD is associated with advanced histology *(p<0.0001)*.

The association between risk factors and outcomes in terms of HG-IPMN and Inv-IPMN was then further analyzed by multivariable logistic regression analysis *(Table 9)*. An MPD dilatation of ≥10 mm was associated with a 3 and 15 times higher risk for HG-IPMN and Inv-IPMN respectively (OR 3.3 and 15.5, respectively). An MPD dilatation of 5-9.9 mm was associated with almost a 6 times higher risk for Inv-IPMN but was not significantly increased for HG-IPMN. On the contrary, the location of a lesion in body/tail was inversely associated with invasive IPMN (OR 0.4).

A second model of multinominal regression analysis was then made focusing on the MPD-dilatation as the only categorical predictor for HG-IPMN and Inv-IPMN, using MPD <5 mm as constant *(Table 10)*. MPD <5 mm showed less risk for HG-IPMN and Inv-IPMN (OR 0.19, 95% CI 0.14-0.2 and 0.08, 95% CI 0.05-0.13 respectively). MPD dilatation of 5-9.9 showed 2.7 times higher for HG-IPMN and 4.4 times higher risk for Inv-IPMN. The risk of HG-IPMN and Inv-IPMNs for patients with MPD-dilatation over 10 mm were almost 7 and 15 times higher. Thus, a pattern can be seen that an increased dilatation of MPD results in an increased risk for advanced histology, as illustrated in Figure 5.

***Table 8*** *- Analysis of the association between risk factors and outcome (LG-IPMN, HG-IPMN and Inv-IPMN respectively.*

[a] *Calculated with Pearson-Chi-Square test, *Significant association to Inv-IPMN, **Significant association to LG-IPMN*

| | **Total n=796** | **LG-IPMN n=476 (59.8) N (%)** | **HG-IPMN n=185 (23.2) N (%)** | **Inv-IPMN n=135 (17) N, %** | **p-value [a]** |
|---|---|---|---|---|---|
| **Sex** | | | | | |
| Females | 413 (51.9) | 261 (54.8) | 95 (51.4) | 57 (42.2) | **0.05*** |
| Males | 383 (48.1) | 215 (45.2) | 90 (48.6) | 78 (57.8) | |
| **BMI** | | | | | |
| < 25 | 329 | 186 (41.6) | 76 (42.2) | 67 (51.5) | 0.15 |
| 25-29 | 274 | 171 (38.3) | 60 (33.3) | 43 (33.1) | |
| ≥ 30 | 154 | 90 (20.1) | 44 (24.5) | 20 (15.4) | |
| **Age** | | | | | |
| ≤ 70 years | 414 (52) | 270 (56.7) | 81 (43.8) | 63 (46.7) | **0.005*** |
| > 70 years | 382 (48) | 206 (43.3) | 104 (56.2) | 72 (53.3) | |
| **Familial history of PC** | | | | | |
| No | 642 (87.9) | 376 (85.1) | 149 (89.8) | 117 (95.9) | **0.004**** |
| Yes | 88 (12.1) | 66 (14.9) | 17 (10.2) | 5 (4.1) | |
| **Personal history of malignancy** | | | | | |
| No | 663 (83.6) | 397 (83.8) | 150 (81.5) | 116 (85.9) | 0.5 |
| Yes | 130 (16.4) | 77 (16.2) | 34 (18.5) | 19 (14.1) | |
| **Diabetes Mellitus** | | | | | |
| No | 601 (78.2) | 383 (80.5) | 149 (80.5) | 96 (71.1) | **0.05*** |
| Yes | 168 (21.8) | 93 (19.5) | 36 (19.5) | 39 (28.9) | |
| **New onset of diabetes (< 1 Year)** | | | | | |
| No | 776 (97.6) | 465 (97.7) | 180 (97.8) | 131 (97) | 0.8 |
| Yes | 19 (2.4) | 11 (2.3) | 4 (2.2) | 4 (3) | |
| **Jaundice** | | | | | |
| No | 712 (89.7) | 443 (93.39 | 168 (91.3) | 101 (74.8) | **<0.0001*** |
| Yes | 82 (10.3) | 32 (6.7) | 16 (8.7) | 34 (25.2) | |
| **Abdominal pain** | | | | | |
| No | 533 (67.3) | 330 (69.5) | 111 (61) | 92 (68.2) | 0.1 |
| Yes | 259 (32.7) | 145 (30.5) | 71 (39) | 43 (31.8) | |
| **Acute pancreatitis** | | | | | |
| No | 602 (82.6) | 365 (84.1) | 133 (78.2) | 104 (83.2) | 0.2 |
| Yes | 127 (17.4) | 69 (15.9) | 37 (21.8) | 21 (16.8) | |
| **Weight loss** | | | | | |
| No | 645 (81.9) | 405 (86) | 143 (78.1) | 97 (72.4) | **0.001*** |
| Yes | 143 (18.1) | 66 (14) | 40 (21.9) | 37 (27.6) | |
| **Incidental diagnosis** | | | | | |
| No | 368 (50.5) | 187 (43.4) | 94 (55) | 87 (68.5) | **<0.0001**** |
| Yes | 361 (49.5) | 244 (56.6) | 77 (45) | 40 (31.5) | |

| | | | | | |
|---|---|---|---|---|---|
| **Patients under surveillance for IPMN** | | | | | |
| No | 525 (67.3) | 284 (65) | 131 (76.6) | 110 (86) | **<0.0001**** |
| Yes | 211 (32.7) | 153 (35) | 40 (23.4) | 18 (14) | |
| **Serum CA 19-9 > 37** | | | | | |
| No | 178 (47.3) | 103 (68.7) | 48 (64) | 27 (30.3) | **<0.0001*** |
| Yes | 198 (52.7) | 47 (31.3) | 27 (36) | 62 (69.7) | |
| **Multifocal IPMN** | | | | | |
| No | 463 (58.2) | 257 (54) | 114 (61.6) | 92 (68.2) | **0.007*** |
| Yes | 333 (41.8) | 219 (46) | 71 (38.7) | 43 (31.8) | |
| **Localization (clinically relevant lesion)** | | | | | |
| Head/Uncinatum | 370 (46.6) | 197 (41.4) | 98 (53.6) | 75 (55.6) | **0.01*** |
| Body/tail | 196 (24.7) | 131 (27.5) | 38 (20.8) | 27 (20) | |
| Whole pancreas | 228 (28.7) | 148 (31.1) | 47 (25.6) | 33 (24.4) | |
| **Mural nodules** | | | | | |
| No | 667 (83.9) | 416 (87.4) | 149 (80.5) | 102 (76.1) | **0.003*** |
| Yes | 128 (16.1) | 60 (12.6) | 36 (19.5) | 32 (23.9) | |
| **Cyst diameter ≥ 40 mm** | | | | | |
| No | 604 (80.1) | 372 (82.3) | 133 (76) | 99 (78) | 0.1 |
| Yes | 150 (19.9) | 80 (17.7) | 42 (24) | 28 (22) | |
| **MPD diameter** | | | | | |
| < 5 mm | 305 (41.2) | 240 (55.2) | 45 (25.6) | 20 (15.4) | **<0.0001*** |
| 5 – 9.9 mm | 286 (38.6) | 152 (34.9) | 78 (44.3) | 56 (43.1) | |
| ≥ 10 mm | 150 (20.2) | 43 (9.9) | 53 (30.1) | 54 (51.6) | |
| **Clinical classification of IPMN** | | | | | |
| BD-IPMN | 361 (49.9) | 279 (63.1) | 56 (31.6) | 26 (24.8) | **0.007*** |
| MD-IPMN | 92 (12.7) | 39 (8.8) | 28 (15.8) | 25 (23.8) | |
| MT-IPMN | 271 (37.4) | 124 (28.1) | 93 (52.6) | 54 (51.4) | |

***Table 9*** *- Multinominal logistic regression analysis of the association with risk factors and HG-IPMN and Invasive IPMN. Adjusted Odds Ratios (OR) and 95% Confidence Intervals (CI) are shown.*

| | HG-IPMN | | Invasive IPMN | |
|---|---|---|---|---|
| **Risk Factor** | Odds Ratio | 95% CI | Odds Ratio | 95% CI |
| **Familial history of PC** (yes) | 0.9 | 0.4-1.7 | 0.4 | 0.1-1.3 |
| **Age** (>70 years) | 1.5 | 0.9-2.3 | 1.6 | 0.9-2.8 |
| **Sex** (males) | 1 | 0.7-1.6 | 1.5 | 0.8-2.6 |
| **Jaundice** (yes) | 0.8 | 0.3-1.9 | 1.7 | 0.7-3.9 |
| **Incidental diagnosis** (no) | 0.7 | 0.4-1 | 0.6 | 0.3-1.1 |
| **Patients under surveillance for IPMN** (yes) | 0.7 | 0.5-1.2 | 0.8 | 0.4-1.6 |
| **Clinical classification of IPMN** | 1.2 | 0.5-3.1 | 1 | 0.4-2.9 |
| Main duct IPMNs | 1.7 | 0.9-3.8 | 0.8 | 0.3-1.9 |
| Mixed-type IPMNs | | | | |
| **Multifocal IPMN** (yes) | 0.9 | 0.5-1.7 | 0.8 | 0.3-1.9 |
| **Location** | | | | |
| Body/tail | 0.7 | 0.4-1.2 | **0.4** | **0.2-0.9** |
| Whole gland | 0.9 | 0.5-1.9 | 1.3 | 0.5-3.3 |
| **Mural Nodules** (yes) | 1.4 | 0.8-2.5 | 1.4 | 0.7-2.8 |
| **MPD diameter** | | | | |
| 5-9.9 mm | 1.5 | 0.6-3.2 | **5.6** | **2-14.9** |
| ≥ 10 mm | **3.3** | **1.3-8.4** | **15.5** | **5-47** |

***Table 10*** *- Odds ratios and confidence intervals for the association between risk factors.*

| | **OR** | **95 % CI** |
|---|---|---|
| **HG-IPMN** | | |
| MPD diameter <5 mm (constant) | **0.19** | **0.14-0.26** |
| MPD diameter 5-9.9 mm | 2.7 | 1.8-4.2 |
| MPD diameter ≥ 10 mm | 6.6 | 3.9-10.9 |
| | | |
| **Inv-IPMN** | | |
| MPD diameter < 5 mm (constant) | 0.08 | **0.05-0.13** |
| MPD diameter 5-9.9 mm | 4.4 | 2.6-7.7 |
| MPD diameter ≥ 10 mm | 15 | 8.2-27.7 |

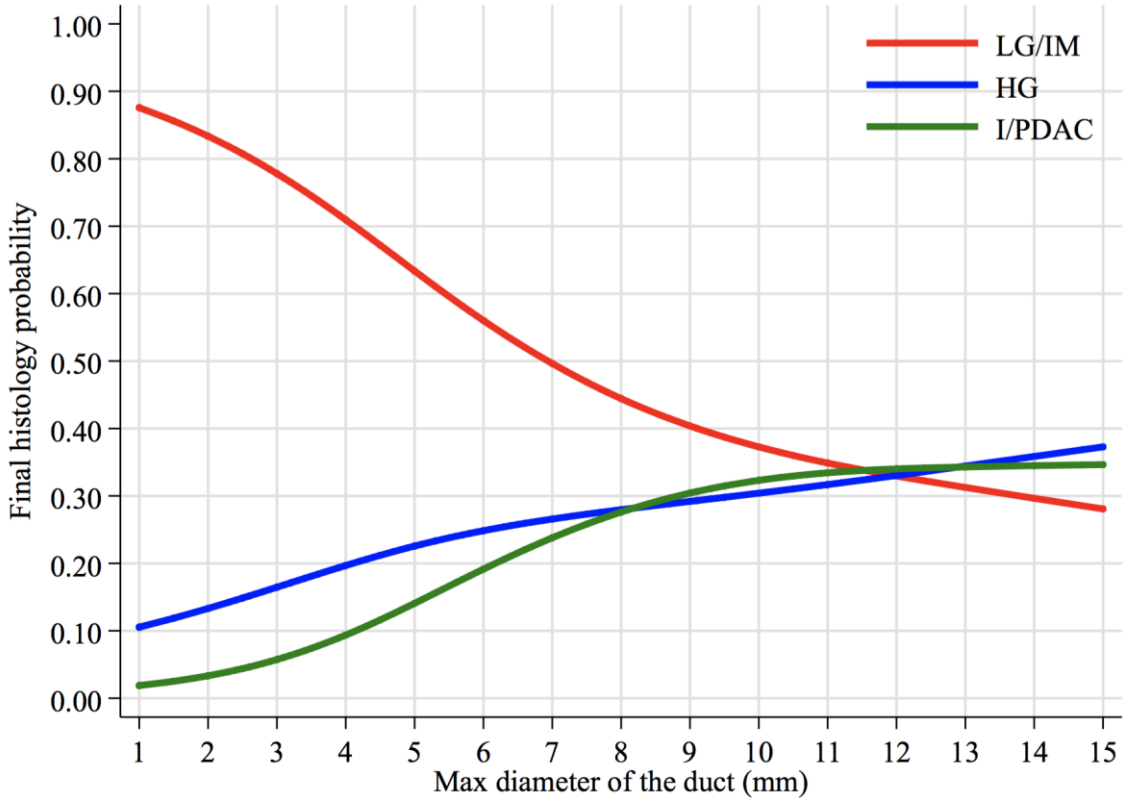


***Figure 5*** *- Predicted probabilities of different IPMN-dysplasia as function of the max MPD-dilatation.*

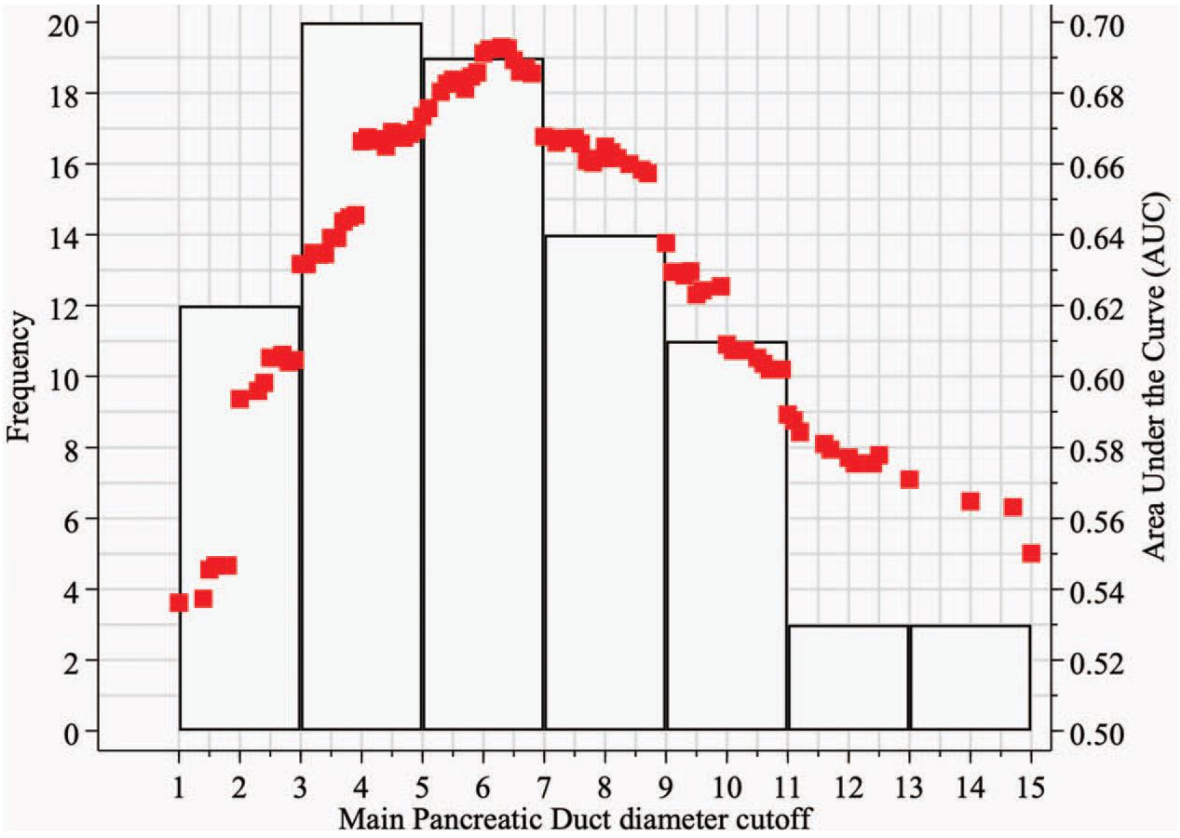


***Figure 6*** *– Area under the curve (AUC), illustrated by the red squares, associated with every cut-points used to dichotomize max diameter of the duct dilatation as the only predictor of the probability of HG/Inv-IPMN. The observed distribution (%) of main duct dilatation is shown in the background.*

In order to find a cut-off value with the best ability to distinguish LG-IPMN from HG-IPMN or Inv-IPMN, a ROC-curve-analysis was performed *(Figure 6)*. The results showed that an MPD-dilatation of 5-7 mm could be used as an accurate cut-off value to identify patients with high-risk IPMN.

## 5.3 STUDY III

From 70 patients who were pre-operatively assessed as eligible for inclusion, 35 patients were selected for final analysis. The excluded patients were due to insufficient cyst fluid in 23 cases and for 12 cases the final histology verified another pathology than SCN or IPMN. From the final 35 patients included in this cohort study, plasma was collected in 21 cases and cystic fluid in 31 cases *(Figure 7)*. The patient characteristics of this final cohort is presented in Table 11.

### 5.3.1 Metabolomics

The metabolite analysis of cystic fluid and plasma was performed by a targeted and (semi)quantitative liquid chromatography-mass spectrometry where 90 and 91 metabolites were measured from respective type of sample. Heatmap of the metabolomic data showed no clear grouping of metabolite profile, however, the principal component analysis (PCA) showed that the metabolic profile was different in cyst fluid from SCN compared to other groups *(Figure 8)*. Next, a quantitative metabolic pathway enrichment analysis was applied which showed several pathways that could distinguish the different types of cystic lesions. In cyst fluid from patients with HGD/cancer, 34 enriched pathways were found and from patients with LGD 12 pathways were identified. The majority of these pathways were lipid pathways. Several of these pathways were also found to be enriched in plasma for instance sphingolipid metabolism, phosphatidylethanolamine biosynthesis and phosphatidylcholine biosynthesis.

### 5.3.2 Lipidomics

Since the first analysis of metabolomics showed alterations in the lipid metabolism, a high definition of lipid profiling was performed. Moreover, patients with PCN might also have an affected pancreatic exocrine function with changes in lipase, which further indicates the need of a lipid analysis. Using the SCIEX Lipidyzer™ technology, 1100 lipids were analyzed of which 430 were detected and quantified in cyst fluid and 941 in plasma. The heatmap showed that triacylglycerol (TAG)-related lipids were in majority in both cyst fluid and plasma whilst the PCA showed differences in projections between the groups, similar to metabolomic analysis *(Figure 9)*. An observation was that the profiles of HGD and Cancer seemed to very similar in the PCA.

Fold change estimations indicate altered TAG-classes in plasma from both LGD and HGD/cancer, whereas free fatty acids (FFA) and ceramides (CER) seem to be in higher concentration in cystic fluid from IPMN patients *(Figure 10)*.

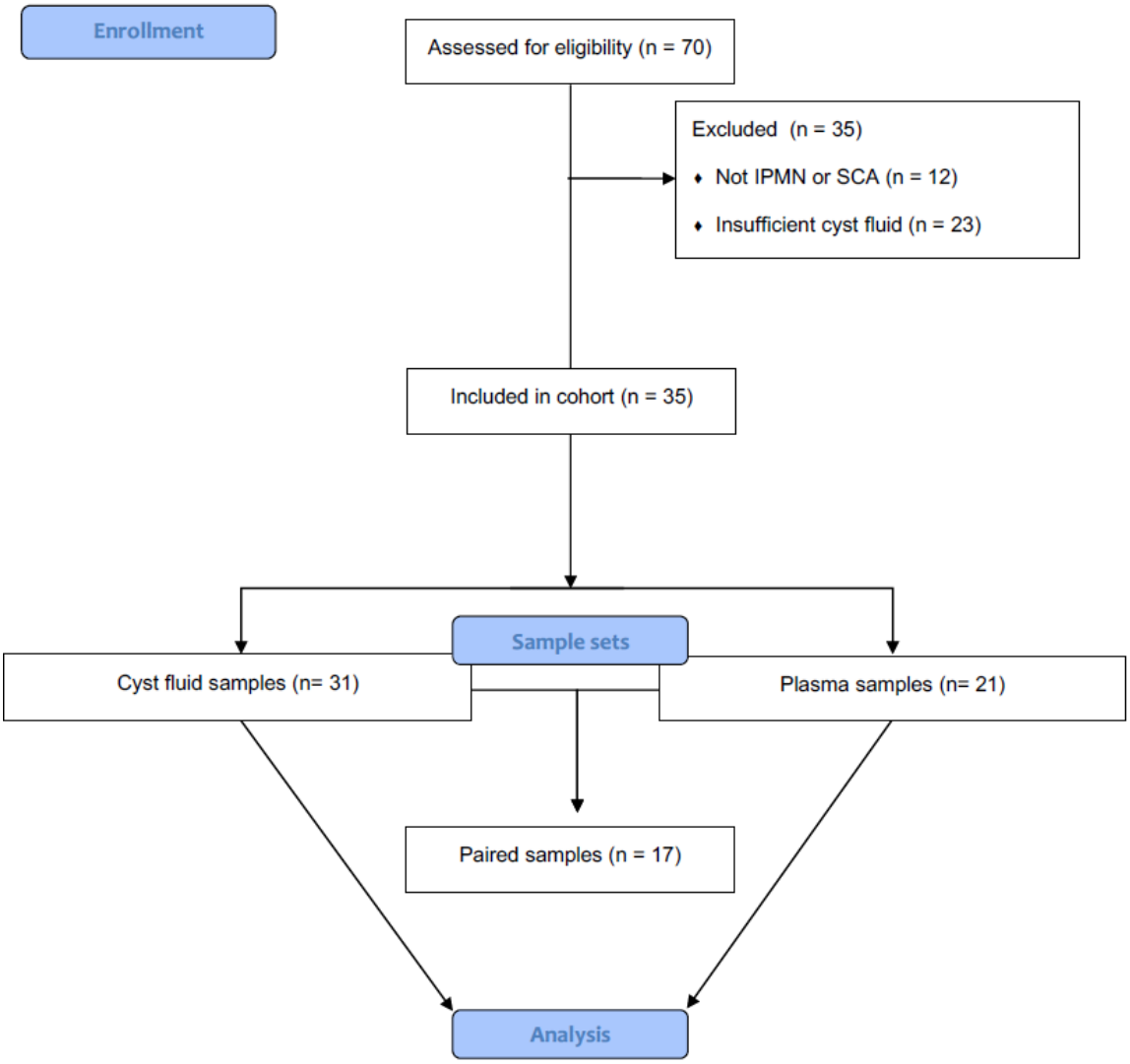


***Figure 7*** *- Flowchart of patients included in the cohort and distribution of samples from cyst fluid and plasma of study III.*

***Table 11*** *– Patient group characteristics. Statistical comparisons between each group and the control group (SCN) were made using one-way ANOVA with Dunnett's multiple comparisons test; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. HbA1c, normal < 52 mmol/mol, S-CA 19-9, normal < 34 kE/L,Amylase, normal <0,15-1,10 µkat/L, Albumin normal 36-45 g/L, ,Bilirubin, normal <5-25 µmol/L, WBC, x $10^9$/L median (range)*

| | CYST FLUID (n = 31) | | | | PLASMA (n = 21) | | | |
|---|---|---|---|---|---|---|---|---|
| | SCN | IPMN | | | SCN | IPMN | | |
| | | LGD | HGD | Cancer | | LGD | HGD | Cancer |
| **Patients** n (%) | 5 (16.1%) | 8 (25.8%) | 7 (22.6%) | 11 (35.5%) | 5 (23.8%) | 5 (23.8%) | 6 (28.6%) | 5 (23.8%) |
| **Female** (%) | 100 | 50 | 37.5 | 27.27 | 100 | 20 | 33.33 | 40 |
| **Alcohol use** (%) | 50 | 25 | 25 | 15.39 | 40 | 20 | 16.67 | 60 |
| **Smokers** (%) | 33.3 | 0 | 0 | 9.09 | 40 | 0 | 0 | 40 |
| **CVD** (%) | 16.67 | 71.43 | 62.5 | 54.55 | 20 | 60 | 50 | 60 |
| **Statins use** (%) | 16.67 | 12.5 | 12.5 | 9.09 | 20 | 0 | 16.67 | 40 |
| **Diabetes** (%) | 0 | 12.5 | 12.5 | 36.36 | 0 | 20 | 16.67 | 20 |
| **Age**, years | 50.5 | **66*** | **71**** | **69***** | 53 | 65 | **72.5**** | **69**** |
| median (range) | (34 - 68) | (56 - 81) | (66 - 75) | (46 - 83) | (34 - 68) | (56 - 76) | (66 - 75) | (65 - 83) |
| **BMI**, kg/m$^2$ | 28.83 | 27.51 | 25.84 | 24.97 | 28.01 | 32.16 | 24 | 25.69 |
| median (range) | (24.09 - 32.01) | (21.77 - 36.61) | (21.5 - 28.34) | (20.16 - 29.7) | (24.09 - 31.01) | (24.80 - 36.61) | (21.5 - 28.34) | (24.08 - 32.88) |
| **HbA1c**, mmol/ mol | 32 | 42.5 | 38 | **43*** | 33 | 44 | 38 | 51.5 |
| median (range) | (30 - 43) | (35 - 48) | (31 - 55) | (31 - 67) | (30 - 43) | (37 - 48) | (31 - 55) | (31 - 81) |
| **S-CA 19-9**, kE/L | 11 | 18 | 16 | **376**** | 11 | 11 | 16 | **285**** |
| median (range) | (6.8 - 62) | (6.4 - 182) | (<1 - 115) | (<1 - 1040) | (7.9 - 62) | (6.4 - 182) | (<1 - 115) | (46 - 480) |
| **Serum amylase**, µkat/L | 0.31 | 0.41 | 0.2 | 0.25 | 0.31 | 0.44 | 0.195 | 0.27 |
| median (range) | (0.19 - 1.64) | (<0.13 - 0.65) | (<0.13-0.93) | (<0.13-0.87) | (0.19 - 1.64) | (<0.13 - 0.54) | (<0.13 - 0.72) | (<0.13 - 0.54) |
| **Albumin**, g/L | 37 | 36 | 34.5 | 31 | 38 | 37 | 34.5 | 31.5 |
| median (range) | (33 - 39) | (26 - 38) | (22 - 39) | (19 - 38) | (33 - 39) | (36 - 39) | (22 - 39) | (28 - 34) |
| **Bilirubin**, µmol/L | 6 | 6.5 | 7.5 | 24 | 6 | 8 | 8 | 30 |
| median (range) | (3 - 18) | (<3 - 13) | (<3 - 315) | (5 - 150) | (3 - 7) | (4 - 13) | (4 - 315) | (12 - 119) |
| **WBC**, x $10^9$/L | 6.65 | 7.45 | 7.6 | 9.8 | 6.3 | 7.5 | 8.3 | **11.2**** |
| median (range) | (4.4 - 9.2) | (5 - 9.4) | (5.6- 12.9) | (5 - 13.9) | (4.4 - 9.2) | (5.3 - 9.4) | (7.2- 12.9) | (8 - 13.9) |

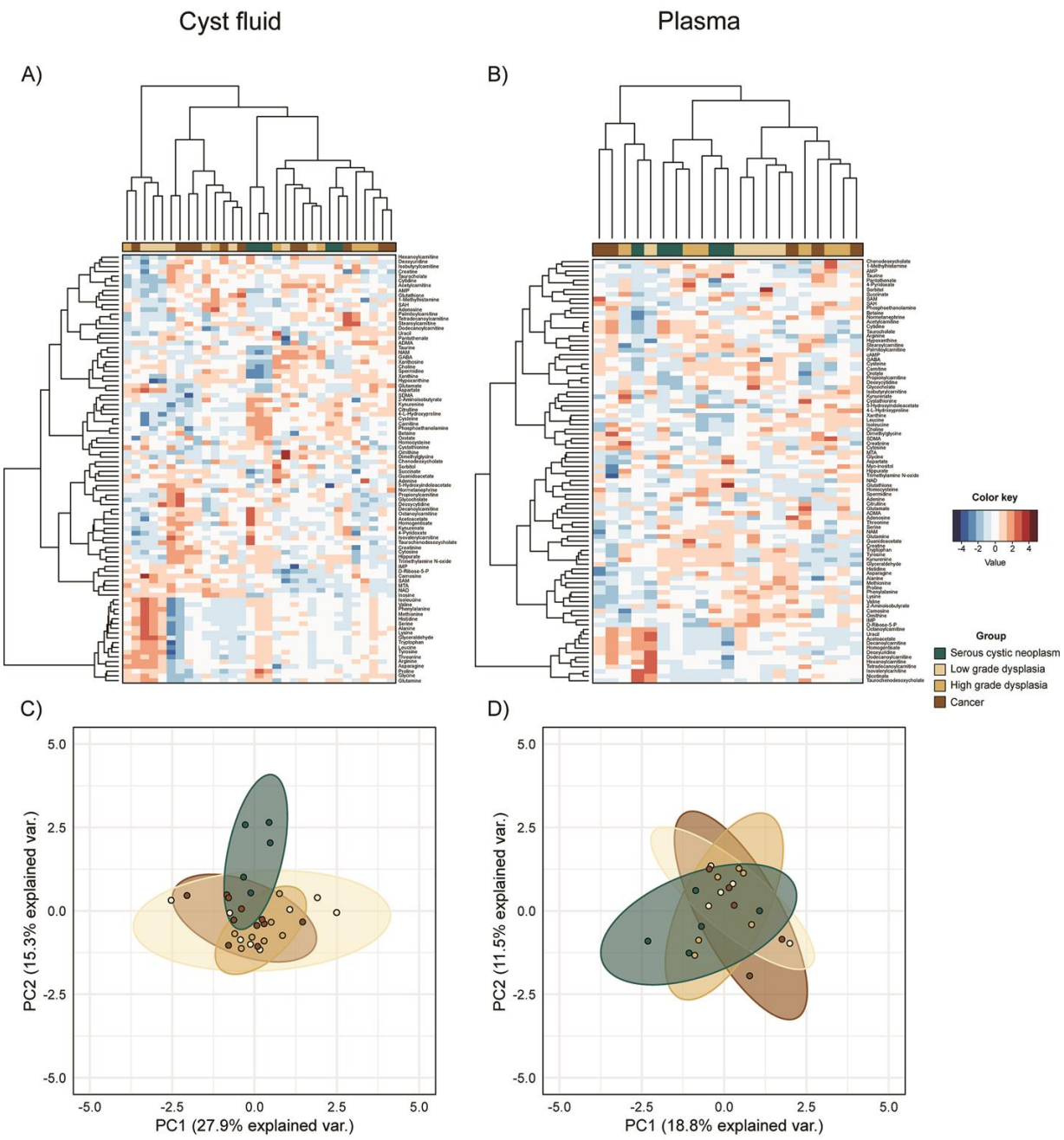


***Figure 8*** *- Metabolomic profile. Heatmap of cyst fluid (A) and plasma (B) metabolite concentrations. Projection of patient samples on the first two principal components (PCA) for cyst fluid (C) and plasma (D) datasets.*

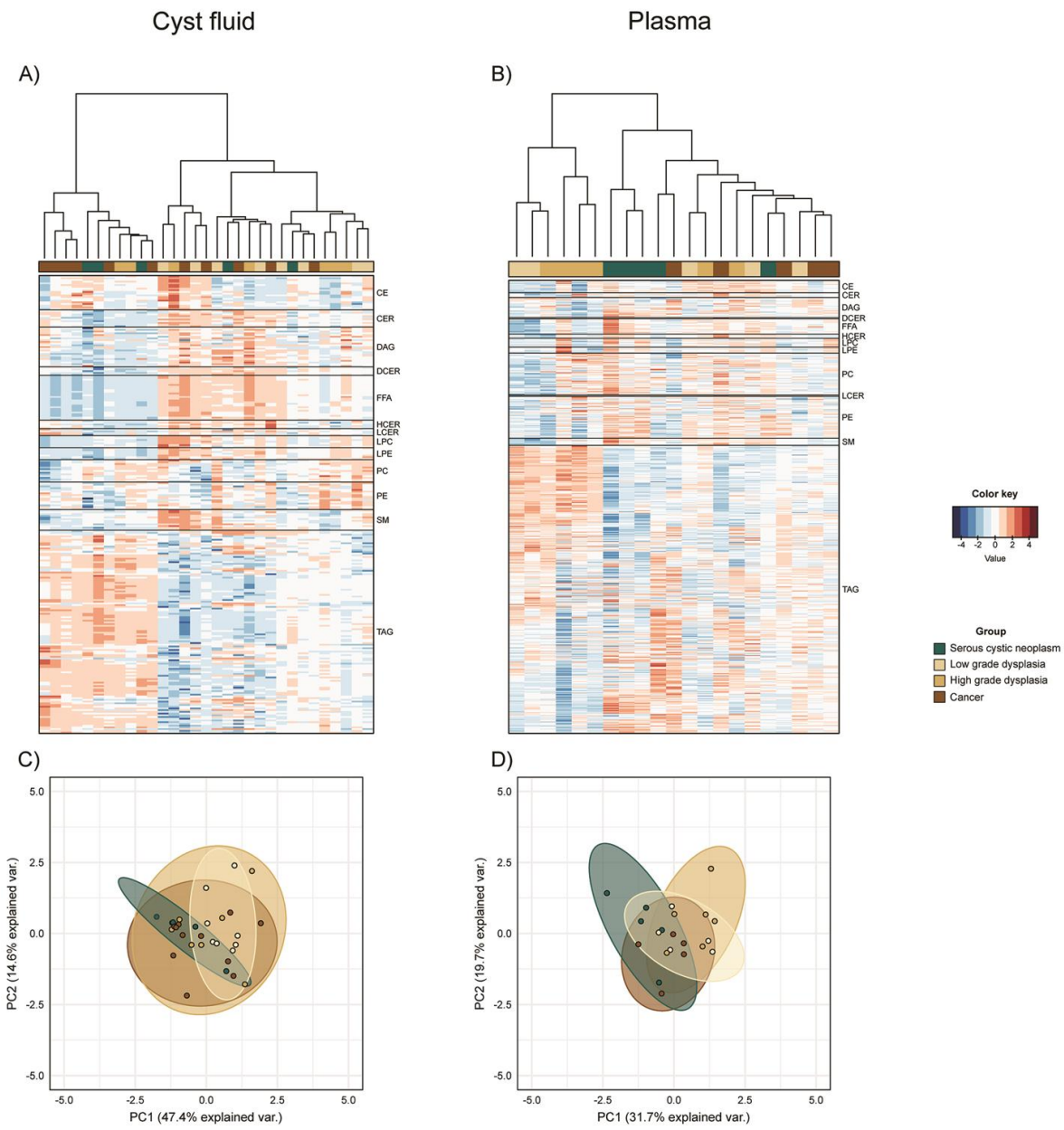


***Figure 9*** *- Lipidomic profile. Heatmap of cyst fluid (A) and plasma (B) lipid concentrations. Projection of patient samples on the first two principal components (PCA) for cyst fluid (C) and plasma (D) datasets.*

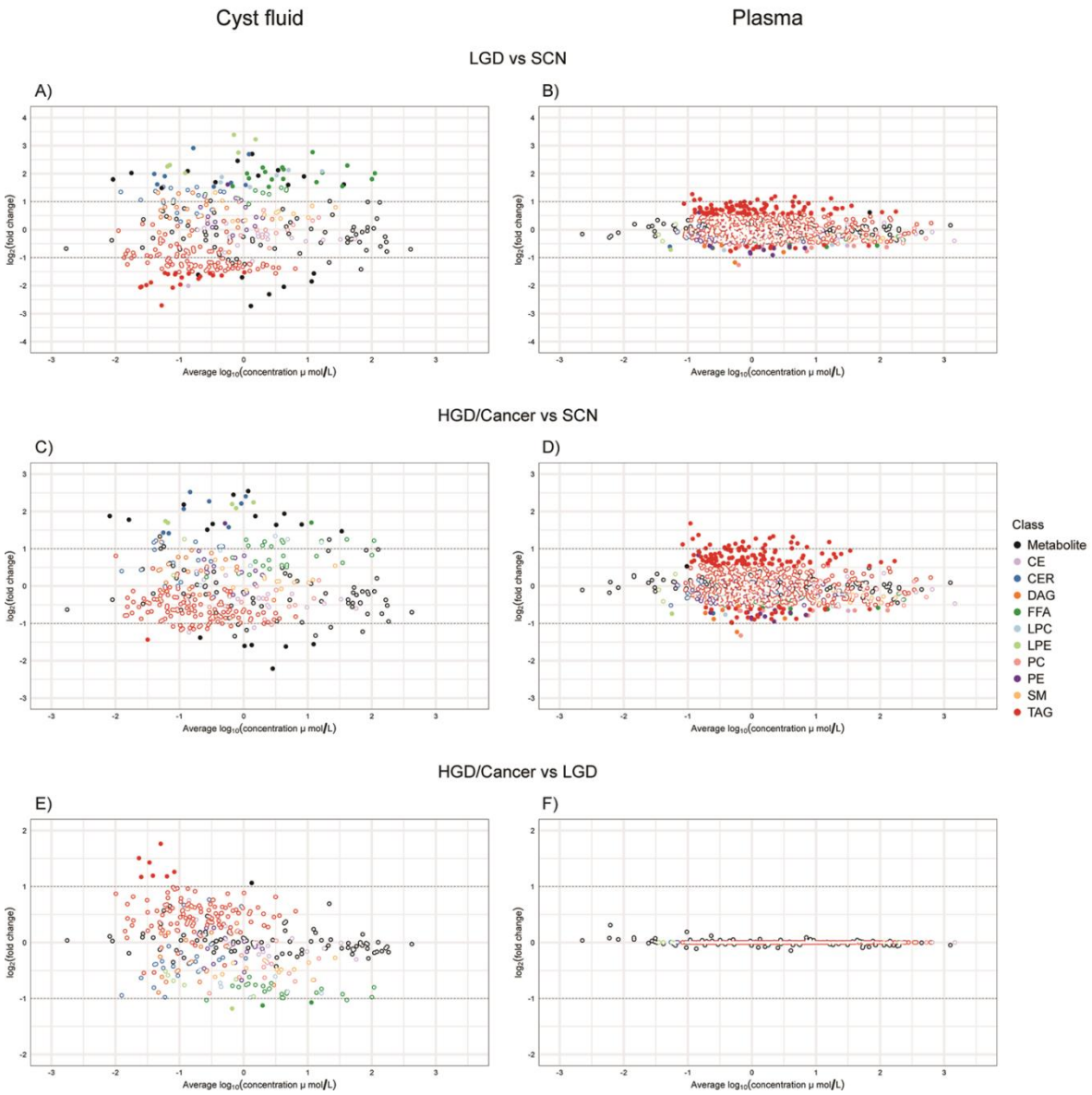


***Figure 10*** *- Estimated fold changes of concentrations of all measured analytes (including metabolite and lipid molecular species) between selected groups. LGD compared to SCN in cyst fluid (A) and plasma (B). HGD/Cancer compared to SCN in cyst fluid (C) and plasma (D). HGD/Cancer compared to LGD in cyst fluid (E) and plasma (F). Filled dots are fold changes whose credibility interval does not overlap with the null reference value of one-fold change, or zero on the plotted log scale.*

### 5.3.3 Integrated Analysis to Predict Lesion and Degree of Dysplasia

The ultimate aim in diagnosis of cystic lesions of the pancreas is not only to distinguish the pre-malignant lesions (IPMN and MCN) from the benign lesions (SCN and pseudocyst) but also to identify those among IPMN and MCN that require surgery, namely the lesions that have high-grade dysplasia or have become invasive. We therefore integrated the results from the metabolomics and lipidomics analyses to test their ability to classify the different types of lesions and the degree of dysplasia/invasiveness. Thus, a Canonical Powered Partial Least Squares Discriminant Analysis (CPPLS-DA) model was used which was able to discriminate

IPMN from SCN in both cyst fluid and plasma *(Figure 11)*. The discriminatory metabolites distinguishing both groups were choline, 2-aminoisobutyrate, trimethylamine n-oxide, glycine, alanine and glyceraldehyde. In addition, dimethylglycine was a discriminatory molecule in cyst fluid whereas serine and GABA were discriminatory metabolites in plasma. The performance of the models was calculated which showed a balanced accuracy of 100% when differentiating IPMN from SCN in samples from both cyst fluid and plasma *(Table 12)*. For the rest of the comparisons the accuracy varied around 90% and was slightly better when testing from cyst fluid than plasma. In regards of discriminating the lesions subject for surgery (HGD/cancer) from others, the balanced accuracy was 90.6 % for cystic fluid and 81.8% for plasma.

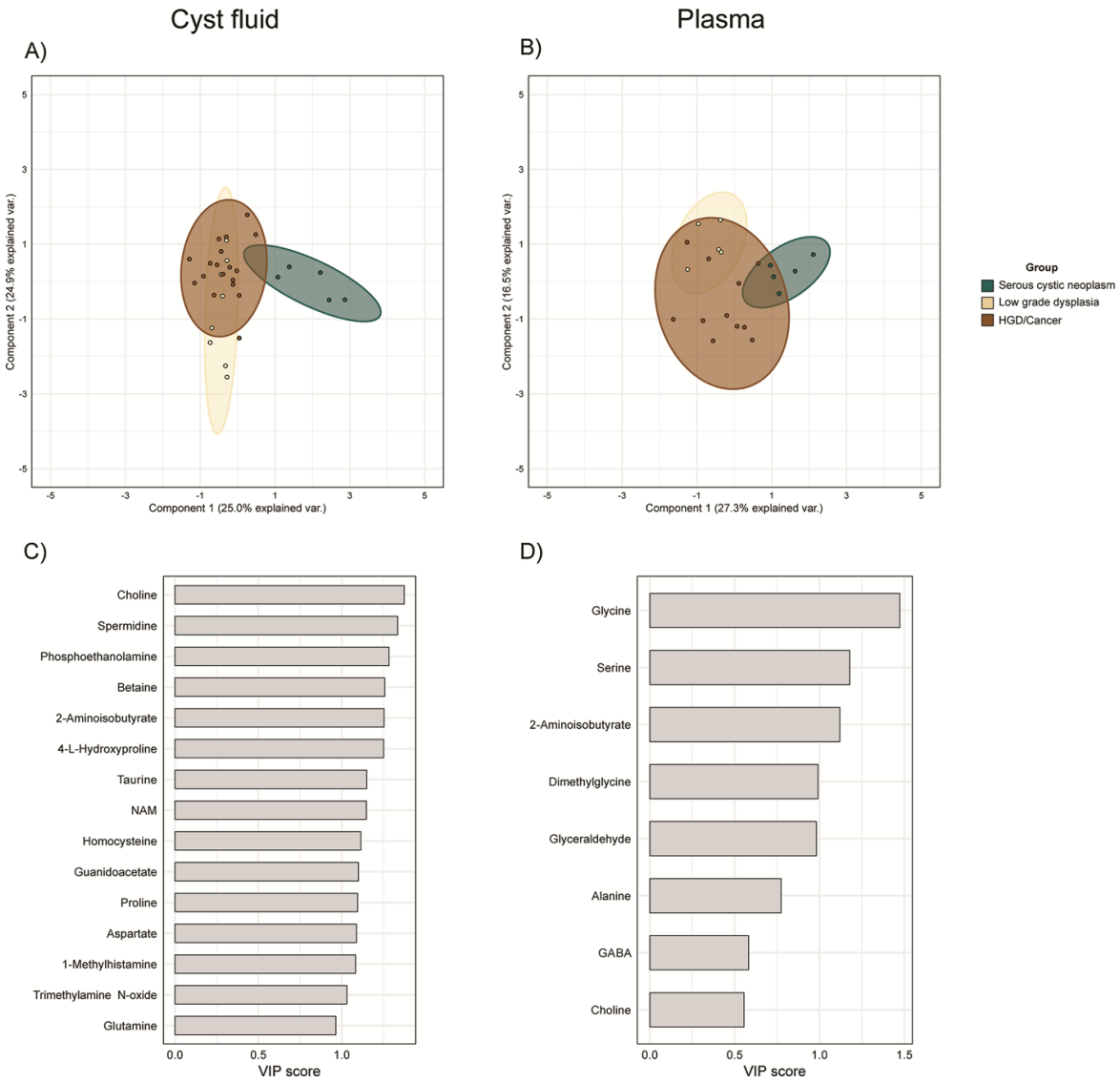


***Figure 11*** *- Canonical Powered Partial Least Squares and Discriminant Analysis (CPPLS-DA) results. Projection of patient samples on the first two principal components in cyst fluid (A) and plasma (B). Highest variable importance in projection (VIP) scores in cyst fluid (C) and plasma (D).*

***Table 12** - Performance measures of binary classifications with the chosen CPPLS-DA model.*
*[a]Area Under the ROC Curve; [b](Sensitivity + Specificity) / 2*

| | Type of sample | AUC[a] | Sensitivity | Specificity | Balanced accuracy[b] |
|---|---|---|---|---|---|
| **SCN vs All** | Cyst fluid | 1.000 | 1.000 | 1.000 | 1.000 |
| | Plasma | 0.950 | 0.800 | 0.875 | 0.837 |
| **LGD vs All** | Cyst fluid | 0.935 | 0.875 | 0.913 | 0.894 |
| | Plasma | 0.825 | 1.000 | 0.812 | 0.906 |
| **HGD-Cancer vs All** | Cyst fluid | 0.949 | 0.889 | 0.923 | 0.906 |
| | Plasma | 0.854 | 0.636 | 1.000 | 0.818 |
| **IPMN vs SCN** | Cyst fluid | 1.000 | 1.000 | 1.000 | 1.000 |
| | Plasma | 1.000 | 1.000 | 1.000 | 1.000 |

## 5.4 STUDY IV

A total of 57 cyst fluid samples and 45 plasma samples were collected. Patient characteristics and the distribution of different types of cystic lesions are described in Table 13.

### 5.4.1 Metabolomics

Untargeted metabolomics analyses were performed on plasma *(Figure 12)* and cyst fluid samples *(Figure 13)*. The plasma analysis was able to identify distinct signatures in all of the cystic lesions *(Figure 12 B)*. Pathway analysis showed metabolites related to amino acid metabolism, carboxylic acids and glycolysis related metabolism *(Figure 12 C, D & E)* to have significant impact on disease progression.

The cyst fluid was analyzed in similar fashion and identified other top metabolism pathways namely purine oxidation, heme metabolism, acetyl carnitine and glycolytic metabolism *(Figure 13 C, D, E & F)*.

Next, to validate these results, targeted metabolomics analyses were performed where 123 metabolites in plasma and 161 in cyst fluid were selected. A partial least square-discriminant analysis (PLS-DA) was performed where the top 25 metabolites are illustrated in Figure 14 by hierarchal clustering. This analysis showed similar changes in top pathways as in the untargeted analysis. This was the case in particular for patients with HGD.

***Table 13*** *- Patient group characteristics. Statistical comparisons between each group and the control group (SCN) were made using Stata 15 software and quantile regression for all quantitative measures; * p≤0.05, ** p≤0.01, *** p≤0.001.*

| | CYST FLUID (n = 57) | | | | PLASMA (n = 45) | | | |
|---|---|---|---|---|---|---|---|---|
| | SCN | IPMN | | | SCN | IPMN | | |
| | | LGD | HGD | Cancer | | LGD | HGD | Cancer |
| **Patients** n (%) | 5 (8.8) | 29 (50.9) | 8 (14.0) | 15 (26.3) | 5 (11.1) | 20 (44.4) | 10 (22.2) | 10 (22.2) |
| **Female** (%) | 100 | 59 | 62 | 53 | 100 | 50 | 70 | 40 |
| **Smokers** (%) | 0 | 19 | 13 | 29 | 0 | 15 | 0 | 20 |
| **CVD** (%) | 40 | 62 | 75 | 53 | 40 | 60 | 70 | 60 |
| **Statins use** (%) | 0 | 21 | 25 | 13 | 20 | 21 | 40 | 20 |
| **Diabetes** (%) | 0 | 21 | 50 | 27 | 0 | 20 | 60 | 20 |
| **Age**, years | 47 | **70***** | **76***** | **71***** | 47 | **68***** | **74***** | **76**** |
| median (range) | (30 - 68) | (47 - 82) | (67 - 84) | (55 - 82) | (30 - 68) | (55 - 79) | (66 - 84) | (65 - 83) |
| **BMI**, kg/m$^2$ | 33.69 | **26.39**** | 28.56 | **24.21***** | 28.01 | 26.87 | 28.04 | 24.08 |
| median (range) | (21.6 - 37.1) | (20.8 - 40.4) | (23.5 - 32.0) | (18.8 - 30.2) | (24.6 - 37.1) | (21.6 - 36.7) | (23.4 - 30.5) | (18.8 - 29.5) |
| **HbA1c**, mmol/ mol | 40 | 40 | 52 | 41 | 41 | 41 | 42 | 40 |
| median (range) | (33 - 49) | (32 - 58) | (28 - 68) | (15.1 - 60) | (36 - 49) | (32 - 62) | (28 - 68) | (31 - 67) |
| **S-CA 19-9**, kE/L | 9.9 | 9.4 | 24 | 39 | 7.9 | 11 | 24 | **255***** |
| median (range) | (<3 - 34) | (<1 - 60) | (9 - 71) | (<1 - 4840) | (<3 - 11) | (<1 - 182) | (<1 - 189) | (<1 - 694) |
| **Serum amylase**, µkat/L | 0.46 | 0.43 | 0.53 | 0.6 | 0.46 | 0.44 | 0.27 | 0.26 |
| median (range) | (0.3 – 0.75) | (<0.13 - 1.45) | (<0.13 - 4.18) | (<0.13 - 11.6) | (0.19 - 0.75) | (<0.13 - 1.45) | (<0.13 - 4.18) | (<0.13 - 2.07) |
| **Albumin**, g/L | 37 | 37 | 36 | 34 | 37 | 36 | 36 | 34 |
| median (range) | (33 - 40) | (31 - 42) | (16 - 39) | (22 - 38) | (33 - 40) | (31 - 42) | (16 - 39) | (22 - 38) |
| **Bilirubin**, µmol/L | 5 | 6 | 8 | 8 | 5 | 6.8 | 5 | **19***** |
| median (range) | (5 - 9) | (<3 - 34) | (<3 - 12) | (4 - 127) | (3 - 9) | (3 - 34) | (3 - 25) | (4 - 119) |
| **WBC**, x 10$^9$/L | 7.6 | 7.0 | 7.6 | 7.9 | 7.1 | 7.1 | 8.4 | 10.2 |
| median (range) | (4.1 - 10.9) | (0.3 - 11.3) | (5.5 - 9.4) | (4.2 - 10.8) | (4.1 - 10.9) | (0.3 - 11.3) | (6.4 - 12.9) | (4.2 - 13.9) |

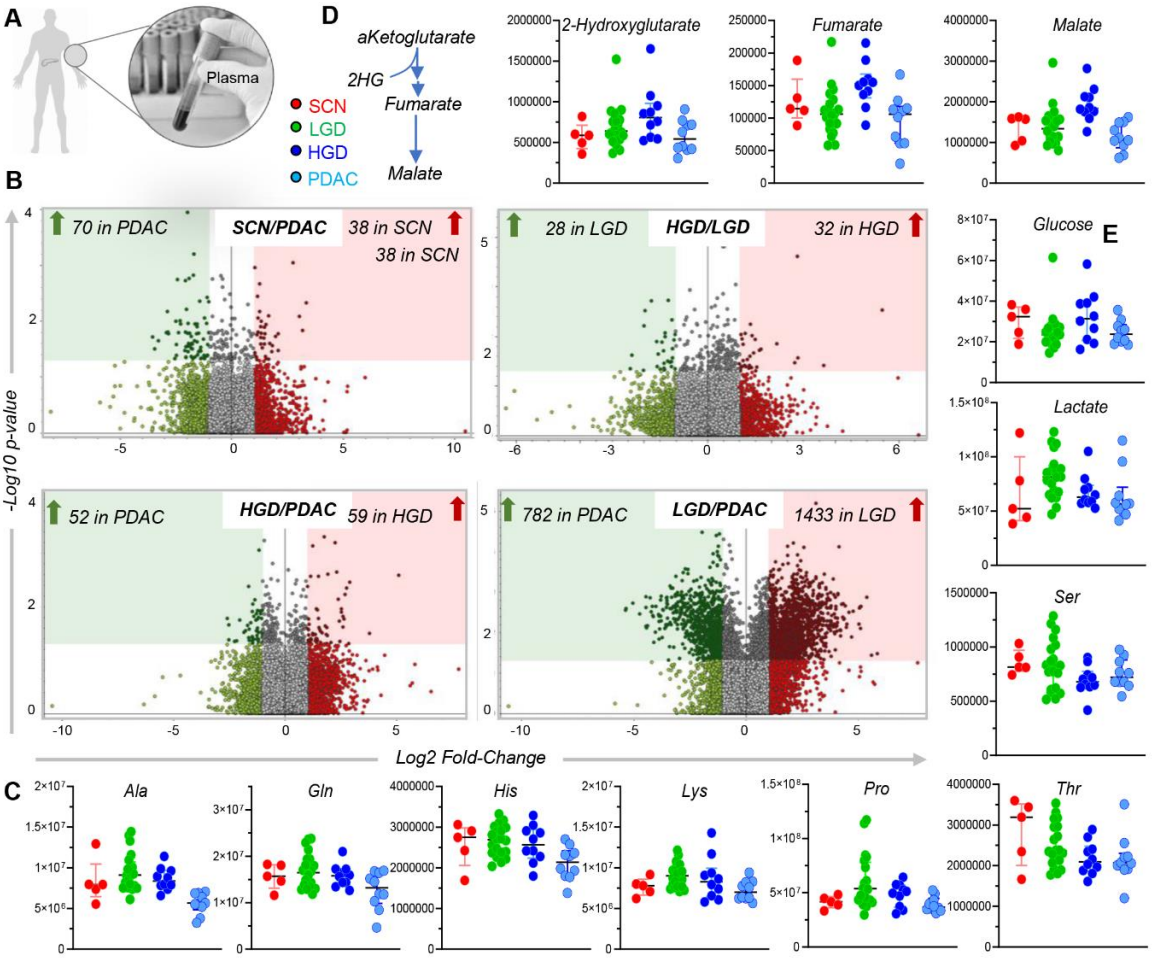


***Figure 12*** *- Untargeted metabolomics analyses of plasma (A) revealed distinct signatures in IPMN and PDAC patients, as shown in the volcano plots from the untargeted metabolomics analyses (B). Top pathways included amino acid metabolism (C), carboxylic acids (D) and glycolysis-related metabolites (glucose and lactate, substrate and product of the pathway, respectively - E).*

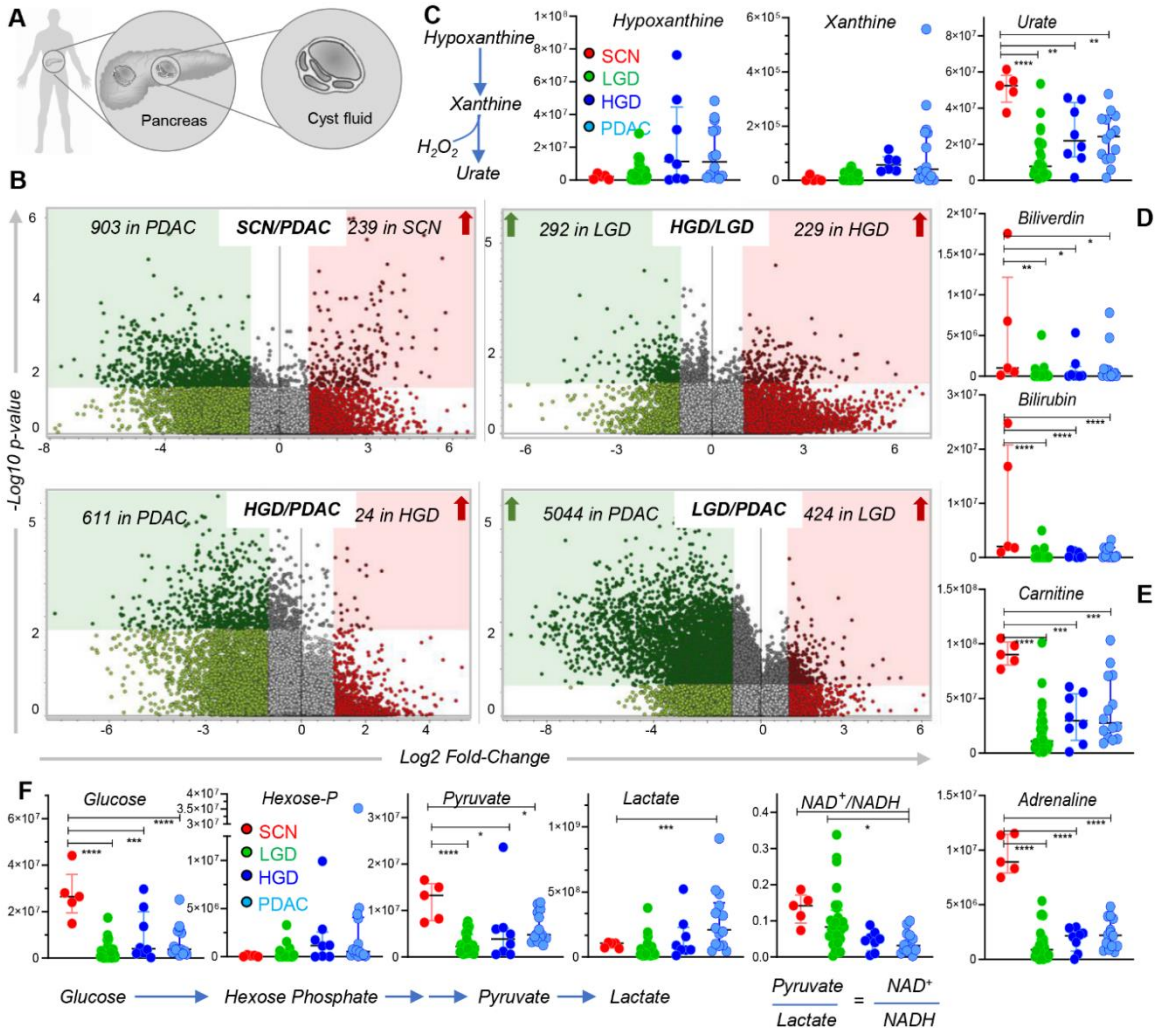


***Figure 13*** *- Untargeted metabolomics analyses of cyst fluid (A) revealed distinct signatures in IPMN and PDAC patients, as shown in the volcano plots from the untargeted metabolomics analyses (B). Top pathways included purine oxidation (C), heme metabolism (D), acyl-carnitines (E) and glycolytic metabolites (F).*

***Table 14*** *- Top metabolic pathways in a) Plasma and b) Cyst fluid from the untargeted metabolomics analysis*

| ***A)*** **Plasma** | | ***B)*** **Cyst Fluid** | |
|---|---|---|---|
| **Metabolic Pathways** | *Metabolite* | Metabolic Pathways | *Metabolite* |
| **Amino Acid** | Alanine | **Glycolytic** | Glucose |
| | Glutamine | | Lactate |
| | Histidine | | Hexose |
| | Lysine | | Phosphate Isobars |
| | Proline | | Pyruvate |
| | Threonine | | |
| | Serine | **Purine** | Hypoxanthine |
| | | | Xanthine |
| **Carboxylic Acid** | 2-Hydroxyglutarate | | Urate |
| | Fumarate | | |
| | Malate | **Heme** | Biliverdin |
| | | | Bilirubin |
| **Glycolytic** | Glucose | | |
| | Lactate | **Acyl-Carnitine** | Carnitine |

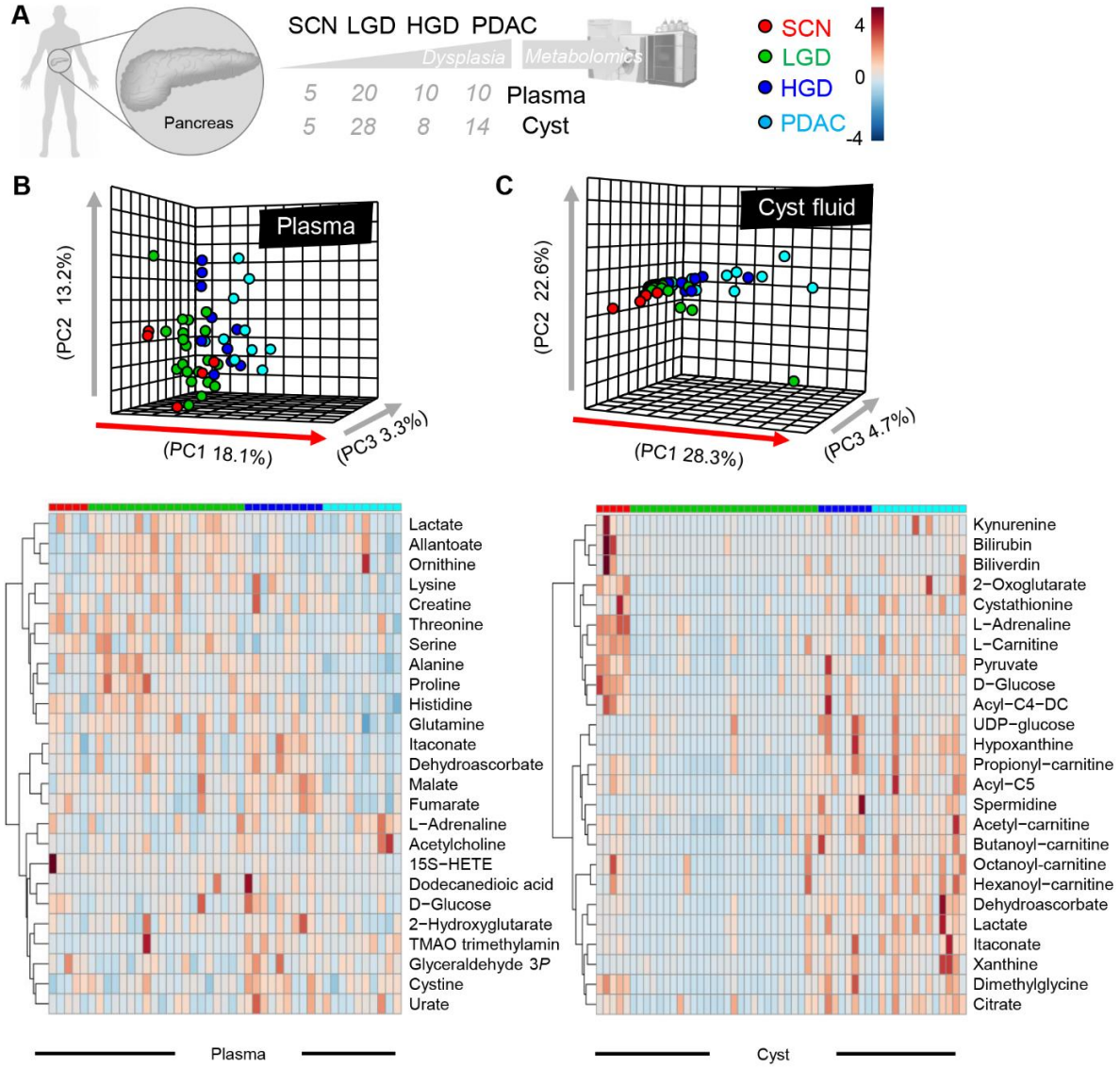


***Figure 14*** *- Metabolomics analyses of plasma and cyst fluid from IPMN and PDAC patients. An overview of the experimental design is provided in A. In B and C, results from partial least square-discriminant analysis (PLS-DA) and hierarchical clustering analysis (top 25 metabolites by ANOVA p-values) in plasma and cyst fluid, respectively.*

### 5.4.2 Biomarker Analysis

The top metabolites were tested with absolute quantitation data to determine the sensitivity and specificity when comparing cancerous lesions (HGD-IPMN and PDAC) with non-cancerous neoplasms (LGD-IPMN and SCN) *(Figure 15).* Amino acids, carboxylic acids and some acyl-carnitines were identified as top biomarkers for HGD and PDAC in cyst fluid *(Figure 15 B).* Carnitines as a class of compounds was the top discriminant between the cancerous and non-cancerous lesions *(Figure 15 C).* The top discriminatory markers between SCN and PDAC were amino acids, sugars, gamma-glutamyl-cycle metabolites, purine oxidation products and carboxylic acids. The top discriminatory biomarkers between HGD and PDAC were carboxylic acids, aromatic amino acids and related metabolites and had a diagnostic accuracy of 80-90%.

Analysis of plasma identified the bacterial metabolite trimethylamine-oxide (TMAO) as the top biomarker along with several conjugated bile acids *(Figure 15 D & E).*

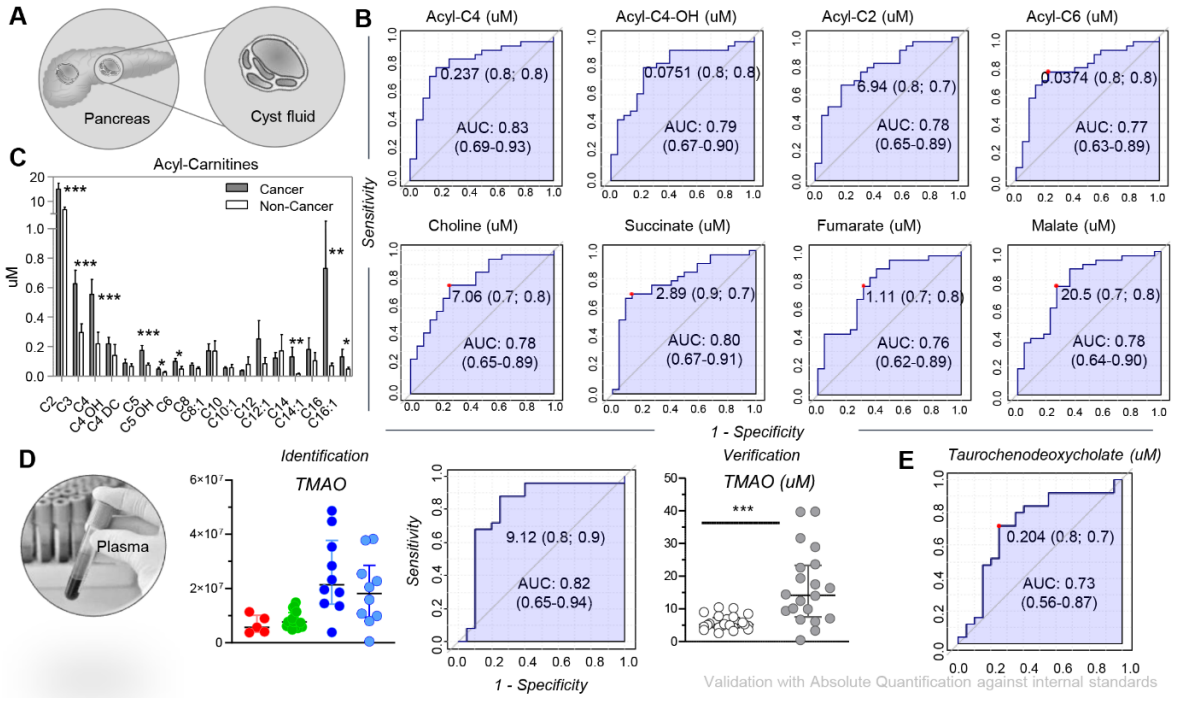


***Figure 15*** *- Most significant metabolites from the exploratory metabolomics analyses were leveraged to determine specificity and sensitivity of top biomarkers in cyst-fluid and plasma. Analysis of cyst fluid (A) identified amino acids, carboxylic acids and – above all – acyl-carnitines (ROC curves in B, bar plots in C) as the top discriminants between non-cancerous IPMN (SCN and LGD) and cysts showing HGD or derived from PDAC patients. Similar analyses in plasma (D) highlighted the bacterial metabolite trimethylamine-oxide (TMAO) as the top biomarker, an observation validated with orthogonal quantitative methods (µM concentration provide in the ROC curve and dot plot in D). Similarly, several conjugated bile acids – that are deconjugated by bacteria in humans – were identified amongst the top plasma markers of cancerous IPMN (E).*

### 5.4.3 Microbiome of Cyst Fluid and Related Metabolites

Analysis of cyst fluid with 16S rRNA gene sequencing identified several families of bacteria of which the top families were *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Alphaproteobacteria*. Copy numbers of 16s gene were then correlated to levels of metabolite which showed strong correlations for several bile acids, odd-chain and oxidized fatty acids, carboxylic acids and arginine/polyamine metabolites *(Figure 16 A & B)*. Correlation curves illustrate that the majority of the correlations was driven by samples with HGD and PDAC which had higher levels of specific metabolites and copy numbers of 16S gene counts *(Figure 16 C)*.

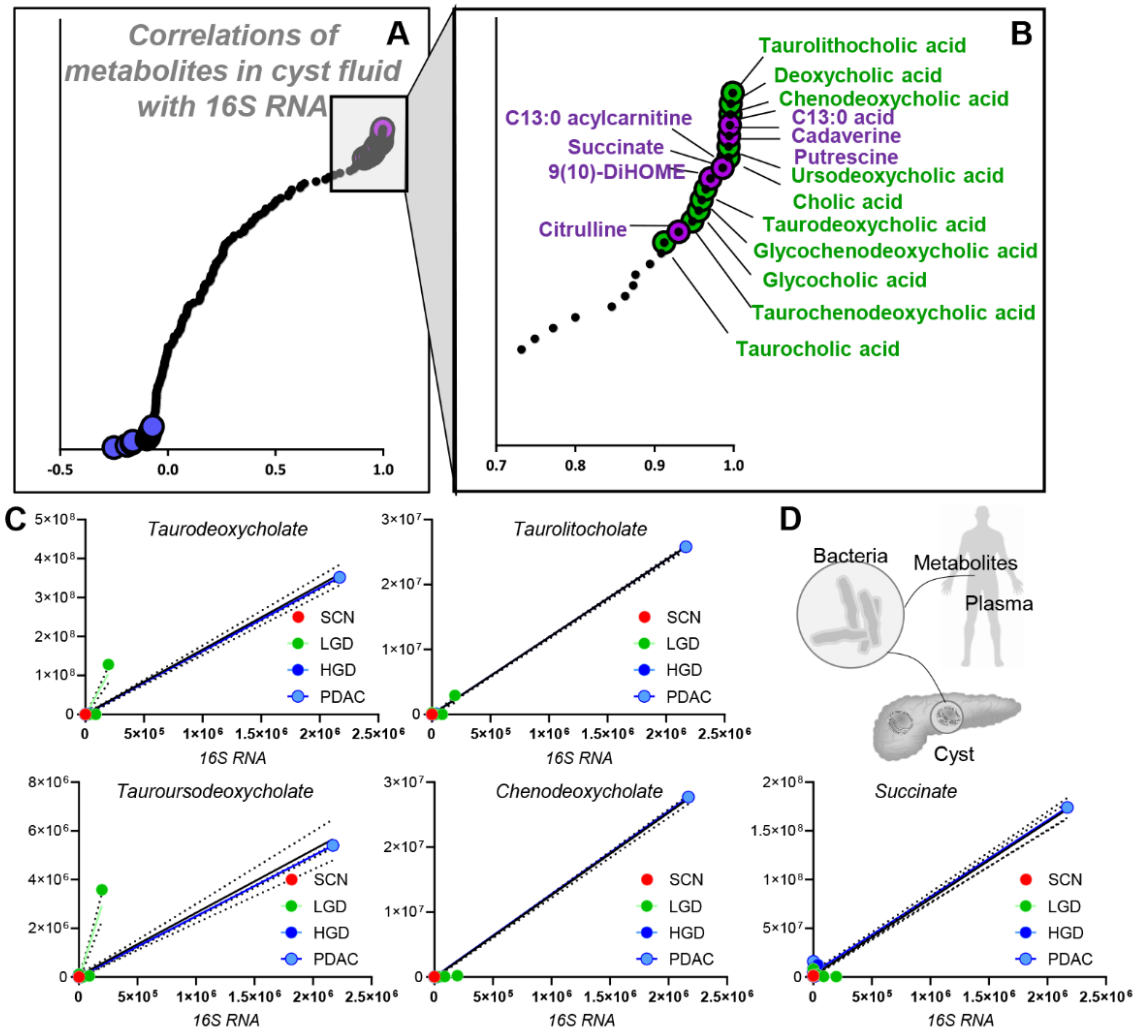


***Figure 16*** *- Correlation of 16S RNA data to cyst fluid and plasma metabolites in this study (A). Several carboxylic acids, polyamines and conjugated bile acids were identified among the top positive correlates to the total 16S RNA levels (B-C), suggestive of the model proposed in D.*

## 5.5 STUDY V

### 5.5.1 Overall Recurrence Rate and Risk Factors

In total, 274 patients underwent pancreatic surgery for IPMN. Fifty were operated with total pancreatectomy and were excluded leaving 224 patients for final analysis (Figure 4). The overall recurrence rate was 44.6% (100/224), but when only taking the clinically significant recurrences into account the rate was 30.8% (69/224) *(Figure 17)*.

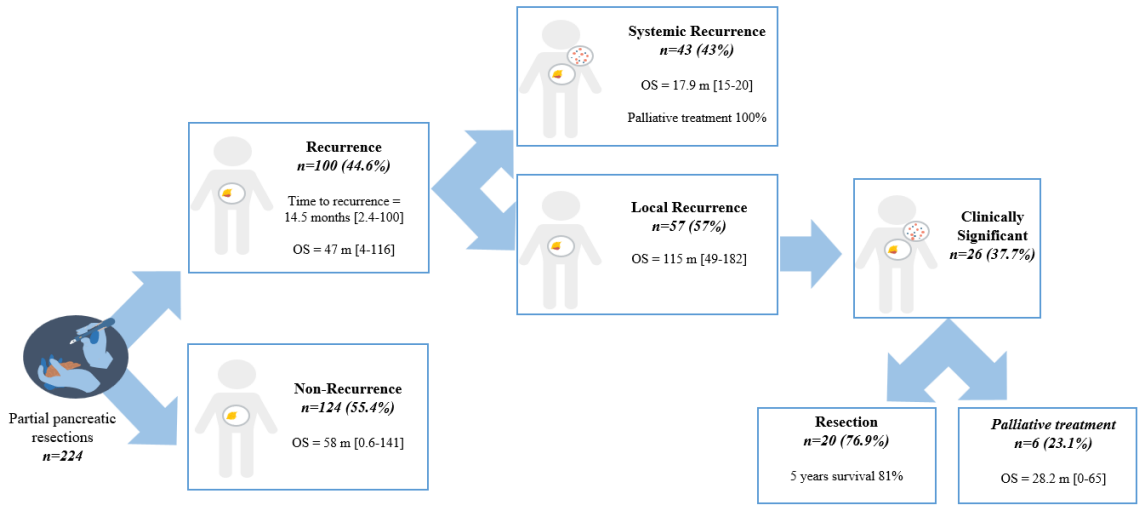


***Figure 17*** *– Flowchart and illustration of recurrence and its distribution among in patents who have undergone partial pancreatic resection. Overall survival (OS) and Time to recurrence is written at median time [range].*

A multivariate logistic regression analysis showed three risk factors to be significant for recurrence *(Table 15)*. Since patients with invasive IPMN or a concomitant PDAC are already prone to recurrence and will be put under surveillance, the aim was to focus on patients with LGD- and HGD-IPMN without concomitant cancer.

***Table 15*** *- Factors associated with risk of recurrence in patients undergoing partial pancreatic resections*

| Variable | OR | Confidence interval |
|---|---|---|
| **CA 19-9 ≥ 100 mU** | 2.2 | 1.2-3.8 |
| **Invasive IPMN** | 2.7 | 1.5-5.3 |
| **Concomitant PDAC** | 2.5 | 1.1-5.7 |

### 5.5.2 Sub-group Analysis of Patients with LGD- and HGD-IPMN

Out of 135 patients, 44 (32.6%) had a recurrence at median time of 19 months (6.8-100.1). All but one was local recurrences out which 14 (10.4%) were clinically significant *(Figure 18)*. The combined (systemic and local) clinical significant rate was 11.1%.

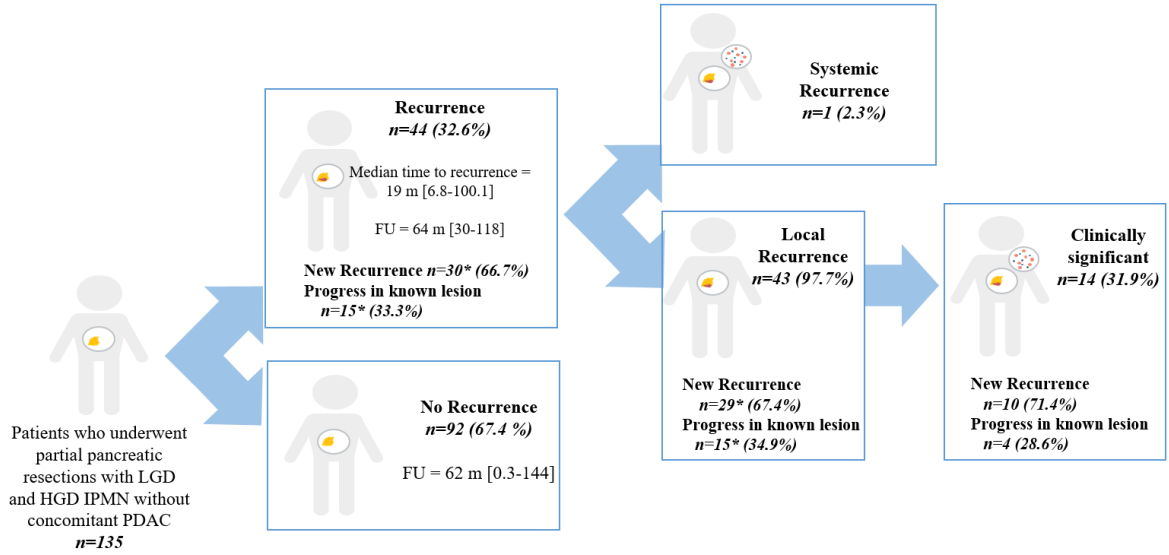


***Figure 18*** *- Flowchart and illustration of recurrence in patients who underwent partial pancreatic resection with histologically confirmed LGD and HGD IPMN at final histology without PDAC. Time to recurrence and Follow-up time (FU) is written in median months (range).* Denotes that one patient had both a new recurrence and a progress in known IPMN lesion in remnant. Both were clinically not significant.*

Comparative analysis revealed two risk factors to be significant for recurrence: "Age over 65 years" ($p$=0.019) and "Known IPMN left in remnant" ($p$=0.036). We therefore continued with a multivariate logistic regression analysis which confirmed the significance of these risk factors

***Table 16*** *- Uni- and multivariate logistic regression analysis of risk factors for recurrence.*
*Values are presented as Odds ratios (CI 95%). *adjusted to age, known IPMN, and Grade of dysplasia*

| Variable | Univariate OR (CI 95%) | Multivariate* OR (CI 95%) |
|---|---|---|
| **Age, years** | | |
| **≥65 years** | 3.3 [1.2-9.3] | 4.4 [1.5-13.1] |
| **<65 years** | Ref | Ref |
| **Known IPMN left in remnant** | | |
| **Yes** | 2.3 [1.1-5.0] | 2.6 [1.12-5.9] |
| **No** | Ref | Ref |

A time-dependent analysis was performed to evaluate the risk of recurrence over time for the two risk factors. As in the previous analysis, the risk of recurrence remained over time with HR of 3.3 (CI 1.3-8.3) for patients over the age of 65 years and 2.0 (CI 1.1-3.7) for patients with a known IPMN left in remnant at the univariate model. The multivariate analysis is illustrated in Figure 19.

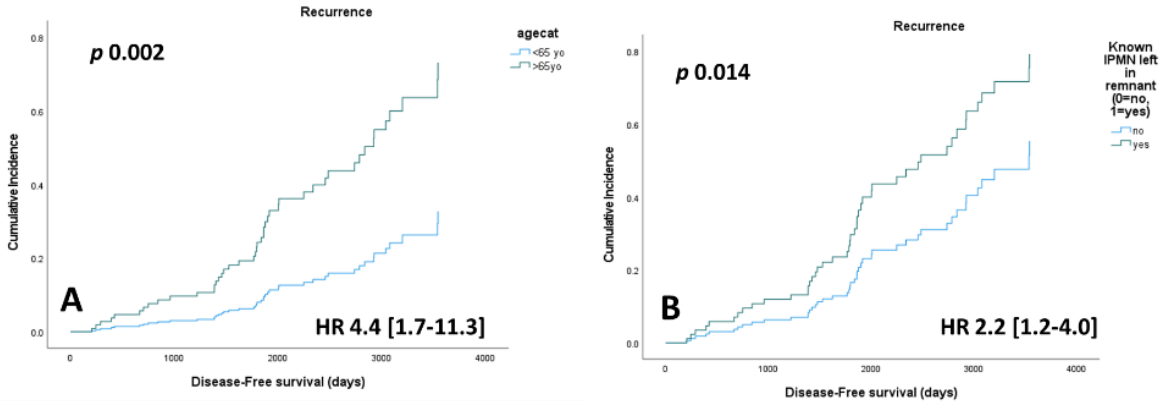


***Figure 19*** *– Multivariate time-dependent analysis illustrating the hazard ratio for recurrence. a) Stratified for age over 65 yes/no. b) Stratified for Known IPMN-lesion left in remnant yes/no.*

### 5.5.3 LGD vs HGD IPMN

Patients with LGD-IPMN at first histology had a similar time to recurrence as the patients with HGD 14.5 and 16 months respectively. This indifference was also seen in the time-dependent analysis in both non-adjusted (HR 1.1 [CI 0.6-2.1]) and adjusted model (HR 1.1 [CI 0.5-2.2]) *(Figure 20).*

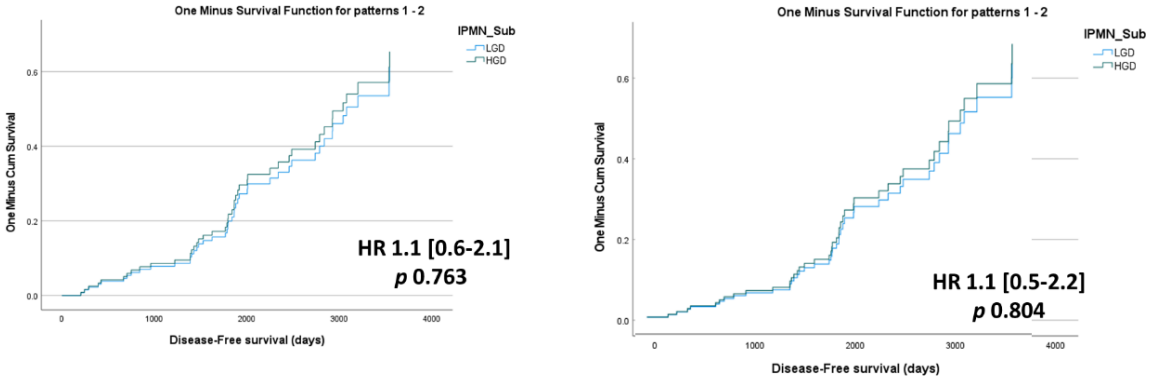


***Figure 20*** *- Illustrates the HR for DFS comparing patients with LGD and HGD at final histology. a) Non-adjusted. b) Adjusted for significant variables.*

### 5.5.4 Re-operations

Twenty (8.9%) of all partially resected patients (n=224) met the criteria for re-operation. Sixteen were due to a new recurrence whereas four were due to a progression of a previously existing lesion.

In the sub-group with patients with LGD- and HGD-IPMN (n=135), fourteen (10.2%) met the criteria for resection. Ten were due to a new recurrence and four due to a progression of a previously existing lesion. The first and second histology of these patients are described in Table 17.

***Table 17*** *- Re-operations – Descriptive table of 14 patients who underwent second surgery. Patients are categorized according to first histology and describes histological findings after second surgery.*
**Denotes missing data about second histology for one of the patients.*

| First Histology | Second Histology | | |
|---|---|---|---|
| | LGD | HGD | Cancer |
| **LGD (n=7)** | 2 | 4 | 1 |
| **HGD (n=7) *** | 2 | 2 | 2 |

# 6 DISCUSSION

## 6.1 GENERAL DISCUSSION STUDY I-IV

Despite advancements in the treatment of pancreatic cancer, the prognosis remains dismal. Therefore, it is of pivotal importance to identify pre-cursor lesions at an early stage when curative surgical resection is still an option (6). It has been well established that the mucin producing cystic lesions, namely IPMN and MCN, are two of such pre-cursor lesions which may progress to PC (45). Since this may be the case for only a few patients (1-3%), there is a need to find methods able to identify those specific lesions that are prone to malignify.

The challenge is not only to distinguish the mucin producing cystic lesion from non-mucinous lesions but more importantly, to identify the degree of dysplasia of the lesion. The optimal target for resection is a lesion with high grade dysplasia (31). Lesions at this stage are one step away from malignant transformation, but the basal membrane has not been breached and the disease is still very local. Surgical resection of the pancreas is associated with high costs and risk of morbidities and complications but for patients with HGD, these associations might overweigh the risk of subsequent cancer. Patients with invasive IPMN are also subject to surgery but for this group it might already be too late. Since it has already reached an invasive stage, it has per definition become systemic with high risk of recurrence. On the contrary, patients with low grade dysplasia have lower risk of malignant transition meaning that a surgery, with its associated risks, may do more harm for this group of patients.

Current available diagnostic methods have low accuracy in diagnosing and distinguishing benign from pre-malignant and malignant lesions (14, 114). Thus, the aim of study I-IV was to find new and to improve existing diagnostic methods and their accuracies in identifying lesions with high malignant potential.

## 6.2 STUDY I AND II

The first two studies aimed at investigating the importance of the main pancreatic duct (MPD) dilatation. Previously, there was a discrepancy concerning the importance of the degree of dilatation amongst different guidelines and mainly the two major ones; the International consensus guidelines (115) and the European evidence based guidelines (7). The International consensus guidelines had MPD-dilatation ≥10 mm as an indication for resection whereas the European consensus guidelines had MPD-dilatation ≥ 6mm. The aim of these studies was thus to further analyze the relevance of MPD-dilatation and correlate it to the outcome in terms of advanced histology (high grade and invasive IPMN).

Study I was a single-institution retrospective study with 152 patients included, which showed a trend that an increasing dilatation of the main pancreatic duct was associated with increasingly advanced histology. However, due to a small cohort it was difficult to draw firm conclusions.

Study II was a continuation of study I with the same aim. In order to address the limitations of study I, in terms of cohort size, we collaborated with Johns Hopkins Hospital and created a common database with 901 patients. Seven-hundred-ninety-six of them were eligible for final analysis.

Since both International Consensus Guidelines (50) and the European Consensus Guidelines (58) were updated in 2017 and 2018 respectively, the sub-categories of MPD-dilatation for this study were changed to a) MPD <5 mm, b) MPD 5-9.9 mm and c) MPD ≥10 mm in order to comply with the revised guidelines. The International guidelines now classifies a 5-9.9 mm dilatation of the MPD as a worrisome feature and ≥10 mm dilatation as a high-risk stigmata. The European guidelines on the other hand, has a 5-9.9 mm dilatation as a relative indication for surgery.

Despite alterations in sub-categories in study II, the results were consistent with the results from study I and showed that an increased dilatation of the MPD is associated with increased risk of advanced histology. Our findings are also consistent with a study by Hackert *et al.* who found that 59.6% of the patients with MPD-dilatation of 6-9.9 mm had an advanced histology i.e. HGD and Inv-IPMN (116). The relevance of a dilatation over 5 mm has been further cemented in a systematic review and meta-analysis by Wu *et al.* (117). A total of 20 studies and 3982 resected cases were analyzed where the results showed that a dilatation of the MPD more than 5 mm was associated with a pooled OR of 5.7 and 7.4 for HGD and invasive cancer, respectively. In contrast, there are a few studies that show that a conservative approach without surgical resection is safe for patients with "Worrisome Features" such as MPD dilatation between 5-9.9 mm (118, 119). This is especially the case if the lesion is assessed as a BD-IPMN.

Since the interval of 5-9.9 mm dilatation might be too broad, the finding of an optimal cut-off value within this interval could aid physicians in the diagnosis and provide a better treatment strategy for the patient. An analysis was performed in study II in order to find an optimal cut-off value that best could discriminate between a high risk lesion and a low risk lesion, and was found to be 5-7 mm. Sugimoto *et al.* performed a similar analysis which suggests an optimal cut-off value of 7.2 mm (120).

Wu *et al.* analyzed the sensitivity and specificity for different cut offs in their meta-analysis. Five mm dilatation as a cut off value was associated with a sensitivity of 74.8% and specificity of 58.6% for classification of "malignancy" which was defined as HGD and Inv-IPMN combined (117). For classification of HGD and Inv-IPMN, the sensitivity and specificity were 72.2% & 70.1% and 75.6% & 69.7%, respectively.

In addition to the MPD-dilatation, other risk factors were also studied. Study I and II found elevated levels of CA 19-9 and jaundice to be associated with higher risk of advanced histology. The importance of CA 19-9 has been varying in the literature in terms of its significance in IPMN. However, our results along with findings by Fritz *et al.* (121), suggest that CA 19-9 could have a role in the diagnosis of IPMN.

The correlation analysis in Study II also found other risk factors to be associated with advanced histology such as age over 70 years, diabetes mellitus, unifocal lesion, location in the pancreatic head and solid component. However, since these risk factors were not the primary subject of this study, they were not further analyzed. Age is widely accepted to be associated with higher risk of cancer development and may not have the same need for further analysis. Tumors in the pancreatic head have been shown to be prone to malignancy according to Ammori *et al.* (122). Thus, our results are in accordance to previous findings.

"Familial history of PC in the family" and "Incidental diagnosis" were risk factors inversely associated to advanced histology. These results may be unexpected, but one explanation might be that these groups of patients are put under an aggressive surveillance strategy at a tertiary referral center. Hence, these groups might also undergo surgery at an earlier stage than other patients as a pre-emptive strategy.

Another risk factor inversely associated to advanced histology was "Cyst size >40 mm". This result may also be unexpected but in fact there is no certain evidence regarding this as risk factor which can be reflected as differences in the various guidelines (50, 58).

Both studies have some common limitations. They were both retrospective with risk of missing data. The patients were collected and underwent surgery over such a long period of time during which several changes may have occurred in terms of knowledge about cystic lesions, imaging quality and histology. This could have resulted in selection bias.

There are also associated strengths of our studies. All patients were managed and operated at tertial referral centers. All radiology and histology were assessed by trained radiologist and pathologist respectively sub-specialized in upper gastro-intestinal diseases.

## 6.3 STUDY III AND IV

Study III and IV aimed to examine the cyst fluid in a novel way. Pancreatic cyst fluid is used in the clinical setting for cytological analysis and for measuring biomarkers such as CEA and CA 19-9. However, these methods have a low diagnostic accuracy, representing the main reason for their limited role as a diagnostic tool.

Metabolomics is an upcoming field within molecular diagnosis. Studies have investigated the metabolic profile in patients with pancreatic cancer. Mehta *et al.* published a meta-analysis where a panel of ten metabolites was found to distinguish PC from normal controls with a high accuracy (123). However, prior to study III, only one published article had studied the metabolomic profile in patients with IPMN with samples from the cyst fluid.

### 6.3.1 Study III

The ultimate aim in the diagnosis of cystic lesions of the pancreas is not only to distinguish the benign from pre-malignant lesions but also to identify those lesions requiring surgery (HG-IPMN and Inv-IPMN).

In study III, we presented a novel method of applying metabolomics in cyst fluid and plasma. Several metabolites and lipids have been discovered to discriminate IPMN from SCN from both types of samples. The molecules we found discriminatory are consistent with findings from other studies and the panel of ten metabolites in the meta-analysis by Mehta *et al.* The metabolites choline, alanine, long-chain fatty acids and sphingomyelins have shown to be correlated to a more progressive disease in our and other studies (123-125).

This study also presented a model where an integrated metabolomics and lipidomics analysis could be used to discriminate IPMN from SCN and to be able to determine the degree of dysplasia of IPMN. The accuracy of discriminating IPMN from SCN was 100%, which itself is major breakthrough. In several clinical cases there is difficulty in discriminating SCN from IPMN; in particular BD-IPMN. This model could be of use for these scenarios since SCN does not require surgery whereas BD-IPMN potentially could.

The next objective was to determine the degree of dysplasia in patients with IPMN. Only patients with HGD or cancer are potential candidates for surgery, thus, being able to distinguish this group from the majority of patients with LGD is highly warranted in order to avoid over-treatment. Our model showed an accuracy of 90.6% for cyst fluid and 81.8% for plasma to differentiate between LGD and HGD/cancer. These accuracy rates are superior to currently available imaging modalities or EUS-FNA but are not as high as the diagnostic accuracy rates in our study for distinguishing IPMN from SCN (58, 126).

Strengths of this study were that all patients underwent surgical resection with histological verification of the diagnosis allowing for accurate matching of sample to diagnosis. Additionally, this was the first study to test 100 metabolites and over 1000 lipids. Previous studies only included between 50 to 100 metabolites without further lipid analysis (124, 127-129).

The limitations of this study consisted of a small and homogenous cohort allowing for potential selection bias and may not be representative for the general population. Moreover, the control cohort consisting of patients with SCN were all female. Although it is acknowledged that SCN is more dominantly represented in women (26), having a control group with only females may have an effect on the results despite adjusting for other confounders.

### 6.3.2 Study IV

This was a validation study of study III (130) in which we reported and correlated metabolites, namely amino acids and some lipids, to IPMN which could in turn be used to discriminate between SCN and IPMN in both plasma and cyst fluid. Other studies have also shown the relation between circulating levels of amino acids and lipids to PDAC, however, only samples of plasma have been tested, not cyst fluid (131-133).

In this study we were able to validate the results from the previous study by using an untargeted metabolomics approach. In addition to the previously identified amino acids and lipids, we found significant metabolites in plasma and cyst fluid which belonged to pathways of

carboxylic acid, heme metabolism, purine oxidation and glycolytic metabolism. In this study, the carnitines as a class of compound was the top discriminant between the cancerous and non-cancerous lesions which has also later been reported by Shi *et al.* (134).

In the previous study we were not able to find metabolites to distinguish HGD from PDAC. However, in this validation study, we found that amino acids, namely tryptophan, and catabolites of potential bacterial origin (indole) could discriminate these groups in cyst fluid. In plasma, the bacterial metabolite TMAO and several conjugated bile acids were the top discriminant metabolites. Since bile acids can be deconjugated by bacteria, a dysregulation of bile acids could serve as marker for bacterial metabolism through inflammation, hemorrhagic shock or iron-induced dysbiosis (135-137). These findings indicate a potential correlation to and impact of bacterial presence.

In another study by our group, we examined the microbiome in cyst fluid which showed enrichment of *Fusobacterium nucleatum* and *Granulicatella adiacens* which are known to inhabit the oral cavity (138). *F. nucleatum,* which has been associated with oncogenesis and related to PC (139), was found in higher abundance in patients with HGD.

Since the metabolic profile in our study was suggestive of microbial metabolism, we analyzed the correlation between the microbiome and the metabolites. The findings of this analysis highlighted the correlation of bile acids and succinate to copy numbers of 16S gene and progressive disease (HGD & PDAC).

The association between microbiome and other cancer types i.e. gastric cancer have been established previously, but several reports, including the ones by our group, have now associated the microbiome to PDAC and progression of IPMN (138, 140, 141). However, the key findings and bacteria that have been associated with PDAC or precursor lesions differ amongst the reports (142).

### 6.3.3 General Discussion Study III and IV

The translational application of metabolomics has gained interest during the last couple of years with increasing areas of application. Metabolomics have been used to analyze patients with PDAC but IPMN patients have rarely been included in those studies. The cyst fluid had, to our knowledge and prior to study III, only been studied by Park *et al.* in 2013 who identified glucose and kynuerenine to be discriminatory metabolites (110). However, their aim was to differentiate the mucinous lesions (IPMN, MCN & cancer) from non-mucinous (SCN & pseudocyst) and were able to reach a diagnostic accuracy of around 90% for each of the metabolites. In our studies, we created an integrated metabolomics and lipidomics analysis model which was able to differentiate SCN from IPMN with 100% accuracy and could differentiate degree of dysplasia with around 80-90% accuracy.

Although metabolomics has shown potential for analysis of samples from resected patients, the ultimate aim is to apply an analytic method in clinical practice as part of the diagnostic workup. Hence, the idea of applied metabolomics is that plasma and cyst fluid are sampled and analyzed

before patients undergo surgical resection. Although blood samples are easy to retrieve, the same cannot always be said for cyst fluid which has to be retrieved through an invasive endoscopic procedure namely endoscopic ultra-sound guided fine needle aspiration (EUS-FNA). Endoscopic ultrasound and EUS-FNA is more commonly used internationally compared to Sweden, however, it is associated with several shortcomings (143). Firstly, it is highly operator-dependent, meaning that only a few centers will be able to perform the procedure and will not always be available as other conventional imaging modalities i.e. CT and MRI. Secondly, due to the invasive nature of the procedure, it is associated with some complications i.e. bleeding, perforation, infection and tumor seeding (144). Thirdly, not all cystic lesion may be available for puncture depending on their size and location, which further limits the application of EUS-FNA (22).

Given the potential difficulties with acquiring samples from cyst fluid, the optimal sample would be plasma. With our studies we have shown that a metabolomics analytical method can be applied to both plasma and cyst fluid samples which are feasible and accurate even though the accuracy is slightly less for the plasma samples.

## 6.4 STUDY V

Knowledge about IPMN has increased over the years since its discovery, especially during the last decade, but a lot remains to be uncovered. Many risk factors and genetic alterations have been associated to IPMN and its malignant transformation, but we still don't know in detail what initiates and drives this progression to malignancy. This is reflected in the differences in guidelines and recommendations for the follow-up of patients with IPMN resulting in only around a quarter of the patients resected for IPMN having a histologically confirmed HGD and Inv-IPMN (145). This means that 75% of the patients are potentially overtreated. The issue that follows a resection is about the post-operative surveillance. The general consensus is that they should be followed in the same manner as before surgery but the evidence for this is lacking (10, 146, 147).

It has been established that IPMN is a multifocal disease and can develop at different times and at different locations in the pancreas, related or unrelated to each other (148). How should this knowledge be taken into account when it comes to the post-operative follow-up? Is there a possibility for less frequent follow-up if the primary lesion has been resected, especially if the first histology confirms it to be LGD-IPMN? Or should the patients be followed more extensively since they have already had a lesion that met the criteria for resection?

The aim of study V was thus to observe the recurrence of IPMN after resection and determine associated risk factors in order gather to more evidence on this less studied area of IPMN management.

The overall recurrence rate in our cohort was 44.6% (100/224), however, the clinical significant recurrence rate was 30.8% (69/224). According to published studies, the recurrence rates vary from 0.7% to 72.5% (57, 149-153). This large range could be due to the differences of study design and definitions of the outcome. Some studies recognize any new lesion or progress as

an event or recurrence which could result in a higher overall recurrence rate. In our study, we further sub-categorized recurrences as non-significant and clinically significant as only the latter will yield a change in the clinical care or treatment. This classification reduced the recurrence rate in our cohort from 44.6% to 30.8%.

The other aim of our study was to examine the recurrence rate in patients with LGD and HGD at first histology. The high risk of recurrences in patients with an invasive disease or concomitant cancer has already been established and was also confirmed in our study as risk factors for recurrence. This group should and will therefore be surveilled as guidelines suggest and as any other cancer. However, the main question, regarding the post-operative follow-up, remains for patients with LGD and HGD. In this subgroup of 135 patients, the recurrence of clinical significance was seen in 15 patients (11.1%). All but one had a local recurrence. The recurrence rate in this groups is seemingly far less than in the cohort of all resected patients (11.1% vs 30.8%), however, it is still not negligible as it means that almost every tenth person will need to undergo a second surgery.

Another question that follows is if patients with LGD have a lower risk for recurrence than patients with HGD as they are further away from the transition into malignancy. The time-dependent analysis in our study was not able to find any significant difference between these groups at both non-adjusted and adjusted model. When comparing to other studies we find different results. Some papers support our result and suggest no significant difference between the groups (151, 154) whereas another paper suggest that patients with HGD have a HR of 2.96 (CI 95 1.10-8.00) for recurrence compared to patients with LGD (155).

The time to recurrence also varies amongst the published articles, however around 75% of the recurrences occur within 5 years (146, 156). The median time to recurrence in our study was shorter than other studies at 16 months which could be due to the patients being followed-up at a tertiary referral center with closer surveillance. Interestingly, seven patients had a non-clinical significant recurrence within the first year of follow-up, however, they were followed for a median time of 81.5 months without any further progression.

The correlation analysis highlighted two risk factors as significant for recurrence which were "age over 65 years" and "known IPMN left in remnant". The correlation of age and malignancy is generally accepted and the recommendations suggest that all patients should be surveilled as long as they are fit for surgery (157, 158). For younger patients this means an increased cumulative risk of recurrence which in turn results in life-long surveillance with annual imaging examinations and increased costs and burden for both patients and the health care system. Could younger patients with high risk of recurrence benefit from pre-emptive total pancreatectomy (TP)? TP is associated with short- and long-term risks and increased morbidities such as exocrine and endocrine deficiencies but for selected patients with high risk of malignancy it could be a safe alternative (159-163).

Patients with known IPMN left in remnant had a higher risk for recurrence which was confirmed by the logistic regression analysis and the time-dependent analysis. However, of the

44 patients that had a known IPMN left in remnant at time of surgery, only 4 progressed into clinical significance (9.1%), which is similar to the overall clinical significant recurrence rate. The conclusion that can be drawn is that although patients with a previously known IPMN in the remnant are at higher risk for recurrence, it does not necessarily mean that the known lesion will progress.

Other aspects and factors that we did not consider in our study, but that can be added in future for a more comprehensive understanding, are other histological parameters such as surgical margin status, histological subtypes and biomarkers. Oncocytic and pancreatobiliary subtypes are associated with a poor prognosis and could potentially have an impact on recurrence as well (50, 53, 164). Biomarkers such as genetic mutations, alterations of miRNA and a high C-reactive protein/albumin-ratio have been associated to higher risk of malignant transformation but little has been studied on their impact on cystic lesions (97, 150, 165, 166).

Given the retrospective nature of the study, it is associated with some limitations. The patients were operated during a long time period (2008-2017), during which several changes have occurred in regards of imaging modalities, knowledge about cystic lesions, guidelines and local clinical protocols. All of these factors may have affected the outcomes and data resulting in selection bias. In addition, the TNM-classifications have also changed during the study period for which this study has not been adjusted for. The TNM-classification, current at the time of surgery has been used. Another limitation is the low number of patients included, especially in the sub-group analysis.

# 7 CONCLUSIONS

**Study I & II**

These two studies support the revised European guidelines and adds to the evidence that even a smaller dilatation of the MPD (≥5 mm) can be associated with malignancy. Although we have not been able to find a method to distinguish HG-IPMN from Invasive IPMN, we have found a cut-off value for MPD-dilatation which distinguishes the LG IPMN from the HG- and invasive IPMN, which are both subject to surgery.

**Study III & IV**

These studies have presented and validated a novel method of distinguishing not only IPMN from SCN, but also distinguishing degree of dysplasia/cancer within IPMN. This has been achieved by mapping the metabolomic and lipidomic profile of the cystic lesions and integrating the results in a model that could be used in diagnosis of cystic lesion and thereby better selection of patients subject to surgery.

The finding from these studies have also indicated and created a hypothesis of bacterial impact in the disease progression of IPMN.

**Study V**

This study shows that patients who have undergone partial pancreatic resection for IPMN have a significant risk for recurrence. The risk is further increased if the patients are older than 65 years and/or have a known IPMN left in the remnant at time of surgery.

Moreover, this study shows that patients with LGD-IPMN at first histology have similar risk for recurrence as patients with HGD-IPMN and should therefore be surveilled according to the guidelines.

# 8 POINTS OF PERSPECTIVE

Ever since the discovery of IPMN around 30 years ago, the knowledge of PCNs has been increasing exponentially. This has certainly been the case in the last decade where several guidelines have been revised which indicate the continuous new flow of studies and evidence (167). This is challenging for physicians managing patients with PCNs. However, the current knowledge is still only the tip of the iceberg, and there is still a long way to go before a full understanding of these entities can be obtained.

Currently, imaging modalities are still a corner stone in the diagnosis whereas the cyst fluid has been used less frequently for further diagnosis. However, there is an increasing interest in cyst fluid analysis and utilizing it more proficiently. As it has been shown in this thesis, molecular analysis, like metabolomics, may be of aid in distinguishing low risk from high-risk lesions and thereby enable optimal selection of patients for surgery. Samples for molecular analysis, i.e. metabolomics, might currently be expensive and difficult to analyze and a challenge to retrieve by EUS-FNA, but further developments could make it more usable in clinical practice.

The cyst fluid can be used in multiple ways, not only for metabolomics. The microbiome mapping as well as analysis of miRNA in the cyst fluid and circulating tumor cells (CTCs) are gaining interest (3, 168-172). Furthermore, with the development of next generation sequencing (NGS), genetic analysis could potentially have a more significant role, especially in more difficult cases where consensus regarding treatment is hard to achieve (173-177).

Another emerging and developing area that could improve the diagnosis is the invasive endoscopy. Single-operator peroral pancreatoscopy (SOPP) is a method that has proven its potential to be a crucial part of the diagnostic arsenal and change the management of lesions (178, 179). However, it has been associated with SOPP-pancreatitis, especially in patients with lower degree of MPD-dilatation (180). Paradoxically, this group might be the one who may benefit the most of pancreatoscopy since it is difficult to associate a normal or only a slightly dilated MPD with advanced histology.

A second developing technique is needle-based confocal laser endomicroscopy (nCLE) which, in similarity to pancreatoscopy, allows for examination of the wall lining at close (181). However, with this technique the probe is passed through the lumen of an EUS-FNA needle which means that the cystic lesion has to be punctured. Napoleon *et al.* performed a prospective multicenter validation study where this method showed a diagnostic accuracy of >95% (182). This has been confirmed by other studies showing that the addition of nCLE to EUS-FNA improves the diagnostic accuracy and changes the therapeutic management for 28% of the patients (183, 184). However, one downside with all endoscopy and especially the above-mentioned procedures, is that they are highly operator-dependent and require to be performed at highly specialized centers, which could limit its availability for the general population (51, 168).

Artificial intelligence (AI) and deep-learning based diagnostics are other upcoming techniques that could be applied to various fields within medicine (12). Several studies have applied AI to CT-imaging and have been able to detect and differentiate PDAC with an accuracy of up to 96.3% (185-189). It has also been proven to be superior and more accurate than experienced radiologists (188). The development of EUS with AI has also been tested and have been able to reach a 94% accuracy to diagnose PDAC (190-192).

AI has also been tested to diagnose and differentiate PCNs. Some models have been able to reach an accuracy of 73-91% (193-196). This is inferior to the accuracy of diagnosing PDAC with CT or EUS but could still prove to be a valid and accurate diagnostic method in the future. Especially since the patients do not have to undergo as an invasive of a procedure as EUS and EUS-FNA.

In current clinical practice the physicians use all clinical and radiological risk factors to make a collected risk assessment. The importance of the risk factors has usually been examined separately or in small groups but have rarely been tested all together as a combination. To take advantage of all available data in a better and more structured way might be a more feasible approach in the near future.

Masica *et al.* have proposed and validated a novel approach with combining clinical and radiological parameters for the diagnosis of pancreatic cystic lesions and achieved an accuracy of 92% in diagnosing IPMN and 87% of MCN (197). Similarly, Kim *et al.* and Hwang *et al.* have created and validated nomograms which could facilitate the diagnosis process (198, 199).

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