The Role of EUS and Proteomic Analysis in the Management and Surveillance of Pancreatic Cystic Lesions.

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Doctorate of Medicine

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Declaration

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Summary of Thesis

Pancreatic cystic lesions (PCL) are fluid filled structures in the pancreas. They can be precursor lesions for pancreatic cancer but not all lesions will progress. Other lesions are sequelae of acute or chronic pancreatitis, these will never progress to malignancy but their management can prove challenging. The combination of an aging worldwide population with an increased reliance on cross sectional imaging means that the incidence of these lesions are increasing. The challenge for clinicians remains that not all PCL will progress to cancer and those which are not at immediate risk need to be kept under surveillance. The literature remains unclear on the rate of progression of PCL. The workup remains primarily confined to specialty centres with expert clinicians. Despite this, post operative histology does not always correlate with preoperative workup.

In this thesis we aim to address the management of PCL through three studies. We reviewed the results and outcomes of all the patients with PCL under surveillance in Tallaght University Hospital over twenty years. We identified that no patients under surveillance progressed to cancer while under surveillance and those patients who required surgery were identified early in the period of surveillance. This surveillance was accompanied with a high cost of €193000 per positive surgical outcome.

We prospectively recruited a group of patients undergoing endoscopic ultrasound assessment of PCL for serum and cystic fluid. We used this to develop the first cystic biobank in Ireland. We performed a discovery proteomic analysis of this fluid for novel proteomic markers which may be additive for the preoperative diagnosis of high risk PCL.

Pancreatic fluid collections can resemble large PCL but their approach remains different. We combined the outcome of three of the largest centres in Ireland managing these lesions endoscopically to assess benefit of newer technologies and overall complication rates of these advanced procedures.

PCL data in European patients is limited and this is the first research performed in an Irish population. We identified that those PCL requiring intervention often progressed quickly to surgery and that long term surveillance comes at a significant cost to the health system.

Abstract

Introduction

Pancreatic cystic lesions (PCL) can be precursors to pancreatic cancer. The incidence of PCL are increasing worldwide with increased patient age and reliance on cross sectional imaging. However, not all PCL will progress and the approach remains unclear on surveillance and intervention. Pancreatic fluid collections remain a challenge for intervention

Methods

This is a three part study. A prospective cohort of PCL undergoing endoscopic ultrasound assessment was recruited for cystic fluid proteomic analysis. We retrospectively identified all PCL under surveillance in a tertiary referral centre over a twenty year period. A multicentre review of outcomes of endoscopic intervention on pancreatic fluid collections.

Results

We identified a low number of PCLs identified over twenty years progressed to surgery, 26 of 28 patients requiring surgery progressed within 2 years of identification of the cyst. This was accompanied by a high cost of surveillance of pancreatic cysts at €193000 per premalignant lesion identified on post operative histology.

We identified 4 proteomic biomarkers. MUC6, PIGR, REG1a and LCN2 showed significant difference in expression in patients with high risk cysts compared to low risk.

Endoscopic drainage of pancreatic fluid collections had a high technical success and clinical success. Complications were similar between stent types. LAMS stents were more commonly performed as day case procedures.

Conclusion

PCL are a diagnostic and management challenge. We identified smaller cysts are at a very low risk of progression. We also identified novel markers to improve pre operative diagnostic approach. Pancreatic fluid collections can be effectively managed at endoscopy.

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2. GS Mellotte, J O'Grady, V Parihar, M Buckley, F MacCarthy, BM Ryan. Multicentre Review of Outcome Of EUS Guided Drainages In Irish Tertiary Referral Centres: A Multicentre Review. Poster Presentation UEG Week 2021.
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6. G Mellotte, V Parihar, N Breslin, BM Ryan. A Retrospective Review To Assess The Value Of Pancreatic Cyst Fluid Analysis. ISG Winter Meeting 2019.

Abbreviations

TUH	Tallaght University Hospital
SJH	St James's University Hospital
MUH	Mercy University Hospital
EUS	Endoscopic Ultrasound
ERCP	Endoscopic retrograde cholangiopancreatography
FNA	Fine Needle Aspirate
MRCP	Magnetic Resonance Cholangiopancreatography
PCL	Pancreatic Cystic Lesion
IPMN	Intraductal Papillary Mucinous Neoplasm
MCN	Mucinous Cystic Neoplasm
SCA	Serous Cystic Adenoma
PFC	Pancreatic Fluid Collection
WON	Walled Off Necrosis
ANC	Acute Necrotic Collection
APFC	Acute Pancreatic Fluid Collection
PDAC	Pancreatic Ductal Adenocarcinoma
LAMS	Lumen Apposing Metal Stent
DPPS	Double Pigtail Plastic Stent

QALY	Quality Assured Life Years
MUC	Mucin
REG	Regenerating islet-derived proteins
REG1a	lithostathine-1-alpha

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Introduction

1.1 Anatomy, Structure and function of the Pancreas

1.1.1 Anatomy

The Pancreas is an retroperitoneal digestive gland. It lies ventral to the L1-L2 vertebrae along the posterior abdominal wall, posterior to the stomach, between the duodenum and the spleen[1]. The pancreas is elongated measuring 12-20cm in length and 3-5cm in height[2]. The gland is macroscopically divided into the head, neck, body, and tail. The head lies within the curve of the duodenum. The uncinate process extends from the inferior pancreatic head posterior to the superior mesenteric artery[1]. The neck of the pancreas overlies the superior mesenteric vessels. The body extends posterior to the stomach. The tail of the pancreas lies adjacent to the hilum of the spleen and the left colic flexure[1].

The ductal system of the pancreas is a major component of the exocrine function of the gland. The main pancreatic duct begins in the tail of the pancreas and extends through

the whole organ to connect to the common bile duct to form the hepatopancreatic ampulla; this is also known as the Ampulla of Vater[2]. This opens into the duodenum at the site of the main duodenal papilla[2]. The accessory pancreatic duct opens into the duodenum at the site of the minor duodenal papilla. This duct is present in 12-82% of people and patent in 52%[3].

The main arterial supply to the pancreas arises from the splenic artery. The Great Pancreatic and Dorsal Pancreatic arteries form branches with pancreaticoduodenal arteries from the gastroduodenal artery and inferior pancreaticoduodenal artery from the superior mesenteric artery. Venous drainage occurs via pancreatic veins which connect to the splenic vein[1].

1.1.2 Structure

The pancreas is a gland with both endocrine and exocrine functions. The exocrine gland is composed of pancreatic acinar cells and ductal cells. The acinar cells are pyramidal shaped cells organised into grape-like clusters. Pancreatic enzymes drain from the acinar cells, via duct cell lined canaliculi into tubules lined with ductal epithelium. These epithelial cells secrete bicarbonate. Branched networks of tubules coalesce into larger tubes before draining into the main pancreatic duct as mentioned above[4].

The endocrine portion of the pancreas is composed of the islets of Langerhans or pancreatic islets. The islets are in turn composed of five cell types; α-cells, β-cells, δ-cells, PP or F-cells and the epsilon cells (ε-cells)[4].

1.1.3 Function

The endocrine and exocrine pancreas are separate but functionally linked systems. Secretory products released by the islets directly act on the acinar cells to affect exocrine functions. This is partly driven by the vascular supply of the pancreas. The arterial blood supply flows first to the islets before acinar cells of the gland[5].

i. Exocrine Function

The acinar cells comprise 80% of the pancreas' total volume[5]. The primary function is to produce, store and secrete the digestive enzymes; amylase, lipase, and protease. These cells have a Golgi apparatus which enables the production of secretory zymogen granules. These granules contain a multitude of enzymes and pro enzymes which are released into the bicarbonate fluid produced by ductal cells[6]. The combination of these products is referred to as pancreas juice. Pancreatic enzymes are proteolytic, glycolytic, or nucleolytic. Proteolytic enzymes trypsin, chymotrypsin, carboxypeptidase, and elastase digest proteins. The glycolytic enzymes targeting carbohydrates are lactase and amylase. Lipolytic enzymes target fats, these include lipase, phospholipase and esterase[6].

ii. Endocrine Function

The islet cells release pancreatic hormones and neuropeptides. The α-cells produce glucagon which plays an important role in the body's energy metabolism and response via gluconeogenesis. It plays an important role for glucose metabolism during periods of reduced energy intake[7]. Insulin is produced by the β-cells in the pancreas. It facilitates glucose uptake in tissues. Insulin also plays a role in pancreatic exocrine function in stimulating basal amylase and secretagogue excretion[8]. Somatostatin is produced by the delta cells of the pancreas. It is an inhibitor of pancreatic insulin and glucagon production. It also inhibits acid secretion by parietal cells of the stomach[5]. The ε-cells produce Ghrelin which plays a role in appetite and lipogenesis[9]. Pancreatic peptide is produced by the F cells. It plays a role in gastrointestinal mobility via the control exocrine pancreatic secretions, acid suppression, and gallbladder motility[5].

1.2 Pancreatic Cystic Lesions

1.2.1. Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) accounts for over 90% of pancreatic cancers[10]. Other less common subtypes of pancreatic cancers include acinar cell tumours, squamous cell carcinoma, adenosquamous carcinoma, and neuroendocrine tumours. PDAC incidence has doubled worldwide over the past two decades[11]. This is primarily related to an aging worldwide population as well as lifestyle factors such as alcohol intake, smoking, obesity, and diabetes. Despite advances in understanding, diagnosis and therapies the outcomes for PDAC remain bleak. 5 year survival is currently 8%[12]. Early

detection of PDAC is key to better outcomes. PDAC has two main groups of precursor lesions. Pancreatic intraepithelial neoplasia (PanIn) is microscopic flat or papillary epithelial neoplasia with cellular atypia. There are three subtypes of PanIn lesions, PanIn 1,2, and 3. The majority of PDAC is thought to arise from PanIn lesions. They cannot be identified by radiological modalities[12,13]. The second group of PDAC precursor lesions are pancreatic cystic lesions (PCL).

1.2.2 Pancreatic Cystic Lesions

PCL are fluid filled structures within the pancreas. They can be unilocular or multilocular, neoplastic or non-neoplastic and some, but not all, can have malignant potential[14]. Pathologically, true cysts in any part of the body are defined as "epithelial lined structures". With the exception of pseudocysts, all PCL are true cysts. The differential diagnosis can range from benign retention cysts to malignant mucosal cysts. It can prove a significant challenge to differentiate these processes. PCL can be broadly divided into epithelial (i.e. true) cysts or non-epithelial cysts. Epithelial cysts can be further separated into serous and mucinous cysts. Only mucinous cysts carry malignant potential.

The WHO released an updated pathological classification of PCLs in 2019. In this classification system, PCL are divided into benign, premalignant, and malignant. Benign lesions include acinar cystic transformation of the pancreas and serous cystadenomas[15]. An outline of the classification of PCL is below in figure 1.1. Pancreatic cyst disease differs from cystic degeneration which may arise in primarily solid lesions such as pancreatic adenocarcinoma[16].

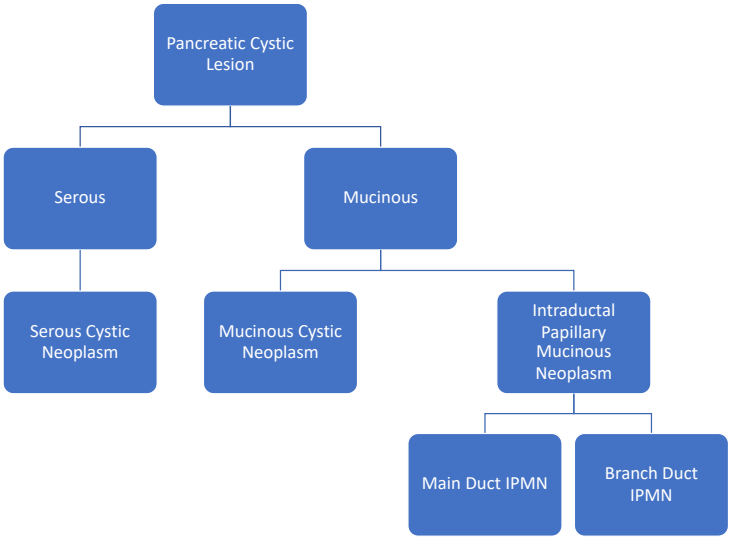

Figure 1.1. Outline of PCL classification by subtype

1.2.3 Retention Cysts

Pancreatic Retention cysts are benign epithelial cystic lesions. They are caused by focal duct obstruction, such as fibrous structure, mucinous plugs, or calculi. These are well defined, rounded lesions within the pancreas. Frequently patient have a history of chronic pancreatitis[17]. They are found in up to 25% of patients with cystic fibrosis[18]. They have no malignant potential.

21.2.4 Serous Pancreatic Cystic Lesions

Serous cystic adenomas (SCA) are benign pancreatic cystic lesions. They predominantly occur in women between the ages of 50 and 70[19]. SCA are usually multiloculated and can have a distinctive “sunburst” appearance on imaging. There are four morphological subtypes; microcystic, macrocystic, mixed microcystic and macrocystic, and solid[20]. There is no risk of progression of these lesions , however larger SCA may cause symptoms. Median growth rate is 0.6cm/year[21].

Histologically, SCAs are defined by the presence of single uniform layer of cuboidal, glycogen rich serous cells. [20]. The main clinical significance of SCAs is that when discovered, the PCL may require some further investigation before it can be definitively be diagnosed as a benign SCA with no malignant potential. SCA do not typically require any long term surveillance.

1.2.5 Mucinous Pancreatic Cystic Lesion

Mucinous cystic neoplasms (MCN) are at risk of developing into pancreatic adenocarcinoma. These occur almost exclusively in women in their 40s and are usually located in the distal pancreas (body and tail)[22]. They do not connect to the main pancreatic duct and the definitive histopathological feature is the presence of ovarian type stroma[23]. The presence of intracystic nodule, larger cysts (>30mm), and elevated serum Ca19.9 is associated with invasion on histology[24].

1.2.6 Intraductal Papillary Mucinous Neoplasms

Intraductal papillary mucinous neoplasms (IPMN) are epithelial cystic lesions arising from the main pancreatic duct or communicating with the ductal system. IPMN are mucinous cysts with the potential for malignant change. They arise equally in men and women with incidence increasing from the 5th decade[25]. They are clinically classified as main duct, branch, or mixed type IPMN. Main duct IPMN are clinically more aggressive with malignancy reported in up to 60% of cases[26].

IPMN can also be classified histologically. IPMN are defined by the development of papillae and can be sub divided into gastric, intestinal, pancreatobiliary, and oncocytic subtypes.

Gastric-type IPMN papillae are composed of tall columnar cells with basally oriented nuclei and abundant pale mucinous cytoplasm. Intestinal- type IPMNs have villous papillae of tall columnar cells with pseudostratified cigar-shaped nuclei and basophilic cytoplasm; mucin volumes are variable and are typically apical. Pancreatobiliary-type IPMNs have complex thin branching papillae consisting of columnar cells with marked atypical nuclei and neutral or basophilic cytoplasm, often mucin produced is thick. Finally, oncocytic-type IPMNs have thick arborizing papillae consisting of cells with enlarged round nuclei and eosinophilic cytoplasm.[16,27]

The 2019 WHO update distinguished intraductal oncocytic papillary neoplasms and intraductal tubulopapillary neoplasms from IPMN. An additional change was the implementation of a two tier grading system of high and low dysplasia based on the highest grade found on examination[15]. The previously used borderline grade is no longer

in use. In addition to traditional H&E staining the use of immunohistochemical staining is additive for the diagnosis of IPMN subtype.

1.2.7 Pseudocysts

A pancreatic pseudocyst is a non-epithelized PCL, hence the prefix “pseudo”, as true cysts are epithelial lined structures. They can occur as a complication of acute or chronic pancreatitis[28]. A pseudocyst is defined by its inflammatory epithelium which distinguish it from true cystic lesions. Pseudocyst fluid is rich in the exocrine pancreatic enzymes, amylase and lipase[28]. Location of a pseudocyst can vary, most commonly they are retrogastric and lie extrapancreatic. Intrapancreatic pseudocyst are most commonly found in the head of the pancreas[29]. Identifying the location of a pseudocyst is vital in determining therapeutic approach.

More on the classification of pancreatitis and pancreatic collections is covered below.

1.2.8 Walled Off Necrosis

Walled off Necrosis (WON) occurs in the setting of acute necrotising pancreatitis. It is a mixed solid and fluid collection within a fibrinous wall[30]. WON are at risk of infection. Infected pancreatic necrosis is associated with mortality rates of 30%[31]. It may also be complicated by splenic vein thrombosis in up to half of cases[32].

1.3 Assessment of Pancreatic cystic Lesions.

Few cases of PCL were described until the 1980's and remained a rare entity [33,34]. Increasing rates of diagnosis occurred with the increased use of cross sectional imaging in an aging Western population. PCLS are frequently asymptomatic and are diagnosed incidentally. Once discovered it is important that lesions are appropriately diagnosed.

PCLs and their management are a challenging issue in healthcare. There are differing guidelines with subtle but important differences in guidance for clinicians. The discovery of a PCLs places a burden of surveillance on healthcare systems with guidelines advocating for lifelong surveillance of lesions.

1.3.1 Epidemiology of Pancreatic Cystic Lesions

The exact incidence of PCL is hard to ascertain. A systematic review of population studies in asymptomatic patients found a range of 2.5-45.9%, the pooled prevalence was 8%[35]. This highlights the variation across both populations and published studies. There is a higher occurrence in the elderly population and this has been seen across a number of studies[36,37]. In an autopsy study it was found that up to 33% of elderly patients had PCL at time of death, with only 3.8% of these showing high grade dysplasia[38]. This presents extra challenges in decision making when faced with an aging population. A population based study performed on 2333 people in Pomerania found an incidence of 49.1% of PCL, with prevalence and size increasing significantly with age[37].

In addition to age, obesity and type II diabetes have been linked with the development of pancreatic cystic lesions.[39] A large prospective population study, PANCY, in an Italian population found no significant links to smoking or alcohol in cyst development[40] but there is limited data in prospective wider populations to confirm this across all populations.

1.3.2 Imaging of Pancreatic Cystic Lesions

Most pancreatic cysts are discovered incidentally in asymptomatic patients[41]. Both computed topography (CT) and Magnetic resonance imaging (MRI) are used for the assessment of PCLs. CT can aid in characterisation of lesions with identification of some of the aggressive features of PCL such as septae, calcification of the cyst wall, mural nodules, and findings suggestive of pancreatitis[42]. However, MRI is more sensitive for characterisation of smaller lesions, internal cyst characteristics, and for identification of communication with the pancreatic duct[43]. MRI with magnetic resonance cholangiopancreatography (MRCP) is more sensitive as it has a high fluid and soft tissue contrast ratio[44]. It also has the added benefit of low radiation exposure which is important for surveillance in young patients.

The accuracy of a single modality of imaging for a PCL can vary and frequently a combined approach is required. The addition of MRI to CT imaging of PCL has been shown to improve accuracy in diagnosis from 61.4% to 80.5% compared to CT alone[45].

1.3.3. Endoscopic Assessment of PCL

Endoscopic Ultrasound (EUS) is the current gold standard for assessment of PCL. ERCP bears higher risks with lower sensitivity/specificity for pancreatic cystic lesions, and while ERCP may have been used in decades gone by it really has a very limited, if any role in the assessment of PCL, with the exception of main duct IPMN and some pancreatic fluid collections[46]. Endoscopic ultrasound allows for both imaging and potential sampling of pancreatic cystic lesions. Routine procedural technique usually involves initial lesion assessment with radial endoscope, although some endosonographers (endoscopists with a special interest in EUS) may prefer to assess solely with a linear EUS scope. An initial assessment to characterise the morphology, size and location of the PCL may be followed by fine needle aspiration (FNA), which is done using a linear EUS scope and a puncture needle. The cyst is localised within the pancreas and a cyst puncture is performed freehand transmurally into the cyst. Fluoroscopic guidance is not required. This can be performed using a 19g, 22g, or 25g needle. Aspiration is through the endoscope into a syringe. While a single study showed a miss rate of pancreatic cysts at 17.5% in linear EUS compared to 33% in radial EUS examination of the pancreas[47]. The difference has not been formally studied in terms of superior diagnostic ability in the characterisation of pancreatic cysts. The EUS assessment and fine needle aspirate of pancreatic cysts is endorsed across the guidelines. ESGE did not find benefit to performing FNA in cysts less than 10mm[48].

Endoscopic imaging of pancreatic cysts has benefits over cross sectional imaging in the diagnosis of PCL. Guidelines are based on imaging criteria for the diagnosis of high-

risk lesions. There is little difference between the accuracy of EUS and MRI in diagnosis of cystic lesions and a combined approach is usually adopted in clinical management [49,50].

Under EUS imaging, characteristics of PCL differ. IPMN by definition are connected to the main pancreatic duct, as either main type or branching ducts. They may appear as macro or micro cystic with thin septations[51]. Endosonographic appearances of MCN appear on imaging as smooth encapsulated lesions with thick walls and without connection to the main pancreatic duct[52]. Serous adenomas classically carry a starburst appearance with central area of calcification[53]. Given emerging concern regarding links of histological subtype with cancer progression one group attempted to identify histological subtype based on EUS images at assessment. Multiple cysts were statistically linked to gastric subtype and MPD dilatation of >10mm correlated with intestinal subtype[54]. The impact of this is unclear on clinical outcome and further studies of imaging correlation with histology are needed.

i. EUS guided FNA to needle not to needle?

EUS-FNA is performed to better characterize a PCL. The objective is to identify premalignant lesions and establish the risk of a lesions transformation to cancer. There is no concrete consensus between guidelines on the use of FNA in the assessment of PCL. The European and The IAP guidelines advocate the use of EUS-FNA where results may change the management for patients. The views on the benefit of EUS-FNA remains mixed in the literature. EUS-FNA of PCL sensitivity has been estimated at 51-52%[55] with accuracy limited by a wide definition of malignancy between studies. This same study felt there was a need for a more accurate algorithm for PCL fluid and we will discuss current

and future markers in the following sections. The assessment of the patient remains critical and EUS-FNA should not be performed where the patient would not be a surgical candidate. Many PCL are diagnosed in elderly frail patients, those with a Charlson comorbidity index of 7 or higher, have an 11 fold higher risk of non PCL related death within three years[56]. Ultimately, the use of EUS-FNA provides benefit where further information may prevent prolonged surveillance or unnecessary surgery in the right candidate. However, it is limited to expert clinicians who are experienced in both performing and interpreting FNA.

Overall complications rates of EUS are an estimated 0.002%[57]. All endoscopy carries the risk of perforation, pain or reaction to sedation. EUS endoscopes differ from OGD in that most are oblique view, have a wider diameter and are generally less flexible. This raises concerns of perforation at intubation leading to a cervical oesophageal dissection. Duodenal perforation is a significant complication seen more frequently in ERCP than EUS. However, a German survey of 100,604 procedures saw 19 duodenal perforations, an incidence of 0.022%. Compared with 8 oesophageal, an incidence of 0.009%[58].

The most controversial complication is the risk of tumour seeding due to needle puncture. This is a more frequently reported complication of solid pancreatic tumours[59]. The picture is less clear in pancreatic cystic lesions as the fear appears to have arisen from reported complications in isolated cases. There are two cases of pancreatic cystic lesions causing tumour seeding in the literature. The first concern was secondary to a case of peritoneal seeding in a 57 year old gentleman in Japan who developed carcinomatosis peritonitis post FNA[60]. The second case is a 75 year old gentleman who had a 30mm cystic lesion with associated solid adenocarcinoma at diagnosis, 3 months

post EUS he developed a cystic mass on the posterior gastric wall on follow up CT. This case was inoperable and he died 29 months later[61]. This was attributed to FNA and as such concerns of seeding PCLs have arisen since. The PIPE study, published in 2014, followed 201 patients undergoing pre-operative EUS-FNA and 82 without sample taken. Prevalence of seeding in the no sample group was 4.4% and 2.3% in the EUS group, the difference was not significant. P=0.403[62]. These results would suggest that the risk of spread to the gastric wall was more related to the inherent behaviour and invasiveness of the IPMN lesion itself, rather than the FNA procedure. A series of 152 mucinous cystic neoplasms in Japan found two incidences of peritoneal seeding but it is unclear whether these patients underwent EUS guided biopsy[23]. There is little evidence for MCN at risk of seeding from FNA biopsy. Interestingly a large proportion of the cases reported are from Japan which may relate to unknown genetic disposition or possible publication bias.

More common complications of pancreatic FNA include bleeding, infection, and pancreatitis. Bleeding post puncture is a recognised complication of FNA. One series of 457 EUS-FNA found that FNA in cystic lesions had higher complication rates than solid lesions[63]. However, this group included pseudocysts as opposed to PCLs and the overall cystic cohort was small at 22. Although there are reported incidence of catastrophic haemorrhage post FNA of PCLs the overall rate is low[64–66]. A meta-analysis of 5124 patients saw 34 post procedure haemorrhages (0.69% of cases)[67]. Stopping of anticoagulation or anti-thrombotics prior to EUS-FNA is complicated and with no clear answer it should be individualised to each patient case[68]. In general, anticoagulation is stopped. Antiplatelets are also usually stopped, apart from low dose aspirin, which can be safely continued. Pancreatitis is a devastating complication with potentially long lasting consequences. Incidence appears to range from 0%-2%[67,69,70] between studies. The use of

rectal NSAID in pancreatic EUS is not well studied, likely due to the low overall incidence. Conversely, antibacterial prophylaxis is common practice in EUS-FNA, it is endorsed by ASGE[71] and European[46] guidelines. The Europeans accept a single shot as adequate but Americans advise 3-5 days of treatment. The evidence for prophylaxis is unclear. Bacteraemia post FNA of cyst is estimated at 0.44%[67]. The benefit of prophylactic antibiotics has been studied extensively in EUS FNA with no clear outcome[72–76]. A recently published randomised control trial of pancreatic cystic lesions undergoing FNA demonstrated no benefit in prophylactic use but also showed no harm arising from use of same[77]. The use of prophylactic antibiotics remains user dependent but endorsed by guidelines.

The limited sensitivity of pancreatic cystic lesion cytology is due to the acellular aspect of cystic fluid. Attempts to improve this include the development of “through the needle biopsy”. These micro forceps (Moray® Micro Forceps, Steris Healthcare, USA) are passed through the gauge of a 19G needle. Under endosonographic guidance, an operator can target the cyst wall to obtain a tissue sample. A prospective trial of this device found an acquisition-yield of 83.3% with micro-forceps[78]. Overall performance of micro forceps in a pooled analysis of 425 cases found a diagnostic yield in 79.60 % with a diagnostic accuracy of 82.76 % and adverse event rate of 1.08%[79]. This is a significant improvement over EUS FNA cytology.

1.3.4 Current Cystic Markers

Current best practice for cyst analysis endorsed by guidelines is the use of Carcino Embryonic Antigen (CEA), Amylase and cytology for assessment and diagnosis of pancreatic cystic lesions.

Cytology is limited in pancreatic cystic lesions by low sensitivity. Pancreatic cystic lesions are largely acellular, as a result many FNA samples are inadequate for cytological assessment. Cytology has been found to be diagnostic in 13-35% of EUS FNA[80–82]. Specificity has found to be high however the low sensitivity of EUS FNA lowers the accuracy in diagnosis of PCL[83].

Cytology has been demonstrated as more sensitive in Main Duct IPMN and in those hypersecreting mucin[84]. In their study. *Yamaguchi et al* found 40% sensitivity in malignant cases. The volume of cystic fluid obtained during EUS-guided FNA is generally very small (often 0.3-1ml), and thus, in clinical practice, it is frequently a choice between sending a sample for cytology or biochemistry for the endoscopist in assessing their patients. *Sahin et al* proposed that all samples should be sent to cytology assessment for centrifugation prior to biochemical assessment in order to improve accuracy[85] as opposed to current practice which frequently involves dividing samples in the endoscopic suite. The limitations of cytological assessment mean that other markers for assessment are important

CEA has been extensively investigated in the assessment of PCL. Levels are elevated in mucinous cysts, both IPMN and MCN, and are generally not elevated in SCAs or pseudocysts. It is a more sensitive marker than cytology for assessment and diagnosis of mucinous cysts[81,86–88]. Currently guidelines advise that levels of >192µml as diagnostic

of IPMN or MCN, this target level was confirmed in a multicentre trial with an accuracy of 75%[81]. A pooled analysis of 12 studies of CEA analysis in cystic fluid found a sensitivity and specificity of 48% and 98% for diagnosis of mucinous cysts when the level was set at 800µg/ml[89]. This same study found that Brugge et al[81] would have found a specificity of 27% and specificity of 95% if they had used the same cut off values.

Amylase is frequently examined in the routine assessment of pancreatic cystic lesions. Found in inflammatory cysts, levels in pancreatic pseudocysts are found to be in the thousands in routine clinical practice[90,91]. As such it can be a strong marker of exclusion for diagnostic purposes. It can be especially useful in the diagnosis of serous cystic adenomas. Amylase levels in serous cystic adenomas are low with low levels of CEA, giving a different picture to that of pseudocysts. It may be considered that amylase would be raised universally in IPMN given the connection to the ductal system by definition. However, this finding is not sensitive enough to be used as a diagnostic marker for the diagnosis of mucinous lesions. As such an amylase level of <250 with low CEA may be assumed to constitute a serous cyst; serous cystic adenoma or macro/microcystic adenoma.[89]

Glucose as a marker is beneficial as it is cheap and quick. First postulated in 2013, mucinous cysts were found to have significantly lower levels of cystic glucose compared with serous cysts.[92] The same group followed this up with a cohort study of 65 banked pancreatic cystic samples. These confirmed the results of their initial study with significantly lower levels of glucose in serous cysts. They found an overall sensitivity and specificity of 95% and 57% respectively[93]. No improvement in accuracy was found in combination with CEA for diagnostic potential. A prospective cohort of 153 patients found that at a threshold of <50mg/dL cyst glucose is 92% sensitive and 87% specific for

pancreatic cysts. Further in combination with CEA there was statistical improvement in diagnostic rates, however area under the curve was similar at 0.95 in combination compared with 0.91 in glucose alone[94]. A recent post hoc analysis of a prospective cohort found that low levels of glucose in a cyst was diagnostic of mucinous cystic lesions. This group's CEA was found to have sensitivity/specificity of 50% & 92% for a CEA >192ng/ml. In the same cohort, an intra cyst glucose threshold of <41 mg/dl found a sensitivity of 92% and a specificity of 92%. Using glucose in combination with CEA yielded a sensitivity of 49% and specificity of 97%[95]. Glucose is a useful additive test for the diagnosis of pancreatic cysts but clear diagnostic threshold is not set and it remains best used in combination with other markers.

At present the use of cytology, CEA, Amylase and glucose in combination provides the bedrock for mucinous cyst diagnosis in pancreatic cyst assessment. This group of markers, even in combination with radiological imaging and EUS findings relies upon experienced clinician's interpretation and is open to error. However it remains the current and best practice for assessment.

1.3.5 DNA PCL fluid markers in clinical practice

The expansion of use of genomic and proteomic markers in numerous medical conditions, particularly in neoplasia and pre-neoplasia, offers the potential for their use in the assessment and diagnosis of PCLs in clinical practice. The use of these techniques may be particularly useful in allowing for more advanced and accurate stratification of PCLs into higher and lower risk groups.

KRAS mediates RAS signalling and has been shown to play a role in the initiation and progression of pancreatic cancer[96]. KRAS gene point mutations on codon 12 are frequently implicated as an triggering event for pancreatic cancer. Mutations may also occur on codons 11, 13, 61, or 146.[97] KRAS mutations are an early marker of change in PCL. The incidence of mutations have been shown to be increased with increasing invasion at post-operative histology of IPMN [98]. The prevalence of KRAS mutations has been studied in PCL with one meta-analysis of 12 studies, with post-operative histology as reference standard. This group found KRAS had a specificity of 97% but a sensitivity of 46% across 731 patients[99]. This was inferior to CEA and cytology in accuracy for both malignancy and significant cysts in their cohort but they did note KRAS was a useful additive test given the limitations of cytology and CEA. A larger meta-analysis of 33 studies found KRAS mutations present in 60.9% of 1253 patients. KRAS was significantly associated with histological subtype but it was not associated with higher grade or progressions[100].

GNAS alterations in the pathway of pancreatic cancer development promotes cyclic adenosine monophosphate (cAMP) production and result in protein kinase A (PKA) activation[101]. A prospective study of 626 patients found GNAS mutant-allele frequencies for GNAS in 55% of pancreatic cysts with high grade dysplasia. The same study found that 100% of mucinous cysts had GNAS and/or KRAS mutations present in cystic fluid[102]. Similar to KRAS the specificity of GNAS has been found to be high for mucinous cysts but sensitivity remains low[103]. A study comparing pancreatic cancer tissue with PCL fluid mutations found the sensitivity of GNAS was 87% with a specificity of 62% for detecting mutation present in the neoplastic tissue[104]. A recent Meta-analysis found that the

accuracy of GNAS alone was 0.5 compared to KRAS alone 0.71 in the diagnosis of pancreatic cysts. Accuracy improved to 0.97 when GNAS and KRAS was combined[105].

Clinical use of DNA markers in assessment of PCL fluid is growing. However, their use has not been widely implemented due to cost and limited availability outside of large academic centres. However, early results show promise in aiding the diagnosis of PCL in clinical settings[106].

1.3.6 Management of Pancreatic Cystic Lesions

Prior to 2006 there were no consensus on how to manage PCLs due to the difficulty in distinguishing between benign and malignant lesions[107,108]. Guidelines for the management of pancreatic cystic lesions were first released in 2006 by the International Association of the Pancreas (IAP) as part of the Sendai conference[109], these were updated in 2012 at the Fukuoka conference and again in 2017 [110]. Since then the American Gastroenterology Society (ACG)[111], the American College of Gastroenterology (AGA)[112], and the European group[46] have all released their own guidelines for management of suspected mucinous pancreatic lesions. Given the high rates of incidental discovery there have also been guidelines released by the American College of Radiology (ACR) in 2017[113] as a follow up to their white paper on incidental findings in cross sectional imaging. [114]

The first step in all PCL assessment is the determination of the type of PCL. Early identification of those without malignant potential or at low risk of progression can

effectively be spared from a prolonged period of surveillance and potentially from an unnecessary surgical procedure.

i. Significant or Worrisome Features

The aim of the guidelines are to provide advice to clinicians for assessment of these pancreatic lesions, surveillance, and intervention when necessary. Across the guidelines PCL radiological or clinical characteristics are referred to as significant features, high risk factors or worrying features. The implication remains the same, to identify those at a higher risk of progression to malignancy. There remains variation between the guidelines in their definition.

While the guidelines are similar overall, there are some differences in their recommendations. These differences are highlighted in Table 1.1

The European guidelines are based on the need for surgical resection of a PCL. They categorise these into absolute indications and relative indications for IPMN, MCN, and other pancreatic cystic neoplasms. SCA are only recommended to intervene where there are compressive symptoms. It is always recommended to intervene in solid pseudopapillary neoplasms.

Absolute indications for surgery in the European guidelines for IPMN are a positive EUS FNA with a malignancy or high grade dysplasia, evidence of solid mass or enhancing mural nodule >5mm, tumour related jaundice and an MPD dilatation in excess of 10mm. A size of >40mm is a relative indication for surgery in IPMN but in MCN surgical

intervention is recommended at this threshold. Other relative indications are a growth rate of >5mm/year, increased levels of Ca19.9, MPD dilatation of 5-9.9mm, new onset of diabetes or pancreatitis.

The IAP guidelines are similar to the European guidelines but with some key differences in language and threshold. The defined “high-risk features” are those which should undergo surgery without further investigation: obstructive jaundice related to a cystic lesion, mural nodule >5mm, MPD of >10mm. Worrisome features are those which are recommended for further investigation with EUS. These are >30mm, enhancing mural nodule <5mm, thickened or enhanced cyst walls, MPD 5-9mm, pancreatic atrophy, lymphadenopathy, raised Ca19.9, and growth >5mm over 2 years. Cystic lesions with worrisome features are recommended to undergo EUS assessment and close surveillance.

The AGA guidelines highlight high risk features as >30mm, dilated main pancreatic duct or presence of a solid component to undergo EUS examination.

The ACG define high risk characteristics for pancreatic cysts by both symptoms, imaging findings and cytology. Presentations of clinical concern are jaundice, pancreatitis, and elevated Ca19.9. Imaging characteristics of concern are mural nodules or solid components, MPD >5mm or change in upstream calibre, size >30mm and growth of >3mm per year.

The ACR guidelines align very closely with the International guidelines but do have one minor difference in the definition of pancreatic ductal dilatation as a worrisome feature when >7mm. Other worrisome features are cyst >3cm, thickened or enhancing cyst wall, non-enhancing mural nodule. High risk stigmata are defined as obstructive jaundice with cyst in head of the pancreas, enhancing solid component within the cyst and a main pancreatic ductal calibre of >10mm in absence of obstruction.

	International association of the Pancreas (2017)	European Guidelines (2018)	AGA (2015)	ACG (2017)	ACR (2017)
Size	>30mm	>40mm	>30mm	>30mm	>30mm
Main Pancreatic Duct	>10mm High Risk >5mm or abrupt change is Worrisome	>10mm absolute indication >5mm relative indication	Dilated MPD	>5mm or abrupt change in size	>10mm High Risk >7mm worrisome
Solid Component/Mass		Absolute indication	High Risk	High Risk	High risk
Enhancing Mural Nodule	>5mm high risk <5mm worrisome	>5mm absolute indication <5mm relative indication	Presence – high risk	Presence – high risk	High risk
Jaundice	High risk	Absolute indication	Not mentioned	High risk	High Risk
Cytology	High risk	Absolute indication	High risk	High Risk	Not mentioned
Growth Rate	>5mm over two years	>5mm per year	Not mentioned	>3mm per year	>2mm per year is concerning but not

					worrisome/high risk feature
Raised level of Ca19.9	Worrisome	Relative indication	Not mentioned	High Risk	Not mentioned
Pancreatitis	Worrisome feature	Relative indication	Not mentioned	High risk characteristic	Not Mentioned
Diabetes	Worrisome	Relative indication	Not mentioned	Not mentioned	Not mentioned

Table 1.1 Summarising differences between guidelines. Green boxes indicate consensus. The term "indication" refers to an indication for surgery, I.e those at a higher risk for malignancy. Thresholds for concerning features for PCL differ between guidelines, for example the cut off for size is higher in European guidelines than elsewhere.

ii. Surveillance Methodology and Frequency

The consensus between guidelines advocates for the use of MRI in surveillance of pancreatic cystic lesions. This is due to a better visualisation of the relationship to the ductal system and in addition it allows for better visualisation of the cyst itself.

The use of CT for follow up is of benefit in certain situations, the ACG and European guidelines advise use of CT for better characterisation of cysts where there is uncertainty in the diagnosis. CT scans involve significant radiation and regular surveillance CT would not be ideal. The European guidelines advise CT may better distinguish pseudocysts, vascular invasion, and evidence of recurrence post operatively.

While there is a general unanimity between groups on the method of surveillance there are wide ranging differences in surveillance intervals. The timepoints within the guidelines can also vary widely based on the characteristics of the cyst identified. The differences in surveillance patterns are summarised in table 1.2 below. The IAP guidelines are based on high risk and worrisome features as outlined above. Where worrisome features are present an EUS assessment of the lesion is advised. If the recommendation for surgery post EUS is still inconclusive then close surveillance with alternating MRI/EUS for 6months; this pathway is recommended for all cysts over 30mm not progressing to surgery.

Where a cyst does not have any worrisome features then surveillance is based on size. 2-3cm require a 3-6 month EUS then alternating MRI with EUS annually thereafter. 1-2cm lesions should undergo cross sectional imaging annually for two years then lengthen intervals to 2 yearly if no change observed. Sub centimetre lesions require a follow up scan in 6 months followed by 2 yearly scans.

The European guidelines advise surgery in patients with absolute indications but in patients with relative indications but not for immediate surgery they advise intensive six month clinical, serum ca19.9, and MRI or EUS. In those patients without any concerning features six monthly evaluation for the first year then annually thereafter.

The ACG also stratifies cyst sized based upon the size of the lesion. >30mm lesions require 6monthly MRI/EUS alternating for three years. 20-30mm lesions are advised to be monitored every 6-12 months for the first three years before endoscopic reassessment. 10-20mm lesions should be scanned annually for three years and sub centimetre lesions scanned every 2 years for the first four years.

The AGA recommends a repeat scan in 1 year then every 2 years from that point in cysts requiring surveillance.

The ACR has a complicated set of 5 algorithms based on a cysts size, patient age, and MPD communication. They are the only set of guidelines which takes into account a patient's age from the time of diagnosis. In patients over 80 year of age it advises a less intensive investigative regimen with a lower threshold for discontinuation than in other patients. In cysts below 15mm the surveillance plan is also age based with under 65 year old patient recommended for annual screening and over 65 year olds recommended to be screened every two years.

In larger cysts, 15-20mm it is recommended to annually survey for five years then reassess on growth pattern and new size. For cysts 20mm-25mm 4 6monthly scans, followed

by 2 annual and then 3 biennial scans. Cysts over 25mm should be assessed as cysts in the 20-25mm cysts in the absence of high risk features, otherwise they should be referred for EUS and surgical opinion.

iii. Can surveillance be stopped? If so, when?

This is the million dollar question. Most PCLs are picked up as incidental findings in patients (usually older), with other comorbidities. But once a PCL is identified, the patient is the enrolled into a surveillance programme with no clear exit point. This is both costly for healthcare systems and anxiety-inducing for patients. Exit from surveillance programmes remains controversial as the various consensus guidelines are not in agreement regarding point of discontinuation. AGA guidelines recommend finishing at 5 years but otherwise no other association gives guidance on limiting time of surveillance. The Europeans advocate for a lifelong surveillance of cysts regardless of size but agrees that surveillance intervals can be extended in stable cysts. The IAP guidelines, however, feel that the risk increases as time proceeds and as such advises that these lesions should have their surveillance intensified as time progresses.

The ACR algorithms are more complicated but given that they are related to age, there is some guidance on discontinuation of surveillance of these lesions. They recommend that in certain situations that with periods of stability of 9-10 years then the surveillance can be stopped. However, this is different in the over 80 cohort where 4 years of stability is sufficient.

The decision on stopping surveillance remains highly individualised to both patient and PCL. Branch duct IPMN which are stable over five years have demonstrated a low rate of progression to malignancy[115]. In addition, growth rate has been demonstrated as a strong indicator of risk of transformation[116]. The evidence would suggest that stable smaller cysts can have their surveillance intervals lengthened or stopped. However, this can be a challenging decision. In patients who are not surgical candidates the decision is clear and surveillance can be discontinued. In younger patients this is not a clear decision and further evidence is needed for ending surveillance.

	International association of the Pancreas (2017)	European Guidelines (2018)	AGA (2015)	ACG (2017)
<10mm	Repeat imaging in 6 months then every 2 years if no change	Re image in 1 year. If stable for 3 years, follow-up may be extended to every 2 years.	pancreatic cysts <3 cm without a solid component or a dilated pancreatic duct undergo MRI for surveillance in 1 year and then every 2 years for a total of 5 years	MRI every 2 years for 4 years then reassess
<15mm	n/a			
>15mm		Repeat imaging in 6months then annually thereafter		
10-20mm	Cross-sectional imaging every 6 months for one year Yearly for two years Follow by biennial	n/a		Annual MRI for 3 years then reassess
20-30mm	EUS within 3-6month			MRI or EUS every 6-12 months for 3

	Alternating annual EUS or MRI			years and reassess
>30mm	Alternating MRI and EUS every 3-6months		MRI surveillance after 1 year and then every 2 years	Alternating EUS and MRI every six months. Also recommends MDT input
End of Surveillance	Intensify after five years	Lifelong as long as surgical candidate	5 years	Recommended until 76years, individualised thereafter

Table 1.2 Surveillance Patterns by Society Guidelines

1.3.7 Potential Cystic Fluid Markers

i. Genetic Cystic fluid Markers

The use of KRAS and GNAS in clinical practice was previously discussed. However, more explorative analysis of PCL fluid are yielding further targets which could improve PCL diagnosis.

Ring Finger Protein 43 (RNF43) is an oncoprotein which was originally associated with colon cancer, but has also been found to contribute to pancreatic, stomach, and endometrial cancers among others[117]. A retrospective study of RNF43 in PCL fluid found a mutation rate of 8%, all in high grade dysplasia or invasive cases[118]. However, further studies question the significance that RNF43 plays in transformation, finding little link between presence of mutations and invasion[117,119].

Smad family member 4 gene (SMAD4) codes for signal transduction protein SMAD4. It is inactivated in up to 50% of pancreatic cancers[120]. The loss of SMAD4 has been linked with poorer overall survival rates in pancreatic cancer[121]. Interpretation of the significance of SMAD4 detection in PCL is limited by small numbers of cases reported in studies however it appears to be frequently associated with invasion or transformation[122–124].

Tumour protein 53 (P53) is tumour suppressor gene implicated in progression of pancreatic cancer. Underexpression has been implicated in progression high grade dysplasia and invasive lesions compared to low grade cysts[125]. *Singhi et al* demonstrated that 63% of mucinous cysts with advanced neoplasia had P53 aberations[102]. P53 appears to be a marker highly associated with malignancy in pancreatic cystic lesions[126] and potential basis for prognostication.[127]

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is a gene which codes for protein 16 (P16). Exhibited in cases with increasing dysplasia it is a potential diagnostic marker for the those cysts at higher risk[119,124].

The von Hippel landau gene has been associated with serous cystic adenomas, it does not occur in mucinous cysts[128]. Molecular studies of radiologically diagnosed IPMN in a cohort of 86 patients allowed clinicians to confirm diagnosis of SCA and avoid unnecessary surgeries[129].

Additional reported targets with unclear use for clinical practice include BRAF[130], PTEN[104] and ATK[130]. A comprehensive analysis of pancreatic cysts by Noe et al demonstrated alteration in ATM, GLI3 and SF3B1[119] however these have yet to be further investigated as potential targets.

Genetic analysis of PCL fluid is still in its infancy but early results are promising. Potential targets for stratification of high lesions are being identified and with further research could be implemented into clinical management.

ii. Proteomic Markers

Proteomic profiling of cystic fluid has frequently focused on mucin expression in pancreatic cystic lesions. Immunohistochemistry staining is used in practice to help identify the histological subtype of IPMN, namely MUC1, MUC2, MUC5AC, MUC6 and CDX2[27,131]. Identified mucin proteins include MUC1, MUC2, MUC5AC, MUC5b, MUC6, MUC16, MUC18[132,133]. Mucin proteins play key roles in normal body processes for lubrication of epithelial surfaces. The proteins MUC1, 3, 5AC, 6, 13, 20 may be found in the stomach wall and 1,2,3,6,17&20 may be found in the duodenal wall[134]. This has direct implications in proteomic profiling when considering the avenue of puncture to obtain PCL fluid (i.e. an FNA needle passes through either the gastric or duodenal wall). A prospective study of 78 cysts found all lesions with malignant potential expressed one of MUC 1,2 or 5AC; MUC1 was found to be more accurate that CEA for detecting malignancy in their cohort[132]. The addition of MUC4, CK20 and villin may be additive in subtype identification but does not provide

additional information for those cysts at high risk[131]. MUC13 levels can increase with increasing dysplasia and is a potential marker of higher risk cystic lesions[135,136].

Proteomic profiling using mass spectrometry allows for a deep investigation of proteomic profiles of cysts. By examining differences between cysts we can identify differing patterns of protein expression between groups. Using this mass spectroscopy to identify and confirm with ELISA assays a prospective group identified CD55 expression significantly higher in IPMN dysplasia[137]. Similar techniques have been used in other studies, and HOOK1 and PTPN6 have also been found to be upregulated in high risk cysts[138]. However these findings are from small cohorts of patients in a research setting and they have not yet been applied in clinical management. Therefore further validation studies of proteomics are needed prior to implementation into clinical practice.

iii. MicroRNA

There are limited studies of micro-RNA (mRNA) in pancreatic cystic fluid. Wang et al identified 15 mRNA targets using next generation sequencing in IPMN samples which were differentially expressed among high and low risk lesions [139]. miR-21 has been documented in pancreatic adenocarcinoma tissue and is a promising potential target in cystic fluid studies[140]. The level of miR-216 was significantly raised in the high risk group compared with the low risk group. Five of the identified mRNA overlap with 37 mRNA identified in 2012 by PCR[138]. These five mRNA are miR-125, miR-195, miR-26, miR-30 and miR-217. However these 5 were not included by this group in their statistical model for separation of high and low grade IPMN. Next generation sequencing (NGS) examination of a small cohort of 13 IPMN samples in a

2021 paper identified MiR10a-5p as upregulated in invasive IPMN[141]. There is limited data on mRNA use in pancreatic cystic lesions and as such it remains an emerging field

The complex picture of pancreatic cysts with varied diagnoses available means that future treatment decisions are likely to incorporate the use of genetic, proteomic, or mRNA biomarkers. The likelihood is that in the future we will see a combination of markers used to accurately assess cystic fluid marker, so-called "multi-omics". A large multicentre group produced a cystic marker based on multiple genetic alterations known to be mutated in pancreatic cysts. This group used a MOCA algorithm to combine the results of genetic testing of cystic fluid but found that the result still resulted in over half the patients receiving surgery which could have been avoided with more accurate pre op diagnostics[142]. The same group progressed to developing the CompCyst classifying test based on combined clinical, imaging and molecular testing in a machine learning approach. This test had a higher accuracy (69% v 56%) of classifying patients into groups for surgery, discharge or surveillance compared to current clinical practice[143]. However, this test was only applied retrospectively in post-operative patients and has not been validated in a prospective cohort to date.

1.3.8 Serological Markers for Pancreatic Cystic Lesions

There are currently no serological biomarkers proven for use in the management and assessment of PCLs. Ca19.9 is a marker of pancreatic (and biliary) transformation / malignancy. Serum Ca19.9 has long been identified as a marker of pancreatic malignancy, with raised levels identified in serum compared to healthy controls[144–147]. However, the sensitivity can fluctuate and it is not a perfect marker of transformation with pooled

sensitivity of 47%[148]. CEA is similarly not sufficiently sensitive, indeed it is not routinely used as a serum marker in clinical practice in pancreatic malignancy. Serum SPAN-1 and DUPAN2 which have been examined in pancreatic cancer have not shown effective sensitivity or specificity for adoption into use in IPMN[149].

Novel biomarkers in pancreatic cystic disease are not yet used in clinical practice. Serum KRAS levels has been identified as a marker in pancreatic cancer, with possible use in prognostication and monitoring of patients[150,151]. A limited study of circulating $KRAS^{G12D}$ and $TP53^{R273H}$ in 7 IPMN patients found a prevalence of 28.63% and 14.7% respectively[152]. This study is limited by low numbers but it raises the possibility of future studies in this area as it is a proof of principle that genetic changes with the PCL may be detected in circulating DNA, so-called 'liquid biopsies'.

Serum mucin levels were examined in a cohort of 40 PCL patients, 21 high risk and 19 low risk by clinical features. Serum levels of MUC5AC were elevated in high risk patients compared to low but this difference was not significantly different for identifying high grade dysplasia compared to low[153]. Serum glycan levels in a cohort of 79 IPMN discovered the glycan 3195 m/z in serum with potential diagnostic value. 3195 exhibited sensitivity and specificity of 92.3% and 66.7% for distinguishing invasive IPMN[154]. A similar study using MALDI to profile protein signatures of PDAC and IPMN was able to distinguish main duct/mixed type from branch duct but sensitivity is unclear[155].

The identification of miRNA in the serum appears more common than cystic fluid. 30 miRNA were identified as possible markers with miR-145-5p showing the most significant association with IPMN compared with healthy controls. This group was able to identify a 30 mRNA panel which diagnosed IPMN from healthy controls with an AUC of 74.4. The same

study found a five mRNA signature which correlated with malignant IPMN, miR-200a-3p, miR-1185-5p, miR-33a-5p, miR-574-3p, and miR-663b [156]. In a study of solid pancreatic lesions plasma miR-223 was shown to be significantly higher in malignant IPMN compared with benign (p=0.0988), in addition there was a significant difference between levels in patients with malignant IPMN and PDAC (p=0.004)[157]. MiR-21 is elevated in serum of 12 IPMN compared to healthy control (p=0.394) but not significantly different to PDAC[158]. Another study of circulating mRNA identified possible diagnostic potential for miR-21-5p, in addition to miR-33a-3p, miR-320a, and miR-93-5p, of a group of 14 mRNA significantly upregulated in IPMN serum[159]. Serum exosomal examination of IPMN patients identified ExmiR-191, ExmiR-21 and ExmiR-451a was significantly up-regulated in patients with pancreatic cancer and IPMN compared to the controls ($p<0.05$)[160]. The potential of exosomal targets for a diagnostic may be more useful than circulating mRNA. Extra-vesicular RNA is a potential pathway to mRNA diagnosis in pancreatic cysts. EV-miR-4539 was found to have sensitivity of 60.5% and specificity 92% for diagnosis of IPMN from healthy controls[161]. It is not just circulating mRNA in extra-vesicular vesicles which provides targets. HULC is a long coding RNA which is demonstrated upregulation in IPMN[162].

While there are few studies on serum or blood based markers in pancreatic cystic disease there are emerging potential targets. Receptor-binding cancer antigen (RCAS1) has previously been identified in elevated levels in pancreatic cancer tissue and cancers, it was demonstrated to be elevated in 60% of IPMN patient's serum in a cohort of 20 IPMN patients[150]. A small discovery cohort of 12 IPMN found higher levels of serum PODXL and SCGB1D2 in advanced IPMN compared to healthy controls[163]. Serum osteopontin and MIA was shown to be effective in differentiating IPMN from acute pancreatitis, a useful adjunct

where there is uncertainty regarding a cyst's diagnosis[164]. Overexpression of EpCAM (epithelial cell adhesion molecule) was demonstrated in the serum of malignant IPMN[165].

There are limited studies on serum in PCL. It remains an important research goal which could significantly reduce endoscopic and surveillance burden but further studies are required prior to application in patient care.

1.3.9 Emerging Endoscopic Approaches

The limited sensitivity of pancreatic cystic lesion cytology is largely due to the acellular aspect of cystic fluid, as was alluded to previously. Attempts to improve this include the development of "through the needle biopsy". These micro forceps (Moray® Micro Forceps, Steris Healthcare, USA) are passed through the gauge of a 19G needle. Under endosonographic guidance, an operator can target the cyst wall to obtain a tissue sample. A prospective trial of this device found an acquisition-yield of 83.3% with micro-forceps[78]. Overall performance of micro forceps in a pooled analysis of 425 cases found a diagnostic yield in 79.60 % with a diagnostic accuracy of 82.76 % and adverse event rate of 1.08%[79]. This is a significant improvement over EUS-FNA fluid cytology.

Contrast enhanced harmonic endoscopic ultrasound (CH-EUS) is a technique aimed at measuring perfusion of tissues and provide better diagnostic images of lesions. This modality is designed to better visualise small perfusing vessels than doppler imaging. The modality is based on use of commercial ultrasound contrast agents like Sonazoid®, Sonovue®, or Definity®. These are all second generation ultrasound contrast agents. They are protein or lipid shelled microbubbles with low solubility gases trapped within[166]. Definity® yields

bubbles 1.3-3.3µm in diameter[166]. Sonovue® mean diameter is 2.5µm[167], and Sonazoid® averages 2.1µm[168]. Being smaller than a red blood cell this allows the bubbles to enter the microvasculature and perfusing vessels of organs and tissues. Contrast-enhanced harmonic imaging visualizes the microcirculation and parenchymal perfusion by selectively depicting the signals from these agents while simultaneously filtering signals originating from tissues[169]. The contrast agent is administered intravenously with saline flush and contrast enhanced examination is performed for ~ 60 seconds. CH-EUS is not widely used in cystic lesion analysis and more evidence is available for use in solid pancreatic lesion assessments. A recent prospective study showed minimal improvements in sensitivity for interpreting the IAP guidelines with the introduction of CH-EUS[170]. Similar results comparing CH-EUS and MRI have found little benefit in pancreatic cysts[171]. A retrospective analysis of 166 patients undergoing CH-EUS prior to surgery found CH-EUS was more sensitive than CT for identifying MD involvement, AUC: 0.8523 vs. 0.7138, P=0.0004 [172]. However, this study did not compare contrast enhanced and non-enhanced EUS so the benefit over traditional EUS imaging is unclear.

Confocal endomicroscopy allows for the endoscopist to perform intraprocedural diagnostic microscopy within the PCL, with the aim of identifying more advanced neoplastic lesions. The technology is based on tissue illumination using low power laser and detection of fluorescence illumination within tissues[173]. It allows for high magnification of GI tract. This can be performed with probes, endoscope based or needle based technology.

For Pancreatic cysts the needle confocal light endomicroscopy probe (nCLE) is combined with EUS (EUS-nCLE). The method for performing EUS-nCLE is similar to standard EUS assessment of lesions. Prior to the imaging a fluorescein sodium 10% solution is administered intravenously. A 19g FNA needle is passed into the identified pancreatic cyst to

contact with the epithelial wall of the cyst[174]. The use of nCLE for pancreatic cysts was first introduced in 2011 with the INSPECT trial[175]. This feasibility study was followed by the DETECT trial which found CLE to have a sensitivity of 80% in diagnosis of mucinous cysts[176]. , CONTACT 1&2 studies described the histological features and postulated diagnostic criteria to aid in diagnosis of SCA in the CONTACT 1 and IPMN, MCN and NET in CONTACT 2 trial[177,178]. These were followed by the INDEX prospective study which compared outcomes in confocal endoscopy with that of ex vivo assessment of pancreatic cystic lesions[179]. A recent meta-analysis of nCLE in PCL has found a pooled sensitivity of 82.4% and specificity of 96.6% across 9 retrospective and prospective studies[180]. The most significant complication seen to date in nCLE is post procedure pancreatitis, with a rate of 1.2% across studies[181]. The use nCLE has been shown to be cost effective in the diagnosis of benign pancreatic cystic lesions by improving diagnostic accuracy over FNA alone[182]. The limitations of nCLE are availability of sufficiently trained endoscopists to perform investigations but it is a viable additive examination and diagnostic tool.

i. Endoscopic Therapy

At present endoscopic treatment options for pancreatic cysts are very limited and remain in research domain. Ablation of cysts has been performed in small numbers of patients but widespread use is not endorsed. Yet, there are a few avenues for treatment options. The benefit of endoscopic interventions is potential blurring of the lines between diagnostic and treatment procedures, reducing patient procedures.

ii. Chemical Ablation

Chemical ablation of pancreatic cysts was first described in 2005 by Gan et al[183]. The first trial was the use of ethanol to lavage the cyst. This procedure involved the introduction of increasingly concentrated ethanol from 5% to 80% via endoscopic needle. This was a double blind crossover trial of ethanol and saline for pancreatic cysts. A single ethanol lavage was found to reduce mean cyst size. However, resolution was seen in 3 of 13 saline lavages before ethanol crossover. Overall cyst resolution rate was 33% for the trial[184]. A trial of 14 ethanol ablations in France reported 85% resolution rate without complications[185]. The next step in cyst ablation was the introduction of chemotherapeutic agents. First described was the use of paclitaxel in addition to the use of ethanol. Paclitaxel is a chemotherapeutic agent which functions through microtubule inhibition. Cysts were ablated by first ethanol lavage followed by paclitaxel. Oh et al described the procedure in a pilot study with resolution in 11 of 14 (78.6%) patients, with one episode of pancreatitis in the group[186]. A follow up of the initial pilot study showed resolution in 29 of 43 (67.4%) patients, with partial resolution in 6 (13.9%)[187]. An American trial of combination lavage reported complete or partial resolution in 75% of patients but adverse events rates of pancreatitis in 10% and abdominal pain in 13%[188]. The largest cohort published to date was a south Korean cohort of 214 patients who underwent ablation with 99% ethanol. Short term response rate in this group was 69% with an adverse event rate of 33.2%[189]. 21 cases of acute pancreatitis related to ablation were reported, 2 duodenal strictures, 1 bleeding event and 1 episode of cholangitis. The overall concern with ethanol ablation of cysts is the rate of adverse events. With significant concerns for the possible development of pancreatitis, a pooled analysis adverse event rate of 21.2% in ethanol alone ablation compared to paclitaxel ablation (±ethanol)[190]. Although most

paclitaxel trials have included ethanol in procedure for ablation the strength and time of exposure to ethanol is usually shorter. The same meta-analysis found a resolution rate of 63.6% in paclitaxel regimes compared with 32.8% in alcohol lavage.[190]

The CHARM trial was a randomised trial with two arms, with and without ethanol for the ablation of cysts. It combined not only a study of a protocol without ethanol, it also combined paclitaxel with gemcitabine for the treatment in place of paclitaxel alone. 12 month ablation rates were 61% in ethanol arm and 67% in the ethanol free arm[191]. All adverse events occurred in the alcohol arm, 1 serious event of pancreatitis requiring admission. This effectively demonstrated that ethanol was not necessary for ablation of these cysts. A long term follow up report on this trial showed persistent resolution in 87% of those showing resolution at one year and 31% of those not completely resolved at one year continued to trend toward a resolution[192].

A single centre cohort performed ablation using lauromacrogol. A sclerosing agent used in management of oesophageal varices and ablation of non-pancreatic cystic lesions. 29 patients were enrolled in the lauromacrogol arm of this group, 7 patients underwent repeat ablations. Overall resolution rate was 37.9% with 2 mild pancreatitis adverse events and one fever[193]. Long term follow up data from this group showed an overall complication rate of 3 in 84 ablations (3.8%). In patients followed up for >12 months, clinical resolution was seen in 51% and partial resolution in 25.7%[194].

We are beginning to see the emergence of longer term follow up cohorts. DeWitt et al reported the follow up of their twelve patient cohort, 9 patients were not lost to follow up and no recurrence was seen at a median of 26 months post ethanol ablation[195]. 164 patients post paclitaxel/ethanol combination ablative therapy were 98.3% recurrence free at 6 years post follow up.

iii. Radiofrequency Ablation

Radiofrequency ablation (RFA) is emerging as a novel technique for the management of PCLs. The technique is similar to that used in chemical ablation but a radiofrequency probe is used to deliver a burst of heat to a focal lesion within the pancreas. A pilot study was performed on porcine tissue to mimic the papillary projections of IPMN. EUS RFA devices consist of a 22g needle with a monopolar electrode at the tip. This pilot study performed 32 procedures on porcine tissues with times ranging between 102-440s and temperatures ranging between 50-70°c. This study found an increased volume of tissue was ablated at higher temperatures but the patterns varied between cysts suggesting an asymmetrical distribution of heat, even in a controlled ex-vivo setting[196]. To date there have been two trials of human pancreatic cystic ablation. A prospective French cohort included 17 PCL patients who underwent RFA. This technique trial performed FNA of cystic fluid to reduce size of the cystic cavity, followed by the introduction of an RFA needle applying 50W until reaching 100 Ohms impedance. At 1 year response rate was 11 of 17 (65%) patients achieved complete remission of the PCL and one patient achieve partial remission[197]. There was one significant adverse event, pneumoperitoneum and duodenal perforation, in an IPMN ablation. *Gai et al* published a pilot study experience investigating the outcome of 6 patients with PCL undergoing RFA. Ablation was applied at varying strength from 5-25 watts for 90 second intervals. Results from this study showed complete resolution in 2 cysts and 48.4% reduction in 3 other patients. No significant adverse events were noted.

At present, the routine use of ablative techniques is not endorsed by any of the guidelines and remains experimental. The concern is that there is a high number of complications seen with lavage, although as mentioned previously this is reduced with the removal of ethanol in place of chemotherapeutic agents although as of yet a regimen has not been developed to the point of routine use.

1.4 Pancreatitis

Pancreatitis is an inflammatory disorder of the pancreas. Acute pancreatitis is one of the leading causes for gastroenterology admissions in Europe and North America [198–200]. The principle causes of pancreatitis are alcohol, biliary (gallstones), hypertriglyceridemia, and iatrogenic post-ERCP. There is no significant association with aetiology of pancreatitis and collection formation[201]. Overall mortality in acute pancreatitis is 5%[202]. Chronic pancreatitis is a recurrent inflammatory condition of the pancreas, which leads to scarring and irreversible damage of the organ. It can lead to both exocrine and endocrine dysfunction[203]. Pancreatic collections can arise in both moderate and severe cases of pancreatitis. They can present management challenges in both the acute and long term management of pancreatitis patients. The approach to these collections has evolved over the recent years with movement away from surgery as the first line intervention and evolution of new approaches for clinicians.

1.4.1 Mechanism of Pancreatitis

i. Acute pancreatitis

Inflammation arises in pancreatitis with obstruction of the pancreatic ductal system. The initiating injury blocks the secretions from the ductal cells. This in turn causes zymogen granules from the acinar cells to coalesce and form vacuoles. These vacuoles are stimulated by lysosomal enzymes to convert trypsinogen to the digestive enzyme trypsin. This premature enzyme activation occurs within the acinar cells of the pancreas leading to autodigestion of the pancreas[204]. This is the hallmark pathological injury of acute pancreatitis. Within the acinar cell, altered regulation of the Ca2+ signalling prompts mitochondrial overload and resulting failure of the cell to generate its own ATP[205,206].

The initial injury causing this disruption may be multifactorial. Alcohol sensitises the pancreas to damage with toxic effects on pancreatic stellate cells [207]. Smoking has been linked but no direct causation has been proven[41]. There may be an underlying genetic component with PRSS1, CFTR, and SPINK among commonly implicated genes [208,209]

Pancreatic necrosis develops when there is disruption of the microvascular components of the pancreas leading to a reduction of the local blood flow. Histological examination of specimens reveals microthrombi and inflammation in capillaries in lobular and ductal

necrosis specimens [210]. This restricted blood flow at the microvascular level induces hypoxic necrosis of the tissues.

The severity of pancreatitis is thought to be determined by cytokines and chemokines which are released following acinar cell injury. Damage to the acinar cell initiates an immune cascade with recruitment of pro-inflammatory cells. Pro-inflammatory mediators including tumor necrosis factor α (TNF), interleukins (IL) 1, 2, and 6, as well nitric oxide and reactive oxygen radicals are recruited and activated. These inflammatory cells play the dual role of increasing acinar cell injury and initiating a systemic inflammatory response[202,211]. Systemic inflammatory response syndrome (SIRS) is a clinical response to inflammation, it can progress to multi organ failure and/or pancreatitis associated lung injury. It is a factor in early mortality from acute pancreatitis[202].

ii. Chronic Pancreatitis

Recurrent inflammatory episodes of acute pancreatitis can lead to replacement of the pancreatic parenchyma with fibrous connective tissue. This disruption leads to both exocrine and endocrine dysfunction of the pancreas[212]. Differing models of recurrent or persisting injury can lead to chronic pancreatitis. Pancreatic ductal disruption or partial obstruction can occur from acute pancreatitis and patients are susceptible to subsequent bouts of pancreatitis and pancreatic fluid collections[213]. Progression of an initial attack of pancreatitis can be driven by either genetic or environmental risk factors. Chronic exposure to alcohol is related to higher risk of chronic pancreatitis[212]. Genetic risk in chronic pancreatitis is primarily associated with genes coding for digestive proteases. The trypsin pathway is mediated by

PRSS1, PRSS2,CTRC, and SPINK1 genes. Dysregulation of these pathways have all been implicated in the development of chronic pancreatitis[214].

1.4.2 Pancreatitis Classification

The revised Atlanta classification of 2012 divides pancreatitis into interstitial oedematous pancreatitis and necrotising pancreatitis.[215]. The natural course of pancreatitis will vary between patients. Oedematous pancreatitis accounts for 85% of acute pancreatitis. It is classified by diffuse inflammation and enlargement of the pancreatic tissue without necrosis. When necrosis develops it can lead to infections, liquefaction of the pancreas, development of peri-pancreatic collections or can simply persist over a long period of time. 15% of pancreatitis presentations are necrotic[215]. This necrosis may remain sterile or it can develop into infected necrosis.

Severity of pancreatitis is stratified into 3 categories. Mild acute pancreatitis will resolve without need for intervention in most cases. It is defined by an absence of local and systemic complications or organ failure. Moderate pancreatitis is characterised by transient organ failure (i.e. <48h) and/or local or systemic complications. Severe pancreatitis is characterized by the presence of persisting organ failure. The driving factor of organ failure in severe pancreatitis is a systemic inflammatory reaction (SIRS) driven by cytokine storm. A cytokine storm is an immune reaction driven by feedback loop between cytokine signals and the immune cells, T-Cells and macrophages. The activated cells produce further cytokine signals driving further activation. When this occurs in target organs or tissues it can result in damage or overload of that organ. Implicated pathway include toll-like receptors, IL-1R, NF-κB, and

macrophage migration inhibition factor [216]. Identifying organ failure in acute pancreatitis is important to define severity. The guidelines advise using a modified Marshall score for organ failure, which aids clinicians in recognising and grading organ failure in pancreatitic patients. [217].

1.4.3 Pancreatic Fluid Collections

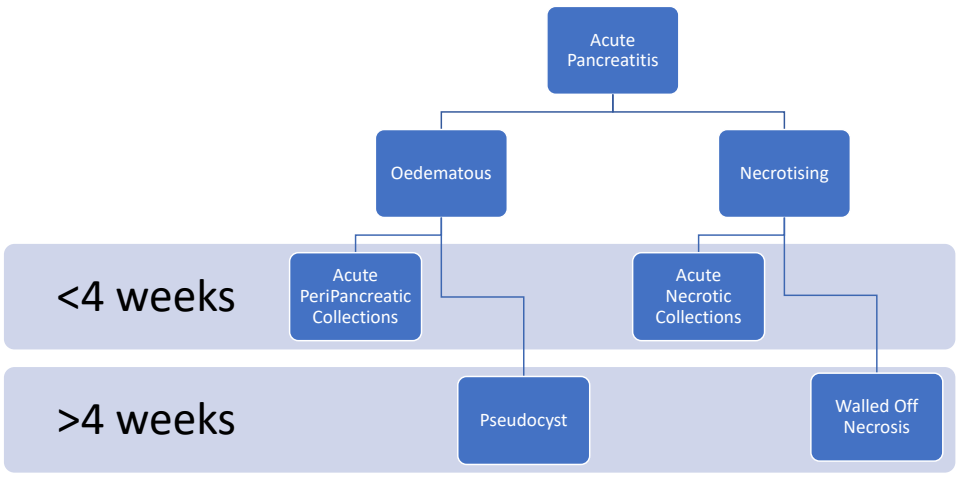

Figure 1.2. Pancreatic fluid collection classification. Timeline to maturation and endothelialisation of fluid collections is important.

The Atlanta classification introduced 4 categories of pancreatic collection: acute peripancreatic fluid collection, acute necrotic collection, pancreatic pseudocyst, and walled off necrosis.[215]

Acute pancreatic fluid collections (APFC) develop early in the onset of interstitial oedematous pancreatitis, within the first 4 weeks of onset. APFC are found in about a third of severe acute pancreatitis [218,219]. They are localised to the retroperitoneum, with poorly defined walls and contain homogenous material.

There is limited evidence as to the cause of acute pancreatic collections. They are less organised than a mature pseudocyst. They do not form walls, they are bound by the fascial boundaries of retroperitoneum within which they develop. It is likely that collections develop from the disruption of the main pancreatic duct or a side branch of the same. This leads to extravasation of the pancreatic juices and results in formation of a fluid collection. The exact incidence of pancreatic fluid collections is unclear. A prospective cohort of 302 patients presenting with acute pancreatitis found APFC in 42.7% of presentations with 14.7% of those patients subsequently developing a pseudocyst[220]. This is similar to the rate reported in a cohort of 4379 acute pancreatitis patients identified retrospectively in China. This group found 17.2% of patients developed a pancreatic pseudocyst but acute pancreatic collection rates were not reported[221].

A pancreatic pseudocyst is a mature collection in the peripancreatic tissues with a well-defined fibrous wall lacking an epithelial lining. Contents are mostly fluid. It is this fibrous

wall which differentiates the pseudocyst from an acute collection. Timing of its formation is usually 3-6 weeks post the initial event of pancreatitis[220].

Pancreatic necrosis is the presence of non-viable pancreatic tissue. Necrotising pancreatitis gives rise to a semi solid/partially liquefied state of matter within the pancreatic fluid collection. This will give a heterogenous appearance of acute pancreatic necrotic collections.

Acute pancreatic necrotic collections (ANC) arise in the first four weeks of presentation. These only occur in the setting of acute pancreatic necrosis. The presence of solid or semi solid contents within the fluid collection usually distinguishes it from acute peri-pancreatic collections (APFCs) but distinguishing these at early stage may prove difficult.

An ANC can develop into walled off necrosis (WON). WON is an encapsulated collection of pancreatic necrosis with a well-defined inflammatory wall. A WON also takes time develop, between 4-6 weeks before maturation and formation of a wall[30]. Both WON and pseudocysts are essentially the result of 'maturation' rather than resolution of an acute collection. They can resolve over time however but can also lead to complications, which may necessitate drainage.

1.4.4 Clinical Assessment of Pancreatitis

There are a number of scoring systems in place to aid clinicians to identify more severe cases of pancreatitis. The first introduced by Ranson in 1974 was "Ranson's Criteria"[222], based on biochemical and clinical factors on a patient's admission and reassessed at 48hours.

The Glasgow-Imrie score is a prognostic scoring aimed at predicting severity of acute pancreatitis within 48 hours of admission[11]. It is a clinical scoring system consisting of 9

parameters allowing for identification of those patients likely to develop more serious disease, and as such who may require intensive management. Its use remains widespread and it has shown to be highly specific for the identification of severe pancreatitis[11].

The APACHE II score (Acute Physiology And Chronic Health Inquiry),[223] was initially developed for use in patients in ICU settings. It has shown favourable results in the assessment of patients with pancreatitis at predicting outcomes in comparison with Ranson's criteria [224]. The drawback of the APACHE score is the difficulty in its calculation.

The BISAP (Bedside Index Of Severity In Acute Pancreatitis) was proposed in 2008 for early recognition of the severity of acute pancreatitis.[225]. The BISAP score is scored 1-5 with categories for **B**UN >25g/dL (blood urea nitrogen), **I**mpaired mental status, presence of ≥2 **S**IRS criteria, **a**ge >60, and **p**leural effusion. Scored at both admission and 48 hours it is designed to be more rapid and accessible for the clinician.

There have been a number of comparisons of these criteria for patient assessment showing no clear superiority of any system. RANSON's criteria, APACHE, and BISAP have all shown similar levels in sensitivity for identification of severe cases of pancreatitis. There is a concern that APACHE may not appropriately predict local complications. A study of scoring systems found APACHE had 43% sensitivity for local complications compared to 57% and 54% in the RANSON and BISAP respectively[226]. Given that APACHE was not specifically designed for pancreatitis this is not a surprising finding and APACHE remains similar or superior to the other scores for identification of severity and mortality in pancreatitis[221]. *Papachristou et al* compared initial scores at 24h from admission with severity, mortality and necrosis development. Little difference in sensitivity was found between scoring systems and BISAP was sufficiently sensitive and more easily implemented in clinical care[227]. This group also

proposed that clinical scoring systems are at maximal utility and further attempts to predict mortality in AP should be investigated.

Emerging techniques in assessment of severity include monitoring of intra-abdominal pressures. Raised intra-abdominal pressure has been demonstrated to be linked with increasing mortality and need for intervention in severe acute pancreatitis[228]. Bedside monitoring is non-invasive and is linked to improved survival[43]. Its use is not routine in clinical setting but adoption into management algorithms could allow for accurate predictors of mortality or identification of need for early intervention.

1.4.5 Imaging in Pancreatitis

The Atlanta Guidelines recommend the use of contrast enhanced computed topography as the imaging modality of choice in acute pancreatitis. There is still a role for the use of MRI in pancreatitis assessment. MRI has superior soft tissue imaging, more accurately differentiating necrotic tissue and collections in addition to assessment of pancreatic duct integrity.[229,230]

Timing of imaging is important, if undertaken too early it may underestimate the degree of pancreatic necrosis present or miss development of collections. Clinical guidelines recommend imaging[231–233] between 48- 96 hours from presentation although the AGA propose that in the absence of clinical concern or complications that patients can be managed without abdominal imaging. Evolving collections can take days to mature and may not be visible on imaging until 5-7 days post initial presentation.

CT severity index and Balthazar scoring system used in management of pancreatitis guide decision making in the acute phase of pancreatitis and allow for the recognition of

possible decline and worsening of patients[234]. US or CT imaging can be used acutely to determine the aetiology of pancreatitis in a patient's presentation, i.e. biliary or autoimmune disease.

Early ERCP has been investigated extensively and current approach is early intervention, within 48-72 hours of onset of illness, where there is gallstone pancreatitis complicated by cholangitis[235]. ERCP should also be performed if there is any radiological or clinical suspicion for bile duct stones and sphincterotomy should be individualised on a patient to patient basis by the treating clinician.

There may be added benefit from use of an EUS assessment in idiopathic pancreatitis where there has been no cause found on MRCP or CT to out-rule biliary microlithiasis [236,237]

1.4.6 Management of Pancreatic Fluid Collections.

Many pancreatic fluid collections will require no intervention and will resolve spontaneously. Current management strategies lean away from early surgical intervention. The Dutch Pancreatitis Study group published the PANTER trial in 2010[238]. In this randomised control trial 378 acute pancreatitis patients with signs of pancreatic or peripancreatic necrosis were studied. 88 patients were assigned to a "Step-Up approach" with the use of minimally invasive drainage prior to open necrosectomy. It was found that 69% of primary open necrosectomy had major complications or death compared to 40% of the minimally invasive group. [239] The change in approach has made the involvement of multi-disciplinary input vital. Part of the difficulty in management of pancreatitis is deciding when to intervene. European and International guidelines both advocate to allow for four weeks prior to intervention on fluid collections[240,241]. This allows for maturation and encapsulation of the collection to form

a pseudocyst or walled off necrosis in the event it does not spontaneously regress. *Chantarojanasiri et al* describe their experience of intervening, where clinically necessary, prior to four weeks with no significant differences between early and late groups. [242]. However, it is noted that all the patients in their cohort had encapsulated collections prior to intervention.

Indications for intervention are infected pancreatic necrosis, either proven or clinically suspected, organ compression (for example compression of the stomach or duodenum, causing gastric outlet obstruction,, or compression of the splenic vein, which can lead to splenic vein thrombosis), or in rare cases raised intra-abdominal pressure[31,240]. The choice of intervention is dependent on a number of factors including presence of necrosis, location of the fluid collection, patient suitability, time since index pancreatitis episode and available expertise.

i. Endoscopic Drainage of PFC

Endoscopic Management of pancreatic fluid collections has advanced drastically since it was first described in 1975.[243] The advent of endoscopic ultrasound in combination with newer stenting technologies offers therapeutic options for patients with pancreatic collections, allowing for intervention of collections not amenable to conventional endoscopy[244 245]. The transmural approach has been shown to be more efficacious than the transpapillary approach [246], to the point where transpapillary is not performed unless absolutely necessary.

There is no evidence for endoscopic management of acute pancreatic fluid collections. As stated above the vast majority spontaneously regress. In addition these are

sterile collections and so do not serve as reservoirs of infection. ANCs, because they do not have a defined wall, are amenable to percutaneous radiological drainage, which is a sterile procedure.

Only mature PFCs are suitable for endoscopic drainage; this includes both pseudocysts and WONs. Immature PFCs do not have an organised wall and insertion of the drain into the collection, either trans-gastrically or trans-duodenally, will introduce infection into the peritoneum.

Endoscopic management of pancreatic collections involves placement of a variety of stents into the collection, using transgastric or transduodenal approach. The sequence for endoscopic management can be described as a needle puncture of gastric or duodenal wall, dilatation of the formed lumen and placement of a stent to allow for drainage of the contents of the collection via the lumen. There are 3 main therapeutic options for stenting, double pigtail plastic stents (DPPS) inserted after needle puncture; self-expanding metal stents (SEMS) which are adapted from their original use of biliary stenting, and most recently developed are lumen apposing metal stents (LAMS). When EUS-guided drainages were first performed, DPPS was the only option, but over the past 15 years, SEMS and LAMS have been become the default treatment option.

LAMS are expanding metal stents specifically designed for the drainage of pancreatic fluid collections. LAMS are optimally designed to allow for adequate drainage of both pseudocysts and walled off necroses. There are different commercial models available but all bear similar overall structure. They are bi-flanged wire structures designed to be deployed trans-gastrically or transduodenally into a fluid collection. Some are designed to be inserted

using a needle or cystotome and wire guidance similar in technique to DPPS, eg NAGI, instrumed. Some are themselves capable of providing electrocautery cutting and insertion in a single movement, eg HOT AXIOS, Boston Scientific.

The main proposed benefit of LAMS was ease and speed of insertion. It was also postulated that they would lead to more effective drainage procedures, shorter procedure times and, importantly in the case of necrosis, facilitate necrosectomy. Reports of experiences with LAMS procedures were finding high technical success rates with good clinical outcomes[247–250]. Overall clinical success rates of LAMS are estimated to be 90.01% compared to 82.56% in DPPS[251]. Clinical success in pseudocyst drainage is 98% and 90% in WON[252]. However, with ongoing practice and implementation of LAMS devices there has been increasing reports of adverse events citing bleeding, buried stents, and biliary strictures in addition to non – benefit of LAMS over double pigtail stenting[253–256]. This rise in concern has raised questions over the benefit of using LAMS compared to DPPS. The PENGUIN trial is an ongoing, double blind randomised control trial to assess for superiority of LAMS over plastic stents[257]. There are actively recruiting trials in China (NCT03808272) and Sweden (NCT02845258) to assess safety and efficacy of drainage of pseudocysts and WON.

ii. Endoscopic Necrosectomy

Endoscopic necrosectomy involves insertion of a gastroscope through the LAMS or SEMS into the pancreatic collection to remove and debride necrotic debris in WON. Necrosectomy can be primary, ie performed at the index intervention on a collection, or secondary, during a subsequent procedure to stent placement. There are no studies in place or ongoing for assessment of most effective tool for performing endoscopic necrosectomy. Cases have been

reported using polypectomy snares, baskets, and high flow water systems. [258,259]. *Nguyen et al* reported use of a laparoscopic Babcock forceps to assist in necrosectomy post radiological drainage and endoscopic metal stent placement in a combination approach with radiology and gastroenterology teams.[260]

During the necrosectomy session high volume lavage has been shown to be effective. Follow up lavage with 3% hydrogen peroxide has been shown to be a safe and effective means of performing secondary necrosectomy.[261] Lavage with hydrogen peroxide has been shown to have a higher success rate than standard necrosectomy. A comparative study of standard necrosectomy and necrosectomy with hydrogen peroxide in WON found a significant improvement in both clinical success and time to resolution with the addition of hydrogen peroxide[262].

Endoscopic intervention is important to prevent the recurrence of collections where a disconnected pancreatic duct has occurred. Usually arising in the presence of necrosis, it may be also caused by trauma[263]. A disconnected duct is a disruption of the main pancreatic duct. It is associated with recurrence of pancreatic fluid collection accumulation. The risk of recurrence can be lessened with the insertion of plastic stents. [263,264]

1.5 Aims and Objectives

From the discussion of pancreatic cystic lesions (PCLs) and pancreatic fluid collections (PFCs), there are a number of important unanswered questions in clinical practice. With regard to PCLs, as discussed, there are a number of consensus guidelines currently in use, which differ on a number of issues. One important question is '*can surveillance of PCLs be safely stopped*

in more patients than current practice dictates?' In our centres alone, there are hundreds of patients (generally older, frailer with co-morbidities) undergoing annual surveillance of low-risk cysts, because this is what the guidelines dictate. Our clinical experience is that few of these patients actually progress to high risk lesions. Yet annual or bi-annual surveillance of these patients places a heavy burden on healthcare systems and on patients. To investigate this further, we aimed to retrospectively identify patients with PCL undergoing surveillance at our institution, and assess progression of these lesions over time.

A second clinical challenge is to accurately stratify PCLs into low and high risk lesions. The methods used at present rely heavily on morphological features such as PCL size. Current biomarkers in use help distinguish mucinous (IPMN and MCN) from other PCLs (such as SCA), but do not help risk stratify the IPMNs, the majority of which, will not progress significantly over time . We planned to investigate PCL fluid to novel biomarkers, which might be associated with risk of progression.

Finally, in a separate study of management of pancreatic fluid collections (PFCs) in this country, we aimed to look at success rates and complications of EUS-guided drainage of PFCs in a retrospective, multi-centric study.

1.5.1 Specific objectives

1. Study 1: To retrospectively assess the rate of progression of a known PCL cohort undergoing active surveillance at a single institution. The cost of this surveillance and the rate of progression to cancer in this large Irish Cohort will be assessed.

2. Study 2: To Prospectively recruit a cohort of patients to pancreatic fluid (PCL and PFC) biobank. Using this pancreatic fluid we will attempt to identify novel biomarkers of high risk cystic lesions.
3. Study 3: Evaluate the outcomes and complications of EUS- guided drainage procedures of inflammatory pancreatic fluid collections (PFCs) in Ireland.

2.

Methodology

2. Sites

Patients were recruited for this study in Tallaght University Hospital (TUH) and St James's Hospital (SJH). Both hospitals are tertiary referral units for hepatopancreatobiliary endoscopy. Samples were processed on site in the Meath Foundation Laboratory on the TUH campus and in the Trinity Translational Medicine Institute (TTMI) on the SJH Campus. Samples were stored on site of collection. Lab work was performed in the TTMI Surgical Laboratory.

Pancreatic cystic drainage data was recruited from the Mercy University Hospital in Cork. This is the regional hepatopancreatobiliary centre for endoscopy.

2. Ethical Approval

Ethics approval was granted by St James's Hospital and Tallaght University Hospital Joint Research Ethics Committee. The research was conducted in accordance with the principles of the Declaration of Helsinki.

Copies of the Patient Information Leaflet and Patient Consent Forms can be found in the appendices.

2.1 –Patients Under Surveillance in Tallaght University Hospital

2.1.1 Identification of patients

Patients were identified retrospectively through the upper gastrointestinal surgical MDT paper and digital records between 2005-2020. This identified all patients discussed at the MDT for purpose of decision making regarding pancreatic cystic lesions.

To widen the net further a radiology search for pancreatic cystic lesions patients was performed with the aid of the NIMIS radiology system. The keyword search terms used were "Pancreatic Cyst", "Pancreatic cystic lesion", "Intraductal papillary mucinous neoplasm", “intraductal papillary mucinous tumour", "IPMN", "IPMT", "Mucinous cystic neoplasm", "MCN", "Serous cystic adenoma", "SCA" or "pancreatic cystic neoplasm".

2.1.2 Cyst Characteristics

Using this combination of search methods, each patient’s electronic records were examined. Patient demographics identified were age, gender, date of initial diagnosis, managing clinical

team, date of exit from surveillance strategy or decision for surgery, date of death was noted if applicable.

Surveillance data recorded was date of initial diagnosis. Timepoints were divided into 6 months, 1 year and annual thereafter. Method of surveillance, CT/MRI/EUS was also recorded.

Cystic characteristics identified were cyst maximal diameter, presence of worrying features. Interval scans had size of pancreatic cyst and development of worrisome features.

Initially worrisome feature criteria used were in keeping with the 2018 European guidelines. However, after collection of results and reviewing of the cohort timing this was adjusted to the IAP definition of worrisome features as this was more in keeping with the prevailing guidelines for the majority of the cohort's time under surveillance.

2.1.3 Assessment of cystic growth

Cystic size was identified in mm at each timepoint available.

For assessment of growth rate of lesions at least two imaging points were required. The largest axial diameter reported was taken as cyst size, in keeping with guidelines and clinical practice. We calculated growth rate in 2 ways; Absolute Delta change (Absolute Δ) and Relative Delta Change (Relative Δ). Relative Δ was calculated by the formula below:

$$\frac{Final\ size - Initial\ Size}{Initial\ Size} \times 100 = \text{growth rate}$$

2.1.4 Calculation of Costs

Costs were attributed to pancreatic surveillance and surgery. Each scan, annual clinical review, EUS with FNA, cyst fluid assessment, annual serum Ca19.9 was accounted for. There was limited data for the pricing of procedures in Tallaght University Hospital and the HSE as a whole in comparison to international databases as seen in Switzerland and the Netherlands.

Initially costs were calculated in consultation with the business managers in Tallaght University Hospital. This gave us an initial starting point of costing for procedures performed in Tallaght, i.e EUS with FNA, CT, MRI and outpatient review. Given that pancreatectomy are not performed in TUH we costed an estimate of a similar surgical procedure (oesophagectomy). In an attempt to cost the processing of cystic fluid and bloods we contact lab managers in TUH, however laboratory estimates of costs were significantly lower than realistic. In place we used the sample cost of blood tests at local GP centres and clinics to account for human factors of phlebotomy and administration staff.

All costs as estimated were then discussed with experts in the field, i.e. MRI costs were discussed with radiologists, endoscopy costs with endoscopists, and surgical costs with surgeons.

The final costings for our assessment is below.

Standard Outpatient appointment	€162 per attendance

Serum Ca19.9	€50 per test
Magnetic resonance cholangiopancreatography	€350 per scan
Computed Topography scan with contrast	€350 per scan
Endoscopic ultrasound with Fine Needle Aspiration	€900 per day case
Cystic Fluid Analysis (Amylase, CEA, Glucose, Cytology)	€120 per case
Pancreatectomy	€50,000 per procedure

Table 2.2.1 Costings for Procedures

2.1.5 Calculation of benefit:

Screening for pancreatic cystic lesion transformation differs from other screening programmes. It does not prospectively recruit patients and patients are only followed when found incidentally. As such benefit was calculated by benefit of finding a positive post operative histology (ie. advanced neoplastic changes including high grade dysplasia or cancer) where a patient under surveillance underwent surgery for a pancreatic cystic lesion.

2.2 Proteomic profiling of Pancreatic Cystic Cohort

2.2.1 Patient Recruitment

Patients were prospectively recruited between July 2019 and June 2022 in Tallaght University Hospital and St James's Hospital. Patient were identified via MDT and direct endoscopy referral. All patients were consented prior to endoscopy. On the day of endoscopy 12mls of blood was taken via venipuncture from the antecubital fossa of the right or left arm. All blood was collected using 2x 6ml vacuum bottles (Greiner VACUETTE® Z Serum Clot Activator) using a standard 22g butterfly needle.

Cystic samples were taken at the time of endoscopy with a 19g, 22g, or 25g needle (Boston Scientific Acquire™, Boston Massachussets). This was performed transgastric or transduodenal at the discretion of the endoscopist and based on cyst location. The cystic sample was collected into a sterile universal container. All samples were divided in two separate containers for clinical lab analysis and for the purpose of this study. No patients were given prophylactic antibiotics prior to cystic or serum collection.

2.2.2 Sample Processing and Storage

All samples were transferred at room temperature to the on campus laboratory within 30minutes of venous or cystic puncture.

Serum and cystic samples were centrifuged at 2500 RPM at 4°c within 90minutes of collection. The serum was divided into 1ml aliquots which were snap frozen in liquid nitrogen prior to storage. Cystic samples were divided into 250µl aliquots and snap frozen in liquid nitrogen. All samples were stored at -80°c.

The sample processing protocol was altered due to concern of the protein concentration between aliquots in larger samples. Following centrifugation of the cystic samples the supernatant was transferred to a fresh aliquot. The protocol was adjusted for shredding of the proteins within the cystic fluid and homogenisation of the aliquots. Post centrifugation the supernatant was transferred to a fresh aliquot. It was then drawn up a 21g – 0.8mm (green) needle into 2ml syringe, repeated x20. This step was then repeated with a 26g 0.45mm (brown insulin) needle into 2ml syringe.

2.2.3 Patient Stratification

Patients were stratified into “high-risk” and “low-risk” groups. This stratification was adapted from the European Evidence based guidelines. Patients were stratified as a high risk category if they fulfilled one of the following criteria:

- Main Duct IPMN
- Solid mass
- MPD >5mm
- Mural nodule >5mm
- Positive cytology for high grade dysplasia or malignancy
- CEA >192

- >40mm diameter
- Clinical jaundice as a result of IPMN

Stratification decision made on assessment of EUS and imaging or biochemical findings.

2.2.4 Identification of Proteomic Preparation

To identify the best means of isolating peptides from cystic fluid two trial runs of three protocols were undertaken. Cystic samples from two high risk patients and two low risk patients were chosen. The protocol was based around single-pot, solid-phase-enhanced sample-preparation technology (SP3). SP3 beads are a para-magnetic bead–based platform which are coated with carboxylate functional groups to capture proteins. The proteins are in turn washed of contaminants, enzymatically digested, and eluted into solution for mass spectrometry analysis. Sample runs comparing the peptide results between 50µg, 100µg of protein using the SDT buffer in addition to a run with 50µg of protein with preceding overnight acetone precipitation of the cystic fluid. There was minimal difference between the methods and as such the use of 50µg of protein from cystic fluid was decided for the isolation of protein.

2.2.5 SP3 Protocol

A cohort of 40 patients were chosen, 15 high risk patients, 15 low risk patients, and 10 pseudocyst fluids for controls. The patients were randomly assorted into two batches to

allow for timing and spacing on the required magnetic rack for SP3 beads. Randomisation was performed using a Wichmann-Hill random number generator.

Prior to the protocol all cystic samples were defrosted at room temperature. Sonication was performed for two 3 second bursts with 5 seconds intervals.

Once the samples had been sonicated the protein concentration was quantified across the samples using a protein BCA assay (ThermoScientific™). This assay was used to quantify the volume of protein within the samples. The requisite volume of sample required for 150µg of proteins was calculated.

The volume of cystic fluid was mixed with a matching volume of SDT lysis buffer and boiled at 100°c for 30 minutes to denature the proteins. Once the proteins had been denatured a urea lysis buffer was added to each Eppendorf to prevent further protein interactions. 20µl of SP3 beads (SpeedBeads, GE healthcare, UK) were added to each aliquot and placed on a rotovator for one hour to allow for thorough mixing of the samples. The supernatant was removed with use of a magnetic rack and beads were resuspended sequentially in ethanol followed by acetonitrile. The beads were subsequently incubated overnight with lyophilized trypsin at 37c.

Following incubation period a further 10µl was added to each aliquot and acetonitrile before placing back on the rotator device. The peptides were eluted from the SP3 beads using MS grade water. Using an assay, the peptide concentration of each sample was calculated to make a final solution of 100ng/µl in 20µl. This was combined with formic acid. The samples were now suitable for mass spectrometry.

2.2.6 Discovery Proteomic Analysis using Data Dependent Acquisition (DDA)

Samples were run on a Thermo Scientific Q Exactive mass spectrometer coupled to a Dionex Ultimate 3000 (RSLCnano) chromatography system. The tryptic peptides were separated on a reversed-phase C18 column packed in-house (8cm x 75µm ID; C 18 , 3.0 µm (ReproSil-Pur 120 Dr Maitsch GmbH.)) and separated at a constant flow rate of 250 nL/min by an increasing acetonitrile gradient. Mobile phases were 0.5% (v/v) acetic acid, 2% (v/v) acetonitrile, 97.5% (v/v) water (phase A) and 0.5% (v/v) acetic acid, 2% (v/v) water, 97.5% (v/v) acetonitrile (phase B). The peptides were separated by a gradient starting from 1% of mobile phase B and increased linearly to 30% for 58 minutes at a flow rate of 250 nL/min. The mass spectrometer was operated in data dependent TopN 12 mode, with the following settings: mass range 320-1600Th; resolution for MS1 scan 70,000; AGC target 3e6; resolution for MS2 scan 17,500; AGC target 5e4.

Data were searched against the Human Reference Proteome (reviewed entries) downloaded from Uniprot.org (21-05-2021), using MaxQuant (version 1.6.17.0). Label Free Quantitation was selected as was the Match between Runs option. The following parameters were selected for the search - Fixed Mod: carbamidomethylation; Variable Mods: methionine oxidation, acetyl (protein N-term); Trypsin/P digest enzyme; Precursor mass tolerances 4.5 ppm; Fragment ion mass tolerances 20 ppm; Peptide FDR 1%; Protein FDR 1%.

2.2.7 Proteomic Analysis

Filtering of the raw MS proteomic data was performed in Perseus (ver 2.7 Max-Planck-Institute of Biochemistry, Germany). IPA software (QIAGEN, USA) was used to perform network analysis of the proteomic data. Pathway analysis transforms the output of high volume proteomic analysis into networks based on the Ingenuity Pathway knowledge base built on existing known literature. It allows for the identification of established and previously identified pathways for disease mechanisms. This crosslinks identified proteins and gene expression which has been shown to be disrupted. The software was used to interpret the differentially expressed data, which included biological processes, canonical pathways, and networks.

2.3 Multicentre review of EUS guided drainages

2.3.1 Identification of patients

Patients were identified retrospectively across three tertiary referral centres in Ireland between 2009 and 2020. Patients were identified using electronic endoscopic records from their specific site. The variables identified were: patient age, gender, aetiology of pancreatitis if available, PFC type, cyst size at both EUS and CT, method of drainage, recurrence, subsequent procedures and any complications arising from same.

Pancreatic pseudocysts and walled off necrosis (WON) were identified in line with the Atlanta classification of 2012.

2.3.2 Procedural technique

All procedures were performed by experienced endoscopists using a linear endoscopic array. Patients were consented prior to the procedure. Prophylactic antibiotics were used at the discretion of the endoscopist. All procedures in this study were performed under conscious sedation.

Endoscopic drainage involves insertion of either double pigtail plastic stents (DPPS) (sometimes multiple DPPS are inserted) or of self-expanding metal stents (SEMS). Lumen-apposing metal stents (LAMS) are a type of SEMS, that has been specifically designed for pancreato-biliary drainage procedures.

The technique for double stent insertion: Under ultrasound guidance a 19G needle or cystotome is passed through the gut wall into the pancreatic collection. A guidewire is passed into the cyst to coil. This is confirmed with fluoroscopy. The tract along the guidewire is dilated using balloon dilation. A DPPS is passed along the guidewire within the tract formed by the needle.

In this cohort drainage was performed using two types of LAMS: the Boston Scientific Hot Axios system™ and the Instrumed Surgical NAGI™. The collection is identified at endoscopy for position, size, and presence of debris or necrosis. The approach for the Hot Axios slightly differs from the NAGI stent. The NAGI requires guidewire introduction with a needle puncture while the Hot Axios is inserted freehand using a proprietary introducer.

Direct endoscopic necrosectomy can be performed after deployment of a lumen apposing stent. Where clinically indicated an endoscope was passed transmurally into the cyst cavity and necrotic tissue was removed under direct visualisation. Concurrent washout and lavage of the cavity was performed at endoscopist's discretion using hydrogen peroxide.

2.3.3 Procedural outcomes

The technical success rates of drainage was defined as the successful insertion of the drain without immediate complication or failure.

Clinical success was defined as resolution of the PFC without need for further radiological or surgical intervention.

Complications were identified as immediate (ie at the time of endoscopy), early (within 7 days) and late (beyond 7 days).

3. Results of 20 Year Experience of Pancreatic Cystic Lesions in a Tertiary Referral Centre

3.1 Patient Cohort

550 patients were identified with PCLs between 2000 and 2020. 243 patients were discussed at MDT regarding management, otherwise PCL surveillance was undertaken by the primary team with guidance from radiology.

300 patients (55%) were female. The mean age at first diagnosis was 69. The PCLs were distributed throughout the pancreas: Uncinate 68 (12.5%), Head 148 (26.81%), Neck 44 (7.97%), Body 127 (23.01%), Tail 124 (22.46%), Unlisted 38 (6.88%).

28 patients had surgical intervention for PCL, this cohort is discussed in more detail below.

The mean cyst size across the whole group at first diagnosis was 14.45mm, Range 1-92mm.

Median length of follow-up was across the cohort was 23.5 months. This ranged from 0 months to 139 months.

We found that patients over 75 had shorter observation period than those under 75 but there was no significance noted in relationship between size or gender on length of follow up for our patients.

Patients	N=550
Female	N= 300
Mean Age (range)	69 years (20-95)
Median Size (IQR)	10mm (14MM)
Size at Diagnosis <10mm 10-29mm >30mm	 238 240 62
Location Uncinate 68 Head Neck Body Tail Unlisted	Location 68 (12.5%) 148 (26.81%) 44 (7.97%) 127 (23.01%)]124 (22.46%) 38 (6.88%)
Worrying Features	
Size Greater than 30mm	62 (11.2%)
MPD dilatation	65 (11.8%)
Nodules	13 (2.4%)

Enhancement	5 (0.9%)
Cyst wall thickening	14 (2.5%)

Table 3.1. Patient Cohort

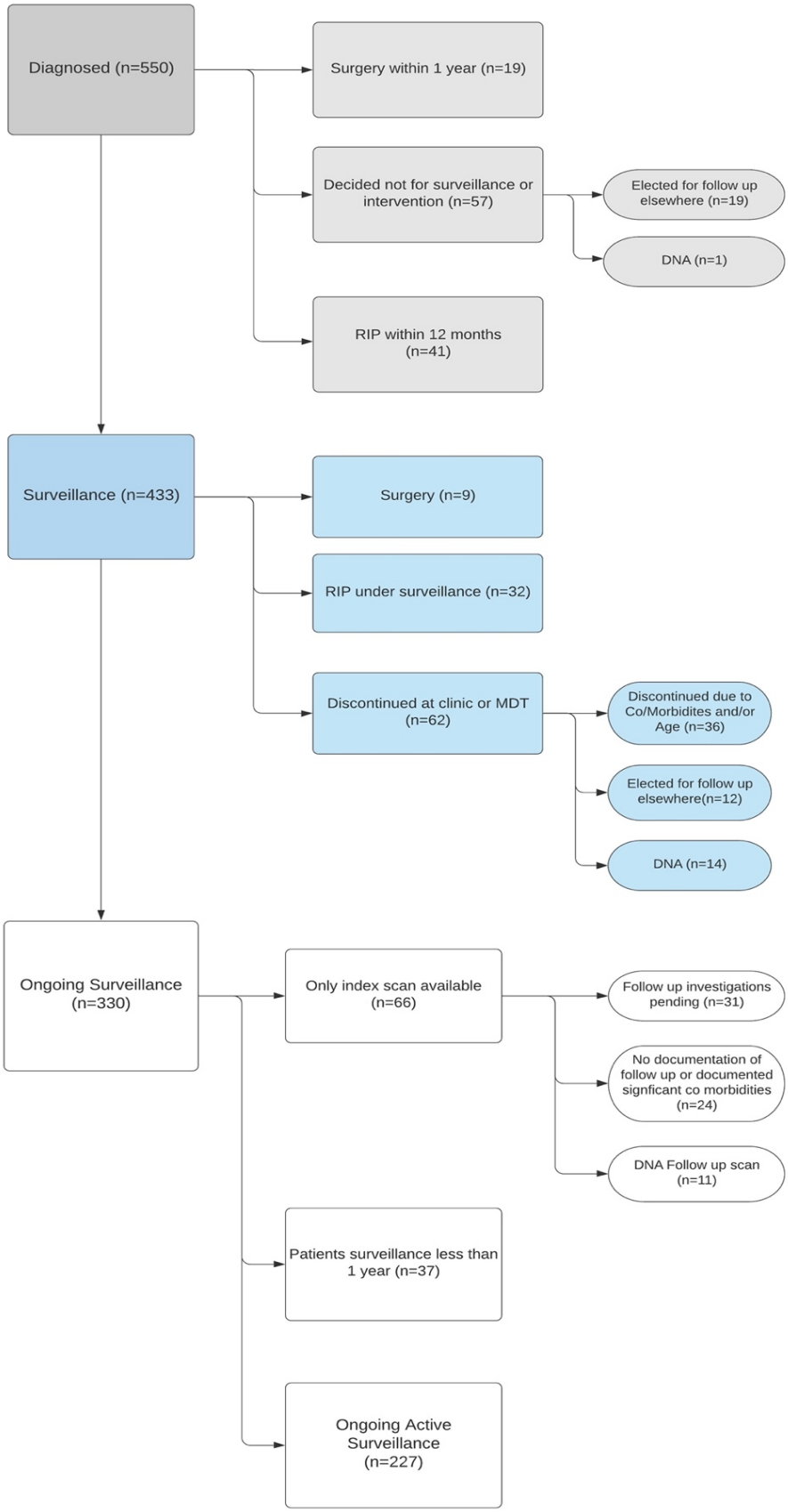

Figure 3.1 Breakdown of patient surveillance

Worrying features were identified in line with the IAP guidelines. 124 of the 550 patients (22.5%) had worrying radiological features at diagnosis. 5 patients had 3 significant features, 20 patients had 2 worrying radiological features. The features identified at imaging were: dilated main pancreatic duct, size greater than 30mm, solid element or nodularity, and cyst wall thickening. There was no overall significant difference in age or length of follow up in the groups with worrying features at diagnosis compared to those without ($p = 0.526$ and 0.359 respectively)

As expected patients PCLs with worrying features were larger than those without significant features, 27.2mm compared with 10.7mm ($p<0.001$). There was no significant difference in age between the groups, 70.1 compared to 69 years ($p=0.392$). In the cohort with worrying features 20 patients proceeded to surgery.

104 patients with worrying features did not proceed to surgery. If we compare these patients as two groups we see the surgical group mean size is 38mm compared with 25mm in the non-surgical, and mean age is 68 compared 71.8 although this is not statistically significant at $p=0.854$ and $p=0.613$ respectively. 59 of these patients remain under active surveillance, 15 patients died due to non-pancreatic cancer related causes, 30 actively discontinued in clinic due to age or co-morbidities.

The median follow up of the group with PCL size >30mm was 12 months compared with 24 months in the smaller cysts ($p=0.001$). 14 of these patients went for surgery (22.5%), 11 were discontinued in clinic due to co morbidity (17.7%), with 6 electing for follow up in other centres(9.7%), 8 died (12.9%) and 23 (37%) remain under active surveillance.

212 of the cohort were >75 at first diagnostic scan. 6 patients over 75 proceeded to surgery (2.8%). Median time to surgery was 2.5 months. In this group of 212 patients there remains 108 still under

active surveillance, 6 patients underwent surgery, 55 patients had their surveillance discontinued due to comorbidities or unsuitability for surgery, 43 died from causes not related to cyst transformation.

In the >75 group, mean size was 15.4mm which was not statistically different to the under 75 group, 13.69mm (p=0.829). Median overall follow up in this group was 18 months, compared with 28 in the under 75 group, this difference was statistically significant (p<0.001).

3.2 Surgical cohort

28 patients progressed to surgery for PCL from our centre. Operative approaches and post-operative histology are listed in table 3.2 below.

The mean age of the surgical cohort was 64.8. Mean cyst size in this group was 33.36mm (+/- 18.72), significantly larger than the whole cohort (p<0.001). 20 patients had worrying features at first diagnosis.

Median time to surgery was 5.5 months from initial diagnosis, range 0 – 57 months of follow up. 26 patients were operated on within two year of diagnosis, with two remaining operations at 27 and 57 months respectively.

Surgical Cohort	N= 28
Female	N=14
Mean age (range)	64.78 (Range 37-83)
Surgical Approach Distal Pancreatectomy	14

Pancreaticoduodenectomy	10
Subtotal Pancreatectomy	2
Total Pancreatectomy	2
Post-Operative Histology	
Within 1 year	
Pancreatic Cancer	2
Acinar Cell tumour	1
Invasive IPMN	1
IPMN with High Grade Dysplasia	3
IPMN with Low Grade Dysplasia	6
MCN	4
SCA	1
Inflammatory mass	1
After 1 year*	
IPMN with High Grade dysplasia	3
IPMN with Low Grade Dysplasia	3
Serous Cyst Adenoma	2
Ductal retention cyst	1

Table 3.2 Surgical Cohort. * After one year references the patients who were brought for surgery after a period of surveillance of at least one year.

Three patients with cancer on histology were operated on at 0, 2 and 7 months respectively.

9 patients were operated on as part of the surveillance programme, i.e. at least a year after diagnosis.

3.3 Cyst Growth

304 patient had two separate imaging points allowing us to assess pancreatic cyst growth and change over the course of surveillance. This excluded patients with early intervention or removal from surveillance.

The mean size of this group at diagnosis was 14.01 mm, SD 11.88 compared to 14.861 in the group deemed not for surveillance (p=0.443). The mean age or the followed group was 67.49 compared with the unfollowed group of 71.7 years, (p=0.362).

We calculated both relative delta and absolute delta but found that relative delta was skewed in smaller cysts compared to larger. The smaller cysts were found to have grossly inflated relative delta changes due to small absolute changes that could be explained by inter user readings between radiologists or scans. Therefore we used absolute change, which we felt was more accurate indication of PCL growth.

Scatter plots of individual cyst growth by size are shown below in figure 3 (a,b,c) by initial size at diagnosis.

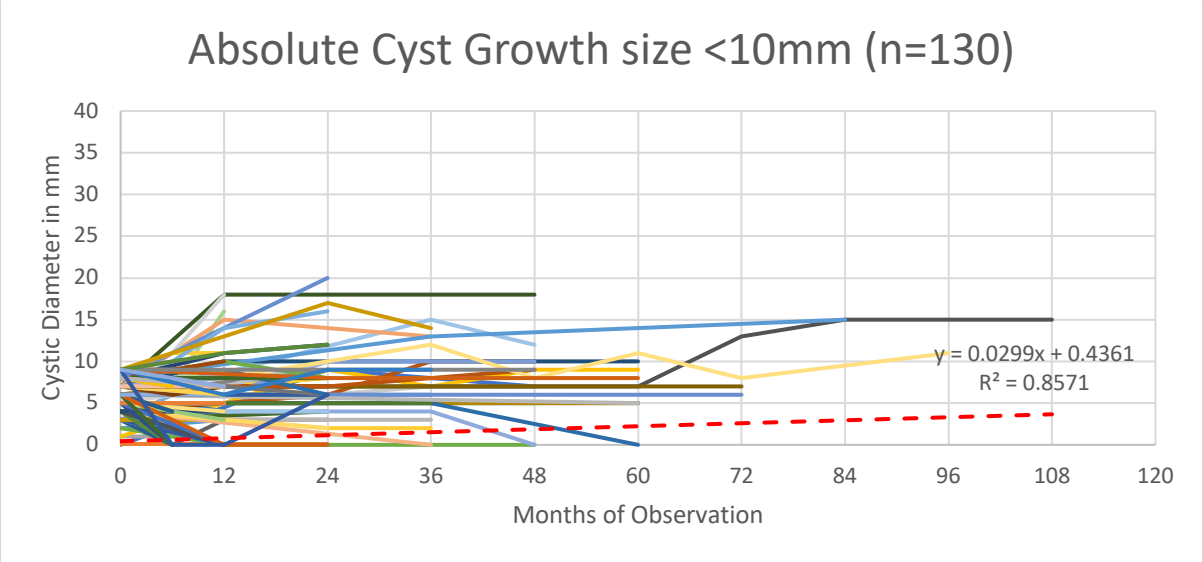

Figure 3.1.2(a)

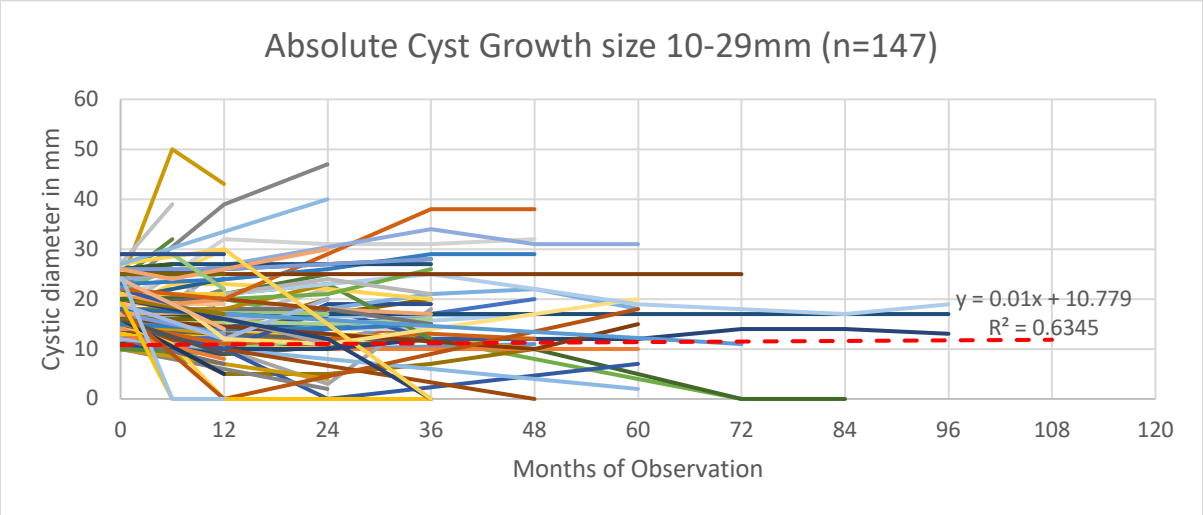

Figure 3.1.2(b)

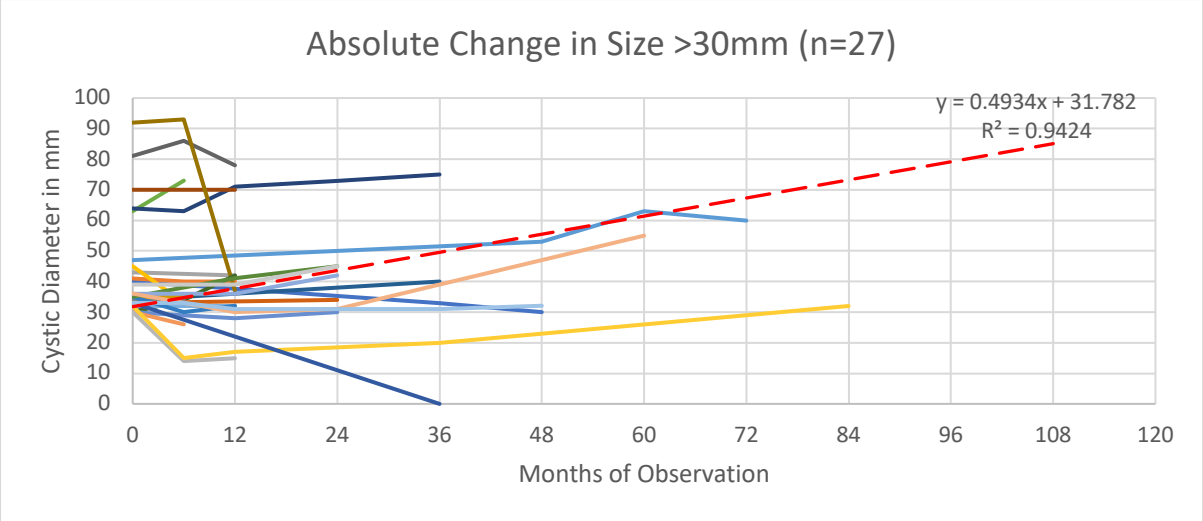

Figure 3.1.2(c)

Assessing the absolute change in size of our PCL over time we found that only initial starting size correlated significantly with absolute change (p<001), however we found the r value in a linear regression model was 0.163 with a standard error of 6.85mm. This model was not strengthened by the addition of age, presence of worrying features, or length of time in the development of a growth prediction model.

3.4 Cost Analysis

With the increasing diagnosis of PCLs and the associated burden on health care systems to survey these lesions, we examined the cost of our centre's PCL surveillance programme. Across the entire cohort we saw a cost of €2,251,564 spent on surveillance and surgery of these patients. This was divided into €593,504 for clinical and radiology surveillance, €258,060 on endoscopic procedures and €1,400,000 in direct surgical costs.

The benefit of surveillance is the detection and prevention of pancreatic cancer. 19 of the surgeries undertaken for PCL were index surgeries, within the first year from detection, i.e not after a period of surveillance.

Only 9 patients progressed from surveillance to surgery beyond the first year of diagnosis. The surveillance programme was deemed to have had benefit if the surgical procedure yielded a positive malignant or high grade histology., ie. a cancer was found at an early stage, or a cancer was potentially prevented. The post-operative histology for these 9 patients were: 6 IPMN, 2 serous cyst adenoma and one ductal retention cyst. As shown in Table 3.2, only 3/9 (33%) of these patients had high grade dysplasia, and there were no cancers. Thus, the other 6 patients essentially underwent unnecessary surgery as a result of the surveillance programme. The cost of surveillance of this total patient cohort beyond the first year was €1,164,440. 3 high grade dysplasia cases were found, which translates into a cost of €388,147 per positive surgical outcome from surveillance. Even if we consider the benefit of a surgery as pancreatic cancer prevention, and include low grade dysplasia as being a possible prevented cancer, 6 patients had potential cancer-preventing surgery and the cost or surveillance per prevented cancer falls to €194,073.

3.5 Discussion

Within our cohort we saw 28 patients progress to surgery. 26 of these patients were operated on within the first two years of PCL discovery. This group was significantly younger and had larger cysts than in the remainder of the cohort. Despite MDT discussion and careful patient selection for surgery, the results of this study clearly highlight the limitations of the current diagnostic approach to pancreatic cystic lesions and current best practice. As shown in Table 3.2, despite implementation of strict surgical selection criteria and MDT discussion, 5 patients with entirely benign lesions underwent surgery; 3 patients with serous cystic

adenomas, 1 with an inflammatory lesion and one with a ductal retention cyst. 19 (67.8%) of our patients underwent surgery within the first year of diagnosis, 16 of these patients (84%) had malignant or premalignant histology. 6 of the 9 (66%) patients who were operated on following a period of surveillance had a pre-malignant histology. None of our patients under surveillance had a pancreatic cyst transform to a malignant or invasive process.

Our outcomes align with other large centres' experience, which have also shown a low risk or progression of PCLs to invasive lesions. A 30 year review of PCL surgery in an American Quaternary referral centre found that 10% of surgeries were performed where lesions were thought to be malignant pre operatively only for post-operative histology to differ[265]. European data on pancreatic lesions is still somewhat limited. A UK cohort from 2000 to 2013 in a tertiary referral centre identified 1090 PCL, including inflammatory cysts which we excluded from our cohort. This group had a similar experience of 570 patients undergoing surveillance following initial diagnosis, of whom only 19 (3%) eventually undergoing surgical intervention and 2 patients showing malignant post-operative histology[265]. Crippa et al, described their experience in an Italian cohort of BD IPMN, finding that those patients at diagnosis with high risk stigmata remain at 10 fold higher risk of IPMN related death but those without are not at high risk of progression of disease[266]. The SHIP cohort identified 1077 PCL in a population based study, only 6% were greater than 1cm and none progressed during the five years of observation[37]. The progression to PDAC of PCL in Japanese cohorts rates are between 17.9% and 41.6%[267,268]. This is higher than that reported in our group and in other European groups, suggesting a possible underlying genetic predisposition in certain populations. Further data is required from more European and Irish cohorts to assess if there are any true population factors contributing to progression.

We attempted to evaluate our PCL surveillance programme in terms of cost per potential cancer prevented and with regard to Quality Adjusted Life Year (QALY). However, calculation of QALY was somewhat limited in this study as there was a zero rate of transformation of pancreatic cysts in this cohort and accordingly, the effect of surveillance and surgical intervention cannot be inferred to have had any benefit on overall health status and longevity.

Instead, we evaluated cost of our PCL surveillance programme, per 'potential cancer prevented' based on the post-operative histology of those progressing to surgery from surveillance. Depending on whether we considered all IPMNs (those with both low grade and high grade dysplasia) as being 'potential cancers prevented', or only those who had high grade dysplasia, we calculated a cost of €193,000 and €388,147 respectively of the surveillance programme per 'potential cancer prevented'. We use the term 'potential' as it is not certain that these patients with dysplasia would have progressed to cancer, although the risk is certainly higher for those with high grade dysplasia.

Willingness-to-Pay (WTP) is the valuation of a health procedure in monetary terms [269]. WTP per QALY is hard to measure, it also varies greatly between countries and health systems, for example NICE guidelines in UK advise for £20,000-30,000 per QALY gained while in the United States this it estimated a willingness to pay of $50-100000 per QALY. In attempts to equate across different systems, GDP has been proposed as a possible means of calculating WTP[270]. In theory each additional year of QALY would be balanced out by a patient's contribution back to the economy following treatment. The rate of private healthcare in a country also has an impact on a WTP threshold[271]. In Ireland the GDP per capita is €72,346[272]. This would equate to a willingness to pay of €72,346 per QALY. The

actual number of life years gained remains hard to quantify in our post-operative patients given the unclear rates of progression of pancreatic cystic lesions to pancreatic cancer.

Aronsson et al compared management strategies using Markov modelling for the management of BD IPMN, finding watchful surveillance the most cost effective models however noting some limitations in their paper in that upfront pancreatectomy could be more efficient in high risk or younger individuals[69].

We found a low rate of progression and growth of PCLs in our study population, particularly in cysts below 30mm. Malignant histology was found only in patients operated on within the 1st year of diagnosis. These patients were index cases as opposed to being picked up through surveillance. Unfortunately we do not have information as to whether or not these patients are symptomatic or are incidental findings. In the 9 patients who underwent surgery as a result of surveillance, only 3 had advanced histology. In addition we found a high cost burden to our centre for the surveillance of these cysts at €193,000 per positive surgical outcome based on histology.

Given the wide range of consensus guidelines in use and the unclear benefits of prolonged surveillance, there is ongoing uncertainty regarding the optimal management of patients with PCL. The current PCL landscape is further confused by the slight differences between the many guidelines available to clinicians[46,111–113]. At present, best practice within guidelines aims to identify those at high risk of progression to cancer. Ultimately, the first challenge is whether a patient's cyst is serous or mucinous. As we are aware, serous cysts, both cystadenomas and pseudocysts do not pose a malignant risk. Mucinous cysts with higher risk features are at risk of transformation. It is the smaller cysts which pose a greater strain for

surveillance and health systems. Within our cohort we did not find any of these cysts progressing to malignancy over the period of surveillance.

This study is the first longitudinal assessment of a population of pancreatic cystic lesions in Ireland. We found that more patients who required surgery did so within a year of diagnosis. None of the population developed pancreatic cancer or suffered from pancreatic related mortality during the period of surveillance. The limitations of the study are that this is a retrospective database of patients. We also may have referral bias as a tertiary referral centre. The strengths of the study are that this is a long period of surveillance with histological outcomes available for all patients undergoing surgery.

4 Results of Discovery Analysis of Novel Proteomic Markers in Pancreatic Cystic Fluid

4.1 Patient Cohort

Between July 2019 and April 2021 a total of 99 patients were prospectively recruited to the study prior to their EUS procedure. All patients had serum drawn prior to the endoscopy procedure. Due to technical factors 51 patients had FNA performed for cyst fluid analysis. Patients were divided into high risk and low risk categories based on clinical features of: main duct IPMN, solid mass, MPD >5mm, mural nodule >5mm, positive cytology for high grade dysplasia or malignancy, CEA >192, >40mm diameter, clinical jaundice as a result of IPMN. To power the analysis appropriately, two groups of 15 high risk and 15 low risk patients were require. 10 patients were pseudocysts were also recruited as control subjects.

Pancreatic cystic aspirates of 42 patients were analysed. These were divided into 15 high risk, 17 low risk and 10 pseudocyst controls. 26 of the group (61.9%) were female, median age was 66 years, ranging from 18 to 81. Patient characteristics are summarised below in table 3.2.1

	High Risk	Low Risk	
N (female)	15 (9)	17 (10)	
Mean Age (range)	72 (39-85)	58 (22-82)	P=0.19
Mean size (SD)	31.33mm (±22.08mm)	23.06mm (±6.4mm)	P=0.58
Mean Amylase	8781.58mm (±17799.8)	4203.3mm (±3698.2)	P=0.225
Mean CEA	9389.4 (±18179.73)	39.4 (±43.9)	P=0.001
Mean Glucose	0.129 (±0.11)	3.07 (±2.45)	P<0.001
Cytological diagnosis	3 (20%)	1	
High Risk Features	CEA (>192) - 9 Solid 2 Size >40mm 5 Cytological 3 Pancreatic ductal dilatation 2		
Family History of Pancreatic Cancer in 1st degree relative	2	0	
Smoking	Active 1 Ex-Smoker 5 Never 9	Active 3 Ex-Smoker 2 Never 12	

History of Alcohol excess	0	2	
History of Pancreatitis	0	2	
Diabetes	1	1	

Table 4.1 High versus Low Risk group demographics

In the pseudocyst group, all patients had a history of pancreatitis. Median age of the cohort was 49. 7 of the group were female (70%). Cyst characteristics and aetiologies are contained in table 3.3.2 below.

N (female)	10 (7)
Median Age (range)	49 years (18-70)
Aetiology of Presentation of pancreatitis	Gallstones 4 Idiopathic 3 Alcohol 1 Pseudocyst index presentation 1 Post Pancreatectomy 1
Mean size (SD)	86mm (±26mm)
Mean Amylase	23941.5 (±42547)
Mean CEA	43.46 (±64)
Mean Glucose	3 (±1)

Table 4.2 Pancreatic Pseudocyst cohort

4.2 Differential Profiling of Pancreatic Cystic Fluid

Discovery analysis of pancreatic cystic fluid from all groups identified 1178 individual labelled proteins with the mass spectrometry profiling. This was filtered for consistency across the group to 126 proteins appearing in at least 70% of all cystic samples. These proteins were then comparatively compared between the groups of high risk, low risk patients and controls (pseudocysts). Figure 3.2.1 shows volcano plots outlining the results.

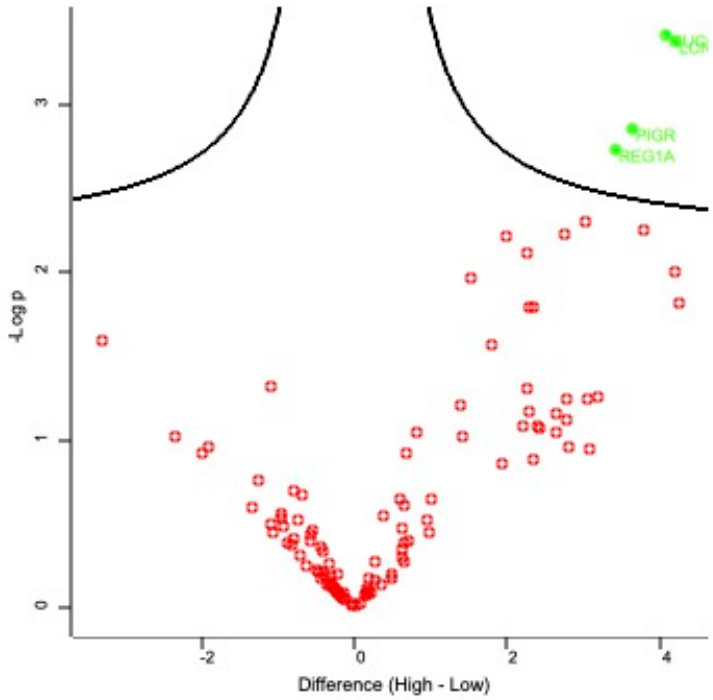

Figure 4.1(a) Volcano plot comparing high versus low risk group. Right side of the chart shows four proteins significantly upregulated highlighted in green text. These proteins were statistically differently expressed in the high risk group compared with the low risk group which did not reach statistical difference between patients.

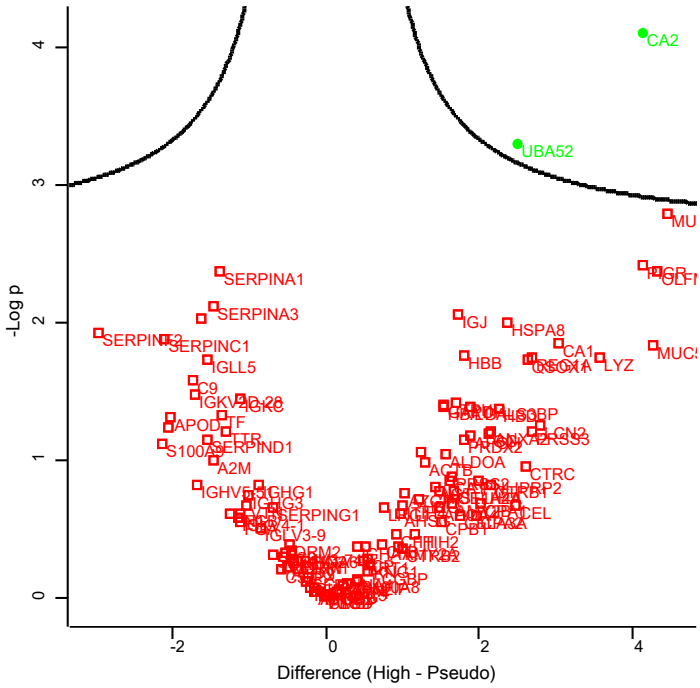

Figure 4.1 (b) Volcano plot comparing high risk versus pseudocyst group. The upregulated proteins identified in differential between the high and low risk group were not identified between high risk and control groups.

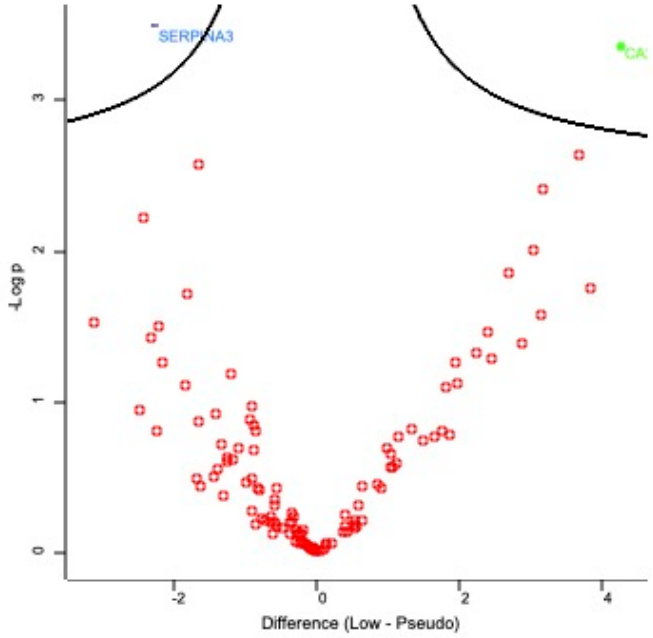

Figure 4.1(c) Volcano plot comparing low risk versus pseudocyst group. We did not find differentially expressed proteins between low risk and pseudo compared to the high risk groups.

4 proteins were significantly upregulated in high risk pancreatic cystic fluid. MUC6, PIGR, REG1a and LCN2 showed significant difference in expression in patients with high risk cysts compared to low risk. CA2 (carbonic Anhydrase 2) was upregulated in both high risk and low risk patients compared to controls but could not distinguish between high and low risk patients.

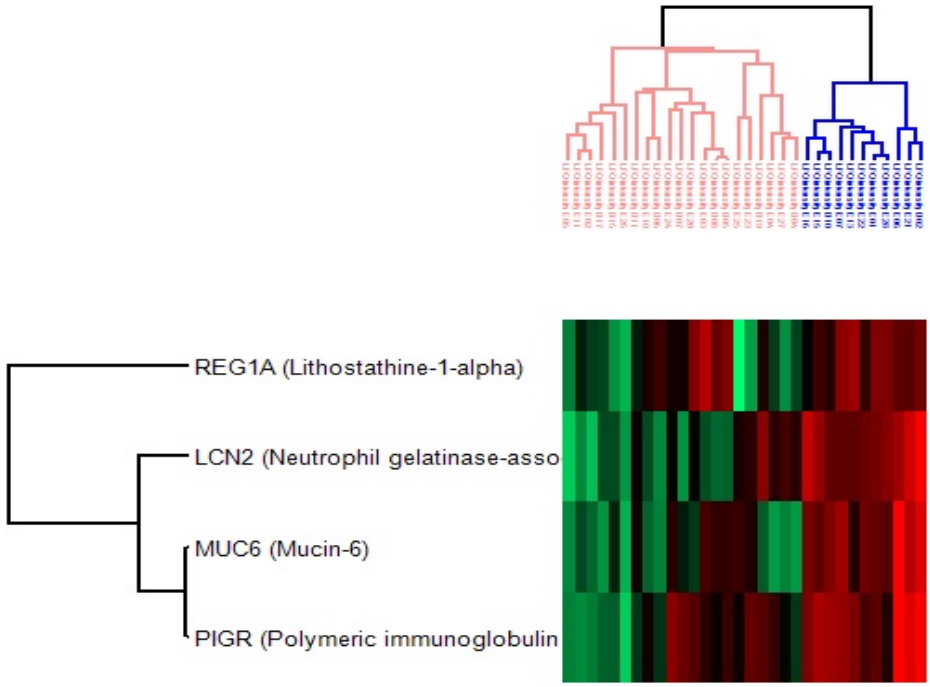

Figure 4.2 heat map of upregulated proteins identified in high risk patients compared with low risk patient cyst. Red reflects upregulated values with the high risk group presented to the right of the graph, showing the upregulated protein groups.

4.3 Ingenuity Pathway Analysis

Using Ingenuity Pathway Analysis (IPA), we examined the relationship between these 1178 highly significant proteins to estimate the most significant canonical pathways and biological networks. Our analysis revealed highly significant overlap canonical pathways differing between our high risk group with the low risk group. The SPINK1 pathway shows the highest affinity with the high risk group with 15 proteins downregulated identified within the dataset.

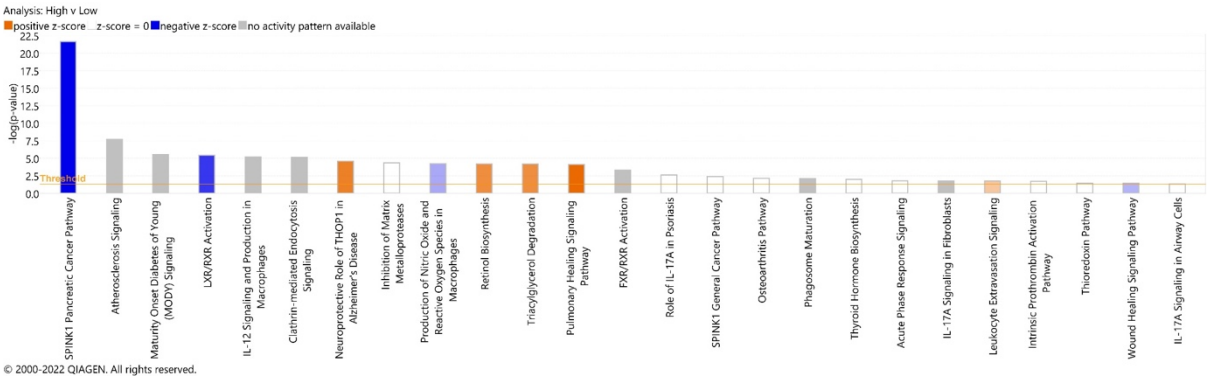

Figure 3.2.3 Canonical pathways of High versus Low Cysts with down regulation of the SPINK1 pathway the most closely associated pathway. Blue indicates that these pathways are inhibited within the dataset, orange indicates upregulation of the pathway. Grey indicates a predicated pathway but not discovered within the dataset.

Within the high risk group in our data set we identify the presence of numerous proteins downregulated in pathways contributing to pancreatic cell injury leading to pancreatitis and ultimately pancreatic cancer.

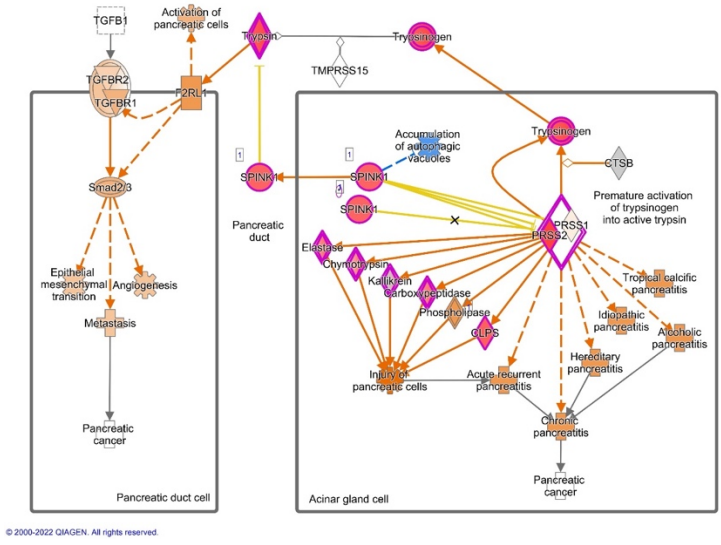

Figure 3.2..4 The SPINK1 pathway within the acinar cell is an enzymatic pathway which is implicated in the disease process of pancreatitis. Purple markers indicate those proteins present within the dataset which are identified by the software.

Comparison of canonical pathways in the high groups, low groups, and the pseudocyst group shows similar clustering of the high risk group compared with the low risk and pseudocyst groups, suggesting that different pathways of activation and development are at play.

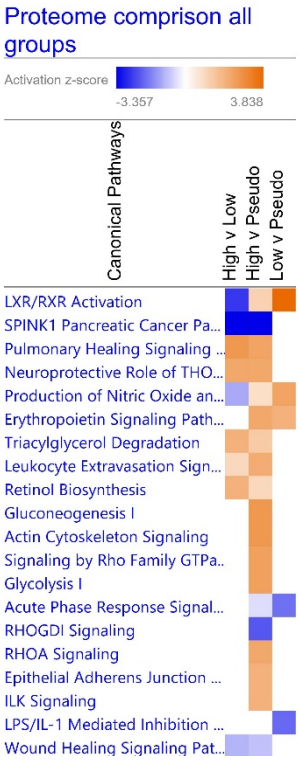

Figure 3.2.5 We can see a similar biological profile evident in the high risk group which differs when the low risk is compared with the pseudocyst control group.

4.4 Discussion

Ultimately if we can improve diagnostic approaches to pancreatic cystic lesions we can reduce the need for surveillance and reduce unnecessary surgeries.

We found upregulated levels of MUC6 in high risk PCL. MUC glycoproteins have been linked to the development of ductal cancers. MUC6 has already been shown to be unregulated in PDAC[273]. Other MUC proteins have also shown to be elevated in high risk IPMN and PDAC. MUC5AC has been found in circulating extravesicular vesicles. This same

group however did not find a similar upregulation of MUC6 in circulating markers[274]. Histological examination of IPMN post resection has found association with oncocytic and pancreaticobiliary subtype of IPMN with MUC6 expression. This differential expression would suggest a pyloropancreatic pathway[275]. The presence of MUC proteins has not been identified in serous cystic aspirates but to date data is lacking.

REG proteins were first described in patients with chronic pancreatitis[276]. They are a group of secreted proteins containing a C-type lectin domain. Their processes include proliferation, differentiation, inflammation, and carcinogenesis of cells of the digestive system[276]. The REG proteins have been implicated in inflammatory conditions of the pancreas, particularly pancreatitis. The exact pathway to this remains unclear. Additionally they play a role in carcinogenesis of the GI system. REG1b has been discovered in the urine of patients with pancreatic adenocarcinoma. In our high risk group we found significantly upregulated levels of REG1a. REG1a has previously been shown to be upregulated in the pancreatic juice of pancreatic cancer patients[277,278]. There are limited proteomic analyses of pancreatic cystic fluid. REG1a has previously been identified as beneficial in distinguishing mucinous and non-mucinous cysts but has not been validated for distinguishing cysts at higher risk of progression.

Lipocalin-2 (LCN-2) is a circulatory protein which plays a role in antibacterial, anti-inflammatory, and protection against cell and tissue stress. LCN2 is increased in the tissue of patients with metabolic syndromes but exact pathway remains unclear[279]. Examination of the pancreatic juice in chronic pancreatitis has shown elevated levels of LCN2. Levels have also been found to be elevated in PDAC biopsies and examination of PDAC tumour microenvironment[280]. Its role in PDAC appears to be in inflammation within tumour tissue. LCN depletion in murine and human models improved survival and slowed tumour invasion.

However, this group was based on PANIN models and not PCL[281]. The identification of LCN2 in pancreatic cysts remains limited.

Polymeric Immunoglobulin Receptor (PIGR) regulates the mucosal immune system within the epithelial cells of mucosal membranes[280].

Current cystic fluid biomarkers are limited. We identified four proteins with previously identified roles in PDAC. The use of proteomic analysis of cystic fluid is limited. At present one other study has shown similar profiles for distinguishing mucinous and non mucinous PCL. However, this study does not try to clinically grade the differing profiles into high and low risk. Another proteomic profiling study attempted to correlate proteins with dysplasia grade in PCL. There was limited crossover in the proteins identified in this study. Further validation studies are needed to identify and confirm these proteomic profiles.

The transformation of pancreatic cystic lesions are complicated biological processes. Patients with pancreatic cystic lesions bear different risks. With network analysis of the proteomic data we can predict pathways of disease processes within the pancreatic cystic fluid. The SPINK1 pathway was the most highly predicted in our samples. The downregulation of SPINK1 is associated with inappropriate trypsin activation in acinar cell injury of chronic pancreatitis[282]. Increased tissue levels of SPINK1 have been demonstrated in pancreatic cancer[283]. The utility of SPINK1 as a circulating biomarker is limited as serum levels have been found to be elevated in both pancreatitis and biliary diseases as well as pancreatic cancer. Its sensitivity is high but it is not specific for malignancy[284]. SPINK1 levels have been previously identified in higher levels in mucinous cystic lesions compared with serous and pseudocyst

lesions[285,286]. The addition of SPINK1 as a biomarker panel may help improve cystic fluid analysis for identification of pre malignant pancreatic cystic lesions.

5

Multicentre Study of Endoscopic Management of Pancreatic Fluid Collections

Endoscopic management of pancreatic fluid collections (PFC) is performed using devices which allow drainage of PFC contents into the bowel lumen. Figure 5.1 below shows an overview of the drainage procedure using both lumen apposing stents (LAMS) and double pigtail plastic stents (DPPS).

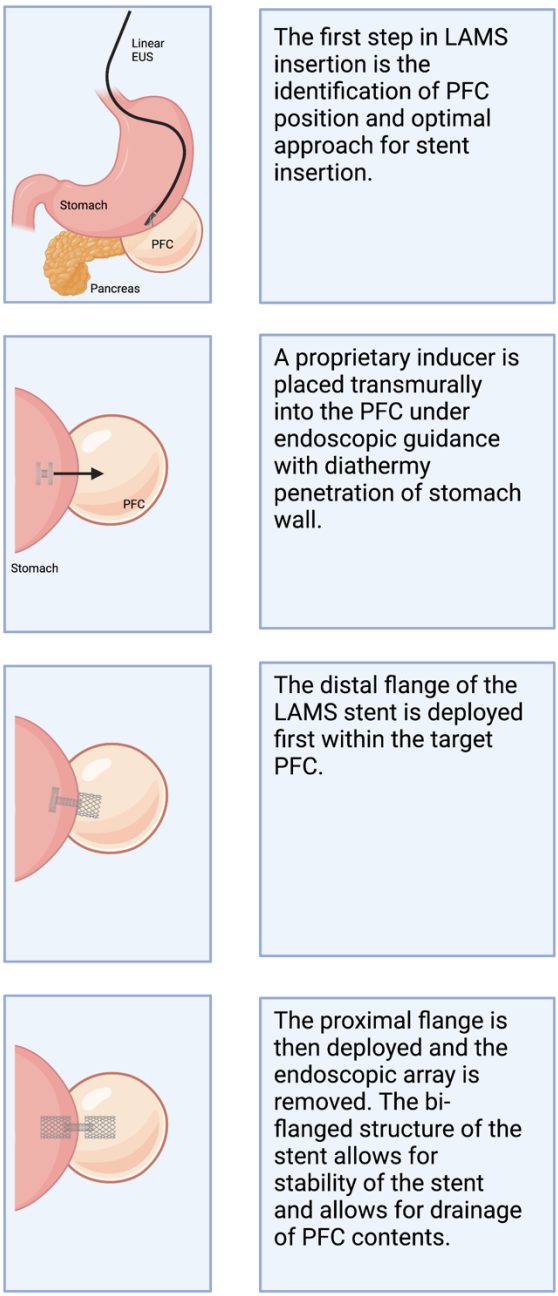

Figure 5.1(a) Technique for transmural deployment of a LAMS stent for drainage of PFC.

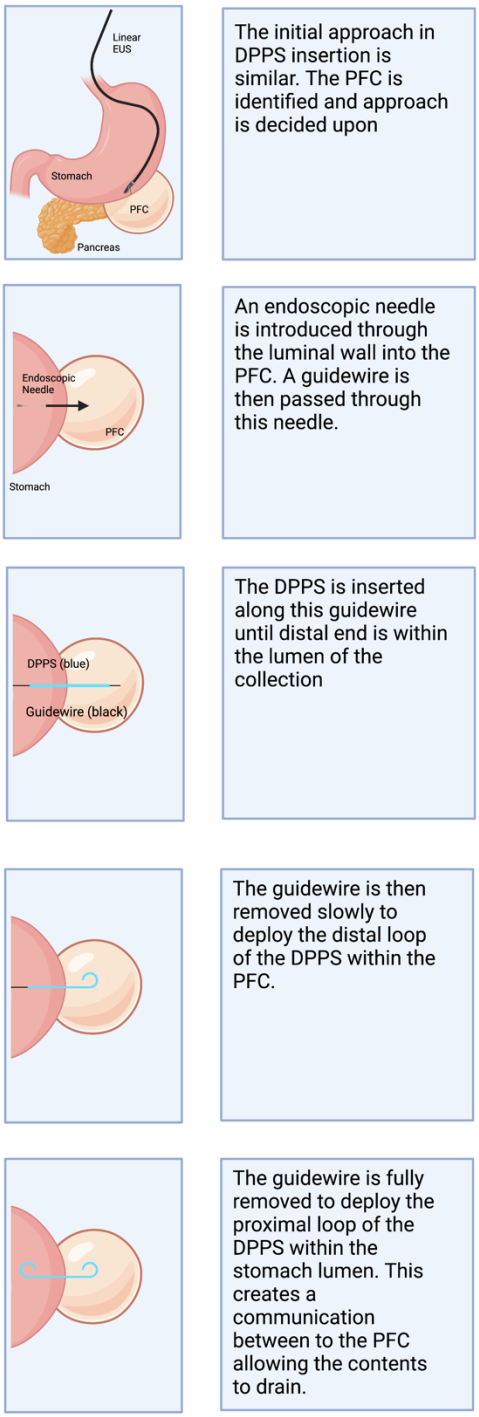

Figure 5.1 (b) Technique for insertion of DPPS stent for PFC drainage.

5.1 Patient Cohort

Across three tertiary referral centres 122 patients (42F) underwent endoscopic ultrasound guided drainages of pancreatic fluid collections (PFCs). This covered a period from 2009 to 2020. The mean age of the group was 53.08 years, range 13-86. We identified no significant differences in PFC size, age, or length of stay between genders or across centres.

N (female)	122 (42)
By Centre	
Mercy University	35
St James's	37
Tallaght University Hospital	50
Mean Age	53.3, range 13-86
Mean cyst size CT	102.23 (SD 46.072)
Mean Cyst size EUS	93.77 (SD 38.397)
Type of Collection	
Pseudocyst	86
Walled off Necrosis	32
Infectious Abscess or Collection	2
GB collection	2
Aetiology of Pancreatitis	
Alcohol	25

Trauma	1
Biliary (Gallstone)	42
Iatrogenic	8
Idiopathic	6
Hereditary	2
Hyperlipidaemia	1
Unknown Aetiology	33
Not related to pancreatitis	4

Table 5.1 Patient Characteristics.

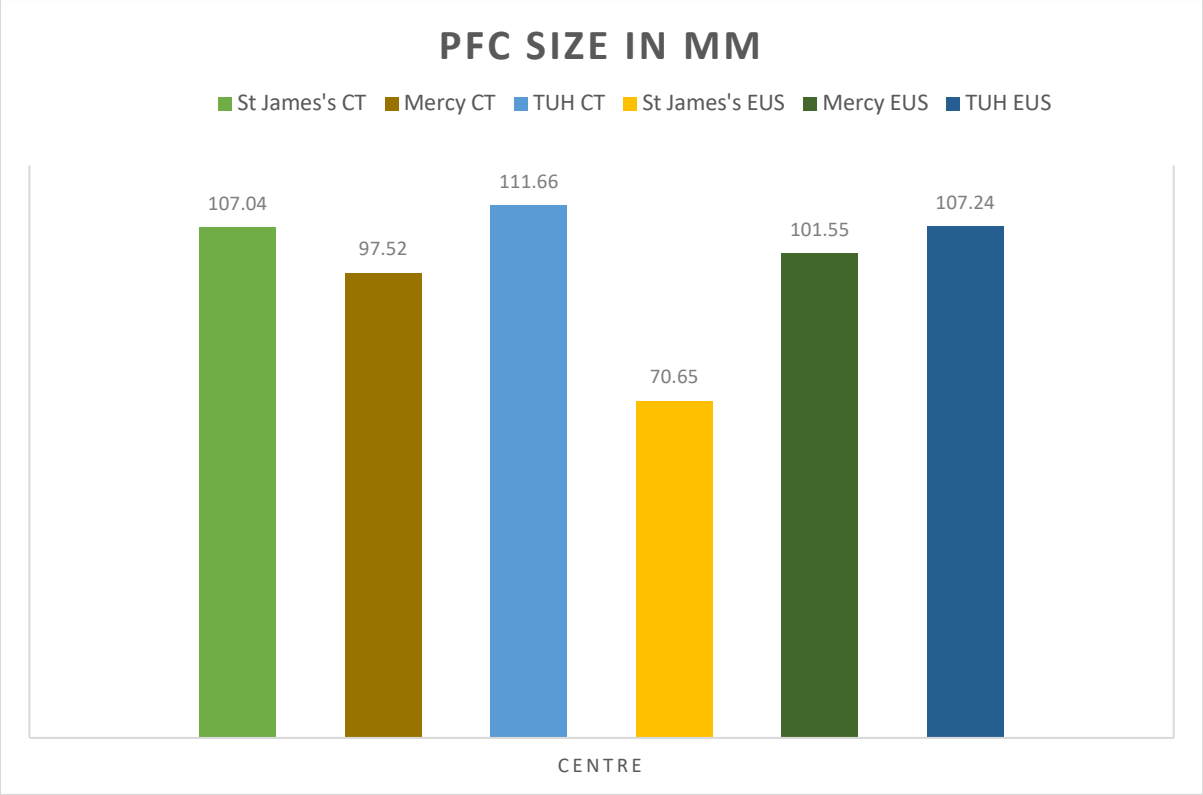

Figure 5.2 PFC size by centre.

Within this group there were 85 drainages performed using lumen apposing metal stents (LAMS) and 37 with double pigtail plastic stents (DPPS). The patient cohort was homogenous between centres with no significant difference in age or PFC sizes between three centres.

5.2 Technical results

LAMS	85
Hot Axios Stents	77

10*10	6
15*10mm	58
Unlisted size	13
NAGI	8
30*26mm	8
DPPS	37
7frx4cm (2)	5
7frx5cm (2)	1
7frx7cm (2)	1
7frx7cm & 7frx4cm	1
7Fr – length unlisted	19
Unstated size	7

Table 5.2 Stent types used in drainages

116 of 122 procedures (95%) were technically successful, 81 of 85 LAMS procedures (95.2%) and 35 of 37 DPPS procedures (94.5%). 74 (87%) of the LAMS stents were inserted freehand, i.e. without needle and wire guidance. All procedures in our cohort were performed under conscious sedation.

Median days to removal of LAMS stents after insertion was 56 days. 20 patients (23.5%) within the LAMS cohort required further procedures for endoscopic necrosectomy prior to removal of the stent.

Clinical success was deemed as the resolution of a collection without need for further percutaneous or surgical procedures. Overall 96 of 122 patients (78.5%) had resolution of their PFC without further intervention. Median time to resolution was 67 days, there was no significant difference between procedures in timing to resolution of LAMS 66.5days compared with DPPS 88 days, p=0.331. However, this timing is imprecise as imaging post drainage is ad hoc and guidelines do not stipulate a timeframe for interval imaging.

5 Patients recurred and underwent subsequent surgical intervention. 1 patient required radiologically guided percutaneous drainage. A recurrence of collection seen in 3 patients overall, 2.5% of population.

5.3 Comparison of the DPPS and LAMS outcomes

The mean age was not significantly different between the LAMS group and the DPP group (p=0.483). PFC size at CT was 102.12mm and 102.47mm respectively but the EUS assessment of sizes differed at 87.68mm in LAMS and 106.88 in the DPP group (p=0.002).

Mean time to PFC resolution was 127.5days, there was no significant difference between procedures in timing to resolution LAMS 123.57 days (SD191.216) compared with DPPS 137.61 (SD 192.058), p=0.331. Of note there was no significant variation between sites in time to resolution (p=0.631).

Mean subsequent procedure numbers were 1.41 in the LAMS group and 0.57 in the DPPS patients, this difference was not significant (p=0.052).

5.3.1 Length of stay

41 of 85 LAMS procedures were performed as day procedures, 13 LAMs required overnight admission. In the DPPS cohort, 7 were performed as day procedures and 10 required overnight admission. LAMs were significantly more likely to be performed as day procedures (p=0.003) but no significant difference between groups requiring and overnight admissions or choice of stent in inpatients.

The median length of stay across the whole cohort was 1 day, (range 0-93 days). The median length of stay for LAMS was 0 and DPPS was 2. This difference was not significant (p=0.612)

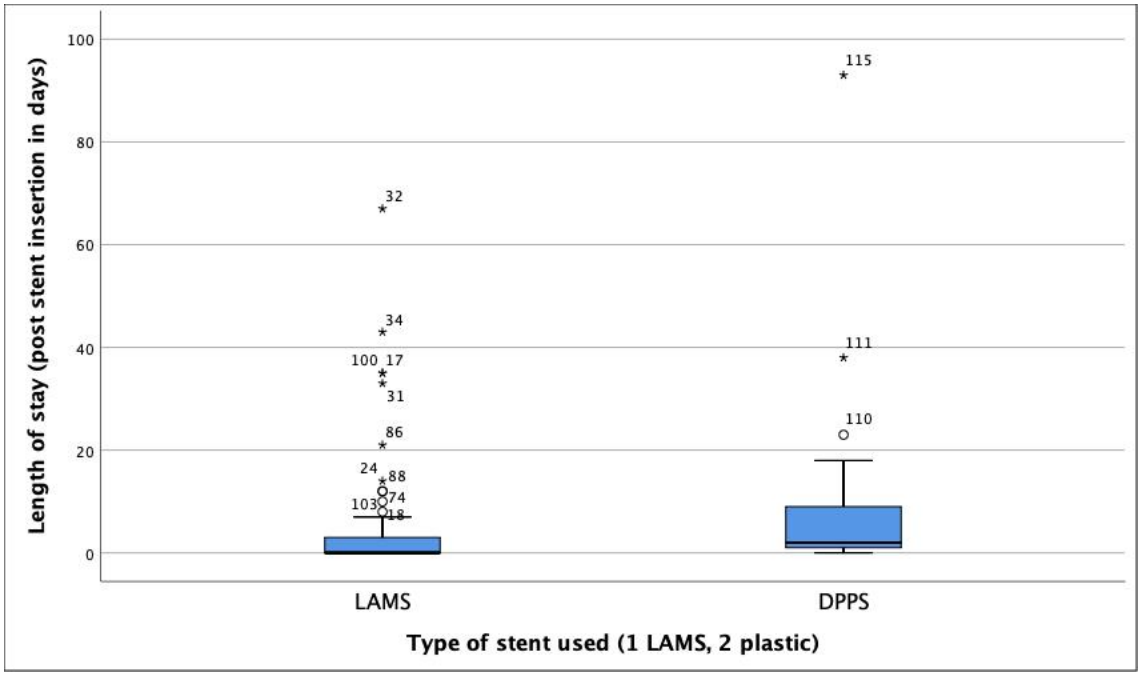

Figure 5.2 Boxplot of length of stay by type of stent.

5.3.2 Complications

Complications were compared between LAMS and DPPS groups. The overall number of complications was 37 (30.3%). These were divided into immediate (at time of endoscopy), early (within 7 days) and late (occurring beyond 7 days). There was no significant difference in complication rates following insertion of LAMS compared to DPPS (p=0.554). The complications are detailed in Table 5.3 below.

There were 10 total immediate complications. The LAMS group saw two perforations, 1 immediate bleed and 1 stent failed to deploy correctly. Immediate complications were significantly (p=0.035) more associated with DPPS compared with LAMS procedures

There was 1 death in the cohort which occurred as a result of a pneumoperitoneum during DPPS drainage.

3 patients in the LAMS cohort had bleeding from cyst within days of insertion of stent, there was no mortality as a result of these bleeds.

Complication	LAMS n=85	DPPS n=37
Overall	24 (28.2%)	13 (35.1%)
Immediate	4 (4.7%)	6 (16.7%)
Perforation	2 (2.4%)	3 (8.1%)
Death (secondary to perforation)	0	1 (2.7%)
Bleeding	1 (1.2%)	1 (2.7%)
Stent failure	1 (1.2%)	0
No drainage from stent	0	1 (2.7%)
Early (<7 days)	16 (18.8%)	4 (10.8%)
Sepsis post drainage	9 (10.5%)	3 (8.1%)
COVID	0	1 (2.7%)
Stent blockage	1 (1.2%)	0
DKA	1 (1.2%)	0
Pain	1 (1.2%)	0
Delayed bleed	3 (3.6%)	0

Perforation at necrosectomy	1 (1.2%)	0
Late (>7 days)	4 (4.7%)	3 (8.1%)
Developed oesophageal stenosis limiting stent removal	1 (1.2%)	0
Infected cystic bed	1 (1.2%)	0
Readmission with pain	1 (1.2%)	0
Buried Stent	1 (1.2%)	0
Stent dislodgement	0	3 (8.1%)

Table 5.3. Complications by stent type and timing

5.4 Pseudocyst & WON cohort

86 of the cysts were pseudocysts, 54 (62.7%) of this group were male. Mean age in this group was 50. Pseudocysts are entirely fluid filled, when assessed at EUS, whereas WONs contain solid debris within the PFC, due to previous necrosis of pancreatic tissue.

52 of these cases were drained using LAMS devices and 34 using DPPS. 81 of 86 (94.1%) were technically successful procedures. 68 of 86 cysts resolved without further intervention. 3 pseudocysts recurred (3.4%). 3 patients required a subsequent surgical drainage and one required drain placement by interventional radiology.

Mean time to resolution was 122 days in the pseudocyst population.

32 WON were drained at endoscopy, 23 males (71.8%). Maximal diameter averaged 118mm at CT and 90.9mm at EUS. Mean estimation of necrotic volume was 30.45% at endoscopy.

30 of these procedures were performed using a LAMS device and 2 using DPPS. All WON procedures were technically successful and 27 (84.3%) were clinically successful. 1 patient proceeded to surgical drainage post endoscopy. Mean time to resolution was 183 days.

There was no significant difference in length of stay, time to resolution or complications between the two groups.

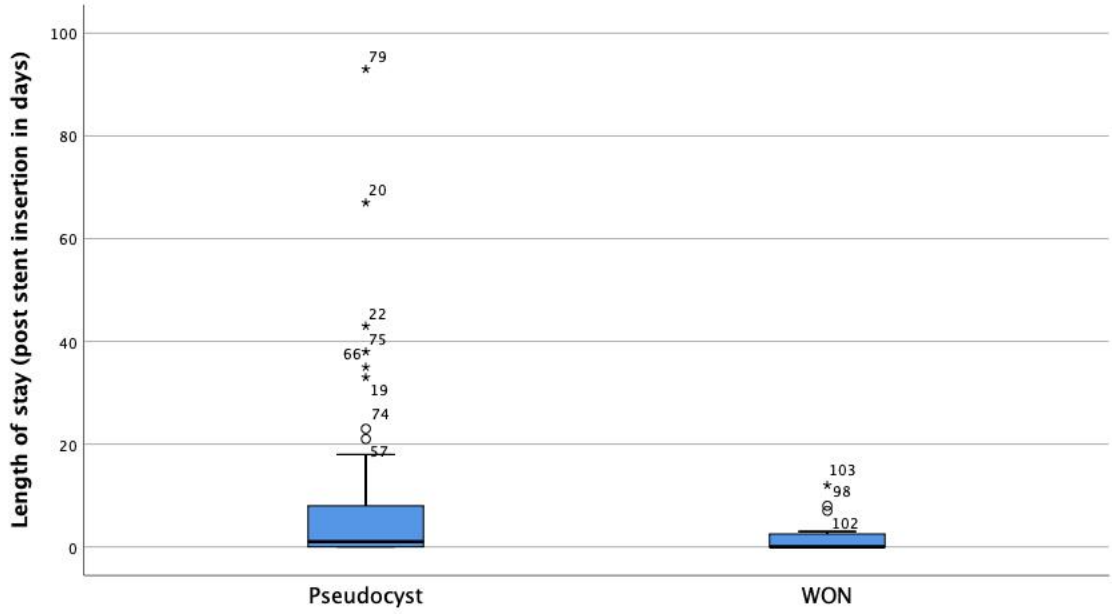

Figure 5.3a. Box plot of median length of stay.

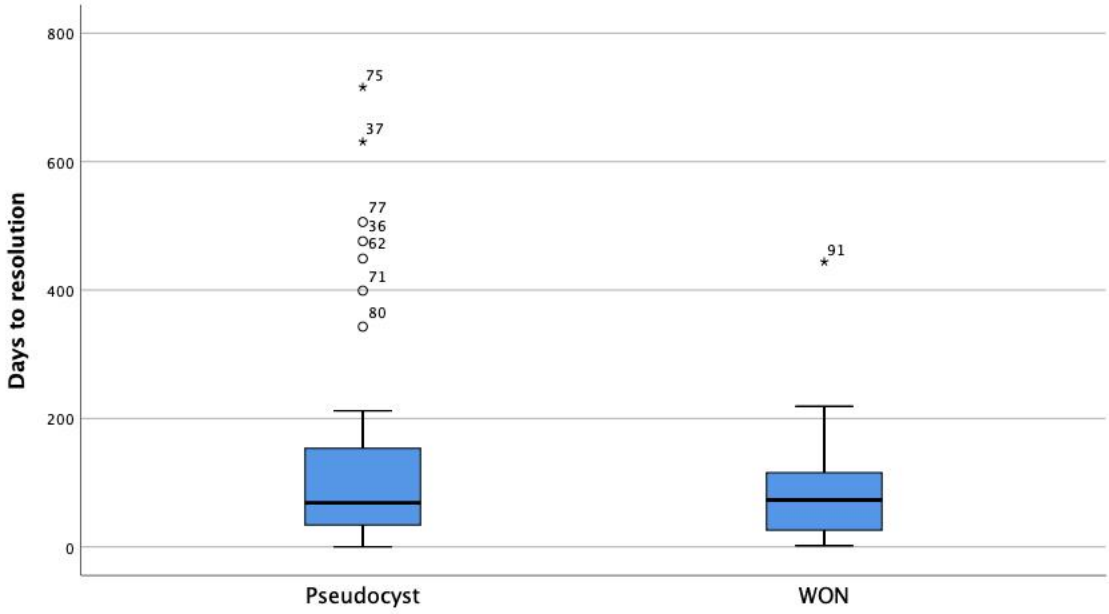

Figure 5.3.b Box plot of median time to resolution by type of collection.

5.5 Discussion

The step up approach to pancreatic collections has shifted clinical care of pancreatitis. LAMS allowed for rapid and effective drainage of PFCs. The added benefit of LAMS over DPPS was they allowed repeat necrosectomy and washout for necrosis or stent blockage. The larger lumen of LAMS devices allows for direct inspection of the PFC cavity with direct endoscopy

on both initial and subsequent procedures. The wider lumen is designed to allow for these procedures, especially were debris within a PFC has been identified radiologically. Early evidence was positive for the use of LAMS with a good safety profile[287,288]. Recently the benefit has been questioned with increasing reports of complications. The most serious of which is catastrophic delayed bleeding[289] and stent migration[290]. Emerging studies on patients undergoing these drainage are conflicting on the best means of intervening and the optimal choice of stent in a given patient[291]. We saw one embedded stent in our group which could still be removed at endoscopy without the need for surgical intervention.

In our cohort we saw one of the benefits of LAMS. 47.6% of our LAMS group could be treated as day cases, freeing up hospital beds and allowing for ease of patient care. We saw one delated bleed in our patient group, fortunately this patient was an inpatient and could be managed without further morbidity.

Life threatening complications occurred in 5 patients from stent placement. 1 LAMS procedure had a large, immediate, haemorrhage and stent failure. Ultimately the patient was stabilised and the procedure abandoned. Another patient developed a procedure related DKA, requiring unexpected hospitalisation. The single procedure related death in our cohort was a MPD leak and pancreaticopleural fistula during insertion of a DPPS. The adverse event rate in our LAMS population was 28.2% compared with 35.1% of our DPPS patients. 4 of these complications occurred as late complications, arising more than a week after endoscopy. None of our late complications caused significant mortality or morbidity. This 28.2% complication rate is in line with numbers reported in a recent meta-analysis' of LAMS procedure in PFC and WON [292,293].

It must be noted that across the 86 patients we only saw one adverse event in the LAMS cohort due to a stent mechanical failure where the stent was unable to pierce the stomach wall to deploy.

We saw a much greater use of LAMS devices in management of WON, 30 cases were managed with LAMS. The larger lumen allows for better drainage of debris in addition to necrosectomy and washout of the WON cavity.

The advent of LAMS has allowed for greater interventional applications beyond that of pseudocysts with evidence emerging for the drainage of gallbladder collections, post-surgical collections and gastric strictures[294]. One EUS guided gallbladder drainage was performed using an AXIOS stent. Our case was unfortunately unsuccessful with the stent dislodging acutely. Although this may raise the question whether the LAMS devices are appropriate for these procedures a recent meta-analysis found favourable outcomes in EUS guided drainage of acute cholecystitis compared with an transpapillary or transcutaneous route[295]. Endoscopic ultrasound guided drainage is still a novel technique not routinely advised in guidelines but benefit in patient comfort can be seen when performed in high volume centres[296]. A recent population study performed in the US showed that the use of percutaneous drainages remains more widespread than endoscopic despite more favourable outcomes in endoscopic patients[297]. This is likely due to endoscopic drainage remaining an emerging field with the need for advanced endoscopic expertise and support, frequently only available in tertiary centres.

Endoscopic management of pancreatic collections is an emerging field. This cohort of patients from the three largest centres in Ireland comprises the bulk of the experience of these procedures in this country. The study is limited by its retrospective nature and unknown degree of severity of pancreatitis in patients undergoing intervention. However, we have demonstrated that technical and clinical success remains high. These results demonstrate that endoscopic management should play a central role in the step up interventional approach to PFCs.

6

Discussion

The incidence of PCLs is rising worldwide. A German prospective population based study of pancreatic cysts found a prevalence of 49%[37]. There have been estimations of prevalence between 2.4% and 2.6% in the United States[298]. In Ireland the prevalence of PCLs remains unknown. The prevalence of pancreatic cysts has been shown to increase with patient age[37]. With an increasingly aging Irish population this surveillance requirement is set to grow rapidly. The population of over 65s in Ireland is 768000 in 2022, this is projected to double to 1.6 million by 2051[299]. This will place a heavy burden of surveillance in future years.

To date there have been no longitudinal studies of PCLs in an Irish population. With confusing guidelines regarding the need for intervention many are referred to tertiary referral centres. Our experience found that the positive yield from surveillance was low. None of our cysts progressed to malignancy during surveillance with a high cost to our hospital systems. Active surveillance and non-surveillance groups were compared in a similar population in two Italian centres. There was no significant difference in the incidence rates of pancreatic cancer and disease related mortality of either group[300]. This would suggest that branch duct IPMN are

stable lesions with low rates of progression to pancreatic cancer. The risk of malignancy in sub 15mm cysts appears to drop over time[301]. Stable lesions with negligible growth rates have demonstrated lower incidence of progression[116]. This indicates that long term surveillance of stable lesions does not provide a benefit to patients.

The gold standard diagnosis of PCL is histological examination post resection. However, this approach would have an unreasonably high cost in patient morbidity and system burden. The diagnostic approach to PCL at present relies on imaging and cystic fluid analysis. The use of proteomic assessment in clinical practice is limited. In our group of high risk patients we found upregulated levels of MUC6, PIGR, REG1a and LCN2. These proteins have been previously identified in pathways of pancreatic cancer and pancreatitis. The altered biological pathways in the development of PCL still remain unclear. Those PCLs with underlying genetic predisposition are at higher risk than those without. In our own cohort of patients, CEA and fluid glucose were significantly different between the high risk and low risk groups. Given the complicated and wide range of pathology which give rise to PCL it is clear that a combination of markers is needed to aid diagnosis as opposed to reliance on single markers for diagnosis and prognostication.

The course of development of pancreatic fluid collections (PFCs), both pseudocysts and WONs is different to that of PCL. PFCs develop exclusively as a complication of acute or chronic pancreatitis. Smaller lesions pose diagnostic challenges, as they can mimic PCLs, particularly in patients with no definite history of pancreatitis. On the other hand, larger pancreatic collections are complex to manage. We demonstrated that PFCs can be safely and effectively managed endoscopically. Our technical success rate in LAMS and DPPS was high at

95% and 94.2% respectively. We also found that many LAMS patients could be managed effectively as outpatients. Complications remain the main concern of LAMS. Overall complications rates were found to be lower in LAMS compared to DPPS in a recent meta-analysis. 20% of LAMS procedures reported adverse events compared to 16% of DPPS[302]. These figures are similar to our complication rates of 28% and 24% respectively.

7

Conclusion

Pancreatic cystic lesions are a challenge for clinicians. The increased use of cross sectional imaging in aging populations has led to an increased burden on our health care system. By examining our practice of surveillance we have found that the benefit of these surveillance programmes are limited. The majority of concerning lesions progress quickly to surgery with good outcomes. It is when we consider the patients under surveillance we question the intervals and length of time they are watched.

Improving diagnostic approaches to PCL will help to reduce the yoke of surveillance. The ability to distinguish mucinous and non-mucinous lesions will allow to discontinue surveillance at the point of diagnosis. We identified candidate proteomic markers which with further study may provide targets to help diagnose and offer a prognosis in terms of risk of malignant transformation of a PCL early in its assessment.

Endoscopic drainages of pancreatic collections in Ireland is safe, effective, and allows for rapid management of collections. We did not find evidence of high rates of delayed

complications recently reported in literature and found that overall outcomes for Irish patients were favourable and in line with international practice.

8

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9.

Appendices

9.1 Participant Information Leaflet

Tallaght University Hospital | Ospidéal Ollscoile Thamhlachta
An Academic Partner of Trinity College Dublin

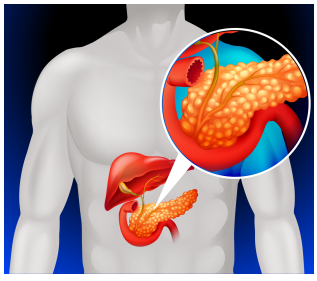
ST JAMES'S HOSPITAL

ST. VINCENT'S UNIVERSITY HOSPITAL
Elm Park

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

PARTICIPANT INFORMATION LEAFLET

Monitoring of patients with Pancreatic Cystic Lesions



Principal Investigator(s) and Co-investigator(s):

- Prof Barbara Ryan, Consultant Gastroenterologist, Tallaght University Hospital (TUH)

- Prof Kevin Conlon, Professor of Surgery, Trinity College Dublin, TUH and St Vincent's University Hospital (SVUH)
- Prof Paul Ridgway, Consultant Surgeon, TUH
- Dr Finbar McCarthy, Consultant Gastroenterologist, St James's Hospital (SJH)
- Prof Dermot O'Toole, Consultant Gastroenterologist, SJH and SVUH
- Dr Stephen Maher, Ussher Assistant Professor, Trinity College, Dublin

Thank you very much for taking the time to read this document. You are being invited to take part in a research study to be carried out at Tallaght University Hospital and St James's Hospital by Prof Barbara Ryan. You are being asked to participate in this study because you have a cyst on your pancreas and are / have been undergoing monitoring and assessment of this cyst. Before you decide whether or not you wish to take part, you should read the information provided in this leaflet carefully. Take time to ask questions – don't feel rushed or under pressure to make a quick decision. You should understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. You may wish to discuss it with your family, friends or GP.

PART 1 – THE STUDY

Why is this study being done?

You have been diagnosed with a cyst on your pancreas, as so-called Pancreatic Cystic Lesion (PCL). Most PCLs are entirely harmless and will never cause you any problems (the cyst may have been discovered entirely incidentally when you had a scan done for another reason).
A small proportion of PCLs have a small risk of developing into a cancer over many years, ie. are pre-cancerous, and as a result we tend to monitor patients who have PCLs to pick up any changes that might indicate that they are at risk of undergoing change.
At the moment we rely on doing MRIs and Endoscopic ultrasound (EUS) of the pancreas to monitor things. You will likely have had one or other of these tests done in the past and may be scheduled for further tests again in the future.

The aim and purpose of this study is to review all (or as many as possible) of the patients attending our hospitals (Tallaght University Hospital, St James's Hospital and also St Vincent's University Hospital) who have been attending for monitoring of their pancreatic cyst over the past 15 years, so that we can determine the outcomes of our surveillance. We would like to see how many patients are still undergoing simple monitoring and how many patients have required surgery etc. We are trying to find better ways of monitoring and assessing these pancreatic cysts.

Why am I being asked to take part?

As you know, you have a cyst on your pancreas, a so-called Pancreatic Cystic Lesion (PCL). We are trying to find better and non-invasive ways of monitoring these cysts that might in the future avoid the need for you to have to undergo repeat endoscopies and scans.

What will happen to me if I agree to take part?

During your routine endoscopy (no extra visits);

1. You will be given this information leaflet to read, have time to discuss any questions with the research team, sign a consent form if happy to do so.
2. Donate:
    - Blood sample (2 tablespoons).
    - Cystic fluid (less than half a teaspoon of fluid usually) taken at endoscopy while under concious sedation. This is a routine part of the clinical assessment of pancreatic cysts.
4. Give us permission to access your healthcare records for research.
5. Samples and data will be used now and into the future to help better understand pancreas diseases

Do I have to take part? What happens if I say no? Can I withdraw?

You do not have to take part in this study. If you decide not to take part it won't affect your current or future medical care. You can change your mind about taking part in the study and opt out at any time even if the study has started. If you decide to opt out, it won't affect your current or future medical care. You don't have to give a reason for not taking part or

for opting out. If you wish to opt out, please contact Prof Barbara Ryan, TUH who will be able to organise this for you.

What happens if I choose to withdraw from the study

To withdraw from the study you can contact any of the principle or co investigators. If you choose to withdraw from the study you will be asked to sign a letter confirming your desire to leave the study. You may be asked your reason for leaving the study for documentation purposes. You will not be contacted for further information by the investigators of this study beyond this time. You may choose to have your data removed from the study if you wish.

How will the study be carried out?

If you agree to participate in the study, we will simply review your medical notes, the results of scans, any endoscopic procedures and blood tests that you may have had since your pancreatic cyst was diagnosed. We will also take note of other factors in your medical history such as your age, gender and things such as smoking. This is all part of your routine clinical care.

As part of the study, we will also ask you some simple questions about yourself, such as age, smoking history etc. We will also access your hospital medical notes to see what other tests (CT or MRI scans) that you have had done to date.

We are approaching all our patients who have cysts on their pancreas to participate in this study.

Are there any benefits to me or others if I take part in the study?

There is no direct benefit to you personally as a result of participating in this study. We do hope that this study will improve our knowledge of pancreatic cysts and possibly lead to improved tests in the future. But this will not benefit you directly at this time.

Are there any risks to me or others if I take part in the study?

There is absolutely no risk to you by participating in this study. Any personal data we document as part of this study will be used for the sole purpose of this study and will be kept on a secure computer and will be fully compliant with GDPR.

Will I be told the outcome of the study? Will I be told the results of any tests or investigations performed as part of this study that relate to me?

Participants in this study will not receive individual results. Unfortunately, it is not practical to notify each person of their individual research result. Research results will not necessarily be approved for clinical use. More studies are needed to validate research finds before we can use them in the clinic.

We might look at your DNA (genetic research) to identify genes which may be involved in how a disease behaves. If this finding might affect your care, your consultant will be informed immediately.

The results of our studies will also be reported in medical/scientific journals and at medical/scientific conferences. Please speak to your study coordinator if you want to know more about study results.

PART 2 – Your DATA PROTECTION

What information about me (personal data) will be used as part of this study? Will my medical records be accessed?

In joining this study, you give the research team permission to access your medical records. We will store some basic information about you including: Your age, sex, smoking and alcohol history. We will also access information regarding the results of your previous scans and the results of the standard biochemical analysis that will be carried out on the cyst fluid. To do this we will need to look through you medical chart.

When storing your data in a secure file, on a secure computer, your details will be anonymised so that you would not be easily identified from the information present in the file.

What will happen my personal data?

We collect your clinical data and use it in two ways;

Identifiable: We need to be able to identify you to follow your care in the hospital. This identifiable information is not shared with those outside of the research team.

Coded: We give every participant a special number or 'code' which hides their identity to anyone outside the research group. We share information that does not identify you with other scientists worldwide to improve and advance our research study. They will only receive this special number or code and not your name.

Data will be stored indefinitely as this type of research takes many years and many thousands of participants to complete. The law on data protection means we have to protect your data to a very high standard (GDPR, 2018). We never share you data with insurance or marketing companies.

Your samples and data may be shared with other research groups worldwide, both within the EU, and outside the EU. Sharing samples and data offers the best attempt at finding meaningful results and sharing them with other patients. All data collected on EU citizens must be kept secure and we will ensure only CODED data is ever shared outside the St. James's Hospital research team.

Will my personal data be kept confidential? How will my data be kept safe?

You will be given an unique study number for this study and all your details will be stored under this unique number. The key to the unique numbers (ie, the link back to you) will be stored in a secure file and only the principal investigators will have access to this.

Your privacy is important to us. We take many steps to make sure that we protect your identity during the study and keep your data safe.

- We store all paperwork in locked cabinets, in locked offices with restricted access.
- We password protect all electronic files with identifiable information on St. James's Hospital computers with restricted access and central nightly backup.

- We store all samples labelled with codes, not names, in locked freezers at St. James's Hospital.
- We make every research team member do data protection training.
- We check data security policies with any other scientists outside our research team before we share samples and data.
- We did a Data Protection Impact Assessment to minimise any potential data breach risks.
- Data security procedures are regularly reviewed by data protection officers.

An assessment of the **data protection implications** of the health research and /or a data protection impact assessment was carried out and the chance of any breach of confidentiality is felt to be very low.

Any **presentation or publication** in relation to the study in the future shall /could not identify you in any way.

What is the lawful basis to use my personal data? What are my rights?

After you have given us explicit permission to do so, we may use your personal data.

The lawful basis for data processing under The General Data Protection Regulation 2018 is Article 6: 6 (1)(e) Public Interest and Article 9: 9 (2) (j) Scientific Research Purpose

Under data protection law (GDPR,2018) you have the following rights as a data subject;

- Right of access
- Right to rectification
- Right to erasure
- Right to restrict processing
- Right to data portability
- Right to object

At any time you can withdraw your consent to participate in the study. If you decide to do this we will erase all information regarding you from the study database. You have the right

to access this data at any time in an accessible format. You have the right to have any errors in your data rectified.

For the duration of the study, the data will be held securely, in pseudoanonymised form, as explained above and will not be shared with any other parties. The data will not be used to profile the participants in any way. The data will not be used for any marketable purposes.

You have the right to object to processing of your data at any time.

Data Protection Officer Contact Details:

- Siobhan Lingwood, Data protection officer, Tallaght University Hospital, Siobhan.Lingwood@tuh.ie
    - Data Protection policy https://www.tuh.ie/About-us/Statement-of-Information-Practice.pdf
- Data protection officer, St James's Hospital, James's Street, D.8 dataprotection@stjames.ie
    - SJH Privacy policy: http://www.stjames.ie/InformationGovernance/PrivacyPolicyFull/
- Data protection officer, secretary's office, Trinity College Dublin, D.2 dataprotection@tcd.ie
    - TCD privacy policy: https://www.tcd.ie/info_compliance/data-protection/policy/

PART 3 – COSTS, FUNDING & APPROVAL

Will it cost me anything if I agree to take part?

There will not be any expense incurred by you for participating in this study.

Who is funding this study? Will the results of the study be used for commercial purposes?

This study is being funded by a research grant awarded by the not-for-profit Meath Foundation. This is a charity based in Tallaght University Hospital that supports research. The investigators are not being paid for running this study. Two junior researchers are undertaking this research with a view to completing a higher degree (a PhD or a MD) and are being paid a salary for the duration of the study.

Has this study been approved by a research ethics committee?

This study has been approved by the Tallaght University Hospital / St James's Hospital Joint Research Ethics Committee on 29/05/2019. In addition to your permission, we must also get ethical approval for any new projects, before we let scientists outside of our research team use your samples and data.

Contact: TUH/SJH REC officer: Dr. Sadhbh O'Neill

Email: ResearchEthics@tuh.ie / Sadhbh.ONeill@tuh.ie Phone: 01-414 2199

The principal investigators of this study work in Tallaght University, St James's and St Vincent's University Hospitals, but do not have any personal links with any members of the Joint Research Ethics Committee.

PART 4 – FUTURE RESEARCH

Will my personal data and/or biological material be used in future studies?

You have only given permission for your data to be used for the current study but we are seeking permission to store the data for use in further investigation of pancreatic cysts for a period of up to 5 years. This includes the use of any endoscopic data, radiology images, medical notes, and biological data obtained in the current study.

If this is the case, the research would be overseen by the same principal investigators as for the current study and would not be shared with any other people or agencies. If the researchers wished to retain the data for a period longer than 5 years, or to perform any additional research, then they would seek additional approval from the Ethics Committee. If no further approval is sought for further studies all data collected for the purposes of this study will also be permanently deleted at this time.

PART 5 – FURTHER INFORMATION

Where can I get further information?

- Principal Investigator(s): Prof Barbara Ryan, Dept of Gastroenterology, TUH (01 414 2000) and Prof Kevin Conlon, TUH (01 414 2000) and Dr Stephen Maher, TTMI, TCD (01 896 3268).
- Data Controllers: Prof Barbara Ryan, Prof Kevin Conlon, TUH and SVUH, Dr Stephen Maher Trinity College Dublin and Dr Finbar McCarthy, St James's Hospital (01 410 3000), Prof Paul Ridgway, TUH (01 414 2000), Prof Dermot O'Toole, St James's Hospital (01 410 3000). Ms Siobhan Lingwood, Data Protection Officer (dpo@tuh.ie).
- MD Student. Dr Gregory Mellotte, Research Fellow, TUH (Gregory.Mellotte02@TUH.ie)

What happens if I wish to make a complaint?

If you wish to make a complaint you can contact Prof Barbara Ryan or her Research Fellow, Dr Gregory Mellotte, at Tallaght University Hospital, 4142000

If you wish to complain about how we processed you data, please contact the Office of the Data Protection Commission on +353 578 684 800 or +353 761 104 800.

Data Protection Commission, 21 Fitzwilliam Square South, Dublin 2, D02 RD28

Will I be contacted again?

Once you have consented to participate in the study, we would not envisage the need to contact you again.

9.2 Consent form

Tallaght University Hospital / Ospidéal Ollscoile Thamhlachta
An Academic Partner of Trinity College Dublin

ST JAMES'S HOSPITAL

ST. VINCENT'S UNIVERSITY HOSPITAL
Elm Park

Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Consent Form

Monitoring of patients with Pancreatic Cystic Lesions

To be completed by the **PARTICIPANT**:

I have read and understood the information leaflet.	YES ☐	NO ☐
I have had the opportunity to discuss the study, ask questions about the study and I have received satisfactory answers to all my questions.	YES ☐	NO ☐
I have received enough information about this study.	YES ☐	NO ☐
I understand that I am free to withdraw from the study at any time without giving a reason and this will not affect my future medical care.	YES ☐	NO ☐
I agree to allow the researchers use my information (personal data) as part of this study as outlined in the information leaflet.	YES ☐	NO ☐

I agree to allow the researchers access my medical records as part of this study	YES □	NO □
I agree to be contacted by researchers as part of this study	YES □	NO □
I consent to take part in this research study having been fully informed of the risks, benefits and purpose of the study	YES □	NO □
I give my explicit consent to have my data processed as part of this research study	YES □	NO □

Participant's Name (Block Capitals):	
Participant's Signature:	
Date:	

To be completed by the **RESEARCHER**:

I have fully explained the purpose and nature (including benefits and risks) of this study to the participant in a way that he/she could understand. I have invited him/her to ask questions on any aspect of the study.	YES □	NO □
I confirm that I have given a copy of the information leaflet and consent form to the participant.	YES □	NO □

Researcher's Name (Block Capitals):	
Researcher's Title & Qualifications:	
Researcher's Signature:	
Date:	