The development of novel diagnostic markers and treatments for cystic lesions of the pancreas

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I, Margaret Geraldine Keane, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Pancreatic cysts are an increasingly common clinical finding, present in 13-49% of patients undergoing magnetic resonance imaging for non-pancreatic reasons. They have a wide differential diagnosis, which includes a small proportion that will ultimately progress to invasive cancer.

Pancreatic ductal adenocarcinoma is the third leading cause of cancer death in Europe. In the UK approximately 10,000 people are diagnosed with the disease annually. In most cases curative surgical resection is not possible, and this is largely attributed to late diagnosis. Approximately 15% of pancreatic cancers arise from precancerous pancreatic cysts (Intraductal Papillary Mucinous Neoplasms or Mucinous Cystic Neoplasms), offering a unique opportunity for early detection and curative intervention, in a disease with a dismal prognosis and five-year survival of less than 7%.

The natural history of pancreatic cystic lesions (PCL) remains poorly understood. Chapter 2 summarizes the surveillance and surgical outcomes from a large UK cohort. Growing numbers of patients are being followed annually. Chapter 3 demonstrates through a questionnaire-based study, that surveillance for PCL with low malignant potential is anxiety provoking and worrisome for patients. In patients referred for surgical resection, only a third are found to have invasive cancer. Better diagnostic tests are therefore needed to more accurately diagnose invasive cancer preoperatively. Chapter 4 provides a systematic review of biomarkers for pancreatic cancer. Chapter 5 evaluates novel cell cycle biomarkers in cyst fluid, for the identification of high-risk lesions. Chapter 6 summarises the results of a phase II study of the safety and utility of endoscopic ultrasound guided needle based confocal endomicroscopy (EUS nCLE) for detection of high-risk PCL. Although improved sensitivity and diagnostic accuracy was demonstrated, this was not significantly better than cyst fluid cytology, which is the current standard of care. Chapter 7 explores if fluoroscopic labeled biomarkers could differentiate high risk PCL and discusses if ultimately these biomarkers could be used to improve the EUS nCLE technique.

Overtreatment remains a concern in patients with PCL, sent for surgical resection.

Chapter 2 highlights less than a third of patients are ultimately diagnosed with invasive cancer. Pancreatic surgery, even when performed in high volume centres, is associated with significant morbidity (up to 40%) and mortality (0-4%). Minimally invasive ablative techniques are an attractive alternative to surveillance in low-risk lesions and for high-risk lesions in those unfit for surgery or who refuse surgery. Chapter 9 summarises the results from a phase II study of the safety and utility of endoscopic ultrasound guided radiofrequency ablation in the treatment of premalignant PCL.

Impact statement

Pancreatic ductal adenocarcinoma (PDAC) is a disease with a dismal prognosis.(Rahib et al., 2014) Despite substantial progress in other gastrointestinal malignancies,(Allemani et al., 2018) 5-year survival in PDAC remains low at 3-15%.(Bray et al., 2018, Arnold et al., 2019) Poor survival figures are largely attributed to late diagnosis.(Kamisawa et al., 2016) The need for earlier diagnosis is recognised globally (Canto et al., 2013) and is advocated by several healthcare organisation's, (WHO) as patients diagnosed with early stage disease have a much improved survival.(Poruk et al., 2013a)

There are number of challenges associated with diagnosing PDAC earlier; namely pancreatic cancer being a relatively rare disease and current diagnostic tests being imperfect, which makes screening of the general population impossible.(Hart and Chari, 2019) Diagnostic tests can lead to inadvertent false positive results, which in the setting of PDAC, would lead to a patient being referred for surgical resection, which is associated with a significant morbidity and mortality.

However screening of high-risk individuals is associated with better detection rates.(Poruk et al., 2013a) Targeted screening is therefore advocated in high-risk groups.(Kimura et al., 2012, Del Chiaro et al., 2013, Vege et al., 2015b) One such group, are patients with a pancreatic cystic lesion (PCL). Although a pancreatic cyst has a broad differential diagnosis, it does include two of the three precursors of PDAC, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs). However accurately diffentiating these lesions from other PCL is a recognised challenge.(Valsangkar et al., 2012, Sahora et al., 2013) Being able to reliably detect high risk PCL would provide an opportunity for early curative intervention in a disease with a dismal prognosis.

In addition being able to offer minimally invasive treatment options as alternatives to surgical resection would also reduce the potential morbidity associated with the treatment of PCL and pancreatic screening programmes. This project therefore aims to explore novel diagnostic and therapeutic strategies for the management of PCL.

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List of abbreviations

Abbreviation	Explanation
BD IPMN	Branch Duct Intraductal papillary mucinous neoplasms
BTC	Biliary Tract Cancer
CA19-9	Carbohydrate antigen 19-9
CDST	Cancer decision support tools
CEA	Carcinoembryonic antigen
CLE	Confocal Laser Endomicroscopy
CT	Computerised tomography
CR	Complete response
CRUK	Cancer Research United Kingdom
ECOG	Eastern Cooperative Oncology Group
ERCP	Endoscopic retrograde cholangiopancreatography
EUS	Endoscopic ultrasound
EUS-FNA	Endoscopic ultrasound guided fine-needle aspiration
EUS-RFA	Endoscopic ultrasound guided radiofrequency ablation
FBC	Full blood count
FNAC	Fine-needle aspiration cytology
GCP	Good Clinical Practice
HIFU	High Intensity Focused Ultrasound
HPB	Hepatopancreatobiliary
ICU	Intensive Care Unit
IHC	Immunohistochemistry
INR	International Normalised Ratio
IPMN	Intraductal papillary mucinous neoplasms
IQR	Interquartile range
IRE	Irreversible electroporation ablation
IV	Intravenous
LAPC	Locally advanced pancreatic cancer
MCN	Mucinous cystic neoplasms
MCM	Minimicrosome maintenance protein

MD IPMN	Main Duct Intraductal papillary mucinous neoplasms
MDM	Multidisciplinary meeting
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
MT IPMN	Mixed Type Intraductal papillary mucinous neoplasms
MW	Microwave
NCI	National Cancer Institute
nCLE	Needle-based Confocal Laser Endomicroscopy
PanIN	Pancreatic Intraepithelial Neoplasia
PanNET	Cystic pancreatic neuroendocrine tumour
PCL	Pancreatic Cystic Lesion
PDAC	Pancreatic ductal adenocarcinoma
PD	Progression of disease (≥20% increase in size)
PFC	Pancreatic Fluid Collections
PR	Partial response (≥30% reduction)
QOL	Quality of life
RF	Radiofrequency
RFA	Radiofrequency ablation
SCN	Serous cystic neoplasm
SD	Stable disease (between PR and PD)
SPPN	Solid pseudopapillary neoplasm
UK	United Kingdom
US	Ultrasound
W	Watts

Publications

The following publications arose from this work:

Original papers:

Pereira SP, SP, Oldfield L, Ney A, Hart PA, **Keane MG**, Pandol SJ, Li D, Greenhalf W, Jeon CY, Koay EJ, Almario CV, Halloran C, Lennon AM, Costello E. Early detection of pancreatic cancer. Lancet Gastroenterol Hepatol. 2020 Mar 2. pii: S2468-1253(19)30416-9.

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1 DIAGNOSIS AND MANAGEMENT OF PANCREATIC DUCTAL ADENOCARCIOMA & CYSTIC LESIONS OF THE PANCREAS

Pancreatic ductal adenocarcinoma

Background and epidemiology

Pancreatic ductal adenocarcinoma (PDAC) is the tenth commonest cancer in the UK with an incidence of approximately 17 per 100,000 population, or 10,800 new cases annually between 2017-2019. Rates vary significantly worldwide, with the highest incidence being in Northern Europe and North America,(Altekruse et al., 2010) which is 3-4 times higher than rates seen in some tropical countries.(Curado et al., 2007) Studies by our group and others show that the incidence of PDAC is also rising in the UK, Europe and North America at a rate of approximately 2% per year [Figure 1.1].(Altekruse et al., 2010, Keane et al., 2014b, CRUK)

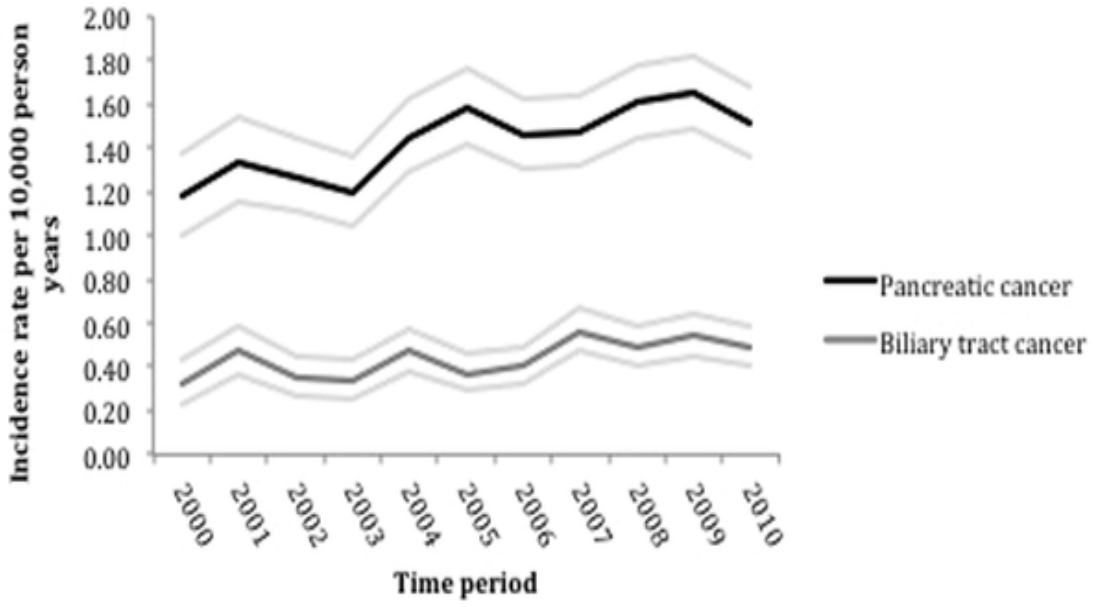


Figure 1.1 Time trends in pancreatic and biliary tract cancer in UK primary care patients between 2000 and 2010 – annual incidence with 95% confidence intervals.(Keane et al., 2014b)

Pathophysiology

Invasive PDAC arises from precursor lesions within the pancreas, primarily from pancreatic intraepithelial neoplasia (PanINs) but also Intraductal Papillary Mucinous Neoplasms (IPMNs) and Mucinous Cystic Neoplasms (MCN) [Figure 1.2]. As PanINs grow they progress from flat to papillary lesions and become increasingly dysplastic (PanIN-1A to PanIN-1B to PanIN-2 to PanIN-3),(Wilentz et al., 2000) before ultimately developing into infiltrating ductal adenocarcinoma [Figure 1.2]. The progression to cancer is promoted by the acquisition of increasing numbers of genetic alterations [Figure 1.2]. Activating point mutations of the *KRAS* oncogene on codon 12 is the most common mutation present in PDAC, occurring in over 90% of tumours. (Singh et al., 2011) HER-2/neu mutations in the encoding *ERBB2* gene are more common in PanIN lesions than invasive PDAC,(Day et al., 1996) and their loss along with the acquisition of mutations in tumour suppressor genes such as *P16*, *TP53* and *DPC4* is believed to drive the progression from PanINs to PDAC.(Wilentz et al., 2000)

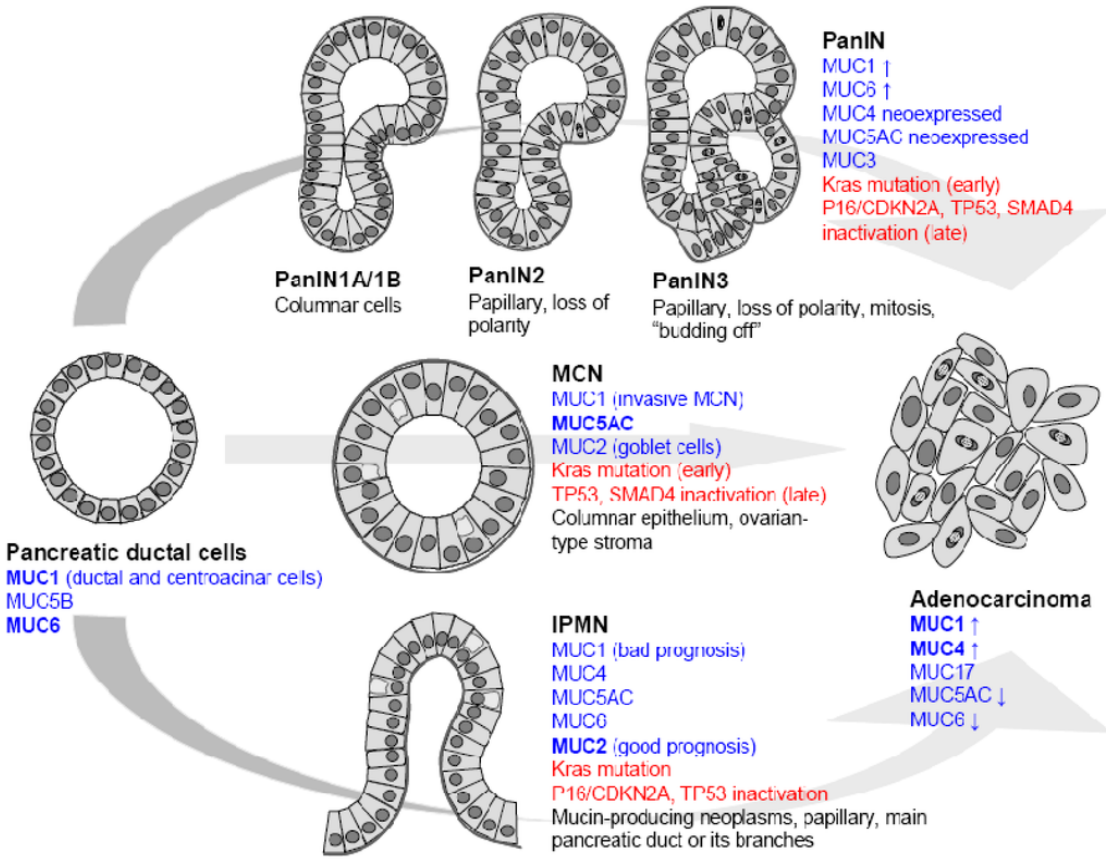


Figure 1.2 Progression of pancreatic precursor lesions (PanIN, MCN, IPMN) to pancreatic ductal adenocarcinoma. Image from **(Jonckheere et al., 2010)**

Risk factors

Most PDAC tumours occur sporadically and therefore variation in incidence seen over time and between populations is largely thought to be the result of differences in an individual's life style and exposure to environmental risk factors.(Lichtenstein et al., 2000) PDAC is more common in the elderly and is slightly more common in men than women.(Wood et al., 2006, Network, 2008, registrations., 2010, Khan et al., 2012b, Shaib and El-Serag, 2004, Keane et al., 2014b, CRUK) Cigarette smoking is strongly associated with PDAC.(Hippisley-Cox and Coupland, 2012, Stapley et al., 2012, Silverman et al., 1994, Fuchs et al., 1996, Muscat et al., 1997, Bonelli et al., 2003, Larsson et al., 2005, Hassan et al., 2007, Keane et al., 2014b) After smoking cessation the frequency of PDAC gradually diminishes, but does not return to baseline for ten years.(Iodice et al., 2008) Chronic medical conditions such as diabetes mellitus, chronic pancreatitis (Hassan et al., 2007, Gullo et al., 2001) and obesity,(Ferlay J, 2008) are also risk factors for PDAC. It is estimated 37% of pancreatic cancers in the UK are preventable.(CRUK)

Individuals with two or more first-degree relatives with pancreatic cancer carry a lifetime risk of risk of around 8-12% of developing PDAC.(Grocock et al., 2007) Several familial cancer syndromes which, although rare, are associated with a higher risk of developing PDAC e.g. Peutz-Jeghers Syndrome (lifetime risk of PDAC of 36% by age 65), familial atypical multiple mole melanoma syndrome (16% lifetime risk), Li-Fraumeni syndrome (*TP53* mutation), Lynch Syndrome (microsatellite instability), Familial Adenomatous Polyposis (*APC* mutation), *BRCA1 and BRACA*2 mutations (5% lifetime risk), hereditary pancreatitis with mutations in the SPINK1 gene (>50% risk by age 75) or cationic trypsinogen (*PRSS1*) gene.(Lowenfels et al., 1997, Rebours et al., 2008) Screening and surveillance is therefore recommended by international guidelines for all individuals with a greater than 5% risk of developing PDAC.(Canto et al., 2013).

Clinical presentation

Overt pancreatic cancer symptoms have traditionally been reported to occur only in the late stages of the disease.(Watanabe et al., 2004, Ambler et al., 2005, Papadoniou et al., 2008)

Prognosis

Most patients with pancreatic cancer are diagnosed with advanced disease, which is not amenable to curative surgical resection. Overall, the long-term prognosis of the disease is poor with a one-year survival rate of approximately 28% and a 5-year survival around 8% if diagnosed between 2016 and 2020 in England. Despite improvements in imaging, surgical techniques and chemotherapy, overall survival has not improved appreciably in the last five decades.(CRUK) One- and five-year survival rates in the UK are also lower than most other European and North American countries, which has been attributed to delays in investigation leading to later diagnosis.(Sant et al., 2009, CRUK) Prognosis in pancreatic cancer is closely associated with disease stage at diagnosis. Survival improves dramatically if a tumor can be identified at an early stage. In comparison to the dismal prognosis outlined above, a recent study found that if patients are diagnosed with stage I disease, 80% of are alive 10 years after diagnosis.(Kanno et al., 2018) There is thus an urgent need to find opportunities to identify pancreatic cancer earlier. One way to do this is to identify individuals with pre-cursor lesions which can progress to pancreatic cancer. Of the three known pre-cursors lesion in pancreatic cancer, Pancreatic intraepithelial neoplasia (PanIN) is the most common but cannot currently be identified on diagnostic imaging. In contrast, the other two precursors, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs), are fluid filled pancreatic cystic lesions that are visible on CT or MRI, and as such, can be followed to screen for the development of invasive cancer.

Cystic lesions of the pancreas

Background and epidemiology

Pancreatic cystic lesions (PCL) have become an increasingly common radiological finding, driven largely by the growing use and greater sensitivity of cross-sectional imaging. They are present in approximately 1.2-2.6% (Laffan et al., 2008, Spinelli et al., 2004) of patients undergoing abdominal computed tomography (CT) and in up to 13.5% of patients undergoing an MRI for non-pancreatic indications. (Lee et al., 2010) Most

cysts are asymptomatic when diagnosed and are being detected at a smaller size than historically.(Nilsson et al., 2016, Keane et al., 2015a)

Cystic lesion subtypes

PCL include a range of subtypes, each with differing malignant potential [Table 1.1]. (Bosman et al., 2010) The seven most common subtypes are classified by the World Health Organisation pathologically as intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasm (MCN), serous cystic neoplasm (SCN), solid pseudopapillary neoplasm (SPPN), cystic pancreatic neuroendocrine tumour (PanNET), cystic degeneration of PDAC or a pseudocyst.(Adsay et al., 2010)

Table 1.1 The World Health Organisation classification of PCL

Epithelial neoplastic (True cysts)	Epithelial non-neoplastic (True cysts)
Intraductal papillary-mucinous neoplasm (IPMN) Mucinous cystic neoplasm (MCN) Serous cystic adenoma (SCA) VHL associated serous cystic adenoma Serous cystadenocarcinoma Cystic neuroendocrine tumour G1–2 Acinar cell cystadenoma Cystic acinar cell carcinoma Solid pseudopapillary neoplasm Accessory-splenic epidermoid cyst Cystic hamartoma Cystic teratoma (dermoid cyst) Cystic ductal adenocarcinoma Cystic pancreatoblastoma Cystic metastatic epithelial neoplasm	Lymphoepithelial cyst Mucinous non-neoplastic cyst Enterogeneous cyst Paraampullary duodenal wall cyst Retention cyst Endometrial cyst Congenital cyst (in malformation syndromes)
Non-epithelial neoplastic (False cysts)	**Non-epithelial non-neoplastic (False cysts)**
Benign non-epithelial neoplasm (e.g. lymphangioma) Malignant non-epithelial neoplasms (e.g. sarcomas)	Pancreatitis-associated pseudocyst Parasitic cyst

1.1.1.1 True cysts

True cysts are differentiated from other pancreatic cysts by the presence of a cyst wall with an epithelial lining, which secretes fluid and proteins into the cyst cavity. Although

there are many types of true pancreatic cysts [Table 1.1], they can be broadly divided into serous cysts (which are almost always benign) and mucinous cysts (which are pre-malignant). In clinical practice, approximately 15% of pancreatic cancers arise from PCL (Le et al., 2008).

1.1.1.1.1 Serous cysts:

- *Serous cystic neoplasm (SCN):* SCNs typically occur in middle-aged women and are almost always benign. They are nearly always located in the body or tail of the pancreas.(Lennon and Wolfgang, 2013, Del Chiaro et al., 2013, Jais et al., 2016) On imaging they can be unilocular, microcystic, oligocystic/macrocystic or occasionally solid. Some will have a central stellate scar, which is pathognomonic. They are usually small, asymptomatic and contain clear watery fluid, but they can grow and compress local structures leading to symptoms such as pancreatitis or pain.(Lennon and Wolfgang, 2013, Del Chiaro et al., 2013, Jais et al., 2016) Rarely they are associated with inherited conditions such as Von Hippel Lindau syndrome.(Charlesworth et al., 2012)

1.1.1.1.2 Mucinous cysts (Pre-malignant):

- *Intraductal papillary mucinous neoplasm (IPMN):* IPMNs arise from the branch or main ducts of the pancreas. The epithelial lining of the cyst secretes thick mucinous fluid leading to the formation of a cyst or dilation of the pancreatic duct. Typically IPMNs present in the sixth and seventh decades of life, however with improvements in the sensitivity of cross-sectional imaging, smaller cysts are being diagnosed at a younger age.(Lennon and Wolfgang, 2013) IPMNs are slightly more common in men. Most patients are asymptomatic and the lesion is often detected incidentally. IPMNs are classified as precancerous lesions that can progress though a spectrum of dysplasia from low-grade dysplasia to high-grade dysplasia and then invasive carcinoma. Branch-duct IPMNs (BD-IPMN) are associated with a lower rate of malignant transformation and are the most commonly detected PCL. BD-IPMN are generally managed by surveillance. In contrast IPMNs which originate from the main pancreatic duct (main-duct IPMN or MD-IPMN) or both the main duct and side branches (mixed-type IPMN or MT-IPMN), are associated with a higher rate of malignant transformation so are nearly always managed by

surgical resection. Surgical resection of a dysplastic lesion without invasive cancer is curative, but patients should remain in surveillance after surgical resection for synchronous lesions. If the resected lesion contains invasive carcinoma 5-year survival is estimated to be between 40-60%.(Lennon and Wolfgang, 2013)

- *Mucinous cystic neoplasm (MCN):* MCNs are mucinous cysts are epithelial lined cystic lesions which unlike IPMNs are not ususally connected to the pancreatic duct. They commonly occur in middle-aged women and typically occur in the body or tail of the pancreas.(Tanaka et al., 2012, Ohtsuka et al., 2024a) Approximately half of these cysts are associated with non-specific symptoms such as abdominal discomfort.(Lennon and Wolfgang, 2013) Invasive cancer has been found in between 0-34% of surgically resected MCN. Due to the rates of associated cancer most guidelines recommend MCNs are referred for surgical resection. However, differentiating MCN from other PCL can be challenging. In this scenario recent series have noted the rate of invasive cancer in small MCNs (<4cm) is low and therefore continued surveillance is an acceptable management option in certain groups (e.g. diagnostic uncertainty, the comorbid or elderly).(Nilsson et al., 2016, Keane et al., 2018)

1.1.1.1.3 Malignant cysts:

- *Pancreatic ductal adenocarcinoma (PDAC):* PDAC can be associated with cystic degeneration.

- *Pancreatic neuroendocrine tumours (PanNET):* These pancreatic tumours arise from the pancreatic endocrine cells, the islets of Langerhans. They are commonly solid in nature but can also rarely present as cystic or solid/cystic lesions. They are equally common in men and women and become more common in older age. These lesions have a much better prognosis than PDAC.

- *Malignant transformation of a mucinous cyst:* Although IPMNs and MCNs are classified as premalignant lesions the overall risk of malignant transformation in most cysts is low, approximately 0.95% per year.(Hruban et al., 2007) In surgically resected lesions rates of associated malignancy are higher; 62.2% (range 36-100%) in main duct IPMNs and 24.4% (range 6-51%) in branch duct

IPMNs.(Allen et al., 2006, Baiocchi et al., 2013, Maguchi et al., 2011, Sawai et al., 2010, Tanaka et al., 2012, Ohtsuka et al., 2024a)

- *Solid pseudo-papillary neoplasm (SPPN):* These are rare cystic lesions that occur almost exclusively in young women. They can range in size from 1 - 30cm. Although classified as malignant, most lesions are indolent and progress very slowly in comparison to PDAC. Management is surgical. Most patients have a very good prognosis following complete surgical resection.(Lennon and Wolfgang, 2013, Law et al., 2014)

1.1.1.2 False cysts

False cysts are pancreatic cysts without an epithelial lined wall. The most common cyst in this group are inflammatory cysts or pseudocysts. They harbor no malignant potential. Pancreatic Fluid Collections (PFC) normally develop weeks or months after an episode of acute pancreatitis or a flare of chronic pancreatitis.(Banks et al., 2013, Banks and Freeman, 2006) They are often connected to the pancreatic ductal system so the cyst fluid contains digestive enzymes, such as amylase. These cysts can occur at any age and can develop in any part of the pancreas or be extra pancreatic. They can occur as a single collection or as multiple cysts. PFCs if small (<6cm), usually do not cause symptoms and no further treatment is generally required. Larger PFCs can cause pain, become infected or cause obstruction of the bile duct or gastric outflow tract leading to vomiting. If any of these symptoms occur, endoscopic (and or percutaneous) drainage of the cyst is recommended.(Keane et al., 2015b, Huggett et al., 2015)

Diagnostic investigations for Pancreas Cancer and PCL

Serum markers

Carbohydrate antigen 19-9 (CA 19-9) was first isolated in 1979.It is a sialylated Lewis antigen of the protein MUC1. CA19-9 has a sensitivity of 70–90% and specificity of 90% in the diagnosis of pancreatic cancer in symptomatic patients.(Vestergaard et al., 1999, Steinberg, 1990, Ghaneh et al., 2007) However, CA19-9 is not secreted by around 7% of the population who do not have the Lewis antigen. It can also be raised in other

conditions such as acute and chronic pancreatitis, liver cirrhosis, cholangitis and obstructive jaundice.(Duffy et al., 2010)

As a screening test for pancreatic cancer, some studies have shown that high levels of CA-19-9 are indicative of invasive cancer.(Testini et al., 2010b, Park et al., 2014, Sperti et al., 1996, Bassi et al., 2002, Yasue et al., 1994, Duffy et al., 2010) However its utility in early pancreatic cancer is limited due to its poor positive predictive value and it's reduced sensitivity; only 65% of patients with resectable pancreatic cancer have an elevated level of CA19-9.(Goggins, 2005) Guidelines on screening for pancreas cancer in PCL have therefore not routinely included CA 19-9 in to their algorithms. Some have recognizing, that when elevated that a rising CA 19-9 is a risk factor for malignant transformation.(Del Chiaro et al., 2013, Tanaka et al., 2012, Vege et al., 2015b, 2018, Elta et al., 2018, Ohtsuka et al., 2024a)

With regards to other gastrointestinal serum biomarkers, a single study from more than 20 years ago explored if an elevated serum CEA could differentiate mucinous from serous cysts.(Bassi et al., 2002) Although it suggested some utility, this has not been validated in any subsequent series and has not been incorporated in to PCL guidelines.

Imaging and endoscopy

1.1.1.3 Transabdominal ultrasonography

Transabdominal ultrasound (US) is the most commonly used initial imaging modality in the evaluation of abdominal pain and obstructive jaundice, the two most common presentations of pancreatic cancer.(Watanabe et al., 2004) In PDAC, US has a sensitivity and specificity of approximately 76-87% and 63-99%, respectively,(Bipat et al., 2005, Karlson et al., 1999, Maringhini et al., 1993) which is limited by the retroperitoneal position of the pancreas, which is often obscured by overlying bowel gas. However, US can also be useful in excluding other causes of biliary obstruction, particularly choledocholithiasis.(Di Stasi et al., 1998) In PCL trans-abdominal US has a very low diagnostic accuracy for PCL subtype (<50%),(Testini et al., 2010b) so is not utilized routinely, unless patients cannot undergo cross-sectional imaging.

1.1.1.4 Computed tomography

Computed tomography (CT) with intravenous contrast is the most commonly used imaging modality for diagnosing and staging pancreas cancer. (Conroy et al., 2023, NICE, 2018, Peddu et al., 2009)

PCL are identified in approximately 1.2-2.6% (Laffan et al., 2008, Spinelli et al., 2004) of patients undergoing abdominal CT for non-pancreatic indications. Differentiating cyst subtype by CT alone is challenging. Some studies have reported that up to 40% of mucinous cysts and 33% of SCNs were misdiagnosed by CT alone resulting in inappropriate management.(Rabie et al., 2014, Brugge, 2015, Scarlett et al., 2011, Testini et al., 2010a)

1.1.1.5 Magnetic resonance imaging

Contrast-enhanced magnetic resonance imaging (MRI) with magnetic resonance cholangiopancreatography (MRCP) is the most commonly recommended method of delineating and surveying PCL in all current guidelines.(Vege et al., 2015, 2018, Elta et al., 2018, Ohtsuka et al., 2024) It provides detailed imaging of the cyst and any relationship with the pancreatic ducts. MRI can also provide useful information to differentiate cyst subtypes, for example MCNs tend to be smooth unilocular structures. SPPNs are often multicystic, lobulated lesions. IPMNs can be unilocular or muticystic with septations or occasionally mural nodules.(Kim et al., 2006) However many of these features overlap and preoperative imaging only correlates with surgical pathology in between 30-74% of cases for cyst subtype.(Del Chiaro et al., 2013, Garcea et al., 2008, Loftus et al., 1996, 2018)

MRI can also be used to identify most high risk stigmata and features of concern e.g. solid component that require surgical management.(Del Chiaro et al., 2013, Garcea et al., 2008, Loftus et al., 1996, 2018) Some high risk and worrisome features such as mural nodules are better appreciated on EUS, so imaging modalities can be used in combination during the evaluation of high risk lesions because they provide complimentary information. Vege et al., 2015, 2018, Elta et al., 2018, Ohtsuka et al., 2024)

1.1.1.6 Positron emission tomography-CT

PET-CT has superior sensitivity to CT for the diagnosis of PDAC in a multicentre randomized controlled trial from the UK (PET-PANC) with a sensitivity 92.7% vs. 88.5%, p=0.010 and specificity 75.8% vs. 70.6%, (p=0.023).(Ghaneh et al., 2016) PET-CT does not reliably differentiate PCL subtypes effectively but can detect malignant transformation.(Sultana et al., 2015) Given most PCL are low risk lesions its overall clinical utility is limited.

1.1.1.7 Endoscopic ultrasonography

Endoscopic ultrasound (EUS) is a sensitive method for the assessment of PCL and early pancreatic tumours. It also enables cytological or histological samples to be obtained via EUS-guided fine needle aspiration (EUS-FNA) or biopsy (EUS-FNB) for diagnosis.(Kochman, 2002) In a meta-analysis of nine studies (total 576 patients) comparing FNB to FNA needles for tissue acquisition in pancreatic cancer, there was no significant difference in diagnostic adequacy (75.2 % vs. 89.0 %, odds ratio [OR] 0.39, P = 0.23), diagnostic accuracy (85.8 % vs. 86.2 %, OR 0.88, P = 0.53) or rate of histological core specimen acquisition (77.7 % vs. 76.5 %, OR 0.94, P = 0.85) between the needles, respectively. The mean number of passes required for diagnosis, however, was significantly lower when using an FNB needle (standardized mean difference - 1.2, P < 0.001).(Bang et al., 2016) Adverse events (pancreatitis, bleeding, infection) following EUS are rare, occurring in approximately 1-2% of cases.(Polkowski et al., 2012, Wang et al., 2011b, Adler et al., 2005)

The utility of EUS over cross sectional imaging in surgical decision for PCL continues to be debated,(Maker et al., 2008, Cho et al., 2013, Del Chiaro et al., 2013, Del Chiaro et al., 2014, Tanaka et al., 2012) (Ohtsuka et al., 2024a). EUS has a substantial learning curve and can be operator dependent.(Nakai et al., 2014) However when the test is performed in high volume centres EUS-FNA in addition to abdominal imaging can significantly improve pre-operative diagnostic accuracy in PCL.(Khashab et al., 2013)

Cyst fluid can be evaluated for a several factors to aid diagnosis in PCL. An initial assessment of the fluid for the presence of the “string-sign” is highly suggestive of a mucinous lesion.(Bick et al., 2015, Leung et al., 2009) Cytologically serous lesions demonstrate glycogen rich cells, whereas mucinous lesions have an abundant mucinous background, with small clusters of flat sheets of relatively bland glandular cells.

Prominent papillary arrangement of the tall columnar cells has been reported in fluid aspirated from IPMNs, although it is almost always impossible to differentiate MCNs from IPMNs by cytology alone.(Recine et al., 2004, Zhai et al., 2006) Biochemical analysis of cyst fluid, demonstrating a carcinoembryonic antigen (CEA) of greater than 192 ng/mL is suggestive of a mucinous cyst.(Al-Haddad et al., 2014, Brugge et al., 2004b) Amylase levels in the cyst fluid can also be measured and when elevated are suggestive of a connection to the pancreatic ductal system, but does not reliably differentiate between cyst subtypes.(Attasaranya et al., 2007, Aljebreen et al., 2007)

Sequencing of the DNA isolated from pancreatic cyst fluid has identified several somatically mutated genes and chromosomal copy number alterations that strongly correlated with cyst subtype.(Wu et al., 2011) The identification of DNA alterations in cyst fluid is therefore substantially improved the evaluation of pancreatic cysts. Panels of cyst fluid molecular markers are now used in many centres to aide the classification of PCL and predict the presence of high-grade dysplasia or invasive adenocarcinoma. (Springer et al., 2019) (Paniccia et al., 2023) (Wu et al., 2011) However all of these tests are dependent on obtaining sufficient fluid for analysis which can be challenging, when lesions are less than 2cm in size, mucinous and the contents are particularly viscous and difficult to aspirate. In a prospective study of 143 patients with PCL at two leading tertiary referral centers; adequate cellular material to enable cytological analysis was only obtained in only 31% and biochemical analysis was possible in less than half of all cases.(de Jong et al., 2011) Novel alternative diagnostic strategies to improve the diagnostic accuracy in PCL are therefore needed.

Screening and surveillance for pancreatic cancer

As pancreas cancer remains a relatively rare disease, screening of the general population would not be cost-effective and could be potentially harmful for patients through over investigation. Guidelines therefore advocate targeted screening of individuals at an increased risk of developing PDAC (>5% risk).(Canto et al., 2013) At present this includes individuals with a family history of pancreatic cancer, hereditary pancreatitis, certain genetic syndromes, or mucinous PCL. The utility of screening other high-risk groups such as newly diagnosed diabetics or those with combinations of risk factors or early symptoms is being evaluated through ongoing clinical trials.

Current screening programmes for PDAC in the UK:

1.1.1.8 EUROPAC:

Patients with a family history of PDAC, hereditary pancreatitis or inherited syndrome in the UK are often screened via the pan-European EUROPAC registry (http://www.europac-org.eu).(Grocock et al., 2007) Patients enrolled in the registry have cross-sectional imaging and blood tests (including tumour markers) at registration and then an annual EUS. If a suspicious lesion is identified during surveillance further investigations and treatment are arranged as clinically necessary.

1.1.1.9 Pancreatic cysts

International, European and American College of Gastroenterology guidelines, recommend that patients with malignant or high-risk PCLs are referred for immediate surgical resection while all other patients undergo regular surveillance with interval cross-sectional imaging. (Vege et al., 2015, 2018, Elta et al., 2018, Ohtsuka et al., 2024) Low-risk lesions are being detected with increasing frequency; as a result growing numbers of patients are entering screening programmes for PCLs every year.

Developing future diagnostic tests for patients with PCL

As outlined earlier in the chapter invasive pancreatic cancer arises from well-defined precancerous lesions; pancreatic intraepithelial neoplasia (PanIN) or mucinous cystic neoplasms. The most common lesion PanINs are not visible on cross sectional imaging. Therefore, there has been a significant interest in developing novel diagnostic biomarkers with improved sensitivity to aid earlier diagnosis. Improved understanding of the molecular and genetic drivers of pancreatic cancer development has led to the identification of key mutations, including *KRAS, TP53, CDKN2A,* and *SMAD4,* that drive tumorigenesis.(Voutsadakis and Digklia, 2023) However, the evolution to effective diagnostic biomarkers in PDAC has been slow.(Tenchov et al., 2024)

Biomarker discovery in pancreas cancer and pancreatic cystic lesions can be approached using either a hypothesis-based or hypothesis-free approach. Traditional hypothesis-based methods, focus on validating known biomarkers, which are used in other cancers (CA199, CA 125, CA-15-39, CA 72-4) or have the potential to become a diagnostic biomarker in pancreas cancer based on a mechanistic understanding of

disease process, have had variable performance and none have not been adopted into routine clinical practice.(Sperti et al., 1996, Bassi et al., 2002, Silverman et al., 2009) In high risk cystic lesions, that have not yet developed into invasive cancer biomarker development is even more challenging and most have performed poorly.(Franses et al., 2018, Rhim et al., 2014)

While hypothesis-free biomarker discovery approaches employ multi-omic technologies, which analyze large genomic, transcriptomic, proteomic, or metabolomic datasets individually or collaboratively to develop diagnostic panels. Artificial intelligence (AI) and machine learning approaches can help analyze these complex datasets to identify correlations not always apparent through conventional methods.(Osipov et al., 2024, Tripathi et al., 2024)

Staging pancreatic cancer

Pancreatic cancer is usually staged by the American Joint Committee on Cancer (AJCC) tumor node metastasis (TNM) classification system [Table 1.2]. The classification is based on three key factors, the tumor size (T) and extent, spread to lymph nodes (N) and if the tumor has metastasized to distant sites (M).

Table 1.2 American Joint Committee on cancer (AJCC) 8th edition staging system for pancreas cancer (Amin et al., 2017)

Primary tumour (T)	
Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ (included PanIN III)
T1	Maximum tumour diameter ≤ 2cm
T2	Maximum tumour diameter > 2, ≤ 4cm
T3	Maximum tumour diameter > 4cm
T4	Tumour involves the coeliac axis, common hepatic artery or the superior mesenteric artery
Regional lymph nodes (N)	
N0	No regional lymph node metastasis
N1	Metastasis in 1-3 regional lymph nodes
N2	Metastasis in ≤ 4 regional lymph nodes
Distant metastasis (M)	

M0	No distant metastasis		
M1	Distant metastasis		
	T	**N**	**M**
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T1-3	N1	M0
Stage III	Any T / T4	Any N	M0
Stage IV	Any T	Any N	M1

The validation of the AJCC 8th edition staging system has been undertaken in PDAC rather than PCL with invasive cancer. A single study using the SEER database, found that tumor size in invasive IMPN did not predict survival in those with a tumour size >4 cm versus >2 and ≤4 cm). The earlier 7th edition appeared to correlate better with prognosis in invasive IPMN. However, it was not clear if the whole tumor or just the cystic component was measured. Further studies are needed to validate this classification system specifically in mucinous cystic neoplasms with invasive carcinoma. (Fan et al., 2019)

Resectable versus unresetable pancreatic cancer

Although the AJCC staging system gives a detailed classification of the tumor, it is based on surgical pathology. Pre surgery patients are given a clinical stage based on their biopsy and cross-sectional imaging findings and classified as resectable, borderline resectable or unresectable (either locally advanced due to major blood vessel involvement or metastatic). Between centres and over time definitions of resectability have varied, which often make comparing outcomes retrospectively challenging.(Hidalgo, 2010)

Other prognostic factors

Following surgery, the radicality of the resection is graded as follows; R0 - all margins of the specimen are histologically tumor-free, R1 - microscopically visible tumor cells are present at the specimen margins, R2 - resection macroscopically visible tumors exist.

The tumour can also be graded in comparison to the surrounding pancreatic tissue:

Grade 1 (G1) looks much like normal pancreatic tissue
Grade 3 (G3) suggests the cancer is very abnormal.
Grade 2 (G2) falls somewhere in between G1 and G3.
G1 cancers grow slower than G3 cancers. G3 cancer have a poorer prognosis than G1/2 cancers.

The ESMO 2023 guidelines endorse the new definition for borderline resectable disease by the International Association of Pancreatology (IAP) that also includes biological criteria based on serum CA19-9 levels, and the patient's performance status, thereby broadening the patient population with indication for neoadjuvant therapy. (Conroy et al., 2023) Although intuitively this appears reasonable, it is not evidence-based since the randomized studies that established neoadjuvant therapy for borderline-resectable patients, including PREOPANC-1 (Versteijne et al., 2020) and ESPAC-5 (Philip et al., 2022), used empirical anatomical staging criteria only.

Treatment of pancreatic cancer

Treatment for pancreatic cancer, is based on cancer stage, performance status and patient preference. In most major medical centres a specialist pancreatic cancer multidisciplinary team, including surgeons, oncologists, radiologist, pathologists and palliative care physicians review the patients case and relevant pathology and imaging to decide on management. If patients are diagnosed in local community hospitals it is recommended care should be delivered in partnership with local cancer units.(NICE, 2018)

Resectable pancreatic cancers are primarily treated by surgical resection. Tumours of the pancreatic head and the periampullary region are treated with a Whipple pancreaticoduodenectomy. Where possible pylorus preservation should always be considered because it leads to a better outcome in terms of postoperative recovery, weight maintenance and lower rates of dumping syndrome. Distal tumours in the body or tail of the pancreas can be resected with a distal pancreatectomy. A total pancreatectomy is indicated in very few patients e.g. if there are positive resection margins after frozen section. The potential benefits of a total pancreatectomy have to be balanced against the high morbidity associated with the procedure, as patients will loose all pancreatic endocrine and exocrine functions and brittle diabetes is inevitable.

Performing pancreatic surgery in regional high volume HPB centres is associated with better outcomes.(Alexakis et al., 2004) As such, since 2001 the National Cancer Plan in the UK has advocated the centralisation of hepatopancreaticobiliary surgery. Postoperative mortality following a Whipple resection in high volume centres is between 0%-6% and <2% following a distal pancreatectomy. Postoperative morbidity remains common occurring in 30-60%.(Bassi et al., 2005, Diener et al., 2007)

In patients with borderline resectable disease neoadjuvunt chemotherapy can be considered prior to surgery with the aim of achieving a curative R0 resection. Using this approach it has been estimated that an additional third of patients can obtain R0 resection. Adjuvant chemotherapy can also be used after patients have recovered from surgery. Typically gemcitabine with or without capecitabine is utilized.(NICE, 2018)

The latest European Society of Medical Oncology (ESMO) guidelines endorse primary resection followed by adjuvant chemotherapy for resectable pancreatic cancer. FOLFIRINOX is recommended based on the PRODIGE 24 study with gemcitabine/capecitabine (according to the ESPAC-4 study) reserved from those unable to tolerate FOLFIRINOX. 5-fluorouracil (5-FU)/folinic acid or gemcitabine monotherapy, is now only indicated for frail patients.(Conroy et al., 2023) The guideline still advises against adjuvant radiochemotherapy outside clinical trials.

For patients with borderline resectable disease, there is a stronger recommendation for neoadjuvant therapy prior to surgery. There is no agreement on the best induction therapy or if radiotherapy should be included. FOLFIRINOX or gemcitabine/nab-paclitaxel are suggested followed by chemoradiotherapy “on a case-by-case basis” without defining the criteria for the radiochemotherapy. Whilst the PREOPANC-1 trial (Versteijne et al., 2020) used neoadjuvant chemoradiation, the ESPAC-5 trial (Philip et al., 2022) with short course neoadjuvant regimens, reported 1-year overall survival rates of 78% [95% confidence interval (CI): 60–100%] for gemcitabine plus capecitabine and 84% (95% CI: 70–100%) for FOLFIRINOX, compared to 60% (95% CI: 37–97%) for capecitabine-based chemoradiotherapy and 39% (95% CI: 24–61%) for immediate surgery (P=0.0028).(Ghaneh et al., 2023) Moreover the 1-year disease-free survival from surgery was 33% (95% CI: 19–58%) for immediate surgery and 59% (95% CI: 46–74%) following neoadjuvant therapies (P=0.016).(Ghaneh et al., 2023) It is also

noteworthy that in the phase II Alliance A021501 study, neoadjuvant radiotherapy after seven cycles of mFOLFIRINOX resulted in inferior 18-month overall survival of 47.3% compared with 66.7% using eight cycles of chemotherapy with FOLFIRINOX without radiotherapy.

In patients with locally advanced pancreatic cancer, the NICE guidelines recommend adjuvant combination systemic chemotherapy is offered first line. Gemcitabine can be used in those not able to tolerate combination chemotherapy. If chemoradiotherapy is considered, capecitabine is advised as a radiosensitiser.(NICE, 2018) For patients with locally advanced disease, the latest ESMO guidelines recommend a paradigm change from "6 months of gemcitabine" in the ESMO 2015 guidelines to a "conversion surgery strategy" with intensive induction chemotherapy. Evaluation for resectability is advised every 2–3 months by the local multidisciplinary team. In addition arterial resection after induction therapy is considered a potential option in experienced centers after induction therapy.

In metastatic disease a combination of leucovorin, fluorouracil, irinotecan, and oxaliplatin (FOLFIRINOX) demonstrated a significant survival benefit of 4.3 months over gemcitabine alone. However grade 3-4 toxicity are more frequent, so only patients with a good performance status can be considered for this treatment. Nab-paclitaxel and gemcitabine show an improved median survival of 1.8 months, compared to gemcitabine alone. This regimen is better tolerated with lower toxicity profile and far fewer adverse events so is suitable for elderly patients and those with co-morbidities. Therefore in metastatic pancreatic cancer FOLFIRINOX is offered to patients with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1.(Christians et al., 2014, Ferrone et al., 2015, NICE, 2018).

The role of immunotherapy in pancreatic cancer is more uncertain.(Hilmi et al., 2023) Currently the role of cellular therapies and chimeric antigen receptor (CAR) T cell cells against mesothelin, claudin 18.2 and carcinoembryonic antigen (CEA) are being evaluated in PDAC. (Kronig et al., 2023) (Wittwer et al., 2023) The first results for individualized neoepitope vaccines are promising, and larger studies are ongoing.(Rojas et al., 2023)

Palliative treatments

1.1.1.10 Biliary drainage

In patients with potentially resectable disease, pre-operative biliary stenting via ERCP is not essential unless the patient is severely jaundiced, has cholangitis, there is diagnostic uncertainty requiring additional investigation or neoadjuvant chemotherapy is planned. This was confirmed in a randomized controlled trial of 196 patients treated with either early surgery or pre-operative biliary drainage followed by surgery. The rates of serious complications were 39% in the early-surgery group compared to 74% in the biliary-drainage group ($p<0.001$).(van der Gaag et al., 2010)

However in patients with unresectable or metastatic disease, ERCP with biliary stenting or EUS guided choledochoduodenostomy is an accepted initial treatment for the palliation of jaundice, with a lower complication rate than percutaneous drainage or surgical bypass.(Huggett et al., 2010, Teoh et al., 2023) At the time of endoscopic retrograde cholangiopancreatography (ERCP) a plastic or self-expanding metal stent (SEMS) can be inserted. SEMS has a longer patency time than plastic stents.

Ablative therapies can be evaluated prior to stent placement to improve patency or following placement when stent blockage occurs. Randomised studies comparing PDT with biliary stenting to stenting alone have had conflicting results. Initial studies reported prolonged stent patency and improved survival after PDT.(Zoepf et al., 2005, Gerhardt et al., 2010) However, a phase III trial from the UK closed early, as overall survival was longer in those treated with stenting alone.(Pereira et al., 2012) The use of RFA in combination with SEMS placement has been reported in two small studies to date. The investigators showed that the median bile duct diameter increased following endobiliary RFA and that 86% (19/22) of SEMSs were patent at 90 days.(Steel et al., 2011, Figueroa-Barojas et al., 2013) Early studies also suggest that endobiliary RFA may confer some early survival benefit in patients with malignant biliary obstruction independent of stent blockage and chemotherapy.(Steel et al., 2011) Occasionally centres have used RFA alone to achieve biliary drainage.(Shariff et al., 2013) Current guidance from the National Institute for Health and Care Excellence in the UK recommends that biliary ablation treatments should only be undertaken in specialist centres, in the context of clinical trials.(NICE, 2013)

1.1.1.11 Gastric outlet obstruction

Approximately 10-20% of patients with PDAC will develop gastric outlet obstruction. (Jeurnink et al., 2010) This can be managed by EUS guided gastrojejunostomy, duodenal stenting or rarely surgical bypass. With better oncological treatments, the life expectancy of many PDAC patients with malignant GOO is now increasing. Surgical gastrojejunostomy can provides good long-term results but its associated morbidity and longer recovery limits its utility. (Khashab et al., 2017) Although duodenal stent placement is associated with the lowest rates of adverse events, stent occlusion is common in patients with a prognosis of more than 3 months. In many centres with experienced therapeutic endosccopists, EUS guided gastrojejunostomy is now the preferred management of malignant GOO. (Keane and Khashab, 2020)

1.1.1.12 Pain control, nutrition and end of life issues

Severe intractable abdominal or back pain in patients with PDAC is unfortunately common. This is best managed by increasing analgesia in line with the WHO analgesic ladder with or without coeliac plexus block via EUS or rarely percutaneously.(Johnson, 2005) Patients with PDAC can also lose weight rapidly and develop symptoms of exocrine insufficiency requiring pancreatic enzyme replacement in addition to nutritional supplementation.(NICE, 2018) In a randomised double blind placebo-controlled of pancreatic enzyme treatment, patients receiving pancreatic enzymes gained more body weight compared to those taking placebo (p=0.02).(Bruno et al., 1998) Depression is extremely common in PDAC patients and specific treatment with regular psychological support is often necessary.(Johnson, 2005)

Management of cystic lesions of the pancreas

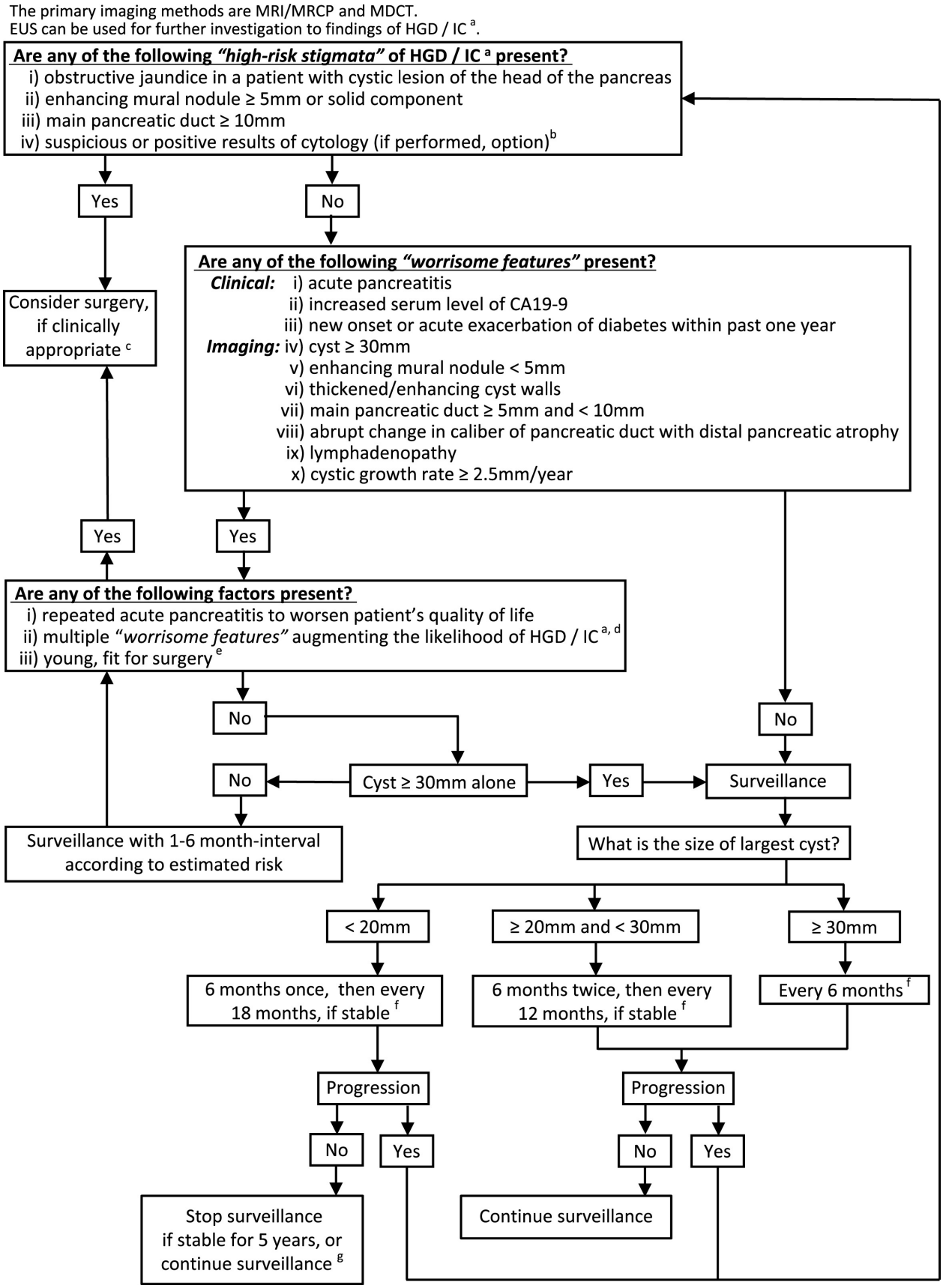
In accordance with international guidance, patients with mucinous PCL that are thought to be malignant or at high-risk of malignant transformation are referred for immediate surgical resection. Patients with a BD-IPMN or indeterminate mucinous PCL with low-risk features, but who are fit for surgical resection if required, enter a surveillance programme.(Del Chiaro et al., 2013, Tanaka et al., 2012, Vege et al., 2015b, 2018, Elta et al., 2018, Ohtsuka et al., 2024a)

Surveillance

BD-IPMNs that are suitable for surveillance, are small lesions (<3cm) that are not associated with high-risk stigmata or features of concern on cross-sectional imaging. High risk stigmata are defined by the International guidelines as obstructive jaundice, enhancing solid component or dilation of the main pancreatic duct to >10mm. Worrisome features are defined as pancreatitis, cyst >3cm, thickened/enhancing cyst walls, main duct size of 5-9mm, a non-enhancing mural nodule, abrupt change in the caliber of the main pancreatic duct with distal atrophy of the gland.(Tanaka et al., 2012) (Ohtsuka et al., 2024a)

Other worrisome features, which have been identified in recent studies and may be included in future guidelines are, a PCL growth rate of >2mm/year,(Kang et al., 2011, Rautou et al., 2008) a raised CA 19-9 or new onset diabetes.(Rodriguez et al., 2007, Pelaez-Luna et al., 2007) The evidence, which informs current guidelines, remains of relatively low quality so variation across the current guidelines exists [Figure 1.3, 1.4 and 1.5].(2018, Elta et al., 2018, Tanaka et al., 2012, Ohtsuka et al., 2024a)

Figure 1.3 Algorithm for the management of suspected BD-IPMN (Kyoto International guidelines) (Ohtsuka et al., 2024a)



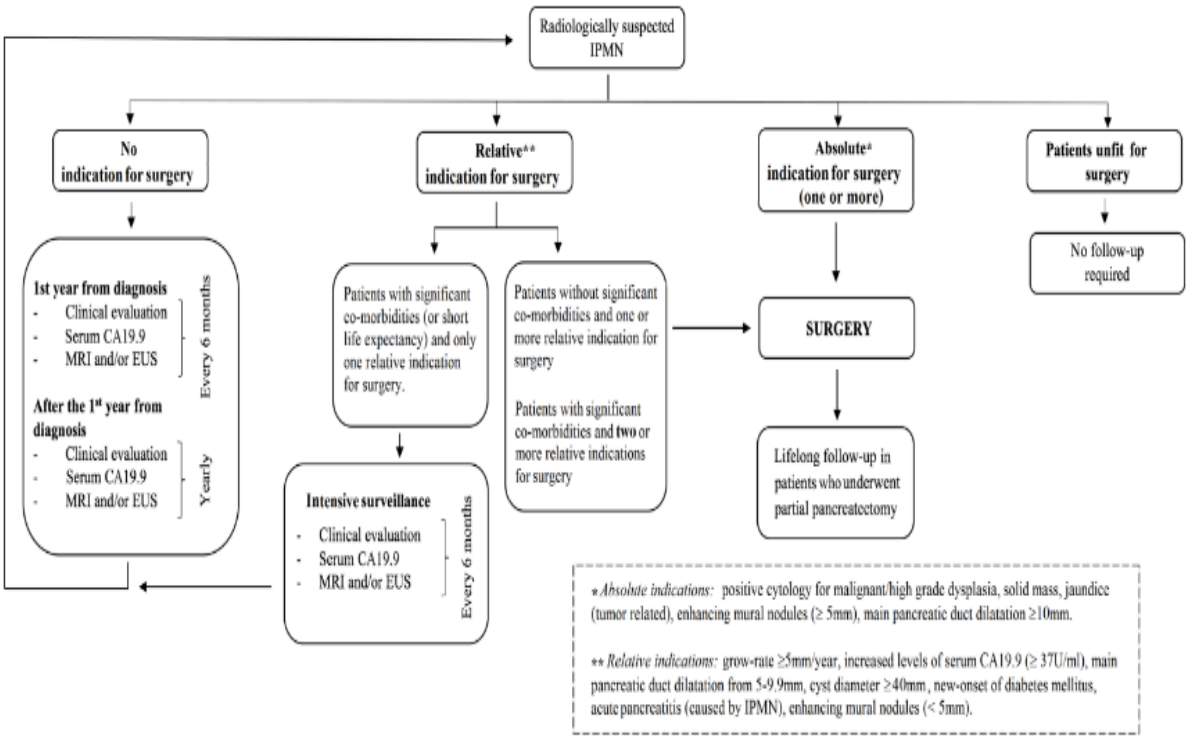


Figure 1.4 Indications for surgery in PCL (European guidelines)(2018)

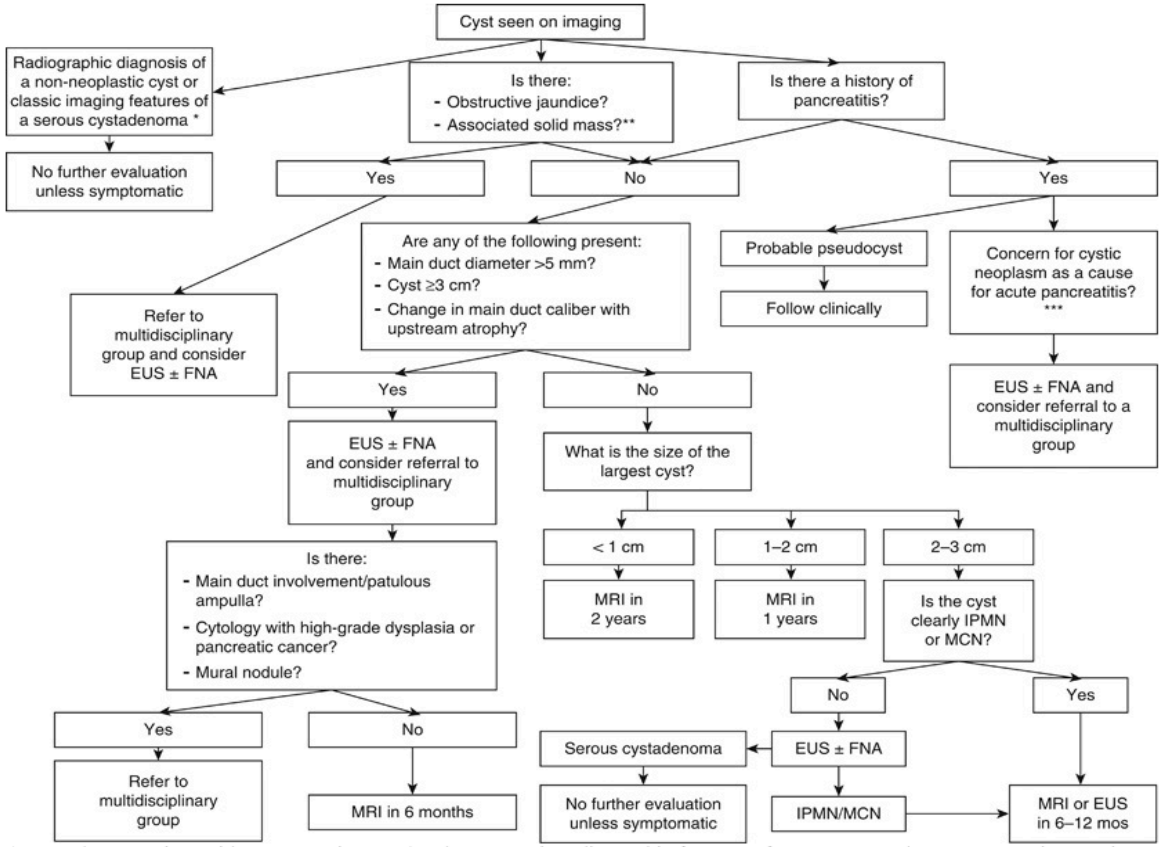


Figure 1.5 American College of Gastroenterology (ACG) approach to a patient with a pancreatic cyst. *Pathognomonic radiographic features of a SCN are a microcystic appearance with a central stellate scar. **Occasionally benign lesions can have a solid appearance. In cases where the diagnosis is unclear EUS±FNA should be performed. ***Unusual cystic features or present at initial onset of acute pancreatitis. EUS, endoscopic ultrasound; FNA, fine needle aspiration.(Elta et al., 2018)

Surgery

Consensus guidelines recommend that all high-risk PCL should be resected in patients fit for surgery.(2018, Elta et al., 2018, Tanaka et al., 2012) (Ohtsuka et al., 2024a) In terms of the surgical technique, according to the current guidelines a patient should undergo an organ-preserving pancreatic resection if the size of the tumour is less than 3-4 cm and has no associated worrisome features or symptoms.(Gagner and Palermo, 2009, Del Chiaro et al., 2013, Tanaka et al., 2012) (Ohtsuka et al., 2024a)

In PCL in the head of the pancreas a pancreaticoduodenectomy (either pylorus-preserving or classic Whipple) is associated with a postoperative mortality is between 0-6% even in high-volume centres, with a morbidity of 40-60%.(Crippa et al., 2007, Kiely et al., 2003) Following a distal pancreatectomy postoperative mortality is close to zero, but postoperative morbidity remains significant, mainly due to the possibility of a postoperative pancreatic fistula, which can occur in 10-30% of the cases.(Bassi et al., 2005) A middle pancreatectomy or an enucleation are more challenging procedures than a distal resection. The incidence of postoperative complications also remains high (30-50 %) e.g. postoperative fistula.(Crippa et al., 2007, Christein et al., 2006, Zhou et al., 2014, Goudard et al., 2014, Del Chiaro et al., 2014, Kiely et al., 2003)

Laparoscopic and robotic procedures shorten the length of hospital stays and minimise the cosmetic impact of the surgical wound,(Ohtsuka et al., 2014). Therefore in addition to the traditional oncological pancreatic resections undertaken for the management of PDAC (Pancreaticoduodenectomy, distal or total pancreatectomy) patients with suspected benign PCL can also be offered a segmental resection (i.e. middle pancreatectomy or enucleation) or robotic procedure, depending on local expertise.(Gagner and Palermo, 2009, Del Chiaro et al., 2013, Tanaka et al., 2012) (Ohtsuka et al., 2024a)

1.1.1.13 Follow–up after surgery

Follow-up after surgery is based on surgical histology and varies by cyst subtype and if an invasive component is present. Complete resection of a benign MCN is considered to be curative, with several studies having reported zero recurrence after complete resection,(Keane et al., 2018) further postoperative surveillance is therefore not

required.(2018, Elta et al., 2018, Tanaka et al., 2012, Ohtsuka et al., 2024a) IPMNs may be associated with other synchronous pancreatic tumours or further IPMNs so any remaining pancreatic tissue should be surveyed with interval imaging.(Crippa et al., 2008, 2018, Elta et al., 2018, Tanaka et al., 2012) Invasive MCN or IPMN should be followed up in the same way as pancreatic ductal adenocarcinoma, with regular CA19-9 tests (when elevated pre-operatively) and at least annual cross-sectional imaging.(2018, Elta et al., 2018, Tanaka et al., 2012, Del Chiaro et al., 2013, Yasue et al., 1994, Duffy et al., 2010, Ohtsuka et al., 2024a) Whether this management impacts prognosis or recurrence, remains unknown.(Tanaka et al., 2012, Ohtsuka et al., 2024a)

In terms of neo-adjuvant or adjuvant chemotherapy regimens in malignant PCL, there is limited data and generally clinicians follow the same management as PDAC.(Del Chiaro et al., 2013) A recent series of patients with invasive IPMNs were treated with adjuvant therapy and a survival advantage was seen, particularly in those with positive resection margins or lymph node metastasis.(Testini et al., 2010b)

2 PAPERPAC: Pilot study of patient's perceptions of pancreatic screening and surveillance

Introduction

The first case reports of an IPMN were only published in the early 1980s.(Ohhashi, 1982) As such surveillance programs in pancreatic cancer are overall relatively early in their evolution, in comparison to other cancers such as colorectal, breast and lung cancer, where the natural history is better understood and national screening protocols are established. In pancreatic cancer surveillance there is also a lack of a simple screening test. Screening programmes therefore are forced to employ expensive, time consuming and potentially invasive tests such as MRCP and EUS to image the pancreas and programmes are only targeted at high-risk individuals [as outlined in Chapter 1].

With growing numbers of patients entering pancreatic surveillance, little is known about how patients perceive these programmes and their willingness to participate. Due to the dismal prognosis in pancreatic cancer, it is hypothesized that anxiety and stress could negatively affect adherence. A pilot questionnaire-based study was therefore undertaken to explore patient perceptions of long-term surveillance in a pancreatic cancer screening (PAPERPAC study).

Methods

2.1.1.1 Study setting and patients

The study was conducted at University College London Hospitals (UCLH) or the Royal Free Hospital (RFH) between May 2015 to May 2017. The study was approved by Institutional Review Board (approval number 1101CESC). The study is consistent with the Declaration of Helsinki.

Patients with cystic tumours of the pancreas, hereditary pancreatitis or a strong family history of pancreatic cancer who are eligible for surveillance for pancreatic cancer were

invited to participate in the study. Patients were contacted during surveillance clinic or endoscopy visits and returned questionnaires in person or by post.

2.1.1.2 Study Aims and Objectives

Primary: To evaluate how patients undergoing pancreatic surveillance assess their level of cancer risk.

Secondary:

- Compare how rates of cancer worry and perceived need for surveillance vary over time in patients enrolled in a surveillance programme for pancreatic cancer.
- Compare differences in rates of cancer worry before and after treatment (ablation/surgery) in patients under surveillance for cystic tumours of the pancreas.
- Compare how rates of cancer worry, perceived need for surveillance and overall surveillance experience differ between those in active surveillance to those who declined surveillance.

2.1.1.3 Inclusion Criteria

- Can provide informed written consent
- Patients under surveillance for cystic lesions of the pancreas
- Asymptomatic high-risk patients enrolled in the EUROPAC registry (including familial pancreatic cancer, hereditary pancreatitis Peutz-Jeghers Syndrome (PJS), hereditary pancreatitis and BRCA2 mutation carriers with a family history of pancreatic cancer, FAMMM)
- Age over 18 years

2.1.1.4 Questionnaire Design

The study questionnaire [Appendix 1] included 8 sections, 7 to be completed by patients and one to be completed by the physician or surgeon looking after the patient. Section A asked patients about perceived benefits and barriers to surveillance. Section B about mood. Levels of anxiety and depression were assess using the hospital anxiety and depression scale. Level of cancer worry was assessed using the Lerman Cancer worry scale. Section C asked patients to assess their risk of developing pancreatic cancer on a scale from 0-10 if participating and if not participating in a surveillance programme.

Section D asked if patients were currently participating in a surveillance programme. Section E asked about a patient's motivation to participate in surveillance. Section F asked patient about their experience of being in a surveillance programme. Section G was completed by patients who dropped out of surveillance. It asked open questions about reasons for not entering the programme or ultimately dropping out. Section H asked about the patients race and ethnicity, highest level of education, employment, risk factors for pancreatic cancer and current symptoms. Section J was completed by the patient's clinician documenting relevant past medical history, reasons for pancreatic surveillance, type and method of surveillance and the clinicians estimated risk of them developing pancreas cancer. Sections A, E and F included predetermined options based on the authors experience and feedback from 4 patients who reviewed the draft questionnaire during the London Cancer Patient forum on Cystic tumours of the Pancreas [Appendix 2 - held on the 3rd October 2014]. All of the sections included an area for open ended patient responses, that did not fit the predetermined answers.

Results

In this initial pilot study, 7 patients were enrolled, 5 were female. Median age 54 (range 43-70). 5 patients were white Caucasian and 2 Afro-Caribbean. 5 were under surveillance for a pancreatic cyst and 2 were in the EUROPAC surveillance program because of a strong family history of pancreas cancer. Five patients were currently employed or two were retired. Based on the hospital anxiety and depression score four patients reported signs of depression and one evidence of anxiety.

Benefit and Barriers to surveillance

All patients felt surveillance offered a sense of security and were reassured after their surveillance appointment. Five patients felt that surveillance was advantageous. Patient sited the following benefits to surveillance:

- "Helps demystify any fear"
- "To be informed"
- "Offers me peace of mind"
- "Surveillance to me means peace of mind. I do feel luck to have had an incidental finding of the cyst when having scans for different problems"

One patient stated they would prefer local surveillance to coming into the Tertiary centre, where most pancreatic surveillance programs are based. All patients reported that surveillance appointments reminded them of their disease. None of the patients reported issues with surveillance programs using blood-based biomarkers or MRI. One patient reported CT to be a barrier to participation. One patient reported EUS to be a barrier to participation. They commented "There are no barriers except for the EUS, which I find very unpleasant. Have had endoscopies before but find the pancreas one very uncomfortable and painful".

Perceived risk of cancer

Patients reported a median cancer risk of 4/10 while participating in a surveillance program but 7/10 without a surveillance programme. When asked if patients worry about pancreatic cancer, two out of seven reported they worry often, three reported they worry sometimes and two reported they worry rarely. Three of the patients reported that knowledge of their pancreatic lesion could affect their mood, and one reported this sometimes affected their daily activities.

Motivations to participate in surveillance

All patients reported participating in surveillance to enable early cancer detection when it is at a stage when it is treatable. Most patients (4/7) felt surveillance decreased their fear of their lesion and three reported feeling it provided control over their medical condition. Most patients (6/7) were referred to the screening program by their GP or local Gastroenterologist. One patient was referred by a family member. Two patients have had family members that have died from pancreas cancer. Two patients reported undertaking surveillance “for their children”. All were motivated by the opportunity to learn more about their condition, having contact with their clinical team and having the opportunity to contribute to research.

Surveillance experience

Patients universally reported that surveillance appointments provide an opportunity to discuss their concerns and worries. All patients reported that providers listened and had enough time during surveillance appointments. Three patients reported feeling nervous before surveillance. One patient reported they did not sleep well during the week prior to surveillance and postponed plans. One patient reported feeling dread commenting

"Although it is a worrying time, it is necessary as my mother died of pancreatic cancer". No patients stated they would like less frequent surveillance appointments.

Discussion

Due to the limited methods to stratify patients with pancreatic cystic lesions, growing numbers of patients are entering long-term surveillance with regular MRI scans or EUS.(Vege et al., 2015, 2018, Elta et al., 2018, Ohtsuka et al., 2024) This initial pilot study of patients perceptions of a pancreatic surveillance programme in a large UK tertiary HPB centre, found higher than expected levels of psychological distress. Rates of depression were reported in 57% compared to 11% in the general UK population.(Arias de la Torre et al., 2021) Several patients also reported feeling anxious and nervous prior to surveillance appointments. Similar levels of anxiety and somatization have been found in other IPMN surveillance cohorts.(Marinelli et al., 2020b) However this has not been a consistent finding, IPMN patients in the PACYFIC international cohort study reported low rates of psychological burden. Authors hypothesized that being in a research programme rather than a clinical programme may bring greater understanding of their condition as well as longer and more frequent clinic appointments, helping then to feel more reassured.(Overbeek et al., 2019a) Recognising the psychological burden that patients in pancreatic surveillance endure and addressing it, is likely to improve adherence and prevent requests for unnecessary medical checks or additional imaging, which can be costly and burdensome and without clear benefit to the patient.

Patients preferred minimally invasive and simple methods of surveillance. They felt blood-based markers and MRI were the most acceptable methods currently. This theme was also reinforced by patients attending the feedback groups at the patient forum on cystic tumors of the pancreas [Appendix 2] who strongly supported simple diagnostic tests for the diagnosis of pancreatic cancer being an important focus of future research.

This study also demonstrated that patients in pancreatic surveillance programmes, vastly overestimated their actual cancer risk which for most patient is between 5-10% over 10 years.(Pergolini et al., 2017, Goggins et al., 2020) This may be driven by an limited patient information about cancer risk in this condition. Multiple patients attending the forum on cystic tumors of the pancreas [Appendix 2] also expressed a

need for better patient resources on PCLs. We therefore collaborated with charity Pancreatic Cancer Action to develop a dedicated pamphlet for patients with pancreatic cysts and cystic tumors [Appendix 3]. This resource is now available for patients visiting surveillance clinics and online via the charity website.

This initial pilot study has several limitations, in particular the sample size is small and insufficient to make reliable conclusions on patient perceptions of current surveillance programmes for pancreas cancer. Validation of the trends raised will come from larger studies. The study is also limited to UK participants in a clinical programme. Future studies would benefit from including patients from multiple centres to be able to make more reliable and reproducible recommendations for modification to future pancreatic surveillance programmes.

3 NATURAL HISTORY OF PANCREATIC CYSTIC LESIONS: A RETROSPECTIVE UK COHORT STUDY

Introduction

Pancreatic cystic lesions (PCL) have become an increasingly common radiological finding, due largely to a greater availability and sensitivity of cross-sectional imaging.(Lee et al., 2010) It is estimated that around 15% of all cases of PDAC arise from a PCL.(Le et al., 2008) Early detection of high risk cysts therefore offers an opportunity for curative intervention in a disease with a dismal prognosis. However the natural history of these lesions remains poorly understood. Malignant transformation of premalignant cysts is estimated to occur at a rate of approximately 0.95% per year (Hruban et al., 2007) and take at least 10 years to progress to invasive cancer.(Handrich et al., 2005, Sohn et al., 2004) Most PCL studies to date have assessed cancer risk in surgical cohorts and on only a handful of studies have followed PCL under surveillance longterm. Cohorts of PCL that have undergone surgical resection will overestimate the cancer risk of most PCL. Therefore further large cohort studies that have undergone careful classification are required to better understand the malignant potential of PCL, outcomes and features that predict malignant transformation.

Study aims and objectives

The primary aim of this study was to assess the rate of malignant transformation in a large cohort of patients with a PCL who were followed in a surveillance programme at a tertiary UK Hepatopancreaticobiliary (HPB) centre. Secondary aims included assessing rates of surgical resection, cancer stage at diagnosis as well as clinical and imaging feature that predicted malignant transformation.

Methods

Ethical consideration

The study protocol was reviewed by the Health Research Authority and was exempt from formal ethical review.

Setting

A large regional hepatopancreaticobiliary cancer centre based across two tertiary-care hospitals; University College Hospital and the Royal Free Hospital, London.

Study design

Retrospective cohort study.

Management

In the UK there are no national guidelines for the management of PCL so management broadly followed International or European guideline recommendations [outlined in Chapter 1].(Tanaka et al., 2006, Tanaka et al., 2012, Ohtsuka et al., 2024a) High risk and complex cases were also discussed a weekly multidisciplinary team (MDT) meeting.

Study definitions

A symptomatic PCL was defined as a lesion identified on imaging undertaken for upper abdominal pain, obstructive jaundice or acute pancreatitis. For malignant lesions, weight loss, back pain and new-onset or deterioration of diabetes were also recognised as associated symptoms.

If multiple PCL were present, the characteristics of the most significant cyst were reported (i.e. the largest cyst or the cyst with associated worrisome features).

In this study, all mixed type IPMNs (MT-IPMN) i.e. IPMN lesions which met criteria of both main duct and branch duct IPMN, were managed as if they were a main duct IPMNs (MD-IPMN). Pathologically tumours were graded as having low (or intermediate) grade dysplasia, high-grade dysplasia including carcinoma *in situ* and malignant when invasive carcinoma was present, in line with the updated WHO classification of PCL.(Adsay et al., 2010)

Length of follow-up in the surveillance group was calculated from the time of the first to the last cross-sectional imaging study.

Inclusion criteria

The cohort included patients diagnosed with a PCL seen at UCLH or RFH between January 1st 2000 and December 31st 2013. Data was collected retrospectively. Patients were primarily identified from the radiology database (PACS: picture archiving and communication system, GE Healthcare, USA) and records of the weekly HPB multidisciplinary team (MDT) meeting. Databases were searched using the following terms; pancreatic cyst, serous cystadenoma, intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, mucinous cyst adenocarcinoma, solid pseudopapillary neoplasm, cystic pancreatic neuroendocrine tumour.

Exclusion Criteria

After initial case review, the following patients were excluded:

- patients < 18 years
- patients with solid lesions
- patients with an inflammatory pancreatic cyst - defined as a cyst measuring more than 4cm on CT/MRCP and located within or adjacent to the pancreas with a documented history of acute or chronic pancreatitis.

Data Recorded

The electronic medical records of each patients were reviewed and the following information recorded in an electronic spreadsheet; demographic information (age, sex), initial symptoms, history of pancreatitis or solid organ malignancy, family history of pancreatic cancer. Laboratory tests including elevations in serum amylase, CEA or CA19-9. Baseline imaging (computed tomography (CT), magnetic resonance cholangiopancreatography (MRCP)), and endoscopic studies (endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasound (EUS) with or without fine needle aspiration (FNA)) were recorded. Imaging features recorded included size (maximal dimension in mm), location and number of cystic lesions, presence of a solid

component, mural nodules, calcification of the cyst or the wall, wall thickening, presence of septations, features of acute or chronic pancreatitis, dilatation of the pancreatic duct and communication of the cystic lesion. For patients undergoing EUS-FNA, cytology and cyst fluid analysis (CEA and amylase) results were recorded. For patients referred for surgery, type of surgery, final histology and adverse events were recorded. Date of last imaging study was recorded to calculate length of follow-up.

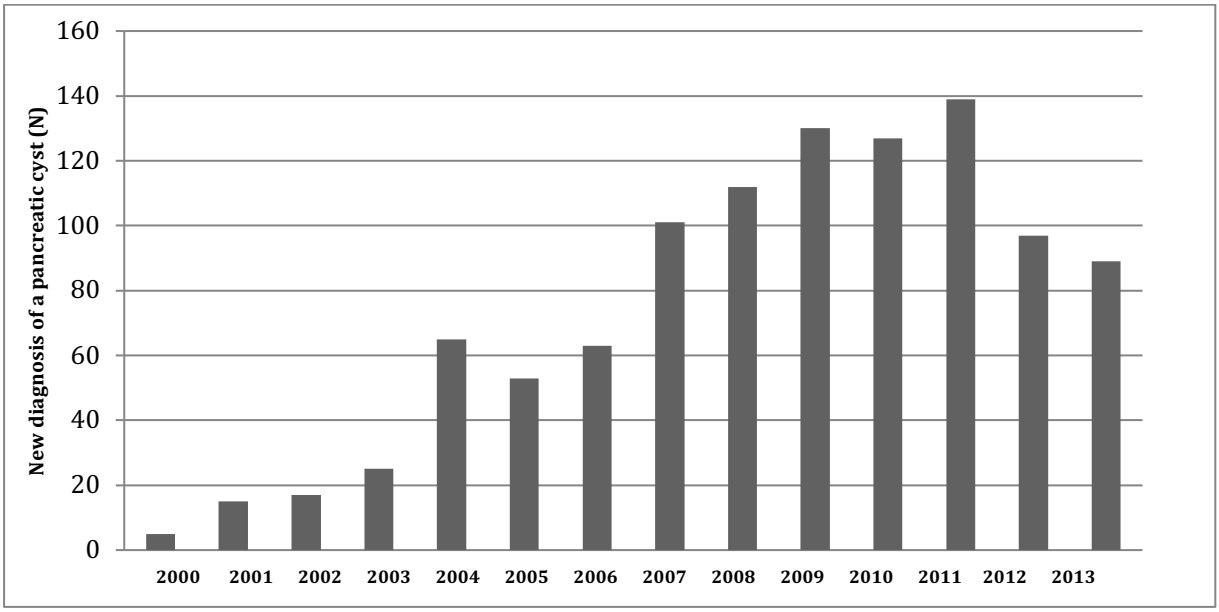
Statistical analyses

Statistical Package for Social Sciences for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Associations between malignancy and various clinical and radiographic characteristics were evaluated using a 2-sample *t* test for continuous variables, and a Chi-squared test for categorical variables.

Results

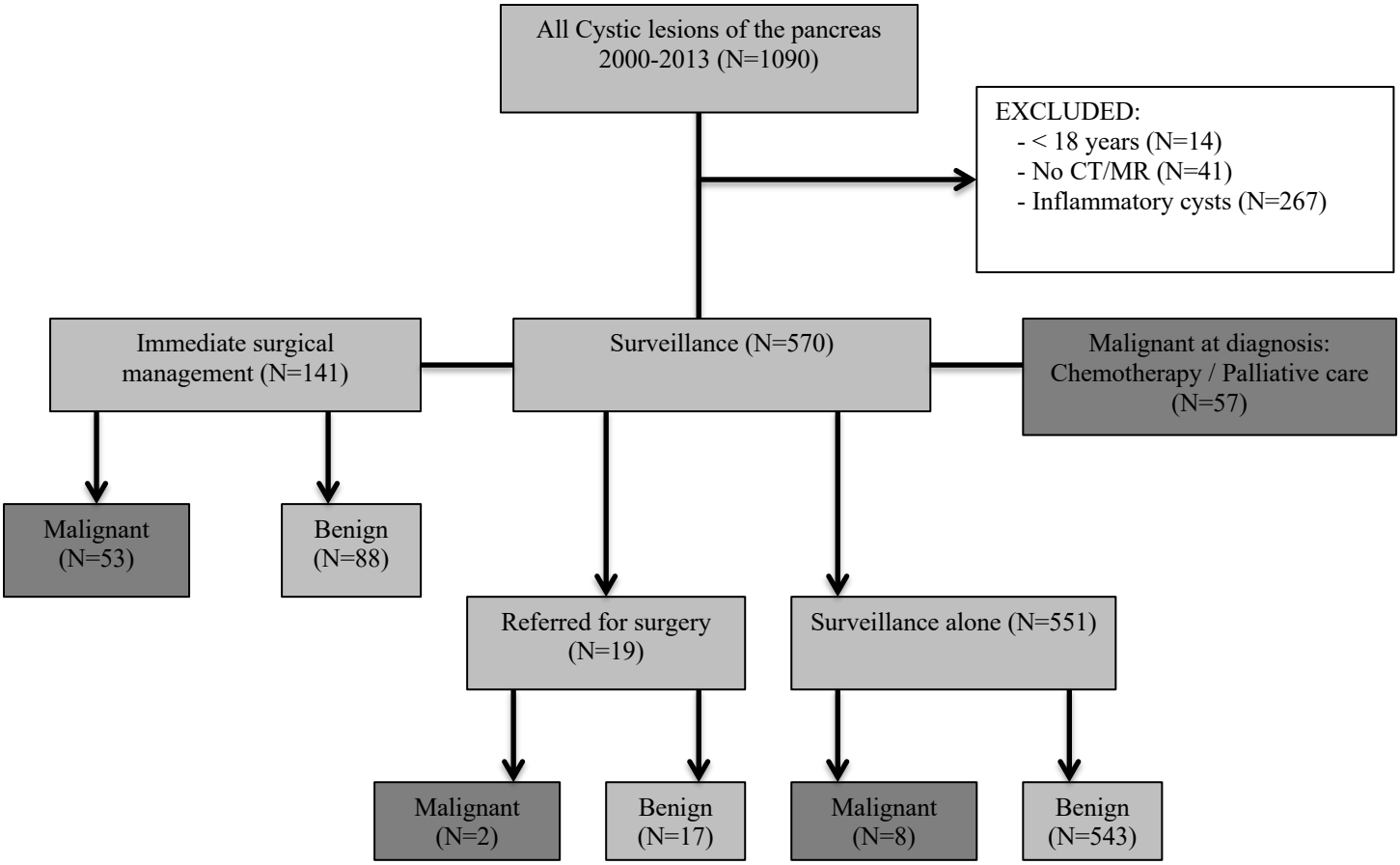
During the 14-year study period, 1090 patients with PCL were evaluated. The number of patients being diagnosed and referred to our unit with a PCL increased annually until 2011 and then plateaued at approximately 90 new referrals per year [Figure 3.1].

Figure 3.1 New patients with a PCL seen in our centre, by year, between 2000 and 2013



Fourteen patients were under 18 years and were excluded from the study, as were 41 patients who had had a PCL identified on EUS but without available cross-sectional imaging. During follow-up of >12 months, 267 cysts were confirmed as pseudocysts, necessitating endoscopic or percutaneous drainage, and were also excluded. The final cohort included 768 patients, with a PCL necessitating surveillance, surgery or oncologic management [Figure 3.2].

Figure 3.2. Study selection flowchart and risk of invasive pancreatic cancer by management subtype (surgery vs surveillance)



Diagnostic work-up prior to MDT

97% (743/768) of patients assessed at the MDT had had a CT; the remaining 3% of patients underwent an MR / MRCP. 34% (259/768) of patients had both a CT and MRI as part of their diagnostic work-up. In patients with an indeterminate PCL, or worrisome feature on cross-sectional imaging, an EUS was performed in 39% (301/768), an ERCP in 9% (67/768) and a percutaneous biopsy in 4% (34/768).

Surgery

Of the 768 patients included in the study, 141 (18%) were referred for immediate surgical resection; a further 19 who were initially managed by surveillance eventually underwent pancreatic resection. 79 patients had an open or laparoscopic distal pancreatectomy with or without splenectomy, 65 had a Whipple's or pylorus-preserving pancreaticoduodenectomy, 10 had a total pancreatectomy and the remaining 6 patients had an enucleation. The 30-day mortality following pancreatic resection for a PCL was 1% (2/160). Post-operatively, patients were followed up for a median of 15 (range 0-121) months.

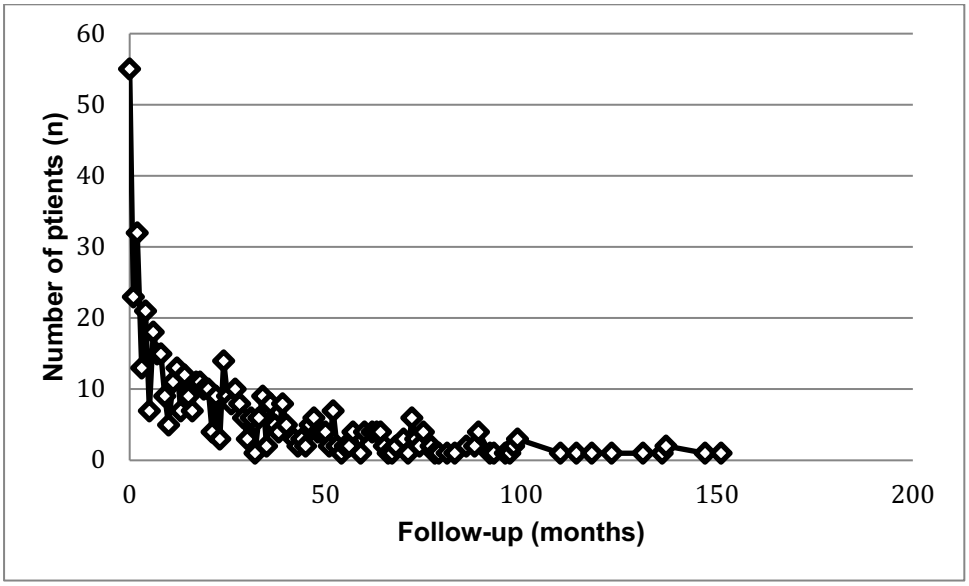
Of the 56 patients who underwent pancreatic resection for malignant disease, 16 received adjuvant chemotherapy and 20% (11/56) died during follow-up. Of these, 9 cases were as a result of pancreatic cancer, one patient died unexpectedly while in hospital from an undetermined cause and one died from metastatic breast cancer.

Median survival following resection of a malignant PCL was 8 (range: 0-19) months for PDAC (no PCL), 16 (range: 0-91) months for a malignant IPMN, 32 (range: 5-84) months for a PanNET, 26 (range: 7-35) months for a SPPN and 43 (range: 11-69) months for a malignant MCN.

Surveillance

During the study period 570 patients entered the surveillance programme. The median follow-up was 18 months (range, 0-151 months) but dropout from surveillance was considerable after 12 months [Figure 3.3].

Figure 3.3 Time spent in active surveillance for a PCL in our centre; if surveyed during the study period 2000-2013



The median age of patients managed by surveillance was 67 years (range 20-92), which was older than those receiving surgical management. The median size of a cyst at entry to the surveillance programme was 20mm (range 3-130), which was smaller than all other management subtypes [Table 3.1a and b].

Table 3.1a Comparison of clinical features by management and cyst subtype for surgically resected lesions

Cyst	N	Median Age (range)	Male	Female	Symptoms	Clinical history of pancreatitis	Previous cancer	Family history of PDAC / syndrome	Median CA 19-9	Range CA 19-9
MANAGEMENT										
Immediate surgical management	141	61 (23-83)	40% (56)	60% (85)	50% (64)	23% (31)	11% (15)	4% (6)	15.3	(1.4-5604)
Surveillance	570	67 (20-92)	47% (266)	53% (304)	36% (182)	27% (153)	25% (142)	5% (30)	11.4	(<1-2102)
Chemotherapy / Palliative care (malignant at presentation)	57	69 (43-95)	63% (36)	37% (21)	76% (40)	20% (11)	16% (9)	4% (2)	106	(<1-4981)
SURGERY – BENIGN										
IPMN (benign)	44	65 (42-82)	52% (23)	48% (21)	49% (18)	33% (13)	12% (5)	0% (0)	15.75	(<1-460)
MCN	20	60 (27-76)	5% (1)	95% (19)	50% (7)	16% (3)	5% (1)	0% (0)	15	(1.4-36)
SCN	24	68 (49-78)	12% (3)	88% (21)	18% (3)	0% (0)	17% (4)	4% (1)	6.5	(1.4-49)
Pseudocyst	11	50 (34-66)	55% (6)	45% (5)	70% (7)	73% (8)	0% (0)	9% (1)	32	(8.8-5604)
SPPN	7	28 (23-49)	0% (0)	100% (7)	33% (2)	14% (1)	0% (0)	0% (0)	6.45	(3.5-17.4)
SURGERY – MALIGNANT										

IPMN (malignant)	17	72 (54-81)	47% (8)	53% (9)	79% (11)	25% (4)	19% (3)	13% (2)	10.8	(8-19.6)
PanNET	12	55 (36-77)	50% (6)	50% (6)	17% (2)	0% (0)	17% (2)	8% (1)	32.3	(16.9-108.2)
PDAC	9	68 (54-77)	55% (5)	44% (4)	75% (6)	33% (3)	0% (0)	0% (0)	37	(6-119.6)
MCN (malignant)	5	53 (41-69)	40% (2)	60% (3)	75% (3)	20% (1)	0% (0)	0% (0)	6	6

IPMN: Intraductal papillary mucinous neoplasm, PDAC: pancreatic ductal adenocarcinoma, MCN: Mucinous cystic neoplasm, SCN: Serous cystic neoplasm, SPN: Solid pseudopapillary neoplasm, PanNET: Pancreatic neuroendocrine tumour

Table 3.1b Comparison of cross-sectional imaging features by management and cyst subtype in resected PCL

Cyst	N	Median Size + range(mm)	Head / Neck	Body / Tail	Multiple pancreatic	Solid component	Septations	Acute pancreatitis	Chronic pancreatitis	PD dilatation	PD com	CBD dilatation	LN enlargement	Vascular compromise	Concomitant cysts in other
MANAGEMENT															
Immediate surgical management	141	33 (3-230)	41% (58)	59% (83)	14% (20)	22% (31)	9% (13)	4% (5)	18% (25)	28% (39)	8% (11)	14% (19)	6% (9)	4% (6)	27% (38)
Surveillance	570	20 (3-130)	56% (315)	44% (245)	35% (197)	10% (59)	11% (65)	11% (64)	27% (154)	27% (151)	10% (56)	13% (72)	6% (35)	3% (14)	35% (202)
Chemotherapy / Palliative care (malignant at presentation)	57	41 (7-250)	70% (40)	30% (17)	23% (13)	42% (24)	7% (4)	9% (5)	25% (14)	40% (23)	2% (1)	39% (22)	12% (7)	9% (5)	21% (12)
SURGERY – BENIGN															
IPMN (benign)	44	30 (11-130)	61% (27)	39% (17)	18% (8)	11% (5)	11% (5)	2% (1)	21% (9)	43% (19)	25% (11)	11% (5)	7% (3)	2% (1)	27% (12)
MCN	20	42.5 (18-120)	20% (4)	80% (16)	0% (0)	35% (7)	30% (6)	0% (0)	10% (2)	10% (2)	10% (2)	0% (0)	0% (0)	5% (1)	25% (5)
SCN	24	42.5 (14-159)	29% (7)	71% (17)	8% (2)	25% (6)	13% (3)	0% (0)	17% (4)	4% (1)	4% (1)	4% (1)	0% (0)	0% (0)	25% (6)
Pseudocyst	11	36 (20-90)	64% (7)	36% (4)	46% (5)	9% (1)	0% (0)	55% (6)	46% (5)	36% (4)	0% (0)	9% (1)	18% (2)	27% (3)	0% (0)
SPPN	7	59 (20-150)	0% (0)	100% (7)	0% (0)	29% (2)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)	14% (1)	0% (0)	14% (1)
SURGERY-MALIGNANT															
IPMN (malignant)	17	23 (15-56)	53% (9)	47% (8)	29% (5)	18% (3)	0% (0)	6% (1)	29% (5)	47% (8)	0% (0)	47% (8)	0% (0)	0% (0)	53% (9)
PanNET	12	23.5 (15-94)	25% (3)	75% (9)	17% (2)	33% (4)	0% (0)	0% (0)	0% (0)	0% (0)	17% (2)	0% (0)	8% (1)	0% (0)	33% (4)
PDAC	9	25 (15-59)	44% (4)	55% (5)	11% (1)	44% (4)	11% (1)	0% (0)	11% (1)	67% (6)	11% (1)	44% (4)	22% (2)	0% (0)	22% (2)
Mucinous cyst adenocarcinoma	5	120 (23-230)	40% (2)	60% (3)	0% (0)	40% (2)	0% (0)	0% (0)	40% (2)	20% (1)	0% (0/	0% (0)	0% (0)	20% (1)	0% (0)

IPMN: Intraductal papillary mucinous neoplasm, PDAC: pancreatic ductal adenocarcinoma, MCN: Mucinous cystic neoplasm, SCN: Serous cystic neoplasm, SPPN: Solid pseudopapillary neoplasm, PanNET: Pancreatic neuroendocrine tumour, PD com: PD communication

Of the 451 patients with serial imaging during surveillance, 76 cysts (17%) increased in size, 272 remained stable, 50 decreased in size and 54 resolved [Table 3.2]. During follow up, 3% (19/570) of patients were ultimately referred to surgery and 2% (10/570) developed pancreatic cancer [Figure 3.2].

Table 3.2 Proportion of patients with a PCL that increased, decreased, remained stable or resolved while under surveillance with interval imaging during the study period.

	N = 452	Number PDAC	Median length of follow up	Range	Referred to surgery	Currently in active follow up
Increased	76	9	29	(0-137)	13 (1 malignant)	21
Stable	272	1	22	(0-151)	5 (1 malignant)	69
Decreased	50	0	24	(2-83)	1	12
Resolved	54	0	26	(3-147)	0	2

Of the 10 patients that underwent malignant transformation, nine had a PCL that increased in size and all developing worrying features [Table 3.3]. Seven of the 10 patients had an EUS; which was non-diagnostic in two cases and suggested benign pathology in the remaining cases. Only two of the 10 patients were ultimately referred for surgical resection; both had R0 resections and one developed recurrence at 13 months. The other eight patients were managed non-operatively, five having been discharged from active surveillance, as they were no longer fit for surgery. Two further patients were discharged from surveillance because the PCL was presumed to be an inflammatory cyst and one patient ultimately refused surgical intervention after developing unresectable pancreatic cancer [Table 3.3].

Of the 3% of patients in surveillance who were ultimately referred for surgery, 47% (9/19) were found to have a non-mucinous, non-malignant cyst on final pathology [Table 3.1b]. These patients had been in a surveillance programme for a median of 37 months prior to surgery (range: 7-64 months).

Table 3.3 Characteristics of the patients and route to diagnosis in those who underwent malignant transformation of a PCL during or following participation in a PCL surveillance programme

Surgical management					
	Age	**Sex**	**Time to malignant transformation from diagnosis (months)**	**Route to diagnosis**	**Management**
1	77	M	18	Investigations for recurrent pancreatitis revealed a 2cm cyst in the uncinate. Entered surveillance, CA 19-9 rising 69.9 IU/ml. EUS-FNA revealed the cyst was communicating with a dilated main PD. Cytology non-diagnostic. ERCP – pathognomonic findings of MD-IPMN.	**Surgery:** Whipple. **Histology:** T2N0MXR0 tumour arising from a MD-IPMN. **Outcome:** No recurrence during 20-months of follow-up.
2	68	F	18	Imaging following acute necrotising gallstone pancreatitis revealed a 5.9cm cyst in the head of the pancreas with dilated main PD. Thought to be a symptomatic pseudocyst so a EUS guided cystenterostomy was performed. Following removal of the stents a small cyst persisted which had a solid component. CA 19-9 rising (1869.0 IU/ml). Repeat EUS-FNA: cytology consistent with a pseudocyst but cyst fluid CEA 105 ng/ml, amylase 1598 IU/L.	**Surgery:** Total pancreatectomy + splenectomy + PV reconstruction **Histology:** T3N1 (1/24) MxR0 PDAC + pseudocyst. **Outcome:** Adjuvant chemotherapy with gemcitabine. 13 months on PET-CT confirms recurrent disease – no further chemotherapy, asymptomatic.
Non-operative management					

	Age	Sex	Time to malignant transformation from diagnosis (months)	Route to diagnosis	Management
1	75	M	18	Right hemicolectomy for a Dukes B colorectal cancer, complicated by an anastomotic leak and prolonged ITU stay. Follow-up imaging revealed an incidental 23mm cyst in the pancreatic tail. EUS-FNA – cytology: atypical cells consistent with IPMN. 14 months later presented with jaundice. Cyst had increased to 3cm + solid component and dilated main PD. CA 19-9 rising (1879.0 IU/ml). Further EUS-FNA; cytology – atypia, histology - IPMN.	Resectable disease but patient refused pancreatic surgery. ERCP + metal stent inserted. Patient died 4 months later.
2	78	F	24	Admitted with deranged LFTs and abdominal pain. Imaging revealed cirrhosis and chronic pancreatitis + 12cm PCL with septations and a solid component. Developed nausea and weight loss so underwent percutaneous drainage of a presumed pseudocyst cyst at a local hospital. Follow-up imaging revealed unresectable PDAC with vascular incasement. EUS-FNA – cytology: well-differentiated PanNET but IHC not supportive, CEA 36223 ug/L. Amylase < 3 IU/L.	Unresectable disease. Palliative care – died 3 months later.
3	71	F	76	Imaging for autoimmune hepatitis revealed multiple incidental PCL with features of chronic pancreatitis. Thought to be multiple pseudocysts and therefore not actively followed-up. Patient requested a second opinion and when reimaged lesions had undergone malignant transformation.	Multiple comorbidities unfit for surgical resection – tissue diagnosis not pursued. Palliative care – subsequently died.
4	70	M	62	Family history of PDAC. Abdominal imaging for renal calculi revealed a 35mm cyst is the head of the pancreas with a dilated main PD and multiple other cysts. EUS-FNA; cytology consistent with low grade IPMN. CA 19-9 66 IU/ml. Discharged from active surveillance because of comorbidity after 18 months. Recommenced after 23 months & had developed a metastatic liver lesion of upper GI origin.	Unresectable disease. Palliative cisplatin + gemcitabine chemotherapy. Died 36 months later
5	81	M	24	Investigated for deteriorating blood sugars (recently diagnosed Type 2 DM). Abdominal CT: dilated PD without cause. EUS-FNA: 8mm multiloculated cyst in the pancreatic tail, with mural nodule. Cytology: possible mucin-secreting tumour but non-diagnostic. CA 19-9 101 IU/ml. Discharged from active surveillance as no longer a fit for surgical resection. Represented with metastatic PDAC 6 months later. ERCP + biliary brushings: IPMN with atypia.	Unresectable disease. Palliative care – subsequently died 36 months later.
6	71	F	19	Background of pancreatic trauma in 1971, requiring pancreatic surgery + drainage. Investigated for faecal inconsonance, colonic polyps and exocrine insufficiency with a CT pneumocolon. Found to a dilated main PD + 14mm cyst in the pancreatic tail, presumed due to trauma. Intermittent surveillance with colonic polyp surveillance via CT over 19 months. Developed significant weight loss and repeat imaging revealed unresectable disease. Cytology from pleural aspirate confirmed metastatic adenocarcinoma (? PanNET).	Unresectable disease, palliative care, died 8 months after diagnosis.
7	78	F	73	Right hemicolectomy for Dukes B tumour, T3N0M0. During follow-up noted to have a dilated main PD. Over time became associated with a cystic and then a solid lesion. CA 19-9 rising (526.2 IU/ml). August 2012 – cytology from EUS-FNA suggestive of chronic pancreatitis but percutaneous biopsy confirmed moderately differentiated PDAC.	Locally advanced disease but unfit for surgical resection because of comorbidities. No chemotherapy, clinically stable 26 months after diagnosis.
8	87	M	12	CT pneumocolon for abdominal pain and diarrhoea revealed a dilated main PD and side branches with retroperitoneal LNs. Stable on imaging for 11 months then represented with jaundice and cholangitis. CA19-9 2102 IU/ml. ERCP + brushings – non-diagnostic.	Unresectable disease. Multiple comorbidities. Refused chemotherapy. Histological diagnosis not pursued. Palliative care – subsequently died.

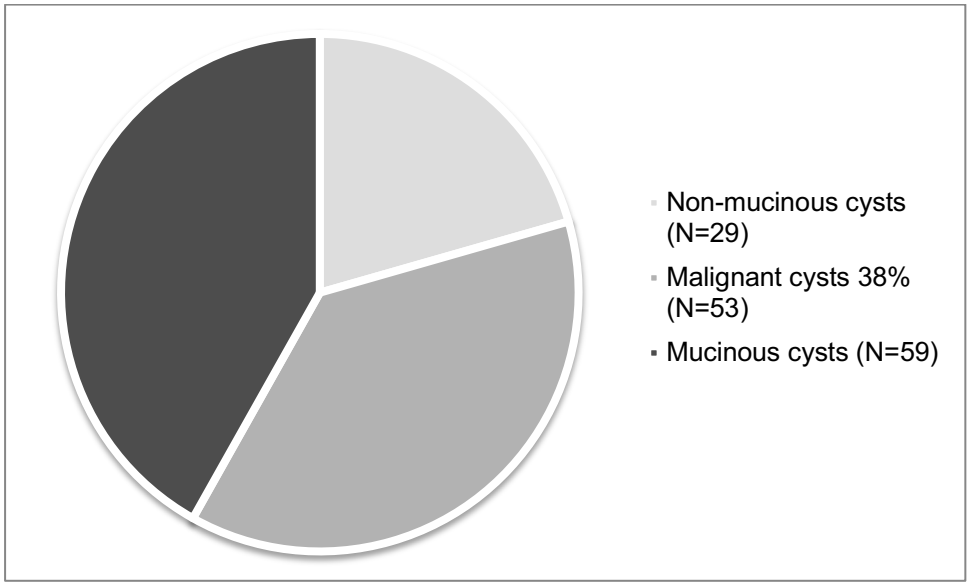
PDAC: Pancreatic ductal adenocarcinoma. PD: Pancreatic duct. EUS-FNA: Endoscopic ultrasound and fine needle aspiration. ERCP: Endoscopic retrograde cholangiopancreatography. LN: Lymph nodes. PCL: Pancreatic cystic lesion, DM: Diabetes mellitus, IHC: immunohistochemisty

Features of malignant transformation

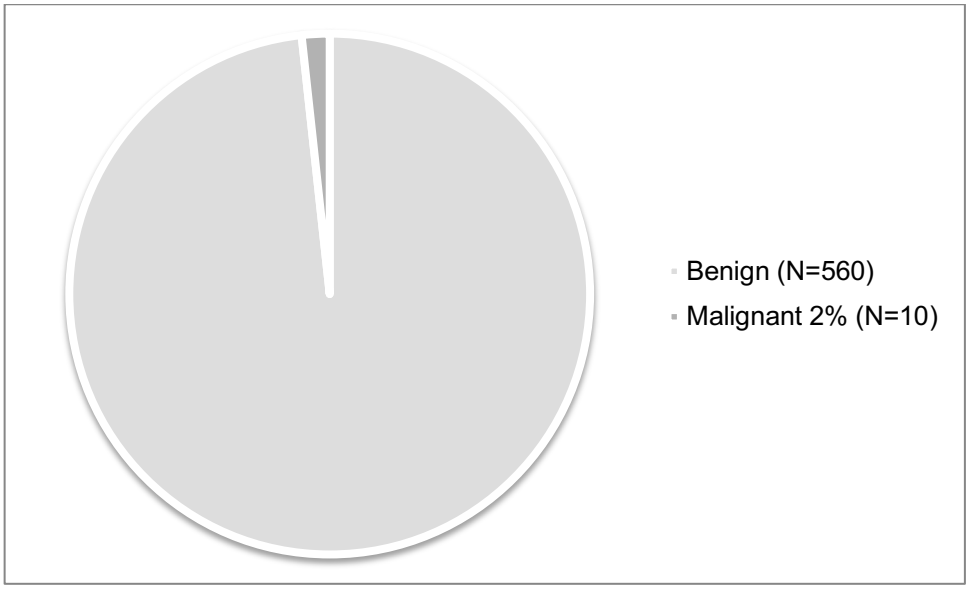
During the study period, 16% (120/768) of patients were diagnosed with pancreatic cancer of whom 46% (55/120) underwent surgical resection. Of the patients initially referred for surgery, 38% (53/141) were diagnosed with a malignant pancreatic cyst compared to 2% (10/570) in the surveillance group [Figure 3.4].

Figure 3.4 Incidence of pancreatic cancer in patients with a PCL managed by immediate surgical resection (a) vs surveillance (b)

a. Immediate surgical management

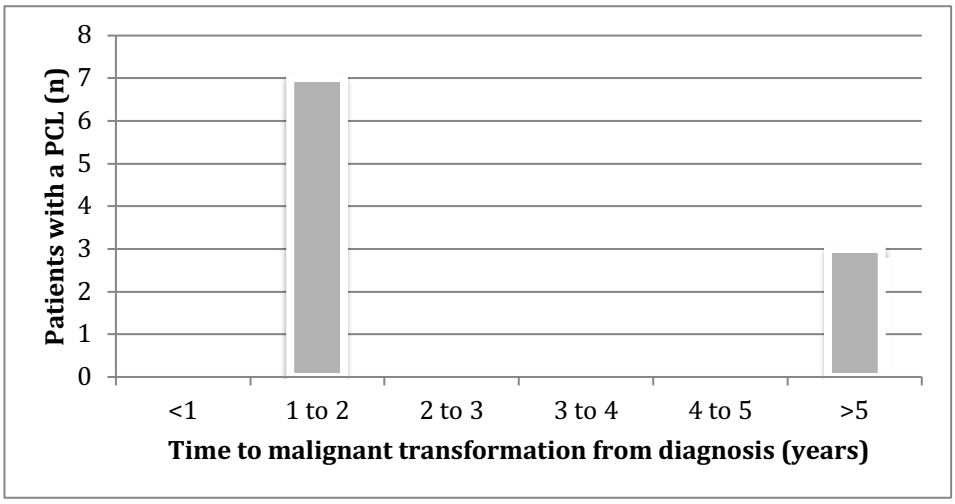


b. Surveillance



92% (110/120) of all patients with malignancy were diagnosed at the time the PCL was detected. The median age at diagnosis for a malignant PCL was 67 (23-95) years. 64% (67/105) were symptomatic. The median size of a malignant PCL at diagnosis was 35 (6-250) mm. 39% (47/120) had an associated solid component and 38% (45/120) had pancreatic duct dilation. Most patients developing malignancy did so within 2 years of diagnosis, but 30% underwent malignant transformation after more than 5 years follow up [Figure 3.5].

Figure 3.5 Time to malignant transformation for patients with a PCL under surveillance

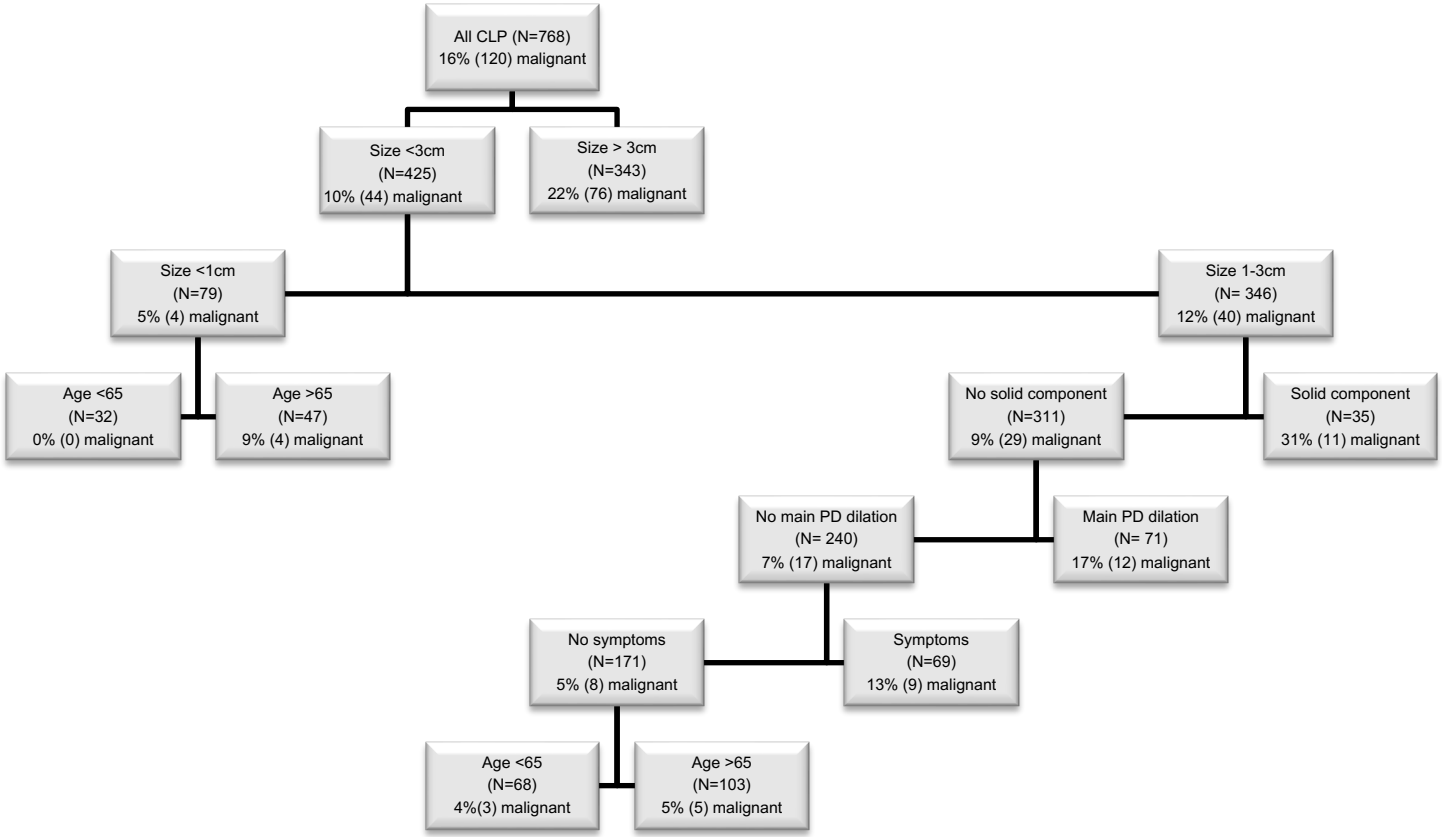


The overall sensitivity of current diagnostic tests leading to immediate surgery for high-risk PCL (malignant or mucinous) was high (92%) but specificity was low (5%). Table 3.1 a. and b, compares cross-sectional imaging features by management and cyst subtype. Cysts that were malignant at diagnosis or were referred for immediate surgical resection were larger than cysts managed by follow-up surveillance. A mural nodule was an exceptionally rare radiological finding in patients in this study, but a solid component was present in 42% of patients with malignant cysts managed by chemotherapy and palliative care compared to 22% of PCL referred to surgery and only 10% of PCL entering surveillance. Pancreatic and common bile duct dilatation along with lymph node enlargement were also common features of malignant cysts managed non-operatively.

International and European guidelines stratify patients based on high-risk stigmata and cyst size. (Tanaka et al., 2006, Tanaka et al., 2012, Ohtsuka et al., 2024a, 2018) The cohort was first stratified by cyst size, as this remains the most common way of

differentiating cysts for surveillance (<3cm) vs. surgery (>3cm). High risk stigmata were then included in turn based on their associate risk of malignancy. High risk stigmata were not used in the first tier of stratification as they are a rare finding in the cohort overall, which contained mostly low risk lesions that were under surveillance. [Figure 3.6].

Figure 3.6 Recursive partitioning analysis decision tree to assess risk of pancreatic cancer based on cyst size and high-risk stigmata in PCL



PCL: pancreatic cystic lesion, PD: pancreatic duct

Discussion

In this large UK cohort study of patients with a PCL managed at a tertiary referral HPB centre, patients with high risk and worrisome features who were referred for immediate surgery and had much higher rates of malignancy than those managed by surveillance with interval imaging (38% vs. 2%). Rates of malignant transformation were overall similar to other international cohorts.(Allen et al., 2006, Das et al., 2008, Walsh et al., 2008, Walsh et al., 2005) ,

Pre-operative investigations had a high sensitivity for detecting malignancy, but were associated with a poor specificity and a substantial proportion of patients underwent unnecessary surgery (21% of immediate and 47% of delayed pancreatic resections had completely benign disease e.g. SCN which would have never undergone malignant transformation). Other groups have reported similar findings. Pre-operative cross-sectional imaging correlates with surgical pathology in only 30-74% of cases.(Khashab et al., 2011) This is significant as pancreatic surgery has an associated morbidity of 20.8-59% and mortality of 0-7.1% (1% in our cohort), even in high volume centres.(Diener et al., 2007) The addition of EUS in this study did not improve the accuracy pre operative diagnosis. However cyst fluid was only sent for cytology and CEA. Low cytological yields from PCL have also been reported by a number of other groups, likely due to the paucity of cells in cyst fluid.(Brugge et al., 2004a, Minami et al., 1989, Koito et al., 1998, Bassi et al., 2003, Gaujoux et al., 2011, de Jong et al., 2011) Improved cyst fluid biomarkers may improve the utility of EUS in the pre operative diagnosis of PCL.

Of the 10 patients in the surveillance group who ultimately developed pancreatic cancer, two were referred for surgery, one underwent a Whipple's resection and the other had a total pancreatectomy and splenectomy. Both were R0 resections. The patient undergoing a total pancreatectomy developed recurrence 13 months after surgery. Most malignant lesions were detected within 1-2 years of diagnosis, but 2 patients were diagnosed more than 5 years after entering surveillance. This has also been reported by other groups (Wu et al., 2014a), supporting long-term surveillance for patients with mucinous PCL who remain fit for surgical resection.

Strengths and Limitations of the study

This study has several strengths; its large size and carefully characterised clinical and radiological characteristics by cyst subtype and management. However due to the retrospective nature of the data collection, symptoms and high risk stigmata or worrisome features may have been underestimated. Serial imaging in this study was reported by a large pool of Radiologists and variation in cyst size measurements and presence of high-risk features were observed. A multivariate analysis was therefore not performed to predict high risk features in PCL, due to concerns about the accuracy of the retrospectively collected data. A recursive partitioning analysis was included instead to reflect the typical clinical decision tree in PCL. Cyst size was included first followed by the presence of high-risk stigmata. The order of high-risk stigmata was based on frequency in the cohort. However, in a better characterized prospective longitudinal cohort of patients with a PCL, artificial intelligence and machine learning techniques (Lavista Ferres et al., 2024) could be employed to develop predictive models for the diagnosis of PCL under surveillance.

Although the study was conducted over a 14-year period, patient drop out rates were high and the median follow-up was only 18 months. This short follow up period is unlikely to have been long enough to capture all cases of interval malignant transformation, potentially underestimating the risk in this cohort. However, the actual rate of malignancy in this study was 16% (120/768). This is considerably higher than rates reported by community based population studies with largely low-risk patients.(Wu et al., 2014a) This probably reflects increased rates of high risk referrals to our HPB centre, which is a trend that has been reported by other groups.(Das et al., 2008, Walsh et al., 2008, Walsh et al., 2005). This will likely bias our cohort reducing the applicability of the recursive partitioning model outside of HPB centres.

Conclusions

In this large surveillance cohort from a tertiary referral HPB centre the overall rate of malignancy in PCL was 16%, which is lower than most surgical series but higher than community-based studies. The majority of malignant lesions (92%) were detected at the time of diagnosis. The sensitivity of current diagnostic tests leading to immediate

surgery for high-risk PCL (malignant or mucinous) was high (92%) but specificity was low (just 5%). Surveillance of PCL without high-risk features was associated with a low incidence of cancer development (2%) supporting the use of worrisome clinical and radiological features (older age, symptoms, increasing size of the lesion and the presence of a solid component) in the initial stratification of PCL.

4 Systematic review of biomarkers for Pancreatic Ductal Adenocarcinoma

Introduction

Despite improved diagnostic techniques, detecting pancreatic malignancy remains a significant clinical challenge. Common symptoms and radiological findings can overlap with benign disease, Endoscopic retrograde cholangiopancreatography (ERCP) or endoscopic ultrasound (EUS) used to obtain pathological confirmation of cancer. (Saluja SS, 2007, Fernandez- Esparrach G, 2007, Sai JK, 2009) However biliary brush cytology and EUS guided fine needle aspirations can have very low sensitivities for malignancy particularly in early disease when tumours are small, (Lee, 2006, Kalaitzakis et al., 2011) Some patients therfore require multiple procedures to obtain a final diagnosis.(De Bellis et al., 2002, Harewood et al., 2004, Moreno Luna et al., 2006)

There has therefore been a growing interest in development of simple tests to streamline the diagnosis to pancreaticobiliary malignancy and guide appropriate and timely therapy for patients. Identifying better diagnostic tools would also make screening and surveillance, for PDAC, particularly in high-risk populations.(Hippisley-Cox and Coupland, 2012, Coupland et al., 2012, Klein et al., 2013) This would enable the detection of tumours at an earlier stage when curative resection is possible, leading to substantial improvements in survival.(Ariyama et al., 1986) This review provides an overview of the diagnostic biomarkers for pancreatic cancer.

Methods

A systematic review of the literature using the PubMed, EMBASE and the Cochrane Library. The search was limited to studies published in the English language between January 2013 and March 2017. MeSH terms were decided by a consensus of the authors and included pancreatic cancer and biomarker. The search was restricted to title, abstract and keywords. Articles that described outcomes for fewer than five patients were excluded. Case reports, abstracts and reviews were excluded. All references were screened for potentially relevant studies not identified in the initial literature search.

The following variables were extracted for each report when available: Number of malignant and benign cases, sensitivity, specificity and AUC. 110 papers were included in the final review.

Results

4.1.1.1 Serum biomarkers and blood tests

Carbohydrate antigen (CA) 19-9 is the most widely used tumour marker in pancreaticobiliary malignancy. Overall sensitivity (78 - 89%) and specificity are low (specificity 67 - 87%) and in around 7% of the population who lack the Lewis (a) antigen, CA19-9 will remain negative.(Locker et al., 2006) In small tumours sensitivity decreases further. The marker can also be elevated in a number of other malignant diseases (e.g., gastric adenocarcinoma) and benign diseases, particularly those causing jaundice (e.g. primary biliary cirrhosis, cholestasis, cholangitis) and in smokers.(Bonney et al., 2008) In addition variation has been reported between commercially available assays, which may impact on interpretation.(Hotakainen et al., 2009) To improve the sensitivity of the marker in current clinical practice it is therefore always interpreted in the context of cross-sectional imaging findings.(Locker et al., 2006)

Other commercially available tumour makers that have a role in diagnosing pancreaticobiliary cancer include carcinoembryonic antigen (CEA) and carbohydrate antigen 125 (CA125). CEA is a glycosyl phosphatidyl inositol cell surface anchored glycoprotein that is involved in cell adhesion. When elevated it is highly suggestive of colorectal cancer, but is also increased in approximately a third of patients with BTC(Abi-Rached and Neugut, 1995, Lazaridis and Gores, 2006, Khan et al., 2012c). CA125 is a protein encoded by the MUC16 gene and is a large membrane-associated glycoprotein with a single transmembrane domain. When elevated it is suggestive of ovarian cancer but is also increased in approximately 40-50% of patients with pancreaticobiliary malignancy, particularly when there is peritoneal involvement.(Khan et al., 2012c)

Due to the limitations of existing biomarkers, over the last few years several studies have evaluated various combinations of biomarkers to supplement or ultimately replace existing biomarkers. Biomarker panels using combinations of markers, often including CA19-9 have been particularly successful in detecting small tumours and early disease. Validation studies have also shown that these markers can differentiate PDAC from relevant benign conditions in some cases detect tumours up to 1 year prior to diagnosis with a specificity of 95% and sensitivity of 68%.[7] [Table 4.1]

Table 4.1 Summary table of serum protein biomarkers for pancreatic cancer, published between 2013 – 2017

Author and year	Biomarker / Combination (Serum)	PDAC	Benign Control	Healthy Volunteer	Sensitivity	Specificity	AUC
Single biomarkers							
Sogawa K et al. 2016(Sogawa et al., 2016)	C4BPA	52	20	40	67	95	0.860
Rychlikova J et al. 2016(Rychlikova et al., 2016)	Osteopontin	64	71	48	-	-	-
Lin C et al. 2016(Lin et al., 2016)	APOA-I	78	-	36	96	72.2	0.880
Lin C et al. 2016(Lin et al., 2016)	TF	78	-	36	75	72.8	0.760
Zhao J et al. 2016	TGF-β1	146	58	-	83	76.4	0.794
Guo X et al. 2016(Guo et al., 2016)	Dysbindin	250	80	150	81.9	84.7	0.849
Han SX et al. 2015(Han et al., 2015)	Dickkopf-1	140	-	92	89.3	79.3	0.919
Qu D. et al. 2015(Qu et al., 2015)	DCLK1	74	74	-	-	-	0.740
Dong H et al. 2015(Dong et al., 2015)	Survivin	80	-	80	-	-	-
Gebauer F et al. 2014(Gebauer et al., 2014)	EpCAM	66	43	104	66.7	77.5	-
Wang X et al. 2014(Wang et al., 2014)	MIC-1	807	165	500	65.8	96.4	0.935
Kendrick ZW et al. 2014(Kendrick et al., 2014)	IGFBP2	84	40	84	22	95	0.655
Kendrick ZW et al. 2014(Kendrick et al., 2014)	MSLN	84	40	84	17	95	0.668
Kang CY et al. 2014(Kang et al., 2014)	COL6A3	44	46	30	-	-	0.975
Willumsen N et al. 2013(Willumsen et al., 2013)	C1M	15	-	33	-	-	0.830
Willumsen N. et al. 2013(Willumsen et al., 2013)	C3M	15	-	33	-	-	0.880
Willumsen N. et al. 2013(Willumsen et al., 2013)	C4M	15	-	33	-	-	0.940
Willumsen N. et al. 2013(Willumsen et al., 2013)	C4M12a1	15	-	33	-	-	0.890
Falco A et al. 2013(Falco et al., 2013)	BAG3	52	-	44	75	75	0.770
Falco A et al. 2013(Falco et al., 2013)	BAG3	52	17 (CP)	-	81	77	0.810
Chen J et al. 2013(Chen et al., 2013)	TTR	40	-	40	91	47	0.730

Gold D et al. 2013(Gold et al., 2013)	PAM4	298	-	79	76	96	-
Gold D et al. 2013(Gold et al., 2013)	PAM4	298	120	-	-	-	0.890
Poruk K. et al. 2013(Poruk et al., 2013b)	OPN	86	48	86	-	-	0.720
Poruk K. et al. 2013(Poruk et al., 2013b)	TIMP-1	86	48	86	-	-	0.770
Lee MJ et al 2014(Lee et al., 2014)	CA 19-9	41	12	44	80.4	70	0.833
Lee MJ et al 2014(Lee et al., 2014)	Human complement factor B (CFB)	41	12	44	73.1	97.9	0.958
Mixed cohorts							
Ince AT et al. 2014(Ince et al., 2014)	CEA	96 (41 PDAC +25 BTC)	129	-	42.7	89.9	0.713
Ince AT et al. 2014(Ince et al., 2014)	CA19-9	96 (41 PDAC +25 BTC)	129	-	49	84.5	0.701
Ince AT et al. 2014(Ince et al., 2014)	VEGFR3	96 (41 PDAC +25 BTC)	129	-	48.4	82.9	0.622
Ince AT et al. 2014(Ince et al., 2014)	Total Antioxidant Capacity	96 (41 PDAC +25 BTC)	129	-	61.1	60.5	0.602
Abdel-Razik A et al. 2016(Abdel-Razik et al., 2016)	IGF-1	47 (25 PDAC + 18 BTC)	62	-	62	51	0.605
Abdel-Razik A et al. 2016(Abdel-Razik et al., 2016)	VEGF	47 (25 PDAC + 18 BTC)	62	-	58.3	57.3	0.544
Biomarker combinations							
Chen J et al. 2013(Chen et al., 2013)	TTR + CA19-9	40	-	40	81	85	0.910
Lee MJ et al 2014(Lee et al., 2014)	CA19-9 + CFB	41	12	44	90.1	97.2	0.986
Sogawa K et al. 2016(Sogawa et al., 2016)	C4BPA + CA19-9	52	20	40	86	80	0.930
Makawita S. et al. 2013(Makawita et al., 2013)	CA19-9 + REG1B	100	-	92	-	-	0.880
Makawita S. et al. 2013(Makawita et al., 2013)	CA19-9 + SYCN + REG1B	100	-	92	-	-	0.870
Willumsen N et al. 2013(Willumsen et al., 2013)	C1M+C3M+C4M+C4M12a1	15	-	33	-	-	0.990
Shaw VE et al. 2014(Shaw et al., 2014)	IL10+IL6 + PDGF + Ca19-9	84	45 (benign)	-	93	58	0.840
Shaw VE et al. 2014(Shaw et al., 2014)	IL8+IL6 +IL-10 + Ca19-9	84	32 (CP)	-	75	91	0.880
Shaw VE et al. 2014(Shaw et al., 2014)	IL8+IL1b + Ca 19-9	127	-	45	94	100	0.857
Brand RE et al. 2011(Brand et al., 2011)	Ca-19 + CEA + TIMP-1	173	70	120	71	89	-
Capello M et al. 2017(Capello et al., 2017)	TIMP1 + LRG1 + Ca19-9	73	-	60	0.849	0.633	0.949
Capello M et al. 2017(Capello et al., 2017)	TIMP1 + LRG1 + Ca19-9	73	74	-	0.452	0.541	0.890
Chan A et al. 2014(Chan et al., 2014)	Ca19-9 + Ca125 + LAMC2	139	65	10	82	74	0.870
Makawita S et al. 2013(Makawita et al., 2013)	CA19-9 + REG1B	82	41	92	-	-	0.875
Makawita S et al. 2013(Makawita et al., 2013)	CA19-9 + SYNC + REG1B	82	41	92	-	-	0.873
Makawita S et al. 2013(Makawita et al., 2013)	CA19-9 + AGR2 + REG1B	82	41	92	-	-	0.869

As outlined in chapter 1 tumour development is driven by a series of cumulative genetic abnormalities; genetic and epigenetic changes have therefore been explored as diagnostic targets in circulating tumour cells (CTC), cell-free DNA (cfDNA) and noncoding RNA. [Tables 4.2, 4.3, 4.4]

Table 4.2 Genetic and epigenetic alterations in circulating tumour cells in PDAC and BTC, 2013 to 2017

Author + Year	Target	BTC	PDAC	Benign Lesion	Healthy Volunteer	Detected	Sensitivity	Specificity	AUC
Ankeny JS et al. 2016(Ankeny et al., 2016)	K-ras	-	72	28	-	-	75	96.4	0.867
Kulemann B et al. 2016(Kulemann et al., 2016)	K-Ras	-	21	-	10	80 (stage IIA/IIB) 91 (stage III/IV)	-	-	-
Singh N et al. 2015(Singh et al., 2015)	ctDNA, K-ras	-	-	-	-	-	65.3	61.5	0.6681
Kinugasa et al. 2015(Kinugasa et al., 2015)	K-ras	-	141	20	20	-	62.6	-	-
Takai E et al. 2015(Takai et al., 2015)	K-ras	-	259	-	-	-	29.2	-	-
Sausen M et al. 2015(Sausen et al., 2015)	ctDNA	-	77	-	-	-	43	-	-
Kulemann B et al. 2015(Kulemann et al., 2015)	CTC K-ras	-	11	-	9	75 (stage IIb) 71 (stage III)	-	-	-
Zhang Y et al. 2015(Zhang et al., 2015)	$DAPI^{+}$, CD45-, $CK^{+,}$ $CEP8 > 2^{+}$	-	22 Validation cohort: 11	6 8	30 10	68.2	 63.6	 94.4	 0.84
Wu J et al. 2014(Wu et al., 2014b)	K-ras	-	36	-	25	-	0	0	-
Bidard FC et al.2013(Bidard et al., 2013)	CK, CD45	-	79	-	-	11%	-	-	-
Bobek V et al. 2014(Bobek et al., 2014)	DAPI, CK, CEA, Vimentin	-	24	-	-	66.7%	-	-	-
Rhim AD et al. 2014(Rhim et al., 2014)	DAPI, CD45, CK, PDX-1	-	11	21	19	78%	-	-	-
Iwanicki-Caron et al. 2013(Iwanicki-Caron et al., 2013)	CTC	-	40	-	-	-	55.5	100	-
Sheng W et al. 2014(Sheng et al., 2014)	CTC	-	18	-	-	94.4%	-	-	-
Catebacci DV et al.	CTC (in portal venous	2	14	-	-	100% (PV blood)	-	-	-

2015(Catenacci et al., 2015)	blood at EUS)					22.2% (peripheral blood)			
Earl J et al. 2015(Earl et al., 2015)	CTC	-	35	-	-	20%	-	-	-
Cauley CE et al. 2015(Cauley et al., 2015)	Circulating epithelial cells	-	105	34	9	49%	-	-	-
Kamande JW et al. 2013(Kamande et al., 2013)	DAPI, CD45, CK	-	12	-	-	100%	-	-	-

Table 4.3. Genetic and epigenetic alterations in circulating cell-free DNA PDAC and BTC, 2013 to 2017

Author + Year	Target	PDAC or BTC	Cancer	Benign Lesion	Healthy Volunteer	Detected	Sensitivity	Specificity
Takai E. et al. 2016(Takai et al., 2016)	K-Ras	PDAC	107 (non-operable)	-	-	59%	-	-
Takai E at al. 2015(Takai et al., 2015)	cfDNA	PDAC	48			29%		
Hadano N. et al. 2016(Hadano et al., 2016)	K-Ras	PDAC	105	-	20	31%	-	-
Zill OA et al. 2015(Zill et al., 2015)	KRAS, TP53, APC, FBXW7, SMAD4	PDAC	26	-	-	-	92.3	100
Earl J et al. 2015(Earl et al., 2015)	K-Ras	PDAC	31	-	-	26%	-	-
Kinusaga H et al. 2015(Kinugasa et al., 2015)	G12V, G12D, and G12R in codon 12 of K-ras gene	PDAC	141	20	20	62%	-	-
Sausen et al. 2015(Sausen et al., 2015)	cfDNA	PDAC	77	-	-	43%	-	-
Wu et al. 2014(Wu et al., 2014b)	K-Ras	PDAC	24	-	25	72%	-	-

Table 4.4 Epigenetics: circulating noncoding RNA and DNA methylation markers in PDAC / BTC in 2013 to 2017

Author + Year	MiRNA	BTC	PDAC	Benign Lesion	Healthy Volunteer	Sensitivity	Specificity	AUC
Circulating noncodeing RNA								
Kishimoto et al. 2013(Kishimoto et al., 2013)	MiR-21 (↑)	94 94	- -	- 23	50 -	85 72.3	100 91.3	0.93 0.83
Wang et al. 2013(Wang et al., 2013b)	miR-27a-3p + CA19-9(↑)	-	129	103	60	85.3	81.6	0.886

Kawaguchi et al. 2013(Kawaguchi et al., 2013)	miR-221 (↑), miR-375 (↓)	-	47	-	30	-	-	0.762
Zhao et al. 2013(Zhao et al., 2013)	miR-192 (↑)	-	70	-	40	76	55	0.63
Li et al. 2013(Zhao et al., 2013)	MiR-1290 (↑)	-	41	53	19	88	84	0.96
Carleson AL at al. 2013(Carlsen et al., 2013)	MiR-375 (↑)	-	48	47	-	-	-	0.72
Que R et al. 2013(Que et al., 2013)	miR-17-5p (↑) miR-21 (↑),	-	22	12	8	-	-	0.887 0.897
Schultz NA et al. 2014(Schultz et al., 2014)	Index I + CA19-9 Index II + CA19-9	-	409	25	312	85 85	88 86	0.93 0.92
Silakit R et al. 2014(Silakit et al., 2014)	MiR-192 (↑)	11	-	-	9	74	72	0.803
Lin et al. 2015(Lin et al., 2014)	MiR-492 (↑) MiR-663a (↑)	-	49	-	27	75 85	70 80	0.787 0.870
Chen Q et al. 2014(Chen et al., 2014)	miR-182 (↑)	-	109	38	50	64.1	82.6	0.775
Wang et al 2015(Wang et al., 2015)	MiR-150 (↑)	15	-	-	15	80	58	0.764
Ganepola GA et al. 2015(Ganepola et al., 2014)	miR-22 (↑), miR-642b (↑) miR-885-5p (↑)	-	11	-	11	91	91	0.970
Voigtlander et al. 2015(Voigtlander et al., 2015) (Serum)	MiR-1281 (↑) MiR-126 (↑) MiR-26a (↑) MiR-30b (↑) MiR-122 (↑)	31	-	40	-	55 68 52 52 32	90 93 93 88 90	0.83 0.87 0.78 0.78 0.65
Voigtlander et al. 2015(Voigtlander et al., 2015) (Bile)	miR-412 (↑) miR-640 (↑) miR-1537 (↑) miR-3189 (↑)	31	-	53	-	50 50 67 67	89 92 90 89	0.81 0.81 0.78 0.80
Abue M et al. 2015(Abue et al., 2015)	miR-21 (↑), miR-483-3p (↑)	-	32	12	30	-	-	0.790 0.754
Salter EP et al. 2015(Slater et al., 2014)	miR-196a (↑), miR-196b (↑)	-	19	10	10	100	90	0.99
Kojima M et al. 2015(Kojima et al., 2015)	miR-6075, miR-4294, miR-6880-5p, miR-6799-5p, miR-125a-3p, miR-4530, miR-6836-3p, miR-4476	98	100	21	150	80.3	97.6	0.953
Xu J et al. 2015(Xu et al., 2016)	miR-486-5p (↑) miR-938 (↑)	-	156	142	65	-	-	0.861 0.693
Madhaven B et al. 2015(Madhavan et al., 2015)	PaCIC + miRNA serum-exosome marker panel	-	-	-	-	100	80	-
Komatsu S et al. 2015(Komatsu et al., 2015)	miR-223 (↑)	-	71	-	67	62	94.1	0.834
Miyamae M et al. 2015(Komatsu et al., 2015)	miR-744 (↑)	-	94	-	68	-	-	0.8307

Alemar B. et al 2016(Alemar et al., 2016)	MiR-21 (↑) MiR-34a (↑)	-	24	-	10	-	-	0.889 0.865
Wu. X et al 2016(Wu et al., 2016)	MiR-150 (↓)	30	30	28	50	-	-	-
Bernuzzi et al. 2016(Bernuzzi et al., 2016)	MiR-483-5p(↑) MiR-194(↑)	40	40	70	40	-	-	0.77 0.74
Kim et al. 2016(Kim et al., 2016)	mRNA – CDH3 (↑) mRNA -IGF2BP3(↑) mRNA – HOXB7 (↑) mRNA – BIRC5 (↑)	-	21	14	-	57.1 76.2 71.4 76.2	64.3 100 57.1 64.3	0.776 0.476 0.898 0.818
Duell et al. 2017(Duell et al., 2017)	MiR-10a (↑) MiR-10b (↑) MiR-21-5p (↑) MiR-30c (↑) MiR-155 (↑) MiR-212 (↑)	-	225	-	225	-	-	0.66 0.68 0.64 0.71 0.64 0.64
DNA hypermethylation								
Branchi et al. 2016(Branchi et al., 2016)	SHOX2 / SEPT9	20	-	-	100	0.45	0.99	0.752

Due to the position and composition of pancreaticobiliary tumours, tissue samples are frequently acellular, making diagnostics challenging. Recently the utility of next-generation sequencing has been explored as a technique that allows the detection of low abundance mutations and abnormalities in small amounts of material.(Malgerud et al., 2017) Changes in the metabalome are also being explored as a potential diagnostic tool in pancreaticobiliary malignancy.(Lindahl et al., 2017)

Bile and biliary brush biomarkers

Patients with an indeterminate stricture on cross-sectional imaging are typically referred for an ERCP and biliary brushing with or without endobilary biopsy to obtain tissue for diagnosis, with or without therapeutic stenting.(De Bellis et al., 2002) Although these techniques do not compromise resection margins in potentially resectable cases, sensitivity remains low (9 – 57%) and patients frequently have to undergo multiple procedures to obtain a diagnosis.(De Bellis et al., 2002, Harewood et al., 2004, Moreno Luna et al., 2006) Bile can be easily obtained at the time of ERCP and due to its proximity to the tumour is a potentially important source of diagnostic biomarkers in these cancers. [Table 6] Unfortunately due to the invasiveness of ERCP, the role of these biomarkers is limited to diagnosis rather than screening or surveillance in these tumours.

Table 4.5. Bile and biliary brush biomarkers for pancreaticobiliary malignancy

Author + Year	Biomarker	PDAC	BTC	Benign Lesion	Healthy controls	Bile or biliary brush	Sensitivity	Specificity	AUC
Single biomarkers									
Dhar et al. 2013(Dhar et al., 2013)	M2-PK	-	88	79	17	Bile	90.3	84.3	-
Navaneethan U et al. 2015(Navaneethan et al., 2015)	M2-PK	-	-	-	-	Bile	52.9	94.1	0.77
Keane MG. 2017(Keane et al., 2017)	MCM5	24	17	47		Biliary brush	55.6	77.8	0.79
Danese E et al. 2014(Danese et al., 2014)	MUC5AC	-	20	20	-	Serum : Bile	-	-	0.94 0.99
Farina A et al. 2014(Farina et al., 2014)	CEAM6	23	6	12	-	Bile	93	83	0.92
Budzynska A. et al 2013(Budzynska et al., 2013)	NGAL	6	16	18	-	Bile	77	72	0.74
Jiao X et al. 2014(Jiao et al., 2014)	Nucleosides		202*	203	205	Bile	95.3	96.4	-
Ince AT et al. 2014(Ince et al., 2014)	CE	41	25	129	-	Bile	57.3	68.2	0.516
Ince AT et al. 2014(Ince et al., 2014)	CA 19-9	41	25	129	-	Bile	74.0	34.1	0.616
Ince AT et al. 2014(Ince et al., 2014)	VEGFR3	41	25	129	-	Bile	56.2	79.1	0.663
Ince AT et al. 2014(Ince et al., 2014)	Total antioxidant capacity	41	25	129	-	Bile	65.6	50.4	0.581
Abdel-Razik A et al. 2016(Abdel-Razik et al., 2016)	IGF-1	25	18	62	-	Bile	91.4	89.5	0.943
Abdel-Razik A et al. 2016(Abdel-Razik et al., 2016)	VEGF	25	18	62	-	Bile	90.3	84.9	0.915
Kim et al. 2016(Kim et al., 2016)	mRNA – CDH3 (↑) mRNA -IGF2BP3(↑) mRNA – HOXB7 (↑) mRNA – BIRC5 (↑)	-	21	14	-	Biliary brush	57.1 76.2 71.4 76.2	64.3 100 57.1 64.3	0.776 0.476 0.898 0.818

* Gallbladder cancer

Urinary biomarkers

Urine provides a very easy and acceptable source for biomarker analysis. In BTC a 42 peptide panel (consisting mostly of fragments of interstitial collagens) correctly identified 35 of 42 BTC patients with a sensitivity of 83% and a specificity of 79%.(Metzger et al., 2013) In PDAC, the three biomarker panel (LYVE-1, REG1A and TFF1) has been validated in a multicentre cohort of 371 samples. When comparing PDAC stage I-IIA (resectable disease) to healthy urines, the panel achieved area under the curve (AUCs) of 0.97 (95%CI 0.93-1.00). The performance of the urine biomarker panel in discriminating PDAC stage I-IIA was superior to the performance of serum CA19.9 (p=0.006).(Radon et al., 2015) [Table 7]

Table 4.6 Summary table of urine protein biomarkers for pancreatic and biliary tract cancer 2013 - 2017

Author + Year	Biomarker / Combination (Urine)	PDAC	BTC	Benign Cancer / Chronic pancreatitis	Healthy Volunteer	Sensitivity	Specificity	AUC
Single biomarker								
Roy R et al. 2014(Roy et al., 2014)	MMP2	51	-	-	60	70%	85%	-
Roy R et al. 2014(Roy et al., 2014)	TIMP-1	51	-	-	60	90%	70%	-
Jiao X et al. 2014(Jiao et al., 2014)	Nucleosides	-	202 (GB cancer)	203	205	89.4	97.1	-
Metzger J et al. 2013(Metzger et al., 2013)	Urine Proteomic analysis	-	42	81	-	83	79	0.87
Biomarker combinations								
Radon TP et al. 2015(Radon et al., 2015)	LYVE-1 + REG1A + TFF1	192	-	-	87	-	-	0.89

Discussion

Currently the most widely used tumour marker in pancreaticobiliary malignancy remains CA19-9. However, its use is limited by its elevation in a number of other benign and malignant conditions. Furthermore, it is not produced in approximately 7% of the population who are Lewis antigen negative and is often undetectable when tumours are small. Over the last few years, a number of promising individual biomarkers and biomarker panels have been identified in pancreatic cancer. Larger validation studies are needed to confirm efficacy. Further studies are needed to evaluate the performance of these markers in small tumours and early-stage disease to ensure they have the ability to detect disease when curative intervention could be possible.

5 EVALUATING CELL CYCLE PROTEINS AS BIOMARKERS IN PANCREATIC CYSTIC LESIONS

The control of DNA licensing in normal tissues and cancer cells

The cell cycle

The eukaryotic cell cycle represents a series of events, which includes the duplication of DNA and the formation of two daughter cells [Figure 5.1].

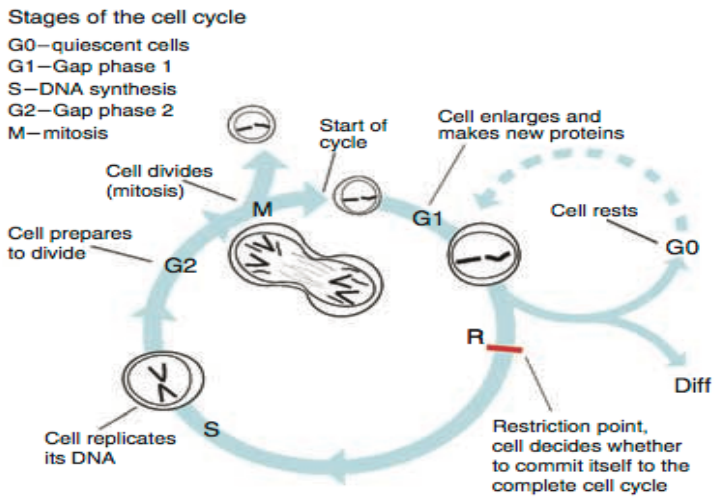


Figure 5.1 The eukaryotic cell division cycle

Progression through each phase of the cycle is tightly controlled by regulatory proteins. In G1 phase the machinery required for DNA replication (pre-replicative complex) is assembled so that DNA can become 'licensed' and the cell can progress into S phase.(Ritzi and Knippers, 2000) During S phase thousands of replication forks are initiated at their origins, which have been licensed in the G1 phase. These forks are necessary for the complete replication of chromosomal DNA.(Blow and Dutta, 2005) During synthesis (S-phase) the cell then undergoes DNA replication. The initiation of DNA replication is a crucial decision point in cell proliferation that lies at the point of convergence of all oncogenic signalling and transduction pathways that trigger proliferation. The second gap phase, G2, is defined by the synthesis of cellular proteins,

which is essential for mitosis. During this time, DNA licensing is tightly down regulated to prevent on-going replication.(Blow and Tanaka, 2005, Li and Blow, 2005) Cells are then able to progress into mitosis (M phase), which is characterised by the steps of nuclear division (prophase, metaphase, anaphase and telophase) and then cytokinesis, leading to the formation of two complete daughter cells.

Initiation of DNA replication

The initiation of DNA replication through the assembly of pre-replicative complexes was initially discovered after studies in *S. cerevisiae* yeast and *Xenopus* frog eggs,(Donaldson and Blow, 1999) but an almost identical processes occur in all eukaryote cell from yeast to mammals.(Tye, 1999)

In early G1 phase, the origin recognition complex (ORC), made up of six subunits, binds to chromatin to initiate the process of replication licensing. ORC is responsible for recruiting the proteins Cdc6 and Cdt1, which are required for loading the minichromosome maintenance proteins 2-7 (Mcm2-7) onto DNA at replication origins.(Blow and Gillespie, 2008, Tanaka et al., 1997, Gillespie et al., 2001, Blow and Dutta, 2005) Once Mcm loading has occurred, the resultant complex of ORC, Cdc6, Cdt1 and Mcm2-7 at the replication origins is termed the pre-replicative complex.(Masai et al., 2005) In late G1 phase, the origin is “fired” by CDKs and Cdc7/ASK kinase. Cdc7 phosphorylates the Mcm 2, 4 and 6 subunits, thereby inducing a change that stimulates Mcm activity and exposes a domain of Mcm5.(Sclafani and Holzen, 2007, Moyer et al., 2006) As DNA replication is initiated, the MCM proteins become detached from the origins, probably travelling ahead of the replication fork.(Blow and Dutta, 2005) To ensure that replicated origins do not become relicensed during S phase, there must be tight down-regulation of the licensing components before entry into S phase. This is done in two ways, firstly, cyclin dependent kinases (CDKs) have a number of key effects, which inhibit the licensing system through the phosphorylation, ubiquitination, and proteolysis of several individual components of the pre-replication complex including Cdc6, ORC and Cdt1.(Blow and Hodgson, 2002, Blow and Tanaka, 2005) Secondly, downregulation of Cdt1 activity by the protein geminin, which is expressed in the cell cycle in S, G2 and M phases, prevents relicensing and replication of DNA.(Blow and Gillespie, 2008, Mihaylov et al., 2002,

Melixetian et al., 2004) Functionally, geminin exerts this effect by binding to Cdt1 and preventing its ability to load Mcm2-7 onto the DNA. Geminin levels fall during late mitosis and early G1 phases when it is ubiquitinated by the action of APC/C, allowing for Cdt1 levels to rise and for licensing to occur again.(Blow and Tanaka, 2005)

Minimicrosome maintenance proteins

Mcm2-7 proteins were originally identified in the budding yeast when their genes were found to be necessary for the progression of the cell division cycle. Mcm2-7 are hydrophilic nuclear proteins, which range in size from 776 to 1017 amino acids. Mcm5 being the smallest and Mcm6 the largest.

Cell cycle markers in benign and malignant tissue

The majority of normal human cell populations exist in non-proliferating 'out-of-cycle' states. Only self-renewing tissues such as cervix, colon or skin are actively cycling.(Potten and Loeffler, 1990) Most cells in functional solid organs reside in a quiescent (G0) non-cycling state or are at the point of terminal differentiation (e.g. neurones or myocytes).(Hall and Watt, 1989) In contrast, cancer cells typically re-enter the cell cycle and exhibit characteristic uncontrolled growth with a high proportion of cycling cells.(Williams and Stoeber, 2007) High levels of Mcm2-7 expression has been demonstrated in tissue lung, breast, ovary, kidney, bladder and prostate tumours, demonstrating their potential utility as cancer biomarkers.(Williams and Stoeber, 2011) In addition DNA licensing and hence the presence of Mcm proteins is restricted to the proliferating cells with absence of the licensing proteins in any normal adjacent cells which are not capable of self renewing. MCM proteins can also be detected in relatively small samples of e.g. when cancer cells are shed from the epithelial surface of the bladder into urine, or from the biliary tract into bile or in cervical smear samples.(Williams and Stoeber, 2011, Freeman et al., 1999)

ANALYSIS OF CELL CYCLE PROTEIN MARKERS IN FORMALIN FIXED PARAFFIN EMBEDDED TISSUE FROM CYSTIC LESIONS OF THE PANCREAS

Introduction

Current diagnostic modalities for identifying pancreatic cyst subtype or high-risk PCL are imperfect [as outlined in Chapter 1 and 2]. In previous work by our group, Mcm proteins are highly expressed in pancreatic, biliary tract and ampullary tumours.(Huggett et al., 2016, Keane et al., 2017) Increased expression has also been seen in some premalignant conditions.(Going et al., 2002) In this study we aimed to assess the potential of a range of cell cycle proteins as diagnostic biomarkers in PCL.

Methods

5.1.1.1 Patients

Between 1/1/2005 and 1/1/2016 all patients, who underwent a surgical resection for a PCL, with available histological material, from University College Hospital, London and the Royal Free Hospitals, were included in the study. Patients were identified via a search of the CoPath histology database (Sunquest, Tucson AZ, USA). For each patient, the original haematoxylin and eosin (H&E) stained slides were first reexamined by an experienced histopathologist to confirm level of dysplasia and presence or absence of malignancy. A representative area was then selected for cutting and subsequent staining.

5.1.1.2 Clinical data

A database was formed comprising of the following clinicopathological fields: sex, age at diagnosis, PCL subtype, symptoms, tumour markers, preoperative investigations (i.e. CT / MRI / EUS) with noteable features of concern, level of dysplasia, presence of malignancy with stage, completeness of resection and residual disease ((R) status), recurrence, time to post-resection recurrence, cause of death and length of follow-up.

5.1.1.3 Immunohistochemistry

Once the formalin fixed paraffin embedded tissue blocks of representative tumour were obtained, consecutive serial tissue sections were cut at a thickness of 4μm onto

Superfrost Plus slides (Visions Biosystems, Newcastle Upon Tyne, UK), dewaxed in xylene and rehydrated through graded alcohol to water. The tissue sections were pressure-cooked in 0.1 M citrate buffer at pH 6.0 for 2 minutes and immunostained using the Bondt Polymer Refine Detection kit and Bondt-Max automated system (Vision Biosystems). Primary antibodies were applied at the following dilutions: Mcm2 (1:1000), geminin (1:150), H3p (1:3000) and Cdc7 (1:100). The slides were then dehydrated with graded alcohol and then washed thrice with xylene (100% concentration). Coverslips were applied with Pertex mounting medium (CellPath Ltd, Newtown, Powys, UK). Incubation without a primary antibody was used as a negative control and tonsil epithelial sections as positive controls.

5.1.1.4 Antibodies

Mcm2 monoclonal antibody (clone 46) was obtained from BD Transduction Laboratories (Lexington, KY, USA), Geminin monoclonal antibody from Leica Microsystems (Newcastle Upon Tyne, UK), Histone H3 phosphorylated on Serine 10 (H3p) polyclonal antibody from Upstate (Lake Placid, NY, USA), Cdc7 monoclonal antibody from MBL International (Woburn, MA, USA).

5.1.1.5 Protein expression analysis

Protein expression analysis was performed by determining the Labelling Index (LI) for each of the markers, in each case, as described previously.(Loddo et al., 2009, Rodriguez-Acebes et al., 2010) Slides were initially evaluated at low-power magnification (100x) to identify the regions with the highest intensity of staining. These areas were identified in conjunction with an experienced pathologist. From these selected areas, 3-5 fields at 400x magnification were captured with a charged-coupled-device camera and analysis software (SIS, Munster, Germany). Images obtained were printed in colour for quantitative analysis. Images were counted by an individual blinded to clinicopathological variables. Both positive and negative cells were counted within each field and stromal and inflammatory cells excluded. A minimum of 500 cells from the epithelial border of the cyst were counted in each case. The LI was then calculated for each marker using the following formula: LI= number of positive cells/total number of cells x100. The reassessment of 10 randomly selected cases by an independent assessor was undertaken to ensure the accuracy of the LI and showed high levels of concordance.

5.1.1.6 Ethical approval

Local research ethics committee approval was obtained from the joint UCL/UCLH Committees on the Ethics of Human Research (REC reference 06/Q0512/106).

5.1.1.7 Statistical analysis

Statistical tests and graphics were generated using the IBM SPSS Statistics package (version 19.0, SPSS Inc., Chicago, IL, USA). Biomarker labeling indices were summarised with the median and range. The level of signal was compared between patient groups using the Mann-Whitney U-test. Spearman's Rank correlation coefficient was used to assess associations between the markers. Kaplan-Meier plots were used to show the estimated predictive effects of markers. All tests were two-tailed, with effects summarised using 95% confidence intervals. A 5% level was used to indicate statistical significance.

Results

5.1.1.8 Patient demographics

The study cohort comprised of 44 patients with a PCL. 23 were male and 21 were female. Median age at diagnosis was 65 (range 34-84) years. An additional 73 patients with pancreatic cancer were used as positive controls. 21 patients with a normal pancreas or chronic pancreatitis were used as negative controls.

5.1.1.9 Immunohistochemistry

For the patients in the study, the pattern of Mcm2, geminin, histone and Cdc7 expression was assessed by immunohistochemistry. Staining of the cell cycle biomarkers was largely restricted to the epithelial lining of the cyst wall and was most intensive in areas of high-grade dysplasia or invasive cancer. Within the adjacent areas of stoma and normal pancreas, staining was extremely low [Figure 5.2].

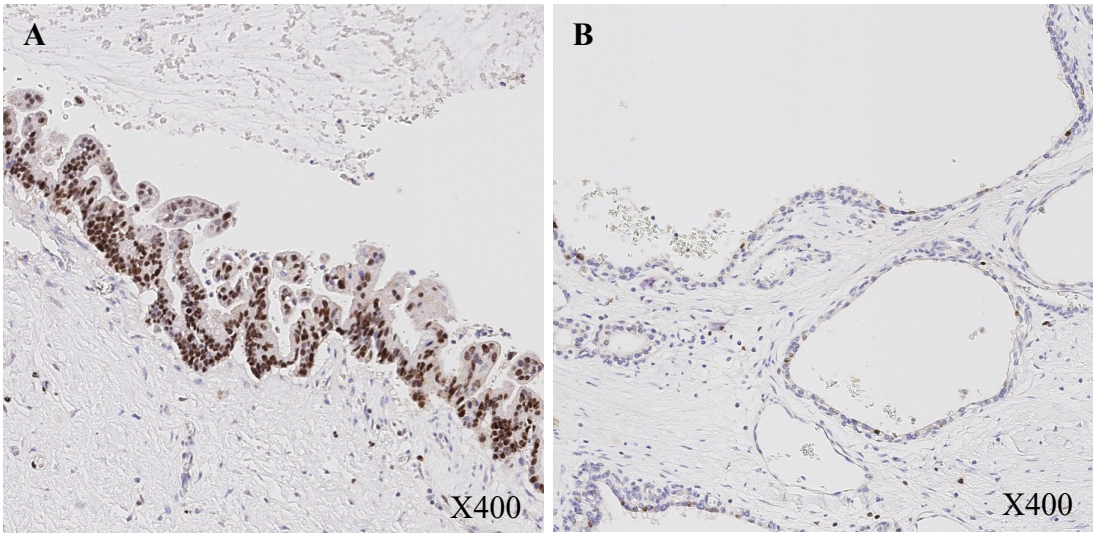


Figure 5.2. Expression of Mcm2 (brown immunostain with blue hematoxylin counterstain) in representative tissue sections from an IPMN with high grade dysplasia (A) vs. serous cystic neoplasm (B). Immunoexpression correlates with the PCL malignant potential with strong expression in high-grade lesions and minimally expression in low grade lesions.

5.1.1.9.1 Mcm2 expression

The percentage of nuclei positive for Mcm2 was significantly higher in malignant tissue than in benign tissue (62.2% vs. 0.4%, (P<0.05)). Patients with HGD had a similar rate of nuclear expression of Mcm2 to patients with PDAC (62.2% vs. 76.4%). Patients with LGD had a reduced level of expression in comparison to patients with IGD or invasive cancer. Benign cysts with no malignant potential e.g. serous cystic neoplasms, had very low levels of Mcm2 positivity. Rare low-grade malignant tumours, solid pseudopapillary tumours also had very low levels of Mcm2 expression, correlating with their good clinical prognosis [Table 5.1].

Table 5.1 Median labeling index for Mcm2 by PCL subtype, with positive (PDAC) and negative (chronic pancreatitis / normal pancreas) controls for comparison of expression.

Tissue	**N**	**Median LI (%)**
Positive Controls: PDAC	73	62.2
IPMN + High grade dysplasia	9	76.4
IPMN or MCN with low grade dysplasia	20	13.8
Serous cystic neoplasm	13	8.1
Solid pseudopapillary neoplasm	2	3.1
Negative Controls: Normal pancreas, Chronic pancreatitis	21	0.4

5.1.1.9.2 Histone (H3p), Geminin and Cdc7 expression

For Histone, Geminin and Cdc7, like Mcm2 there was a significant difference in expression between PDAC and IPMN/MCN with HGD from benign controls (chronic pancreatitis and pseudocysts). Histone (20.70 vs. 5.21 (P=0.005)) and Geminin (27.55 vs. 5.00 (P=0.005)) could also differentiate IPMN/MCN with HGD from benign SCN. All three biomarkers could also differentiate solid pseudopapillary neoplasms, which have low malignant potential, from PDAC or IPMN / MCN with HGD [Table 5.2].

Table 5.2 Median labeling index with range for Histone, Geminin and Cdc7 by PCL subtype. Pancreatic cancer was used as positive control and chronic pancreatitis / normal pancreas as a negative control.

Tissue	**N**	**Histone (H3p)**		**Geminin**		**Cdc7**	
	N	**Median LI (%)**	**Range**	**Median LI (%)**	**Range**	**Median LI (%)**	**Range**
Positive Controls: PDAC	73	20.70	0.9-51.49	27.55	17.87-32.62	22.45	0.40-37.55
IPMN or MCN with HGD	9	75.46	53.76-85.36	39.97	5.36-47.35	30.81	21.39-43.25
IPMN or MCN with LGD	20	22.5	4.58-81.12	9.54	1.43-50.68	26.47	8.78-69.71
SCN	13	5.21	3.31-6.09	5.00	1.06-9.44	12.29	1.19-34.31
Solid pseudopapillary neoplasm	2	0.84	0.17-1.51	0.18	0.17-0.18	1.85	0.16-3.53
Negative Controls: Normal pancreas, Chronic pancreatitis	21	0	0-0.31	0.15	0-0.96	0.17	0-3.01

Discussion

This study demonstrated that the cell cycle proteins are significantly overexpressed in pancreatic cancer and pancreatic cysts with invasive cancer compared with benign controls. In Mcm2, intermediate expression was seen in PCL with low grade dysplasia. This is consistent with prior studies in other HPB cancers and precancerous lesions when dysplastic cells were shows to lose their out-of-cycle phenotype when they begin to replicate again.(Huggett et al., 2016, Going et al., 2002) This study demonstrates the potential of cell cycle biomarkers to identify high-risk PCL. For these biomarkers to be useful clinically, the same expression needs to be present in pre-operative diagnostic samples such as pancreatic cyst fluid obtained during EUS. This is therefore explored in subsequent work [section 5.3].

MINICHROMOSOME MAINTENANCE PROTEIN 5 (MCM5) IN CYST FLUID FOR THE DIAGNOSIS OF HIGH-RISK PCL

Introduction

As shown in sections 5.1 and 5.2 cell cycle proteins such as Mcm2 are expressed in pancreatic cancers and dysplastic area of cystic lesions of the pancreas. In prior work by our group it was shown that Mcm5 can be detected in tumour cells shed in to the bile or collected by brush cytology in patients with PDAC.(Ayaru et al., 2008, Keane et al., 2016) In this pilot study, Mcm5 expression was measured by immunofluorometric assay in cyst fluid from patients with a range of PCL.

Methods

5.1.1.10 Inclusion criteria

Commencing June 2011, patients over the age of 18 years referred for EUS to University College Hospital, London (UCLH) or The Royal Free Hospital (RFH) were eligible to participated in the study if they had a pancreatic cyst requiring fine needle aspiration.

5.1.1.11 Ethical approval

The study was approved by the Joint UCLH/UCL ethical committee and all patients gave written informed consent (NRES: 06/Q0512/106).

5.1.1.12 Clinical Data Recorded

For each patient recruited, the electronic medical records were reviewed and information was recorded in an electronic spreadsheet. Data was recorded from the Pathology (CoPath histology database, Sunquest, Tucson AZ, USA), Endoscopy (GI reporting tool, Unisoft medical systems, UK) and Imaging (PACS: picture archiving and communication system, GE Healthcare, USA) database systems. Data collected included demographic information (age, sex, hospital number), history of acute or chronic pancreatitis or malignancy, family history of pancreatic cancer or relevant clinical syndrome. Cross-sectional imaging (computed tomography (CT) and/or magnetic resonance cholangiopancreatography (MRCP)) features were recorded.

Details of the EUS procedure along with cytology and histology results were recorded. For patients referred for surgery, date of the operation, type of resection and final histology were recorded. Length of follow-up was calculated from first procedure to last clinic appointment attended, or date of clinic discharge, or death. Diagnosis was established by surgical resection, if undertaken or a combination of imaging, cytology and cyst fluid biochemistry. Benign disease was confirmed by follow up of at least 12 months.

5.1.1.13 EUS procedure and sample collection

Endoscopic ultrasound (EUS) was undertaken after informed written consent was obtained from the patient. The procedures were performed under conscious sedation or general anaesthesia using a linear array echoendoscope (Olympus, UK). Fine-needle aspiration (FNA) was performed using either a 19- or 22-gauge FNA needle (Cook Medical or Boston Scientific). The collected cyst fluid was divided and sent for cytology, biochemistry (including CEA and amylase) and the remaining fluid was agitated by inversion and then immediately snap-frozen on dry ice in the procedure room. At the end of the EUS list, the samples were transferred to a -80°C freezer for short to medium term storage. All samples were blinded, coded and anonymised prior to dispatch to the laboratory for the Mcm5 assay. The cytology samples were prepared by placing a drop of the cyst fluid on to a slide, which was smeared and dried and later stained for malignant cells using the standard Papanicolaou technique. These slides were analysed by a Consultant Cytopathologist at UCLH or RFH.

5.1.1.14 In Vitro Diagnostic Assay Development & Validation

In conjunction with a commercial partner (VarleighDx (UK) Ltd, London) and under license from the Cancer Research United Kingdom (CRUK) licensing group Cancer Research Technologies (CRT), a diagnostic MCM5 ELISA assay was developed in conformity with the requirements of the IVD Directive EC 98/79/EC. Validation studies were designed to test required aspects of assay performance including clinical performance, assay precision, interference, cross reactivity and stability.

5.1.1.15 Antibody Development

Monoclonal antibodies (MAbs) 12A7 and 4B4, directed against non-overlapping epitopes, were raised against His-tagged human MCM5 protein and were protein A-purified from hybridoma supernatants as described previously.(Stoeber et al., 2002) For

use in the ELISA assay the protein A-purified MAb 12A7 was labeled with horseradish peroxidase using conventional conjugation techniques, while capture antibody 4B4 was adsorbed to the surface of microtitre plate wells.

5.1.1.16 Clinical Validation Studies

Fifteen of the 23 samples were performed in duplicate as per the manufacturer's instructions. The assay was controlled using a) lysis buffer as the blank representing the cell lysate matrix and b) recombinant Mcm5 antigen positive analyte control (concentration ~0.60ng/mL). The assay was further controlled using a calibrator of recombinant Mcm5 antigen (batch specific calibrator concentration 0.29ng/mL). Optical Density (OD) measurements were recorded for all samples and controls tested. On completion of the pilot study, patient data and the Mcm5 ELISA results were compared with cytology results and where available final clinical diagnosis after follow-up.

5.1.1.17 Assay Precision Studies

Precision studies were conducted utilising recombinant Mcm5 antigen. A 12-day study was conducted using 3 reagent kit lots (12 runs with each reagent lot) and 4 replicates of each control sample per run.

5.1.1.18 Assay Cross Reactivity

In order to ensure specificity of the ELISA for the Mcm5 target protein cross reactivity studies were performed against other members of the Mcm family (Mcm2-7). Each Mcm protein was tested in duplicate over the range of concentrations 0.05 – 100 ng/mL.

5.1.1.19 Data analysis

Statistical Package for Social Sciences for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Associations between various clinical and radiographic characteristics were evaluated using a 2-sample *t* test for continuous variables and a 5% level was used to indicate significance. The sensitivity of cytology was compared with that of the immunofluorometric Mcm5 test using McNemar's test for paired proportions.

Results

Sample collection is ongoing but results from an interim analysis of the first 23 samples is presented below.

5.1.1.20 Patients

One patient had metastatic PDAC (with cystic degeneration), 1 patient had a cystic PanNET, 6 patients had benign mucinous PCL (BD IPMN) and 15 had benign PCL (SCN, pseudocyst, lymphoepithelial cyst). Fifteen patients were male and eight were female. Median age was 64 (range 28-84) years.

5.1.1.21 Mcm5 expression in pancreatic cyst fluid

Cut off for test positivity was extrapolated from prior work in biliary-brush samples in indeterminate biliary strictures.(Keane et al., 2017)

The Mcm5 test on cyst fluid was positive in the single patient with metastatic PDAC. The 7 patients with premalignant lesions (IPMN, MCN and PanNET), with varying degrees of dysplasia had mixed results, three had a positive and four a negative test result. In the benign group the test was negative in all patients with an SCNs. However, the test was unexpectedly positive in the one patient with a benign lymphoepithelial cyst and 4/12 of the patients with a benign pseudocyst [Table 5.3].

Table 5.3 Mcm5 expression in cyst fluid from a range of PCLs. A cut off of 1.2 was used to determine a positive test. Table shading: dark grey = pancreatic cancer (positive control), light grey = precancerous lesion (IPMN, MCN or PanNET) and white = benign PCL (SCN, pseudocyst, lymphoepithelial cyst)

Sex	Age	Cyst size (mm)	Final diagnosis	Basis of diagnosis	Mcm5	+ Test (>1.2)
M	84	Large	Metastatic PDAC	Perc-US	0.445	+
F	46	58	MCN (HGD)	Surgery	0.056	-
F	64	20	MCN (no dysplasia)	Surgery	0.233	+
F	77	30	BD IPMN	Imaging / cytology	0.207	+
M	77	10	BD IPMN	Imaging	0.189	+
M	77	27	BD IPMN	Imaging	0.06	-
M	79	19	BD IPMN	Imaging	0.06	-
F	77	17	PanNET	Imaging / cytology	0.06	-
F	34	58	SCN	Imaging / cytology	0.09	-
F	70	45	SCN	Imaging / cytology	0.058	-
M	52	Large	Pseudocyst	Imaging	0.053	-
M	64	163	Pseudocyst	Imaging	0.06	-

F	76	130	Pseudocyst	Imaging	0.06	-
M	64	130	Pseudocyst (infected)	Imaging	0.072	-
M	28	110	Pseudocyst	Imaging / cytology	0.062	-
M	45	70	Pseudocyst	Imaging	0.06	-
F	36	60	Pseudocyst	Imaging / cytology	0.06	-
M	48	57	Pseudocyst	Imaging	0.06	-
M	76	200	Pseudocyst	Imaging	0.246	+
M	50	195	Pseudocyst	Imaging	0.191	+
M	61	100	Pseudocyst	Imaging	0.181	+
M	64	80	Pseudocyst	Imaging	0.133	+
M	56	36	Lymphoepithelial cyst	Surgery	0.187	+

Discussion

In patients undergoing surgical resection of a PCL, those with malignant and precancerous cysts demonstrated increased expression of cell cycle biomarkers, in the epithelial lining of the cyst wall, in comparison to benign cysts. This expression mirrored findings in other dysplastic precancerous lesions such as Barretts osophagus where Mcm2 expression could effectively differentiate Barretts osophgagus with LGD, HGD or invasive cancer. (Choi et al., 2024)

However, most patients with a PCL are not managed surgically. High risk lesions are typically referred for EUS and fine needle aspiration of cyst fluid. Therefore, in the second part of this study [Chapter 5.3] levels of Mcm5 expression were measured in a pilot study of 23 patients with a range of PCL. In the single patient with pancreatic cancer the test performed well and was positive. In patients with precancerous PCL with or without dysplasia the test as positive in around half of patients. Levels of expression did not appear to correlate with increasing levels of dysplasia, but this would require further validation in a carefully characterized larger cohort of patients with PCL

In patients with benign cysts, the test performed poorly being positive in 5/15 cases. Four out of five of these cysts were pseudocysts. It is unclear if cyst contamination or the presence of inflammation affected the test result. Further studies to evaluate the test in these common clinical situations are needed, as this is important for interpretating test outcomes.

This study has several limitations, particularly the small size of the cohort and that very few had final diagnosis based on the gold standard surgical pathology. Pseudocysts are typically diagnosed based on serial imaging so in this group, the false positive tests could have been due to incorrectly classified lesions. Larger carefully characterized cohorts of patients with pancreatic cysts and ideally match cyst fluid and surgical pathology would be needed to validate the findings in this initial pilot study.

Conclusion

In this pilot study, Mcm5 expression in cyst fluid from patients with a range of pancreatic cysts, performed well in detecting patients with malignant PCL. Expression was variable in patients with precancerous cysts, with or without dysplasia. The test performed poorly in benign cysts with a third of patients having a false positive result. Further assay precision and cross reactivity testing in PCL fluid samples is needed to optimize the current Mcm5 test and cut offs in PCLs. Then further validation in larger carefully characterized PCL cohorts is necessary to confirm the clinical applicability of this test.

6 A PHASE II MULTICENTRE STUDY OF NEEDLE-BASED CONFOCAL LASER ENDOMICROSCOPY IN CYSTIC TUMOURS OF THE PANCREAS (CONCYST-01)

Introduction

Pancreatic cystic lesions (PCL) have a broad differential diagnosis (Adsay et al., 2010) and standard diagnostic tests are imperfect [as outlined in Chapter 1]. Improved diagnostic test to accurately detect high risk PCL are therefore urgently needed.

Confocal laser endomicroscopy (CLE)

Confocal laser endomicroscopy (nCLE) provides real-time optical imaging of the cyst wall, during EUS-FNA. A laser transmits a low-power laser beam via a probe which is introduced to the cyst via the FNA needle. The probe also allows the detection of fluorescent light, which is returned to the operating system to form the image.

The Cellvizio® Endomicroscopy System (Cellvizio; Mauna Kea Technologies, Paris, France), consists of the following components:

- Confocal endomicroscopy probes (gastroflex™, coloflex™, cholangioflex™ or AQ-flex™ probes (Mauna Kea, Paris)): probes contain optical fibres which generate a dynamic images with a scanning field of 30 000 pixels. The probe used to image PCLs is the AQ-Flex miniprobe. [Figure 4.1].
- Laser Scanning Unit: that enables light illumination, signal detection and rapid scanning device capable of delivering up to 12 images / second. The semiconductor laser oscillates at 488 nm.
- Control and acquisition software and Viewer: the Cellvisio system reconstructs the video frames obtained using a computer algorithm (‘‘mosaicing’’) to form an image with an enlarged field of view (4 mm × 2 mm), providing the clinician with images so they are able to make a real-time histological assessment.

PRODUCT NAME	GASTROFLEX™ UHD	COLOFLEX™ UHD	CHOLANGIOFLEX™	AQ-FLEX™ 19*
DISTAL TIP VISUAL				
COMPATIBLE OPERATING CHANNEL	≥ 2.8 mm	≥ 2.8 mm	≥ 1.2 mm	≥ 0,91 mm
LENGTH	3 m	4 m	4 m	4m
MAXIMUM NUMBER OF USES PER PROBE	20	20	10	10
USUAL CORRESPONDING PROCEDURE	Eso-Gastro-Duodenoscopy (EGD)	Colonoscopy (compatible with EGD)	Endoscopic Retrograde Cholangio-Pancreatography (ERCP)	Endoscopic UltraSound Fine Needle Aspiration (EUS-FNA)
MAIN PATHOLOGY OF INTEREST	Barrett's Esophagus	- Colonic polyps - Inflammatory Bowel Disease	Indeterminate pancreatico- biliary strictures	Pancreatic cysts
TECHNICAL SPECIFICATIONS (WITH LSU 488nm)				
FIELD OF VIEW	∅ 240 microns	∅ 240 microns	∅ 325 microns	∅ 325 microns
CONFOCAL DEPTH	55 to 65 microns	55 to 65 microns	40 to 70 microns	40 to 70 microns
RESOLUTION	1 micron	1 micron	3.5 microns	3.5 microns

Figure 6.1 Commercially available confocal endomicroscopy probes for use during endoscopy. The Gastroflex, coloflex and cholagioflex probes are passed down the working channel of an endoscope. The AQ-FLEX 19 probe, used in the CONCYST 01 study below, is advanced into PCL via a 19G FNA needle during EUS FNA procedure.

Prior nCLE studies

6.1.1.1 Animal studies in nCLE

Two types of needle-based miniprobes were developed for use during EUS procedures. The miniprobes were 300µm and 650µm in diameter and could fit though 19G and 22G needles respectively. They were tested on 10 pigs at three centres (Klinikum rechts der Isar, Munich; Mayo Clinic, Jacksonville; and AMC, Amsterdam). This study confirmed that microscopic structures could be visualised when the device was inserted into solid organs such as the pancreas, liver, lymph nodes, spleen and ovaries [Figure 6.2]. The prototype miniprobes lacked a protective coating and were found to be too fragile for clinical use at this stage, as they were breaking while inside the organ.(Becker et al., 2010, Buchner et al., 2010)

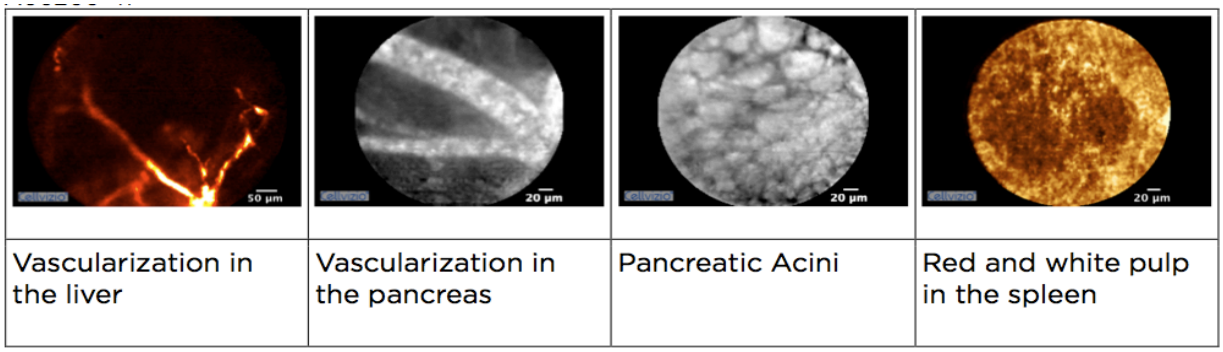


Figure 6.2 nCLE images obtained during feasibility studies from the porcine liver, pancreas and spleen (Becker et al., 2010)

6.1.1.2 Clinical studies in nCLE

1.) The first study of EUS-nCLE in humans was conducted at four centres, Yale New Haven Hospital; Institut Paoli Calmettes, Marseille; Mayo Clinic, Jacksonville and the University of Chicago. The prototype miniprobe was used in 18 patients referred for EUS-FNA of a pancreatic lesion (16 patients with a PCL and 2 with solid pancreatic mass). There were no device malfunctions. Technical challenges were encountered in 6/18 procedures and were attributed to the post-loading technique (loading the nCLE probe after the EUS-FNA needle had been inserted in the organ – which is no longer advised) and using a transduodenal rather than a transgastric imaging approach. nCLE imaging were obtained in 17/18 cases with good or very good quality images being obtain in 10/17. Two serious adverse events occurred; both were post-procedural pancreatitis requiring hospitalization.(Konda et al., 2011)

2.) **INSPECT:** This study aimed to assess the safety and efficacy of EUS guided nCLE in PCL. In Stage 1 of the study descriptive terms for structures visualized were determined during an off-line, unblinded consensus review (reviewers included a gastrointestinal pathologist to enable correlation of histology with nCLE images). Stage 2 of the study assessed whether the criteria defined in Stage 1 could identify PCL subtypes such as IPMN, MCN or adenocarcinoma when the images were reviewed in an off-line blinded consensus review. Sixty-six patients were recruited from eight referral centers. nCLE imaging was available in 65 patients, 8 of which were subsequently excluded due to insufficient information. The presence of epithelial villous structures on nCLE identified a PCL (P=0.004); sensitivity 59%, specificity 100%, positive predictive value 100%, and negative predictive value of 50%. Adverse events occurred in 9% and included pancreatitis (1 mild case, 1 moderate case), transient abdominal pain (n=1), and intracystic bleeding not requiring further intervention (n=3). This study confirmed EUS guided nCLE in PCL was relatively safe and feasible, with a high specificity.(Konda et al., 2013)

3.) **CONTACT 1:** The aim of the study was to develop a comprehensive nCLE image classification for PCL. Thirty-three patients with a lone pancreatic cyst were included. Diagnosis was based on either pathology result (Group 1, n = 20) or an adjudication

committee consensus (Group 2, n = 13). Using the images generated formal criteria for interpreting nCLE were defined for the first time:

- SCN (superficial vascular network),
- IPMN (papillary projections),
- MCN (thick gray line),
- Pseudocyst (field of bright particles), and
- PanNET (black neoplastic cells clusters with white fibrous areas).

In this small case series, the nCLE criteria demonstrated high specificity (>90 % for mucinous cysts, 100 % for non-mucinous cysts).(Giovannini et al., 2014, Napoleon et al., 2015a, Napoleon et al., 2015b) This Phase II study is ongoing and aims to include patients with indeterminate PCL, lymph nodes and pancreatic masses.

4.) **DETECT:** This study evaluated using a combination of cystoscopy (using a through-the-needle fiberoptic probe (SpyGlass, Boston Scientific, USA)) followed by nCLE under EUS guidance for the diagnosis of PCL. The study recruited 30 patients with a range of PCL located throughout the pancreas. The procedure was technically successful with the exception of 1 probe exchange failure. Two patients (7%) developed post-procedure pancreatitis. Criteria for defining mucinous cysts were identified as: mucin on cystoscopy and papillary projections or dark rings on nCLE. The sensitivity of cystoscopy individually was 71% and 77% for nCLE alone (77%), but improved considerably when both modalities were used together (93%). By comparison the sensitivity and accuracy of pancreatic cyst fluid CEA in this study was 33% and 61%, respectively.(Nakai et al., 2015)

These initial studies demonstrated EUS-nCLE to be a safe test. Although specificity was shown to be high (>80 %), sensitivity varied by cyst subtype and was generally lower (69% for SCN, 59-80% for IPMN and 67% for MCN).(Napoleon et al., 2015a, Jais et al., 2015, Nakai et al., 2015, Kamboj et al., 2017, Napoleon et al., 2018) Concerns remain about the ease of image interpretation and the reproducibility of the test outside of expert centres with experienced operators. Initial studies of inter and intra observer variation has not been seen in expert endosonographers,(Krishna et al., 2017) but further studies are needed in endoscoographers with less experience of nCLE. To date EUS-nCLE has not been evaluated in a UK population with indeterminate

cystic lesions; therefore a Phase II study was conducted to assess the safety and utility of this technology in this population (CONCYST-01).

Methods

Study aim

The primary outcome of the study was to define the safety and efficacy of nCLE in the diagnosis of indeterminate PCL in an UK population.

Study design and inclusion criteria

This phase II prospective study was conducted in three large regional HPB centres in the UK; Royal Free NHS Foundation Trust, London, Cambridge University NHS Trust, Cambridge, Freeman Hospital, Newcastle.

Patients with a PCL for which EUS-FNA was indicated, based on multidisciplinary (MDT) review of cross-sectional imaging. The PCL had to be >1cm in size. Patients had to be >18 years, with an ECOG performance status 0, 1 or 2, an estimated life expectancy of at least 12 weeks and capable of giving written informed consent. They should not have pancreatitis within the previous 3 months and women of childbearing potential should have a negative pregnancy test in the week before nCLE.

Data Recorded

The electronic medical records of the included patients were reviewed, and information was recorded in an electronic spreadsheet. Data collected included demographic information (age, sex, hospital number), initial symptoms, and history of acute or chronic pancreatitis or malignancy, family history of pancreatic cancer or relevant clinical syndrome. Cross-sectional imaging (computed tomography (CT) and/or magnetic resonance cholangiopancreatography (MRCP)) was obtained from PACS (picture archiving and communication system, GE Healthcare, USA) and relevant features recorded. Details of the endoscopic procedure were obtained from the GI reporting tool. Pathology reports including cytology were obtained from the electronic histology database, in each centre for patients ultimately referred for surgery, date of the operation, type of resection and final histology were recorded. Length of follow-

up was calculated from first procedure to last clinic appointment attended, or date of clinic discharge, or death.

Study definitions of PCL subtype by EUS-nCLE

Definitions were established from previous EUS-nCLE publications [Figure 6.3]:

Intraductal Papillary Mucinous Neoplasm (IPMN): Papillary projections.(Khan et al., 2012a, Napoleon et al., 2015a, Jais et al., 2015)

Serous Cystic Neoplasm (SCN): Superficial vascular network (SVN).(Napoleon et al., 2015a, Jais et al., 2015)

Mucinous Cystic Neoplasm (MCN): The epithelial cyst border appears as a gray band delineated by a thin dark line.(Napoleon et al., 2015a, Jais et al., 2015)

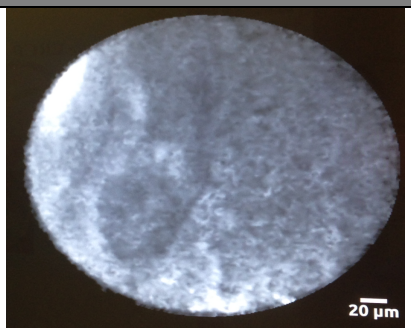
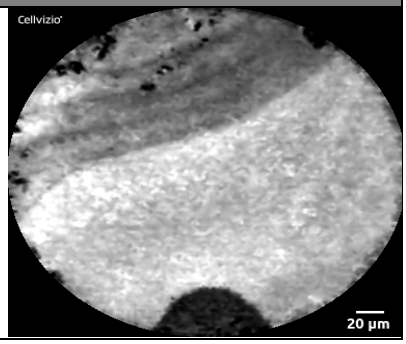
Pseudocyst: A pseudocyst was identified by bright, gray and black particles.(Napoleon et al., 2015a, Jais et al., 2015)

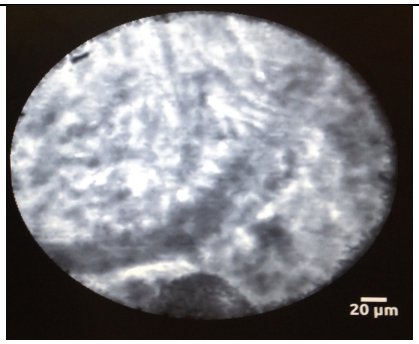
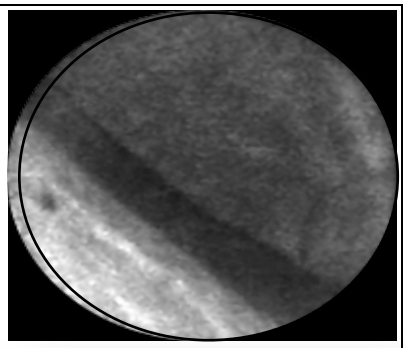
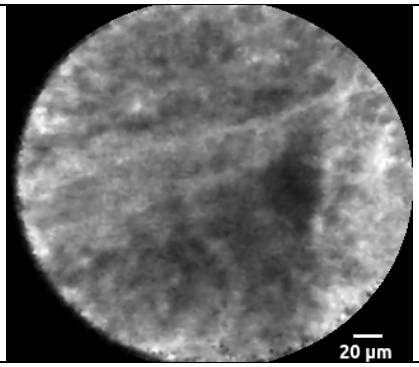
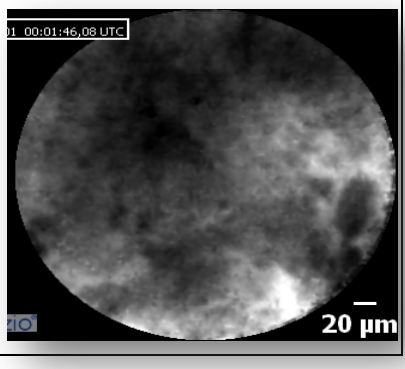
Pancreatic cancer (PC): PC was identified by the presence of black clumps.(Napoleon et al., 2015a, Jais et al., 2015)

Cystic Pancreatic Neuroendocrine Tumour (PanNET): Dark irregular clusters of cells, surrounded by various quantities of gray tissue.(Napoleon et al., 2015a, Jais et al., 2015)

Indeterminate PCL: Lesions that after review of nCLE images did not display recognisable features of any of the PCL listed above.

Figure 6.3: Description and representative image of common nCLE pancreatic cyst subtypes for image interpretation standards in the CONCYST 01 study

PCL subtype with nCLE features	nCLE image	PCL subtype with nCLE features	nCLE image
IPMN: Papillary projections		**Pseudocyst:** Fibrous strands and debris	

SCN: superficial vascular network		**MCN:** The epithelial cyst border appears as a gray band delineated by a thin dark line	
PDAC: Disorganised appearance with black clumps		**Cystic PanNET:** Dark irregular clusters of cells, surrounded by various quantities of gray tissue	

Final Diagnosis

Final diagnosis was based on pathology in those undergoing surgical resection. In all others final diagnosis was based on MDT consensus with at least 12 months follow up.

Procedures

Endoscopic Ultrasound guided needle based Confocal Laser Endomicroscopy (EUS-nCLE): Informed written consent for the procedure and study participation was obtained. The procedures were performed under conscious sedation or general anaesthesia using a linear array echoendoscope (Olympus, UK or Hitachi Pentax). Once the cyst had been visualised from the stomach or duodenum patients received 2.5ml of 10% fluorescein. The cyst was then punctured with a 19G fine-needle aspiration (FNA) needle (Cook Medical or Boston Scientific), which had been preloaded with the AQ-flex 19 miniprobe (Mauna Kea Technologies, Paris, France). Once in the cyst the probe was gently advanced past the bevel of the needle and on to the cyst wall to begin nCLE imaging. Once the nCLE imaging had been completed the probe was removed from the FNA needle and the cyst aspirated to dryness. Cyst fluid was sent for cytology, fluid CEA, fluid amylase levels or gram stain and culture as clinically indicated. Patients were discharged within 4 hours from the recovery unit as long as they were clinically stable. A single dose of antibiotics was given to each patient during the procedure.

Patients were then followed up by telephone clinic at 1 month and then seen as per routine in outpatients. Clinical records were reviewed at 12 months to confirm clinical outcome, and all patients were discussed at the HPB multidisciplinary team (MDT) meeting following EUS to determine cyst subtype and the subsequent management plan.

Ethical approval and consent to participate

The CONCYST-01 study protocol was approved by the UK National Health Research Authority *(14/LO/0040)* and all patients gave written informed consent. The protocol was registered on ClinicalTrials.gov (13/0572).

Data analysis

Statistical Package for Social Sciences for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Associations between various clinical and radiographic characteristics were evaluated using a 2-sample *t* test for continuous variables, and a Chi-squared test for categorical variables.

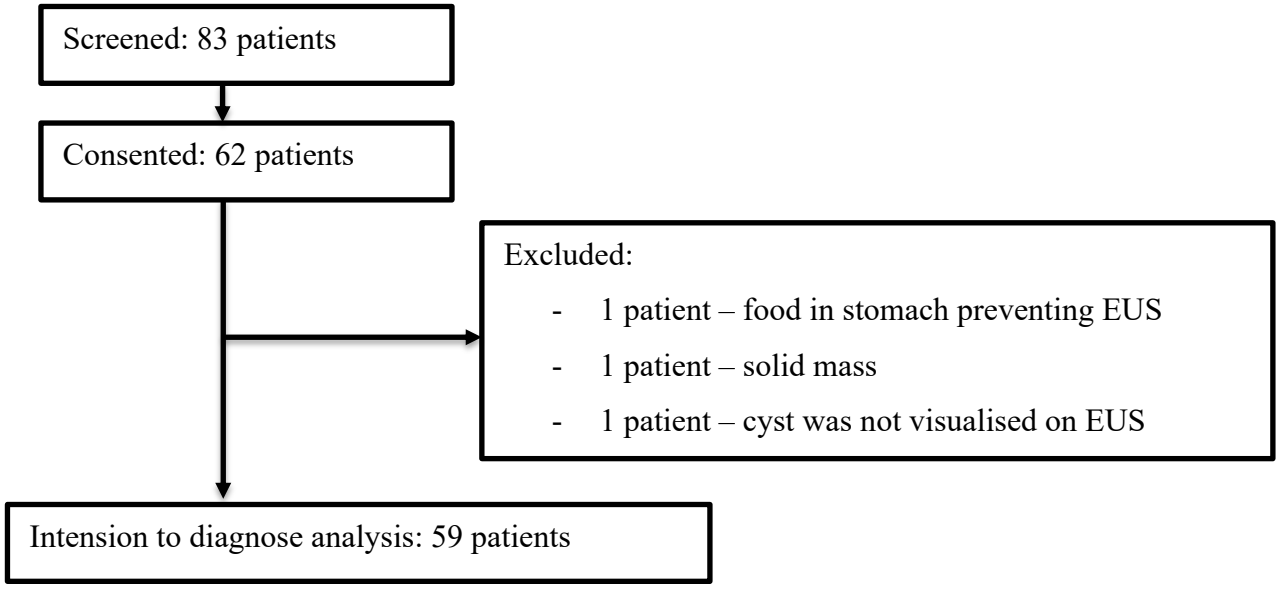
The study sample size was based on cytology being diagnostic in 31% of cases of PCL (de Jong et al., 2011) and nCLE in between 59 and 91% of cases, based on previous studies (Napoleon et al., 2015a, Jais et al., 2015, Nakai et al., 2015, Kamboj et al., 2017, Napoleon et al., 2018). Assuming, a 5% significance level and 85% power, it is calculated that 61 patients are required for the study.

Results

83 patients were screened, and 62 patients were ultimately consented to participate in the study. Three patients were excluded from the analysis during endoscopy, one because of a gastric residue despite fasting making it unsafe to proceed with EUS under conscious sedation, one because the cyst could not be visualised at EUS and one because the lesion was determined to be solid at EUS so the patient no longer met the study criteria. nCLE was not performed in a further 3 cases, in two cases because the

cyst could not be punctured with a 19G FNA needle, and in one case because of extravasation of the fluorescein preventing imaging [Figure 6.4].

Figure 6.4. Flowchart for patients included in the CONCYST 01 study and the intension to diagnose analysis



59 patients were included in the final analysis, 30 male, 29 female; median age 68 (range 28-80). 43% (24/42) of patients were symptomatic and in the remaining cases the lesions were found incidentally. 26% (11/42) had a history of pancreatitis. One patient had known von Hippel Lindau syndrome but no others had a family history of pancreatic cancer or associated syndromes. Seven patients had a history of a non-pancreatic solid organ malignancy. One patient had previously undergone a Whipple's procedure for a 3cm SCN. During assessment all patients had had a CT scan, 23/59 had an MRI and 5/59 patients had had a prior EUS (with indeterminate findings) before entering the CONCYST-01 study. 36% of cysts were in the head or uncinate of the pancreas with the remaining lesions in the body or tail [Table 6.1].

Table 6.1: Patient demographics for the CONCYST 01 study

Patients	%	n
Median age (range), years	68 (28-80)	
Sex		
Male	51%	30/59
Female	49%	29/59
Cyst morphology		

Location		
Head	36%	21/59
Uncinate	3%	2/59
Body	24%	14/59
Tail	37%	22/59
Median lesion size (range), mm	25 (10-70)	
Associated symptoms	43%	18/42
Solid component / mural nodule	15%	9/59
Septations	32%	19/59
Dilated MPD (>5mm)	27%	16/59
Final diagnosis definition		
Surgery	5%	3/59
MDT consensus / cytology	95%	56/59

Final diagnosis was determined by surgical pathology in 3 cases and MDT consensus and follow up in all other cases [Table 6.2]. 4/59 cases were lost to follow up. The remaining cases were followed up for >12 months.

Table 6.2: Final diagnosis of participants in the CONCYST 01 study (based on surgical resection or MDT consensus with 12 months follow up)

Final Diagnosis	**N**
PDAC	3
BD IPMN	29
MD IPMN	2
Multifocal IPMN + LGD	1
PanNET	1
GIST	1
Pseudocyst	12
SCN	9
Indeterminate cystic lesion	1

Determining diagnosis by clinical history and radiology alone, performed poorly in comparison to EUS with cytology or EUS with nCLE (5% vs. 63% vs. 73%; P=0.001). Most cases, unless they had undergone malignant transformation remained indeterminate after cross sectional imaging, warranting further investigations.

Recognisable confocal images were obtained in 48/59 cases. Median nCLE scanning time was 5 minutes and did not exceed 10 minutes in any case. EUS-nCLE findings correlated with final diagnosis (based on imaging, cytology and multidisciplinary team

review) in 43/59 (73%) of cases, compared with 37/59 (63%) for cytology alone (P=0.199). In IPMN cases though nCLE performed significantly better than routine cytology (82% vs 63%, p=0.05) [Table 6.3].

Table 6.3: Comparing diagnostic accuracy of EUS nCLE to clinical history, radiology and EUS + cytology

Cyst subtype	EUS nCLE vs. final diagnosis	History + radiology vs. final diagnosis	P value	EUS + Cytology vs. final diagnosis	P value
All	73% (43/59)	5% (3/59)	**P <0.001**	63% (37/59)	0.199
IPMN	82% (26/32)	0% (0/32)	**P <0.001**	63% (20/32)	**0.050**
SCN	56% (5/9)	0% (0/9)	**P <0.001**	44% (4/9)	0.621
Pseudocyst	67% (8/12)	0% (0/12)	**P <0.001**	92% (11/12)	0.138
PDAC	100% (3/3)	100% (3/3)	-	67% (2/3)	0.322

EUS-nCLE had an overall sensitivity of 79.6 %, which improved to 90% for IPMN, and 100% for PDAC [Table 6.4]. When enough cyst fluid was obtained to measure fluid CEA it was only diagnostic in cases with positive cytology so was not compared separately.

Table 6.4 Sensitivity, PPV and NPV for EUS nCLE by PCL subtype in the CONCYST 01 study

Cyst subtype	Sensitivity (%)	Accuracy (%)	PPV (%)
All PCL (subtype)	79.63 (66.47-89.37)	76.79 (63.58-87.02)	95.56 (94.95-96.09)
IPMN	89.66 (72.65-97.81)	86.67 (69.28-96.24)	96.3 (05.83-96.71)
PDAC	100 (29.24-100)	100 (29.24-100)	100
SCN	55.56 (21.20-86.30)	38.46 (13.86–68.42)	55.56 (41.07-69.16)
Pseudocyst	66.67 (34.89-90.08)	66.67 (34.89-90.08)	100

The rate of associated adverse events was 5.1% (3/59), all were graded as mild-moderate in severity. One patient experienced mild pruritus immediately after the procedure (probable allergy to fluorescein) and one developed mild bruising on their hand due to extravasation of the fluorescein, both were graded as mild events. The final adverse event was a case of an infected pseudocyst, which resolved with IV antibiotics and a short hospital admission. This case was graded as moderate severity. There were

no significant differences were seen in nCLE performance or adverse outcomes between the individual 3 centres in the study.

Discussion

Early experience of EUS-nCLE using the AQ-Flex probe has shown it to be a safe technique and a useful adjunct to EUS-FNA.(Konda et al., 2011, Napoleon et al., 2015a, Jais et al., 2015, Nakai et al., 2015) In this study, nCLE was found to have a similar diagnostic accuracy to routine cytology (73% vs. 63%; p=0.199). Although there was a trend towards statistical significance, our final patient number (n=59) was smaller than the planned 61 patients. Three study patients were excluded for clinical or technical reasons. In addition, the diagnostic accuracy of cytology in this study was substantially higher than that reported in previous retrospective studies from our centre or other published series. The improvement in cytology in this study may be attributable to the cytopathologist being present in the endoscopy room for a proportion of the cases, ensuring the slides were prepared correctly and assessed immediately.

The study used the criteria defined by the international INSPECT, CONTACT and DETECT studies to identify cyst subtype.(Napoleon et al., 2015a, Jais et al., 2015, Nakai et al., 2015, Kamboj et al., 2017, Napoleon et al., 2018) These studies showed these criteria to have a high specificity (>80 %) but a lower and somewhat variable sensitivity.(Napoleon et al., 2015a, Jais et al., 2015, Nakai et al., 2015, Kamboj et al., 2017, Napoleon et al., 2018) In this study we had similar findings with sensitivities ranging between 55 and 100%. Somewhat unexpectedly the sensitivity of SCN in this study was only 55% which is lower than that reported in the CONTACT 1 study (69% sensitivity and 100% specificity).(Napoleon et al., 2015a) This may reflect the operator's learning curve or alternatively the technique used for performing nCLE. In the French CONTACT 1 study, a lower sensitivity for nCLE in SCN (69%) was also observed.(Napoleon et al., 2015a) In these cases the probe was "brushed or walked" along the wall during imaging, possibly resulting in epithelium being dislodged. In the subsequent CONTACT 2 study, a different technique was used to obtain nCLE images; with operators placing the probe on two points on the cyst wall only. In this subsequent study there was an improved sensitivity for nCLE in SCN group (>95%). A rise in the cytology yield was also seen compared to the CONTACT 1 study which may be due to

the epithelium being dislodges and therefore improving yields.(Napoleon et al., 2018) In this study, the probe was used in a similar way to the CONTACT 1 study so imaging technique may have also explain our lower sensitivity in SCN and improved cytology findings. Importantly in this study in IPMNs, nCLE was significantly more accurate at detecting IPMNs than routine cytology. Arguably this is the most important group to detect because of their pre-malignant potential.

Adverse events in this study (5.1%), were similar to those reported in prior studies. We encountered no episodes of post procedure pancreatitis, which has been reported in around 4% in other studies. (Napoleon et al., 2015a, Jais et al., 2015, Nakai et al., 2015, Kamboj et al., 2017) The highest rate was seen in the DETECT study (6.6%), which required longer needle access time as the technique was combined with Spyglass cystoscopy as well as nCLE imaging.(Nakai et al., 2015) Increased adverse advents are potentially attributable to prolonged procedure time and manipulation.(Nakai et al., 2015, Kamboj et al., 2017) Limiting both in this study may account for the low rates of associated adverse events observed.

In this study substantial differences were not seen between different IPMN with low- or high-grade dysplasia. This may be due to the relatively small sample size of patients and a predominance of small lesions with likely low grade dysplasia. Emerging reports suggest that different subtypes of IPMN may have different criteria when imaged by EUS-nCLE,(Kamboj et al., 2017) which could have prognostic significance. Further studies in patients who ultimately undergo surgical resection would be needed to evaluate this further.

This UK based outpatient study further confirmed the safety profile of nCLE in the assessment of pancreatic cystic lesions, it did have certain limitations. This study did not demonstrate better diagnostic accuracy than cytology, the current diagnostic standard. As this technology is expensive and increases the length of the procedure, its place in diagnostic algorithms for PCL, needs to be further defined. A recent study by the CONTACT authors looked at the cost effectiveness of this technology in a French population and the potential for EUS-nCLE to prevent unnecessary over treatment or surveillance, especially in patients with an SCN. They found that EUS-nCLE would reduce the rate of surgical intervention by 23%, with 4 in 1000 patient deaths prevented

due to unnecessary surgery. Given the lower diagnostic accuracy for nCLE in SCN in this study it is unclear if similar benefits and cost savings would be found in a UK population and further studies would be needed to explore these hypotheses.

Conclusion

In a UK population EUS-nCLE under conscious sedation in the day case setting is safe adjunct to EUS FNA for the assessment and diagnosis of pancreatic cysts. However, its diagnostic utility over current standard tests remains uncertain. Larger adequately powered studies are needed to validate these promising findings.

7 DEVELOPING REAL-TIME MOLECULAR IMAGING TO DETECT HIGH-RISK PRECURSOR LESIONS FOR PANCREATIC CANCER

Introduction

Molecular fluorescence-guided biomarkers have been used in cancer surgery for many years.(Hernot et al., 2019) It requires a camera to excite an (intravenously injected) fluorophore conjugated to a tumor-specific targeting molecule and detect its emitted fluorescence.(Mieog et al., 2022). The technique has the potential to be able to distinguish tumor from surrounding benign tissue. In pancreatic surgery this improves rates of R0 resection and the quality of pancreatic surgery.(de Muynck et al., 2023)

As outlined in Chapters 1 and 4 one of the most studied biomarkers in pancreatic cancer and IPMN is carcinoembryonic antigen (CEA). CEA is a glycoprotein involved in cell adhesion and is overexpressed in more than 90% of pancreatic cancers. (van Manen et al., 2020b, van Manen et al., 2020a) (Hammarström, 1999) CEA is also found at high concentration in the cyst fluid of mucinous cysts and is currently one of the main ways of differentiating mucinous and non-mucinous PCL, with a greater accuracy than cytology alone.(Utomo et al., 2015, Nagula et al., 2010) Studies have demonstrated an anti-CEA monoclonal antibody conjugated to a 700 nm fluorophore (SGM-101) can clearly visualize pancreatic tumors during surgery.(Hoogstins et al., 2018) CEA was therefore selected as the primary candidate biomarker for targeted fluorescence imaging in this study.(Vuijk et al., 2020)

S100P is a member of the S100 protein family, localized in the cytoplasm or nucleus of a range of cells. It is a calcium-binding protein that is involved in the regulation of cellular processes such as cell cycle progression and differentiation. It is upregulated in both pancreatic cancer and IPMN, and is expressed in the early stages of pancreatic carcinogenesis, so is a promising target for the detection of early stage disease.(Ohuchida et al., 2006) Anterior gradient protein 2 homolog (AGR-2) is a secreted cement gland protein. High levels of AGR2 correlate with downregulation of the p53, cell migration, and cell transformation and proliferation AGR2 is upregulated

in pancreatic cancer and its precursor lesions, and its expression is correlated with the development of pancreatic cancer as well as poor survival.(Qu et al., 2024) With the potential to provide stratification of more severe tumors it was selected as the last marker for evaluation in this study.

Fluorescence-guided markers for diagnosis have been evaluated infrequently in pancreas cancer. In this study, we will assess the expression of three biomarkers with potential for fluoroscopic labelling, in a range of PCL. If a fluoroscopically labelled biomarker can differentiate high risk PCL, there is the potential they could be used to enhance emerging diagnostic techniques such as needle based confocal endomicroscopy [Chapter 6] to improve the accuracy of image interpretation

.

Methods

Data recorded

For each patient recruited, the electronic medical records were reviewed and information was recorded in an electronic spreadsheet. Data was recorded from the Pathology (CoPath histology database, Sunquest, Tucson AZ, USA), Endoscopy (GI reporting tool, Unisoft medical systems, UK) and Imaging (PACS: picture archiving and communication system, GE Healthcare, USA) database systems. Data collected included demographic information (age, sex, hospital number), history of acute or chronic pancreatitis or malignancy, family history of pancreatic cancer or relevant clinical syndrome. Cross-sectional imaging (computed tomography (CT) and/or magnetic resonance cholangiopancreatography (MRCP)) features were recorded. If performed, details of the EUS procedure along with cytology and histology results were recorded. Surgical details included, date of the operation, type of resection and final histology were recorded. Length of follow-up was calculated from first procedure to last clinic appointment attended, or date of clinic discharge, or death. Diagnosis was established by surgical resection. Benign disease was confirmed by follow up of at least 12 months post-resection.

Immunohistochemistry

Formalin fixed paraffin embedded tissue blocks of representative tumour were obtained from patients with a range of PCL. Consecutive serial tissue sections were cut at a thickness of 4μm onto Superfrost Plus slides (Visions Biosystems, Newcastle Upon Tyne, UK), dewaxed in xylene and rehydrated through graded alcohol to water. The tissue sections were pressure-cooked in 0.1 M citrate buffer at pH 6.0 for 2 minutes and immunostained using the Bondt Polymer Refine Detection kit and Bondt-Max automated system (Vision Biosystems). Primary antibodies for CEA, S100p and AGR2 were applied. The slides were then dehydrated with graded alcohol and then washed thrice with xylene (100% concentration). Coverslips were applied with Pertex mounting medium (CellPath Ltd, Newtown, Powys, UK). Incubation without a primary antibody was used as a negative control and gastric sections as positive controls.

Image Analyses and Quantification

Image acquisition and analysis were performed using the TissueGnostics (microscope, AxioImager Z.2; Zeiss) or Qupath software. Regions of interest were selected which were representative of the strength of staining within the epithelial lining of the cyst wall. Cell-based analysis was performed using automated cell segmentation based on colour. A threshold for minimum cell area, number of cells detected (>500 cells) and variance of staining was set based on previous work.(Vassileva et al., 2015) Positive cells were detected on the basis of mean and maximum intensity of staining. Data were generated by calculating the percentage of positively stained cells over the total number of cells in the regions of interest.

Data analysis

Statistical Package for Social Sciences for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. Associations between various clinical and radiographic characteristics were evaluated using a 2-sample *t* test for continuous variables and a 5% level was used to indicate significance. The sensitivity of the biomarker in comparison to final diagnosis was compared using McNemar's test for paired proportions.

Ethical approval

The study was approved by the Joint UCLH/UCL ethical committee and all patients gave written informed consent (NRES: 06/Q0512/106).

Results

Patient demographics

The study cohort comprised of 63 patients with a range of surgically resected PCL [Table 7.1]. 21 participants were male and 42 were female. Median age at diagnosis was 63 (range 20-84) years. 10 patients with an IPMN with high-grade dysplasia or invasive cancer were used as positive controls.

Cyst subtype	n
IPMN + HGD or invasive cancer	10
IPMN + LGD or IGD	18
MCN + LGD or IGD	11
Mucinous non neoplastic cyst + no dysplasia	1
SPN	5
Serous cystic neoplasm	12
Pseudocyst	7

Table 7.1 Pancreatic cyst subtype for study participants. Representative tissue samples were stained for CEA, S100p and SGR2. Positive controls were IPMN + HGD or invasive cancer. SCN and pseudocysts were used as a benign control.

Immunohistochemistry

For the 63 patients included in the study, the pattern of CEA, S100p and AGR2 expression was assessed by immunohistochemistry. Staining of the biomarkers was largely restricted to the epithelial lining of the cyst wall and was most intense in areas of high-grade dysplasia or invasive cancer [Table 7.2]. Within the adjacent areas of normal pancreas and in benign lesions, staining was extremely low.

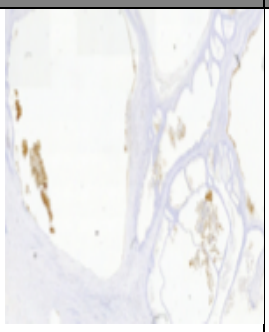
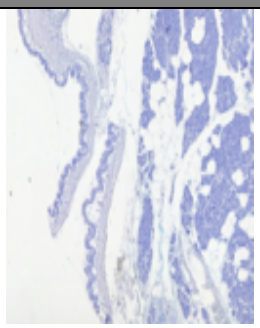
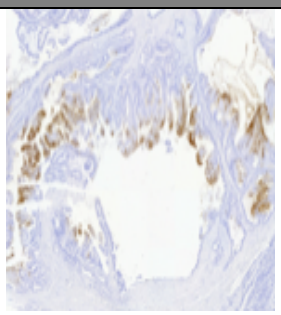
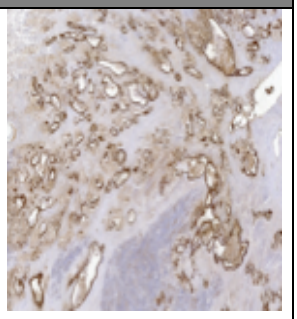
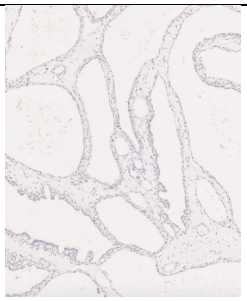
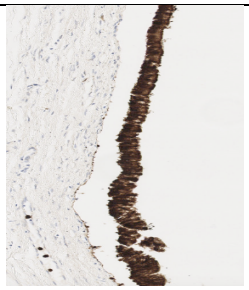
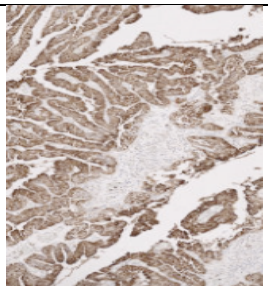
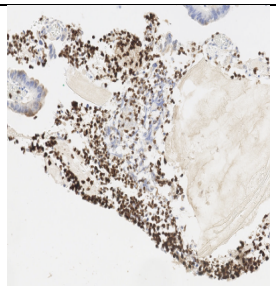
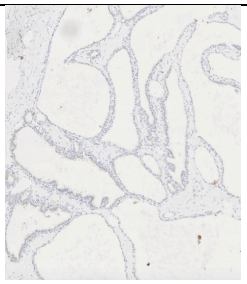
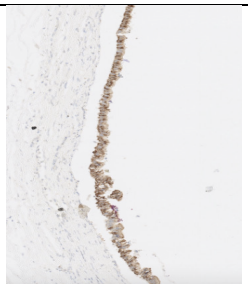
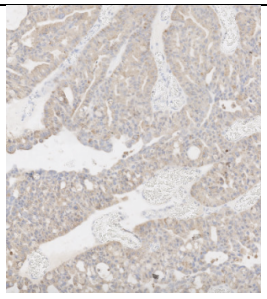
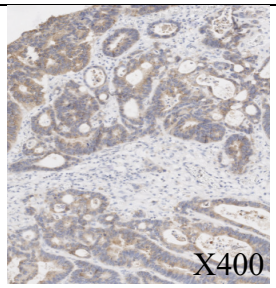
	SCN	IPMN + LGD	IPMN + HGD	PDAC
CEA				
S100p				
AGR-2				

Table 7.2 Representative photomicrographs illustrating the expression of CEA, S100p, AGR-2 in a range of PCL (immunostains x400). Expression of all three markers was strong in the epithelial wall of IPMN with HGD or invasive cancer. Minimal expression was seen in benign serous cystic neoplasms. S100p and AGR2 expression was also present in IPMN + LGD

CEA, S100p and AGR2 expression in a range of PCL

In this cohort of 63 patients with a range of PCL all three biomarkers were expressed strongly in PCL with HGD or invasive cancer. Unlike CEA, S100p and AGR2 continued to be expressed strongly in IPMN/MCN with LGD. S100p and AGR2 are expressed at very low levels in benign disease (SCN and pseudocysts) and CEA was not expressed at all. Mucinous non-neoplastic cysts are rare lesions that are not thought to have malignant potential. Expression of CEA, A100p and AGR2 were consistently

low. Expression was also low in SPPN lesions, consistent with their low malignant potential [Table 7.3].

	CEA - median LI (%)	Range	P value	AGR2 - median LI (%)	Range	P value	S100p median LI (%)	Range	P value
Positive control: IPMN + HGD or invasive cancer	8.84	0.43-48.27	-	79.65	4.48-93.52	-	84.55	4.73-96.9	-
IPMN + LGD	3.166	0.77-37.76	P=0.36	71.37	27.27-98.61	P=0.60	93.8	2.14-99.95	P=0.06
MCN + LGD	2.02	0.14-26.41	P=0.17	69.51	14.54-92.53	P=0.35	63.9	10.51-95.92	P=0.41
Mucinous non neoplastic cyst	4.09	-	P=0.08	28.63	-	**P=0.034**	71.08	-	**P=0.05**
SCN	0	0	**P=0.005**	15.69	1.12-50.04	**P=0.006**	22.61	8.97-53.24	**P=0.014**
SPPN	-	-	**P=0.005**	22.78	0.68-29.4	**P=0.023**	30.46	1.62-90.57	P=0.194
Negative control: Pseudocyst	0	0	**P=0.005**	21.05	0.74-75.44	**P=0.04**	33	0.06-96.92	P=0.307

Table 7.3 CEA, S100p and AGR2 expression in the epithelial wall of a range of PCLs. Median LI and range are reported. Differences in expression are compared to the positive controls (IPMN with HGD or invasive cancer)

Discussion

In this study, three biomarkers were evaluated for their potential as fluoroscopically labeled targets to differentiate high-risk PCL. CEA, S100p and AGR2 all demonstrated increased expression in the epithelial wall of PCL with HGD or invasive cancer in comparison to benign lesions.

In future work, we would aim to evaluate if fluoroscopically labelled CEA (e.g. huA5B7), S100p and AGR2 when incubated with IPMN or PDAC cell lines (e.g.

AsPC-1, BxPC-3, Capan-1, T3M-4, HPAF-II and SU.86.86), could detect dysplastic or cancer cells. Optical imaging would then be used to detect the markers; typically near-infrared fluorescence (NIRF) imaging as the fluorescent molecular probe has emissions in the near-infrared region (650–900 nm). If successful a feasibility in vivo study could be performed in a IPMN animal model. Nakai et al. demonstrated feasibility of in vivo, real-time visualization of fluoroscopically labelled EGF-R and survivin in the porcine pancreas following local injection of FITC-labeled antibodies via EUS-guided needle based confocal laser-induced endomicroscopy (EUS nCLE).(Nakai et al., 2012)

If a fluoroscopically labelled CEA could be detected by nCLE, it will likely make differentiation of mucinous cysts during EUS nCLE considerably easier. Expression of CEA in this study varied by level of dysplasia, being strongly expressed in invasive cancer, and had very low expression in low grade dysplasia. Quantification of maker expression in real time during EUS, would make the detection of dysplasia in high-risk lesions much easier. At present there is a significant learning curve and intra operator variation in the EUS nCLE technique.(Krishna et al., 2017) Fluoroscopically labelled marker have the potential to simplify the technique and reduce the learning curve of this emerging technology.

Conclusion

This initial study explores the potential for in vivo visualization of fluroscopically labelled markers (CEA, S100p and AGR2) in pancreatic cysts via EUS guided needle-based confocal laser endomicroscopy. Further in vitro and in vivo studies are needed, but if this technique proves to be feasible, it may lead to wider adoption of this technology outside of expert centres, and by providers that perform this procedure less frequently.

8 Systematic review of minimally invasive ablative treatments for pancreatic cancer and cystic tumors of the pancreas

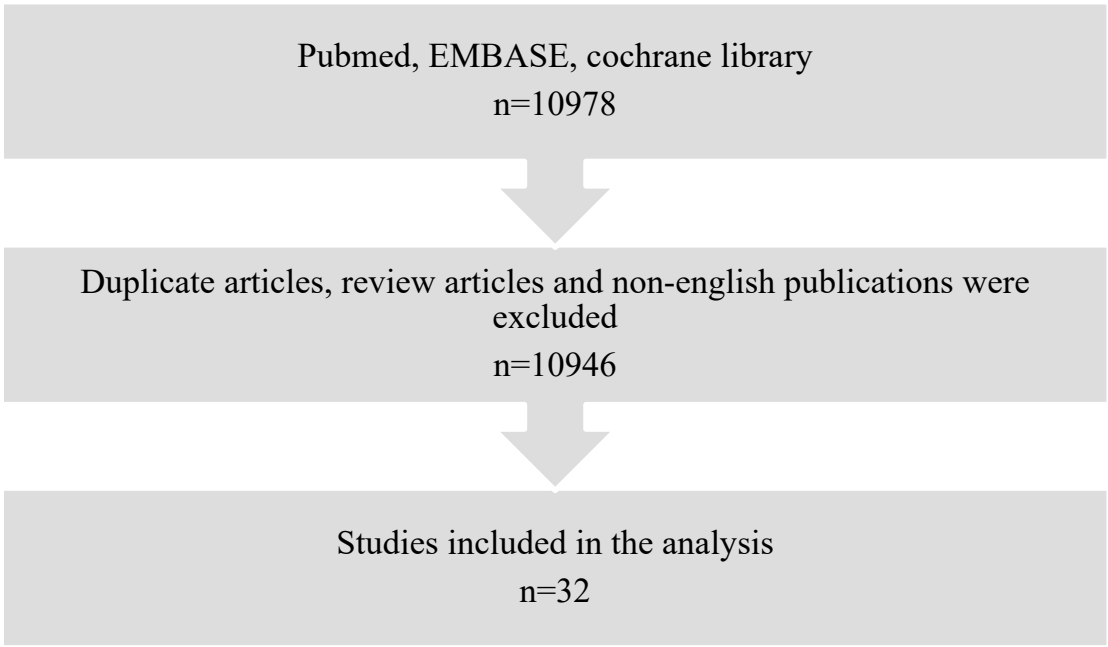
Ablative treatments in locally advanced PDAC

Given that a proportion of patients with locally advanced PDAC that are unsuitable for surgery due to co-morbidity and that most have only a limited response to chemotherapy, there has therefore been a growing interest in the utility of minimally invasive cytoreductive therapies.

A systematic review of the literature was performed. The primary aim was to assess safety and efficacy of each ablation therapy in the treatment of locally advanced or metastatic PDAC. Secondary endpoints included improvements in overall survival, changes in symptoms, tumour markers or performance status where available. A search was performed using the PubMed, EMBASE databases and the Cochrane Library for studies published in the English language up to 1st October 2013. MeSH terms were decided by a consensus of the authors and were (radiofrequency ablation, catheter ablation, photodynamic therapy, PDT, cryoablation, cryosurgery, laser, high intensity focused ultrasound ablation, microwave, electroporation) and (pancreas OR pancreatic), and were restricted to the title, abstract and keywords. Only articles, which described ablation in unresectable PDAC, were included. Articles that described the use of ablative therapies in premalignant pancreatic disease were excluded. Similarly, studies that included non-ablative therapies were excluded. Any study with fewer than four patients and those reporting on tumours that did not originate in the pancreas were excluded. In cryoablation and high frequency focused ultrasound of the pancreas, many of the largest case-series are published in non-English language medical journals. Although articles not published in the English language were excluded from this systematic review, if an English language abstract was available the results were included in the summary tables. All references were screened for potentially relevant studies not identified in the initial literature search. The following variables were extracted for each report when available: number of patients, disease extent, device used and settings, distance of probe from surrounding structures, duration of therapy

and number of ablations applied, additional safety methods used. Thirty-two papers were included [Figure 8.1].

Figure 8.1 Systematic review flow diagram outlining the databases used, screening articles and studies included.



Thermal ablative techniques

8.1.1.1 Radiofrequency ablation

Radiofrequency ablation (RFA) causes tissue destruction through the application of a high frequency alternating current that generates high local temperatures leading to a coagulative necrosis. The technique has been widely used in many solid organ malignancies and is now part of standard therapy in several tumours including hepatocellular carcinoma.(Llovet et al., 1999) The first application of RFA in the normal porcine pancreas was described in 1999. Although this application was performed under EUS guidance,(Goldberg et al., 1999) it has since, nearly always been delivered intraoperatively (rarely percutaneously) in combination with palliative bypass surgery.(Date and Siriwardena, 2005) Although RFA was deemed to be feasible and safe in animal studies,(Goldberg et al., 1999) early clinical applications in the pancreas were associated with unacceptably high rates of morbidity (0-40%) and mortality (0-25%) [Table 4].(Matsui et al., 2000, Girelli et al., 2010, Elias et al., 2004, Hadjicostas et al., 2006, Wu et al., 2006, Spiliotis et al., 2007) However, most RFA of pancreatic

tumours had been performed using the Cool-tip™ RF Ablation system (Radionics). Many of the complications arose as a result of inadvertent damage to structures adjacent to the zone of ablation such as the normal pancreas, duodenum, biliary tree or peri-pancreatic vasculature. These early studies applied high temperatures (>90°C) and multiple rounds of ablation to treat large tumours in the head of the pancreas in one session.(Elias et al., 2004) An ex-vivo study of the thermal kinetic characteristics of RFA found that the optimal settings for RFA in the pancreas to prevent injury to the adjacent vicera was 90°C applied for 5 minutes.(Date et al., 2005) Subsequent clinical studies that reduced the RFA temperature from 105 °C to 90 °C, reported only minimal RFA-related complications.(Girelli et al., 2010) Active cooling of the major vessels and duodenum with saline during intraoperative RFA and observing at least a 0.5cm area between the zone of ablation and major structures, reduced complications.(Varshney et al., 2006, Tang et al., 2008) Since most of the mortality resulted from uncontrollable gastrointestinal haemorrhage from ablated tumours in the head of the pancreas, some authors have recommended this probe should only be employed in body or tail tumours.(Wu et al., 2006, Tang et al., 2008)

All studies have demonstrated that RFA leads to tumour necrosis and a decrease of tumour volume.(Matsui et al., 2000, Date and Siriwardena, 2005, Varshney et al., 2006, Siriwardena, 2006) Some studies have also observed an improvement in tumour related symptoms, in particular a reduction of back pain and analgesia requirements. Tumour markers (i.e. CA 19-9) also decrease following effective ablation.(Tang et al., 2008) Although all patients treated with RFA ultimately developed disease progression (Spiliotis et al., 2007, Date and Siriwardena, 2005, Matsui et al., 2000, Varshney et al., 2006, Siriwardena, 2006) when compared to those at the same stage who received standard therapy in a non-randomised cohort study, patients who received combination therapy had prolonged survival (33 months vs. 13 months, P=0.0048).(Spiliotis et al., 2007) However, this was a single centre study including 25 patients (12 receiving RFA) and larger studies will be required for validation. An earlier non-randomised study did not demonstrate the same survival advantage.(Matsui et al., 2000)

Recently two new RFA probes have been developed that can be placed down the working channel of an endoscope, enabling RFA to be administered under EUS guidance. Twenty-two patients with locally advanced PDAC were treated with the

cryotherm probe (CTP) (ERBE Elektromedizin GmbH, Tübingen, Germany) that incorporates radiofrequency ablation with cryogenic cooling. The probe was sited successfully in 16 patients (72.8%); stiffness of the gastrointestinal wall and tumour prevented placement in the others. Following the procedure three patients reported mild abdominal pain and one experienced minor gastrointestinal bleeding, not requiring transfusion.(Arcidiacono et al., 2012) In a further study 7 patients with unresectable PDAC have received EUS guided RFA using the monopolar radiofrequency (RF) catheter (1.2mm Habib EUS-RFA catheter, Emcision Ltd, London). The tumour was shown to decrease in size in all cases and only one patient developed mild pancreatitis.(Pai et al., 2013b) Long-term follow up date is not available on the efficacy of these new catheters [Table 8.1].

Table 8.1 Outcomes and adverse events from studies of radiofrequency ablation for the treatment of PDAC

Study	Patients	N	Route of administration	Device	RFA Temp (˚C)	RFA duration (Min)	Outcome	Adverse events
(Matsui et al., 2000)	Unresectable PDAC	20 LA:9 M:11	At laparotomy 4 RFA probes were inserted into the tumour 2cm apart	A 13.56-MHz RFA pulse was produced by the heating apparatus	50	15	Survival: 3 months	Mortality: 10% (septic shock and gastrointestinal bleeding)
(Hadjicostas et al., 2006)	Locally advanced and unresectable PDAC	4	Intraoperative (followed by palliative bypass surgery)	Cool-tip™ RFAblation system	NR	2-8	All patients were alive one year post-RFA	No complications encountered
(Wu et al., 2006)	Unresectable PDAC	16 LA:11 M:5	Intraoperative	Cool-tip™ RFAblation system	30-90	12 at 30˚C then 1 at 90˚C	Pain relief: back pain improved (6/12)	Mortality: 25% (4/16) Pancreatic fistula: 18.8% (3/16)
(Spiliotis et al., 2007)	Stage III and IV PDAC receiving palliative therapy	12 LA:8 M:4	Intraoperative (followed by palliative bypass surgery)	Cool-tip™ RFAblation system	90	5-7	Mean survival: 33 months.	Morbidity: 16% (biliary leak) Mortality: 0%
(Girelli et al., 2010)	Unresectable locally advanced PDAC	50	Intraoperative (followed by palliative bypass surgery)	Cool-tip™ RFAblation system	105 (25 pts) 90 (25 pts)	Not reported	Not reported	Morbidity 40% in the first 25 patients. Probe temperature decreased from 105°C to 90°C. Morbidity 8% in second cohort of 25 patients. 30-day mortality: 2%.
(Girelli et al., 2011)	Unresectable locally advanced PDAC	100	Intraoperative (followed by palliative bypass surgery)	Cool-tip™ RFAblation system	90	5-10	Median overall survival: 20 months	Morbidity: 15%. Mortality: 3%.

(Giardino et al., 2013)	Unresectable PDAC. 47 RFA alone. 60 had RFA + radiochemotherapy (RCT) &/or intra-arterial systemic chemotherapy	107	Intraoperative (followed by palliative bypass surgery)	Cool-tip™ RFAblation system	90	5-10	Median overall survival: 14.7 months in RFA alone but 25.6 months in those receiving RFA + RCT and/or IADC (P= 0.004)	Mortality: 1.8% (liver failure and duodenal perforation) Morbidity: 28%
(Arcidiacono et al., 2012)	Locally advanced PDAC	22	EUS-guided	Cryotherm probe; bipolar RFA + cryogenic cooling	NR	2-15	Feasible in 16/22 (72.8%) Median survival: 6 months	Pain (3/22) Minor bleeding (1/22)
(Pai et al., 2013b)	Locally advanced PDAC	7	EUS-guided	Habib EUS-RFA catheter	NR	Median 3 (range 2-4)	2/7 tumours decreased in size	Mild pancreatitis: (1/7)

*LA: Locally advanced PDAC. M: Metastatic PDAC. SEMS: Self-expanding metal stent. RFA: Radiofrequency ablation. EUS: Endoscopic ultrasound. ERCP: Endoscopic retrograde cholangiopancreatography

8.1.1.2 Microwave ablation

Microwave (MW) current is produced by a generator connected via a coaxial cable to 14-gauge straight MW antennas with a 3.7cm or 2cm radiating section. One or two antennae are then inserted into the tumour for 10 minutes. The largest case series of microwave ablation in locally advanced PDAC included 15 patients. Although MW ablation can be performed percutaneously or intraoperatively,(Carrafiello et al., 2013) in this series it was performed intraoperatively at the time of palliative bypass surgery. All tumours were located in the head or body of the pancreas and had an average size of 6cm (range 4-8cm); none had distant metastasis on imaging. Partial necrosis was achieved in all patients and there was no major procedure-related morbidity or mortality. However minor complications were seen in 40% (mild pancreatitis, asymptomatic hyperamylasemia, pancreatic ascites, and minor bleeding). The longest survival of an individual patient in this series was 22 months.(Lygidakis et al., 2007)

8.1.1.3 Cryoablation

The successful use of cryoablation in the pancreas was first reported in primate experiments in the 1970s.(Myers et al., 1970) However its potential application as a therapy in PDAC was not described for a further 20 years.(Patiutko Iu et al., 1991) Cryoablation is most commonly performed intra-operatively under ultrasound

guidance. Small lesions (<3 cm) can be reliably frozen with a single, centrally placed probe but larger tumours require the placement of multiple probes or sequential treatments. Most studies have used the argon-gas-based cryosurgical unit (Endocare, Inc., CA, USA) and employ a double "freeze/thaw" cycle. The tumour is cooled to –160°C and the resulting iceball monitored with ultrasound to ensure the frozen region encompasses the entire mass and does not compromise local structures. The tissue is then allowed to slowly thaw to 0°C and a second cycle of freezing is performed after any necessary repositioning of the cryoprobes. Like in many of the RFA studies, the authors advocated a 0.5cm margin of safety from major structures and that ideally the procedure is performed at the same time as palliative bypass surgery or endoscopic biliary and duodenal stenting. Ablation of liver metastases can also be performed simultaneously.(Xu et al., 2008b)

The largest experience of intraoperative and percutaneous cryoablation in pancreatic cancer has been reported from Asia.(Patiutko et al., 1991, Kovach et al., 2002, Li et al., 2004, Wu et al., 2005b, Yi et al., 2006, Xu et al., 2008a, Li et al., 2011, Xu et al., 2013, Niu et al., 2013) To date more than 200 patients with unresectable PDAC have undergone cryoablation alone or in combination with other therapies. Effective control of pain, normalisation of CA 19-9, improvement in performance status, and prolonged survival have all been reported following cryoablation. Rates of significant complications appear to be lower than in other methods of ablation. Although some patients did encounter delayed gastric emptying following the treatment, this commonly settled with conservative management within a few days.

8.1.1.4 Laser based ablative therapy - Photodynamic Therapy

Photodynamic therapy (PDT) results in tumour ablation by exposure to light following an intravenous injection of a photosensitiser (e.g., *meso*-tetra(hydroxyphenyl)chlorin, porfimer sodium or verteporfin), which is taken up, by cells. It leads to a predictable zone of ablation within the tumour. To date, light has been delivered via small optic fibers which have nearly always been positioned percutaneously under image guidance (e.g. CT).(Bown et al., 2002, Huggett et al., 2013b, Huggett et al., 2013a)

The first Phase I trial of PDT in locally advanced PDAC was conducted in 2002. Substantial tumour necrosis was achieved in all 16 patients included in the study. Median survival after PDT was 9.5 months (range 4-30 months). 44% (7/16) were alive one year after PDT. Two of the patients who had a pancreatic tumor which involved the gastroduodenal artery developed significant gastrointestinal bleeding following the procedure. However both were managed endoscopically with transfusion, without the need for surgery.(Bown et al., 2002) A significant drawback of the early PDT treatments was that patients had to spend several days in subdued lighting following the treatment to prevent complications from skin necrosis. However, newer photosensitisers with a shorter drug-light interval and faster drug elimination times have been developed (e.g. verteporfrin) and have been shown in preclinical and early clinical studies to have a similar efficacy and safety profile to mTHPC.(Ayaru et al., 2007) A Phase I study by our group evaluated verteporfin-mediated PDT in 15 patients with unresectable locally advanced pancreatic cancer (Vertpac-01) [Table 8.2].(Huggett et al., 2013b, Huggett et al., 2013a) The study was designed in 2 parts: the first 13 patients were treated with a single-fibre, with the following 2 patients being treated with light from multiple fibers. A predictable zone of necrosis surrounding the fibers was achieved. No instances of photosensitivity were reported and only one patient developed cholangitis. Patients went on to receive palliative gemcitabine chemotherapy 28 days after ablation.

Table 8.2. Outcomes and adverse events from studies of Photodynamic Therapy for the treatment of PDAC

Study	N	Study	Photosensitiser	Number of fibres	Number of Ablations	Outcome and Survival	Adverse Events
(S. G. Bown)	16	CT guided percutaneous PDT to locally advanced but inoperable PDAC without metastatic disease	mTH-PC	Single	1	Tumour necrosis: 16/16. Median survival: 9.5 months. 44% (7/16) survived > 1 year.	Significant gastrointestinal bleeding: 2/16 (controlled without surgery).
(Huggett et al., 2013b) (Huggett et al., 2013a)	13+ 2	CT guided percutaneous PDT to locally advanced but inoperable PDAC without metastatic disease	Verteporfrin	Single (13) Multiple (2)	1	Technically feasible: 15/15. Dose dependent necrosis occurred.	Single fiber: No complications. Multiple fibers: CT evidence of inflammatory change anterior to the pancreas, no clinical sequelae.

8.1.1.5 Non-thermal, non-laser methods of ablation

Many of the studies of thermal and light ablation techniques in locally advanced and metastatic PDAC have suggested that cytoreduction may improve survival. However in the initial clinical studies some of the techniques were associated with unacceptably high rates of complications. This has led to a search for non-thermal alternative ablative therapies for use in PDAC.

8.1.1.5.1 High-intensity Focused Ultrasound

High intensity focused ultrasound (HIFU) therapy is a non-invasive method of ablation. Ultrasound energy from an extracorporeal source is focused on the pancreatic tumour to induce thermal denaturation of tissue without affecting surrounding organs.(Leslie et al., 2012) Multiple non-randomised studies and case series, largely from Asia, have reported preliminary clinical experiences of using HIFU in PDAC. They have demonstrated that the technique is able to achieve tumour necrosis with relatively few side effects [Table 8.3]. Recently a HIFU transducer has been designed which can be attached to a EUS scope to deliver HIFU locally to pancreatic tumours, thus preventing occasional burns to the skin. Initial animal studies have demonstrated that it can successfully abate the normal pancreas and liver.(Hwang et al., 2011)

Table 8.3 Outcomes and adverse events from studies of High Intensity Focused Ultrasound for the treatment of PDAC

Study	N	Study	Outcome and survival	Adverse Events
(Wang and Sun, 2002)	15	HIFU monotherapy in late stage PDAC	Pain relief: 13/13(100%)	Mild abdominal pain (2/15)
(Xie et al., 2003)	41	HIFU alone vs. HIFU +gemcitabine in locally advanced PDAC	Pain relief: HIFU (66.7%), HIFU + gemcitabine (76.6%)	None
(Xu et al., 2003)	37	HIFU monotherapy in advanced PDAC	Pain relief: 24/30 (80%)	None
(Yuan et al., 2003) (Non-English article)	40	HIFU monotherapy	Pain relief: 32/40 (80%)	None
(Wu et al., 2005a)	8	HIFU in advanced PDAC	Median survival: 11.25 months Pain relief: 8/8	None
(Xiong et al., 2009)	89	HIFU in unresectable PDAC	Median survival: 26.0 months (stage II), 11.2 months (stage III) and 5.4 months (stage IV)	Superficial skin burns (3.4%), subcutaneous fat sclerosis (6.7%), asymptomatic pseudocyst (1.1%).
(Zhao et al., 2010)	37	Phase II study of gemcitabine + HIFU in locally advanced PDAC	Overall survival: 12.6 months (95% CI, 10.2-15.0 months). Pain relief: 78.6%.	16.2% experienced grade 3 or 4 neutropenia, 5.4% developed grade 3 thrombocytopenia, 8% had nausea vomiting.

(Orsi et al., 2010)	6	HIFU in unresectable PDAC	Pain relief: 6/6 (100%)	Portal vein thrombosis (1/6)
(Sung et al., 2011)	46	Stage III or IV PDAC	Median survival: 12.4 months. Overall survival at 12 months was 30.4%.	Minor complications (abdominal pain, fever and nausea): 57.1% (28/29). Major complications (pancreaticoduodenal fistula, gastric ulcer or skin burns): 10.2% (5/49).
(Wang et al., 2011a)	40	Advanced PDAC	Median overall survival: 10 months (stage III) and 6 months (stage IV). Pain relief: 35/40 (87.5%).	None
(Lee et al., 2011)	12	HIFU monotherapy in unresectable PDAC (3/12 received chemotherapy)	Median overall survival for those receiving HIFU alone (9/12 patients): 10.3 months	Pancreatitis: 1/12
(Li et al., 2012)	25	Unresectable PDAC	Median overall survival: 10 months. 42% survived more than 1 year. Performance status and pain levels improved: 23/25.	1st degree skin burn: 12% Mortality: 0%
(Wang et al., 2013a)	224	Advanced PDAC	Not reported	Abdominal distension, anorexia and nausea: 10/ 224 (4.5%). Asymptomatic vertebral injury: 2/224.
(Gao et al., 2013)	39	Locally advanced PDAC	Pain relief: 79.5%. Median overall survival: 11 months. 30.8% survived more than one year.	None

8.1.1.5.2 Irreversible electroporation

NanoKnife® (Angiodynamics, Inc., NY, USA) or irreversible electroporation (IRE) is an emerging non-thermal ablative technique which uses electrodes, placed in the tumour, to deliver up to 3kV of direct current. This induces the formation of nanoscale pores within the cell membrane of the targeted tissue, which irreversibly damages the cell's homeostatic mechanism, causing apoptosis. The US Food and Drug Administration have recently approved the technique for use in the pancreas.

One of the major advantages of this technique is that it can be used in tumours that are in close proximity to peri-pancreatic vessels without risk of vascular trauma. The largest series of percutaneous IRE in PDAC includes 14 patients who had unresectable tumours and were not candidates for, or were intolerant of standard therapy.(Narayanan et al., 2012) The procedure was performed under general anaesthesia with complete muscle paralysis. Two patients subsequently underwent surgery after IRE and both had margin-negative resections; both remain disease-free after 11 and 14 months, respectively. Complications included spontaneous pneumothorax during anaesthesia (n = 1) and pancreatitis (n = 1); both patients recovered completely. No deaths were related

to the procedure but the three patients with metastatic disease subsequently died from disease progression.

Novel minimally invasive ablative treatments in PCL

Given the morbidity associated with pancreatic surgery and uncertainties of long-term surveillance for indeterminate PCL, minimally invasive ablative therapies have therefore been explored as an alternative. A systematic literature search was performed using the PubMed, EMBASE databases and the Cochrane Library for studies published in the English language up to 31st December 2016. MeSH terms used were (EUS radiofrequency ablation, EUS alcohol ablation, EUS paclitaxel) and (pancreas OR pancreatic cyst), and were restricted to the title, abstract and keywords. Only articles, which described ablation of PCL, were included. Articles that described the use of ablative therapies in pancreatic cancer were excluded. Similarly, studies that described non-ablative therapies were excluded. Any study with fewer than four patients and those reporting on tumours that did not originate in the pancreas were excluded. Articles not published in the English language were also excluded. All references were screened for potentially relevant studies not identified in the initial literature search. The following variables were extracted for each report when available: number of patients, lavage or device used, cyst size and subtype, presence of septations, follow up and rate of complete ablation. Eleven papers were included, in the systematic review of EUS guided ablation for PCLs sumarised below.

8.1.1.6 Ethanol or chemotherapy lavage for PCL

EUS-guided injection of alcohol or chemotherapy agents has been reported to achieve complete ablation of PCL in 35-62% of cases, partly limited by the presence of septations. Success does drop further with longer-term follow-up, due to re-growth or recurrence of the cyst. Adverse events (pain and pancreatitis) occurred in between 4-20% of cases, especially if there was any connection to the main pancreatic duct [Table 8.4].(Gan et al., 2005, Oh et al., 2011a, Oh et al., 2008, Oh et al., 2009, DeWitt et al., 2009, DiMaio et al., 2011, Caillol et al., 2012, DeWitt et al., 2014, Oh et al., 2014, Gomez et al., 2016)

Table 8.4 Studies of EUS guided ethanol or chemotherapy lavage for PCL

	N	Lavage used	Median size in mm (range)	Septations	Subtype	FU (mo)	Complete resolution
(Gan et al., 2005)	25	5-80% ethanol	19.4 (6-30)	2nd half of study	MCN 52%, BD-IPMN 16% Indeterminate 32%	NR >6-12	35% (8/23)
(Oh et al., 2008)	14	88-99% ethanol + paclitaxel	25.5 (17-52)	21% (3)	MCN 14%, SCN 14% Lymphangioma 21% Indeterminate PCL 43%	9 (6-23)	79% (11/14)
(Oh et al., 2011a)	10	99% ethanol + paclitaxel	29.5 (20-68)	100% (10)	MCN 30% SCN 40% Indeterminate PCL 30%	9 (6-18)	60% (6/10)
(DeWitt et al., 2009)	42	DBRCT: 80% ethanol (n=25) vs. Saline (n=17)	22.4 (10-58)	41% (17)	MCN 41%, BD-IPMN 41% SCN 12% Pseudocyst 7%	NR but >3-4	33% (12/36) (Saline 0% vs. Ethanol 33-75%)
(Oh et al., 2011b)	52	99% ethanol + paclitaxel	31.8 (17-68)	39% (20)	MCN 17% SCN 29% Pseudocyst 4% Indeterminate 50%	22 (12-44)	62% (29/47)
(DiMaio et al., 2011)	13	R: 2x 80% Ethanol	20.1	70% (7)	BD-IPMN 100%	NR	38% (5/13) (0% after 1st and 38% after 2nd EUS-EL)
(Caillol et al., 2012)	13	R: 99% ethanol +/- lipidol	24 (11-50)	NR	MCN / IPMN 100%	26 (4-118)	85% (11/13)
(DeWitt et al., 2014)	22	100% Ethanol + paclitaxel	24 (15-46)	64% (14)	BD-IPMN 55%. MCN 27% SCN 18%	27 (17-42)	50% (10/20)
(Oh et al., 2014)	10	99% Ethanol + paclitaxel	39.5 (27-119)	80% (8)	NR	12 (7-20)	NR
(Gomez et al., 2016)	23	80% ethanol + 1% lidocaine	27.5 (15-49)	47% (10)	BD-IPMN 65%, MCN 17%, Indeterminate 17%	41	9% (2/23)

8.1.1.7 Laser and thermal ablation

Small case series have demonstrated EUS guided RFA can be used safely for this indication with just 2/8 patient reporting mild abdominal pain that resolved within 3 days with conservative management.(Pai et al., 2013a) Further validation will come from larger Phase II studies.

9 MULTICENTRE TRIAL OF EUS GUIDED RADIOFREQUENCY ABLATION IN CYSTIC TUMOURS OF THE PANCREAS (RADIOCYST-01)

Introduction

The prevalence of pancreatic cysts is estimated to be between 13-49% in asymptomatic adults (de Jong et al., 2010) (Kromrey et al., 2018). Although the overall risk of developing pancreas cancer is low, approximately 15% of pancreatic ductal adenocarcinomas develop from Intraductal papillary mucinous and mucinous cystic neoplasms (IPMNs and MCNs, respectively) (Vincent et al., 2011).

International guidelines for the management of pancreatic cysts recommend surgical resection where there is concern for high-grade dysplasia or early cancer, based on the presence of worrisome clinical and imaging findings (Elta et al., 2018) (Vege et al., 2015a) (2018) (Ohtsuka et al., 2024b). However, current diagnostic tests are imperfect. At least 10% of patients referred for surgery will be ultimately diagnosed with benign lesions and would never have developed cancer (Keane et al., 2020). An additional third will have low grade dysplasia, so surgery could have been safely deferred (Keane et al., 2020). Mortality following pancreatic surgery is 0-3% and morbidity up to 30%, even in high volume centres (Keane et al., 2020).

Most patients with pancreatic cysts are diagnosed with a precancerous low-risk IPMN. The overall risk of malignant transformation in this cohort is considered to be low (Handrich et al., 2005). The most recent International Kyoto guidelines support discharging patients with a BD IPMN less than 2cm in size that has been stable for 5 years (Ohtsuka et al., 2024b). However, recent large surveillance cohort studies, including 1404 patients with a clinically defined IPMN reported an incidence of malignant transformation of 2.9%, 5.9% and 14% at 5,10 and 15 years respectively (Oyama et al., 2020). Therefore, in reality, very few patients are actually discharged from pancreatic cyst surveillance programmes unless unfit for surgical resection. With improved imaging and an ageing population, the number of patients entering pancreatic surveillance annually is also increasing exponentially (Keane et al., 2020). This is financially

burdensome on healthcare systems as well as psychologically distressing for patients (Marinelli et al., 2020a) (Sharib et al., 2020) (Overbeek et al., 2019b).

There has been a growing interest in minimally invasive alternatives to surgery and long-term surveillance for patient with precancerous pancreatic cysts. Endoscopic-Ultrasound (EUS)-guided ablative techniques allow the delivery of high-frequency alternating current to the cyst wall, that results in a thermally induced coagulative necrosis (Younis et al., 2022, Papaefthymiou et al., 2023, Barthet et al., 2019). This prospective multicentre study will assess the efficacy and safety of EUS-RFA for the management of PCLs, 1 year post treatment.

Methods

Study design and inclusion criteria

The RADIOCYST-01 study (NCT02343692) was a phase II multicentre, efficacy and safety trial of EUS-RFA of cystic lesions of the pancreas, which was sponsored by University College London, UK (Ethics number 13/LO/1837). The study was conducted between 2016-2020 at the following participating centres: University College London Hospital, The Royal Free Hospital, Kings College Hospital NHS Foundation Trust and Homerton University Hospital NHS Foundation Trust (London, UK), Nottingham University Hospitals NHS Foundation Trust (Queens Medical Centre; Nottingham, UK), Leeds Teaching Hospitals NHS Trust, (St. James's University Hospital; Leeds, UK), Glasgow Royal Infirmary (Glasgow, UK) and the Royal Melbourne Hospital (Melbourne, Australia). The study was carried out in accordance with the Helsinki Declaration and in line with Good Clinical Practice (GCP) guidance in human research and reported in line with STROBE (reporting of observational studies in epidemiology) guidelines (Cuschieri, 2019).

The study included adult patients (ECOG performance status of 0, 1 or 2) with pancreatic cystic lesions ranging from 5-30mm in size (or >30mm if unfit for or declined for surgical resection), at least 5mm from major vascular structures or pancreaticobiliary ducts, as determined by pre-procedural cross-sectional imaging.

Patients with a main duct IPMN, pregnant patients or those with acute pancreatitis in the preceding four weeks were excluded.

Objectives and endpoints

The primary objective of the RADIOCYST 01 study was to evaluate pancreatic cyst ablation rate at 12-months following EUS-guided radiofrequency ablation. The secondary aims were to assess the frequency of adverse events following EUS-RFA and rate of retreatment.

The efficacy of EUS-RFA was determined radiologically (MR-cholangiopancreatography; MRCP) at 3 months, (and 6 months for those undergoing a 2nd EUS-RFA) and 12 months post treatment. A second EUS-RFA treatment was offered when completion of the ablation was not possible on the first occasion or there was evidence of incomplete treatment on the 3 month MRCP. Symptom registration, physical examination, quality of life assessment (EQ5D questionnaire) and adverse event monitoring occurred at each of the follow-up timepoints.

Treatment efficacy was classified based on the percentage reduction in cyst size, as defined by the longest cyst diameter on follow-up imaging, compared to the pre-ablation scan. These were classified as complete resolution; CR (100%), partial response; PR (≥30% reduction in size), Progressive disease (PD; ≥20% increase in size) or stable disease (between PR and PD) according to a modified RECIST 1.1 classification (16,26). The secondary outcome of this study was the safety of EUS-RFA in management of pancreatic cysts. To ensure the accuracy of the data including adverse events (AEs), an independent safety committee was established. Adverse events (AEs) were graded according to the American Society for Gastrointestinal Endoscopy (ASGE) lexicon (Cotton et al., 2010).

Nine patients were lost to follow-up. Complete follow-up data (i.e. MRI imaging at 3 and 12 months post ablation) was available for 28 of 55 patients. For 14 patients who underwent EUS-RFA ablation in the first half of 2019, a 3-month MRI could not be conducted. This was primarily due to the prioritization of imaging resources and the suspension of research activities during the lockdowns implemented in response to the COVID-19 pandemic.

Technique

Procedures were performed under routine midazolam and fentanyl or propofol sedation, or general anaesthetic if clinically indicated. All endoscopic examinations were performed with a linear therapeutic EUS scope (Olympus, Keymed UK Ltd.; Pentax, Hitachi Medical Systems UK Ltd.). Fine-needle aspiration (FNA) was undertaken with a 22 or 19 G needle (EchoTip,

Cook UK; Expect, Boston Scientific). Aspirated cyst fluid was sent for biochemical (amylase and CEA) and cytological analysis. In multiloculated cysts, septations were disrupted and each locule aspirated in turn.

EUS-RFA was then performed with the Habib^{TM} EUS-RFA 1cm probe (Emcision Ltd, UK) or the EUSRA probe (STARmed, Taewoong, Korea). If the Habib probe was used the FNA needle was not removed from the cyst and positioned in the deepest part of the cyst after aspiration. The Habib EUS-RFA 1cm probe was then introduced through the needle channel until 1cm beyond the needle bevel. 10W of monopolar radiofrequency current was then administered for 90 seconds. The needle and probe were then withdrawn and repositioned to allow for sequential treatments depending on cyst size. Up to 10 treatments were performed during each EUS session.

If the EUSRA probe was used, a small amount of fluid was left in the cyst as a target for ablation. After removal of the EUS-FNA needle, an 18G RFA needle (STARmed, Taewoong, Korea) was placed in the deepest part of the cyst and 50 W current administered in Continuance Mode. RFA was stopped either when the operator saw “white bubbles” on the EUS screen or when the impedance exceeded 100 Ohms.

Following treatment, patients were kept nil by mouth for 4 hours and remained in hospital overnight. Antibiotic prophylaxis (ciprofloxacin 200 mg i.v.) was given peri-procedurally and continued for up to 48 hours. In case of a known allergy, an alternative prophylactic regimen was given in line with local protocols. Contrast-enhanced CT was performed at 24-48h following ablation to assess for complications prior to discharge.

Sample size and statistics

The sample size was based on a Simon's two-stage design to assess the ablation rate. An ablation rate of 32% was assumed, taking into account the findings of previous research (Gan et al., 2005). A lower level of acceptability was considered if the ablation rate was 20%. By assuming a minimax design, a significance level of 5%, and a power of 80%, it was calculated that the first stage of the study would require 42 patients. If at least 20% of the patients in the first stage had successful ablation of their cysts (at least 9 out of 42), the study would have continued, including a total of 82 patients. To account for potential dropouts or incomplete data, an additional 15% of patients would have been recruited, resulting in a total of 97 patients.

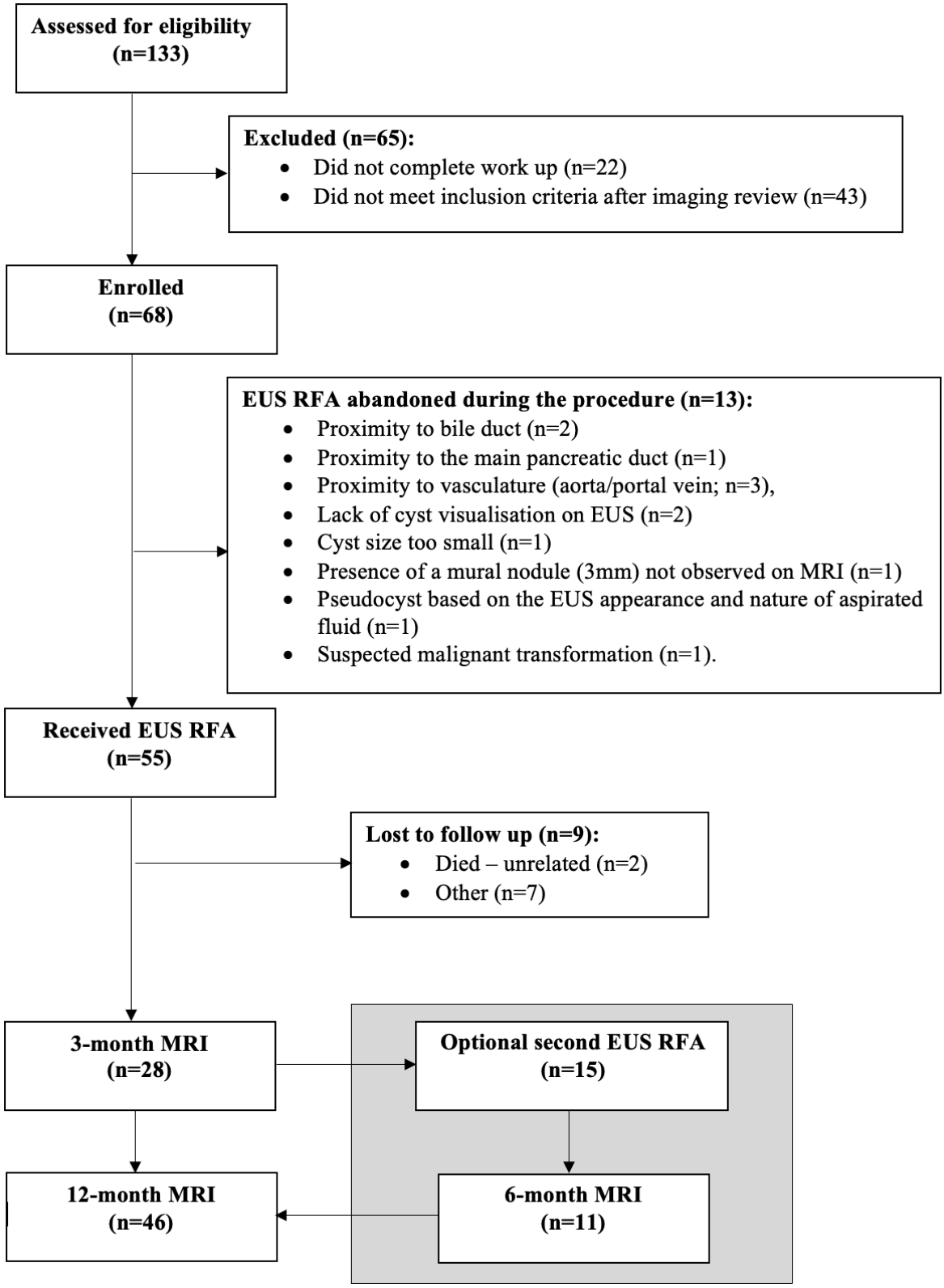
The study planned to recruit 97 patients, however, was closed early after recruitment of 68 patients due to the COVID-19 pandemic.

The relationship between the number of intraprocedural repeat RFA applications and the outcomes was assessed using a non-parametric student's t-test, considering cut-off values of ≥50% and <50%.

Results

Sixty-eight patients with PCLs were consented for the RADIOCYST01 study. EUS-RFA was not performed in 13 patients, due to clinical/anatomical safety concerns; including proximity to bile duct (n=2), main pancreatic duct (n=1) and vasculature (aorta/portal vein; n=3), lack of cyst visualisation on EUS (n=2), and cyst size too small (n=1), presence of a mural nodule (3mm) not observed on MRI (n=1), pseudocyst based on the EUS appearance and nature of aspirated fluid (n=1), suspected malignant transformation (n=1). In the case of suspected malignant transformation, the patient was referred for an elective laparoscopic distal pancreatectomy and died post-operatively due to a pulmonary embolism. In the final patient the procedure was abandoned due to scope-induced duodenal perforation before EUS-RFA was administered [Figure 9.1].

Figure 9.1 CONSORT diagram for the RADIOCYST01 study



EUS-RFA was performed in 55 patients (15 of which underwent a 2nd ablation treatment). The mean age was 63 years (range 37-78) and 39 (57%) were female. The mean cyst size of 16.1mm (range 5-40mm). The cysts were all classified as a BD-IPMN based on pre-procedure review of clinical history and imaging [Table 1]. 18 were treated with the Habib probe, 37 with the EUSRA probe. Median follow-up of 11.85 months (range 1.28-16.5 months), 34% of patients were followed for more than 1 year post procedure.

Table 9.1 Demographics and characteristics of the RADIOCYST cohort

Demographics	**(n)**
Age (years)	63 (mean) IQR: 37 - 78
Gender	
Male	29
Female	39
Presenting symptoms	
Abdominal pain	12
Weight loss	4
Jaundice	1
Pancreatitis	2
Incidental/asymptomatic	34
*** some patients reported more than one symptom**	
Cyst features	
Cyst diameter (MRI)	16.1 mm (mean) IQR: 5 - 40mm
Location:	**(n)**
Head	9
Uncinate	6
Neck/body	35
Tail	18

46 patients underwent an MRCP at 12 months. Complete resolution was observed in 37% (n=17), 17.4% (n=8) had a partial response (≥30% reduction), 39.1% (n=18) had stable disease, while in 6.5% (n=3) the cyst increased in size (progressive disease; >20% increase) [Table 9.2].

Table 9.2. Treatment response to EUS-RFA in the RADIOCYST01 cohort. This table described the overall radiological response (MRI) following EUS-RFA. CR – complete response; PR – partial response (≥30% reduction); PD – progression of disease (≥20% increase in size); SD – stable disease (between PR and PD).

	CR (n;%)	PR (n;%)	SD (n;%)	PD (n;%)	Overall response (%; median, IQR; range)
12 months post EUS RFA					
Overall n (%)	17 (37%)	8 (17.4%)	18 (39.1%)	3 (6.5%)	-48.1% (IQR, 100%; range 0-100%)
Single EUS-RFA	11 (35.5%)	5 (16.1%)	13 (41.9%)	2 (6.5%)	-40% (IQR, 100%; range 43% increase-100% reduction)
2nd EUS-RFA	6 (40%)	3 (20%)	5 (33%)	1 (6.7%)	-76.2% (IQR, 109%; range 15% increase-100% reduction)

15 patients went on to have a second EUS RFA treatment. The average number of intra-procedural RFA applications was higher in significant responders in which 3.65 (mean; SD ± 2.23, 95% CI, ±0.91) compared to 2.35 (SD ± 1.65, CI, ±0.67) performed in non-responders (p=0.026).

Nine procedure related adverse events occurred (12.5%), 1.3% were classified as severe; three cases of abdominal pain (managed with opiate analgesia and a less than 72-hour hospital stay), four cases of mild acute pancreatitis based on cross sectional imaging, one case of biliary obstruction necessitating ERCP and one case of scope-induced duodenal perforation requiring laparoscopic omental patch repair [Table 9.3].

Table 9.3. EUS-RFA complications in the RADIOCYST01 study. Overall adverse event rate of 12.5%. Nine complications (7 mild, 1 moderate and 1severe; by ASGE AE lexicon (Cotton et al., 2010)

	Abdominal pain	Pancreatitis	Biliary obstruction	Duodenal perforation	Severity (ASGE)	Comments
1	x					Transaminitis (ALT 216 AST 290) normal CT
2		x			Mild	
3	x				Mild	Presented 4 days post ablation, unremarkable bloods and CT. Admitted for observation
4	x				Mild	
5		x			Mild	Mild pancreatitis of tail on CT, conservatively manage
6		x			Mild	Focal pancreatitis on CT, amylase 168 IU/L
7		x			Mild	Amylase 852 IU/L, conservatively managed
8			x		Moderate	Required admission, ERCP with stent placement
9				x	Severe	Managed by laparoscopy and omental patch repair

Discussion

Minimally invasive treatments for the management of pancreatic cysts are an attractive alternative to long-term surveillance for low-risk patients with pancreatic cysts and as an alternative to surgery in high-risk patients, who are unfit or refuse surgery. Most ablation studies to date have explored EUS-guided ethanol ablation (with/without paclitaxel), but rates of complete ablation have been variable. A recent meta-analysis of 840 patients, reported a pooled clinical success rate (complete cyst resolution) of 44% (95%CI: 31–57; I2 = 93.7%) and a partial response rate of 30% (95%CI: 20–39; I2 = 86.1%) across all EUS guided ablative techniques (Papaefthymiou et al., 2023). By subgroup ethanol/paclitaxel (70%; 95%CI: 64–76; I2 = 42.3%) was superior to lauromacrogol (44%; 95%CI: 33–54; I2 = 0%), ethanol (32%;

95%CI: 27–36; I2 = 88.4%), and RFA (13%; 95%CI: 4–22; I2 = 95.8%) in terms of complete cyst resolution rates (Papaefthymiou et al., 2023).

In this phase II study, we assessed the efficacy and safety of EUS-RFA in a cohort of patients with low-risk pancreatic cysts (BD-IPMNs without worrisome features, in long-term surveillance). Complete resolution at 1 year was observed in 37% (n=17) and a further 17% (n=8) had ≥30% reduction in cyst size. Fifteen patients received a second EUS-RFA treatment.

A case series by Pai et al. reported outcomes in 6 patients with PCLs treated with EUS FRA (four MCNs, one IPMN and one serous cystadenoma) (Pai et al., 2015). Two (33.3%) had a complete response and four (66.7%) had a partial response on interval imaging at 3-6 months post procedure. Mild transient abdominal pain was the only adverse event reported in 2 patients (33.3%) (Pai et al., 2015). Barthet et al. reported outcomes from a cohort study of EUS-RFA in 17 high-risk PCLs (with mural nodules or thickened cyst walls), with follow-up evaluations conducted at 6- and 12-months following treatment. Mean cyst size was 28mm (range 9–60)]. At the 12-month follow-up, 64.7% of patients had complete cyst resolution, with a further 5.9% having a >50% reduction (Barthet et al., 2019).

Younis et al., reported outcomes of EUS RFA in a series of 6 patients with PanNETs and 5 patients with PCLs [1 mucinous cystic neoplasm, 4 intraductal papillary mucinous neoplasms; mean size 36 mm (range 12-60)] (Younis et al., 2022). All patients presented with worrisome features, and three of them had mural nodules. The response to RFA was assessed at 6 months by EUS and 12 months by cross-sectional imaging. In the PCL group, complete radiologic response was achieved in 60% (3/5), partial response (at ≥ 50% cut-off) in 20% (n=1). Three patients required a second RFA session to complete the treatment, in two of which complete resolution occurred subsequently. The median cyst size was 36 mm (range 12-60).

When EUS-RFA was undertaken in 13 microcystic serous cystic neoplasms with a mean size of 50mm (34.2-52.5mm) results were less favourable, with no episodes of complete cyst resolution observed. 8 patients (61.5%) had a partial response. Mild AE were reported in 8%, with a single case of post-procedural abdominal pain (Oh et al., 2021).

The secondary outcome of this study was to evaluate the safety of EUS-RFA in the management of PCLs. A systematic review of EUS guided ablation reported a median rate of

adverse events of 14% (95%CI: 8–20; I2 = 87.2%). The majority of AEs (10%; 95%CI: 5–15; I2 = 86.7%) were mild with 4% described as severe (95%CI: 3–5; I2 = 0%) (Papaefthymiou et al., 2023). In a subgroup analysis EUS guided ethanol ablation was associated with the highest rate of AEs at 16% (95%CI: 13–20; I2 = 91.0%) compared to 7% (95%CI: 0–12; I^2 = 0%; p = 0.08) for EUS-RFA (Papaefthymiou et al., 2023). In EUS guided ethanol ablation most adverse events were attributed to alcohol leaking into the pancreatic duct and causing acute severe pancreatitis or outside the pancreas causing a chemical peritonitis or venous thrombosis. EUS guided radiofrequency ablation uses thermal energy, so potentially could avoid this complication. However, AEs in this study were frequent, occuring in 12.5% (9 patients), 1.3 % were classified as severe. Barthet et al. noted a similar rate of AEs in their initial cases, but this dropped to just 3.5% when patients were given rectal diclofenac for post procedure pancreatitis prophylaxis, which was not employed in our protocol (Barthet et al., 2019).

This study has several limitations, of 68 consented 19% (13 patients) had the procedure abandoned intra-operatively due to anatomical or safety concerns not appreciated on preprocedural imaging and workup. This may in part be attributed to inter-modality differences in spatial resolution between MRI and EUS (Uribarri-Gonzalez et al., 2018) and/or operator hesitancy particularly early on in an individual's learning curve of the EUS-RFA procedure. The final cohort size was around half of the size of the original planned study based on sample size calculations, however this still represents the largest single cohort study of EUS RFA in PCLs to date. Other obvious limitations include the observational design of the RADIOCYST01 study and the over representation of BD-IPMNs. While the latter restricts our conclusions regarding the efficacy of EUS-RFA across various cyst subtypes, on the other hand, we were able to provide further evidence on the efficacy of EUS-RFA specifically in cases of BD-IPMNs.

Conclusion

In conclusion, EUS-RFA of low-risk BD-IPMN was technically feasible and associated with complete resolution in over a third of patients at 1 year post procedure. However adverse events were common so EUS-RFA should not be considered to be the standard treatment for patients with low-risk pancreatic cysts.

10 Summary and Future Directions

Chapter 1

Pancreatic ductal adenocarcinoma (PDAC) is the tenth most common cancer in the UK with an incidence of approximately 17 per 100,000 or 10,800 new cases annually between 2017-2019. The condition is characterized by late-stage diagnosis, resulting in poor survival rates of around 28% at one year and 8% at five years in the UK. Pancreatic cancer carcinogenesis is driven by series of genetic mutations, predominantly in the KRAS oncogene. Most cases are sporadic but family history as well as certain lifestyle factors like smoking and obesity are thought to be contributory. Clinical symptoms often manifest late, complicating early detection and diagnosis.

Cystic lesions of the pancreas (PCL) are increasingly common clinical finding. PCL subtypes, include intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN), which are considered precancerous as well as benign cysts like serous cystic neoplasms which have no malignant potential. International and European clinical guidelines broadly recommend surgical intervention for high-risk lesions and surveillance for low-risk lesions. PCL with invasive cancer are managed like PDAC.

Chapter 2

The PAPERPAC pilot study explores patient perceptions of pancreatic cancer screening programmes. The study included patients with pancreatic cystic tumors, hereditary pancreatitis, or a strong family history of pancreatic cancer. The study aimed to evaluate patients' perception of their cancer risk, levels of cancer-related anxiety, and their overall experience.

Findings from the initial seven patients enrolled in the study, revealed surveillance provided a sense of security and reassurance, but there were notable psychological impacts. A significant proportion reported symptoms of depression and anxiety and patients frequently overestimating their cancer risk. Less invasive surveillance methods were preferrable, such as blood tests and MRIs over endoscopic procedures.

The study highlights the psychological burden experienced by patients in surveillance. Addressing these issues may improve adherence with surveillance protocols. Better patient

education resources are needed for patients with PCL [patient booklet: Appendix 3]. This initial pilot study was limited by the small sample size. A larger multicenter study across two centres is planned to validate these initial trends and inform recommendations and design of future pancreatic screening programmes.

Chapter 3

This retrospective cohort study investigates the natural history of pancreatic cystic lesions within a surveillance program at a UK hepatopancreaticobiliary (HPB) center. The study aimed to characterize the rate of malignant transformation in patients under surveillance for a PCL or who were referred for surgery. Secondary aims included and identifying clinical and imaging features that could be predictive of malignancy.

The cohort included 768 patients diagnosed between 2000 and 2013. 16% developed pancreatic cancer, with a significant disparity in malignancy rates between those referred for immediate surgery (38%) and those in surveillance (2%). The study underscores the need for better diagnostic tests in PCL. A proportion of patients with benign PCL were mistakenly subjected to surgery due to high sensitivity (92%) but low specificity (5%) of current diagnostic tests. Most episodes of malignant transformations occurred within the first two years after diagnosis, but some patients were under surveillance for more than 5 years also developing invasive cancer, supporting the need for long-term surveillance in patients with PCL who are fit for surgical resection.

The UCL PCL registry is now being maintained prospectively to improve the quality of the data captured. It is an important resource for future longitudinal studies on PCL.

Chapter 4

The systematic review summarizes the literature on biomarkers for pancreatic cancer and describes some of the challenges to their development using current approaches. High risk patients in screening programmes are typically followed with MRI or Endoscopic Ultrasound (EUS) annually, which are expensive and invasive tests. EUS is also needed for pathological

confirmation in suspected PDAC. Accurate simple tests that can detect pancreatic cancer at an early stage are needed, but remain elusive.

Carbohydrate antigen (CA) 19-9, has utility in monitoring for recurrent disease, but is a poor diagnostic biomarker. Genetic and epigenetic alterations in circulating tumor cells and cell-free DNA show promise but further validation studies are needed to substantiate these findings. The best diagnostic accuracy will likely come from panels of biomarkers, which have shown some promise in detecting early-stage tumors and differentiating PDAC from benign conditions.

Pancreatic tumors are characterized by pronounced desmoplasia. The pancreatic tumor microenvironment (TME) is therefore an important source of biomarkers in pancreatic cancer. Tumor stroma is a source of genetic and epigenetic mutations that can act as cancer promoter cells which can become biomarkers for PDAC,(Xie and Xie, 2015) although not widely explored within this thesis, but could be a focus of future work.

Pancreatic screening is currently offered for those with a strong family history of pancreatic cancer, a known genetic predisposition or a precancerous PCL. Studies from our group and others, have used large GP datasets to demonstrate that vague symptoms can herald a diagnosis of PDAC, many months prior to diagnosis. As patients, visit their GP regularly during this period, this is another potential high risk cohort, ideal for pancreatic screening.(Hippisley-Cox and Coupland, 2012, Stapley et al., 2012, PCUK, 2011, Lyratzopoulos et al., 2012, Keane et al., 2014a) Certain symptoms such as back pain (OR 1.33 [95%CI: 1.18,1.49] P<0.001), lethargy (1.42 [95%CI:1.25,1.62] P<0.001) and new onset diabetes (OR 2.46 [95%CI:2.16,2.80]) are more suggestive of PDAC than other pancreaticobiliary cancers.(Keane et al., 2014a) Grouping symptoms allows the development of symptom based cancer decision support tools (CDST) to aid diagnosis. First generation CDST have been introduced into primary care practices across 15 cancer networks in the UK.(Hippisley-Cox and Coupland, 2012) Their development and impact on referral practice is subject being evaluated through ongoing prospective studies. Combining these tools with novel biomarker panels may better stratify high risk patients.

Chapter 5

The eukaryotic cell cycle involves a series of tightly phases of DNA replication, with licensing mechanisms governed by proteins such as minichromosome maintenance (Mcm) proteins. Uncontrolled proliferation, typical of malignant cells, is associated with Mcm protein expression. This study explored is a range of cell cycle biomarkers could detect high risk PCL.

The first part of the study evaluated expression of Mcm2, geminin, histone and Cdc7 in a cohort of patients who have undergone surgical resection for PCLs. Mcm2 expression was significantly higher in patients with pancreatic cancer and IPMN with high grade dysplasia. The second part of the study assessed Mcm5 expression in pancreatic cyst fluid samples obtained during endoscopic ultrasound. In this small cohort of patients the test performed well at detecting patients with PCL and invasive cancer. Expression was variable in patients with precancerous lesions. The test performed poorly in patients with benign cysts, with a third having a false positive result.

Further work is needed to more fully assess the utility of Mcm5 as a biomarker in high risk PCL. Further assay precision and cross reactivity testing would inform if concomitant inflammation or infection affects the validity of the test. The test cut off in this study has been extrapolated from levels in bile from prior studies and will need optimizing specifically for PCL, where fluid is frequently acellular. Ultimately further validation in larger carefully characterized PCL cohorts is necessary to confirm the clinical utility of this test, before moving on to prospective studies in patients.

Chapter 6

The phase II CONCYST-01 study investigates the safety and efficacy of needle-based confocal laser endomicroscopy (nCLE) in diagnosing indeterminate PCL among a UK population. Traditional methods of diagnosing PCL are often inadequate, necessitating advanced endoscopic techniques to detect high-risk lesions. EUS nCLE allows real-time imaging of cyst wall during endoscopic ultrasound (EUS) by utilizing a laser probe to capture detailed images, akin to pathological images in real time.

The CONCYST 01 study enrolled 62 patients, with 59 ultimately being included in the intension to diagnose analysis. Most participants were symptomatic, and the median lesion

size was 25 mm. Final diagnoses were largely determined by multidisciplinary team consensus and follow up. EUS-nCLE demonstrated a diagnostic accuracy of 73% although this was not significantly better than standard of care cytology (63%). However, EUS nCLE did outperforming cytology in IPMN cases (82% vs 63%; 0.05). The technique was found to be safe, with a low adverse event rate of 5.1%.

Further studies are needed to evaluate inter and intra observer variation in nCLE image interpretation, particularly in less experienced endosonographers. Compare nCLE to cyst fluid molecular markers, which have become the standard of care in many centres. In IPMN, further characterization of features of low- and high-grade dysplasia are needed to help identify high risk lesions accurately. Carefully characterized cohorts of patients that have undergoing nCLE and were ultimately referred to surgery will be needed to these studies. The ongoing CLIMB study (NCT03492151; for which I am the current site PI) is working to develop a functional AI program (nCLE-AI) for automatic risk stratification in IPMNs. The nCLE-AI tool will aid easier interpretation and risk stratification of IPMNs.

Chapter 7

The study investigated the use of real-time molecular imaging with fluorescence-guided biomarkers to enhance the detection of high-risk PCL. Molecular fluorescence techniques have been employed in cancer surgery for many years, to differentiate between malignant and benign tissue, with improved surgical outcomes.

The study included 63 patients who underwent surgical resection for a PCL. Carcinoembryonic antigen (CEA), S100P, and anterior gradient protein 2 homolog (AGR2), all demonstrated increased expression in the epithelial wall of high-grade lesions. Further research will explore in vitro if these biomarkers can be detected by near-infrared fluorescence imaging. Ultimately in vivo studies will be undertaken to test if the markers can enhance image interpretation during EUS nCLE. If feasible the technology could simplify the identification of dysplastic PCLs and enhance the accuracy of PCL diagnostics.

Chapter 8

This systematic review evaluates minimally invasive ablative therapies for locally advanced pancreatic ductal adenocarcinoma and pancreatic cystic lesions. As most patients with advanced PDAC are not surgical candidates, there is a growing interest in alternative treatments.

The review included 32 studies of thermal and non-thermal ablative techniques, including radiofrequency ablation (RFA), microwave ablation, cryoablation, and photodynamic therapy (PDT). RFA demonstrated significant tumor necrosis and often improvement in pancreatic cancer symptoms. However early studies revealed high morbidity and mortality rates, prompting modifications in technique to enhance safety. Similarly, microwave ablation showed promising results in achieving necrosis with relatively low complication rates. Cryoablation, has only been evaluated in a few studies but yielded positive outcomes for pain management, although validation studies are needed. In PCL, EUS-guided ablation using alcohol or chemotherapy has been explored, achieving complete resolution in 35-62% of cases, albeit with recurrence over time. EUS guided radiofrequency ablation may provide a more definitive treatment, with potentially lower adverse events, but larger studies are needed. Overall, minimally invasive ablative therapies are promising, but further research is necessary to establish their effectiveness and safety through larger prospective studies.

Chapter 9

Endoscopic ultrasound (EUS)-guided radiofrequency ablation (RFA) is a minimally invasive alternative for managing low-risk pancreatic cysts (PCLs), particularly in patients who face burdensome surveillance or who are high risk for surgery or refuse surgery.

This multicenter Phase II study aimed to evaluate the efficacy and safety of EUS-RFA in patients with benign BD-IPMNs measuring 5-40 mm. Out of 68 recruited patients, 55 underwent EUS-RFA, after 13 cases were excluded during endoscopy because of clinical or anatomical concerns. The primary outcome assessed the rate of cyst ablation at one year. Complete resolution was reported in 37% of patients, with an additional 17.4% displaying a >30% reduction in cyst size. However, the procedure was associated with a 12.5% rate of adverse events, including one severe AE (perforation necessitating surgical repair). These findings suggest that while EUS-RFA is technically feasible and offers some therapeutic

benefit, but the prevalence of adverse events precludes it from becoming an established treatment for low-risk PCLs, at present.

Future work should explore optimal needle placement, energy settings and ablation duration to optimizing outcomes with existing devices. Development of alternative ablation devices, through collaboration with engineering and industry partners. e.g. with irreversible electroporation (IRE), may decrease the thermal effect on adjacent structures and vasculature (as outlined in Chapter 8), reducing the rates of associated AEs.

Appendix 1: PAPERPAC Questionnaire

PAPERPAC Questionnaire Version 2.0 (5th August 2015)

PANCREATIC CANCER SURVEILLANCE QUESTIONNAIRE

You have been invited to take part in this questionnaire study to explore the impact and perceived usefulness of screening for pancreatic cancer. You have been chosen to take part in this study because you have at some point been offered the opportunity to take part in a surveillance programme for pancreatic cancer. Surveillance means that you attend your hospital frequently for imaging appointments to monitor for any early changes that may occur in your pancreas. Surveillance is offered to individuals at increased risk of pancreatic cancer compared to the general population, and includes patient with cysts in the pancreas or those who have a strong family history of pancreatic cancer or pancreatitis.

Before you complete the questionnaire please ensure you have read the patient information leaflet, discussed it with others if you wish and signed the consent form. Please contact the clinical research team if anything is unclear or you would like more information, our contact details are in included the patient information leaflet.

Thank you for participating in this study.

Patient identification

ID Number (to be completed by the researchers)_____

Name and Surname _____

Date form completed |___|___| |___|___| |___|___|___|___|

Hospital attended for surveillance appointments _____

Identification of person completing form (if not the patient)

Name and Surname _____

Relationship to patient _____

Section A: Benefits and Barriers to Surveillance

Read each question and tick the box you feel is appropriate box on the right

Benefits (Reassurance)	*Yes*	*No*	*Don't Know*
1. Do surveillance visits to the hospital convey to you a sense of security?	☐	☐	☐
2. Would you be reassured if you had a surveillance appointment?	☐	☐	☐
3. Do the advantages of surveillance outweigh the disadvantages?	☐	☐	☐
4. Would you worry more about your disease if not in surveillance?	☐	☐	☐

5. Are there any others benefits of surveillance for you:

_____

_____

_____

Barriers (General disadvantages)	*Yes*	*No*	*Don't Know*
6. Would you prefer (if possible) to have surveillance visits in a hospital closer by?	☐	☐	☐
7. Do you think the following surveillance investigations are burdensome?			
a. Blood tests	☐	☐	☐
b. Outpatient appointments	☐	☐	☐
b. CT scan	☐	☐	☐
c. MRI scan	☐	☐	☐
d. Endoscopic ultrasound (EUS)	☐	☐	☐
8. Does surveillance remind you each time of your disease?	☐	☐	☐

9. Are there any other barriers that would prevent you participating in a surveillance programme:

_____

_____

_____

Section B: How You Generally Feel

Below are a series of statements, which people have used to describe themselves. Read each statement and then tick the appropriate box on the right to indicate how you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer, which seems to best describe how you generally feel.

	Yes, definitely	*Yes, sometimes*	*No, not much*	*No, not at all*
1. I wake early and then sleep badly for the rest of the night.	☐	☐	☐	☐
2. I get very frightened or have panic feelings for apparently no reason at all.	☐	☐	☐	☐
3. I feel miserable and sad.	☐	☐	☐	☐
4. I feel anxious when I go out of the house on my own.	☐	☐	☐	☐
5. I have lost interest in things.	☐	☐	☐	☐
6. I get palpitations, or sensations of 'butterflies' in my stomach or chest.	☐	☐	☐	☐
7. I have a good appetite.	☐	☐	☐	☐
8. I feel scared or frightened.	☐	☐	☐	☐
9. I feel life is not worth living.	☐	☐	☐	☐
10. I still enjoy the things I used to.	☐	☐	☐	☐
11. I am restless and can't keep still.	☐	☐	☐	☐
12. I am more irritable than usual.	☐	☐	☐	☐
13. I feel as if I have slowed down.	☐	☐	☐	☐
14. Worrying thoughts constantly go through my mind.	☐	☐	☐	☐

Have you recently?				
	Better than usual	*Same as usual*	*Less than usual*	*Much less than usual*
1. Been able to concentrate on what you're doing?	☐	☐	☐	☐
	Not at all	*No more than usual*	*Rather more than usual*	*Much more than usual*
2. Lost much sleep over worry?	☐	☐	☐	☐
	More so than usual	*Same as usual*	*Less useful than usual*	*Much less useful*
3. Felt you were playing a useful part in things?	☐	☐	☐	☐
	More so than usual	*Same as usual*	*Less than usual*	*Much less capable*
4. Felt capable of making decisions about things?	☐	☐	☐	☐
	Not at all	*No more than usual*	*Rather more than usual*	*Much more than usual*
5. Felt constantly under strain?	☐	☐	☐	☐
	Not at all	*No more than usual*	*Rather more than usual*	*Much more than usual*
6. Felt you couldn't overcome your difficulties?	☐	☐	☐	☐
	More so than usual	*Same as usual*	*Less than usual*	*Much less than usual*
7. Been able to enjoy your normal day-to-day activities?	☐	☐	☐	☐
	More so than usual	*Same as usual*	*Less than usual*	*Much less able*
8. Been able to face up to your problems?	☐	☐	☐	☐
	Not at all	*No more than usual*	*Rather more than usual*	*Much more than usual*
9. Been feeling unhappy and depressed?	☐	☐	☐	☐
	Not al all	*No more than usual*	*Rather more than usual*	*Much more than usual*
10. Been losing confidence in yourself?	☐	☐	☐	☐
	Not at all	*No more than usual*	*Rather more than usual*	*Much more than usual*
11. Been thinking of yourself as a worthless person?	☐	☐	☐	☐
	More so than usual	*About same as usual*	*Less than usual*	*Much less than usual*
12. Been feeling reasonably happy, all things considered?	☐	☐	☐	☐

Section C: **Perceived Risk of Cancer and Level of Cancer Worry**

For the following questions, circle the most appropriate number on a scale of 0-10, where 0 represents no risk of developing pancreatic cancer and 10 represents that you will definitely develop pancreatic cancer.

1. In your lifetime, how likely do you think it is that you will develop pancreatic cancer with surveillance?

0	1	2	3	4	5	6	7	8	9	10
Definitely will not get pancreatic cancer										*Definitely will get pancreatic cancer*

2. In your lifetime, how likely do you think it is that you will develop pancreatic cancer without surveillance?

0	1	2	3	4	5	6	7	8	9	10
Definitely will not get pancreatic cancer										*Definitely will get pancreatic cancer*

For the following questions please tick the box that best applies to your level of worry frequency or impact.

	Not at all	*Rarely*	*Sometimes*	*Often*	*Almost all the time*
3. How worried are you about getting pancreatic cancer someday?	☐	☐	☐	☐	☐
4. How much does your worry affect your mood?	☐	☐	☐	☐	☐
5. How much does your worry affect your ability to perform your daily activities?	☐	☐	☐	☐	☐

Section D: Currently Participating in Surveillance

1. Are you currently participating in a surveillance programme? ☐ Yes (go to ***Section E***)
   ☐ No (go to ***Section G***)

Section E: Motivations to Participate in Surveillance

Read each statement and tick the appropriate box on the right which best represents your reasons for participating in surveillance. Tick as many or few statements as are applicable to you.

	Yes	*No*	*Don't Know*
1. Cancer might be detected early and still be treatable	☐	☐	☐
2. Because of surveillance my fear of cancer decreases	☐	☐	☐
3. Surveillance gives me a sense of control over my body	☐	☐	☐
4. A physician referred me for surveillance.....	☐	☐	☐
5. A family member asked me to undergo surveillance	☐	☐	☐
6. Because relatives died of pancreatic cancer	☐	☐	☐
7. For my children	☐	☐	☐
8. More information on my condition	☐	☐	☐
9. Keep clinical contact	☐	☐	☐
10. To contribute to scientific research	☐	☐	☐

11. Are there additional reasons why you would participate in surveillance that have not been outlined above:

_____

_____

_____

Section F: Surveillance Experience

In order to improve our communication with patients in surveillance, as well as their overall clinical experience, please read the following questions and tick the appropriate box on the right, which best represents your surveillance experience to date.

Communication	*Yes*	*No*	*Don't Know*
1. At a surveillance appointment, can you discuss with your doctor matters that are of concern to you or that worry you?	☐	☐	☐
2. Can you ask about things at surveillance visits?	☐	☐	☐
3. Do people in the hospital listen/pay attention to what you say?	☐	☐	☐
4. Do the physicians at a surveillance appointment have enough time for you?.....	☐	☐	☐

Nervous anticipation	*Yes*	*No*	*Don't Know*
5. Are you nervous before a surveillance visit?	☐	☐	☐
6. Do you sleep less well in the week before a surveillance visit?	☐	☐	☐
7. Do you postpone plans till after a surveillance visit?	☐	☐	☐
8. Do you normally dread surveillance visits?	☐	☐	☐
9. Would you rather have surveillance visits less frequently?	☐	☐	☐

(Please, go to Section H)

Section G: FOR PATIENTS WHO DECLINED OR DROPPED OUT OF SURVEILLANCE

1. Did you decide not to enter the surveillance programme? ☐ Yes (go to *item 2.*) ☐ No (go to *item 3.*)

2. Please describe any reasons which made you decide not to participate in surveillance: *(please, go to Section H)*

_____

_____

_____

3. Did you commence surveillance and decide to stop? ☐ Yes (go to *item 4.*) ☐ No (go to *Section H*)

4. Please describe any reasons which made you decide not to continue surveillance:

_____

_____

_____

Section H: About you?

1. What ethnic or cultural groups do you consider yourself to belong to? **White European [ ] White other [ ] Asian Indian [ ] Chinese [ ] Japanese [ ] Filipino [ ] Other Asian [ ] Black African [ ] Black Caribbean [ ] Mixed Race [ ] Other**……

2. What was the highest level of education you have completed?

   O-level or equivalent [ ] A-level or equivalent [ ] University degree [ ] Other ……..

3. What is your current professional status:

   Employed…….. ☐
   Unemployed…….. ☐
   Retired……... ☐
   Disabled…….. ☐

4. Do you participate in any other screening programmes:

   Bowel Cancer Screening Programme…….. ☐
   Cervical……..…….. ☐
   Breast……... ☐
   Other……

5. What is your height? _____ FEET/INCHES or _____ METRES/CM

6. What is your weight? _____ STONE/POUNDS or _____ KG

Section J: **Clinical History** (to be completed by clinician responsible for care)

Does the patient have…?

Type 1 diabetes [] Type 2 diabetes [] Acute pancreatitis [] Chronic pancreatitis []

History of cancer? Y [] N [] If yes where was the primary site (e.g. lung) ……

Has any members of the individuals family had pancreatic cancer? …… Y [] N [] Don't know []

Number of first-degree relatives affected?…….. Number of second-degree relatives affected?.....

Blood tests results (if available):

CA 19-9…… U/ml Date taken (DDMMYY): ……

Reason for pancreatic surveillance…?

1.) EUROPAC study …… Y [] N []

Reason for inclusion in the EUROPAC study:

- Family history []
- Hereditary pancreatitis [] (were any of the following blood tests positive: PRSS1 [] SPINK1 [] CFTR [])
- Known germline mutation: Peutz-Jeghers Syndrome [] BRAC2 [] familial atypical multiple mole melanoma syndrome (FAMMM) []
- Pancreatic cyst []

2.) Pancreatic cyst …… …… Y [] N []

What type of pancreatic cyst does the patient have? SB-IPMN [] MD-IPMN [] MCN [] SCA [] Indeterminate []

What size is the cyst?.....mm

3.) Other……

Type and method of surveillance surveillance…?

Date surveillance commenced (DDMMYY)? ……

Is surveillance on-going? Y [] N [] If not, when was it discontinued (DDMMYY)?.....

What was the reason surveillance was discontinued? ……

.....

.....

Which of the following imaging modalities were used for surveillance?

CT [] MRI [] Endoscopic ultrasound [] Endoscopic retrograde cholangiopancreatography []

How often is the patient currently surveyed?

0-3 monthly [] 3-6 monthly [] 7-12 monthly [] >12 monthly [] Other……

What do you perceive is the risk of your patient developing pancreatic cancer?

For the following questions, circle the most appropriate number on a scale of 0-10, where 0 represents no risk and 10 is the absolute likelihood of developing pancreatic cancer.

1.) In your patient's lifetime, how likely do you think it is that they will develop pancreatic cancer <u>with surveillance</u>?

0	1	2	3	4	5	6	7	8	9	10
Definitely will not get pancreatic cancer										*Definitely will get pancreatic cancer*

2.) In your patient's lifetime, how likely do you think it is that they will develop pancreatic cancer <u>without surveillance</u>?

0	1	2	3	4	5	6	7	8	9	10
Definitely will not get pancreatic cancer										*Definitely will get pancreatic cancer*

Appendix 2: Patient Forum on PCL

London Cancer

Presents the

Patient Forum on Cystic Tumours of the Pancreas

Friday, 03 October 2014 from 09:00 to 13:00

The Atrium, Royal Free Hospital, Pond Street, London NW3 2QG

Please join London Cancer and the HPB Pathway Board for a half day dedicated to cystic tumours of the pancreas. This event will give patients and the public the chance to learn more about these tumours and an opportunity to input into research.

Agenda for the Day

Time	Topic
8.30-09.00	Breakfast
9.00-09.10	Welcome
9.10-10.00	Short talks
	1. An introduction to cystic tumours of the pancreas: diagnostic and management strategies 2. London Cancer: role of the GP, Commissioners and the MDT 3. Role of the charities 4. Living with a pancreatic cyst: a patient's perspective 5. Support networks for patients and relatives 6. Summary and plan for workshops
10.00-10.30	Coffee
10.30-11.30	Workshops
	1. PATIENT RESOURCES – Review proposed patient information leaflets and websites 2. PATIENT SURVEYS AND QUESTIONNAIRES: Review of current proposals (e.g. PAPERPAC). What are the important topics for future study? 3. CLINICAL STUDIES: Review of current research projects (e.g. RADIOCYST, CONCYST). What are the important topics for future research?
11.30-11.45	Coffee
11.45-12.30	FEEDBACK from the workshops
	1. PATIENT RESOURCES 2. PATIENT SURVEYS AND QUESTIONNAIRES 3. CLINICAL STUDIES
12.30-13.00	Discussion and Wrap-up
13.00-13.30	Lunch (questionnaire completion, collection of expenses forms)
13.30	Close of meeting and Lunch

Do you have questions about Patient Forum on Cystic Tumours of the Pancreas? Contact London Cancer

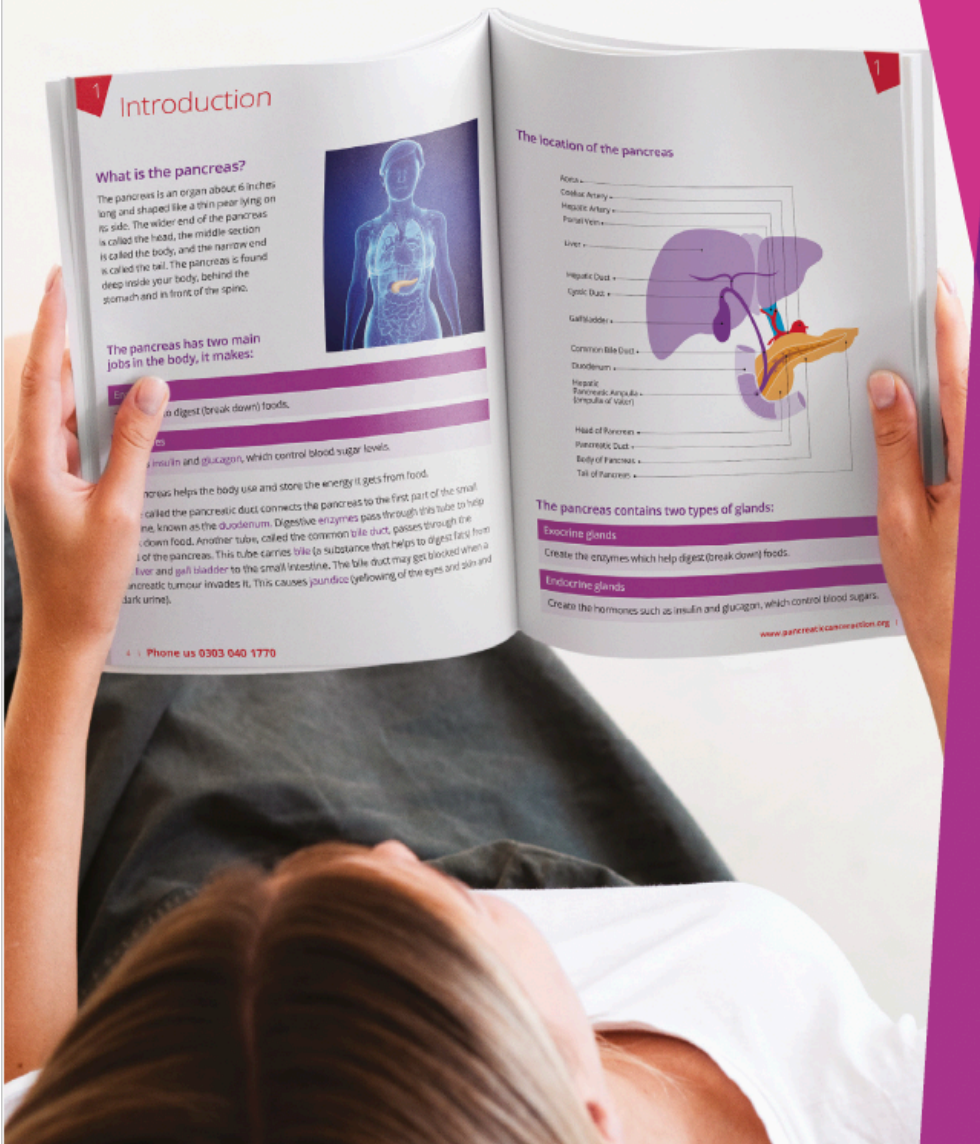
To register and for more information please visit:
http://www.eventbrite.co.uk/e/patient-forum-on-cystic-tumours-of-the-pancreas-tickets-12634645565

Excellent care through partnership

Appendix 3: Pancreatic Cancer Action booklet for PCL patients

Pancreatic cysts and cystic tumours

In this booklet you will find information about pancreatic cysts, the different types and how they are diagnosed and treated.



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Introduction

What is the pancreas?

The pancreas is an organ about 6 inches long and shaped like a thin pear lying on its side. The wider end of the pancreas is called the head, the middle section is called the body, and the narrow end is called the tail. The pancreas is found deep inside your body, behind the stomach and in front of the spine.

The pancreas has two main jobs in the body, it makes:

Enzymes

These help to digest (break down) foods.

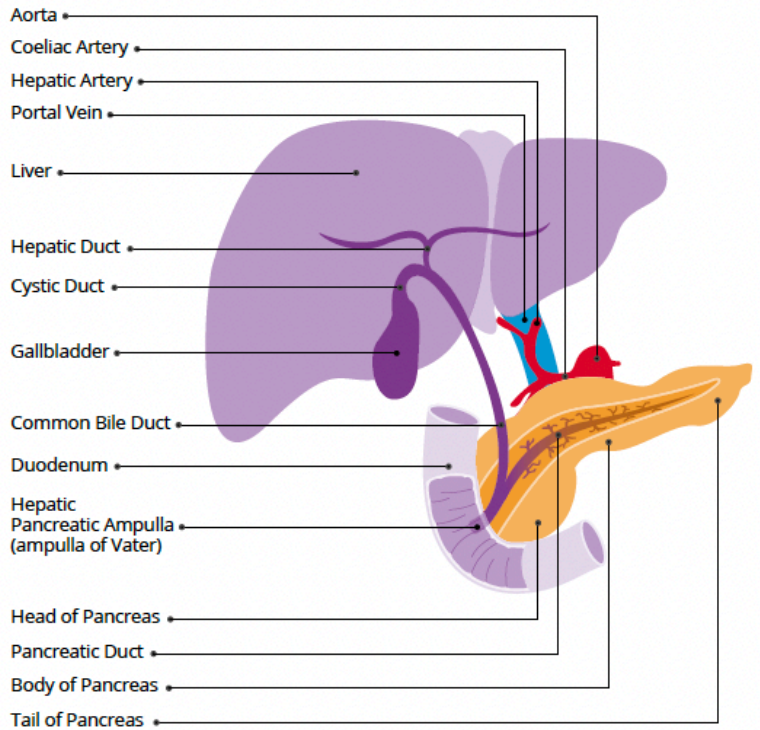
Hormones

Such as insulin and glucagon, which control blood sugar levels.

The pancreas helps the body use and store the energy it gets from food.

A tube called the pancreatic duct connects the pancreas to the first part of the small intestine, known as the duodenum. Digestive enzymes pass through this tube to help break down food. Another tube, called the common bile duct, passes through the head of the pancreas. This tube carries bile (a substance that helps to digest fats) from the liver and gall bladder to the small intestine. The bile duct may get blocked when a pancreatic tumour invades it. This causes jaundice (yellowing of the eyes and skin and dark urine).

The location of the pancreas



The pancreas contains two types of glands:

Exocrine glands

Create the enzymes which help digest (break down) foods.

Endocrine glands

Create the hormones such as insulin and glucagon, which control blood sugars.

Pancreatic cysts

2

What is a pancreatic cyst?

A pancreatic cyst is a fluid-filled sac that forms on or within the pancreas. Pancreatic cysts can range in size from just a few millimetres to several centimetres wide. However the majority are small and less than a centimetre or two in diameter. Most cysts are identified when a patient is given a CT scan or an MRI scan of their abdomen.

Why is it important to diagnose pancreatic cysts?

Many cysts do not cause symptoms and pose no particular risk to health. Some cysts however, contain cancer cells or cells that may turn into cancer over time. It is therefore important to detect and investigate all pancreatic cysts, and to treat the ones which pose a risk.

Types of pancreatic cyst

There are more than 20 different types of pancreatic cyst, which are categorised by their shape and contents. They fit into two main groups:

False cysts - of which the most common type is an inflammatory pseudocyst;

True pancreatic cysts - of which there are many types.

As far as possible it is important to determine which type of cyst you have. Different cysts have different risks and require different management.

Inflammatory pseudocysts (False cysts)

Pseudocysts or inflammatory cysts are common lesions of the pancreas. They normally develop some weeks or months after an episode of acute pancreatitis or a flare of chronic pancreatitis. Inflammatory Pseudocysts can occur at any age and in any part of the pancreas. They may occur as a single cyst or as multiple cysts and can range in size. They are not cancerous.

The term pseudo means false. Pseudocysts are not true cysts – they are distinguished from true cysts because they lack a specialised lining to the cyst wall. They contain fluid that is full of digestive enzymes.

Small inflammatory pseudocysts do not usually cause you symptoms and as long as they can be clearly distinguished from true cysts, no further follow up or treatment is required.

Larger pseudocysts can be more troublesome. They can cause you pain, they can block your bile duct leading to jaundice and they can compress your stomach and small bowel causing vomiting. They can become infected leading to fever. If you experience any of these symptoms, drainage of the cyst may be recommended.

True pancreatic cysts

There are many types of true pancreatic cyst. They are all lined by a special layer of cells that secrete fluid into the cyst and are broadly divided into two groups: mucinous cysts which are filled with mucus and non-mucinous cysts.

Mucinous cysts

Intraductal papillary mucinous neoplasm (IPMN)

IPMNs result from abnormal growth of cells in the ducts of your pancreas. As the abnormal cells grow they secrete a thick fluid called mucin leading to the formation of a cyst. These cysts are equally common in men and women. They can occur at any age but are more commonly found in older people. These cysts often produce no symptoms. Sometimes they can cause you abdominal pain, jaundice, or pancreatitis. Over time a small proportion of these cysts may change and develop into pancreatic cancer.

The number and location of your IPMNs will determine how you are treated. If your IPMNs are in the side branches of your pancreatic duct (branch-duct IPMN) they carry a low risk of becoming cancerous so are generally monitored with regular imaging.

In contrast, if your IPMNs come from your main pancreatic duct (main-duct IPMN) or from both your main duct and side branches (combined-type IPMN), they are more likely to become cancerous. In this case you will nearly always be referred for immediate surgery to remove them (see surgical treatment on page 19).

Sometimes, multiple IPMNs can develop simultaneously in different parts of your pancreas. In this situation each cyst will be assessed and you will be treated according to the risk each one may pose.

Pancreatic cysts

2

Mucinous cyst neoplasms (MCN)

These cysts normally occur in the body or tail of your pancreas. The space within these cysts is filled with mucus but, unlike IPMN, the cyst is not connected to the pancreatic ducts. These cysts are almost always found in middle-aged women. They normally cause non-specific symptoms such as abdominal discomfort, but can also cause nausea, vomiting, weight loss or on rare occasions jaundice. Over time, a proportion of these cysts may change and develop into a cancer.

Non-mucinous cysts

Serous cystadenoma (SCA)

These are almost all benign (non-cancerous) cysts that commonly occur in middle-aged women. They are usually located in the body or tail of your pancreas. Typically they are small, cause no symptoms and contain a watery clear fluid. If these cysts grow very large, they can give you compressive symptoms (because they press on other important organs) so may need to be removed by surgery.

Occasionally these cysts are associated with rare inherited conditions e.g. Von Hippel Lindau syndrome.

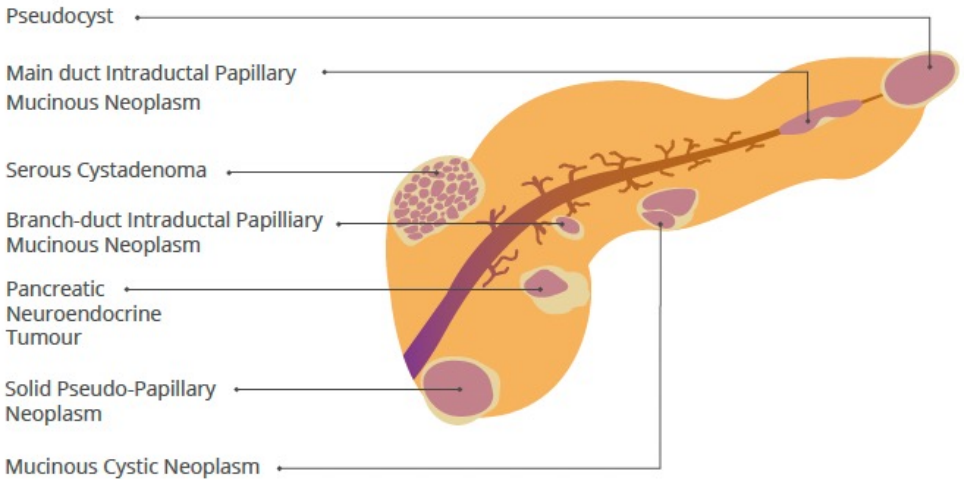
Solid pseudo-papillary neoplasm (SPPN)

These are rare cystic growths most commonly found in young women. They can be large (1-30cm) and are associated with non-specific symptoms such as abdominal pain, nausea, vomiting and a feeling of fullness.

These cysts are malignant (cancerous) tumours. However, they develop very slowly and patients have a very good prognosis once they are removed.

Pancreatic neuroendocrine tumour (PNET)

These are tumours of the hormone-producing parts of the pancreas – the Islets of Langerhans. Rarely, these tumours contain fluid-filled cavities (cysts) within them. These cysts are equally common in men and women and are more common in older age. Although these cysts are pre-malignant or malignant, patients have a very good prognosis once they are removed.



What is an indeterminate pancreatic cyst?

Although every effort is made to identify which type of cyst you have, it is sometimes not possible to do so unless surgery is performed. Cysts which cannot be classified are called indeterminate cysts. As they generally do not pose an immediate risk, indeterminate cysts tend to be managed by surveillance in the same way as small mucinous cystic lesions (see Section 9: Treatment - Surveillance on page 21).

Risk factors for developing a pancreatic cyst

Pseudocysts almost always occur following a bout of acute pancreatitis, a painful condition in which digestive enzymes become prematurely active and irritate the pancreas. Common risk factors for pancreatitis include heavy alcohol use and gallstones.

The risk of developing many pancreatic cysts e.g. IPMN, increases with age. Some cysts are seen almost exclusively in women, like mucinous cystic neoplasms (more than 90%) and serous cystadenoma (more than 70%).

3

Symptoms

What symptoms might I notice from my pancreatic cyst?

Although pancreatic cysts can cause symptoms such as jaundice, acute pancreatitis, back or abdominal pain, the majority of patients experience few or no symptoms, and increasingly many pancreatic cysts are found by chance during investigations for an unrelated complaint.

Pain

Small cysts e.g. less than 2cm, usually do not cause pain. However as cysts enlarge they may exert pressure on the surrounding tissues and nerves, leading to abdominal or back pain.

Acute or recurrent pancreatitis

Pancreatitis is a painful inflammatory condition of the pancreas. Repeated bouts of pancreatitis can be caused by the cyst or mucus secreted by the cyst blocking the pancreatic ducts.

Jaundice

Jaundice is a condition in which the skin and whites of the eyes become yellow and the urine becomes darker. It happens due to a build-up of bilirubin (a chemical constituent of bile) in the blood. Jaundice can occur if a cyst in the pancreatic head compresses or blocks the ducts carrying bile from the liver. Jaundice is a rare symptom in patients with pancreatic cysts (less than 1%), however it is a more common feature if cysts are very large or if cysts contain cancer cells.

Weight loss

Weight loss is a relatively rare feature in most patients because their cyst is only a few centimetres in size. However if cysts are very large they can reduce the ability of the pancreas to produce digestive enzymes. This can cause poor digestion of food resulting in weight loss. Large cysts can also cause compression of the stomach leading to vomiting and a loss of appetite which in turn can cause weight loss. Weight loss is more common in patients with cysts that have undergone cancerous change.

4

Diagnosis and Investigations

Diagnosis and referral

Your general practitioner or hospital consultant who has identified the pancreatic cyst will generally refer you to see a hepatobiliary surgeon or a gastroenterologist to investigate your cyst and recommend further treatment as necessary. Hepatobiliary surgeons and gastroenterologists are specialists in diseases and disorders of the digestive system, including the liver and pancreas.

What will happen when I attend outpatients?

The doctor will begin by asking you questions about your health in general, focusing on any symptoms you may currently be experiencing. They will also ask about any medical conditions you have, medications you are taking, and if you are aware of any diseases that run in your family, in particular any pancreatic problems. They will then go on to examine you, which will include an examination of your abdomen to look for any tenderness or lumps.

Blood tests

At your outpatient appointment you are likely to be asked to provide a sample of blood for various tests to check your blood count, liver and kidney function. Blood tests can also be performed to check levels of pancreatic tumour markers. These are chemical substances produced by cancers that can be detected in the blood. For example, CA19-9 is a tumour marker linked to pancreatic cancer. However, not all pancreatic cancers produce it and it may also occur with other illnesses that are not cancer. This means that blood tests are not used alone to make a diagnosis.

Diagnosis and Investigations

4

Imaging

Pancreatic cysts are usually first diagnosed on an ultrasound or computerised tomography (CT) scan of the abdomen. To try and identify which type of pancreatic cyst you have, further investigations may be arranged, such as a magnetic resonance imaging (MRI) or endoscopic ultrasound (EUS).

Ultrasound scan

An abdominal ultrasound uses sound waves to create a picture of your pancreas and the area surrounding it, including your liver. This is normally done in the x-ray department of the hospital.

The procedure is completely painless. You will be taken into a scanning room and asked to lie on the couch next to the ultrasound machine.

A clear gel will be spread onto the skin of your abdomen. A small device called a transducer will be moved across your abdomen. The transducer creates sound waves that echo when they meet an organ or tumour. The computer will turn these echoes into pictures, which the radiographer or doctor will interpret and the results will be sent to either your GP or your specialist.

CT scan

CT stands for computed tomography. It is really a more detailed and specialist type of x-ray. The CT unit is linked to a sophisticated computer that builds up lots of very detailed images from inside your body. Having a CT scan is completely painless.

What will happen?

Preparation for a CT scan can vary from patient to patient. In the x-ray department, your doctor or nurse will tell you what you need to do before you go for your scan.

Please tell your doctor or nurse if you:

- Have any allergies
- Have asthma
- Have diabetes
- Have kidney problems
- Are taking any medication
- Are pregnant
- Are afraid of needles
- Have had any problems before with any type of x-ray or radiology examination

You might be asked to drink and/or have an injection of dye. This allows the doctor to see the area being scanned more clearly.

If you are allergic to iodine, fish or dyes, you need to tell the person doing the CT scan in advance, as you may not be able to have the dye, drink or injection.

The scanner is shaped like a doughnut. It is about three feet wide and open at both ends. All you need to do is lie still on a table, which slides into the scanner. If necessary, your head and neck will be supported. The scan usually lasts from 15 to 45 minutes, but it depends on the area to be examined. If you need a CT scan, your local scanning department will offer you more detailed written information. When you go for your scan the radiographers will do their best to help you relax.

Remember, you will not be enclosed in any way and most people do not have a problem with having a CT scan.

Diagnosis and Investigations

4

MRI scan

MRI stands for Magnetic Resonance Imaging. This type of scan is quite common. It produces detailed pictures of the body. Instead of x-rays it uses a large magnet and radio waves that are fed into a computer. The computer then builds up cross-sectional images of your body. If you need an MRI scan, more written information will be available from your local scanning department. There are only a certain number of appointments for MRI scans, so it is very important that you do not miss your appointment.

Safety

There is no special preparation for an MRI. However, because of the powerful magnet used to produce the scans, safety guidelines must be followed. You may have to fill in a questionnaire before the scan can be carried out.

It may not be possible to have an MRI scan if you have:

- A heart pacemaker
- Some types of surgical clips inside your head
- Metal fragments in your eyes or elsewhere
- Neuron-stimulator implants

Please tell your doctor or nurse if you:

- Have any allergies
- Have asthma
- Have diabetes
- Have kidney problems
- Are taking any medication
- Are pregnant
- Have had any surgery in the past 12 weeks
- Are in any doubt about your suitability for an MRI scan
- Have had any problems before with any type of x-ray or radiology examination

For the scan:

- Wear something loose without metal zips or buttons
- Remove all metal objects, including rings, before scanning
- Remember to check that you do not have credit cards in your pockets as the magnetic strip is affected by the scan

If necessary, you will have an injection of dye into a vein in your arm. This can help improve the images. You will be asked to lie on a scan table in a type of tunnel. The table contains the magnet and the part of your body to be scanned lies directly over its centre. The table moves into position inside the tunnel and although you will not feel anything, there will be quite a lot of noise. All you need to do is try to relax and keep still while the pictures are being taken.

Because of the noise, you will be given earplugs and headphones may be available so you can listen to your own music or an audiobook. An MRI scan usually takes about 20-30 minutes, but it may be much shorter or can take up to an hour. The radiographer stays outside the room but you can talk with them through a microphone. The radiographers are very experienced and will do all they can to help you relax.

If you are allergic to iodine, fish or dyes, you need to tell the person doing the MRI scan in advance, as you may not be able to have the dye, drink or injection.

4

Diagnosis and Investigations

You may be able to bring a friend or relative with you when you go to the clinic. They can wait in the waiting room while you have your scan. Please check with the radiology department first in case the clinic is very busy.

When will I get the results?

At the end of the scan there could be up to 100 images for the radiologist to look at. Once these have been carefully studied, a report will be sent to your consultant. Make sure you have an appointment to get the result.

Endoscopic ultrasound (EUS)

Endoscopic ultrasound (EUS) is a type of endoscopy where the doctor uses a thin flexible camera with a small ultrasound probe at the tip. During the test, the doctor will look at the lining of your pancreas, as well as examining the lymph nodes. Everything will be magnified on a television screen and images will also appear on the ultrasound machine.

The test takes between 20-30 minutes.

If necessary, your doctor will take samples of cells from the area, by means of a fine needle aspiration (FNA), and send them to the laboratory for examination.

You cannot eat or drink for several hours before an endoscopy. You will have a sedative and a local anaesthetic to make you feel as comfortable as possible. Because of the sedative you should not drive or operate machinery within 24 hours.

When will I get the results?

If you have had biopsies taken it may take 7 to 10 days before the results are available. Before you leave the hospital after your endoscopy, make sure you have an appointment to go back and see your doctor for the results.

If, after the procedure you develop a high temperature, have difficulty swallowing or have increasing throat or chest pain, contact your doctor immediately.

5

Deciding on the best treatment

Multi-disciplinary team

The hepatobiliary surgeon or gastroenterologist you will have seen in clinic will have primary responsibility for your care. However, they work as part of a much larger Multi-disciplinary team (MDT) who will also be involved in arranging some of your tests and guiding your overall care. When planning your care, your doctor will wish to discuss your medical problem at a weekly meeting with other specialists. This means that your planned treatment is a joint decision by your doctor and several other specialists. Members of the MDT include:

- **Hepatobiliary surgeons** (doctors who specialise in operations involving the liver, pancreas and biliary tree)
- **Gastroenterologists** (medical doctors who look after conditions involving the liver, pancreas, biliary tree and gut)
- **Radiologist** (a specialised x-ray doctor)
- **Pathologist** (a doctor who studies body tissues)
- **Oncologist** (a specialist cancer doctor)
- **Clinical Nurse Specialists**

How is treatment decided?

Management of pancreatic cysts depends on the type of cyst you have, the results of your investigations and your general health and fitness as well as your treatment preferences after careful discussion with your doctor.

The most important aspect of the initial assessment and management of a pancreatic cyst is to determine if the cyst contains any cancer cells. However this is a rare occurrence, as the majority of cysts are benign and usually just require monitoring with imaging from time to time.

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Treatment of cysts without cancer

The way non cancerous cysts are treated depends on whether they cause you significant symptoms and whether they carry a risk of developing into pancreatic cancer. Only a few pancreatic cysts have the potential to develop cancer (in particular mucinous cystic neoplasms (MCN) and intraductal papillary mucinous neoplasms (IPMN).

Cysts that require further management

Mucinous cysts (MCN or IPMN) may develop into cancer over time, so they require regular surveillance and sometimes require surgery to remove them.

Serous cysts that are not causing any symptoms have an extremely low risk of turning into a cancer. Further surveillance, surgery and follow up is therefore not needed and so patients are usually discharged.

Treatment of cancerous cysts

What is pancreatic cancer?

Pancreatic cancer occurs when a malignant tumour forms in the pancreas.

Worldwide there are around 496,000 new cases each year; in Europe that figure is more than 104,000. In the UK, approximately 10,500 people are newly diagnosed each year.

Pancreatic cancer affects men and women equally with incidence increasing from the age of 45. The average age at diagnosis is 72.

There are two main types of pancreatic cancer:

Exocrine tumours

These make up the vast majority of all pancreatic cancers (around 95%) and come from the cells that line the ducts in the pancreas which carry digestive juices into the intestine.

These are called pancreatic ductal adenocarcinomas.

Other exocrine tumours of the pancreas are rarer, and include adenosquamous carcinomas and undifferentiated carcinomas.

Endocrine tumours

These are also known as neuroendocrine tumours, (NETS) and are much less common. The neuroendocrine tumours we discuss here are found in the pancreas and are called pancreatic neuroendocrine tumours (pNETS).

These are tumours that develop in our endocrine glands that release hormones (which regulate some processes in our bodies), these are then circulated around the body.

Other rare tumours that can affect the pancreas include pancreatic lymphoma, a cancer that arises from the lymphatic tissue in the pancreas; various cystic tumours and pancreatic sarcomas, which develop in the tissue that holds cells in the pancreas together.

Tumours that arise from tissues close to the pancreas, such as the bile duct (cholangiocarcinoma), Ampulla of Vater (ampullary adenocarcinoma), or duodenum (duodenal adenocarcinoma), may cause similar symptoms to pancreatic cancer but have different treatments and outcomes.

Treatment

What proportion of pancreatic cancers develops from a pancreatic cyst?

Of all cases of pancreatic cancer less than 10% develop from a pancreatic cyst.

Surgery

The only treatment for curing pancreatic cancer is to have surgery to completely remove the cancer and to stop it returning. If an operation is possible the site of the tumour and the extent of the spread of the disease will determine what type of operation your surgeon performs.

Chemotherapy

After your operation, your consultant will discuss the need for further treatment with an oncologist, who may prescribe chemotherapy for you, which is the use of anti-cancer drugs to destroy any remaining cancer cells. If this is needed an appointment will be made for you to attend outpatients once you go home.

More detailed information about treatment for pancreatic cancer can be found at panact.org

Which cysts require surgery?

If there is evidence of cancer or a strong likelihood of the cyst becoming cancerous, surgery is usually recommended. Surgery may be suggested for non-cancerous cysts if significant symptoms need to be relieved.

Features of a cyst that suggest it is likely to become cancerous

- Size (3cm or over) or cysts with rapid increase in size
- Dilatation of the main pancreatic duct to greater than 1cm
- Symptomatic cysts (eg. causing jaundice, diabetes, acute pancreatitis)
- Cysts with a solid component
- Elevated levels of CA19-9 in the blood

What operation might I need?

Whipple's operation (also known as a pancreatoduodenectomy)

During this operation, the head of the pancreas, gall bladder, duodenum and part of the bile duct are removed. The remaining pancreas, stomach and bile duct are joined up to the intestine, so that bile, pancreatic juices and food are able to flow normally and digestion can take place, following the operation.

Distal or left pancreatectomy

Removal of the tail and/or body of the pancreas. This procedure may include a splenectomy (removal of the spleen) and can be done by open or laparoscopic (keyhole) surgery.

Total pancreatectomy

This operation removes the entire pancreatic gland.

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Completion pancreatectomy

This operation removes the remaining pancreas e.g. if you develop recurrence of your cystic lesion or pancreatic cancer in the remaining pancreas after your original operation, your surgeon may recommend the removal of the remaining gland.

You can read more details about surgery in the booklet: Understanding Pancreatic Cancer: Surgery for Pancreatic Cancer.

The booklet is available online at panact.org/patient-booklets

Follow up after surgery

After your operation, the pancreas that is removed is sent to the laboratory for careful examination by a pathologist. They will determine the exact type of cyst you had and if malignant cells were present or not. You will be informed of the results by your surgical team as soon as they are available, which is generally before you leave hospital.

The results will determine further treatment and how often you are followed up. Your first outpatient appointment is generally a few weeks after discharge and, at this appointment, your doctor will go through with you in detail what further management is needed - if any.

If you are found to have a serous cystadenoma or a mucinous cystic neoplasm (MCN), without any cancerous cells, which has been completely removed, this surgery is curative, so no further treatment is required. You will likely be given one further follow up appointment a year later, and if you are well, then you will be discharged.

If you are found to have had an IPMN, further IPMNs may occur in your remaining pancreas so, if you have not had a total pancreatectomy, follow-up with an MRI scan is usually recommended every 6-12 months to detect any new lesions that may develop.

If cancer cells are found in the pancreas at the time of the surgery, you will be referred to an oncologist for consideration for chemotherapy and regular follow-up will be needed over the next few years.

Which cysts require surveillance?

Intraductal papillary mucinous neoplasm (IPMN), mucinous cystic neoplasms (MCN) and Indeterminate cysts.

If I have a pre-malignant cyst, what is the risk of it developing into cancer?

The natural history of pre-malignant cysts (MCNs and IPMNs) is poorly understood but patients are thought to develop cancer at a rate of less than 1% per year.

How will surveillance be performed?

There are various methods of performing pancreatic surveillance and guidelines differ on what tests should be used and how often surveillance should be done, so there may be small differences in how your cyst is monitored depending on the hospital.

However in most hospitals, when you are first diagnosed your doctor will recommend an MRI scan every 6-12 months. If your cyst changes during follow up and needs closer observation, sometimes scans are done sooner (e.g. in 3 months' time for a while).

Once your cyst has been shown to be stable for a period, scans may be carried out less frequently (e.g. every 1-2 years). In some cases your doctor may recommend performing a CT or EUS in addition to your MRI. These tests can also be used instead of MRI if you cannot tolerate this test.

How long do I need to be followed up for?

Surveillance is generally undertaken for as long as you are fit to undergo an operation on your pancreas, if needed. In most people this is for a period of several years.

Will I need surgery in the future?

Patients with larger cysts require more frequent follow-up. If the cyst does grow or change during follow up you may require surgery to remove it.

7

Research, clinical trials and new treatment

Doctors are still learning which treatments work best for pancreatic cysts. There are many studies underway testing different treatments. For example, some are looking at better ways of diagnosing pancreatic cysts, while others will ask you about your experience as a patient or will be testing new minimally invasive treatments, which may be an alternative to surgery.

The only way you can get some of these treatments is to take part in a clinical trial or study. Your doctor will be able to tell you if there are trials going on in your area which might be suitable for you. When considering participating in a clinical trial, you have to bear in mind that you may not get the new treatment as studies usually compare a new treatment with a standard treatment. Nobody knows before the study which treatment will come out best. For more information visit: panact.org/clinical-trials

8

Questions

What questions should I think about asking when I attend outpatients with a pancreatic cyst?

- What type of pancreatic cyst do I have?
- Where in the pancreas is the cyst?
- What size is my cyst?
- Does it have any features which are worrying?
- Am I likely to need an operation?
- Do I need further follow up?

Please write down any questions you may have and bring this with you to your next appointment.

Questions

8

Please write down any questions you may have and bring this with you to your next appointment.

11

Acknowledgements

We would like to thank the following for their contribution to the development of this booklet:

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For sources and references used in the compilation of this booklet, please contact us at the address overleaf.

Pancreatic Cancer Action

This booklet has been funded through the generosity of supporters of Pancreatic Cancer Action, a UK charity founded by a pancreatic cancer survivor, Ali Stunt, who was diagnosed with pancreatic ductal adenocarcinoma in 2007. With a focus on early diagnosis, it is Pancreatic Cancer Action's mission to improve survival rates by raising awareness of pancreatic cancer and its symptoms among the public, medical education, improved information and by funding research specifically to improve early diagnosis of the disease.

If you would like to support us or find out more, please contact us at:
enquiries@panact.org or visit panact.org

If you are unsure of anything at any time please consult your own doctor, dietitian or Cancer Nurse Specialist (CNS)

If you would like to help us by either holding an awareness event or by fundraising, please **email enquiries@panact.org** or call **0303 040 1770**. For more information or to donate directly please visit **panact.org**

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Saving lives through early diagnosis

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Trusted Information Creator

Patient Information Forum

Appendix 4: RADIOCYST01 Trial protocol

RADIOCYST

A PHASE II MULTICENTRE STUDY OF ENDOSCOPIC ULTRASOUND GUIDED RADIOFREQUENCY ABLATION OF CYSTIC TUMOURS OF THE PANCREAS

Version 5.0 Dated 09 January2018

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ClinicalTrials.gov Identifier: NCT02343692

2. Signature Page

The Chief Investigator and the JBRU have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol except in case of medical emergency (See SPON/S15 "SOP for the recording and reporting of deviations, violations, potential serious breaches and **urgent safety measures**") or where departures from it are mutually agreed in writing.

The investigator agrees to conduct the study in compliance with the protocol, GCP and UK Regulations for CTIMPs, the Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the Research Governance Framework (2005), the Sponsor's SOPs, and other regulatory requirements as appropriate.

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This document describes the RADIOCYST study and provides information about procedures for entering patients into it. The protocol should not be used as a guide for the treatment of patients outside the study. Every care was taken in drafting this protocol, but corrections or amendments may be necessary which will be circulated to the known investigators in the study. Clinical problems relating to this study should be referred to the SITU in the first instance.

This study will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 and MRC Good Clinical Practice guidelines. It

will be conducted in compliance with the protocol, the Data Protection Act (DPA Z6364106) and other regulatory requirements as appropriate.

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4. List of abbreviations and definitions

Abbreviation	Explanation
AE	Adverse Event
APR	Annual Progress Report
AR	Adverse Reaction
ASR	Annual Safety Report
CA19-9	Carbohydrate antigen 19-9
CEA	Carcinoembryonic antigen
CI	Chief Investigator
CLE	Confocal Laser Endomicroscopy
CRF	Case report form
CT	Computerised tomography
ECOG	Easter Cooperative Oncology Group
ERCP	Endoscopic retrograde cholangiopancreatography
EUS	Endoscopic ultrasound
EUS-FNA	Endoscopic ultrasound-guided fine-needle aspiration
FBC	Full blood count
FNAC	Fine-needle aspiration cytology
GCP	Good Clinical Practice
hCG	human Chorionic Gonadotropin
HPB	Hepato-Pancreato-Biliary
INR	International normalised ratio
IPMN	Intraductual papillary mucinous neoplasms
IV	Intravenous
JRO	Joint Research Officer
Main REC	Main Research Ethics Committee
MCN	Mucinous cystic neoplasms
MDM	Multidisciplinary meeting
MHRA	Medicines and Healthcare products Regulatory Agency

	(www.mhra.gov.uk)
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
nCLE	needle-based Confocal Laser Endomicroscopy
PAS	Periodic acid shift
PI	Principal Investigator
QOL	Quality of life
REC	Regional ethics committee
RF	Radiofrequency
RFA	Radiofrequency ablation
SAE	Serious Adverse Event
SDV	Source data verification
SITU	Surgical & Interventional Trials Unit
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse event
TMF	Trial Master File
US	Ultrasound
W	Watts

5. Summary

TITLE	RADIOCYST: EUS guided radiofrequency ablation of cystic tumours of the pancreas.
DESIGN	A multicentre phase II study to determine the safety and efficacy of EUS-guided radiofrequency ablation in patients with pre-diagnosed cystic tumours of the pancreas. Patients will be recruited sequentially to undergo radiofrequency ablation followed by standard surveillance. The study will follow a Simon two-stage design. The first part of the study will recruit 42 patients. If a 20% ablation rate is achieved the study will continue to recruit 97 patients in total.
AIMS	**Primary:** To evaluate pancreatic cyst ablation at 12 months following EUS-guided radiofrequency ablation therapy in patients with pre-diagnosed cystic tumours of the pancreas. **Secondary**: To assess the safety and efficacy of EUS-guided radiofrequency ablation. To evaluate surrogate markers of response using imaging (CT, MRI/MRCP, EUS) and perform a health economics assessment.
ENDPOINTS	***Primary*:** -Presence of pancreatic cyst at one year following EUS-RFA. ***Secondary*:** **-**Mortality -Morbidity -Progression following treatment -Rates of surgical resection -Local complication rate -Assess surrogate markers of response: imaging (CT, MRI/MRCP, EUS) and serum markers -Health Economics

POPULATION	Patients with cystic tumours of the pancreas, without evidence of malignant transformation by imaging or EUS-guided sampling criteria.
TRIAL SITES	Procedures will be undertaken at UCLH, the Royal Free Hospital and other participating centres.
ELIGIBILITY	-Cystic tumours of the pancreas 0.5-3cm in size, or >3cm in size if unsuitable for surgical resection. -Cystic tumours of the pancreas that do not arise from the main pancreatic duct and are situated more than 5mm from major vascular or biliary structures. -Patients should be suitable to return to the surveillance program. -Life expectancy of at least 12 weeks. -ECOG performance status 0, 1 or 2. -Over 18 years. -Informed written patient consent. -Negative pregnancy test in pre-menopausal women. -Has not had acute pancreatitis in the previous 4 weeks.
TREATMENT	Treatment is via a single monopolar probe placed under EUS guidance by an experienced endoscopist into the cystic lesion of the pancreas. Ablation is then achieved using an RFA generator (ERBE VIO 300D, Dolby medical products, Scotland) to deliver sequential doses of electrical energy at 10W for a total of up to 25 minutes (10x90 second applications with 60 seconds rest between applications) to ablate the cystic lesion. If complete ablation is not achieved at 3 months, patients will be considered for one further EUS guided RFA treatment. All patients following treatment will undergo standard surveillance.
DURATION	Start date July 2015. Accrual of the 97 patients within 2 years and at least 1 year

	follow-up.
SPONSOR	UCL

6. Background

In order to avoid the uncertainty of follow up of cystic pancreatic lesions and to provide an alternative to surgical resection, some small studies of ablative techniques have been piloted. EUS-guided injection of alcohol has reported reasonable efficacy (35% to 62%) in achieving complete ablation on follow up. However, this technique did not achieve total cyst ablation in cysts with septations and was associated with complication rates (pain and pancreatitis) of between 4% to 20% (Gan SI et al. 2005, Oh HC et al. 2011). A major potential advantage of EUS guided RF for the ablation of cystic tumours is that it could be done in a minimally invasive way, with the likelihood of fewer complications than alcohol injection because the area of ablation can be assessed and monitored in real-time by EUS. We expect the technique to become an ideal therapy for those who are either unfit for surgery or who have cystic tumours with low malignant potential who would otherwise require long-term surveillance.

Definition of cystic lesions of the pancreas for this study

Defining the nature of pancreatic cystic lesions is not straightforward but we will use previously published criteria and consensus opinion of the HPB multi-disciplinary meeting to categorise each lesion. Those without a clear diagnosis will be categorised as indeterminate. The most common diagnoses are:

- Branch-duct IPMN (intraductal papillary mucinous neoplasm) (inclusion criteria)

Pleomorphic wall, communicates with a pancreatic branch duct. Imaging frequently reveals associated dilatation of the pancreatic duct. Typical cyst fluid findings: amylase >800U/ml, CEA >192ng/ml. Cytology reveals periodic acid shift (PAS) positive mucinous epithelial cells with variable atypia.

- Mucinous cystadenoma (inclusion criteria)

Multilocular or unilocular lesion that does not communicate with the pancreatic duct. Typical cystic fluid findings: amylase <250U/ml, CEA >192ng/ml. Cytology reveals periodic acid shift (PAS) positive mucinous epithelial cells with variable atypia.

- Indeterminate cystic lesion

No clear diagnosis by standard criteria.

However if at multidisciplinary review there are atypical features on CT, EUS, amylase, tumour markers or cytology which are sufficiently suggestive of a mucinous lesion then they will be included in this study.

- Pancreatic pseudocyst (exclusion criteria)

Simple thin walled cyst without septations or a solid component. Typically there is communication with the pancreatic ductal system and cystic fluid amylase > 800U/ml and CEA <5ng/ml. Aspirates for cytology are acellular with negative PAS staining. Evidence of previous pancreatitis and/or parenchymal changes of pancreatitis are supportive but not required for diagnosis.

- Pancreatic adenocarcinoma or other malignant cystic lesion (exclusion criteria)

On imaging a rapidly growing lesion, evidence of an invasive solid component, foci of calcification in solid components, larger size, regional lymphadenopathy or metastases suggest malignant transformation of the cyst. Presence of adenocarcinoma cells within cytologic or histologic samples confirms the diagnosis. Typically cyst fluid samples show high CEA levels.

- Main-duct IPMN (exclusion criteria)

Pleomorphic wall, communicates with the main pancreatic duct. Imaging frequently reveals associated dilatation of the main pancreatic duct. Typically cyst fluid amylase >800U/ml, CEA >200ng/ml. Cytology reveals periodic acid shift (PAS) positive mucinous epithelial cells with variable atypia.

- Serous cystadenoma (exclusion criteria)

Usually multicystic lesion with septations (but can be oligocystic) and may have central calcified scar. Typical cystic fluid findings: amylase <250U/ml, CEA <5ng/ml. Cytologically PAS staining for mucin will be negative but aspirates will be acellular and glycogen rich.

Study objectives and purpose

Primary Objectives

To evaluate pancreatic cyst ablation at 12 months following EUS-guided radiofrequency ablation therapy in patients with pre-diagnosed cystic tumours of the pancreas.

Secondary Objectives

To evaluate the treatment with respect to the following:

- Mortality.
- Morbidity.
- Progression-free survival.
- Surgical resection rates.
- Local complication rate.
- Assess surrogate markers of response: imaging (CT, MRI/MRCP, EUS)
- Health economics

7. Study design

Multicentre phase II study of EUS-guided RF cyst ablation. A total of 97 patients with pre-diagnosed cystic tumours of the pancreas will be recruited sequentially to undergo RF followed by surveillance.

The following assessments will be completed. Initial radiological investigations should be performed within 3 months of treatment, as part of routine assessment of disease. A summary of all other investigations and assessments is provided below and summarised in Appendix 3.

Pre-treatment/ Baseline

Month -3 to day 0 (before treatment on Day 1):

Confirmation of diagnosis at multidisciplinary review

- MRI/MRCP (month -6 to day 0) as per local practice
- Pancreatic protocol CT (month -3 to day 0) (optional depending on local practice)

Day -28 to 0 (before treatment on Day 1):

- Informed consent
- Symptom and adverse event monitoring
- Physical examination (including height, weight and ECOG performance status)
- FBC, U&E, LFTs ,INR and biochemical profile including CEA/CA19-9, serum amylase, glucose
- Pregnancy test
- Copy of reports of other prior investigations (eg endoscopy reports, histology or cytology reports)
- Baseline questionnaires – Symptom, health diary, EQ5D
- AE monitoring

Treatment

Visit 1: Day 1 (treatment day):

- EUS-FNA (if sufficient send for cytology and biochemical analysis) and EUS-RFA of cystic lesion.
- Symptom and adverse event monitoring
- Physical examination

Visit 2: Day 2 (after treatment on Day 1):

- Symptom and adverse event monitoring (including QOL score)
- Physical examination (including ECOG performance status)

- FBC and biochemical profile including amylase, glucose
- Pancreatic protocol CT

Follow-up & response assessment

Visit 3: Month 3:

- Symptom, EQ5D, health diary and adverse event monitoring
- Physical examination (including weight and ECOG performance status)
- FBC and biochemical profile including CEA/CA19-9
- MRI/MRCP
- Patient offered further EUS-RFA if incomplete cyst ablation

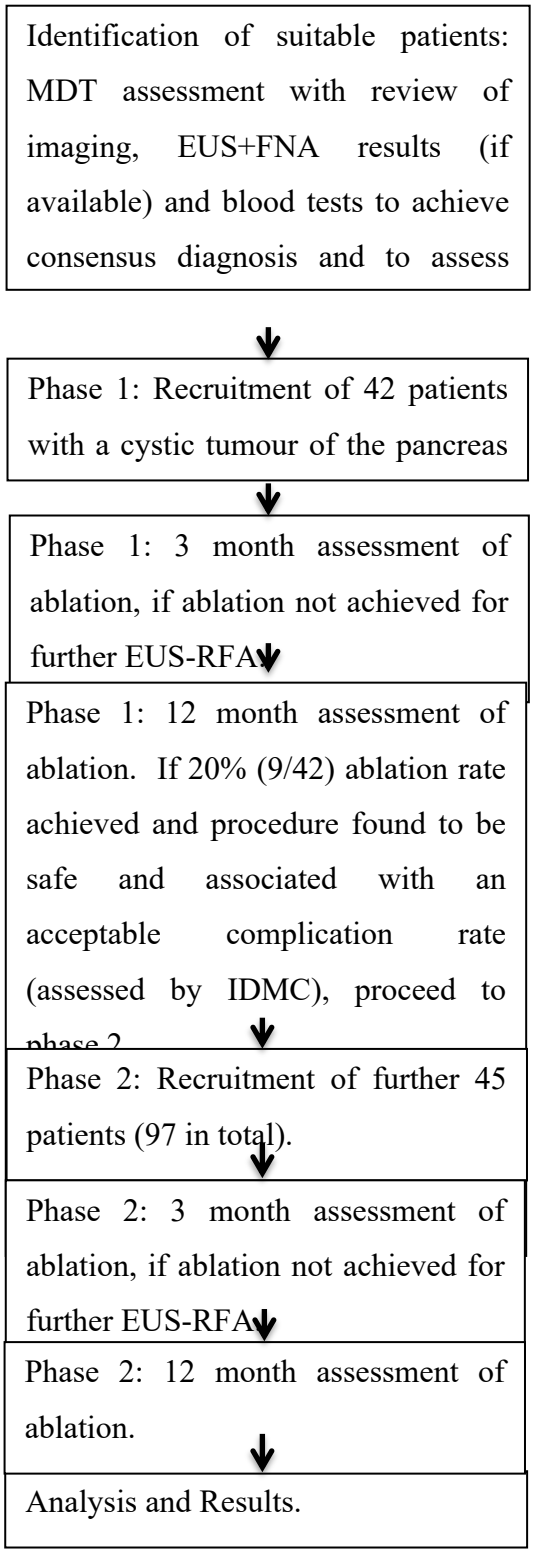
Visit 4: Month 6 (if repeat EUS-RFA performed after month 3 assessment):

- Symptom, EQ5D, health diary and adverse event monitoring
- Physical examination (including weight and ECOG performance status)
- FBC and biochemical profile including CEA/CA19-9

Visit 5: Month 12:

- Symptom, EQ5D and adverse event monitoring
- Physical examination (including weight and ECOG performance status)
- FBC and biochemical profile including CEA/CA19-9
- MRI/MRCP

Study Schema



Outcome measures:

The primary outcome of the study will be evaluate pancreatic cyst ablation at 12 months following EUS-guided radiofrequency ablation therapy in patients with pre-diagnosed cystic tumours of the pancreas. Blood and imaging (CT, MRI/MRCP, EUS) reports will be recorded for each patient along with any complications or side effects encountered and health economics.

Assessment and follow up

Following RFA and discharge from hospital, patients will be followed up at 3 and 12 months in outpatients. Patients who undergo a second EUS-RFA after their Month 3 assessment will also undergo a Month 6 review. All patients will be followed up at 12 months. Following the study period longer term follow up of patients recruited to this study will occur at an interval based on local MDT practice.

Assessment of efficacy/effectiveness:

Depending on local practice, patients will undergo an MRI/MRCP at 3 months. If ablation of the cystic tumour has not been achieved, patients will be considered for a further EUS-RFA treatment. The frequency of patients requiring repeat ablation will be carefully monitored throughout the study.

Assessment of safety:

The safety of this treatment and integrity of the equipment will be assessed following each case.

Annually the sponsor will provide the main REC with an annual progress report (APR). The APR will be prepared, using the REC's APR form, by the Chief investigator or a delegated PI.

Site Monitoring

SITU will attend UK sites after at least 3 patients have been consented to monitor Investigator Site Files for completeness and source data verification for consent and adherence to inclusion/exclusion criteria.

8. Subject selection

If confirmed as eligible, the patient will be consented and allocated a unique study number and the treatment regimen defined. Ethnicity data will be collected. All study data will be recorded on case report forms. All research staff who enter data onto the CRF will have signed the study signature and delegation of duties log before undertaking data entry.

Inclusion criteria

1. A diagnosis of a pancreatic cystic tumour based on multidisciplinary review of imaging, for which further surveillance with non-invasive imaging is indicated.
2. Pancreatic cystic tumour between 0.5 and 3cm in size. Cysts greater than 3cm or with mural nodules can be included only if patients are unsuitable for surgical resection.
3. ECOG performance status 0, 1 or 2.
4. Estimated life expectancy of at least 12 weeks.
5. Age >18 years.
6. Capable of giving written informed consent.
7. Women of child-bearing potential must have a negative pregnancy test (serum/urine) in the week before treatment, AND be using an adequate contraception method, which must be continued for at least 1 week after RF.

Exclusion criteria

1. A diagnosis of a pancreatic cystic tumour where surgical resection is indicated.
2. Pancreatic cysts greater than 3cm or less than 0.5cm in size.
3. Benign pancreatic cysts (e.g. pseudocyst).
4. Serous cystadenomas.
5. Pancreatic cysts with malignant transformation.
6. Cysts involving or in close proximity to vessels or the biliary tree where the zone of ablation is likely to compromise these structures.
7. Cysts with morphology that in the view of the investigator make it undesirable for the patient to participate in the study, e.g. exophytic cysts.
8. Cysts arising from the main pancreatic duct.
9. History of active or prior malignancy that will interfere with the response evaluation (exceptions include in-situ carcinoma of the cervix treated by cone-biopsy/resection, non-metastatic basal and/or squamous cell carcinomas of the skin, any early stage (stage l)

malignancy adequately resected for cure greater than 5 years previously).

10. Acute pancreatitis within the previous 4 weeks.
11. Any evidence of severe or uncontrolled systemic diseases or laboratory finding that in the view of the investigator makes it undesirable for the patient to participate in the study.
12. Any psychiatric disorder making reliable informed consent impossible.
13. Pregnancy or breast-feeding.
14. ECOG performance status 3 or 4.

9. Subject recruitment

At the weekly Cancer Network Hepatobiliary MDTs of participating hospitals, patients who have cystic tumours of the pancreas who are identified as needing follow up with serial imaging will be considered for recruitment into this study. MDT patient identification can be 12 months in advance. Suitable patients will then be invited to the Hepatobiliary outpatient department of the participating hospital to discuss the study. Patients will be recruited voluntarily and formal written consent will be gained prior to the EUS-RFA treatment.

10. Study interventions

10.1 General information

Introduction to Probes

Generic text to cover all probes, in which Habib and STARmed are a subset of.

Only probes via Trial Unit can be used in this trial.

Radiofrequency ablation

Radiofrequency ablation is achieved through a high frequency alternating current which generates high temperatures causing a coagulative necrosis. It has been used widely, percutaneously, intraoperatively and endoscopically to treat primary and secondary cancers in the liver, lung, kidney, bone and oesophagus.

The EUS RFA catheter is a single use sterile catheter for use during endoscopic ultrasound. It is an endoscopic monopolar catheter that has been designed to ablate cystic tumours of the pancreas and has EU European Conformity approval for this purpose. Following identification and puncture of the pancreatic cyst, the EUS RFA catheter can be introduced through a standard EUS-FNA needle. The catheter has either a 1cm or 2cm long active metallic electrode, and can be used with a variety of commercially available RF Generators, such as the RITA 1500, RITA 1500X, or ERBE VIO200D/300D. The catheter used (1cm or 2cm electrode) will be at the discretion of the endoscopist according to the size of cyst treated, and the tip will be placed at the most distal part of the pancreatic cyst under EUS-guidance. A dose of 10W for 90 seconds will be administered to each cystic tumour on up to ten occasions during one EUS guided ablation, with a 60 second cooling time between applications. Participating endoscopists performing the procedure will be experienced (at least 5 years), carrying out regular EUS interventions (approximately 150 cases per year).

Animal studies of pancreatic RFA

A bipolar EUS RFA probe has been used to ablate the pancreas in a porcine model. A modified EUS needle and a commercial RF needle were utilised. The study found

that RFA could provide localised tissue ablation within a 1cm zone from the needle catheter. Complication rates were acceptable with one of the thirteen pigs treated developing pancreatitis (Goldberg SN et al, 1999).

In 2008 Carrara et al. demonstrated the feasibility and efficacy of EUS-guided RFA using a newly developed bipolar ablation probe combining RFA and cryotechnology in 14 pigs. The size of the ablation area was related to the duration of ablation. Complications were less common than for use of conventional RFA needles (Carrara S et al. 2008).

EUS-RFA of the pancreas was attempted on 10 adult mini pigs using an 18-gauge endoscopic RFA electrode (STARmed, Korea). 50W for 5 minutes was administered to the body and tail of the pancreas. A spherical necrotic lesion surrounded by fibrous tissue was observed on histopathologic examination. The ablated tissue had a mean diameter of 23.0 x 6.9 mm. No major procedure-related complications were noted. They concluded EUS-RFA of the pancreatic body and tail was feasible, effective, and relatively safe in a porcine model (Kim HJ et al. 2012).

Animal studies of pancreatic RFA using EUS RFA probe

The safety and efficacy of the monopolar EUS RFA catheter has been examined in the porcine model. Five Yucatan pigs underwent EUS-guided radiofrequency ablation of the head of their pancreas. Using an EUS-needle, RFA was applied with 6mm and then 10mm of the probe exposed at specific wattage for preset durations. Only one pig showed moderate levels of pancreatitis (20% proximal pancreatitis). The other animals showed much lower areas of tissue damage. In 3 of the 5 pigs, the proximal pancreas showed greater levels of tissue injury than the distal pancreas, consistent with the proximity of the tissue to the procedure site. In 1 pig, both proximal and distal pancreas showed minimal pancreatitis (1%). There was minimal evidence of fat necrosis in intra-pancreatic and/or extra-pancreatic adipose tissue. EUS-guided RFA of the pancreatic head with the monopolar probe through a 19-gauge needle was well tolerated and the ablation

area was proportionally related to the catheter length, power or time to which the tissue was exposed (Gaidhane M et al. 2012).

Generator	Power	Time	Length	Depth	Width	Comments
Rita 1500	1 watt	------	---------	------	----------	120Impedance
6mm exposed	4	0.5	6.36	0.55	4.82	230 Impedance
6mm exposed	5	0.9	10.38	1.00	4.63	190 Impedance
6mm exposed	6	0.2	7.03	0.66	3.79	221 Impedance
10mm exposed	4	4.3	13.33	2.31	7.02	183 Impedance
10mm exposed	5	1.4	11.71	1.56	7.51	174 Impedance
10mm exposed	6	0.8	13.83	0.99	4.29	182 Impedance
15mm exposed	4	5.9	17.52	2.24	7.58	146 Impedance
15mm exposed	5	4.1	16.82	1.22	7.54	142 Impedance
15mm exposed	6	6.0	20.86	1.25	7.73	160 Impedance
Date	26.03.2010			Signed	S.M.B.McColm	

Table 1. Porcine power / time ablation studies for Habib EUS probe (unpublished table)

Clinical studies of radiofrequency ablation in pancreatic cancer

Since 2000 RFA has been utilised as a palliative ablative treatment in 106 patients with unresectable locally advanced and metastatic pancreatic adenocarcinoma. RFA was administered intraoperatively in all cases except one in which it was CT guided. The seven studies to date all demonstrate RFA to be a feasible treatment in pancreatic adenocarcinoma. However a number of early studies have demonstrated complications secondary to the RFA treatment (gastrointestinal hemorrhage, pancreatic fistula, biliary leak, portal vein thrombosis and pancreatitis) in up to a third of cases (Pezzilli R et al. 2011).

One potential advantage of EUS-guided RFA is that it is possible to assess the position of the cyst in relation to other structures such as the common bile duct and blood vessels at the time of RFA under real-time EUS guidance, thereby reducing complication rates. Indeed, Arcidiacono and colleagues (2012) treated 22 patients with locally advanced pancreatic adenocarcinoma, using a novel radiofrequency probe with cryogenic cooling inserted under EUS guidance. The probe was successfully inserted in 16 patients (72.8%); in 6 it was not possible because of stiffness of the gastrointestinal wall and of the tumour. With respect to

early complications (within one week of treatment), three patients reported postinterventional abdominal pain, which responded to analgesic drugs. One patient experienced minor bleeding in the duodenal lumen after the procedure, which was treated by endoscopic placement of hemostatic clips and did not require blood transfusion therapy. Amylase arose in 3 of 16 patients; none had clinical signs of pancreatitis. Late complications (within three months) arose in 4 cases, 3 of which required intervention with a biliary or duodenal stent one months after treatment (Arcidiacono PG et al. 2012).

RFA in Pancreatic Cysts

Although RFA is an established ablative technique in a number of premalignant and malignant lesions, to date it has only been used to ablate cystic tumours of the pancreas in a few cases. Figure 1 below is from a 73 year old woman who was diagnosed with a mucinous cyst in the body of the pancreas. She underwent EUS guided radiofrequency ablation using the Habib EUS RFA probe and complete ablation was achieved and confirmed by endosonography at the time of the procedure. She encountered no side effects or complications from the treatment.

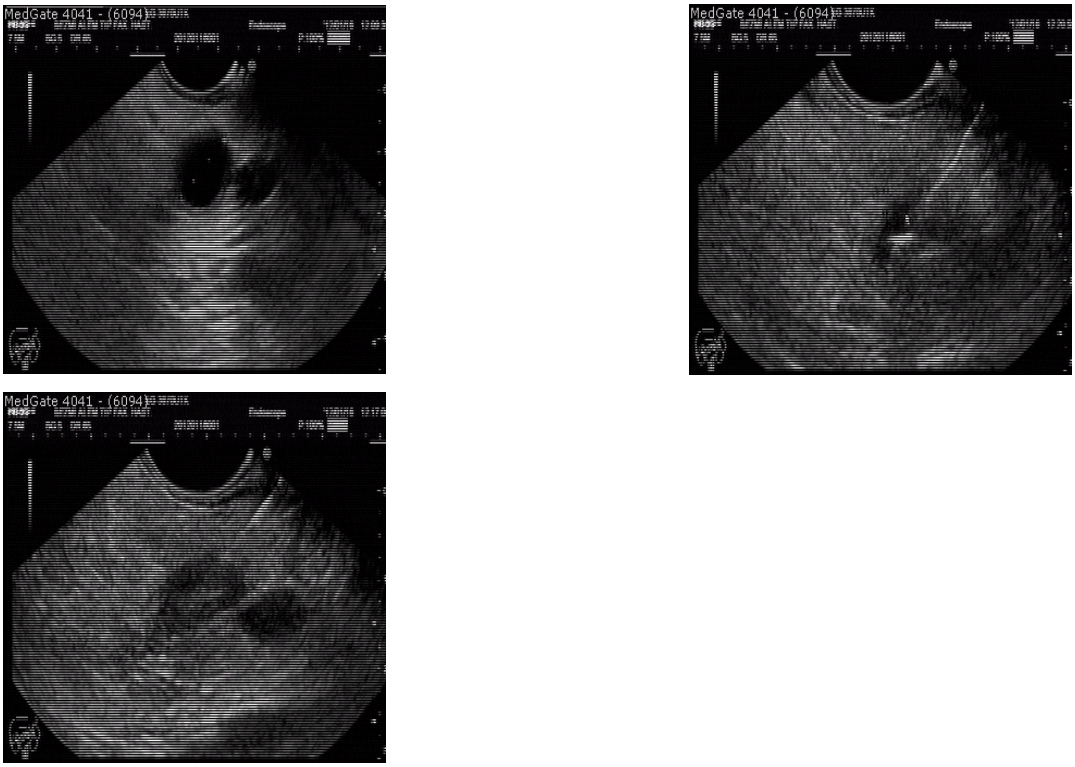


Figure 3. EUS guided RF ablation of a mucinous cystic tumour of the pancreas. Images courtesy of Professor N

In the published experience of six cystic tumours and two pancreatic neuroendocrine tumours ablated by the Habib EUS-RFA catheter to date all tumours were in the head of the pancreas and had a mean size of 36.5 mm. In this initial series a range of energy settings (5-25W) and frequency of applications (2-7 x 90 seconds) were applied to the individual tumours. There were no episodes of acute pancreatitis, perforation or bleeding; two patients had mild abdominal pain which resolved within 3 days of the procedure (Pai M et al. 2015).

10.2 Therapeutic protocol for radiofrequency ablation

Patients will be treated with a monopolar radiofrequency current at 10W for 90 seconds on up to 10 sequential occasions during one EUS-RFA procedure.

EUS -guided radiofrequency ablation

Under routine midazolam, fentanyl and/or propofol sedation, or under general anaesthetic if clinically indicated, endoscopic ultrasound (EUS)-guided fine needle puncture of the cystic lesion followed by radiofrequency ablation will be performed. A disposable 19G or 22G fine needle aspiration device (eg. EchoTip, Cook UK; Expect, Boston Scientific) will be used in conjunction with a linear-array electronic echoendoscope with at least a 2.8 mm accessory channel (Olympus, Keymed UK Ltd.; Pentax, Hitachi Medical Systems UK Ltd.).

Prior to commencing the procedure the patient will receive the first dose of antibiotics which will be continued for up to 48 hours according to local practice. During sedation and standard preparation for endoscopic ultrasound a patient electrode / grounding pad will be placed on the patient. The echoendoscope will then be introduced to the stomach or duodenum to identify the target cyst. After visualization of the cystic lesion under real-time EUS guidance and Doppler examination, the cyst will be punctured using a Cook EUS FNA needle (incorporating a stylet). A 19G to 22G needle may be used at the discretion of the endoscopist depending on location and ease of access to the lesion; in general a 19G needle will be used if the echoendoscope is deployed in the stomach and a

22G needle when the scope is deployed through the duodenum or a single treatment is contemplated Cyst fluid will then be aspirated to dryness and sent for biochemical (amylase & CEA) and cytological analysis. The tip of the needle will then be placed at the deepest part of the cyst. The biopsy needle stylet will then be removed and replaced with a Habib™ EUS RFA probe. Whilst maintaining the position of the probe the needle will be withdrawn to disengage contact with the probe. 10W of monopolar radiofrequency current will then be administered for 90 seconds. After one minute the probe can be repositioned and the procedure repeated sequentially, which will be done on up to 10 occasions in one EUS guided RF ablation (Appendix 4). Tumour ablation will be assessed in real-time by EUS at the end of the ablation procedure. Cyst ablation will be reviewed in each patient following their 3 month scan. If complete ablation has not been achieved patients will be considered for one further EUS-RF ablation treatment (10Wx90s on up to 10 sequential occasions).

Antibiotic prophylaxis

Antibiotic prophylaxis (eg ciprofloxacin 200 mg i.v.) will be given 1 hour before or during the EUS procedure and continued for up to 48 hours according to local practice. If an allergy is present, an alternative prophylactic regimen will be given as per local protocols.

Follow-up of RFA treated patients

Following treatment, patients will be kept nil by mouth for 4 hours and monitored closely on the ward for 24 hours. Contrast-enhanced spiral CT will be performed on treatment day 2 (acceptable CT window day 2–4), to assess any subclinical changes to the pancreas prior to discharge. Subsequent surveillance will be undertaken with an MRCP at 3 and 12 months. A second EUS-RFA treatment will be offered to patients in whom complete ablation has not been achieved at 3 months. Other investigations such as ERCP will be performed as clinically indicated.

11. Registration

This will be an open treatment non-randomised study.

Following a verbal and written explanation of the study, consenting patients will be registered as follows:-

- Contact RADIOCYST Study Coordinator to check eligibility.
- The Study Coordinator will allocate a study number.

Surgical & Interventional Trials Unit (SITU) , Division of Surgery & Interventional Science

Office hours: Mon-Fri 9.00am-5.30pm

Registration

Fax: +44 (0)20 7679 9290

With the patients' permission, the GP will be informed of their study participation. All study data will be recorded on case report forms provided for the study.

12. Blinding & other measures taken to avoid bias

12.1 Blinding

Participants or clinicians involved in this study will not be blinded to the treatment provided.

12.2 Other measures taken to minimise / avoid bias

Since this is a non-randomised study there is an increased potential for bias, however every effort will be made to minimise this. Suitable patients will be identified by the consensus opinion of the multidisciplinary team.

13. Data

13.1 Data to be collected

If confirmed as eligible, the patient will be allocated a unique study number and the treatment regimen defined. Ethnicity data will be collected. All study data will be recorded on case report forms. All research staff who enter data onto the CRF will have signed the study signature and delegation of duties log before undertaking data entry.

A password-protected, computer-based electronic database record of the study will be kept to facilitate statistical analysis. Data collection will be compliant with data protection Act 1998. The study will be registered with the UCL data protection officer. Trial notes and source document may be reviewed by the sponsor as part of internal audit or inspected by the regulatory authorities.

13.2 Adverse Events

Any adverse event or concurrent illness experienced by a patient during any portion of the study will be described in detail, fully evaluated and recorded in the hospital notes and on an adverse event form in the CRF by the investigator, and reported as outlined below.

Adverse event means any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

Adverse reaction means any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

Unexpected adverse reaction means an adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out:

(a) in the case of a product with a marketing authorization, in the summary of product characteristics for that product,
(b) in the case of any other investigational medicinal product, in the investigator's brochure relating to the trial in question.

Events which do not require reporting to the SITU and Regulatory agencies:
The following events do not require reporting to the SITU/Sponsor, but must be recorded in the relevant section(s) of the CRF and medical notes:

- expected adverse events,
- disease related deaths,
- hospitalisation for elective treatment.

Pre-existing medical conditions
Any medical conditions present at baseline, which worsen after exposure to study treatment, must be assessed and recorded as an AE on the adverse event form of the CRF.

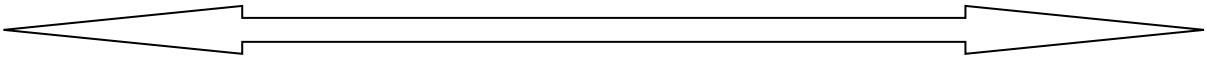
Treatment-emergent adverse event
A treatment–emergent adverse event (TEAE) is defined as any event not present prior to exposure to device being tested in the study or any event that worsens in duration, intensity or frequency following treatment. The adverse event form of the CRF will be completed for all TEAEs as well as the medical notes and AE log.

Part of the adverse event documentation will involve the investigator making an assessment. To promote consistency between investigators, the following elements should be taken into consideration along with good clinical judgment when determining the relationship of study medication to adverse event.

1. Existence of a temporal relationship between the event and the use of device during the study
2. The relationship of the any adverse event and time of treatment, should the study device be withdrawn
3. Reappearance or worsening of the event during retreatment
4. Influence of a pre-existing condition, concomitant disease or medication, or other environmental factors

The number of elements met and good clinical judgement should be used as a guide for determining the device-related event assessment. A binary assessment scale will be used to assess causality.

Not related Definitely Related



Laboratory abnormalities

During the course of the study the investigator will be required to comment on any laboratory values outside the reference range. A laboratory abnormality will be regarded as an AE and recorded on the adverse event form of the CRF and medical notes if according to the investigators judgement the value is significantly worse than at pre-treatment (significantly worse is defined by grade 3 or 4 by the NCI Toxicity criteria – appendix 2).

Serious Adverse Events

Serious adverse events (SAEs) are defined as any event that is fatal; life threatening; causes or prolongs hospitalisation; causes disability or incapacity or requires medical intervention to prevent permanent impairment or damage, any grade 4 toxicity.

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that results in:

- Death
- A life-threatening event
- Inpatient hospitalisation or prolongation of hospitalisation
- Severe or permanent disability
- Cancer (other than cancers diagnosed prior to enrolment in studies involving patients with cancer)
- Congenital anomaly

Recording and Reporting

All AEs and SAEs regardless of causality and expectedness occurring during treatment and up to one month post-treatment will be recorded in the hospital notes and CRF (AE log only for AEs. For SAEs complete both the AE log and the SAE Form)

All serious adverse events will be reported to SITU by fax: +44 (0)20 7679 9290. SAEs must be reported whether or not considered to be treatment related on an SAE form (apart from the expected AEs listed in section 10.2). The Chief or Principal Investigator will complete the sponsor's serious adverse event form and the form will be faxed or email to the sponsor on 020 3108 2312, email sae@ucl.ac.uk , within one working day of the PI becoming aware of the event. The Chief or Principal Investigator will respond to any SAE queries raised by the sponsor as soon as possible.

All SUSARs must be notified to SITU immediately (or at least within one working day of PI being made aware). SITU will notify the main REC of all SUSARs. SUSARs that are fatal or life-threatening must be notified to the REC within 7 days after SITU has learned of them. Other SUSARs must be reported to the REC within 15 days after SITU has learned of them.

Any SAE's occurring after this time will also be reported if thought to be treatment related.

All AEs will be assessed for the following:

- Severity (according to NCI toxicity criteria- appendix 3)
- Causality (see 10.3)
- Expectedness (see below)
- Seriousness (as defined above)

Pregnancy

Any pregnancy or fathering of a child during treatment must be reported by the Investigator SITU. The pregnancy should then be followed-up by the investigator

to determine outcome, including spontaneous or voluntary termination, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. All initial and follow-up must be recorded in the medical notes, CRF, SAE log and the pregnancy form.

If the father has undergone RFA in the last 3 months, informed consent to report information regarding pregnancy outcome must be obtained from the mother.

Adverse Events Reporting Requirements

Regulation 30 of the Medicines for Human Use (Clinical Trials) Regulations 2004 [Statutory Instrument 2004/1031], as amended by Statutory Instrument 2006/1928 states "the Sponsor and the Investigator may take appropriate urgent safety measures in order to protect the subjects of a clinical trial against any immediate hazard to their health or safety. If measures are taken, SITU on behalf of the Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the relevant REC of the measures taken and the circumstances giving rise to those measures."

In order to prevent any delays in the reporting timelines the sponsor has delegated this responsibility to each PI site. Therefore the PI must report any urgent safety measures to the sponsor via SITU.

Period of Observation

For the purpose of this trial the period of serious adverse event (SAE) observation extends from the time of registration onto the trial until 12 months following treatment.

SSARs (Suspected Serious Adverse Reactions)

Suspected Serious Adverse Reactions are adverse reactions (or events) that are thought to be related to the research procedure. For the purpose of this trial the following is a list of potential SSARs:

Related to midazolam

1. Drowsiness and confusion
2. Anterograde amnesia
3. Nausea and/or vomiting
4. Respiratory depression
5. Hypotension
6. Pain at injection site

Related to ciprofloxacin

1. Nausea and/or diarrhoea
2. Rash
3. Headache
4. Rarely Achilles tendon rupture

Related to EUS delivered Radiofrequency ablation

1. Abdominal pain
2. Nausea and vomiting

Related to tissue necrosis

1. fever
2. anorexia, nausea, vomiting
3. abdominal pain
4. pancreatitis
5. bleeding
6. Duodenal perforation
7. Biliary obstruction

Related incomplete resolution of pancreatic cyst

1. cholangitis
2. pancreatitis
3. growth of cyst
4. malignant transformation

SSARs should be reported as described in 14.2

SUSARs (Suspected Unexpected Serious Adverse Reactions)

Suspected Unexpected Serious Adverse Reactions are adverse reactions (or events) that are thought to be related to the research procedure but do not appear on the list of SSARs. SUSARs should be reported as described in 14.2, but certainly within 24 hours.

Deaths

All deaths, with date and cause, must be reported as soon as possible by the PI to SITU for notification to sponsor and the REC committee.

14.3 Data handling and record keeping

The dataset will be used for the sole purpose of the Radiocyst Trial.

Only authorised individuals will have access to personal identifiable data. The Radiocyst Operations Group makes a commitment to maintaining the confidentiality, safety, security and integrity of all confidential and sensitive data, which is held under its guardianship.

Staff in the Radiocyst Operations Group are obliged to fully comply with The Data Protection Act 1998, together with all relevant rules of the sponsor organisation (UCL, London).

All such electronic personal identifiable data is kept in a dedicated database in a secure data vault, separate from anonymised data. The study database is held on a dedicated SITU database server. The SITU network, a subnet of the UCL network, is protected by a SITU firewall and is behind UCL's institutional firewall. Both firewalls are managed by UCL's network group. Access to server and the SITU network is password & access right controlled. Access to identifiable data is controlled by staff roles and passwords.

Data transfer and storage

The data will be sent to the Radiocyst Operations Group via one of three routes: (1) a secure online file transfer system, (2) to a fax machine located in a locked room within a locked building, (3) through the post, (4) Email for non-identifiable data only. Note that the Radiocyst Operations Group can only accept responsibility for the data after it has arrived in their custody.

The data will be retained by the Radiocyst Operations Group until 20 years after the final publication from the trial.

Specific details regarding data storage and destruction are covered in a separate document available from the Radiocyst Operations Group on request.

14. Statistical considerations

All patients included in this study will require long term surveillance of their pancreatic cyst. For RFA to be considered a clinically relevant treatment we would expect 40% of patients to have had complete ablation of their cyst at one year.

Between 2008 and 2011, an average of 76 patients per annum with pancreatic cysts were seen at UCLH and the Royal Free Hospitals who met RADIOCYST recruitment criteria. A study consent rate of 63% (48 of 76) would allow all patients for the study to be recruited from RFH and UCLH within the study period. Most of these patients are currently being followed up with serial imaging in accordance with international guidance, and will be contacted about the study. We also expect that the numbers of new referrals of patients with pancreatic cystic lesions referred to UCLH/RFH will continue to increase. In addition, this will be a UK multicentre study on the national trials portfolio.

14.1 Sample size calculation

The sample size is based on using Simon's two-stage design to assess the ablation rate. An ablation rate of 32% is assumed based on the results of previous research (Gan SI et al. 2005). An ablation rate of 20% would be considered to be the lower level of acceptability. Assuming a minimax design, a 5% significance level and 80% power, it is calculated that 42 patients are required for the first stage of the study. Assuming that at least 20% of the patients in the first stage have successful ablation of their cysts (at least 9/42), then the study will be continued to include 82 patients. To allow for drop outs or the possibility of incomplete data an additional 15% will be recruited resulting in 97 patients in total.

The study will therefore have the following hypothesis:

$H0: p \leq 0.2$, $H1: p \geq 0.32$ where p = proportion of patients with ablation.

In the first stage, complete ablation in 9 or more of the first 42 patients will be a milestone for proceeding to the second stage. At the end of the study if ablation

occurs in 23 or more of the 82 patients, the null hypothesis will be rejected. Complete response will be defined radiologically as absence of pancreatic cyst with or without replacement by a fibrous scar. Persisting or enlarging cysts will be further evaluated with a repeat EUS-FNA and if confirmatory of a cystic tumour, patients will be offered one further EUS-RFA treatment.

14.2 Statistical analysis

Planned Statistical Analysis: An interim analysis of stage 1 data is planned at 12 months following completion of phase 1 recruitment. The main statistical analysis of the primary and secondary endpoints will be performed 12 months following recruitment of the last patient in each stage of the trial. The Trial Statistician has primary responsibility for the statistical aspects of the trial.

15. Compliance and withdrawal

15.1 Subject compliance

Written informed consent will be obtained from all patients according to standard guidelines 'Consent for research on human subjects'. All patients will have the trial explained to them and be provided with an information sheet and given adequate time for questions. When agreed by both parties the patient will sign a consent form and be provided with a copy.

The original copy will be kept in the TMF, a copy will be provided to the patient and a further copy will be placed in the medical notes and the consent process documented. Consent will be obtained by one of the clinical members of the RADIOCYST team.

15.2 Withdrawal / dropout of subjects

Patients who withdraw consent or are lost to follow up will be replaced to maintain the accrual of 94 patients in total.

Withdrawal of Consent

Patients will be consented to the trial voluntarily and may "opt out" of the trial at any time.

16. Interim analysis and data monitoring

16.1 Stopping and discontinuation rules

A patient will discontinue the study under the following circumstances:

- If the physician thinks it would be in the best interests of the patient
- If the patient requests discontinuation
- If unacceptable toxicity is seen, as defined by an underlying grade 4 or more toxicity rate of ≥ 10% (Appendix 3) or there is any treatment-related death, in which case the trial will be stopped.

16.2 Monitoring, quality control and assurance

Throughout the period of the trial there will be continuous monitoring to ensure quality control and assurance.

Quality Assurance

The procedures will be performed in tertiary Hepatobiliary centres who perform in excess of 150 EUS procedures per year. The RFA catheter that has been designed to ablate cystic tumours of the pancreas and has U.S Food and Drug Administration and EU European Conformity approval for such an indication.

Parameters to be collected for QA Audit

For each trial treatment delivered the following will be collected:

- Length of procedure.
- Number of sequential applications.
- Impedance.
- Number of patients requiring a repeat procedure at 3 months.
- Malfunction of catheter or equipment.

16.3 Assessment of safety

Data on safety will be gathered by the following methods:

- From Serious Adverse Event forms.
- Clinical notes (primary and secondary care).
- Endoscopy reports.

Procedures for dealing with the above are contained in relevant Standard Operating Procedures.

At the end of Phase 1, if a 20% (9/42) ablation rate has been achieved and the procedure is found to be safe and associated with an acceptable complication rate (assessed by IDMC) we will proceed to phase 2 of the study. In Phase 2, hospital admission and day 2 CT will only be undertaken if clinically indicated.

17. Ethical considerations

Risks and benefits

The original trial protocol and supporting documents have been reviewed by the UCL/UCLH Hospitals NHS Trust Joint Ethics Committee in Summer 2013.

Informing potential study participants of possible benefits and known risks

Suitable patients will be identified at multi-disciplinary team meetings and approached by a member of the clinical team who will provide verbal and written information about the study including the possible benefits and known risks. If patients agree to participate in the study, written consent will be obtained on the day of the procedure.

Research Governance

The sponsor for this clinical study is UCL. The overall research governance of the study is determined largely by the sponsor, including Standard Operating Procedures and Data Protection.
Each centre takes responsibility for the collection and management of its own data.

The study is being conducted according to the recommendation of the Declaration of Helsinki), ICH Good Clinical Practice guidelines, the Medicines for Human Use (Clinical Trials) Regulations 2004 and the Research Governance Framework for Health and Social Care April 2005 as amended from time to time.

Data Monitoring Committee (DMC)

An independent DMC will be appointed. All Serious Adverse Events (SAEs) will be reported to the DMC and Sponsor who will decide whether the complication rate is in excess of previous reports and would justify suspension of the study to allow full investigation. If unacceptable toxicity is seen, as defined by an underlying grade 4 or more toxicity rate of ≥ 10%, or there is any treatment-related death, the study will be suspended whilst full investigation proceeds.

The study will be monitored according to a monitoring plan agreed by the Trial Management Group. Risk adapted strategies will be used to determine the level of monitoring required.

It is the PI's responsibility to ensure that any findings identified in the site monitor's monitoring report are actioned in a timely manner and any violations of GCP or the protocol reported to the sponsor as soon as possible.

Monitoring will include, but is not limited to, source document verification of eligibility, consent and procedures as per protocol. Copies of all monitoring visit reports will be made available to the sponsor.

18. Financing and Insurance

The sponsor of this study does not indemnify sites for negligent and non-negligent harm, as this is the responsibility of individual participating institutions.

The Principal Investigator will secure funding for the supply of RF probes. The costs of usual treatment (e.g. initial and surveillance CT, EUS, routine clinic reviews) will be met by the hospital. Additional unexpected costs will be supported by the principal investigator.

University College London holds insurance to cover participants for injury caused by their participation in the clinical study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical study is being carried out in a hospital, the hospital continues to have a duty of care to the participant. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or not. This does not affect the participant's right to seek compensation via the non-negligence route.

Participants may also be able to claim compensation for injury caused by participation in this clinical study without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical study shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

19. Reporting and dissemination

Results from this study will be submitted to a peer review journal following completion of the study. A publication policy will be written and agreed by all investigators prior to submission.

In line with the regulations, at the end of the study data will be securely archived for a minimum of 20 years. Arrangements for confidential destruction will then be made.

Intellectual property related to the RF catheter belong to Imperial College London.

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APPENDIX 1 - ECOG PERFORMANCE STATUS

ECOG	0	Fully active, carries on all pre-disease performance without restriction
	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
	2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
	3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
	4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
	5	Dead

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

http://www.ecog.org/general/perf_stat.html

APPENDIX 2 - NCI Common Toxicity Criteria (Version 2.0)

Toxicity	0	1	2	3	4
WBC	≥4.0	3.0 - 3.9	2.0 - 2.9	1.0 - 1.9	≤1.0
Platelets	WNL	75.0 –normal	50.0 - 74.9	25.0 – 49.9	<25.0
Haemoglobin	WNL	1 0.0 – normal	8.0 - 10.0	6.5 - 7.9	<6.5
Granulocytes	≥2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	<0.5
Lymphocytes	≥2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	<0.5
Haemorrhage (clinical)	None	Mild, no transfusion	Gross, 1-2 units transfusion per episode	Gross, 3-4 units transfusion per episode	Massive,>4 units transfusion per episode
Infection	None	Mild	Moderate	Severe	Life-threatening
Nausea	None	Able to eat reasonable intake	Intake significantly decreased intake but can eat	No significant intake	----
Vomiting	None	1 episode in 24 hours	2-5 episodes in 24 hours	6-10 episodes in 24 hours	> 1 0 episodes in 24 hrs or requiring parenteral support
Diarrhoea	None	Increase of 2-3 stools/day over pre-treatment	Increase of 4-6 stools/day, or nocturnal stools or moderate cramping	Increase of 7-9 stools/day, or incontinence or severe cramping	Increase of ≥1 0 stools/day, grossly bloody diarrhoea, or need for parenteral support
Stomatitis	None	Painless ulcers, erythema, or mild soreness	Painful erythema, oedema, or ulcers, but can eat	Painful erythema, oedema, or ulcers and cannot eat	Requires parenteral or enteral support
Bilirubin	WNL	-------	<1.5 x N	1.5 - 3.0 x N	>3.0 x N
Transaminase (SGOT, SGPT)	WNL	≤2.5 x N	2.6 - 5.0 x N	5. 1 - 20.0 x N	>20.0 x N
Alkaline Phosphatase or 5' nucleotides	WNL	≤2.5 x N	2.6 - 5.0 x N	5.1 -20.0 x N	>20.0 x N
Liver (clinical)	No change from baseline	-----	-----	Pre-coma	Hepatic coma
Creatinine	WNL	<1.5 x N	1.5 - 3.0 x N	3.1 - 6.0 x N	>6.0 x N
Proteinuria	No change	1+ or <0.3g% or <3g/1	2 – 3+ or 0.3 - 1.0g% or 3 - 10 g/1	4+ or >1.0g% or >10 g/l	Nephrotic Syndrome
Haematuria	Neg.	Micro only	Gross, no clots	Gross + clots	Requires transfusion

Toxicity	0	1	2	3	4
Alopecia	No loss	Mild hair loss	Pronounced or total hair loss	-----	-----
Pulmonary	None or no change	Asymptomatic, with abnormality in PFTs	Dyspnoea on significant exertion	Dyspnoea at normal level of activity	Dyspnoea at rest

Cardiac dysrhythmias	None	Asymptomatic, transient, requiring no therapy	Recurrent or persistent, no therapy required	Requires treatment	Requires monitoring; or hypotension, or ventricular tachycardia or fibrillation
Cardiac function	None	Asymptomatic, decline of resting ejection fraction by less than 20% of baseline value	Asymptomatic, decline of resting ejection fraction by > 20% of baseline value	Mild CHF, responsive to therapy	Severe or refractory CHF
Cardiac-ischaemia	None	Non-specific T-wave flattening	Asymptomatic, ST and T wave changes suggesting ischaemia	Angina without evidence for infarction	Acute myocardial infarction
Cardiac-pericardial	None	Asymptomatic, effusion, no intervention required	Pericarditis (rub, chest pain, ECG changes)	Symptomatic effusion; drainage required	Tamponade; drainage urgently required
Hypertension	None or no change	Asymptomatic transient increase by greater than 20 mm Hg (D) or to> 1 5011 00 if previously "L. No treatment required	Recurrent or persistent increase by greater than 20 mm Hg (D) or to>1501100 if previously "L. No treatment required	Requires therapy	Hypertensive crisis
Hypotension	None or no change	Changes requiring no therapy (including transient orthostatic – hypotension)	Requires fluid replacement or other therapy but not hospitalisation	Requires therapy and hospitalisation; resolves within 48 hrs of stopping the agent	Requires therapy and hospitalisation for >48 hrs after stopping the agent
Neuro-sensory	None or no change	Mild paraesthesias loss of deep tendon reflexes	mild or moderate objective sensory loss; moderate paraesthesias	Severe objective sensory loss or paraesthesias that interfere with function	-----

Toxicity	**0**	**1**	**2**	**3**	**4**
Neuro-motor	None or no change	Subjective weakness; no objective findings	mild objective weakness without significant impairment of function	Objective weakness with impairment of function	Paralysis

Neuro-cortical	None	Mild somnolence or agitation	Moderate somnolence or agitation	Severe somnolence, agitation, confusion, disorientation, or hallucinations	coma, seizures, toxic psychosis
Neuro-cerebellar	None	Slight incoordination Dysdiadocho-kinesis	Intention tremor, dysmetria, slurred speech, nystagmus	Locomotor ataxia	Cerebellar necrosis
Neuro-mood	No change	Mild anxiety or depression	Moderate anxiety or depression	Severe anxiety or depression	Suicidal ideation
Neuro-headache	None	Mild	Moderate or severe but transient	Unrelenting and severe	-----
Neuro-constipation	None or no change	Mild	Moderate	Severe	ileus >96 hrs
Neuro-hearing	None or no change	Asymptomatic, hearing loss on audiometry only	Tinnitus	Hearing loss interfering with function but correctable with hearing aid	Deafness not correctable
Neuro-vision	None or no change	-----	-----	Symptomatic sub-total loss of vision	Blindness
Skin	None or nochange	Scattered macular or papular eruption or erythema that is asymptomatic	Scattered macular or papular eruption or erythema with pruritus or other associated eruption symptoms	Generalised symptomatic macular, papular, or vesicular	Exfoliative dermatitis or ulcerating dermatitis
Allergy	None	Transient rash, drug fever <38'C 100.4 F	Urticaria, drug fever ≥38'C 100.4 F mild	Scrum sickness, broncho-spasm requiring parenteral medications	Anaphylaxis
Fever in absence of infection	None	37.1 - 38.0 ^{o}C 98.7 - 100.4 ^{o}F	38.1 - 40.0 ^{o}C 100.5 -104.0 ^{o}F	>40.0 ^{o}C >104.0 ^{o}F for less than 24 hours	>40.0 ^{o}C (104.0 ^{o}F) for more than 24 hrs or fever accompanied by hypotension

Toxicity	**0**	**1**	**2**	**3**	**4**
Local	None	Pain	pain and swelling, with inflammation or phlebitis	Ulceration	Plastic surgery indicated
Weight gain/loss	<5.0%	5.0 - 9.9%	10.0 - 19.9%	>20.0%	-----
Hyperglycaemia	<116	116 – 160	161 – 250	251 – 500	>500 or ketoacidosis
Hypoglycaemia	>64	55 – 64	40 – 54	30 – 39	<30
Amylase	WNL	<1.5 x N	1.5 – 2.0 x 1N	2.1 - 5.0 x N	>5. 1 x N
Hypercalcaemia	<10.6	10.6 - 11.5	11.6 - 12.5	12.6 - 13.5	≥13.5
Hypocalcaemia	>8.4	8.4 - 7.8	7.7 - 7.0	6.9 - 6.1	≤6.0

Hypomagnesaemia	>1.4	1.4 - 1.2	1.1 - 0.9	0.8 - 0.6	≤0.5
Fibrino1	WNL	0.99 - 0.75 x xN	0.74 - 0.50 x N	0.49 - 0.25 x N	≤0.24 x N
Prothrombin time	WNL	1.01 - 1.25 x N	1.26 - 1.50 x N	1.51 - 2.00 x N	>2.00 x N
Partial thromboplastin time	WNL	1.01 1- 1.66 x N	1.67 - 2.33 x N	2.34 - 3.00 x N	>3.00 x N
* Musculo-skeletal	None	Aches and pains, no restriction of activity	pain causing restriction of activity	Pain and presence of nodules or clinically inflamed joints or tendons	pain and presence of contracture

APPENDIX 3 - Summary of Investigations and assessments – Radiofrequency ablation

Stage 1:

(X) only applies to Patient offered further EUS-RFA if incomplete cyst ablation

Investigation	Pre-treatment/Baseline		Treatment		Follow-up & response assessment		
			Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
	Month -3 to day 0	Days -14 -0	Day 1	Day 2	3 months	6 * months	12 Months
Confirmation of diagnosis at multidisciplinary review	X						
Informed Consent		X					
Clinical assessment, FBC, U&E, LFTs		X		X	X	X	X
CEA & CA19.9		X			X	X	X
Serum amylase, glucose		X		X			
Prothrombin time / INR		X					
Pancreatic/abdominal CT (optional)	X			X			
Abdominal MRI/MRCP (as per local practice)	X				X		X
Height		X					
Weight		X			X	X	X
Negative pregnancy test (if applicable)		X (-7to0)					
EUS-FNA (if sufficient send for cytology and biochemical analysis) and EUS-RFA of cystic lesion.			X		(X)		
ECOG performance status		X	X	X	X	X	X
AE monitoring		X	X	X	X	X	X
Symptom, QOL, EQ5D questionairres & cost diaries (health economics)		X			X	X	X (no further cost diaries will be issued, but EQ5D will be completed)

* Only patients who undergo a second EUS-RFA after their Month 3 assessment will also undergo a Month 6 review. All patients will be followed up at 12 months.

Stage 2:

In Stage 2 of the study if the EUS-RFA is found to be safe procedure and associated with a low complication rate, patients will continue to be treated as a day case. They will therefore no longer require routine hospital admission after their EUS-FNA or day 2 clinical assessment, blood tests, AE monitoring, or CT, unless felt to be clinically indicated by the investigator. The rest of their treatment and follow up will be as outlined above for Stage 1.

APPENDIX 4 - Habib™ EUS RFA Step by Step Procedure Guide

EMcision™
ENHANCING QUALITY OF LIFE

Habib™ EUS RFA Procedures: For ablation of pancreatic, liver and lymph node malignancies

1. Patient should be prepared for the endoscopic ultrasound scan (EUS) biopsy as per standard hospital protocol.
2. This is a monopolar device and a patient electrode/grounding pad must be placed on the patient.
3. Introduce echoendoscope to the stomach or duodenum and identify the target tumour with EUS.
4. Under EUS control, introduce the EUS biopsy needle (incorporating a stylet) into the target tumour. A 19G biopsy needle should be used if the echoendoscope is deployed in the stomach. A 22G biopsy needle is more appropriate when the scope is deployed through the duodenum.
5. If the tumour is a cyst aspirate the fluid to empty the cyst.
6. Position the tip of the biopsy needle over the deepest part of the tumour.
7. Remove the biopsy needle stylet.
8. Replace with the Habib™ EUS RFA device and push to the end of the biopsy needle until it cannot be pushed any further (NB: the Habib™ EUS RFA is not seen clearly on EUS).
9. Whilst maintaining the position of the Habib™ EUS RFA withdraw the biopsy needle by 3 cm in order to **DISENGAGE CONTACT** with the active part of the Habib™ EUS RFA needle from the biopsy needle. This is a monopolar needle and contact with the metal biopsy needle is not recommended.
10. Connect the Habib™ EUS RFA device to the RF generator and set parameters.

11. Apply RF energy for 90 seconds/2 minutes (depending on generator) at **10 Watts**.
12. Wait 1 minute before repositioning the Habib™ EUS RFA needle and repeat procedure as many times as needed to ensure complete ablation of the tumour (see steps 6 - 12).
13. At the end of the procedure remove all equipment.
14. The patient should be recovered as per standard hospital practice for EUS procedures.

Notes: It is advisable to cover the patient with IV antibiotics for 48 hours according to local practice.

As with other RF procedures the patient can become restless when the RF energy is applied – a top up of sedation maybe required. Ablation zone is about 2.5cm by 0.5cm.

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