Can we Improve the Detection of Pancreatic Exocrine Insufficiency in Secondary Care?

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ABSTRACT

Pancreatic exocrine insufficiency (PEI) is defined as a reduction in the pancreatic enzymes available in the small bowel for digestion to a level that results in malabsorption and maldigestion. PEI can lead to fat malabsorption, micronutrient deficiencies, weight loss and malnutrition-related complications such as osteoporosis and loss of skeletal muscle. Fat malabsorption in severe PEI can cause steatorrhoea but milder symptoms include diarrhoea, abdominal pain, and abdominal bloating termed 'maldigestion' are non-specific and may not trigger testing for PEI causing the diagnosis to be overlooked. Early detection and treatment of PEI can improve patient's symptoms, quality of life and reduce complications. The two major causes of PEI are chronic pancreatitis (CP) and pancreatic cancer, but other diseases have been reported to be associated with PEI termed 'at-risk' conditions and include diabetes mellitus, people living with human immunodeficiency virus, and patients admitted to hospital with high alcohol intake, however, there is a lack of guidance about testing for PEI in 'at-risk' conditions. Gold standard tests to directly measure exocrine secretion to diagnose PEI are invasive, and not widely available. A stool sample to test for faecal elastase-1 (FEL-1) is currently the most common test used in practice to diagnose PEI. FEL-1 is inexpensive, easy to handle by laboratory staff and widely available, however, it has been shown to lack sensitivity especially in mild PEI.

The null hypothesis set out is: pancreatic exocrine insufficiency is adequately recognised in 'at-risk' groups and other methods to test for the consequences of PEI are unhelpful. This body of work aims to quantify the prevalence of PEI in at-risk group and identify positive and negative predictive parameters in order to aid diagnosis, investigation and treatment; this will be done by performing studies to answer the following four questions:

Study 1: What is the current practice and yield of testing for pancreatic exocrine insufficiency in 'at-risk' patients?

Study 2: Is there a role for micronutrient deficiencies in diagnosing pancreatic exocrine insufficiency?

Study 3: Can a skeletal muscle index measurement be used as diagnostic tool for the detection of pancreatic exocrine insufficiency?

Study 4: Can a skeletal muscle index measurement in patients with pancreatic cancer at high risk of pancreatic exocrine insufficiency predict prognosis and aid treatment plan?

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LIST OF ABBREVIATIONS

^{13}C-MTG Mixed 13 C-triglyceride breath test

BIA Bioimpedance analysis

BMI Body mass index

BSC Best supportive care

BSG British society of Gastroenterology

CCK Cholecystokinin

CP Chronic pancreatitis

CT Computed tomography

DM Diabetes mellitus

ECOG-PS Eastern Cooperative Oncology Group performance status

ELISA enzyme linked immunosorbent assay

ESPEN European Society of for Parenteral and Enteral Nutrition

ERCP Endoscopic retrograde cholangiopancreatography

EUS Endoscopic ultrasound

EUS-B Endoscopic ultrasound guided biopsy

HAI high alcohol intake

FEL-1 faecal elastase

HIDA hepatobiliary iminodiacetic acid

HU Hounsfield units

IBD inflammatory bowel diseases

IBS irritable bowel syndrome

IBS-D diarrhoea predominant irritable bowel syndrome

HAART Highly Active Antiretroviral Therapy

LAPC Locally advanced pancreatic cancer

MetS Metabolic syndrome

MRI Magnetic Resonance Imaging

NPV Negative predictive value

MUST Malnutrition universal screening tool

NAFP Non-alcoholic fatty pancreas

NAFPD Non-alcoholic fatty pancreas disease

PDAC Pancreatic ductal adenocarcinoma

PSGBI Pancreatic Society of Great Britain and Ireland

PEI Pancreatic exocrine insufficiency

PEI-MD PEI associated micronutrient deficiencies

PERT Pancreatic enzyme replacement therapy

PFT Pancreatic function test`

PLHIV People living with human immunodeficiency virus

PPV Positive predictive value

RBP Retinol-binding protein

SATI Subcutaneous adipose tissue index

sMRCP Secretin enhanced-magnetic resonance cholangiopancreatography

SMI Skeletal muscle index

VATI Visceral adipose tissue index

VFA visceral fat area

CHAPTER 1: OVERVIEW OF PANCREATIC EXOCRINE INSUFFICIENCY

1.0 Introduction

The pancreas is a vital organ for maintaining physiological digestion and metabolism. Pancreatic exocrine insufficiency (PEI) is defined as a reduction in the pancreatic enzymes available for digestion to a level that results in malabsorption and maldigestion [1-3]. Diseases causing irreversible pancreatic parenchymal or ductal changes can cause PEI including chronic pancreatitis (CP), cystic fibrosis, pancreatic cancer, acute necrotising pancreatitis, and those undergone pancreatic surgery [1, 4-6]. These conditions will be given the label 'primary causes' of PEI in this thesis. However, there is increasing evidence that PEI can be caused or associated by conditions other than 'primary causes'. Any causes other than 'primary causes' will be labelled 'at-risk' conditions in this thesis. 'At-risk' conditions reported in the literature including high alcohol intake (HAI), diabetes mellitus (DM), people living with human immunodeficiency virus (PLHIV), inflammatory bowel diseases (IBD), coeliac disease, or those resulting in postprandial asynchrony, for example, post gastric surgery [4]. Despite the reported association between 'at-risk' conditions and PEI, there is a paucity of guidance about testing for PEI in these groups.

Early diagnosis of PEI can be challenging because symptoms are non-specific, and available tests can lack sensitivity. The pathogenesis, associated diseases, symptoms, tests, and treatment of PEI are discussed in this chapter.

1.1 Anatomy and physiology of the pancreas

The function of the pancreas remained a mystery to scientists for centuries until Johann Georg Wirsung described the main pancreatic duct for the first time in 1642. The first description of pancreatic endocrine function was mentioned in the literature in 1889 in dogs experiencing

hypoglycaemia post-pancreatectomy, however, the role of insulin was not fully understood until 1922 [7].

The pancreas lies in the upper abdomen and posterior to the stomach. It is largely retroperitoneal, divided into five parts: head, uncinate process, neck, body and tail, figure 1.1. The head lies close to the duodenum and the tail extend toward the spleen. The main pancreatic duct (Wirsung's duct) runs across the pancreas to meet the common bile duct before opening into the duodenal wall via the major papilla. Some humans have another duct called the accessory (Santorini's) duct that opens into the duodenal wall via the minor papilla- separate from major papilla. The arterial supply of the pancreas is through the coeliac trunk and the superior mesenteric artery, and both arise from abdominal aorta.

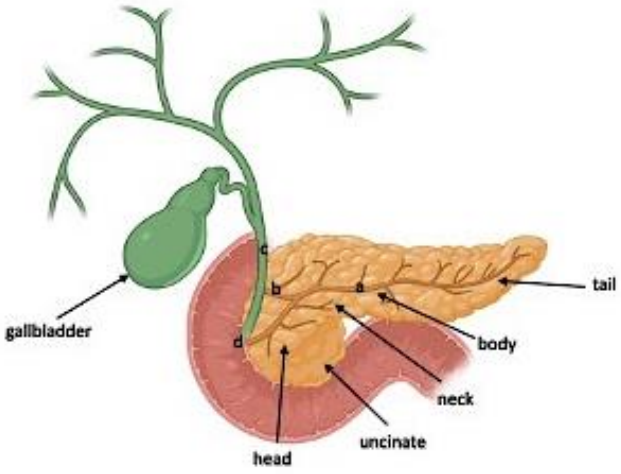

Figure 1.1 Schematic representation of the pancreas showing (a) main pancreatic duct, (b) accessory pancreatic duct, (c) common bile duct traverses the head of pancreas and joins the main pancreatic duct before opening in the duodenal wall via major papilla (d).
Created using BioRender.com

The histology of the exocrine pancreas composed of a complex system of acinar and duct cells. An acinus is a cluster of acinar cells that store pancreatic enzymes, figure 1.2. The ductal system is the main pancreatic duct and its branches called interlobular duct. The Acinar cells secrete pancreatic enzymes while the duct epithelial cells secrete bicarbonate rich fluid [8].

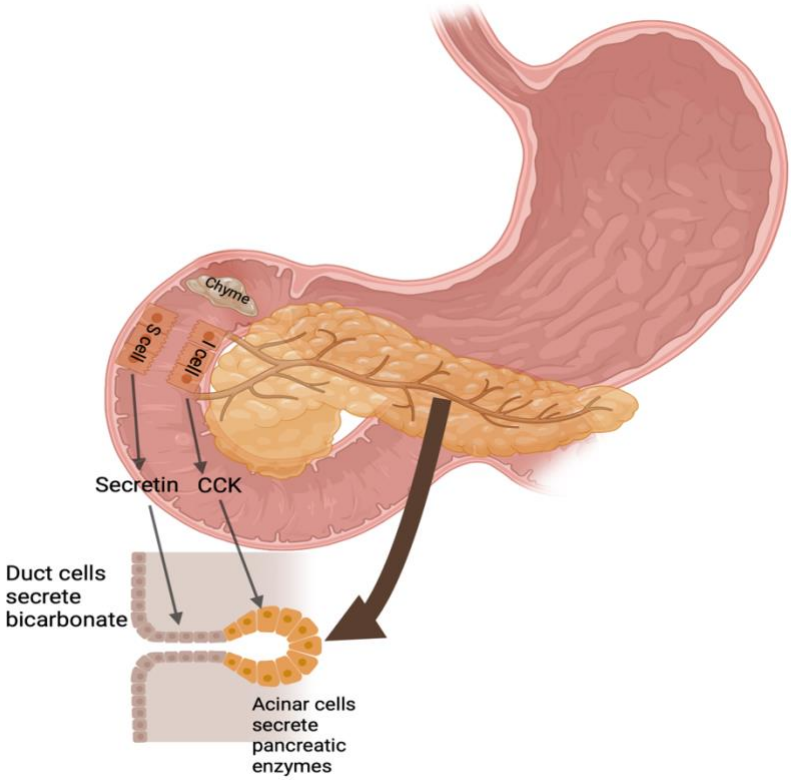

Figure 1.2 Intestinal phase of pancreatic exocrine function. **CCK**, cholecystokinin. Created using BioRender.com

Pancreatic secretion is stimulated via three phases: cephalic, gastric, and intestinal phases. Cephalic and gastric phases result in indirect stimulation of pancreatic secretion through the autonomic and central nervous system. Most of the pancreatic stimulation occurs via the intestinal phase when chyme enters the duodenum. During the intestinal phase, duodenal S-cells produce secretin which in turn stimulates pancreatic duct cells to produce bicarbonate in the pancreatic duct, figure 1.2. Bicarbonate protects duodenal mucosa by neutralising gastric acid and it also optimises duodenal PH for activation of pancreatic enzymes. Cholecystokinin (CCK) released by duodenal-I cells stimulates the acinar cells to produce pancreatic enzymes [1, 9]. The acinar cells produce several enzymes that play important roles in the digestion process, these enzymes are summarised in table 1.1.

Table 1.1 List of enzymes produced by pancreatic acinar cells

Enzyme type	Examples of individual enzymes
Proteases	Chymotrypsinogen, trypsinogen, and elastase
Lipase	Pancreatic triglyceride lipase
Amylase	Pancreatic amylase

Pancreatic enzymes secreted in the duodenum are in an inactive form and require an alkaline pH to be activated, therefore, conditions that cause an increase in gastric acid secretion may result in the inactivation of pancreatic enzymes and cause PEI.

Reduction in pancreatic enzyme secretion can result in malabsorption of nutrients including protein, carbohydrate, and fat. The most affected enzyme in the digestive tract is lipase because it is nearly all produced by the pancreas [10], also the reduction in bicarbonate secretion by the duct epithelial cells can result in failure to buffer the acidic gastric fluid and fail to activate lipase and other pancreatic enzymes [1].

Primary causes of PEI are conditions that cause parenchymal and/or ductal changes leading to exocrine dysfunction and reduction in the amount of pancreatic enzyme available for digestion in the small intestine. The most common 'primary causes' of PEI are CP, cystic fibrosis, pancreatic cancer, acute necrotising pancreatitis, and patients who undergone pancreatic surgery. In addition to primary causes, there are reports of PEI caused by, or associated with other conditions, including: high alcohol intake (HAI), diabetes mellitus (DM), coeliac disease, inflammatory bowel diseases (IBD), and human immunodeficiency virus (HIV) [4, 11]. These 'at-risk' conditions can cause PEI despite a normal appearing pancreas, figure 1.3. Low CCK release as a result of duodenal pathology can cause PEI including Crohn's disease and coeliac

disease. Zollinger-Ellison syndrome, characterized by hypersecretion of gastric acid secondary to the ectopic secretion of gastrin by a gastrinoma, is another condition associated with PEI due to excessive gastric acid secretion and inactivation of pancreatic enzymes [12].

Furthermore, PEI has been reported in patients without morphological changes of CP and without the presence of primary or 'at-risk' conditions. One example of this is a study that found a PEI prevalence of 35.5% in patients with pancreatic steatosis on MRI without any radiological features of CP [13].

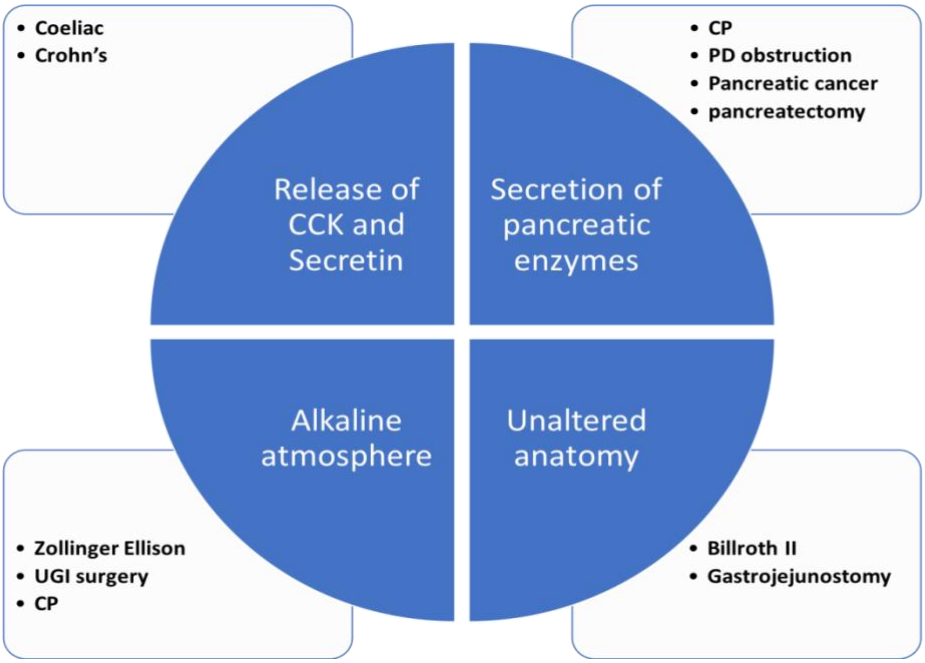

Figure 1.3 Causes of pancreatic exocrine insufficiency. All four components (quadrants) are required for release of pancreatic enzymes and digestion of food. Disruption of this process at any step can cause PEI. Free texts within the rectangles summarise the common pathologies that affect each stage although some overlap can occur. **CCK**, Cholecystokinin; **CP**, chronic pancreatitis; **PD**, pancreatic duct; **UGI**, upper gastrointestinal.

1.2 Prevalence of pancreatic exocrine insufficiency

1.2.1 Prevalence of pancreatic exocrine insufficiency in primary conditions

The reported PEI prevalence in CP is estimated to be 29-64%, table 1.2[14-17]. The incidence of CP in the United Kingdom is estimated to be 8.6 per 100,000 population per year [18]. The global prevalence of CP is estimated between 13.5 and 52.4 per 100,000 population [15, 19-21]. In a multicentre French survey, the prevalence of CP was re-calculated based on the annual incidence rate of 7.8 per 100,000 population (similar to UK) and an average life expectancy of 20 years (for CP) resulting in a new estimated annual prevalence of 120-143 per 100,000 population [15]. Furthermore, high prevalence of morphological changes in historical post-mortem series reporting a significantly higher prevalence of 2.1 to 13% support the hypothesis that CP and PEI are underdiagnosed conditions [22, 23]. Recently, post-mortem CT scan evaluation was carried out in our centre evaluating the pancreas, found that 28.1% showed features consistent with CP including 13.5% with calcification [24].

The British Society of Gastroenterology (BSG) guidelines recommend testing patients presenting with steatorrhoea [25], however if PEI is discovered only when features of advanced malabsorption and pancreatic disease are present then cases of subclinical PEI and early CP can be missed [26]. Therefore, early detection of PEI and CP – as a precursor of PEI, are important to prevent the consequences of PEI including malabsorption and malnutrition.

Screening for PEI in cystic fibrosis is well established due to the known high risk in up to 85% of patients [27]. PEI can occur in pancreatic cancer due to parenchymal destruction and ductal obstruction with estimated prevalence of 66-92% [4, 28]. Acute pancreatitis is another risk factor for developing PEI with prevalence of 30-33%, but it is reported to be higher in acute necrotising pancreatitis 46.2%-58.5% [29, 30], with a further long term follow-up study (mean

43 months) in post-acute pancreatitis showed PEI prevalence of 35% [31]. Steatorrhoea was observed in 82% patients following a Whipple's procedure [32], although the pancreatic head is removed in this operation, PEI is worsened by the anatomy post-surgery which can result in asynchrony between pancreatic enzyme and chyme [33].

1.2.2 Prevalence of pancreatic exocrine insufficiency in 'at-risk' conditions

There is increasing evidence that PEI can be associated with other conditions, table 1.2. HAI is an established risk factor for benign pancreatic diseases. Pancreatic exocrine insufficiency can affect up to 55-72% of patients with history of HAI and alcohol related liver disease [34, 35]. PEI has been shown to develop an average 12 years after the diagnosis of chronic alcohol dependence [36].

The reported prevalence in the literature in DM 1 is 26-74% whilst it is slightly lower in DM2 12-36% [37-41]. Autopsy studies have shown evidence of chronic inflammatory changes and pancreatic atrophy in 11-19% patients with DM [38]. Some of the reported risk factors of developing PEI in DM included patients with poor glycaemic control, high insulin requirement and long disease duration [42]. Morphological changes of pancreas have been reported in patients with DM based on both histological and imaging tests [43, 44].

The reported prevalence of PEI in PLHIV is estimated to be 32-45% [11, 45]. PEI is suggested to be one of the causes of non-infective diarrhoea in PLHIV [45]. Chronic diarrhoea in PLHIV is common and it is associated with decreased quality of life, therefore, testing for PEI should be considered. The mechanism of PEI in PLHIV is still not confirmed yet. Price et al [46] found association with didanosine however, their study was small (n=8) and this was not confirmed in a larger study (n=104) [45]. A post-mortem study of relatively young HIV patients found that up to 90% of cases had pancreatic morphological changes [47]. This study

suggests parenchymal destruction as a cause for PEI, however, such high prevalence may not be valid nowadays due to the improvement in survival since the introduction of Highly Active Antiretroviral Therapy (HARRT).

It is recommended to look for non-inflammatory causes of diarrhoea in IBD patients including PEI. PEI has been shown to be present in 14-30% of IBD [48, 49]. Coeliac diseases can cause PEI by causing a reduction of CCK release [50]. The risk of PEI in coeliac disease is higher amongst those with unresolved chronic diarrhoea in up to 30% [51].

PEI has also been considered as part of an aging process with a low FEL-1 reported in 21.7 % of patients >60 years old without gastrointestinal symptoms [52, 53], similar finding in another study of less elderly patients using FEL-1 showed high prevalence of low FEL-1 in patients > 50 years old [52, 53].

PEI can develop after upper GI surgery with steatorrhoea being observed in 67% of patients who underwent a gastrectomy [54]. Similar findings were observed in patients with Billroth II (gastrojejunostomy) due to inadequate stimulation of CCK and secretin in addition to poorly synchronised pancreatic enzymes secretions causing the development of PEI [55]. Furthermore, PEI has been observed post bariatric surgery including post distal and proximal Roux-en-Y gastric bypass where 31% of patients were found to have PEI after a mean follow up of 12 months [56].

Table 1.2: Prevalence of pancreatic exocrine insufficiency in primary and 'at risk' conditions

Group	Rate of PEI
Pancreatic cancer	66-92% [4, 28]
Cystic fibrosis	85% [27]
Chronic pancreatitis	29-64% [14-17]
Alcohol excess and alcohol related liver disease.	55-72% [34, 35]
Diabetes mellitus type 1	26-74% [37-41]
Diabetes mellitus type 2	12-36% [37-41]
Inflammatory bowel disease	14-30% [48, 49]
~~**Diarrhoea predominant irritable bowel syndrome**~~	~~6.1% [57]~~
Coeliac disease with chronic diarrhoea	30% [58]
People living with Human Immunodeficiency Virus	32-45% [11, 45]
Acute pancreatitis	30-35% [29-31]
>60 yrs old without GI diseases	11.5-21.7% [52, 53]

PEI: pancreatic exocrine insufficiency

1.3 Consequences of pancreatic exocrine insufficiency

1.3.1 Gastrointestinal symptoms

Insufficient pancreatic enzymes can lead to maldigestion and malabsorption. Maldigestion is defined by symptoms from the inability to effectively break down food in the intestine, whilst malabsorption is caused by inability of food absorption by intestinal mucosa [59]. Maldigestion of carbohydrate can lead to the development of bloating, abdominal pain and excessive flatulence, whilst fat and protein maldigestion can cause diarrhoea [60]. Significant fat maldigestion and malabsorption can cause steatorrhoea which occurs when the reduction in lipase secretion falls below 10% of normal. The majority of lipase in the body is produced by the pancreas compared to amylase which is also produced by salivary glands in significant

amounts. The majority of lipase undergoes destruction in the gut with less than 5% of lipase reaching the ileum compared to 20% trypsin and 35% amylase [61].

The majority of symptoms of patients with PEI are non-specific causing a delay in diagnosis or missing it: abdominal pain, diarrhoea, bloating, and weight loss can also be caused by a number of gastrointestinal disorders including IBS, peptic ulcer disease, or functional dyspepsia [62]. Steatorrhoea is more specific and can present in 23-70% in both PEI and CP, 44% in pancreatic cancer, and 15% in cystic fibrosis [63]. Malnutrition can eventually develop in PEI even without the presence of steatorrhoea [63], therefore, if PEI is not suspected, then patients with non-specific symptoms may not undergo pancreatic function tests or cross-section imaging of the pancreas.

1.3.2 Weight loss and malnutrition

Patients with PEI are at risk of malnutrition and weight loss because of protein and fat malabsorption [64, 65]. Several markers have been shown to be deficient in PEI including fat soluble vitamins (A,D,E and K), magnesium, haemoglobin, albumin, prealbumin and retinol binding protein [66] these are discussed further in chapter 3.

1.3.3 Osteoporosis

Decreased bone mineral density has been reported in patients with PEI [67].There is no data available in literature that support direct association between PEI and osteoporosis. However, osteoporosis and increased risk of fracture have been reported in CP. A study of 26 patients with CP and PEI found 69% of patients had osteopenia measured by DEXA scan compared to 56% in CP without PEI [65]. Another small study of 14 patients with CP and PEI found that 71% had osteopenia and 21% had osteoporosis [68]. The National Institute for Health and Care Excellence recommend regular surveillance with DEXA scan every two years [69].

1.3.3 Pancreatic exocrine insufficiency in pancreatic cancer

Patients with pancreatic ductal adenocarcinoma (PDAC) commonly present with weight loss (70%) and cachexia (40%) and both are associated with reduced survival rate[70]. PEI, diagnosed with FEL-1, has been found in 92% of patient with PDAC when the tumour is in the head of the pancreas [28]. A low FEL-1 (<20µg/g) in PDAC has been shown to be associated with a lower survival (7 months) compared to a FEL-1 >20µg/g (11 months) [71]. Retrospective studies showed improvement in survival [72, 73], however a recent metanalysis of randomised controlled trials showed no improvement in survival [74].

1.4 Diagnosis of pancreatic exocrine insufficiency

1.4.1 Direct test:
Quantification of stimulated pancreatic secretion of enzymes and bicarbonate

Direct pancreatic function tests (PFT) are considered the gold standard test for PEI. In this test, a large bore tube is placed in the duodenum to aspirate the pancreatic juice, at the same time, a dose of secretin is given to patient to the stimulate pancreatic secretion. The agreed cut off level of bicarbonate for PEI is below 80 mEq. Tolerability is one of the limitations of this test as the large bore tube may need to stay for up to 60 minutes to obtain the samples. In addition to its high cost and the challenges of tolerability, it is not widely available and used mainly in research setting [1, 62].

1.4.2 Indirect tests:

1.4.2.1 Three-day fat quantification and determination of the coefficient of fat absorption

In this test, patient is required to keep a strict diet of 100g of fat for 5 days and collect faeces for the last three days. It is considered the gold standard test for indirect testing in PEI [75],

its diagnostic value decreases in mild to moderate PEI and requires a period of stopping pancreatic enzyme replacement therapy (PERT) because it can affect the results [62]. It can be a cumbersome test and unpleasant to patients and laboratory staff and rarely used nowadays [1]

1.4.2.2 Mixed 13 C-triglyceride (^{13}C-MTG) breath test

A labelled ^{13}C substrate/butter is given orally, ^{13}C is released after lipids are hydrolysed by pancreatic lipase with the release of free fatty acids with further metabolism in liver releasing ^{13}CO2 in the expired air. The amount of ^{13}C-MTG is measured by mass spectrometry. Subjects with decreased lipase activity as in PEI have decreased ^{13}CO2 recovery in the expired air [76]. It has high sensitivity in mild to severe PEI and correlates well with FEL-1 and the three-day fat quantification test [77]. However, substrate currently not licenced or approved for use in the UK and it has not been shown to be superior to FEL-1 [78].

1.4.2.3 Secretin enhanced-magnetic resonance cholangiopancreatography

The advantage of this test is that both the morphology and the exocrine function of the pancreas can be assessed at the same time. Secretin is injected to stimulate duct cells to secrete water and bicarbonate during MRCP (sMRCP). Subsequently, images of the pancreas taken every 30 seconds for 10 minutes. sMRCP has sensitivity of 77% and specificity of 83% [79, 80]. Current challenges of using sMRCP is the lack of expertise in this test and its high cost [81].

1.4.2.4 Faecal elastase

FEL-1 has been used in the last two decades to diagnose PEI. The test involves patients collecting a small amount of faeces for analysis. Elastase is a proteolytic enzyme produced by the acinar cells of pancreas and binds to bile salts. FEL-1 acts as a surrogate marker for pancreatic enzyme production because it travels through the gut without degradation [62]. It is stable at room temperature for one week and for four weeks when stored at 4°C [62]. It

only detects the human form of elastase therefore the main advantage of FEL-1 is that it does not interrupt PERT treatment [62]. It has become acceptable first line investigation for PEI because it is non-invasive, widely available and inexpensive [62].

False positive results can occur when stool is mixed with water, therefore patients should be advised and instructed on how to obtain a stool sample without contamination with water or urine[62]. FEl-1 >200 µg/g in stools indicates a normal exocrine pancreatic function, concentrations between 100 and 200 µg/g indicated mild-moderate exocrine pancreatic insufficiency, and a level <100 µg/g indicates severe exocrine pancreatic insufficiency [43]. However, the treatment is usually the same for those who has mild, moderate, or severe PEI. FEL-1 has a sensitivity between 54-90% and a specificity of 80% in mild-moderate PEI [46, 82, 83] [84]. In severe PEI, FEL-1 sensitivity increases to 100% and specificity to 93% compared to a faecal fat collection gold standard [79].

Given the suboptimal accuracy of FEL-1, 'low faecal elastase' was referred to those with FEL-1 <200 µg/g throughout the thesis.

1.5 Diagnosis of chronic pancreatitis

The gold standard test for CP is histology, but this is rarely performed due to its invasive nature, risk of complications and poor intra-observer agreement of CP features [85]. Imaging tests are the investigation of choice in diagnosing in CP. Transabdominal ultrasound is non-invasive and widely available but can only detect up to 40% of calcifications when present and its use is limited by poor views due to bowel gas overlying the pancreas[86]. Endoscopic retrograde cholangiopancreatography (ERCP) has sensitivity and specificity 90% and 100%, respectively [80]. ERCP can detect small ductal changes which can be features of early CP, however it is invasive and labour-intense with higher rate of complications including post-ERCP pancreatitis (7%) and is not used as a diagnostic test anymore[87]. ERCP has been

superseded by magnetic resonance imaging (MRI) which can detect parenchymal and ductal changes and it has the advantage over ERCP as it is non-invasive and carries no risk of radiation compared to ERCP. The sensitivity of MRI in detecting CP is about 75% which can be further improved to 90% by introducing intravenous secretin to enhance the quality of ductal images [88].

Computed tomography (CT) scan is another main modality for pancreas imaging. Contrast enhanced CT scan has become the test of choice for patients suspected of CP [89, 90]. It is non-invasive, and it can be used to rule out other causes like pancreatic cancer. Its main limitation is the risk of radiation. It has a sensitivity 74-90% and specificity 80-90% in moderate-severe CP [79, 80]. The most common features of CP found on CT are calcifications of parenchymal tissue, main pancreatic duct dilatation and pancreatic atrophy [91]. Cambridge classification has been developed to standardise radiological diagnosis of CP and reduce inter-observer variability [92]. The classification has been modified to be used with other imaging modalities like MRI and endoscopic ultrasound (EUS), table1.3.

Table 1.3 Modified Cambridge classification for diagnosing chronic pancreatitis.

Classification	Severity	Radiological features
1	Normal	Main PD <2 mm, normal gland size and shape, parenchyma
2	Equivocal	1 only of the following signs: • Main pancreatic duct enlarged (between 2 and 4 mm) • Slight gland enlargement • Heterogeneous parenchyma • Small cavities (<10 mm) • Irregular ducts • Focal acute pancreatitis
3	Mild	2 or more of the above listed criteria
4	Moderate	2 or more of the above listed criteria, with 1 of the following • Increased enhancement of main pancreatic duct wall • Irregular head / body contour
5	Marked changes	As above, with 1 or more of the following: • Large cavities (>10 mm) • Gross gland enlargement (>2 X normal) • Intraductal filling defects or calculi • Duct obstruction, stricture or gross irregularity • Contiguous organ invasion

CP, chronic pancreatitis**; MPD,** main pancreatic duct

EUS has sensitivity of 81% and specificity of 90% in diagnosing CP [93]. EUS can detect early morphological and ductal changes of CP with high accuracy [94]. The EUS features for the diagnosis of CP is divided into parenchymal and ductal changes (table 1.4) the Rosemont criteria was introduced in 2007 (table 1.4) to standardise EUS reporting of CP, it further grades EUS features into major and minor features however, other studies used the total number of EUS features listed without major or minor weighting [95, 96].

Table 1.4 Endoscopic ultrasound features of chronic pancreatitis and Rosemont classification [97]

Parenchymal changes	**Criteria classification**	**Ductal changes**	**Criteria classification**
Hyperechoic foci with shadowing	Major A	**MPD stones**	Major A
Lobularity with honeycombing	Major B	**Irregular MPD**	Minor
Lobularity without honeycombing	Minor	**Side branch dilatation**	Minor
Hyperechoic foci without shadowing	Minor	**MPD dilatation**	Minor
Cystic change	Minor	**Hyperechoic MPD border**	Minor
Stranding	Minor		
EUS diagnosis of CP based on Rosemont criteria			
Consistent with CP	**Suggestive of CP**	**Indeterminate for CP**	**Normal**
1 major A feature (+) ≥3 minor features or 1 major A feature (+) major B feature or 2 major A features	1 major A feature (+) <3 minor features or 1 major B feature (+) ≥3 minor features or 5 minor features (any)	3 to 4 minor features, no major features or major B feature alone or with <3 minor features	<2 minor features, no major features

MPD, main pancreatic duct; **CP**, chronic pancreatitis

The M-ANNHEIM scoring system is another tool used for diagnosis and grading the clinical severity of CP [98]. In addition to radiological features of CP, it includes other important features for example endocrine and exocrine insufficiencies, and requirement for pain control, table 1.5. Depending on the presence or absence of clinical features in M-ANHEM

criteria, the points added together to categorise patients according to M-ANNHEIM severity index, table 1.6**.**

Table 1.5 The M-ANNHEIM scoring system for grading of severity of CP[98]

Clinical features	**points**
Patient report of pain	
No pain without therapy	0
Recurrent acute pancreatitis	1
No pain with therapy	2
Intermittent pain	3
Continuous pain	4
Pain control	
No medication	0
Use of nonopioid drugs or use of mild opioids (WHO step 1 or 2)	1
Use of potent opioids (WHO step 3) or endoscopic intervention	2
Pancreatic surgical intervention for any reason	4
Exocrine insufficiency	
Absence of exocrine insufficiency	0
Presence of mild, moderate, or unproven exocrine insufficiency not requiring enzyme supplementation	1
Presence of proven exocrine insufficiency (according to exocrine function tests) or presence of steatorrhoea, normalized or markedly reduced by enzyme supplementation	2
Morphologic status on pancreatic imaging	
Normal	0
Equivocal	1
Mild	2
Moderate	3
Marked	4

Severe organ complications	
Absence of complications	0
Presence of possibly reversible complications	2
Presence of irreversible complications	4

WHO: World Health Organisation

Table 1.6 The M-ANNHEIM severity index of chronic pancreatitis [98]

Severity index	Severity level	Point range
M-ANNHEIM A	Minor	0-5
M-ANNHEIM B	Increased	6-10
M-ANNHEIM C	Advanced	11-15
M-ANNHEIM D	Marked	16-20
M-ANNHEIM E	Exacerbated	>20

1.6 Management of pancreatic exocrine insufficiency

The main aims in the management of PEI are achieving normal nutritional status, control maldigestion-related symptoms, and replenish micronutrient deficiencies.

1.6.1 Nutrition assessment

Classically, a low-fat diet was advised in order to avoid steatorrhoea and maldigestion, however, this practise is discouraged now, and a normal healthy diet is recommended. A balanced diet has been shown to enhance the nutritional therapy in patients with PEI so early dietician input is vital [99]. Patients with PEI secondary to CP may require nutritional supplement in addition to the standard nutritional requirement of 25 to 35 kcal/kg and 1.2 to

1.5 g/kg protein because weight adjusted calculation does not account for the degree of malabsorption and the high energy expenditure seen in CP patients [100, 101].

A specific concern for PEI is the malabsorption of micronutrients. There has been a variation of the frequency and the type of deficient micronutrients identified in studies of patients with PEI to date, with a majority including patients with PEI due to CP. Micronutrient deficiencies have been identified in patients with CP and PEI but also in patients with CP without PEI [4, 46, 83]. Assessment of micronutrient deficiencies in PEI is covered in more details in chapter 3.

Anthropometric measurements like BMI, waist circumference, mid upper arm circumference, and skinfold measurement are non-invasive tests that provide information about the health and nutritional status of a patient[102]. However, these tests unable to tests measure body compositions like skeletal muscle. Other available tools to measure body compositions including bioimpedance analysis (BIA), Dual-Energy X-ray Absorptiometry (DEXA), and CT scan images. CT scan images have the advantage over BIA and DEXA with better quality and high validity[103].

Baseline bone density assessment is recommended and then two yearly [69]. Advice regarding exercise, smoking and alcohol cessation and adequate diet should be provided [104].

Patients with PEI should be carefully assessed for malnutrition. Weight loss >10% in the last six months and body mass index (BMI) <18.5kg/m^2 are considered the most significant markers of malnutrition [105, 106]. The malnutrition universal screening tool (MUST) is another commonly used tool in clinical practice to identify malnourished patients or those at risk of malnutrition. It uses BMI alongside other measures to identify those at risk of malnutrition. However, a single BMI measurement can be of limited diagnostic use without the pre-morbid state or muscle mass [64, 107]. The recent development of new software enabled the assessment of different body compositions including skeletal muscle and fat mass by using

patients' cross section images [108]. The term sarcopenia defines patients with loss of skeletal muscle mass [109, 110]. Loss of skeletal muscle can cause loss of muscle function, increase risk of frailty, and poor prognosis in patients with medical, surgical and oncological conditions including pancreatic cancer [111].

1.6.2 Pancreatic enzyme replacement therapy

Patients with PEI should be treated with PERT [112-114]. Table 1.7 summarises the practical steps in using PERT in PEI. It is estimated that ~2,000 lipase units is required to digest 1 g of fat (1,000–4,000 U/g) [61]. Starting dose of 50,000 units with meals and 25,000 units with snacks is recommended [106]. Enzymes in minispheres or microspheres enteric-coated of less than 2 mm are preferable to allow dispersing and mixing with food in the stomach. The majority of patients can be managed with normal diet and PERT but if calorie intake is inadequate protein supplementation should be considered [115]. A higher doses is recommended for meals with high fat intake[1]. In order for PERT to work effectively it should mimic normal postprandial environment [106]. Therefore, delayed gastric emptying or acidic duodenal PH should be corrected and these should be considered when prescribing PERT or when reviewing non-responsive patients. Response to treatment should be assessed by monitoring improvement in weight, nutritional status and symptoms [61]. Patients with low nutritional markers may benefit from PERT in the absence of weight loss or maldigestion symptom.

PERT has been shown to improve GI symptoms, nutritional status and quality of life [106]. Unresectable pancreatic cancer with treated PEI has been shown to be associated with longer survival compared to untreated group [72].

Table 1.7 Guidance for pancreatic enzyme replacement therapy *[116]*

PERT should be started in secondary care and prescribing transferred to primary care with agreement from General Practitioner
Starting dose of 50,000 units with meals and 25,000 units with snacks recommended but may need increasing
Gastric acid suppression with proton pump inhibitors or H2 receptor antagonists may be necessary to improve absorption
Most enzyme supplements are porcine but have been approved for use by both Jewish and Islamic communities

1.6.3 Follow-up of patients with pancreatic exocrine insufficiency

Follow up consultations should aim to evaluate symptoms, stabilise weight and improve nutritional status. Patients should remain on at least yearly review where routine blood tests should be checked including markers of malnutrition.

Six-monthly screening for diabetes mellitus with HbA1c should be offered in CP [69]. Additionally, 2-yearly osteoporosis screening should be considered for those with malnutrition or previous low trauma fracture [69].Complete abstinence from alcohol and smoking is essential for all patients with CP and PEI [117-119].

1.7 Conclusion

The current evidence on the pathogenesis, diagnosis, and management of PEI was presented in this chapter. The evidence of PEI risk in primary and 'at-risk' conditions was summarised. The current evidence showed that PEI is likely to be underdiagnosed specially in 'at-risk' conditions. The gold standard tests for diagnosing PEI are invasive, expensive, and not widely available in clinical practice. FEL-1 offer a suitable non-invasive test for PEI. Management of patient with PEI should include identification of cause including pancreatic imaging,

nutritional assessment including anthropometric test and micronutrient markers, and treatment with PERT.

1.8 Aim of this thesis

The findings in this chapter have led to questions that I aim to answer in this thesis. The current awareness and testing in 'at-risk' conditions may still not be established and without a reliable test may miss cases of PEI. Giving the difficulty in testing for PEI, alternative indirect identification of PEI using the conditions endpoints such as malabsorption or malnutrition could be used. Lastly, if alternative tests to assess for malnutrition are available can they help the management of patients with PEI related conditions

To build on the available evidence in this field I have asked the following questions with hypotheses in italics

What is the current practice and yield for investigating pancreatic exocrine insufficiency in 'at-risk' patients?
Testing with faecal elastase can identify those 'at-risk' of pancreatic exocrine insufficiency

What is the role of testing for micronutrient markers in pancreatic exocrine insufficiency?
Micronutrient marker deficiency is common in pancreatic exocrine insufficiency

Can a skeletal muscle measurement be used as diagnostic tool for the diagnosis of pancreatic exocrine insufficiency?
Skeletal muscle index is feasible and low skeletal mass is common in pancreatic exocrine insufficiency

Can the 'skeletal muscle index' measurement in patients with pancreatic cancer at high risk of pancreatic exocrine insufficiency predict prognosis and aid treatment plan?

Skeletal muscle index is a useful technique to predict non-uptake of chemotherapy in pancreatic cancer.

The questions will be addressed in the following four chapters and each chapter can be read independently of others and answers each question in turn.

These studies seek to provide useful information regarding the prevalence of PEI in 'at-risk' conditions and improve the diagnosis of PEI using a relatively new technique.

Changes in clinical practice may be influenced because of the work contained in my thesis. As a result of writing the introduction and conducting the literature review on PEI and CP (chapter 1) a review article has been published in "*Frontline Gastroenterology*" [120]. Chapter 2 has been presented as poster at *British society of Gastroenterology (BSG) annual meeting 2019 and BSG Campus 2021[121, 122]*. Chapter 3 has been accepted in *World Journal of Clinical Cases and presented as a poster at BSG annual meeting 2019 [123]*. Chapter 4 has been published in *Digestive Diseases Journal [124]* and as an oral presentation at Pancreatic Society of Great Britain and Ireland (PSGBI) annual meeting 2019 [125]. Chapter 5 has been published in *Journal of Gastrointestinal Cancer* [126] and accepted as an oral presentation at BSG 2019 [127].
Chapter 6 summarises the projects and suggests future directions of research.

1.9 Patient recruitment and methodology

Each chapter has different patient recruitment and methods. Each chapter contains section which explains recruitment processes, methodology and analysis.

1.10 Extent of assistance

I am very grateful to several people for their advice, guidance and support during the studies contained within my thesis. I conceived and designed all studies contained in the thesis. I am especially grateful to Dr. Ragu Vinagarayam (Consultant Radiologist, Royal Hallamshire Hospital) for his input in analysing CT scans for chapter 4. I am grateful for the following consultants: Mr Ahmed Al-Mukhtar (Consultant HPB surgeon, Northern General Hospital) for his help in recruiting patients for chapter 4, Prof. Solomon Tesfaye (Consultant in Diabetic

Medicine at the Royal Hallamshire Hospital) and Dr. Ann Tunbridge (Consultant in Infectious Diseases, Royal Hallamshire Hospital) for their help in recruiting patients for chapter 2, and Prof. Jonathan Wadsley (Consultant Clinical Oncologist, Weston Park Hospital) for his input in chapter 5.

CHAPTER 2: WHAT IS THE CURRENT PRACTICE AND YIELD FOR INVESTIGATING PANCREATIC EXOCRINE INSUFFICIENCY IN AT-RISK PATIENTS? A PROSPECTIVE OBSERVATIONAL STUDY.

2.1 Summary

Introduction: Each year in the UK, around 11,000 new cases of PEI are diagnosed [128]. The majority of these cases are diagnosed from three main conditions: chronic pancreatitis (CP), pancreatic cancer and cystic fibrosis. However, there is cumulative evidence that PEI is under-recognised and can occur in other 'at-risk' conditions. High alcohol intake (HAI), diabetes mellitus (DM), and people living with HIV (PLHIV) are some of the 'at-risk' conditions that have been reported to be associated with PEI. The main aim of the study to assess the current practice and yield of requesting FEL-1 in 'at-risk' conditions.

Methods: Prospective recruitment of patients attending secondary care clinics with DM, PLHIV, and a third group of inpatients admitted with HAI. Patients with PEI were contacted and offered a follow up review in gastroenterology clinic.

Results: In total, 188 patients were recruited, HAI (n=78), DM (n=64), PLHIV (n=46). The return rate for samples ranged between 56.5-76.6%, HAI 67.9% (53/78), DM 76.6% (49/64), and PLHIV 56.5% (26/46). Sample return was less likely if patients lived outside Sheffield city compared to those lived within city for outpatients, 37.5% versus 71.6% p=0.007. The presence of PEI (FEL-1<200) was shown in 20.4% of patients with DM, 15.4% in PLHIV and 22.6% in HAI. Diarrhoea and bloating were the most reported symptoms in followed-up patients with low FEL-1, 31.8% and 22.7%, respectively. Follow-up CT scans identified CP changes in 13.6% and pancreatic atrophy in 31.8% of low FEL-1 patients.

Conclusion: There is still lack of testing and difficulty for patients to return stool samples in 'at-risk' conditions. The diagnostic yield of PEI by requesting FEL-1 in DM, PLHIV and HAI is high, therefore, case finding and testing with FEL-1 should be considered because 1 in 5 patients may have PEI.

2.2 Introduction

There is cumulative evidence that PEI is under-recognised and can occur in 'at-risk' conditions. HAI, DM, and PLHIV have been reported to be associated with PEI. Patients with history of alcohol excess and alcohol related liver disease are at risk of developing PEI and can affect up to 55-72% [34, 35]. PEI has been shown to develop after an average of 12 years after diagnosis in alcohol related chronic pancreatitis [36].Therefore, it is important to identify those at higher risk in order to plan for lifestyle modification advice and initiate PERT. Morphological changes of the pancreas have been reported in patients with DM on radiological 36.6% and histological assessment 59% [43, 129], also chronic inflammatory changes and pancreatic atrophy have been shown in autopsy studies in 11-19% patients with DM [38]. The estimated prevalence of PEI in DM1 is (26-74%) with less prevalence in DM2 (12-36%) [37-41]. PEI is also shown to be present in 32-45% of PLHIV with good response to PERT [11, 45]. The mechanism of PEI in PLHIV is still not confirmed yet. Historically, this was linked to didanosine, however, this finding was not confirmed later in a larger study [45, 46]. A post-mortem study of relatively young HIV patients found that up to 90% of cases had pancreatic morphological changes [47]. This study suggests parenchymal destruction as a cause for PEI, however, such high prevalence may not be valid nowadays due to the improvement in survival since the introduction of HAART.

Despite the evidence that PEI can occur in 'at-risk' conditions, there is still lack of clear guidance on testing for PEI, also these selected cohorts (HAI, DM and PLHIV) are usually cared for by different medical specialties, therefore, the level of awareness of PEI may be variable. Early diagnosis of PEI can be challenging because symptoms are non-specific and steatorrhoea can be a sign of late-stage disease and develop after significant reduction in lipase enzyme secretion >90% [10], therefore, relying on steatorrhoea as the main symptom may result in delaying diagnosis and missing early stages of PEI.

The gold standard tests are expensive, time consuming and not widely available [62]. FEL-1 is non-invasive and widely available [84]. The process of requesting FEL-1 requires patient providing a small amount of stool sample and then returning the sample to healthcare centres. The burden of the process of returning stool sample for FEL-1 is often overlooked. The evidence in literature showed low stool sample return rate when investigating medical conditions with stool samples including stool test for bowel cancer screening programmes with rate of <60% [130-132]. Some of the barriers for returning stool sample include embarrassment and lack of information on how to obtain and return samples [132].

With the evidence available, current clinical practice needs to be assessed to see which patients are being tested.

2.3 Aims of the study

The main aim of the study to assess the current practice and yield of requesting FEL-1 in 'at-risk' conditions. The secondary aim was to assess stool sample return rate and if there is a difference in return rate between those living within (Sheffield city) compared to those living outside.

2.4 Methods

Consecutive prospective recruitment of patients with DM and PLHIV attending secondary care clinics, and a third group recruited were inpatients admitted with HAI resulting in withdrawal symptoms. Patients were given information about the study. Instructions were given for obtaining a stool sample and kits were provided with a labelled pot, request form, kidney-shape disposable tray, plastic pathological bag, and non-transparent cardboard bag, figure 2.1. Our hospital receives referrals within Sheffield and from regional hospitals. For outpatients, those

lived in ‘Sheffield’ were asked to return samples to our hospital’s specimen reception in person, or to their GP, those living outside Sheffield city ‘non-Sheffield’ patients were asked to return the samples to our hospital’s specimen reception in person.

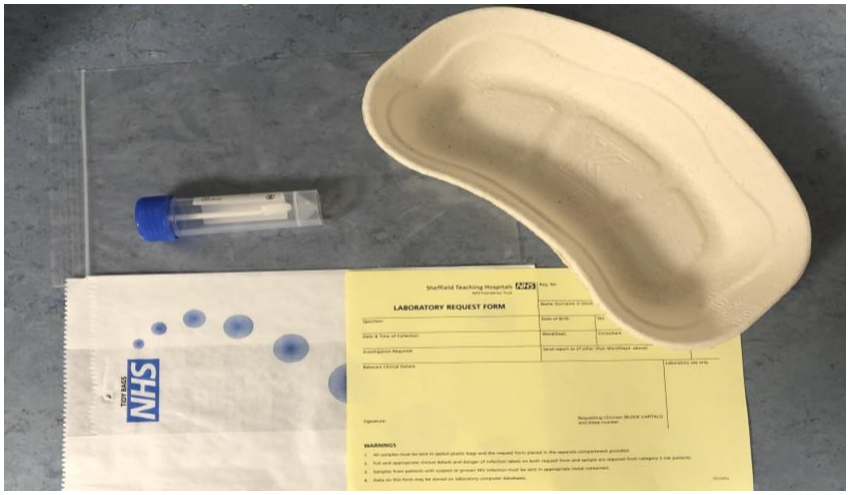

Figure 2.1 PEI sampling kit. Patients were supplied with a disposable tray, labelled pot, request form, plastic bag, and a discrete bag.

A follow up-call planned after 4 weeks to report results or a reminder to return the sample (for those recruited from outpatients). ~~Patients with a previous diagnosis of PEI or a FEL-1 test result were excluded from the study.~~ Inclusion and exclusion criteria are summarised in table 5.

All FEL-1 samples were analysed in our centre with a commercially available enzyme linked immunosorbent assay (ELISA) test which uses two monoclonal antibodies against specific epitopes of human pancreatic elastase (ScheBo- Tech, Wettenberg, Germany). FEL-1 <200µg/g was considered diagnostic of PEI.

2.4.1 Setting

2.4.1.1 DM patients*:* were consecutively recruited between Dec 2018- Oct 2019 from outpatient clinics at Royal Hallamshire Hospital. Both DM1 and DM2 were recruited. The study was conducted in collaboration with the Academic Unit of Diabetes and Endocrinology.

Table 2.1 Inclusion and exclusion criteria for 'at-risk' conditions recruitment study

Inclusion criteria	**Exclusion criteria**
Patients > 18 years old	Patients < 18 years old
Diabetes mellitus patients, or	History of watery acute diarrhoea or proven infectious diarrhoea (would give false positive)
People living with HIV, or	Patients unable to provide stool sample due to clinical or physical disability
Inpatients with high alcohol intake	Patients with confirmed liver cirrhosis (to assess the prevalence of PEI in those without cirrhosis or at early stages)

2.4.1.2 PLHIV: were consecutively recruited between Sep 2019- Feb 2020 from Infectious disease clinics at Royal Hallamshire hospital. The study was conducted in collaboration with the Department of Infectious Diseases and Tropical Medicine.

2.4.1.3 Inpatients with HAI on the gastroenterology ward at Northern General Hospital were recruited between April 2018-Oct 2018. Patients included were those with alcohol withdrawal consequences.

2.4.3 Data collection

In addition to FEL-1 result, other data collected were: age, sex, height, weight, BMI, pack-year smoking history, amount of alcohol consumption per week, onset of disease in DM and PLHIV. Ultrasound findings of liver for inpatients with HAI were also collected if it was performed during same admission spell. CT scan findings of follow up patients with low FEL-1 was collected.

2.4.4 Follow up

Patients with low FEL-1 were contacted and offered a follow up in gastroenterology clinic. Patients were seen in my clinic and their symptoms were reviewed. Instructions were given to patients on how to collect the sample including avoiding contaminating the sample with urine or water. A patient with a low FEL-1 was informed of the requirement for further tests and follow up including a CT scan [133, 134]. A final radiological diagnosis of CP was made if patients had a Cambridge classification of ≥3 on CT [96]. CP diagnosis using CT is summarised in the next chapter, table 3.1.

2.3.5 Ethical approval

This study was reviewed and approved by NHS Research Ethics (STH20142/ IRAS239539), Appendix 2.

2.4.6 Statistical analysis

Data comparisons were reported as median and range for continuous variables using Mann Whitney U test. Categorical data were compared using the Fisher's exact test. Number of patients recruited with number of samples returned were presented in bar graph. Distribution of faecal elastase was presented in box plot graph. Frequency of gastrointestinal symptoms was

presented in bar graph. Statistical significance was considered when p <0.05. Statistical analyses were performed using SPSS (IBM SPSS statistics version 25, Chicago, Illinois, USA).

2.5 Results

In total, 188 patients were recruited and given FEL-1 sampling kit, HAI (n=78), DM (n=64), PLHIV (n=46). The return rate for samples ranged between 56.5-76.6%, HAI 67.9% (53/78), DM 76.6% (49/64), and PLHIV 56.5% (26/46). 'Non-Sheffield' patients were less likely to return stool samples compared to 'Sheffield patients', 37.5% (6/16) versus 73.9% (68/92), p=0.007, figure 2.2.

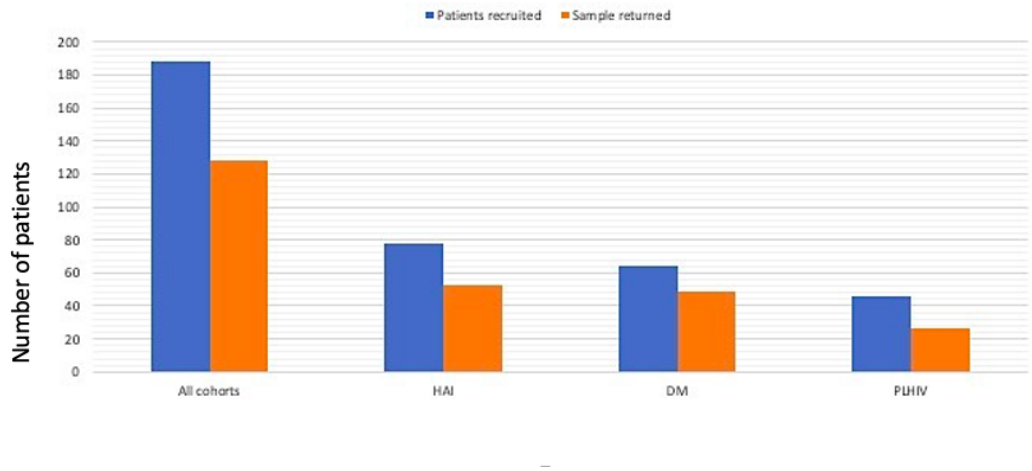

Figure 2.2 Number of patients recruited with number of samples returned in high alcohol intake (HAI), diabetes mellitus (DM), and people living with HIV (PLHIV).

The presence of PEI (FEL-1<200µg/g) was found in 20.4% (10/49) of patients with DM, 15.4% (4/26) in PLHIV and 22.6% (12/53) in patients with HAI, figure 2.3. I found 53.8% (14/26) patients with low FEL-1 had a level of <100 µg/g; further details of patients and diseases characteristics were given in tables 2.2, 2.3, and 2.4

Diarrhoea and bloating were the most reported symptoms in followed-up patients with low FEL-1, 31.8% (7/22) and 22.7% (5/22), respectively, figure 2.4.

HAI patients with low FEL-1 were older compared to those without, 51 vs 45 years, and 66.7% (8/12) were female. The median BMI of low FEL-1 patients was higher than normal FEL-1 26.4 versus 23.8 kg/m^2. The median alcohol intake was not different between low FEL-1 and normal FEL-1 patients. The majority of patients were either current or past smoker with median pack-year smoking history of 9.5. Also, most patients with low FEL-1 had ultrasonographic diagnosis of liver cirrhosis during their admission, 72.7% (8/11). Two out of 8 followed up patients had morphological changes suggestive of CP.

The median BMI of DM with low FEL-1 was 28.5 kg/m^2 and 70% (7/10) of patients were male. Median pack-year smoking was 20 compared to 12 in normal FEL-1. Furthermore, cross section images of DM with low FEL-1 identified high prevalence of pancreatic morphological changes in 60% (6/10).

We compared the prevalence of low FEL-1 in our cohorts to a control cohort of healthy individuals recruited preciously in our centre using the same method (FEL-1). The prevalence of low FEL-1 in the control group was 3.2% (3/95). The prevalence was significantly higher in all groups compared to the control group: HAI p= 0.0003; DM compared P=0.001, and PLHIV p=0.04.

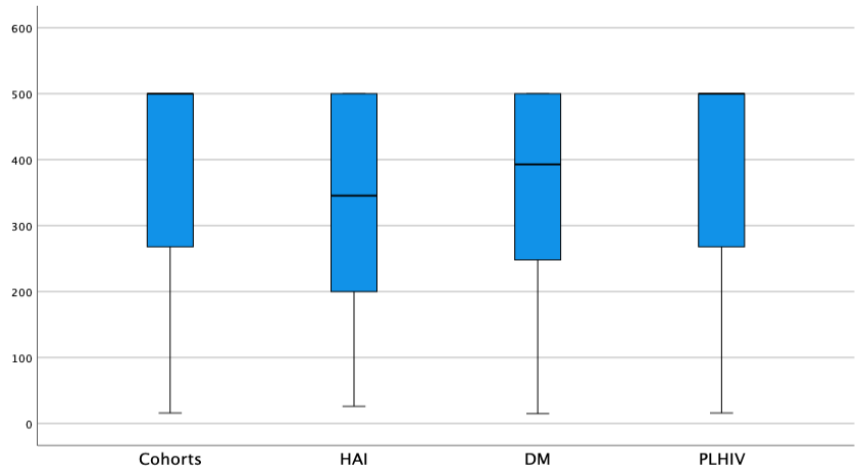

Figure 2.3 Box plot showing the distribution of faecal elastase level (Y axis) in all cohorts, high alcohol intake (HAI), diabetes mellitus (DM), and people living with HIV (PLHIV).

Table 2.2 Diagnostic yield of requesting faecal elastase in inpatients with high alcohol intake and characteristics of population

	Inpatients with high alcohol intake N=53	
	Normal FEL-1 (n) or median (range)	***FEL-1 <200 µg/g*** (n) or median (range)
Number	41	12
Age, years	45(28-74)	51(20-66)
Sex M/F	25/16	4/8
BMI kg/m^2	23.8 (16.5-66.3)	26.4 (20.1-41.1)
Pack-year smoking	11.25 (0-60)	6.25 (0-35)
Alcohol u/week	140 (35-420)	140 (78-315)
Presence of heterogenous livers on ultrasound, % (n)*	39 (16/41)	72.7 (8/11)
CT scan for patients with low FEL-1 **Total:** **Normal** **Chronic pancreatitis** **Atrophy**	NA	8** 6 2 0

* 52 patients had ultrasound of liver during admission
**4 patients did not respond to clinic invitation

Table 2.3 Diagnostic yield of requesting faecal elastase in patients with diabetes mellitus and characteristics of population

	Diabetes Mellitus N=49	
	Normal FEL-1 (n) or median (range)	***FEL-1 <200 µg/g*** (n) or median (range)
Number	39	10
Age, years	58 (28-84)	66 (44-76)
Sex M/F	20/19	7/3
BMI kg/m^2	30.3 (23.9-46.1)	28.5 (24.5-38.6)
Pack-year smoking	12.5(1-72)	20 (0.5-120)
Alcohol u/week	10 (2-20)	6 (4-14)
Disease duration, months	234 (53-589)	189 (61-732)
Diabetic patients on insulin, % (n)	76.9 (30/39)	80 (8/10)
CT scan for patients with low FEL-1 **Total:** **Normal** **Chronic pancreatitis** **Atrophy**	NA	10 4 1 5

Table 2.4 Diagnostic yield of requesting faecal elastase in patients living with HIV and characteristics of population

	People living with HIV N=26	
	Normal FEL-1 (n) or median (range)	***FEL-1 <200 µg/g*** (n) or median (range)
Number	22	4
Age, years	56 (38-68)	47 (42-58)
Sex M/F	13/8	4/0
BMI kg/m^2	28.4 (19.4-45.9)	27.4 (24.2-31.9)
Pack-year smoking	20 (3.7-27.5)	7.5 (1-11)
Alcohol u/week	2 (0-42)	7 (2-14)
Disease duration, months	183 (47-264)	110 (33-153)
CT scan for patients with low FEL-1 **Total:** **Normal** **Chronic pancreatitis** **Atrophy**		 4 2 0 2

All four PLHIV with low FEL-1 were male with median BMI of 27.4 kg/m^2. Low FEL-1 patients had lower alcohol intake, but higher pack-year smoking compared to normal FEL-1. Follow up CT scan identified pancreatic atrophy in 50% (2/4).

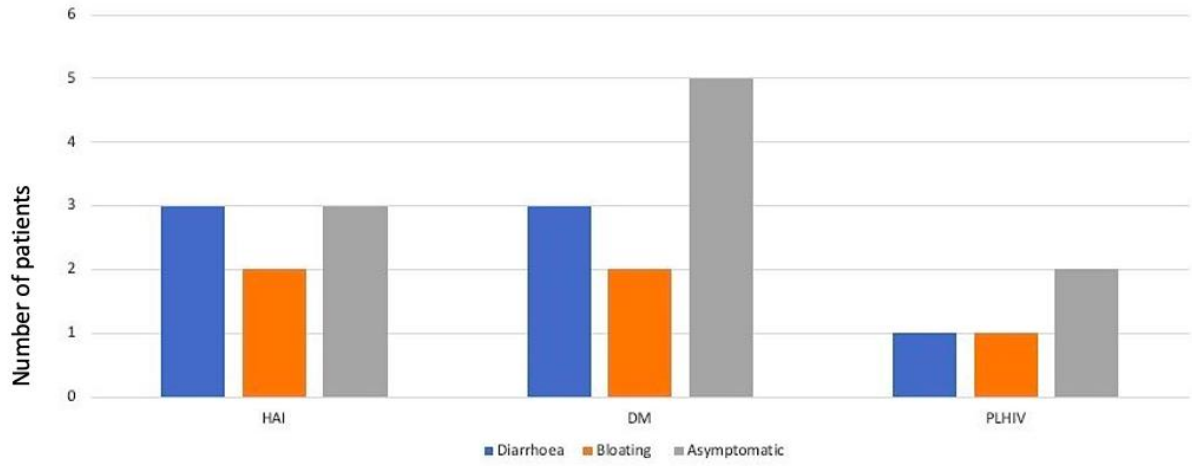

Figure 2.4 Frequency of gastrointestinal symptoms in patients with high alcohol intake (HAI), diabetes mellitus (DM) and people living with HIV (PLHIV).

2.6 Discussion

The findings in this study showed that none of the 188 patients recruited for the study had a previous FEL-1 performed. The study also showed that FEL-1 testing has a significant yield in these 'at-risk' groups. It is common to encounter patients with DM, PLHIV, and HAI in clinical practice across different medical specialties; therefore, a case-finding practice is encouraged. Case-finding practice should be aimed at 'at-risk' group who may clinically be at risk of PEI and prompt testing with FEL-1 to initiate early treatment.

A large primary care-based study showed annual presentation ratio of male to female to be 3:1 which was similar to our cohort [135]. Risk of alcohol related pancreatitis increases with the increase in alcohol consumption. The median weekly intake in HAI group was 140 U which is 10 times higher than the recommended alcohol consumption and currently is 14 U per week for men and women in the UK [136]. HAI is a known risk factor for development of PEI and has been historically linked to CP. The risk of CP increases with the amount of daily alcohol consumed: intake of 3 U/day (OR=1.33) and with 13 U/day (OR= 3.19) [137]. However, despite HAI median intake was 20U/day, the proportion of PEI in HAI was comparable to DM who had significantly lower alcohol intake. This variation in PEI susceptibility could be multifactorial including environmental and genetic factors [137].

A large multicentre study reported 42% PEI prevalence in DM and correlated with duration of the disease [44]. This study has shown PEI prevalence of 20% in DM, however, I was unable to demonstrate a correlation with duration of disease which may be explained by the smaller number of cases in our study compared >1000 patients in their study.

Pancreatic morphological changes have been previously reported in 45% of PLHIV who underwent pancreatic assessment with MRI [138]. In a study of 100 patients with PLHIV with a median disease duration of 173 months, 32 were found to have FEL-1 <200µg/g with improvement in symptoms were achieved in 75% of patients received PERT [11].

To ascertain the findings in this study, the prevalence in the three cohorts were compared with another control group of healthy volunteers who were tested with FEL-1 and recruited in our centre (conference abstract) [139]. The prevalence of PEI in the control group was 3.2% (3/95). The prevalence was significantly higher in all groups compared to the control group: HAI p= 0.0003; DM compared P=0.001, and PLHIV p=0.04. Although this was not incorporated into the original study design, this control group was using the same test/analyser and population so could be viewed as historical controls and strengthens findings.

PEI symptoms are non-specific making the diagnosis of PEI more challenging based on symptoms alone without taking into account premorbid state. However, most patients with low FEL-1 were asymptomatic in our cohort with no report of steatorrhoea or weight loss making it challenging to diagnose PEI by only relying on symptoms and there is a risk of missing the diagnosis. Therefore, a composite assessment for these patients is required based on symptoms, presence of 'at-risk' condition, FEL-1 result, and nutritional assessment.

The majority of low FEL-1 patients were overweight and 77% had BMI ≥25kg/m^2 for all cohorts, therefore, relying on a single BMI measurement for nutritional assessment is inadequate and may miss cases of malnutrition and opportunity of early intervention especially in obesity. High BMI with increased visceral adiposity can cause fatty replacement of the pancreas and atrophy with consequences of exocrine insufficiency [140]. Ten out of 22 followed up patients showed morphological changes in the pancreas with pancreatic atrophy being the most common 70% (7/10). The risk association between PEI and atrophic pancreas may be overlooked, as the finding of atrophic pancreas on radiological reports may not carry the same clinical status compared to calcified chronic pancreatitis.

A significant proportion of inpatients with HAI did not return a sample 32.1% (25/78) despite the request being made on a medical ward. Similar poor returns reported in outpatients with 23.4% (15/64) of DM and 40.9% (18/44) of PLHIV not returning the sample despite providing

kits. The reason for returning the samples was not investigated but the clinical nature of this study reflects the challenges in returning stool samples which can be encountered in routine practice and its burden is often overlooked. The analysis showed those who lived outside Sheffield city had a lower sample return rate compared to those lived within. Similar reports of low stool sample return have been reported [130-132]. A study of women invited for bowel cancer screening programme in England over a period of six years showed that a quarter of patients did not return stool samples despite repeated invitation and re-sending kits for faecal occult test (FOBt) [130]. A systematic review showed that return of samples for FOBt for bowel cancer screening programmes across different countries rarely reached 60% [131]. Another paper studied the barriers to stool sample return for microbiology tests requested by General Practitioners found that embarrassment, hygiene concerns, privacy and lack of information were some of the barriers behind no-return of stool samples [132]. Therefore, it is very important to take these factors in consideration when requesting FEL-1 test.

The main limitation in this study is the lack of follow up to assess the response to PERT, this would strengthen the accuracy of the PEI diagnosis with FEL-1 as false positives results can occur in low probability conditions in up to 11% [141]. To reduce false positive results with FEL-1 checks were made with patients to make sure there is no contamination with water or urine.

In conclusion, despite the significant evidence that PEI is prevalent in DM, PLHIV and HAI, this study demonstrated a lack of testing for PEI by medical teams caring for these ‘at risk’ groups. When requested, there was poor return of FEL-1 stool samples in both outpatient and inpatient settings. It would appear that the FEL-1 test is being under-used as when successfully used the diagnostic yield of FEL-1 is high in DM, PLHIV and HAI. Therefore, case-finding practice should be encouraged to detect PEI by testing with FEL-1 in at ‘at-risk’ group.

CHAPTER 3: WHAT IS THE DIAGNOSTIC YIELD OF TESTING FOR MICRONUTRIENT DEFICIENCIES ASSOCIATED WITH PANCREATIC EXOCRINE INSUFFICIENCY IN A CLINICAL SETTING? AN OBSERVATIONAL PROPECTIVE STUDY

3.1 Summary

Background: PEI can be difficult to diagnose and cause maldigestion symptoms and malabsorption. There have been a number of studies that have identified PEI associated micronutrient deficiencies (PEI-MD), however there is variation in both the frequency and type of PEI-MD reported with the majority of studies including patients with PEI due to CP or CP without PEI. There is a paucity of information for PEI-MD prevalence in patients with PEI but without CP and for the yield for testing for these PEI-MD in a clinical setting of suspected benign pancreatic diseases. The aim of this study was to assess the yield and type of PEI–MD in patients with and without PEI secondary to benign diseases.

Methods: Patients investigated with FEL-1 for maldigestion symptoms and suspected or proven benign pancreatic disease were prospectively identified. At the time of FEL-1 testing serum samples were taken for micronutrients identified by previous studies as PEI-MD: prealbumin, retinol binding protein, copper, zinc, selenium, magnesium and later in the study lipid adjusted vitamin E. FEL-1 recorded with a result <200µg/g was considered diagnostic of PEI. Patients underwent CT imaging, when there was a clinical suspicion of CP, a new diagnosis of PEI made on the stool sample or recurrent pancreatic type pain (epigastric abdominal pain radiating to back with or without previous acute pancreatitis attacks).

Results: After exclusions, 112 patients were recruited that underwent testing for FEL-1 and PEI-MD. PEI was identified in 41(36.6%) patients and pancreatic CT was performed in 82 patients. Overall, a PEI-MD was identified in 21/112 (18.8%) patients. The yield of PEI-MD was 17/41(41.5%) if PEI was present which was significantly higher than those without 4/71(5.6%) (p=0.0001). The yield of PEI–MD was significantly higher when PEI and CP were seen together 13/22 (59.1%) compared to CP without PEI and PEI without CP (p<0.03).

Individual micronutrient assessment showed a more frequent occurrence of prealbumin 8/41(19.5%), selenium 6/41 (14.6%) and magnesium 5/41(12.2%) deficiency when PEI was present (<0.02). The accuracy of using the significant micronutrients as a predictor of PEI showed a positive predictive value of 80-85.7% (95% CI:38-100%) and a low sensitivity of 9.8-19.5% (95% CI: 3.3-34.9%).

Conclusion: Testing for PEI-MD in patients with suspected pancreatic disease has a high yield, specifically when PEI and CP were found together. PEI-MD testing should include selenium, magnesium and prealbumin.

3.2 Introduction

Findings from chapter 2 support the hypothesis that PEI is under-recognised. If PEI is left untreated, it can cause malabsorption and gastrointestinal symptoms termed 'maldigestion'[106]. A specific concern for PEI is the malabsorption of micronutrients. Micronutrients are essential vitamins and trace elements that are important for normal physiological function, if left untreated, a micronutrient deficiency can cause malnutrition, poor growth, and increased risk of morbidity and mortality[142]. Micronutrient deficiencies can be reversed by treating the underlying condition and by the provision of the deficient micronutrient[142].

There have been several studies aimed to identify individual micronutrients associated with PEI, table 3.1. Fat soluble vitamins (vitamin A,E,D) have been a particular focus because lipase is one of most affected enzymes in PEI[143]. An associated incidence of low vitamin A (14.5-35.2%) and more frequently low vitamin E (17.7-75%)[64, 144, 145] levels have been reported with vitamin E demonstrating a correlation with faecal fat excretion in one study[146]. Vitamin D is one of the most common markers studied in patients with PEI and CP however a sole link to PEI has not been demonstrated with a high prevalence reported in control groups and healthy individuals [64, 147]. Along with fat soluble vitamins, the other trace element deficiencies that have been associated with PEI include prealbumin, retinol binding protein (RBP), zinc, selenium, and magnesium[63, 106, 144, 146, 148-150].

There is variation in both the frequency and type of PEI associated micronutrient deficiency reported, with most studies including patients with PEI due to CP, table 3.1. Micronutrient deficiencies have been identified in patients with CP and PEI but also in CP without PEI [46, 83]. However, there is increasing evidence that PEI can also occur in patients due to causes other than CP with little information regarding micronutrient deficiency prevalence in this cohort.

Table 3.1 Summary of previous studies on micronutrients deficiency in adult patients with pancreatic exocrine insufficiency.

Author(s), year	Number of PEI Patients.	Main cohort of patients	Method of PEI diagnosis	Micronutrients tested	Abnormal micronutrients
Kalvaria et al, 1986 [144]	Pancreatic steatorrhoea=23	CP=44	Fat malabsorption	Vitamin E	Low vitamin E in 75% in CP with significantly lower in pancreatic steatorrhoea.
Glasbrenner et al, 1991 [151]	123	CP=137	indirect pancreatic function test using fluorescein dilaurate	Folic acid and Vitamin B12	7 patients (5.7%) with CP and PEI had vitamin B12 serum levels below 190 pg/ml. 5 patients (3.6%) with chronic pancreatitis had folate serum levels below 2.4 ng/ ml; 3 of these had severe and 2 had moderate exocrine insufficiency.
Nakamura et al, 1996 [146]	12	CP=12	NA	Vitamins A,D,E and K	Vitamin E level showed a significant correlation with the faecal fat excretion or the fat absorption rate.
Dutta et al, 1998 [152]	8	CP=28	(a) faecal fat excretion (b) secretin test or histological evidence of chronic pancreatitis	Zinc	Reduced zinc absorption and increased urinary zinc excretion in PEI
Haaber et al, 2000[65]	26	CP=32	meal-stimulated intraduodenal lipase <10% of lowest normal range). All had steatorrhoea	Serum levels of total calcium, phosphate, 25 (OH)D, $1.25(OH)_2D$, alkaline phosphatase, and parathyroid hormone	Low vitamin D level in both groups

Laszity et al, 2002 [153]	NA*	CP=35	NA*	Serum transthyretin, albumin, transferrin and C-reactive protein	Transthyretin levels decreased in 37%, transferrin in 27%, and albumin in 12% of CP patients.
Vaona et al, 2005 [154]	27	CP=38	Collection of duodenal juice	Selenium	Low serum level in CP, significantly low in selenium serum levels in severe and moderate PEI.
Girish et al, 2009 [155]	67	CP=101	FEL-1	Zinc	Zinc was lower in CP and correlated with FEl-1
Sobral_Oliveira et al, 2011 [156]	NA*	CP=20	NA*	Haemoglobin, serum albumin, liver and pancreatic enzymes, serum lipids, iron, zinc, phosphate, magnesium, calcium, vitamin D and vitamin B12 white blood cell count, C-reactive protein, serum amyloid A and leptin	Low Magnesium, cholesterol, LDL in CP.
Lindkvist et al, 2012[150]	38	CP=114	(13)C-mixed triglyceride breath test	Haemoglobin, mean corpuscular volume, lymphocytes, prothrombin time, and serum levels of total protein, albumin, prealbumin, retinol binding protein, cholesterol,	Magnesium below 2.05 mg/dL was significantly associated with PEI. Also, haemoglobin, albumin, prealbumin and retinol binding protein below lower limit of normal and HbA1C above upper limit of normal were associated with PEI in univariate analysis.

				triglycerides, amylase, folic acid, vitamin B12, HbA1C, transferrin, ferritin, magnesium and zinc	
Duggan et al, 2014 [64]	16	CP=62	FEL-1	Vitamins A, D and E	Vitamin A deficiency= 14.5%. Vitamin E deficiency= 24.2% (in CP including PEI)
Min et al, 2018 [145]	77	CP=91	Based on symptoms including steatorrhoea	Vitamin A, D, and E	35.2% (19/54) with vitamin A deficiency, 62.5% (55/88) with vitamin D deficiency, and 17.7% (9/51) with vitamin E deficiency
Vujasinovic et al, 2019 [157]	90	CP=150	FEL-1	Zinc	26% zinc deficiency. In the group of patients with zinc deficiency, 76.7% of patients had an exocrine pancreatic insufficiency (FE-1 < 200 μg/g).

PEI, pancreatic exocrine insufficiency; **CP**, chronic pancreatitis; NA, Not stated or not tested

The diagnosis of PEI is commonly made with FEL-1 which has grown in acceptance as a less sensitive, but acceptable alternative to direct 'gold standard' tests which can be time-consuming, challenging to tolerate for the patient and difficult to standardize [62, 158]. FEL-1 testing for PEI is recommended in patients with diarrhoea or steatorrhoea and in primary pancreatic diseases with a high risk of PEI such as CP, cystic fibrosis [159] and other diseases associated with a lower pre-test probability [44, 46, 57, 83, 129, 160]. It has sensitivity 63% for mild, and 100% for moderate and severe PEI with specificity of 93%[84]. The very high pre-test probability of PEI in pancreatic cancer and the benefits of nutritional support has led to guidelines advocating treatment with enzyme replacement and omission of investigations for PEI [159].

3.3 Aims of the study

The primary aim of this study was to prospectively assess the yield of PEI-MD in patients with suspected benign pancreatic diseases. The secondary aim was to assess if micronutrient deficiencies could be used as a tool to test for PEI and prompt further testing in patients with suspected pancreatic pathology.

3.4 Methods

Patients referred to our centre with maldigestion symptoms investigated with FEL-1 testing for suspected or proven benign pancreatic disease were prospectively identified. Patients were excluded if under the age of 18, taking PERT, and those who had a pancreatic mass or CT scan suspicious of cancer or requiring a biopsy.

3.4.1 Micronutrients markers

At the time of FEL-1 testing, PEI associated micronutrient serum levels were obtained for: prealbumin, RBP, copper, zinc, selenium, magnesium and later in the study lipid adjusted

vitamin E. Vitamin A level testing was not available for the study, however we indirectly represented this with RBP levels (the carrier for retinol in the blood) which has been shown to predict vitamin A deficiency with high sensitivity and specificity when low [161, 162].

3.4.2 Data collection

Demographic information was collected for age, gender, BMI, presence of DM, smoking and alcohol intake. FEL-1 was recorded with a result <200µg/g considered diagnostic of PEI. A second sample was performed for patients who did not have a FEL-1 result available within the last 3 months or when no cause was identified for a PEI diagnosis. All FEL-1 samples were analysed in our centre with a commercially available enzyme linked immunosorbent assay (ELISA) test which uses two monoclonal antibodies against specific epitopes of human pancreatic elastase (ScheBo- Tech, Wettenberg, Germany).

As part of a clinical pathway patients underwent further investigations including CT imaging when there was a clinical suspicion of CP, a new diagnosis of PEI made on the stool sample, recurrent pancreatic type pain (epigastric abdominal pain radiating to back with or without previous acute pancreatitis attacks) [90]. CT scans of the pancreas were examined for evaluation of CP changes and severity using Cambridge classification [92, 98, 163].

3.4.3 Statistical analysis

All results were reported as mean and standard deviation (SD) or 95% confidence interval for continuous variables. Micronutrients were reported as proportion of abnormal value. Categorical variables were analysed using Fisher's exact test. Binary logistic regression was used to assess association of variables with PEI. Statistical significance was considered when $p \leq 0.05$. Statistical analyses were performed using SPSS (IBM SPSS statistics version 25, Chicago, Illinois, USA). The study was approved by local research ethics committee (STH20142/ IRAS239539) and informed consent was obtained from all patients, appendix 2.

3.5 Results

Cohort characteristics

After exclusion, 112 patients were recruited that underwent FEL-1 testing and micronutrient assay, as shown in table 3.2. PEI was identified in 41 patients and pancreatic imaging was performed in 82 patients. There were 19 patients recruited also that underwent Vitamin E assay when it became available.

Table 3.2 Demographics of patients with suspected or proven benign pancreatic disease investigated with Faecal Elastase 1.

	PEI (FEL-1<200µg/g) **n=41** **Mean ± (SD) or n (%)**	**No PEI** (FEL-1>200µg/g) **n=71** **Mean ± (SD) or n(%)**	**P value**
Age, years	58.2 (13.2)	57.4 (5)	0.8
Female	14 (34%)	44 (61%)	***0.006***
BMI kg/m^2	26.1± (5.9)	29.9 ± (7.1)	***0.004***
DM1 **DM2**	9 (22.0%) 13 (31.7%)	11 (15.4%) 26 (36.6%)	0.4
Alcohol unit/week	17.7 ± (22.9)	12.3 ± (10.5)	0.2
Pack year smoking	22.3 ± (21.5)	21.6 ± (20.5)	0.9
Morphological changes of CP*	22/41 (53.7%)	10/41 (24.3%)	***0.02***

PEI, pancreatic exocrine insufficiency
*All patients with PEI and 41 of patients without PEI but suspected pancreatic disease underwent cross-sectional imaging.

The aetiology of PEI was attributed to alcohol-induced chronic pancreatitis, (n=9), idiopathic chronic pancreatitis (n=4), pancreatic atrophy (n=9), and (n=19) with no CP changes or identifiable cause were labelled as ‘idiopathic PEI’. The patients with ‘idiopathic PEI’ were noted to have a history of smoking in 10/19 and alcohol consumption in 11/19 (mean 25.1 ± 28.3 units per week).

Prevalence of micronutrients deficiency

Overall, a micronutrient deficiency was identified in 21/112 (18.8%) patients, table 3.3. There were significantly more patients with a micronutrient deficiency if PEI was present 17/41(41.5%) than those without 4/71(5.6%), $p<0.0001$. Individual micronutrient assessment showed a significantly higher prevalence of prealbumin, selenium and magnesium deficiency when PEI was present.

The micronutrient deficiency occurred as a solitary finding in 16 patients, 2 micronutrient deficiencies in 4 patients and 3 micronutrient deficiencies in 1 patient.

There was a significant higher prevalence of patients with a micronutrient deficiency if severe PEI (FEL-1 0-100µg/g) was present: 56.5% (13/23), compared to when moderate PEI (FEL-1=100-200 µg/g) was present: 22.2% (4/18), p=0.05.

A significantly higher prevalence of patients with a micronutrient deficiency with a low or normal BMI (<25kg/m^2): 29.7% (11/37) was identified compared to a raised BMI (>25kg/m^2): 13.3% (10/75), p=0.04.

C-reactive protein (CRP) was measured in all patients with no significant differences in mean CRP values between patients with PEI (5.6 ± 8.2) and those without PEI (5.1 ± 7.7), p=08. Similarly, albumin level was not significantly different between PEI (44.9 ± 4.4) and those without PEI (44.9 ± 4.4), p=0.8.

Table 3.3 Prevalence of micronutrients deficiency* in 112 patients with suspected pancreatic disease. Grouped according to the presence of pancreatic exocrine insufficiency (PEI) (FEL-1<200µg/g) and chronic pancreatitis (CP). Significant differences identified are shown if p value ≤0.05 (fishers test).

	PEI	**no PEI**	***P value***	**CP and PEI**	**CP no PEI**	**PEI no CP**	**no CP no PEI**
n in group	41	71		22	10	19	61
Micronutrients							
Prealbumin	8 (19.5%)	2 (2.8%)	*0.005*	6 (27.3%)	0	2 (10.5%)	2 (3.2%)
RBP	0	0		0	0	0	0
Selenium	6 (14.6%)	1 (1.4%)	*0.01*	5 (22.7%)	0	1 (5.3%)	1 (1.6%)
Zinc	0	0		0	0	0	0
Copper	2 (4.9%)	0		1 (4.5%)	0	1 (5.3%)	0
Magnesium	5 (12.2%)	1 (1.4%)	*0.02*	3 (13.6%)	0	2 (10.5%)	1 (1.6%)
Vitamin E (n=19)	5/12 (41.7%)	1/7 (14.3%)		5/8 (62.5%)	0/5	0/4	1/2 (50%)
Total patients with at least 1 deficiency	17(41.5%)	4 (5.6%)	*0.0001*	13 (59.1%)	0 *p=0.002***	4 (21.1%) *p=0.03****	4 (6.6%)

*Micronutrient deficiency defied as level below defined laboratory range: prealbumin 0.2–0.5 g/l; retinol-binding protein (RBP) 20-40 mg/l; copper 11.0–27.2µmol/l; zinc 7.2–20.43 µmol/l; selenium 0.61–1.24 µmol/l; magnesium 0.7-1.0 mmol/l; and lipid adjusted vitamin E 3.9-5.9 µmol/l.

**Comparing patients with CP and PEI to patients with CP without PEI

***Comparing patients with CP and PEI to patients with PEI without CP

Micronutrient deficiency as a prediction of pancreatic enzyme insufficiency

The accuracy of using the significant micronutrients identified as a predictor of PEI showed a positive predictive value of 80-85.7% and a low sensitivity of 9.8-19.5%, table 3.4.

Table 3.4 Assessment of significant micronutrients to predicting PEI compared to FEL-1 <200µg/g

	Pre-albumin	**Selenium**	**Magnesium**
Sensitivity	19.5% (CI=8.8-34.9)	14.6% (CI=6.6-33.7)	9.8 % (CI=3.3-21.4)
Specificity	97.2% (CI=90.2-99.7)	98.5% (CI=92.1-99.7)	98.6% (CI=92.4-100)
PPV	80.0% (CI=47.1-94.7)	85.7% (CI=42.1-99.6)	83.3% (CI=37.6-97.7)
NPV	67.7% (CI=64.2-71)	66.7% (CI=56.8-75.6)	60.3% (CI=58.1-62.2)
Accuracy	68.8% (CI=59.3-77.2)	67.9% (CI=58.4-76.3)	61.5% (CI=52.2-70.1)

PPV=positive predictive value; NPV=negative predictive value. CI= 95% confidence intervals

3.6 Discussion

Findings in this prospective, clinical based study showed that specific testing for micronutrients associated with PEI in patients with suspected pancreatic disease has a high yield, with (42%) of patients showing at least one deficiency when PEI is present. The prevalence of micronutrient deficiencies was significantly higher in patients with PEI than those without (7%) confirming the association from previous studies but was predominantly higher when PEI and radiological CP changes co-existed. The significant, individual micronutrient deficiencies identified when PEI was present were selenium, magnesium and pre-albumin, and further analysis identified them as a strong predictor of PEI in patients with suspected pancreatic disease. Data suggest including selenium, magnesium and prealbumin when testing for malnutrition in patients with or with suspected PEI.

The association of the micronutrient deficiencies used in this study with PEI is also strengthened by the increased prevalence of PEI-MD with lower levels of FEL-1 suggesting that malabsorption is a key component. Malabsorption as a possible cause of PEI-MD would also be supported by the higher prevalence of micronutrient deficiencies when a patient has a low or normal BMI compared to a raised BMI.

The micronutrients chosen to represent PEI-MD in this study were identified from previous studies and based on clinical availability. The results are supported by previous studies showing an association of low selenium, magnesium and prealbumin in patients with PEI [150, 154, 156]. There was variation in findings for zinc deficiency when compared to other studies with no patients showing a low zinc level. A recent study of 150 patients with CP including 80 with PEI identified a zinc deficiency in 26% with a deficiency significantly associated with age and smoking but not PEI [157]. The absence of zinc deficiency in CP patients may be in part due to our cohort containing only 28% (32/112) of patients with CP identified on CT and a lower recommended laboratory cut off for zinc deficiency of 7.2 µmol/l used in our study compared

to 11µmol/l used in this recent study of CP patients [157]. Although I was unable to recommend zinc to be included, previous studies would suggest testing to have a high yield in CP patients and be included in nutritional assessment in patients with CP. Re-analysing data using 11µmol/l as a cut off would have identified 23 patients with low Zinc levels but with a greater proportion in the no PEI group (16/23). Therefore, this would not change the recommendation in our study and reflect the different analyser calibration used by our centre.

Only 2 patients were identified with a low copper level (both with PEI). The utility of copper as a micronutrient has been studied previously and a high level rather than low level being observed in a cohort of patients with alcohol-induced CP compared to healthy control, reflecting the inflammatory state of CP rather than malabsorption nature of PEI therefore I feel this is unhelpful to be included as a PEI-MD[164].

A significant yield of low prealbumin levels was detected in PEI (20% in PEI compared to 3% without PEI) which has been previously reported but in the same studies low RBP levels were also noted in patients with CP [150, 153]. I did not detect any low levels of RBP. Both RBP and prealbumin are proteins produced by the liver, pancreas, and visceral adipocytes [165, 166]. Increased levels of RBP have been shown to be associated with DM [167, 168]. The high prevalence of obesity and DM may explain the absence of RBP deficiency in our cohort.

RBP was included because vitamin A or retinol was unavailable to the patients in the study, and it was felt more appropriate to use RBP in a clinical based or practical study. Retinol is unstable when exposed to heat or light and early studies showed good correlation between concentrations of RBP and those of retinol [169], RBP also has been shown to have a reasonable sensitivity and specificity in predicting vitamin A deficiency [161, 170]. RBP has been used as a surrogate marker for vitamin A status in patients and may be a simple, inexpensive tool for assessment of vitamin A deficiency in population studies [171] [162], however RBP was unhelpful as a PEI-MD in our patients.

We gain access to a vitamin E testing for patients later in the study with a deficiency noted again in the PEI and CP group however the low numbers prevented any significant conclusions and a further study required to test this fully. Low levels of vitamin E would be expected given the reduction of pancreatic lipase [172], also the lower production of pancreatic bicarbonate would further reduce lipase activity [173]. Most previous studies of vitamin E were in patients with CP with or without PEI [64, 144-146] with a similar picture developing in this cohort.

Although vitamin D is a fat soluble vitamin I chose not to class it as a PEI-MD as it has been shown to be non-specific to PEI and significantly prevalent in the adult population in general, therefore although it may be a tool to include for general malnutrition assessment I did not class it as a PEI-MD [64, 147].

Although both groups had high mean record of BMI, patients with no PEI demonstrated a higher BMI which could reflect the malabsorption consequences of PEI. We found higher prevalence of female patients with no PEI. This could reflect the higher prevalence of CP in PEI group compared to no PEI with the known associated higher incidence rate of male in CP [174].

The strength of the study is in the nature of the cohort because patients were recruited in a clinical setting according to their symptoms rather than pancreatic morphology which allowed to include other 'at-risk' groups for PEI including DM. In contrast to the previous studies investigated the prevalence of micronutrient deficiency in PEI which were exclusively in CP patients. The findings of PEI despite no radiological features of CP or pancreatic atrophy are not unique. No radiological CP features (on EUS or CT) reported in 5%-32% of patients with a low FEL-1 [43, 175, 176].

We acknowledge that using FEL-1 to diagnose PEI is less accurate compared to 'gold standard' tests, but it was considered an acceptable alternative. FEL-1 has become the first line investigation for PEI because it is inexpensive, relatively easy to perform, and widely available.

Furthermore, direct 'gold standard' tests can be challenging to patients and staff, time-consuming, and expensive [158, 177-179]. The limited sensitivity of FEL-1 to detect PEI in mild to moderate PEI [84] could be supported by the high PPV and specificity of PEI-MD (selenium, magnesium and prealbumin), therefore, the deficiency of these micronutrients would consider retesting or follow up for possible PEI developing as in the 4 patients with a deficiency but without PEI or CP which may be a focus of further studies.

We used laboratory cut off for micronutrients abnormal level in our analysis rather than measuring means. We felt the use of binary report (normal or abnormal) is more relevant in clinical practice.

We also acknowledge that given the clinical nature of the study a number of patients did not have CT scans performed given the lack of clinical findings and normal FEL-1. These patients were presumed to not have CP, however, few patients with radiological changes of CP could have been missed as changes of CP has been found in up to 12% in post-mortem prevalence studies[23, 180]. The data with the patients without CP and no PEI that had a CT (31 of the 71 patients without PEI) were reanalysed as they could have been in a more symptomatic group, but this also showed no new differences or findings.

In conclusion, micronutrient deficiency is common in PEI, especially in those with CP. We recommend testing for these markers in PEI and consider retesting for PEI if deficiency occur Whilst micronutrient deficiency appears to be less frequent in PEI without CP, further larger studies is required to determine if micronutrient levels should be measured in all patients with PEI.

CHAPTER 4: CAN SKELETAL MUSCLE INDEX MEASUREMENT BE USED AS AN ADJUNCTIVE TOOL FOR THE DIAGNOSIS OF PANCREATIC EXOCRINE INSUFFICIENCY AND MALNUTRITION?

4.1 Summary

Introduction: PEI and subsequent malnutrition can be difficult to diagnose but lead to sarcopenia and increased mortality and morbidity even in benign diseases. Sarcopenia is defined as the loss of muscle mass which in turn results in loss of muscle strength and a decline in functional quality. Digital skeletal muscle analysis has been increasingly recognised as a tool to diagnose sarcopenia. The aim of this chapter is to assess the prevalence of sarcopenia in patients with PEI secondary to benign pancreatic diseases using novel skeletal muscle recognition software.

Methods: Prospective recruitment of patients referred for EUS with suspected pancreatic pathology. Patients with suspected pancreatic cancer on initial CT were excluded. The diagnosis of CP was based on CT and EUS findings. PEI was assessed with FEL-1. Digital measurement of skeletal muscle mass identified sarcopenia, with demographic and comorbidity data also collected.

Results: PEI was identified in 45.1% (46/102) of patients recruited and 29.4% (30/102) had changes of CP. Sarcopenia was significantly more prevalent in PEI 67.4% (31/46) compared to no PEI 21/56 (37.5%) regardless of CP changes ($p<0.003$). The prevalence of sarcopenia (67% versus 35%; $p=0.02$) and sarcopenic obesity (68.4% versus 25%; $p=0.003$) were significantly higher when PEI was present without a radiological diagnosis of CP. Multivariate analysis identified sarcopenia and diabetes to be independently associated with PEI (odds ratio 4.8 and 13.8 $p<0.05$). The sensitivity, specificity, positive predictive value of using sarcopenia as diagnostic tool for PEI in CP was 71.4%, 88.5% and 83.3% respectively.

Conclusion: Sarcopenia was strongly associated with PEI in patients undergoing assessment for suspected benign pancreatic pathology. Digital skeletal muscle assessment can be used as a tool to aid identification of sarcopenia in patients undergoing CT scan for pancreatic symptoms.

4.2 Introduction

PEI is associated with malabsorption and malnutrition. Sarcopenia has been widely used as a marker of diagnosing malnutrition [181]. It is associated with loss of muscle strength and decline in physical performance [110]. Historical anthropometric tests such as mid-arm circumference have been associated with low accuracy and significant inter-observer variability [182]. Also, historical anthropometric tests failed to assess for myosteatosis, which is a marker of muscle quality defined by an increased amount of fat within skeletal muscle [183]. Sarcopenia has been shown to be associated with increased mortality and poor outcomes for patients with cancer and those undergoing surgery, but it also has been shown to be reversible with interventions such as physical exercise or chemotherapy in patients with pancreatic cancer [184, 185]. In the last decade the 'digital' recognition of skeletal muscle mass by using CT images has been developed. Coupling the digital skeletal muscle recognition technique with the guidance that CT imaging is recommended for the assessment of patients with suspected pancreatic disease makes digital identification of sarcopenia an intuitive method in assessing the nutritional status of patients with PEI [108].

BMI is widely used to assess for malnutrition, however, sarcopenia is independent of BMI and can be present in underweight and overweight patients [111]. There is a risk of overlooking malnutrition in obese patients due to the longstanding misconception that all malnourished patients are underweight, this is compounded by that prevalence of obesity in the UK which has almost doubled over the last two decades [186].

4.3 Aims of the study

The primary aim of this study was to prospectively assess the prevalence of sarcopenia using a novel 'digital' skeletal muscle recognition software in patients with and without PEI secondary to benign diseases. The secondary aim was to assess if 'digital sarcopenia' is associated with PEI in patients with suspected pancreatic pathology.

4.4 Methods

4.4.1 Patients and data collection

Patients referred to our unit for further assessment of suspected or proven benign pancreatic disease undergo an EUS as part of their clinical assessment pathway. Patients attending for EUS were prospectively identified if there was a previous diagnosis or clinical suspicion of CP or recurrent pancreatic type pain (epigastric abdominal pain radiating to back with or without previous acute pancreatitis attacks) as part of the referral criteria. Patients were recruited if they had already undergone a CT scan of the pancreas and completed the EUS examination as part of their existing clinical pathway [90].

4.4.1.1 Inclusion criteria for study:

- Age 18 or over
- Patients referred for EUS for chronic pancreatitis assessment based on CT and FEL-1 testing.
- Patients referred for EUS for investigation of abdominal pain without a cause found on assessment with CT

4.4.1.2 Exclusion criteria for the study group include

- Patients under 18 years old
- Patients with known solid pancreatic lesion
- Pancreatic enzyme replacement therapy (PERT) for longer than 1 month
- Patients referred for EUS with indications other than abdominal pain or suspicion of chronic pancreatitis
- Patient without a CT scan or with significant artifact seen at the 3rd lumbar vertebrae (L3) level.

4.4.3 Data collection

Demographic information was collected for age, gender, BMI, presence of diabetes mellitus (DM), smoking and alcohol intake. A stool sample tested for FEL-1 was recorded with a result < 200 µg/g considered diagnostic of PEI. A second sample was performed for patients who had not had a recent FEL-1 sample sent (<3 months) or a second sample requested when no cause was identified for a PEI diagnosis. All FEL-1 samples were analysed in our centre with a commercially available enzyme linked immunosorbent assay (ELISA) test which uses two monoclonal antibodies against specific epitopes of human pancreatic elastase (ScheBo- Tech, Wettenberg, Germany).

4.4.2 Pancreatic morphology assessment

Patients underwent the EUS examination by 2 independent endosonographers with the number of EUS features of CP recorded for each patient along with other relevant findings of the examination, see table 1.4. EUS was performed under conscious sedation using a linear array echoendoscope (Olympus GF-UCT260) with full visualisation and documentation of pictures of the pancreatic head, uncinate process from the duodenum and the pancreatic body and tail from the stomach with any features of CP recorded during the exam. The CT scans of the pancreas were re-examined by an independent radiologist blinded to the indication and findings of EUS for evaluation of CP changes and severity using Cambridge classification [92, 98, 163]. A final radiological diagnosis of CP was made if patients had ≥ 5 EUS features of CP or 3- 4 EUS features and a Cambridge classification of ≥3 on CT. Patients with 3 or 4 EUS criteria and equivocal CT analysis were classed as ‘indeterminate for CP’ for the purposes of the study. Those with 0-2 EUS features were considered normal [96]. The information obtained also allowed presence of CP to be classified using the Rosemont EUS classification and M-ANNHEIM severity index, see table 1.6.

4.4.3 Computerised Tomography image analysis and body composition measurements

All CT scans were performed in our centre within 3 months of the EUS examination (Canon Aquilion One scanners,120 kv, slice thickness 1mm) using intravenous contrast and examined in the venous phase.

I analysed CT images for sarcopenia using a commercially available software (*sliceOmatic* 5.0 developed by Tomovision, Quebec, Canada). The procedure of body compositions analysis performed in the following stepwise manner:

1. Choose portal venous contrast phase axial slices.
2. Count downwards starting at T12 and scroll down to the midlevel of the L3 vertebra. At the midlevel of the vertebra transverse processes and spinous process are usually both seen, figure 4.1

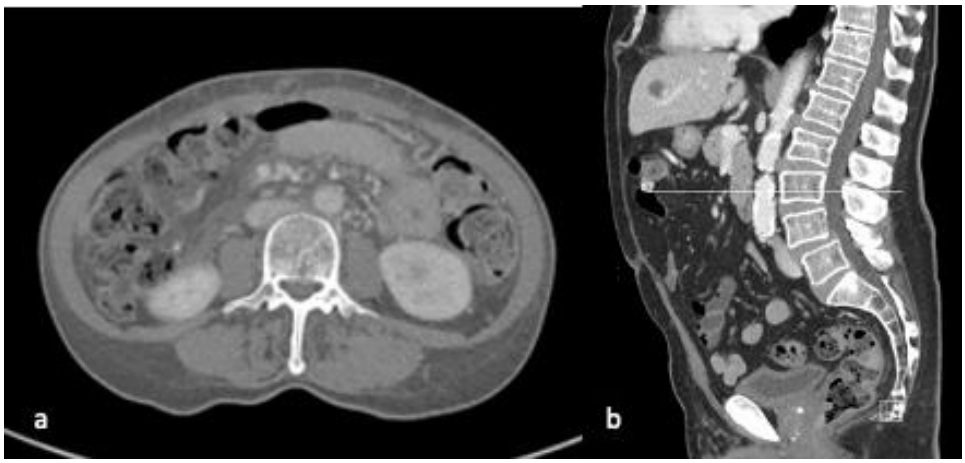

Figure 4.1 Slice of computed tomography at the third lumbar vertebra showing (**a**) axial view and (**b**) sagittal view at the third lumbar vertebra

3. Remove patient details and export the chosen slice as DICOM. Each CT slice should have a study number
4. Import chosen slice into sliceOmatic for body segmentation.
5. Conduct segmentation based on anatomical landmarks and Hounsfield units (HU), figures 4.2, 4.3, and 4.4

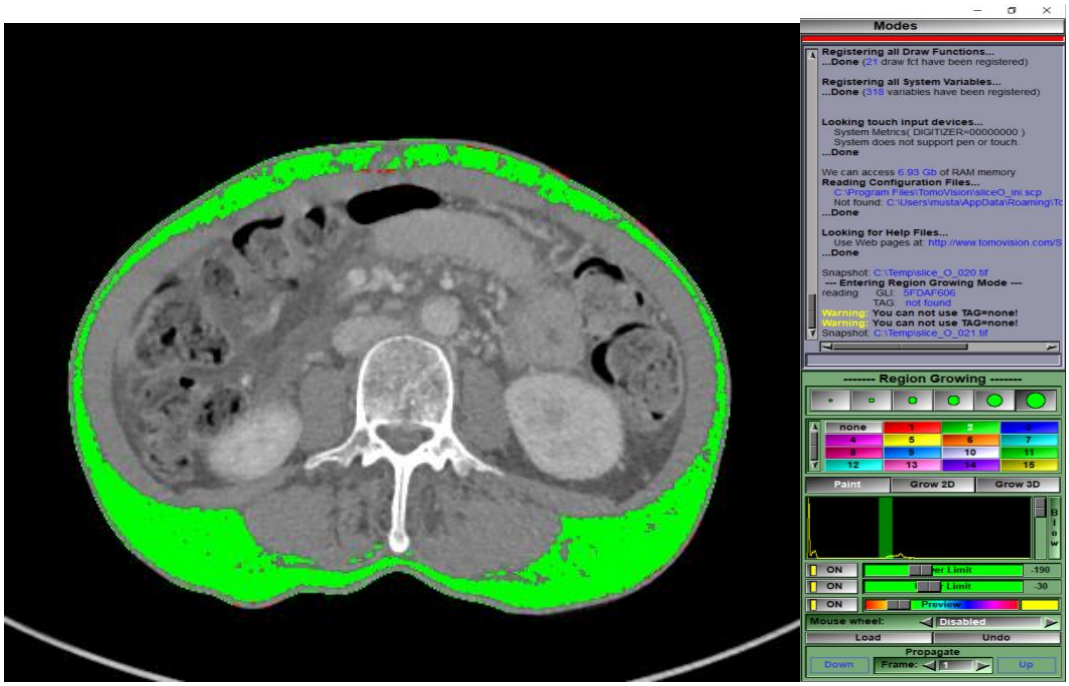

Figure 4.2 Digital body composition analysis showing subcutaneous adipose tissue (SAT) (green colour): Tissue between skin and superficial fascia between -190 and -30 HU.

Figure 4.3 Digital body composition analysis showing skeletal muscle (SM) (red colour): psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal oblique abdominis, and rectus abdominis muscles between -29 and 150 HU. It also shows subcutaneous adipose tissue in green.

Figure 4.4 Digital body composition analysis showing visceral adipose tissue (VAT) (yellow colour): Tissue between transversalis fascia and peritoneum between -150 and -50 HU. It also showing subcutaneous adipose tissue in green and skeletal muscle in red.

6. The surface areas of skeletal muscle, subcutaneous adipose tissue and visceral adipose tissue were then normalised using the square of patient's height to calculate the relevant body composition index measurements: skeletal muscle index (SMI), subcutaneous adipose tissue index (SATI) and visceral adipose tissue index (VATI) (expressed as cm^2/m^2).

7. Myosteatosis (as a measure of muscle attenuation) was measured as the mean Hounsfield units (HU) of all skeletal muscle area at L3.
8. Sarcopenia was defined as a SMI <41 cm^2/m^2 for women, and SMI <43 cm^2/m^2 if BMI <25 kg/m^2 or <53 cm^2/m^2 if BMI ≥25 kg/m^2 for men. Myosteatosis was measured as the mean Hounsfield units (HU) of all skeletal muscle area at L3 and was defined as <33 HU in patients with BMI ≥ 25, and <41 HU in patients with BMI <25

kg/m^2 [187]. Using previous accepted definitions, sarcopenic obesity in this study was referred to the presence of both sarcopenia and a BMI ≥25 kg/m^2, figure 4.5 [188].

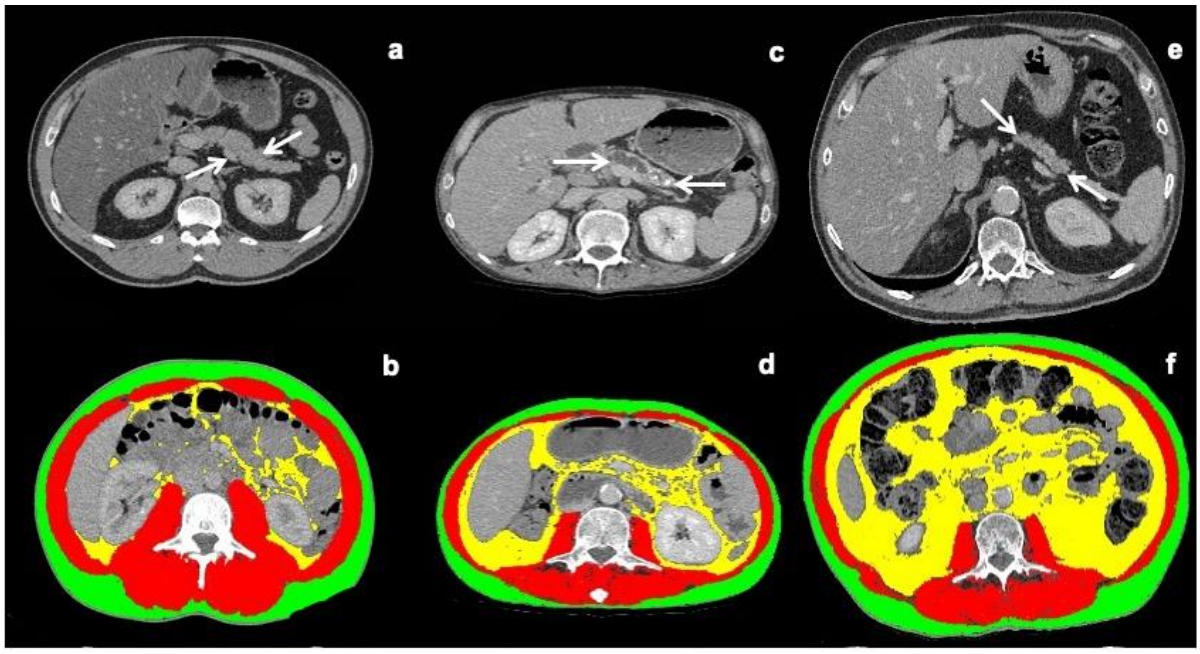

Figure 4.5 Digital body composition analysis: (a) Patient with abdominal pain and normal pancreas. (b) Digital muscle mass assessment at the L3 level showing normal ratios of skeletal muscle (red) subcutaneous adipose tissue (green)1and visceral adipose tissue (VAT) (yellow) (c) Patient with chronic pancreatitis on CT and PEI on testing, (d) analysis showing low skeletal muscle index diagnosing sarcopenia. (e) Patient with PEI but raised BMI and atrophic pancreas. Analysis (f) shows raised VAT and low skeletal muscle index diagnosing sarcopenic obesity and raised muscle fat content 'myosteatosis' (grey areas within the red).

4.4.4 Ethical Approval

The study was approved by local research ethics committee (IRAS 210710, STH 19471) and informed consent was obtained from all patients, appendices 3 and 4.

4.4.5 Statistical analyses

All results were reported as median and IQR for continuous variables. The Mann Whitney U test was used for comparison of continuous variables. Categorical data were compared using the Fisher's exact test. Chi square test was used to calculate the difference between groups by prevalence of sarcopenia according to Rosemont criteria and M-AANHEIM criteria. Linear

regression analysis with Pearson's correlation coefficients (r) was used to analyse the correlation of FEL-1 as a continuous variable with SMI and BMI. Binary logistic regression was used to assess association of variables with PEI. Statistical significance was considered when p <0.05. Statistical analyses were performed using SPSS (IBM SPSS statistics version 25, Chicago, Illinois, USA).

4.5 Results

4.5.1 Patient demographics

A total of 102 patients were prospectively identified and included in the final analysis with 17 other patients excluded due to no available CT scan or no adequate stool samples for FEL-1 measurement. There were 29.4% (30/102) of all patients identified as having radiological evidence of CP, table 4.1. PEI was identified in 45.1% (46/102) of all patients using FEL1<200 (median 59, IQR 20-115) µg/g. The causes of PEI in the 46 patients were attributed to alcohol-induced CP (n=15), idiopathic CP (n=6), hereditary CP (n=1), pancreatic atrophy (n=10) and a remaining group with no CP changes or identifiable cause which were labelled as 'idiopathic PEI' (n=14). The patients with 'idiopathic PEI' were noted to have a history of smoking in 10/14 and alcohol consumption in 11/14 (median 24 units per week; range 10-140). Immunoglobulin G4 levels were negative (<1.3 g/L) in all 14 patients with idiopathic PEI and all 6 patients with idiopathic radiological CP changes. The median BMI of all patients was (25.6, IQR 22.7- 32.8) kg/m^2, and 56.9% (58/102) of patients had a BMI>25 kg/m^2 and 32.4% (33/102) had a BMI of >30 kg/m^2.

Of the patients recruited, 16 were identified with DM. Sarcopenia was prevalent in 66.7% (4/6) of DM1 patients, and 77.8% (7/9) of DM2 patients and 1 patient had pancreatogenic diabetes (type 3) due to severe CP with an FEL-1 of 20 µg/g and sarcopenia. Four patients with DM were taking metformin at time of recruitment and all of them were DM2 with FEL-

1 <200µg/g. Three patients with DM had bloating and abdominal pain and one patient had abdominal pain and diarrhoea.

Table 4.1 Demographics and anthropometric characteristics of patients based on presence or absence of radiological evidence of chronic pancreatitis

	Radiological evidence of chronic Pancreatitis %(n) or median (IQR)		**P value**	**No radiological evidence of chronic pancreatitis %(n) or median (IQR)**		**P value**
	Low FEL-1	**no PEI**		**Low FEL-1**	**no PEI**	
n	22	8		24	48	
Age, years	53 (46-65)	76 (56-80)	0.05	57 (51-66)	48 (42-64)	0.1
Sex, F/M	6/16	5/3	0.4	10/14	31/17	0.08
BMI	21.6 (19.2-24.4)	33.2 (22.7-35.5)	***0.01***	26.3 (25.1-30.9)	27.9 (23.6-34.6)	0.5
BMI<18.5 kg/m^2	13.6 (3)	0 (0)	0.5	0 (0)	2.1 (1)	1.0
BMI >25 kg/m^2	9.1(2)	62.5 (5)	***0.007***	79.2 (19)	66.7 (32)	0.4
BMI >30 kg/m^2	9.1 (2)	50 (4)	***0.03***	45.8 (11)	33.3 (16)	0.3
Sarcopenia	68.2 (15)	50 (4)	0.4	66.7 (16)	35.4 (17)	***0.02***
Myosteatosis	54.5 (12)	37.5 (3)	0.7	50 (12)	31.3 (15)	0.07
Sarcopenia and myosteatosis	40.9 (9)	25 (2)	0.7	37.5 (9)	12.5 (6)	***0.03***
Sarcopenic obesity*	50 (1/2)	20 (1/5)	1.0	68.4 (13/19)	25 (8/32)	***0.003***
SATI	31.5 (12.5-61.7)	84.3 (43.9-118.1)	***0.05***	57.2 (45.9-66.76)	75.1 (53.3-151.4)	0.2
VATI	29.6 (4.8-52.5)	44.9 (61.2-234.3)	0.2	49.7 (26.7-66.8)	35.8 (13.9-63.9)	0.1
DM	30 (7)	12.5(1)	0.4	25 (6)	4.2 (2)	***0.009***
Smoking (Current or past) **Pack-year history**	76.2 (16) 16.5 (14-30)	50 (4) 10 (8-50)	0.4 0.4	54.2 (13) 20 (8.5-32.5)	47.9 (23) 12.5 (7.75-19)	1.0 0.4
Alcohol (units per week)	3 (1-38)	1 (0-16)	0.9	10 (3-18.5)	5 (1-10)	0.6
Pancreas atrophy on radiology				41.7 (10)	4.2 (2)	***0.0002***
Median number of EUS features	5 (4-5)	4 (3-4)	***0.04***	1 (0-2)	1 (0-1.75)	0.5

PEI, pancreatic exocrine insufficiency is defined when FEL-1 <200µg/g; **F**, female; **M,** male; **BMI**, body mass index; **SATI**, subcutaneous adipose tissue index; **VATI**, visceral adipose tissue index; **DM**, diabetes mellitus; **EUS**, endoscopic ultrasound. *Sarcopenic obesity is defined by the presence of sarcopenia and BMI >25 kg/m^2

4.5.2 Digital body composition analysis

The mean time taken for analysis of one slice of CT with the sliceOmatic software was 2.5 minutes. Looking at the whole cohort, significant increases in the prevalence of body

composition abnormalities were identified in PEI compared to no PEI regardless of CP changes, this included sarcopenia: 67.4% (31/46) versus 37.5% (21/56); p<0.003, myosteatosis: 52.2% (24/46) versus 10.7% (18/56); p=0.046, and 'sarcopenic obesity' in: 66.7% (14/21) versus 24.3% (9/37); p=0.002.

The comparison of FEl-1 with SMI and BMI as a continuous variable is shown in figure 4.6. There was no correlation between FEL-1 and SMI r=0.08, p=0.4, and a significant slightly positive-association with BMI (r=0.3, p=0.002).

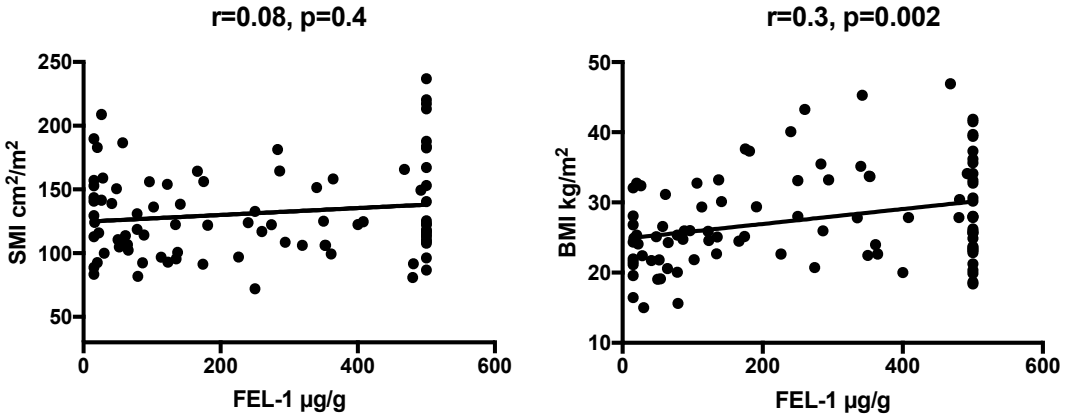

Figure 4.6 Correlation of FEL-1 level with skeletal muscle index (SMI) and body mass index (BMI) in patients being assessed for suspected benign pancreatic disease.

4.5.3 Patients with radiological evidence of chronic pancreatitis

In total, 30 patients had radiological evidence of CP on EUS and/or CT scan with 73.3% (22/30) found to have low FEL-1, table 4.1. Patients with CP and low FEL-1 had a significantly lower median BMI (21.6 versus 33.2) kg/m^2 and lower prevalence of obesity (BMI>30: 9.1% versus 50.0%). In CP the prevalence of sarcopenia was not significantly higher between low FEL-1 and no PEI: 68% versus 50%. The median number of EUS features of CP was higher in low FEL-1 compared to no PEI: 5 versus 4, (p=0.04). The prevalence of sarcopenia was similar between calcified CP and non-calcified CP, 75% (15/20) and 66.7% (4/6), P=1.0.

When using other classifications of CP, the prevalence of sarcopenia was significantly higher in those with a 'consistent with CP' Rosemont classification and lower in those with a 'Normal' pancreas, table 4.2. Similar findings were identified using M-ANNHEIM with 'Marked CP' having a significantly higher prevalence of sarcopenia than those with a Minor M-ANNHEIM grading. No patients were classified as M-ANNHEIM grade 'E'.

Table 4.2 The prevalence of sarcopenia according to Rosemont classification and M-ANNHEIM severity index

Rosemont Criteria	**Normal**	**Indeterminate**	**Suggestive**	**Consistent**	**P value**
n	67	6	6	24	
Sarcopenia % (n)	40.3 (27)	60 (3)	50 (3)	70.8 (17)	0.08
M-ANNHEIM grading	**A (Minor)**	**B (Increased)**	**C (Advanced)**	**D (Marked)**	**P value**
n	29	37	28	8	
Sarcopenia % (n)	27.6 (8)	51.4(19)	57.1 (16)	87.5 (7)	***0.01***

4.5.4 Patients with no radiological evidence of chronic pancreatitis

In total, 72 patients had no radiological evidence of CP on CT and/or EUS with 33.3% (24/72) found to have low FEL-1, table 4.1. Sarcopenia was more prevalent in patients with low FEL-1 (67% versus 35%; p=0.02). The presence of both sarcopenia and myosteatosis was significantly higher in patients with low FEL-1 (37.5% versus 12.5%; p=0.03). Patients with low FEL-1 had a significantly higher proportion of sarcopenic obesity compared to patients without low FEL-1 (68% versus 23%; p=0.003). A higher prevalence of DM and pancreatic atrophy on radiological tests in patients with no radiological evidence of CP.

The final diagnosis made in patients identified with no PEI and no CP (n=48) was most commonly found to be due to non-specific (functional or unexplained) abdominal pain (n=18), irritable bowel syndrome (n=7), gall stone disease (n=6), gastritis and peptic ulcer disease (n=5), bile duct dysfunction (n=7) or previous acute pancreatitis with no residual pancreatic disease (n=4). Sarcopenia was identified in 35% (17/48) of patients with no PEI and no CP; of these 17 patients with sarcopenia co-morbid conditions known to cause

sarcopenia were present in 7 (chronic obstructive airways disease, Crohn's disease and previous colorectal cancer, renal failure on dialysis, heart failure, liver cirrhosis and chronic autoimmune arthropathy), and a further 2 patients were identified with pancreatic atrophy.

4.5.5 Multivariate analysis

Univariate and multivariate logistic regression analysis is shown in table 4.3, only sarcopenia and a history of diabetes were strongly associated with low FEL-1 (odds ratio 4.8 and 13.8 respectively).

Table 4.3 Univariate and binary logistic regression analysis of factors associated with pancreatic exocrine insufficiency in patients undergoing assessment for suspected benign pancreatic pathology

	Univariate analysis		**Multivariate analysis**	
	Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value
Age, years*	1.02 (0.99-1.05)	0.2		
Sex, Female	0.3 (0.12-0.61)	***0.002***		
Presence of CP	6.0 (2.2-16.0)	**<0.0001**		
BMI*	0.9 (0.84-0.96)	***0.003***		
BMI >25 kg/m^2	0.46 (0.2-1.06)	0.07		
Presence of sarcopenia	3.6 (1.5-8.8)	***0.005***	4.8 (1.2-19.1)	***0.02***
Presence of myosteatosis	2.4 (0.9-2.6)	***0.05***		
SATI*	0.99 (0.98-1.0)	***0.04***		
VATI*	1.01 (0.99-1.02)	0.3		
Presence of diabetes	8.8 (2.4-33)	***<0.0001***	13.8 (1.2-158.8)	***0.03***
Pack-year smoking*	1.01 (0.99-1.04)	***0.04***		
Alcohol U/week*	1.02 (0.99-2.04)	0.14		

***Using these variables as continuous variables.
F, female; **M,** male; **CP**, chronic pancreatitis; **BMI**, body mass index; **SATI**, subcutaneous adipose tissue index; **VATI**, visceral adipose tissue index.

4.5.6 Accuracy of using sarcopenia as a diagnostic test for PEI

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of using CP, sarcopenia, or both sarcopenia and CP as diagnostic tool for diagnosing PEI, table 4.4.

Table 4.4 Assessment of accuracy of using sarcopenia in predicting PEI in patients with suspected benign pancreatic pathology

Statistic	CP Value (95% CI)	Sarcopenia Value (95% CI)	Sarcopenia and CP Value (95% CI)
Sensitivity	47.8% (32.9-63.1%)	67.4% (52-80.5%)	71.43% (47.8-88.7%)
Specificity	85.7% (73.8-93.6%)	67.9% (54-79.7%)	88.46% (69.9-97.5%)
PPV	73.3% (57.5-84.8%)	53.3% (52.8-72.6%)	83.3% (62.5-93.8%)
NPV	66.7% (59.8-72.9%)	71.7% (61.7-79.9%)	79.3 % (65.8-88.4%)
Accuracy	68.6% (58.7-77.5%)	67.7% (57.7-76.6%)	80.85% (66.7-90.9%)

PPV, Positive Predictive Value; **NPV**, Negative Predictive Value; **CP**, Chronic pancreatitis

4.6 Discussion

The study showed that the identification of sarcopenia using digital skeletal muscle analysis in patients with CP and suspected pancreatic pathology is feasible. The presence of sarcopenia is significantly associated with

PEI

in patients undergoing assessment for pancreatic type pain and symptoms even when morphological alterations of the pancreas are absent on radiological investigations. Results support digital skeletal muscle analysis as an adjunct nutritional assessment tool in this group of patients.

The importance of sarcopenia as a marker of malnutrition has been increasingly recognised [181], so too has its subsequent association with a poor prognosis in a number of medical and malignant conditions. The poor prognosis of sarcopenia identified on CT imaging has been previously demonstrated in a group of patients with mainly pancreatic cancer and following pancreatic surgery, and although investigators were using a CT scan to calculate the skeletal muscle mass a more 'manual outlining', labour intensive method was used to identify skeletal muscle [189]. The patients in this pancreatic cancer study had a low BMI in both PEI and no PEI groups, 21 kg/m^2 and 22 kg/m^2 respectively (extrapolated from given median height and weight), this is in contrast to our (non-cancer/surgery) cohort with benign pancreatic diseases with a median BMI of (25, IQR 21.8-28.7) kg/m^2 in PEI and (28, IQR 23.4- 34.6) kg/m^2 in no

PEI. These findings suggest that although sarcopenia is associated with PEI in benign and malignant pancreatic conditions, the underlying mechanism of sarcopenia in pancreatic cancer is multifactorial and progressive, and a more typical phenotype of malnutrition is presented leading to guidelines suggesting to start PERT soon after a diagnosis of pancreatic cancer with the benefits outweighing FEL-1 testing [28].

FEL-1 to diagnose PEI in were reanalysed in a number of ways. The initial analysis was similar to a clinical setting where the dichotomous use of FEL-1 to diagnose PEI was with a cut off of 200µg/g. However, as FEL-1 is a continuous variable and severity of PEI reflected by a lower FEL-1 level, a further analysis of sarcopenia rates using lower FEL-1 levels to reflect mild, moderate and severe PEI and a correlation of FEL-1 levels as a continuous variable with SMI and BMI using linear regression was performed. I identified no difference in the rates of sarcopenia within the 3 groups of low FEL-1 levels, and no correlation between FEL-1 and SMI when compared a continuous variable. This may reflect that sarcopenia may be multifactorial and not just due a consequence of PEI, an argument strengthened by the significant prevalence of sarcopenia in the group without CP or PEI. FEL-1 levels reported by our assay is between <15 µg/g and > 500g/g, therefore those with levels more than 500 µg/g were reported similarly which may weaken the use of FEL-1 as a continuous variable in analysis.

In addition to the methods used in this study in diagnosing CP, the prevalence of sarcopenia according to other popular validated grading systems including Rosemont classification and M-ANNHEIM severity index was assessed and have shown similar results of higher prevalence of sarcopenia in patients with Rosemont grade ‘consistent with CP’ and M-ANNHEIM ‘grade D’. The M-ANNHEIM system which encompasses all features of CP, has been increasing in use and also incorporates PEI as part of the grading. EUS-elastography is another possible modality to aid a diagnosis CP at EUS, however, given the limited single centre studies that

have reported its use so far with varying cut offs for diagnosis I did not incorporate it in the study design [96, 190].

Patients had CT scans performed in our centre using the same model of CT scanner. A bias for recruitment could have affected results as patients with more significant symptoms could lead to a more recent CT and favour recruitment. Other potential biases including using different scanners machines and images are reported by different radiologists. Our images were reanalysed by a single radiologist and all images were performed in our centre using the same CT scanners.

The finding of malnutrition in patients with PEI may be expected, however identification of malnutrition itself can be challenging in particular in overweight or obese patients. Patients with PEI and high BMI may conceivably look well-nourished, however, 'sarcopenic obesity' was a prevalent finding in PEI patients with no radiological evidence of CP (66.7%). BMI is commonly used in assessing nutritional status; however, it does not take into account different body compartments and therefore increases the risk of overlooking sarcopenia in overweight patients, therefore in this group 'digital sarcopenia' can aid the identification of sarcopenia and malnutrition. Patients with PEI but without radiological features of CP demonstrated a higher BMI and prevalence of sarcopenic obesity and pancreatic atrophy compared to patients with PEI and CP. There is some evidence to suggest that increased BMI can lead to pancreatic fat deposition and destruction of pancreatic acinar cells causing PEI, with one study reporting a PEI prevalence of 35.5% in patients with pancreatic steatosis based on MRI without any radiological features of CP [13, 191]. An atrophic pancreas on CT was a finding in patients with PEI. Radiological changes associated with atrophic pancreas may not qualify for the diagnosis of CP, however, previously reported studies have established an association between fatty replacement and atrophic pancreas with increased incidence of PEI [140, 192].

The association of DM in PEI has been increasingly recognised [43, 44] and both form part of the M-ANNHEIM classification system to grade the severity of CP [98]. Four patients with DM were taking metformin however only one had diarrhoea which although could lead to a false positive FEL-1 sample, a second sample was obtained when as no radiological cause of PEI was identified. There was an association between PEI and DM, however, the study was not designed to investigate if there was an association between sarcopenia and DM, nor was this association examined in multivariate analysis.

Patients with a FEL-1< 200 µg/g were identified in CP and in atrophic pancreas, but also in patients when no radiological diagnosis of CP or when pancreatic atrophy was present. The low FEL-1 could well be due a false positive tests for PEI when a cut off of 200 µg/g is used, and if this situation is encountered, significant efforts should be made to recheck or look for a secondary cause of PEI that has been previously described [193]. Another possible explanation is that PEI could precede the development of radiological changes and represent the early 'microscopic' changes of CP [194]. This phenomenon of low FEL-1 in non-CP has been reported previously in 3 studies demonstrating 5%-32% of patients with a low FEL-1 having no radiological CP features identified (on EUS or CT) [43, 175, 176]. Although it may not be correct to describe this as PEI due to the lack of pancreatic disease, a further study from our centre has shown an improvement in symptoms in 84.7% (72/85) with PERT when this situation is encountered [193].

Sarcopenia was shown to be associated with PEI on multivariate analysis however the mechanism of this has not been demonstrated. PEI could contribute to sarcopenia as a consequence of fat malabsorption and although it was not measured in this study, protein maldigestion in PEI has been demonstrated in porcine models [195]. A more multifactorial aetiology for sarcopenia would be supported by sarcopenia being identified in 35% of patients without PEI or CP. Sarcopenia has been demonstrated in other chronic diseases that were

identified in group without CP or PEI, these include chronic pulmonary disease, Crohn's disease, dialysis dependent renal failure, chronic liver disease and cardiovascular disease. These other causes should be considered as a cause for sarcopenia in patients along with advancing age, low physical activity, and alcohol dependence to contribute to sarcopenia when identified.

Digital analysis of sarcopenia using a patient's own CT scan has the advantage of providing rapid and objective measurement of muscle quantity in suspected cases of pancreatic pathology. This has been facilitated by the development of digital novel analysis software coupled with the abundant use of CT scanning globally which is increasing by 10% per year [196]. Consequently, nearly all patients referred for EUS examination for suspected pancreatic pathology had undergone a CT scan prior to referral as part of their standard care. One limitation of recruitment to study could be a number of patients with CP changes identified on a CT that were not referred to our unit (possibly due to lack of symptoms) and therefore not be identified or undergo EUS to be included in the study.

This study has a number of limitations. We did not assess muscle function using standard methods like hand grip, gait speed, or the new Newcastle SarcScreen proforma[197]. The most recent European guidelines defined Sarcopenia by the loss of muscle strength, quantity and quality[198]. Applying this definition, we would expect lower prevalence than the cuurent finding assuming not all patients have poor muscle function. Another limitation is using FEL-1 in diagnosing PEI. FEL-1 has a low sensitivity to detect mild to moderate reductions in pancreatic enzyme secretion, however the definition of PEI implies that pancreatic secretion needs to be low enough to disrupt normal digestion and absorption of nutrients therefore patients with a mild reduction may not be symptomatic or have developed sarcopenia. Alternative, more sensitive, direct 'gold standard' tests for PEI can be time-consuming, challenging to tolerate for the patient and difficult to standardize [158] but may have identified

further cases of mild reduced enzyme secretion. Given the clinical endpoint of 'significant reduction in muscle mass', not detecting the additional patients with mild reduction in enzyme secretion would only have a minor effect on results and identified more patients with low enzyme secretion rather than true PEI. Therefore, using FEL-1 to detect PEI in this study setting was a less sensitive, but acceptable alternative. To reduce the false positive results in diarrhoea, a second FEL-1 sample was performed when no cause was identified for a PEI diagnosis.

We acknowledge that the pathophysiology of sarcopenia is complex and multifactorial including malnutrition, age, early life influences, physical activity, presence of chronic diseases and use of toxin including alcohol and certain drug treatment. Alcohol was a cause in only a third of PEI cases 32.6% (15/46) and the median intake was 3-10 U/week. Sarcopenia caused by age is known as primary sarcopenia whereas any cause other than age is known as secondary sarcopenia. It is possible that our patients had both primary and secondary sarcopenia at varied degree. However, the age difference between PEI and no PEI patients was not significant but PEI was associated with higher prevalence of sarcopenia suggesting that PEI is a risk of development of secondary sarcopenia. Early recognition of malnutrition and sarcopenia in PEI is important to prevent long term complications including frailty.

To the best of our knowledge, this is the first study to show that identification of 'digital sarcopenia' in patients with and suspected benign pancreatic pathology or CP is feasible. The presence of 'digital sarcopenia' is common and significantly associated with PEI in patients undergoing assessment for pancreatic type pain and symptoms even when morphological alterations of the pancreas are absent on radiological investigations. Given results I would conclude digital skeletal muscle assessment can be used as a tool to aid identification of sarcopenia in patients that have undergone CT scanning for suspected pancreatic pathology.

CHAPTER 5: CAN 'SKELETAL MUSCLE INDEX' MEASUREMENT IN PATIENTS WITH PANCREATIC CANCER AT HIGH RISK OF PANCREATIC EXOCRINE INSUFFICIENCY PREDICT PROGNOSIS AND AID TREATMENT PLAN?

5.1 Summary

Introduction Pancreatic cancer has a poor prognosis, and most patients are considered for best supportive care (BSC) only. There is emerging role for using CT scan to assess presence of sarcopenia. The aim of this study was to assess if presence of sarcopenia using diagnostic CT images could predict uptake of palliative chemotherapy in locally advanced pancreatic cancer (LAPC).

Methods Patients with diagnosis of LAPC referred for endoscopic ultrasound guided biopsy (EUS-B) by the regional cancer network between Jan 2016 and Dec 2018 were identified. Age, BMI, and Eastern Cooperative Oncology Group performance status (ECOG-PS) were collected. CT images were analysed for skeletal muscle index (SMI) and the presence of sarcopenia. Decision outcomes on receiving chemotherapy or not were collected from the regional oncology database.

Results Of the 204 patients recruited, 75% (153) received at least one dose of a chemotherapy agent compared to 25% (51) who had BSC. Sarcopenia was more prevalent in the BSC group. SMI was significantly lower in BSC group compared to chemotherapy group. Overall median survival was 10.1 months from index CT scan. Sarcopenic patients in the cohort had lower survival compared to non-sarcopenia, 8.9 vs 11.4 months, $p = 0.02$. Logistic regression analysis demonstrated that a low SMI was the only associated factor identifying patients for BSC and not uptake chemotherapy after adjusting for age and ECOG-PS.

Conclusions This study has shown that performing SMI measurement at the time of a diagnostic CT for pancreatic cancer is feasible and can identify sarcopenia and malnourished patients who are less likely to take up chemotherapy. These patients could be triaged to oncology assessment prior to EUS-B to avoid unnecessary investigations.

5.2 Introduction

PDAC is the 11th most common cancer in the UK, and it accounts for 3% of cancer cases per year [199]. PDAC has a poor prognosis with 80% of patients presenting with local or advanced disease at diagnosis [200]. I have shown that sarcopenia was common in PEI of benign pancreatic diseases. However, recent reports showed that loss of skeletal muscle mass during neo-adjuvant chemotherapy for borderline resectable pancreatic cancer was associated with poor survival and with lower resection rate [201, 202]. Locally advanced PDAC (LAPC) is a local disease with involvement of regional vasculature but without evidence of distant metastasis [203]. The majority of LAPC are referred for rapid oncology assessment for consideration of chemotherapy to improve survival and possibly downstage to resectable disease [204].

Sarcopenia is common in PDAC with reported prevalence of 25- 63% in PDAC and it has shown to impact adversely on prognosis in those undergoing resection or palliative therapy [111, 202, 205-208]. Sarcopenia can still occur in obese patient and the combination of both of them often given the term ‘sarcopenic obesity’ which was shown to adversely impact on survival in PDAC [111].

Given its aggressive course, pancreatic cancer has been described as a ‘medical emergency’ and patients undergo a rapid sequence of tests to stage and confirm the diagnosis [209]. Unfortunately, only 8% of patients present with resectable pancreatic cancer, therefore, most are considered for palliative chemotherapy only [199, 210]. Endoscopic ultrasound (EUS) guided biopsy (EUS-B) is recommended prior to palliative chemotherapy [211-213]. However, the availability of EUS-B services are often limited to tertiary centres and university hospitals requiring some patients to travel significant distances for an invasive procedure that requires significant sedation [214]. It has been recorded by cancer support charities that in all patients with local and metastatic advanced cancer who undergo a biopsy from the primary or a

metastatic site only 28% of patients go on to receive chemotherapy [215]. Therefore, an EUS-B can be an unnecessary and onerous procedure for many patients with PDAC and delay them receiving supportive and beneficial treatments that focus on nutrition, pain and psychological support termed best supportive care (BSC) [210, 216, 217].

There is emerging role for using CT scan to measure body composition and assess presence of sarcopenia including image analysis software to enable objective assessment of skeletal muscle mass [108]. Patients undergo CT scanning as part of the standard care for suspected PDAC [218], and therefore presents the opportunity to gain prognostic information about a patient.

5.3 Aims of the study

The aim of this study was to assess if presence of 'digital sarcopenia' on the diagnostic CT scan could predict patients who do not go on to receive palliative chemotherapy in LAPC after a EUS-B. The secondary aim was to assess the impact of sarcopenia on survival.

5.4 Methods

5.4.1 Patients cohort

Patients referred for endoscopic ultrasound fine needle aspirate or biopsy EUS-B by the regional cancer network between Jan 2016 and Dec 2018 were considered for the study.

Inclusion criteria were diagnosis of LAPC based on histological diagnosis or with a clinical progression compatible with the diagnosis. Patients with resectable PDAC, metastatic disease, ampullary carcinoma, cholangiocarcinoma, neuroendocrine tumors, lymphoma or secondary cancer were excluded. Additional inclusion criteria were availability of CT scan image for analysis.

Patient information collected included: sex, age, BMI, and Eastern Cooperative Oncology Group performance status (ECOG-PS). Decision outcomes on receiving chemotherapy or not

were collected from the regional oncology database. Date of death was recorded or until the date of censoring 1st May 2019.

5.4.2 Computerised Tomography image analysis and body composition measurements

The same method described for CT scan analysis and body composition measurements in Chapter 4, section 4.4.3. Skeletal muscle was analysed from a single axial CT image at the level of the third lumbar vertebra using a commercially available software (sliceOmatic V5, Tomovision software). Cross-sectional area (cm^2) of skeletal muscle was identified by specific colour tagging as shown in figure 5.1.

5.4.3 Ethical Approval

The study was approved by local research ethics committee (IRAS301193, STH218885), appendix 5

5.4.4 Statistical analyses

Data were presented as median and range for continuous variables. Normality distribution was assessed using skewness, kurtosis and shapiro-wilk tests. Fischer's exact test was used to compare categorical variables. Mann Whitney U test were used to compare continuous variables. Survival was determined from the index CT scan until death or censor date of 1st May 2019. Survival outcomes were evaluated using Kaplan–Meier method and comparison of the curve performed using Long-Rank analysis. Factors associated with death were analysed in chemotherapy and BSC separately using Cox regression hazard model. A p value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (IBM SPSS statistics version 25, Chicago, Illinois, USA).

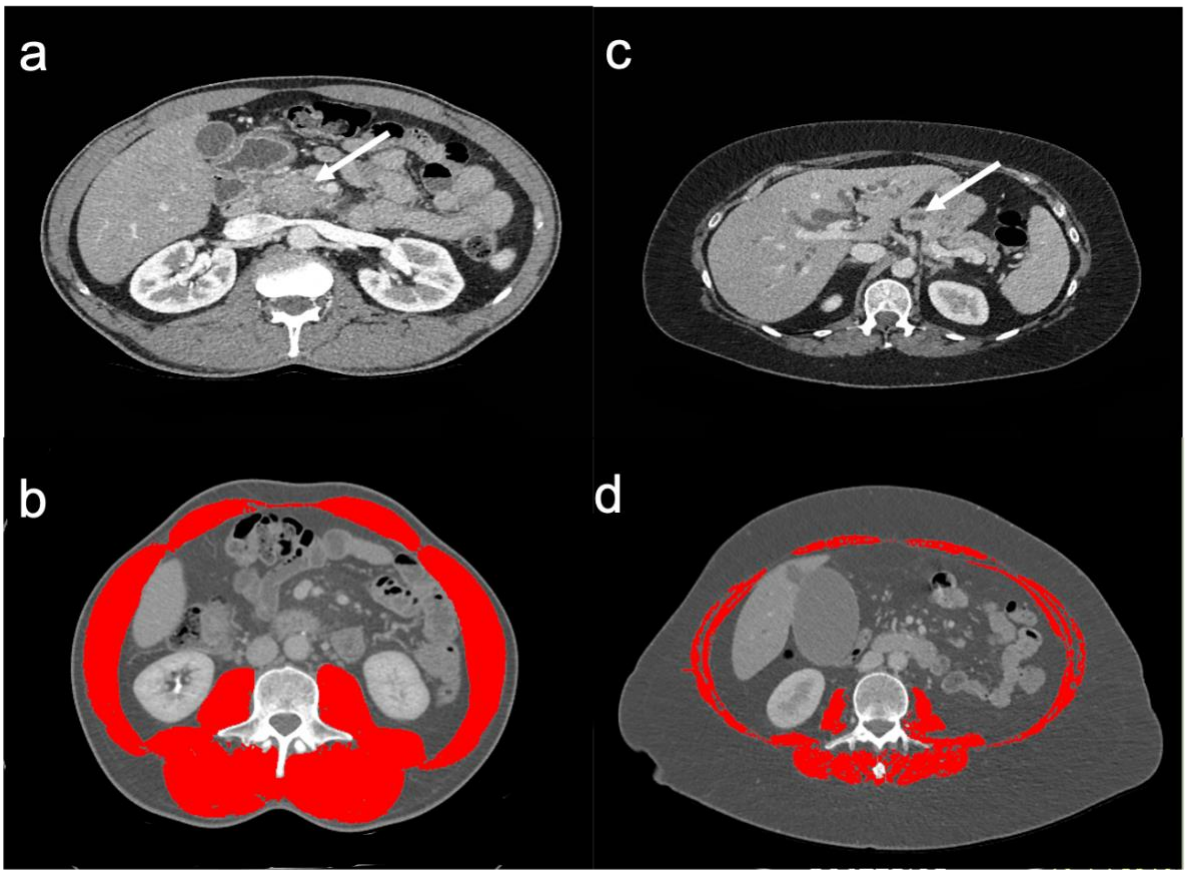

Figure 5.1: Digital skeletal muscle analysis: (a) locally advanced pancreatic cancer in the uncinate process (white arrow) in a patient with a BMI 21.6 kg/m^2 (b) Digital muscle mass analysis at the L3 level showing normal ratios of skeletal muscle (red) SMI 50.1 cm^2/m^2 (c) patient with a locally advanced pancreatic cancer in the head and a dilated main pancreatic duct (white arrow) with high BMI 32.1 kg/m^2 (d) analysis showing very low skeletal muscle index diagnosing sarcopenia. The second patient did not receive chemotherapy. The presence of high BMI and sarcopenia defines 'sarcopenic obesity'[188]

5.5 Results

5.5.1 Demographics and anthropometric characteristics

A total of 204 patients were included, 114 males with median age 69 (42-84) years. Head of pancreas was the most common cancer site 66.2% (135) followed by body of pancreas 30.4% (62), then tail of pancreas 3.4% (7).

Sarcopenia was present in 54.4% (111) patients and was significantly higher in female 63.3% compared to male patients 47.4%, *p*=0.03. Sarcopenic obesity was present in 27% (55). Most patients had PS ≤1 85.8% (175) compared to PS ≥2 14.2% (29).

Of the 204 patients recruited, 75% (153) received at least one dose of a chemotherapy agent compared to 25% (51) who had BSC, table 5.1. SMI was significantly lower in BSC group compared to chemotherapy group. Using the predefined cut off, sarcopenia was more prevalent in the BSC group. Age ≥ 75 years old and ECOG-PS 2-3 were more common in BSC. There were no significant differences in comparing the median BMI, BMI <20 kg/m^2, or sarcopenic obesity between BSC and Chemotherapy group.

Table 5.1 Comparison of demographics and anthropometric assessments between best supportive care only and chemotherapy taken up by patients with locally advanced pancreatic cancer.

Parameter	**Best supportive care** **% (n) or median (range)**	**Chemotherapy** **% (n) or median (range)**	**P value**
Number of patients	25% (51)	75% (153)	
Age, years	71 (49-84)	68 (42-83)	***0.02***
Age ≥75 years	37.3% (18)	19.6% (30)	***0.03***
Sex,			
Male	45.1% (23)	59.5% (91)	
Female	54.9% (28)	40.5% (62)	NS
ECOG-PS 0-1	74.5% (38)	89.5% (137)	
ECOG-PS 2-3	25.5% (13)	10.5% (16)	***0.01***
BMI kg/m^2	23.6 (17.7-42.2)	25 (15.4-50.8)	NS
BMI <20 kg/m^2	15.7% (8)	8.5% (13)	NS
Sarcopenia	76.5% (38)	47.8% (73)	***0.0003***
SMI	40.5 (26.3-60)	44.2 (26.5-80.9)	***0.02***
Sarcopenic obesity	25.5% (13)	27.5% (42)	NS

NS: not significant; **ECOG-PS**: Eastern Cooperative Oncology Group performance status; **BMI**: body mass index; **SMI**: skeletal muscle index.

Performing logistic regression analysis demonstrated that a low SMI was the only associated factor identifying patients to choose BSC and not uptake chemotherapy after adjusting for age, and ECOG-PS. The estimated odds ratio favoured a decrease of nearly 4.5% for opting into BSC for every one unit increase of SMI.

5.5.2 Survival

Overall median survival was 10.1 months from index CT scan. Forty-five patients were alive on the censor date (1st May 2019) including 43 (95.6%) patients receiving chemotherapy. Median survival in chemotherapy group was significantly higher compared to no-treatment group (12.7 vs 6.6 months, $p<0.0001$), figure 5.2. Sarcopenic patients in the cohort had lower survival compared to non-sarcopenia, 8.9 vs 11.4 months, $p= 0.02$. However, no significant differences in survival between sarcopenic vs non-sarcopenia in chemotherapy group 15.5 vs 13.2 months, $p=0.2$ and in BSC group 7.2 vs 6.7 months, $p=0.9$, figure 5.3

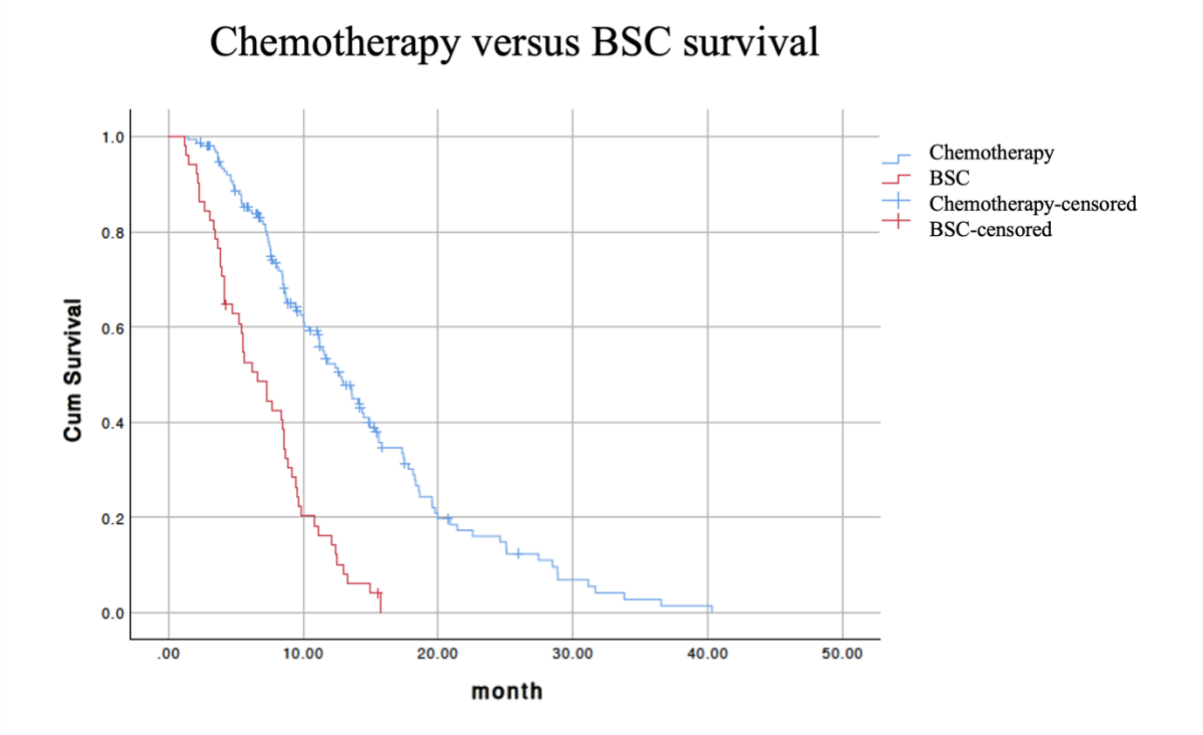

Figure 5.2 Kaplan Meier Survival curve in chemotherapy compared to no-chemotherapy groups. **BSC**: best supportive care

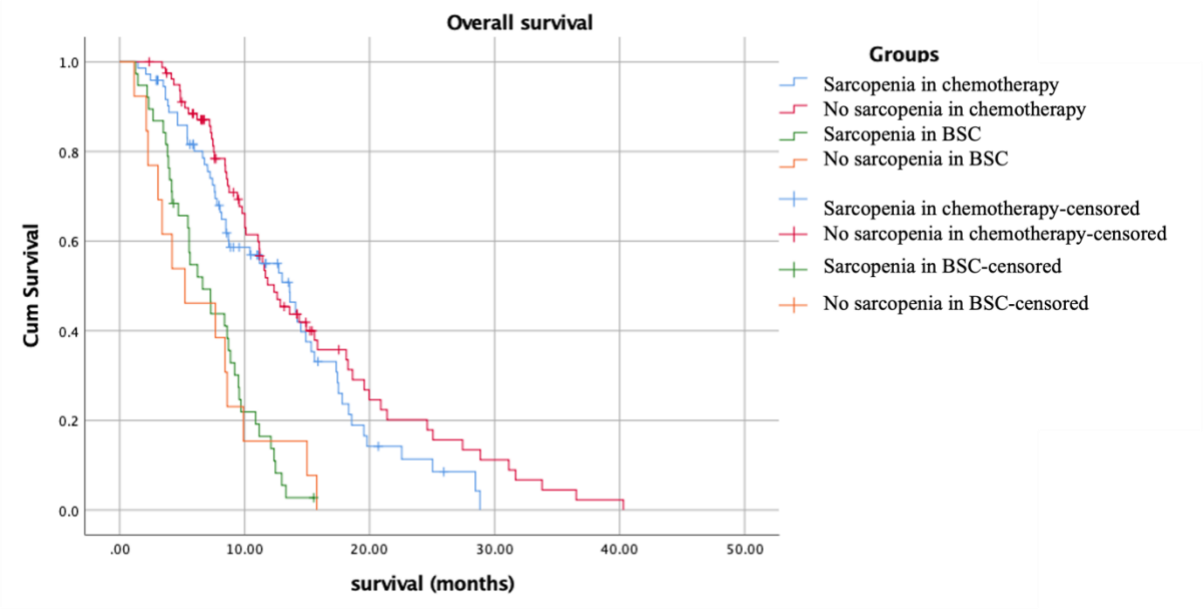

Figure 5.3 Kaplan Meier Survival curve in sarcopenia compared to non-sarcopenia patients. **BSC**: best supportive care

Cox Regression proportional hazard showed that age was the most significant factor associated with poor survival in the BSC, whilst ECOG PS 2-3 was associated with poor survival in chemotherapy group, tables 5.2 and 5.3

Table 5.2 Cox Regression Hazard analysis for BSC group

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	-.053	.018	8.301	1	.004	.948	.915	.983
ECOOG 2-3	-.259	.369	.491	1	.483	.772	.374	1.592
Sarcopenia	.327	.389	.710	1	.399	1.387	.648	2.971

Table 5.3 Cox Regression Hazard analysis for chemotherapy group

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	-.004	.012	.108	1	.743	.996	.973	1.020
ECOOG 2-3	.964	.302	10.163	1	.001	2.621	1.450	4.741
Sarcopenia	.318	.205	2.401	1	.121	1.374	.919	2.055

5.6 Discussion

This study has shown that SMI measurement at the time of a diagnostic CT for pancreatic cancer is feasible and can identify sarcopenia. We have also shown that the presence of 'digital sarcopenia' objectively identifies malnourished patients who are much less likely to take up chemotherapy.

The prevalence of sarcopenia in this study was in congruent with the published literature in unresectable PDAC receiving chemotherapy, table 5.2. However, to our knowledge, the association between sarcopenia and low uptake of palliative chemotherapy in patients with LAPC has not been described before and could facilitate a patient's care pathway. The average time for SMI measurement for a single patient was 2.5 minutes so could make up part of an initial clinical or multidisciplinary team assessment.

A notable benefit of SMI assessment for patients with PDAC from the diagnostic CT is that it performs an early, objective nutritional assessment tool. Patients presenting with suspected malnutrition can be assessed with alternative methods such as percentage weight loss [219], but a single BMI measurement is of limited diagnostic use on its own [220]. Malnutrition screening tools are available which do have a high inter-rater reliability (κ=0.67–1.00) [221-223] and recording specific anthropometric measurements is possible with serial readings [64, 224]. However alternative nutritional methods should also be interpreted with caution in an obese population [64, 107] and current study showed that nearly half of patients were overweight, and approximately 1 in 4 patients had sarcopenic obesity. The average weight-losing patient in PDAC is overweight which highlights the reported link of PDAC with obesity [111, 225].

Table 5.4 Summary of prevalence and outcomes of digital sarcopenia in unresectable pancreatic adenocarcinoma patients receiving chemotherapy.

Study	Number of patients	cohort	Age years	Prevalence of sarcopenia	Outcomes
Current study	204	LAPC	69	54.5	Sarcopenia was prevalent in BSC group compared to chemotherapy group
Cho et al[226] ***Tumori*** **2020: 300891620937795**	299	LAPC	62	9.7%	sarcopenia was a significant factor for progression-free survival
Uemura et al[227] ***Br J Nutr*** **2020: 1-8**	69	Advanced PDAC	63	48%	Sarcopenia had no bearing on survival
Kurita et al[228] ***Pancreatology*** **2019;** 19**(1): 127-135**	82	PDAC receiving FOLFIRINOX	64	51.2%	Sarcopenia was significant in multivariate analysis for OS
Basile et al [229] ***J Cachexia Sarcopenia Muscle*** **2019;** 10**(2): 368-377**	94	Locally advanced and metastatic PDAC	48% of patients < 70 years	73%	Sarcopenia not significant in multivariate analysis
Park et al[230] ***Cancer Res Treat*** **2016;** 48**(4): 1264-1273**	88	Metastatic or recurrent PDAC	65	86.3%	Sarcopenia was one of the independent prognostic factors for OS, HR 2.97
Naumann et al[231] ***Cancers (Basel)*** **2019;** 11**(5)**	147	Unresectable locally advanced PDAC	64	67.3%	Sarcopenia was not significant in multivariate analysis for OS
Choi et al [232] ***PLoS One*** **2015;** 10**(10): e0139749**	484	PDAC receiving palliative chemotherapy	60	21.3%	Sarcopenia was one of the poor prognostic factors for OS
Dalal et al [208] ***J Pain Symptom Manage*** **2012;** 44**(2): 181-191**	41	Inoperable, locally advanced pancreatic cancer	59	63%	Sarcopenia in obese patients was associated with poorer survival
Tan et al[111] ***Clin Cancer Res*** **2009;** 15**(22): 6973-6979**	111	PDAC receiving chemotherapy	64	55.9%	Overweight/obese sarcopenia (hazard ratio, 2.07, *P*= 0.006) was identified as one of the independent predictors of survival on multivariate analysis.
Rollins et al [206] ***Clin Nutr*** **2016;** 35**(5): 1103-1109**	228	Unresectable PDAC	Chemotherapy group: 65 No-treatment group: 73	61%	Sarcopenia had no bearing on survival

LAPC: locally advanced pancreatic cancer**; BSC:** best supportive care**; PDAC**: pancreatic adenocarcinoma; **OS:** overall survival; **NS**: Non-significant

A further advantage of sarcopenia assessment is that patients with sarcopenic obesity may be at higher risk of chemotherapy toxicity due to dosing currently being based on body surface area which does not take into account body compositions [218]. The benefits of nutritional assessment and subsequent intervention has been demonstrated in one study showing an independent association with survival among patients with unresectable pancreatic cancer (HR 2.12) regardless of chemotherapy treatment [216].

The association between PEI and PDAC has been well established. The prevalence of PEI in PDAC is estimated to be up to 92% [4, 28]. PEI can develop as consequences of PD obstruction and/or parenchymal cell destruction. Due to the high prevalence and the consequences of PEI, it is recommended that PDAC patient start on PERT at time of diagnosis [233].

Despite undergoing a procedure to obtain a biopsy to confirm diagnosis, 51 (25%) patients with LAPC did not receive chemotherapy. Although still high, this proportion is much lower than other reported data would predict [199], and lower than initial data collection published in abstract form [234]. Current findings would be supported by exclusion of patients with metastatic disease (who would undergo liver biopsy locally) and our cancer network awareness of the initial abstract findings prompting attempts to improve patient assessment prior to EUS-B referral.

Although patients with sarcopenia had poorer survival compared to those without sarcopenia, when adjusting for age and frailty we found that age and frailty were associated with poor survival in BSC and chemotherapy group, respectively. The subjective assessment of a patients ECOG-PS is challenging and can alter quickly, in this study it proved to be a limitation because the inter-observer variability of ECOG-PS was not eliminated, this is in contrast to digital SMI assessment which has been shown to have good intra-observer agreement [108, 235, 236]. ECOG-PS is an important functional assessment and can be combined with digital skeletal muscle analysis if required. Another limitation is the association between sarcopenia and

increasing age [237], however, the regression analysis identified low SMI only to be associated with non-uptake of chemotherapy.

The study has shown that performing SMI measurement at the time of a diagnostic CT for pancreatic cancer is feasible and can identify sarcopenia and malnourished patients who are less likely to take up chemotherapy. This information could help predict patients that are unlikely to take up palliative chemotherapy in LAPC. These patients could be triaged to initial oncology assessment for nutritional assessment prior to EUS-B referral to gain nutritional support, best supportive care and avoid unnecessary investigations.

CHAPTER 6: SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Can we improve the detection of pancreatic exocrine insufficiency?

I have shown that testing in 'at-risk' groups gives a significant yield when considered. The current status showed lack of testing and awareness which means PEI is going undetected. The findings from chapter 2 confirm the available literature suggesting high prevalence of PEI in the three cohorts of patients included in the study: HAI, DM and PLHIV [11, 34, 35, 37-41, 45]. The findings showed lack of testing despite the known association because none of the patients approached were tested for FEL-1. The work from this study supports the hypothesis that testing with faecal elastase can identify those 'at-risk' of PEI. Therefore, the case finding in 'at-risk' groups can be improved by testing with FEL-1 and awareness is required by cross-specialty working and education to promote testing in these groups. Due to the fact the test is a stool sample requiring patient to pick up a pot then return it (as opposed to a one stop blood test) may itself be a logistical barrier and coupled with the tests accuracy we have shown that alternative methods can be helpful to identify PEI. Findings from chapter 3 support the hypothesis that micronutrient deficiencies are common in patients with suspected PEI including patients from 'at-risk' conditions. Three markers showed high prevalence in PEI including prealbumin, selenium and magnesium. These markers can be included in PEI assessment and aid nutritional assessment. In chapter 4, sarcopenia identified on a CT scan is common in PEI and with the increased number of patients undergoing CT assessment it could be a promising tool to guide testing for PEI. I have shown that the identification of sarcopenia using digital muscle analysis is feasible and should be used in future studies to aid its integration into clinical practice. The method of digital sarcopenia can be part of patient's care and can be performed without subjecting patient to additional test as most patients with suspected pancreatic pathology undergo CT scan.

In chapter 5, I showed that digital sarcopenia analysis in patients with PDAC was feasible. PEI is very common in PDAC and forms part of the nutritional assessment that is vitally important to aid survival. The digital analysis of the CT scans of patients with PDAC identified patients

who were sarcopenic (malnourished) and that should be triaged to alternative pathways. Those with sarcopenia may benefit from formal nutritional assessment, best supportive care and avoid unnecessary investigations. With further studies reproducing digital skeletal muscle assessment to identify malnutrition (including PEI) could be possible to integrate this analysis as part of patient's assessment with PDAC. The identification of sarcopenia and malnutrition using digital analysis would offer a different route to diagnose PEI.

Therefore, in answer to the question posed by the thesis, I believe I have shown that we *can* improve the detection of PEI.

6.2 Future directions:

6.2.1 Improving sample return

The overall stool sample return rate in this thesis was 74.9% (230/307). Therefore, PEI could have been missed in those who did not return the sample given the high diagnostic yield. The reasons for no-return of samples were not explored. The process of providing and returning stool sample can be unpleasant and troublesome for patients especially the elderly, patients with disability, and those living far away from healthcare centres. In addition to technical and logistic barriers, social embarrassment could be another barrier to sample return.

Providing returned envelopes to send back samples may improve sample return. Another example is a home test kit, similar to the current bowel cancer screening programme, which can provide alternative method of performing and sending stool samples without the need to visit healthcare centres. A newly available rapid test that use stool sample to diagnose PEI is the pancreas Elastase-1 Quick™ Test (ScheBo® Biotech AG, Giessen, Germany) which is based on the same monoclonal antibodies as the standard FEL-1. The advantage of Pancreas Elastase-1 Quick™ Test is that it is easy to perform and provide results within minutes, however, it is unable to provide quantitative report and therefore unable to identify those with severe PEI.

In the test, elastase bound to the monoclonal antibodies and the complex travel across the test membrane to reach test line (T) which has another monoclonal antibodies attached, figure 6.1

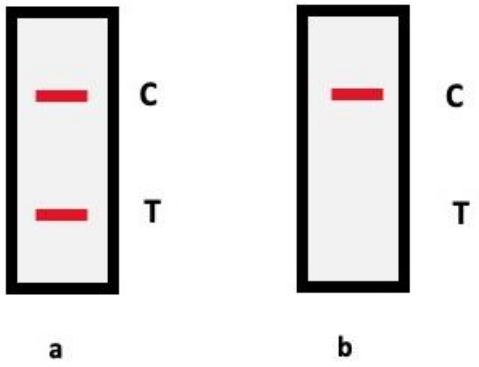

Figure 6.1 Point of care test of Pancreas Elastase-1 Quick™ Test showing (a) normal result and (b) pancreatic exocrine insufficiency

Studies in children with CF found the Pancreas Elastase-1 Quick™ Test to have high sensitivity 93% and specificity 97% compared to standard FEL-1 [238]. However, the accuracy was found to be less in another study with adult population with pancreatic resection, CP, and PDAC with sensitivity of 50% and specificity of 84% [239]. Therefore, careful patients' selection is important due to limited sensitivity.

In order to explore reasons for low return of stool samples, future studies are required to survey patients' experience and use the result to improve stool sample return.

6.2.2 Is visceral obesity a new 'at-risk' condition?

Overweight and obesity were common findings in all cohorts in this thesis including patients in chapter 5 with PDAC. There is evidence to suggest that increased BMI can lead to pancreatic fat deposition and destruction of pancreatic acinar cells causing PEI [13, 191]. Pancreatic steatosis and fatty pancreas are interchangeable terms used to describe fat accumulation in the pancreas. Non-alcoholic fatty pancreas disease (NAFPD) is a term used to describe the combination of fatty pancreas and presence of obesity and/or metabolic syndrome [191].

The presence of visceral fat can cause DM and metabolic syndrome and have been linked to fatty pancreas [140]. I have shown that visceral fat can be quantified using digital body composition analysis, table 4.2. The additional advantage of digital body composition analysis is that it can measure the circumference of the cross-section slice as a surrogate method for waist circumference measurement used in the diagnostic criteria for metabolic syndrome. There has been attempts in literature to use a cut off for visceral fat area in those with metabolic syndrome using digital body composition analysis [240]. Further research is required in this area to study the association of visceral obesity with PEI in those with metabolic syndrome and compared to those without and using MRCP to quantify pancreatic fat and FEL-1 or sMRCP to assess the exocrine function.

In addition to genetic predisposition and smoking, obesity has been shown to be another independent risk factor for development of PDAC [225, 241, 242]. It can also have impact on the prognosis of cancer [243]. Data from chapter 5 showed that approximately half of patients were at least overweight and 1 in 4 patients had sarcopenic obesity. Several reports showed that obesity and visceral fat obesity are associated with poor prognosis in PDAC [244, 245], however, there is scarce of information on the impact of visceral obesity on outcomes in PDAC in western-based population. Future large cohort studies are required to explore the impact of visceral fat area on survival in PDAC.

6.2.3 Other accessible alternative tests for diagnosing PEI?

Of the available tests in diagnosing PEI, sMRCP offers a potential alternative to FEL-1. Studies showed good correlation between sMRCP and FEL-1 with high sensitivity and specificity [79, 80, 88, 246, 247]. However, the main barriers in introducing sMRCP in clinical practice is the lack of the secretin substrate, its high cost, and lack of standardisation [81]. Despite these challenges, sMRCP is an appealing alternative to FEL-1 because it provides assessment of both parenchymal and exocrine function at the same time. Dynamic tests that use substrate and take

serial images are common in clinical practice, for example, hepatobiliary iminodiacetic acid (HIDA) scan and gastric emptying study.

^{13}C-MTGT is another non-invasive indirect pancreatic function test, however, its frequent sampling and long duration of test are major barriers for implementation in clinical practice [78].

In conclusion, PEI is a common disease that causes morbidity and is treatable with PERT. With increased awareness, vigilant testing in appropriate groups and adjunct nutritional assessments less cases can be overlooked enabling earlier intervention.

APPENDIX 1: PUBLICATIONS, ORAL PRESENTATIONS, AND ABSTRACTS.

Peer review articles related to this thesis:

Jalal M, Campbell JA, Tesfaye S, Al-Mukhtar A, Hopper AD.
'Yield of testing for micronutrient deficiencies associated with pancreatic exocrine insufficiency in a clinical setting: An observational study'
In press. Accepted in World Journal of Clinical Cases. Nov 2021

Jalal M, Rosendahl J, Campbell JA, Vinayagam R, Al-Mukhtar A, Hopper AD,
'Identification of 'digital sarcopenia' can aid the detection of pancreatic exocrine insufficiency and malnutrition assessment in patients with suspected pancreatic pathology'
Dig Dis 2021. doi: 10.1159/000517554

Jalal M, Campbell JA, Wadsley J, Hopper AD.
'Computed Tomographic Sarcopenia in Pancreatic Cancer: Further Utilization to Plan Patient Management' J Gastrointest Cancer. 2021 Jul 22. doi: 10.1007/s12029-021-00672-4.

Jalal M, Campbell JA, Hopper AD
'Practical guide to the management of chronic pancreatitis'
Frontline Gastroenterology 2019;**10:**253-260. DOI: 10.1136/flgastro-2018-101071

Peer review articles unrelated to this thesis:

Hopper AD, **Jalal M**, Munir A on behalf of Sheffield Teaching Hospitals European Neuroendocrine Tumour Centre
'Recent advances in the diagnosis and management of pancreatic neuroendocrine tumours.'
Frontline Gastroenterology 2019;10:269-274. DOI: 10.1136/flgastro-2018-101006

M. Jalal, AD Hopper 'Diagnosis and management of oesophageal cancer' The Practitioner *2018;262(1812):21-25*

Oral Presentations at national conferences:

British Society of Gastroenterology annual meeting 2019
OWE-29 Can digital skeletal muscle index predict palliative chemotherapy uptake before patients undergo endoscopic pancreatic biopsy?
Gut 2019;68:A154. *DOI: 10.1136/gutjnl-2019-BSGAbstracts.289*
Jalal M, Campbell J, Wadsley J, *Hopper AD*

Pancreatic Society of Great Britain and Ireland annual meeting 2019
Sarcopenia assessed by using novel digital software is prevalent in pancreatic exocrine insufficiency: A prospective study. **Mustafa Jalal**, Jonas Rosendahl, Jennifer Campbell, Ragu Vinagarayam, Ahmed Al-Mukhtar, Andrew Hopper

O40 High prevalence of sarcopenic obesity in pancreatic exocrine insufficiency patients: a prospective study *Gut* 2021;**70:**A22. Jalal M, Campbell J, Vinayagam R, *Al-Mukhtar A, Hopper AD. DOI:*10.1136/gutjnl-2020-bsgcampus.40

Abstracts

PTU-023 Endoscopic ultrasound biopsy prior to palliative treatment for pancreatic cancer: can we prevent unnecessary procedures?
Gut 2018;**67:**A155-A156. DOI: 10.1136/gutjnl-2018-BSGAbstracts.308
Jalal M, Wadsley J, Hopper A

PWE-067 Can serum nutritional markers be used for diagnosing pancreatic exocrine insufficiency in at risk groups? *Gut* 2019;**68:**A158-A159. DOI: 10.1136/gutjnl-2019-BSGAbstracts.298 **Jalal M,** Campbell J, Hopper A

PWE-066 Are we missing cases of pancreatic exocrine insufficiency in hospitalised patients with high alcohol intake? *Gut* 2019;**68:**A158. DOI: 10.1136/gutjnl-2019-BSGAbstracts.297
Jalal M, Hopper A

P254 Are we still missing cases of pancreatic exocrine insufficiency and pancreatic atrophy in diabetes mellitus? *Gut* 2021;**70:**A173. **Mustafa Jalal**, Solomon Tesfaye, Andrew Hopper. DOI: 10.1136/gutjnl-2020-bsgcampus.328

APPENDIX 2: HEALTH RESEARCH AUTHORITY APPROVAL LETTER FOR CHAPTERS 2 and 3

Health Research Authority

Dr Andrew Hopper
Consultant Physician, Gastroenterologist Sheffield Teaching Hospitals NHS FT Royal Hallamshire Hospital
Room P39 P Floor
Glossop Road Sheffield S10 2JF

Email: hra.approval@nhs.net

27 February 2018

Dear Dr Hopper,

Letter of HRA Approval

Study title:	**Prevalence of exocrine pancreatic insufficiency in chronic diseases**
IRAS project ID:	**239539**
REC reference:	**18/HRA/0838**
Sponsor:	**Sheffield Teaching Hospitals NHS FT**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to

participating organisations to opt out of the study, or request additional time, before their participation is assumed.

- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from the HRA website.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The attached document *"After HRA Approval – guidance for sponsors and investigators"* gives detailed guidance on reporting expectations for studies with HRA Approval, including:

- Working with organisations hosting the research
- Registration of Research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics and is updated in the light of changes in reporting expectations or procedures.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England. If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found through IRAS.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details on the HRA website. Your IRAS project ID is **239539**. Please quote this on all correspondence. Yours sincerely,
Emma Stoica Senior Assessor

Email: hra.approval@nhs.net

APPENDIX 3: HEALTH RESEARCH AUTHORITY APPROVAL LETTER FOR CHAPTER 4, SUBSTANTIAL AMENDMENT

NHS
Health Research Authority
Yorkshire & The Humber - Sheffield Research Ethics Committee

NHS Blood and Transplant Blood Donor Centre
Holland Drive Newcastle upon Tyne Tyne and Wear
NE2 4NQ Tel: 0207 104 8079

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

04 September 2018 Mr Luke Barron
Directorate Research Coordinator for the Academic Directorate of Gastroenterology Research Coordinator for Specialised Cancer & Haematoncology
Sheffield Teaching Hospitals NHS Foundation Trust Royal Hallamshire Hospital
Room P39 P Floor Glossop Road, Sheffield S10 2JF

Dear Mr Barron

Study title:	**Elastography in the diagnosis of chronic pancreatitis**
REC reference:	**16/YH/0315**
Amendment number:	**Substantial Amendment 1, 15-08-18**
Amendment date:	**15 August 2018**
IRAS project ID:	**210710**

The above amendment was reviewed by the Sub-Committee in correspondence.

Summary of Amendment

Change to study protocol regarding the use of CT scans of participant's abdomen prior to referral.

Changes to participant information sheets and consent forms. Revised date for end of study.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper	Letter from Luke Barron	15 August 2018
Notice of Substantial Amendment (non-CTIMP)	Substantial Amendment 1, 15-08-18	15 August 2018
Participant consent form	1.3 Tracked	01 May 2018
Participant information sheet (PIS)	1.3 Tracked	01 May 2018
Research protocol or project proposal	2 Tracked	20 April 2018

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

16/YH/0315: Please quote this number on all correspondence

Yours sincerely Pp

Professor Basil Sharrack Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

APPENDIX 4: PATIENT CONSENT FORM

(Form to be on headed paper)

Study Number: STH19471 IRAS ID: **210710**

Participant Identification Number for this trial:

CONSENT FORM version 1.3, 01/05/2018

Title of Project: Can elastography be used as a test for chronic pancreatitis?

Name of Researchers: Dr Andrew Hopper, Dr. Mustafa Jalal, Dr Jennifer Campbell

Please initial box

1. I confirm that I have read the information sheet dated 01/05/2018 (version 1.3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that by taking part in this study, the ultrasound could indicate that I may hav diagnosis of chronic pancreatitis, in which case I will be followed up in the gastroenterology clinic ☐

5. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐

6. I agree to take part in the above study ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

APPENDIX 5: HEALTH RESEARCH AUTHORITY AND HEALTH AND CARE RESEARCH WALES APPROVAL LETTER FOR CHAPTER 5

Prof Andrew Hopper Consultant Gastroenterologist
Sheffield Teaching Hospitals NHS Foundation Trust
P39 Royal Hallamshire Hospital
Glossop Road
Sheffield S102JF

Email:
approvals@hra.nhs.uk
HCRW.approvals@wales.nhs.uk

17 June 2021 Dear Prof Hopper

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Influence of abdominal fat distribution and muscle parameters on the mortality of pancreatic and lung cancer patients - a retrospective study**
IRAS project ID:	**301193**
Protocol number:	**STH21885**
REC reference:	**21/HRA/2546**
Sponsor	**Sheffield Teaching Hospitals NHS FT**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?

HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?

HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The *"After HRA Approval – guidance for sponsors and investigators"* document on the HRA website gives detailed guidance on reporting expectations for studies with HRA and HCRW Approval, including:

- Registration of Research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?

Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **301193**. Please quote this on all correspondence. Yours sincerely,

Barbara Cuddon Approvals Specialist

Email: approvals@hra.nhs.uk

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