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Keywords:	chronic pancreatitis, inflammation, fibrosis, oxidative stress, pain processing

Characterization of the fibro-inflammatory process involved in progression from acute to chronic pancreatitis: study protocol for a multicentre, prospective cohort study

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Abstract

Introduction: Chronic pancreatitis (CP) is thought to present the end-stage of a continuous disease process evolving from acute pancreatitis (AP), over recurrent acute pancreatitis (RAP), to early and end-stage CP. Due to the irreversible nature of CP, early detection and prevention is key. Prospective assessment based on state-of-the-art imaging modalities as well as biochemical markers of inflammation, fibrosis and oxidative stress may provide a better understanding of the underlying pathological processes and help identify novel biomarkers of disease with the ultimate goal of early diagnosis, intervention and prevention of disease progression. This paper describes the protocol of a prospective multicentre cohort study investigating the fibro-inflammatory process involved in progression from acute to chronic pancreatitis using state-of-the-art diagnostic imaging modalities and circulating biomarkers of inflammation, fibrosis and oxidative stress.

Methods and analysis: We plan to include adult patients (50 in each group) with either first time AP, RAP, early CP (preserved pancreatic exocrine and endocrine function) or end-stage CP (exocrine insufficiency with or without endocrine insufficiency) recruited from outpatient clinics at the participating sites. Included patients will be followed prospectively for 15 years with advanced MRI and contrast enhanced EUS with elastography, assessment of endocrine and exocrine pancreatic function, biochemical and nutritional assessment, and evaluation of pain processing using quantitative sensory testing. Blood for a biobank will be obtained. The purpose of the biobank is to allow analyses of potential biomarkers for the progression of disease eventually leading to CP including interleukins, transforming growth factor beta-1, soluble fractalkine, monocyte chemoattractant protein 1, matrix metalloproteinases and markers of oxidative stress.

Ethics and dissemination: Permissions from the Regional Science Ethics committee and the Regional Data Protection Agency are obtained. We will submit the results of the study for publication in peer-reviewed journals regardless of whether the results are positive, negative or inconclusive.

Article Summary

Strengths and limitations of this study

- The study design enables prospective, long-term characterization of patients in different phases of the pancreatitis disease spectrum.
- The project will lead to a better understanding of the fibrogenic process underlying CP and improve the understanding of changes in pain perception and nutritional aspects during the course of disease.
- The combination of diagnostic imaging, functional and biochemical parameters may enable early identification of patients at increased risk of developing chronic pancreatitis.
- As there are emerging inhibitors of pancreatic fibrosis, which potentially could impair the ongoing fibrogenesis of pancreas, this project may lead to new strategies for patient management and disease prevention.
- A limitation of the study is that “State-of-the art” imaging, functional, endoscopic and biochemical tools may change during the study period, thus making comparisons over time difficult and potentially based on outdated methods.

Introduction

Chronic pancreatitis (CP) is a progressive fibro-inflammatory disease associated with persistent pathologic response to parenchymal injury or stress [1]. Over time, the fibro-inflammatory process can lead to irreversible fibrosis with morphological changes and destruction of acinar cells with subsequent loss of pancreatic function. Due to the irreversible nature of end-stage pancreatitis, early detection and prevention is key.

A recent epidemiological study found an annual CP incidence of 7.8 per 100,000 [2]. Assuming a survival of 15–20 years, the annual prevalence should be between 120 to 143 per 100,000 [3]. However, accumulated evidence suggests that the incidence of CP is rising [4,5] and a Danish nationwide population-based cohort study showed a five-fold increased mortality rate with a life expectancy that was approximately 8 years less among CP patients compared with population controls [6]. Taken together, this illustrates the great impact CP has on health, economic, and social aspects.

Alcohol and smoking are the major aetiological risk factors of CP [7], and there seems to be an additive effect of alcohol and smoking. Other causes include hypercalcemia, hyperlipidaemia, obstruction of the biliary or pancreatic duct, autoimmune conditions, and hereditary conditions. About 20% of cases are idiopathic, but this number is likely to decrease as the understanding of the pathophysiology underlying CP evolves.

Currently the diagnosis of CP is based on a combination of symptoms, biochemical tests, and imaging. Abdominal pain is the cardinal symptom of CP and present in the majority of patients during their disease course, but the intensity and temporal pattern varies substantially. At later disease stages patients may develop diabetes, steatorrhea and weight loss, but the disease course is unpredictable in the majority of patients, thus making symptom based assessment unreliable. The biochemical tests used for assessment of CP include glycated haemoglobin [HbA_1C] (endocrine pancreatic function) and faecal elastase [FE], breath test or direct pancreatic function tests (exocrine pancreatic function tests). Diagnostic imaging is used for characterisation of typical morphological changes including parenchymal lobulation, calcifications, parenchymal atrophy, pseudocysts and main pancreatic duct abnormalities. Various imaging modalities are used for assessment including computed tomography (CT), magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS)[8].

Some patients are diagnosed with CP without preceding attacks of acute pancreatitis (AP). These patients typically present with symptoms of end stage disease including steatorrhea, diabetes and chronic abdominal pain. However, a large proportion of patients diagnosed with CP have a history of AP or recurrent acute pancreatitis (RAP), especially when aetiology is toxic (Fig. 1). A retrospective study found that among 669 patients admitted with their first episode of AP, 17% developed RAP and 8% progressed to CP within 5 years [9]. These findings attest to the understanding of CP as a continuous disease process evolving from AP, over RAP, to early and end-stage CP as also outlined in a recent international consensus draft on a mechanistic definition of CP [1]. Importantly, this understanding provides a theoretical framework for studying the fibro-inflammatory processes involved in the development of CP by prospectively assessing patients admitted with AP and RAP during their course of disease towards early and end-stage CP. Hence, prospective assessment based on state-of-the-art imaging modalities as well as biochemical markers of inflammation, fibrosis and oxidative stress may provide a better understanding of the underlying pathological processes and help identifying novel biomarkers of disease with the ultimate goal of early diagnosis, intervention and prevention of progression.

Overall, there is a substantial and unmet need for a better understanding and characterisation of the pathological processes underlying inflammatory pancreatic disorders. The present protocol provides a unique opportunity to study such changes in well defined, and well characterized prospective patient cohorts.

Current standards and challenges in assessment of pancreatitis

Imaging

Various imaging techniques can be used to characterize pancreatic morphology. CT is in most cases used for the initial work-up and is particularly useful for detection of advanced disease characteristics of CP including parenchymal and intraductal calcifications, gross duct pathology and parenchymal atrophy [10]. Furthermore, CT is key in ruling out relevant differential diagnosis including pancreatic cancer. Despite CT being the imaging modality of choice for initial investigation, it cannot exclude a diagnosis of CP, nor can it be used to exclusively diagnose ‘early CP’.

MRI based techniques including magnetic resonance cholangiopancreatography (MRCP) are more accurate for detection of pancreatic duct abnormalities (dilatations and strictures) and subtle changes of the pancreatic parenchyma and side branches, which can be attributed to early signs of CP [11]. The MR based techniques can be further refined by assessment of pancreatic gland volume and administration of secretin whereby a combined quantitative morphological and functional assessment of the pancreatic gland can be obtained. This includes characterization of subtle ductal abnormalities and pancreatic secretion. In addition, diffusion weighted imaging (DWI) can provide a proxy of fibrotic changes and emerging methods like the DIXON technique can be used to assess fat signal fraction (FSF) for evaluation of fatty infiltration of the pancreatic parenchyma.
The gold standard for establishing a diagnosis of early CP imaging is currently endoscopic ultrasound (EUS). Diagnostic criteria related to the parenchyma and the pancreatic duct have been established, and in 2009 a weighted scoring system was proposed by a panel of experts, the Rosemont score [12]. Emerging endoscopic methods with assessment of pancreatic blood flow and tissue stiffness by elastography may further improve the diagnostic utility of EUS [13].
Taken together, a multimodal approach combining advanced MRI and EUS techniques will likely provide novel and complementary information on pancreatic morphology and function that may reflect unique aspects of the pathology underlying progressive pancreatitis disorders (AP → RAP → CP) that have not previously been identified using conventional imaging modalities.

Circulating biomarkers of inflammation, fibrosis and oxidative stress

Most of our knowledge about physiological and pathophysiological mechanisms in healthy and diseased pancreas comes from experimental studies. Due to the anatomy, complexity and risk associated with direct sampling from pancreatic tissue, most of our information on human pancreas arises from circulating marker of pancreatic physiology or injury.

Inflammation: The inflammatory profile characterising early stages of pancreatitis may reflect the trajectory of disease and subsequent development of fibrosis. Hence, patients with CP have increased serum levels of interleukin (IL)-6, IL-4, IL-18 [14][15][16], which

are implicated in the wound healing response and the progression of fibrotic disease. Also, tumour necrosis factor (TNF)-α may be implicated in disease progression [17].

Fibrosis: Patients with CP have elevated plasma levels of fibro genesis markers such as transforming growth factor beta-1 (TGF-β1), soluble fractalkine (s-FR) and monocyte chemoattractant protein 1 (MCP-1) [18][19][20]. Decreasing MCP-1 and TGF-β1 levels with anti-inflammatory drugs was shown to reduce the severity of CP in a caerulein-induced CP mouse model [21]. Matrix metalloproteinases (MMPs) are a group of enzymes involved in the degradation of collagen, proteoglycans, elastin and fibronectin, all of which play an important role in the remodelling of the extracellular matrix. The role of MMPs in the development of progressive fibrosis and cirrhosis is well established [22] and emerging evidence suggests that MMPs may have a similar role in CP [23]. Elevated serum MMP-9 levels have been found in patients with both AP and CP, and active form of MMP-9 could be involved in the development of diabetes mellitus associated with CP [24].

Oxidative stress is a result of imbalance between reactive oxygen species and their neutralizing mechanisms, which may result in cell or tissue damage. Increased oxidative stress with the resulting formation of free radicals has been implicated in the pathophysiology of CP [25] and treatment with antioxidants has been shown to reduce disease and pain severity in randomized controlled trials [26,27], although the effects may be related to the underlying aetiology [28,29]. Recent studies have shown that oxidative stress may also be implicated in RAP [30]. Free radicals do not only exert local effect in pancreas, but are also released in systemic circulation and can thus be quantified.

Pain processing and quantitative sensory testing

The processing of pain in central pain pathways undergoes changes in a large proportion of patients with painful CP. These include sensitization of central pain pathways, impaired capacity of pain modulation as well as structural and functional changes in the brain [31]. Although the presence of these changes is well documented in numerous cross-sectional studies, the temporal aspects of changes in pain processing during disease progression have not previously been investigated in the context of pancreatitis. Quantitative sensory testing (QST) can be used to map the pain system; the technique is based on the rationale

that different neural pathways and networks can be explored using standardized stimulation with simultaneous recording of the evoked pain response by psychophysical and/or objective methods [32]. Due to spinal convergence between visceral afferents from the pancreas and somatic afferents from upper abdominal skin dermatomes, somatic QST can be used to assess if the pain system is sensitized by nociceptive input from the pancreas [33]. Together with specific test paradigms (temporal summation and assessment of pain modulation) QST can be used to characterize the state of the pain system and to document if patients have abnormal central pain processing.

Nutrition

The risk factors associated with underweight and malnutrition in patients with CP are complex and likely multifactorial with the most frequently reported being pancreatic exocrine insufficiency. However, many patients lose weight early in their disease course and before evolution of pancreatic exocrine insufficiency. For example, postprandial pain, which is seen in many patients, may also limit food intake and lead to underweight and malnutrition. There is a paucity of data on this important topic, and continuous characterization of nutritional status in patients going through phases of AP, to RAP to CP may provide us with essential knowledge on metabolic processes related to inflammation and fibrosis. A combined use of bioelectrical impedance, hand-grip strength (HGS), timed up and go test (TUG), assessment of endocrine- (Hb1AC) and exocrine insufficiency (FE), and biochemical tests enables qualitative and quantitative assessment of nutritional status.

Hypotheses

Current knowledge on the progression from AP to CP is mostly based on cross-sectional studies including patients with different stages of RAP and CP. By prospectively following well characterised cohorts of patients with pancreatitis at different stages (AP → RAP → early and late CP) with serial imaging (advanced MRI and EUS), profiling of fibro-inflammatory pathways using circulating biomarkers and assessment of nutritional status and pain processing using QST, this study will help elucidate the underlying pathophysiology of pancreatitis.

The hypotheses of this study are:

- Prospective assessment of patients progressing from AP to CP, using advanced imaging techniques and circulating biomarkers of inflammation, fibrosis and oxidative stress, can provide new mechanistic insight to the underlying fibro-inflammatory process. This knowledge may be used to design future clinical studies focusing on prevention of inflammation and fibrosis in the pancreas and other organ systems.
- Changes in pain processing over time are poorly understood in patients progressing from AP to CP. This information may be used to identify patients at risk for developing a chronic pain syndrome with irreversible neuroplastic changes in the central nervous system.
- Changes in nutritional state over time are poorly understood in patients progressing from AP to CP. This information may be used to identify patients at risk for developing malnutrition.

Aims

Primary aim: To prospectively investigate the fibro-inflammatory process involved in progression from acute to chronic pancreatitis using state-of-the-art diagnostic imaging modalities (contrast enhanced EUS, EUS guided elastography and advanced MRI) and circulating biomarkers of inflammation, fibrosis and oxidative stress.
Secondary aim: To prospectively characterize pain processing and nutritional status in patients progressing from acute to chronic pancreatitis.

Methods

Study design

Prospective cohort study including adult patients with either first time AP, RAP or CP recruited from outpatient clinics at the participating sites. The study is approved by the Danish Data Protection Agency (VD-2018-298; I-suite no.: 6542) and the Regional Committee on Health Research Ethics (Journal-no.: H-18017705). All study participants will provide written informed consent and the study will be conducted according to the Declaration of Helsinki. The study is non-interventional; all participants will be treated and

monitored according to the current best clinical evidence-based practice. The patient inclusion begins in February 2019, and the anticipated completion date is February 2034.

Patient inclusion criteria

- Age 18-70 Years
- **Cohort 1**: Patients with CP (N=50) of any aetiology except gallstone induced CP: 25 patients with CP without exo- or endocrine insufficiency (early CP cohort); 25 patients with CP and exocrine insufficiency with or without endocrine insufficiency (end-stage CP cohort). The M-ANNHEIM diagnostic criteria will be used for the definition of CP [34]; both patients with probable and definitive CP will be included to cover the full spectrum of disease.
- **Cohort 2**: Patients with their first attack of AP of any aetiology except gallstone induced AP (N=50). The revised Atlanta criteria for acute pancreatitis will be used as diagnostic criteria [35].
- **Cohort 3:** Patients with RAP (N=50) except gallstone induced RAP. RAP is defined as two or more cases of AP as diagnosed by the revised Atlanta Criteria [35].

Patient exclusion criteria

Patients with the following conditions will be excluded from participation in the study:

a. Pregnant or lactating patients
b. Patients in whom MRI is contra-indicated (metallic implants, pacemaker, implantable cardioverter defibrillator, claustrophobia)
c. Patients with chronic liver disease, chronic renal failure, malignancy, chronic inflammatory bowel syndrome, chronic obstructive lung disease, pulmonary fibrosis. If the patient develops any of the listed diseases during the study period they will continue their participation in the study.
d. Patients treated with anti-inflammatory drugs of any kind at the time of inclusion: local or systemic corticosteroids, NSAID´s, salazopyrins or other. However, once included, they will continue their participation even though they receive treatment with anti-inflammatory drugs.

Healthy controls: Fifty sex- and age matched healthy controls without any previous history of pancreatic or gastrointestinal disease will be included.

Sample size, time schedule and follow up

The present study is observational. No previous long-term studies have evaluated the fibro-inflammatory process associated with the development of CP and we were therefore unable to undertake a valid sample size calculation. Consequently, the sample size was set to 50 participants in each cohort, assuming that 20-30% of participants in cohort 2 and 3 would develop CP [36,37] . Based on previous cohort studies from our departments, we expected that we would be able to reach our sample size during an inclusion period of two years.

Included patients will be followed up prospectively for 15 years. An interim analysis, assessing both clinical, functional, imaging and biochemical parameters will be performed 5 years after inclusion. All three cohorts will follow the same time schedule and plan for follow up as outlined in Table 1.

Included patients will be evaluated during the quiescent phase; an acute attack of pancreatitis or exacerbation in CP requiring admission will lead to a quarantine period of 4 weeks before evaluation by the protocol can be performed. Resolution of pancreatitis will be documented by assessment of plasma amylase and CRP.

On inclusion, review of medical history including medication and physical examination will be performed. The following patient characteristics will be recorded: age at inclusion; gender; date the patient was diagnosed with AP/RAP/CP; aetiology of pancreatitis; previous and current use of tobacco and alcohol; presence of diabetes mellitus and other comorbidities; medications. Advanced MRI and contrast enhanced EUS with elastography will be used to characterize pancreatic morphology (Table 2). Endocrine and exocrine pancreatic function will be characterized by Hb1AC and faecal elastase and routine biochemical tests including white blood count, haemoglobin, platelets, sodium, potassium, urea, creatinine, C-reactive protein, albumin, ALT, amylase, alkaline phosphatase, bilirubin, INR, calcium, phosphate, magnesium, cobalamin, vitamin-D, PTH will be obtained. In addition to routine biochemical tests, five blood samples (5x14 ml of EDTA blood) will be deposited in a biobank for future evaluation of biomarkers of inflammation, fibrosis, and oxidative stress (Table 3). Nutritional assessment and evaluation of pain

processing by QST will be performed, and the measurement variables reported in Table 4 will be recorded.

On subsequent yearly visits review of medical history including medication and physical examination will be performed. Endocrine and exocrine pancreatic function will be reassessed together with the routine biochemical tests outlined above. Five blood samples (5x14 ml of EDTA blood) will be deposited in a biobank for future evaluation of circulating biomarkers and nutritional assessment and evaluation of pain processing by QST will be performed as described for the inclusion visit. In addition to the yearly assessments, patients will undergo advanced MRI and contrast enhanced EUS as described at the inclusion visit every second year.

Biobank

Five blood samples (5x14 ml of EDTA blood) will be deposited in a biobank on the day of inclusion, and subsequently on a yearly basis for 15 years. The purpose of the biobank is to allow analyses of potential biomarkers for the progression of disease eventually leading to CP. Analyses of biomarkers will only be conducted following approval from the Regional Committee on Health Research Ethic. The analyses will be conducted before January 1th, 2040 (the termination date). Any remaining blood will be destroyed.

Study procedures:

Magnetic resonance imaging

MRI is performed in collaboration with the radiological departments of the participating institutions. A common MRI protocol is used at all participating sites; the protocol has been described in detail previously [8,40].

Endoscopic ultrasound

EUS is performed under conscious sedation with fasting (min 6 hours) patient lying on the left side, and under continuous monitoring of pulse, blood pressure and oxygenation. EUS B-mode is applied to calculate Rosemont score (performed before further examination). Ten seconds sequences from the body and head of the pancreas are recorded. From

stomach, elastography is applied on pancreatic body: 10 seconds loop will be recorded with three images and a histogram. Contrast enhanced EUS is performed by a bolus administration of 2,4 ml SonoVue®. A film sequence of 90 seconds is recorded.

Circulating biomarkers

Venous blood samples are drawn from an antecubital vein in 14 mL EDTA tubes (15% 0.084 mL) and gently mixed. Following this procedure, the plasma is isolated and stored at -80° C until subsequent analysis.

Quantitative sensory testing

Pressure algometry: The pressure pain detection threshold (pPDT) and pressure pain tolerance threshold (pPTT) are determined on six different sites, corresponding to the somatic dermatomes above the clavicle (C5), middle of the anterior axillary fold (T4), dorsum (T10(D)), spina illiaca anterior superior (L1), rectus femoris (L4), and the abdominal pancreatic area (T10(P)). Pressure algometry is performed using an electronic pressure algometer (Somedic AB, Stockholm, Sweden), with a surface area of 1 cm^2. Pressure is increased at a rate of 30 kPa/sec until pPDT and pPTT is reached, and subjects are instructed to press a button at this point, which stops the stimulation, and the corresponding pressure (kPa) is recorded.

Conditioned pain modulation (CPM): To induce CPM, a cold pressor test is applied as the conditioning stimulus and pressure stimulations are used as test stimuli. Pressure stimulation is applied at the on the non-dominant rectus femoris until subjects reach the PTT using the equipment and procedure described above. The cold pressor test is performed by asking the subject to immerse their dominant hand in cooled water (2 °C) for 2 minutes, or less if the evoked pain is considered to be intolerable. The CPM effect is assessed as the absolute and relative change in PTT before and after the cold pressor test.

Temporal summation: In this test, the perceived intensity of a single pinprick stimulus

of 256 mN, is tested over the epigastric area (T10) and the dominant forearm and compared with that of a series of 10 repetitive pinprick stimuli of the same physical intensity (1/s applied within an area of 1 cm^2). The subject is asked to give a pain rating representing the single stimulus, and the estimated mean over the whole series of 10 stimuli using a '0–10' numerical rating scale (NRS). The temporal summation ratio is calculated as the absolute and relative change in pain scores to single and repetitive pinprick stimulations. Pin pricks are applied using a modified von Frey hairs (Optihair2-Set, Marstock Nervtest, Germany).

Nutritional assessment

Body composition: Bioelectrical impedance analysis enables a fast and accurate measurement of body compartments. For the purpose of this study, bioelectrical impedance will be assessed by the seca medical Body Composition Analyzer 514/515 (seca gmbh & co. kg, Hamburg) or BioScan 920-II (Maltron, Essex, UK). The analyser consists of a platform with an integrated scale, a handrail system, and a display and operation unit. The device uses four pairs of electrodes that are positioned at each hand and foot, with one electrode in each pair through which the electrical current enters the limb and the other electrode detects the voltage drop. Analysis time is 20-30 seconds.

Hand grip strength: Muscle strength is determined by hand grip strength measured to the nearest kilogram using a hydraulic hand dynamometer (NC70142, North Coast Medical, Arcata, CA, USA or Jamar® Smart Hand Dynamometer, Patterson medical, Warrenville, Il, USA). The dynamometer is held in the second handle position and the patient is instructed to sit on a chair with the shoulder neutrally rotated, holding the elbow bend 90º and the wrist in neutral position. Hand grip strength is measured 3 times for each hand; assessments are separated by intervals of approximately 10 seconds. The highest value for each hand is recorded and the mean value is calculated.

Timed up-and-go test: Muscle function is characterized by the timed up-and-go test (TUG). This test is performed and reported (seconds) as the time it takes a patient to get up from sitting position on a chair, walk three meters, turn around, walk back to the chair, and sit down.

Questionnaires

Quality of life: The EORCT QLQ-C30 questionnaire is used to document life quality, physical function, and a number of other health-related parameters [41]. The questionnaire has been validated specifically for assessment of patients with CP and is composed of single-item measures and multi-item scales with scores ranging from 0 to 100 after linear transformation of the raw score [42]. A high score for a functional scale represents a high level of functioning, as does a high score for the global health status, while a high score for the symptom items represents a high level of symptomatology.

Pain symptoms: The brief pain inventory short form is used to document the patients pain symptoms and its interference with daily activities (interference score) [43].

Ethics and dissemination

This study will be conducted according to the Danish national legislation on health. Permissions from the Regional Science Ethics committee and the Regional Data Protection Agency are obtained. The project will lead to better understanding of fibrogenic process that leads to CP and better understanding of changes in pain perception and nutritional aspects during the course of the disease. The combination of diagnostic imaging and biochemical parameters will enable improved identification of those patients who are at particular risk of developing early chronic pancreatitis. The improvement in identification is vital, as there are emerging inhibitors of fibrosis, which potentially could impair the ongoing fibrogenesis of pancreas. Hence, the project will lead to new strategies for patient management. Socio-economically, the results of this study may lead to reduced costs for hospitalizations and accidents related to CP. The techniques developed in the project period may also be used to understand and evaluate the fibrogenic processes in other organs, primarily the liver.

The investigations and examinations that are planned in present protocol are all a part of existing routine work-up. The major change associated with the participation in the study is that the examinations are scheduled at regular time intervals. Blood for the purpose of biobank will be drawn simultaneously with the routine blood tests. Faecal elastase, bioelectrical impedance, hand-grip test, timed-up-and-go test, quantitative sensory testing and MRI are not associated with any risks. The endoscopic ultrasound can be associated

with discomfort and in very rare cases with serious complications such as perforation of esophagus, stomach or duodenum.

The study may contain several combinations of data that may be written as papers in peer reviewed international journals. We will publish the study results regardless of whether the results are positive, negative or inconclusive.

Author contributions

All authors made substantial contributions to the design of the study. **SN**, **LLG** and **SSO** contributed to drafting the protocol and revising it critically for important intellectual content. **ABJ**, **JBF** and **FKJ** contributed with important aspects on diagnostic imaging. **DK**, **PNS** and **EFH** contributed with important aspects on endoscopic techniques. **MW**, **AB**, **CN**, **MBH**, **LNJ** and **AMD** contributed critical revisions to the draft for important intellectual content. All authors reviewed and approved the final version submitted for publication.

Conflicts of interest

None to declare.

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References

[1] Whitcomb DC, Frulloni L, Garg P, Greer JB, Schneider A, Yadav D, et al. Chronic pancreatitis: An international draft consensus proposal for a new mechanistic definition. Pancreatology 2016;16:218–24. doi:10.1016/j.pan.2016.02.001.

[2] Levy P, Barthet M, Mollard BR, Amouretti M, Marion-Audibert AM, Dyard F. Estimation of the prevalence and incidence of chronic pancreatitis and its complications. Gastroenterol Clin Biol 2006;30:838-44

[3] Lévy P, Domínguez-Muñoz E, Imrie C, Löhr M, Maisonneuve P. Epidemiology of chronic pancreatitis: Burden of the disease and consequences. United Eur Gastroenterol J 2014;2:345–54. doi:10.1177/2050640614548208.

[4] Jupp J, Fine D, Johnson CD. The epidemiology and socioeconomic impact of chronic pancreatitis. Best Pract Res Clin Gastroenterol 2010;24:219–31. doi:10.1016/j.bpg.2010.03.005.

[5] Nøjgaard C, Bendtsen F, Becker U, Andersen JR, Holst C, Matzen P. Danish patients with chronic pancreatitis have a four-fold higher mortality rate than the Danish population. Clin Gastroenterol Hepatol 2010;8:384–90. doi:10.1016/j.cgh.2009.12.016.

[6] Bang UC, Benfield T, Hyldstrup L, Bendtsen F, Beck Jensen JE. Mortality, cancer, and comorbidities associated with chronic pancreatitis: A Danish nationwide matched-cohort study. Gastroenterology 2014;146:989–94. doi:10.1053/j.gastro.2013.12.033.

[7] Etemad B, Whitcomb DC. Chronic pancreatitis: Diagnosis, classification, and new genetic developments. Gastroenterology 2001;120:682–707. doi:10.1053/gast.2001.22586.

[8] Madzak A, Olesen SS, Wathle GK, Haldorsen IS, Drewes AM, Frøkjær JB. Secretin-Stimulated Magnetic Resonance Imaging Assessment of the Benign Pancreatic Disorders: Systematic Review and Proposal for a Standardized Protocol. Pancreas 2016;45:1092–103. doi:10.1097/MPA.0000000000000606.

[9] Ahmed Ali U, Issa Y, Hagenaars JC, Bakker OJ, van Goor H, Nieuwenhuijs VB, et

al. Risk of Recurrent Pancreatitis and Progression to Chronic Pancreatitis After a First Episode of Acute Pancreatitis. Clin Gastroenterol Hepatol 2016;14:738–46. doi:10.1016/j.cgh.2015.12.040.

[10] Löhr JM, Dominguez-Munoz E, Rosendahl J, Besselink M, Mayerle J, Lerch MM, et al. United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). United Eur Gastroenterol J 2017;5:153–99. doi:10.1177/2050640616684695.

[11] Robinson PJA, Sheridan MB. Abdominal radiology Review article Pancreatitis : computed tomography and magnetic resonance imaging 2000;408:401–8.

[12] Catalano MF, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W, et al. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. Gastrointest Endosc 2009;69:1251–61. doi:10.1016/j.gie.2008.07.043.

[13] Kawada N, Tanaka S. Elastography for the pancreas: Current status and future perspective. World J Gastroenterol 2016;22:3712–24. doi:10.3748/wjg.v22.i14.3712.

[14] Pedersen N, Larsen S, Seidelin JB, Nielsen OH. Alcohol modulates circulating levels of interleukin-6 and monocyte chemoattractant protein-1 in chronic pancreatitis. Scand J Gastroenterol 2004;39:277–82. doi:10.1080/00365520310008296.

[15] Sandström A, Andersson R, Segersvärd R, Löhr M, Borrebaeck CAK, Wingren C. Serum proteome profiling of pancreatitis using recombinant antibody microarrays reveals disease-associated biomarker signatures. Proteomics - Clin Appl 2012;6:486–96. doi:10.1002/prca.201200051.

[16] Schneider A, Haas SL, Hildenbrand R, Siegmund S, Reinhard I, Nakovics H, et al. Enhanced expression of interleukin-18 in serum and pancreas of patients with chronic pancreatitis. World J Gastroenterol 2006;12:6507–14.

[17] Sri Manjari K, Jyothy A, Shravan Kumar P, Prabhakar B, Uma Devi M, Ramanna M, et al. A single-nucleotide polymorphism in tumor necrosis factor-α (-308 G/A) as a biomarker in chronic pancreatitis. Gene 2014;539:186–9. doi:10.1016/j.gene.2014.02.014.

[18] Kamath MG, Pai CG, Kamath A, Kurien A. Monocyte chemoattractant protein-1, transforming growth factor-β1, nerve growth factor, resistin and hyaluronic acid as serum markers: comparison between recurrent acute and chronic pancreatitis. Hepatobiliary Pancreat Dis Int 2016;15:209–15. doi:10.1016/S1499-3872(15)60029-

7.

[19] Ceyhan GO, Deucker S, Demir IE, Erkan M, Schmelz M, Bergmann F, et al. Neural fractalkine expression is closely linked to pain and pancreatic neuritis in human chronic pancreatitis. Lab Investig 2009;89:347–61. doi:10.1038/labinvest.2008.170.

[20] Ito T. Can measurement of chemokines become useful biological and functional markers of early-stage chronic pancreatitis? J Gastroenterol 2007;42:72–7. doi:10.1007/s00535-006-1929-4.

[21] Bai H, Chen X, Zhang L, Dou X. The effect of sulindac , a non-steroidal anti-inflammatory drug , attenuates inflammation and fibrosis in a mouse model of chronic pancreatitis 2012.

[22] Roeb E. Matrix metalloproteinases and liver fibrosis (translational aspects). Matrix Biol 2017. doi:10.1016/j.matbio.2017.12.012.

[23] Kurzepa J, M A, Czechowska G, Kurzepa J, Celiński K, Kazmierak W, et al. Role of MMP-2 and MMP-9 and their natural inhibitors in liver fibrosis, chronic pancreatitis and non-specific inflammatory bowel diseases. Hepatobiliary Pancreat Dis Int 2014;13:570–9. doi:10.1016/S1499-3872(14)60261-7.

[24] Descamps FJ, Van den Steen PE, Martens E, Ballaux F, Geboes K, Opdenakker G. Gelatinase B is diabetogenic in acute and chronic pancreatitis by cleaving insulin. FASEB J 2003;17:887–9. doi:10.1096/fj.02-0725fje.

[25] Tandon RK, Garg PK. Oxidative Stress in Chronic Pancreatitis: Pathophysiological Relevance and Management. Antioxid Redox Signal 2011;15:2757–66. doi:10.1089/ars.2011.4115.

[26] Uden S, Bilton D, Nathan L, Hunt LP, Main C, Braganza JM. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. Aliment Pharmacol Ther 1990;4:357–71.

[27] Bhardwaj P, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. Gastroenterology 2009;136:149–159.e2.

[28] Forsmark CE, Liddle RA. The challenging task of treating painful chronic pancreatitis. Gastroenterology 2012;143:533–5.

[29] Siriwardena AK, Mason JM, Sheen AJ, Makin AJ, Shah NS. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study.

Gastroenterology 2012;143:655–63.e1. doi:10.1053/j.gastro.2012.05.046.

[30] Bopanna S, Nayak B, Prakash S, Shalimar, Mahapatra SJ, Garg PK. Increased oxidative stress and deficient antioxidant levels may be involved in the pathogenesis of idiopathic recurrent acute pancreatitis. Pancreatology 2017;17:529–33. doi:10.1016/j.pan.2017.06.009.

[31] Poulsen JL, Olesen SS, Malver LP, Frokjaer JB, Drewes AM. Pain and chronic pancreatitis: a complex interplay of multiple mechanisms. World J Gastroenterol 2013;19:7282–91. doi:10.3748/wjg.v19.i42.7282.

[32] Bouwense SA, de Vries M, Schreuder LT, Olesen SS, Frokjaer JB, Drewes AM, et al. Systematic mechanism-orientated approach to chronic pancreatitis pain. World J Gastroenterol 2015;21:47–59. doi:10.3748/wjg.v21.i1.47.

[33] Olesen SS, Graversen C, Bouwense SAW, van Goor H, Wilder-Smith OHG, Drewes AM. Quantitative sensory testing predicts pregabalin efficacy in painful chronic pancreatitis. PLoS One 2013;8:e57963. doi:10.1371/journal.pone.0057963.

[34] Schneider A, Löhr JM, Singer M V. The M-ANNHEIM classification of chronic pancreatitis: Introduction of a unifying classification system based on a review of previous classifications of the disease. J Gastroenterol 2007;42:101–19. doi:10.1007/s00535-006-1945-4.

[35] Banks P a, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013;62:102–11. doi:10.1136/gutjnl-2012-302779.

[36] Kalaria R, Abraham P, Desai DC, Joshi A, Gupta T, Abraham P. Rate of recurrence in Indian patients presenting with acute pancreatitis and identification of chronicity on follow up : Possible risk factors for progression 2018:10–5.

[37] Sankaran SJ, Xiao AY, Wu LM, Windsor JA, Forsmark CE, Petrov MS. Frequency of Progression from Acute to Chronic Pancreatitis and Risk Factors: A Meta-analysis. Gastroenterology 2015;149:1490–1500.e1. doi:10.1053/j.gastro.2015.07.066.

[38] Schreyer AG, Jung M, Riemann JF, Niessen C, Pregler B, Grenacher L, et al. S3 guideline for chronic pancreatitis - Diagnosis, classification and therapy for the radiologist. RoFo Fortschritte Auf Dem Gebiet Der Rontgenstrahlen Und Der Bildgeb Verfahren 2004;186:1002–8. doi:10.1055/s-0034-1385005.

[39] Kuwahara T, Hirooka Y, Kawashima H, Ohno E, Ishikawa T, Kawai M, et al. Quantitative diagnosis of chronic pancreatitis using EUS elastography. J Gastroenterol 2017;52:868–74. doi:10.1007/s00535-016-1296-8.

[40] Madzak A, Olesen SS, Lykke Poulsen J, Bolvig Mark E, Mohr Drewes A, Frøkjær JB. MRI assessed pancreatic morphology and exocrine function are associated with disease burden in chronic pancreatitis. Eur J Gastroenterol Hepatol 2017;29. doi:10.1097/MEG.0000000000000955.

[41] Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 1993;85:365–76.

[42] Fitzsimmons D, Kahl S, Butturini G, Van Wyk M, Bornman P, Bassi C, et al. Symptoms and quality of life in chronic pancreatitis assessed by structured interview and the EORTC QLQ-C30 and QLQ-PAN26. Am J Gastroenterol 2005;100:918–26. doi:10.1111/j.1572-0241.2005.40859.x.

[43] Mendoza T, Mayne T, Rublee D, Cleeland C. Reliability and validity of a modified Brief Pain Inventory short form in patients with osteoarthritis. Eur J Pain 2006;10:353–61. doi:10.1016/j.ejpain.2005.06.002.

Tables

Table 1. Schedule of Events.

		On inclusion	Yearly	Every second year
Informed consent and assessment of eligibility criteria		X		
Review of medical history including medication		X	X	
Physical examination	Anthropometrics	X	X	
	Vital Signs	X	X	
Clinical Lab and Biobank		X	X	
F-elastase-1 and HbA_1C		X	X	
Diagnostic Imaging	Advanced MRI	X		X
	Contrast enhanced EUS and elastography	X		X
Pain Processing	Quantitative Sensory Testing	X	X	
Questionnaires		X	X	
Body composition: Bioelectrical impedance		X	X	
Muscle strength and function: Hand grip strength and timed up and go test		X	X	

Table 2. Imaging assessment parameters.

Method	Modality	Measurement variables
MRI	T1, T2, MRCP	Cambridge classification with modification for MRCP [38]
		Main pancreatic duct diameter
		Pancreatic dimensions and volume
	DWI	Apparent diffusion coefficient (ADC)
	DIXON	Fat signal faction (FSF)
EUS	EUS B-mode	Rosemont score [12]
	Contrast enhanced	90 sec film recorded from stomach of pancreatic body will be recorded for later analysis
	Elastography	Histogram analysis and measurement of strain ratio [39]

Table 3. Circulating biomarker assessment parameters.

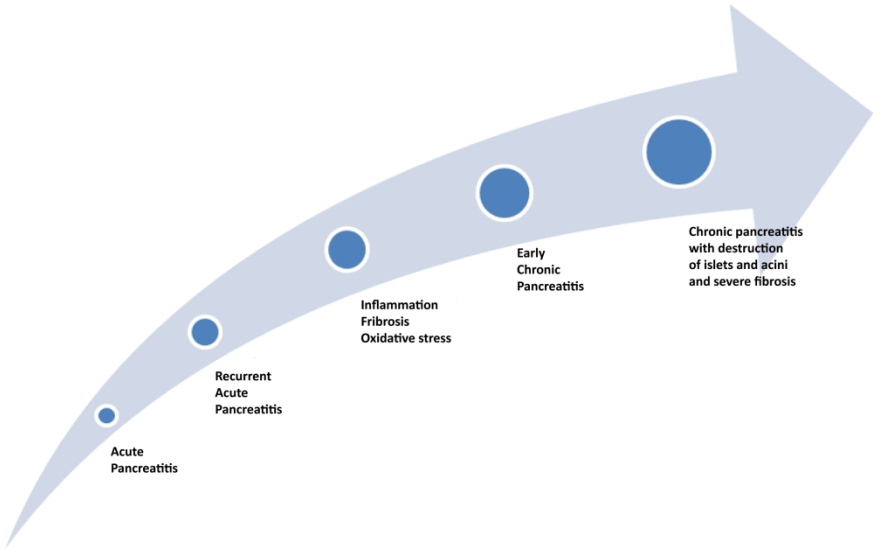
	Measurement variable
Inflammation	Interelukin(IL)-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-alpha
Fibrosis	Transforming growth factor beta-1 (TGF-β1), Soluble fractalkine (s-Fr), Monocyte chemoattractant protein 1 (MCP-1), Matrix metalloproteinases (MMPs)
Oxidative stress	Glutathione peroxidase, Vitamin C, Ferric reducing ability of plasma, Malondialdehyde, 4-hydroxynonenal, superoxide dismutase, nitric oxide

Table 4. Nutritional and QST assessment parameters

	Method	Measurement variable
Nutritional assessment	Muscle strength: Hand held dynamometer	Muscle strength (kg)
	Muscle function: Timed up and go test	Seconds
	Body composition: Bioelectrical impedance	Various bioelectrical impedance parameters
Assessment of pain processing	Pressure algometry	Pressure pain and tolerance thresholds (kPa)
	Temporal summation	Absolute and relative change in pain scores (NRS) to single and repetitive pinprick stimulations
	Conditioned pain modulation	Absolute and relative change in pressure pain tolerance thresholds (kPa) before and after cold pressor test

Figure legend

Figure 1. A large proportion of patients develop CP after an episode of acute pancreatitis. The development of CP is associated with continued or recurrent inflammation and progressive development of fibrosis.


Acute
Pancreatitis
Recurrent
Acute
Pancreatitis
Inflammation
Fribrosis
Oxidative stress
Early
Chronic
Pancreatitis
Chronic pancreatitis
with destruction
of islets and acini
and severe fibrosis

Figure

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Characterization of the fibro-inflammatory process involved in progression from acute to chronic pancreatitis: study protocol for a multicentre, prospective cohort study

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Characterization of the fibro-inflammatory process involved in progression from acute to chronic pancreatitis: study protocol for a multicentre, prospective cohort study

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Abstract

Introduction: Chronic pancreatitis (CP) is thought to present the end-stage of a continuous disease process evolving from acute pancreatitis (AP), over recurrent acute pancreatitis (RAP), to early and end-stage CP. Due to the irreversible nature of CP, early detection and prevention is key. Prospective assessment based on advanced imaging modalities as well as biochemical markers of inflammation, fibrosis and oxidative stress may provide a better understanding of the underlying pathological processes and help identify novel biomarkers of disease with the ultimate goal of early diagnosis, intervention and prevention of disease progression. This paper describes the protocol of a prospective multicentre cohort study investigating the fibro-inflammatory process involved in progression from acute to chronic pancreatitis using state-of-the-art diagnostic imaging modalities and circulating biomarkers of inflammation, fibrosis and oxidative stress.

Methods and analysis: Adult control subjects and patients at different stages of CP according to the M-ANNHEIM system will be recruited from outpatient clinics at the participating sites and form three cohorts: controls (n=40), suspected CP (n=60) and definitive CP (n=60). Included patients will be followed prospectively for 15 years with advanced MRI and contrast enhanced EUS with elastography, assessment of endocrine and exocrine pancreatic function, biochemical and nutritional assessment, and evaluation of pain processing using quantitative sensory testing. Blood samples for a biobank will be obtained. The purpose of the biobank is to allow analyses of potential circulating biomarkers of disease progression including markers of inflammation, fibrosis and oxidative stress.

Ethics and dissemination: Permissions from the Regional Science Ethics committee and the Regional Data Protection Agency have been obtained. We will submit the results of the study for publication in peer-reviewed journals regardless of whether the results are positive, negative or inconclusive.

Article Summary

Strengths and limitations of this study

- The prospective and repetitive assessment of imaging, functional and biochemical parameters in the present study may provide clinical useful biomarkers for the identification of patients at increased risk of developing chronic pancreatitis.
- The relatively homogenous population, both genetically, socially and economically provide us with a unique and unbiased framework for studying the natural course of pancreatitis.
- A limitation of the study is that “state-of-the art” imaging, functional, endoscopic and biochemical assessment parameters may change during the study period, thus making comparisons over time difficult and potentially based on outdated technology.

Introduction

Chronic pancreatitis (CP) is a progressive fibro-inflammatory disease associated with persistent pathologic response to parenchymal injury or stress [1]. Over time, the fibro-inflammatory process can lead to irreversible fibrosis with morphological changes and loss of pancreatic function. Due to the irreversible nature of end-stage pancreatitis, early detection and prevention is key.

The global pooled incidence of CP is 10 per 100,000 general population per year [2] and prevalence rates are reported to be between 40-50 per 100,000 persons [3]. However, cumulating evidence suggests that the incidence of CP is rising [4,5] and a Danish nationwide population-based cohort study showed a five-fold increased mortality rate with a life expectancy that was approximately 8 years less among CP patients compared with population controls [6]. Taken together, this illustrates the great impact CP has on health, economic, and social aspects.

Alcohol and smoking are the major aetiological risk factors of CP [7], and there seems to be an additive effect of alcohol and smoking. Other causes include hypercalcemia, hyperlipidaemia, obstruction of the biliary or pancreatic duct, autoimmune conditions, and hereditary conditions. About 20% of cases are idiopathic, but this number is likely to decrease as the understanding of the pathophysiology underlying CP evolves.

Currently the diagnosis of CP is based on a combination of symptoms, biochemical tests, and imaging parameters. Abdominal pain is the cardinal symptom of CP and present in the majority of patients during their disease course, but the intensity and temporal pattern varies substantially. At later disease stages patients may develop diabetes, steatorrhea and weight loss, but the disease course is unpredictable in the majority of patients, thus making symptom-based assessment unreliable. The biochemical tests used for assessment of CP include glycated haemoglobin [HbA_1C] (endocrine pancreatic function) and faecal elastase [FE], breath test or direct pancreatic function tests (exocrine pancreatic function tests). Diagnostic imaging is used for characterisation of typical morphological changes including parenchymal lobulation, calcifications, parenchymal atrophy, pseudocysts and pancreatic duct abnormalities. Various imaging modalities are used for assessment including computed tomography (CT), magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS)[8].

Some patients are diagnosed with CP without preceding attacks of acute pancreatitis (AP). These patients typically present with symptoms of end stage disease including steatorrhea, diabetes and chronic abdominal pain. However, a large proportion of patients diagnosed with CP have a history of AP or recurrent acute pancreatitis (RAP) (Fig. 1). In keeping with this, a metanalysis found that 10% percent of patients with a first episode of AP and 36% of patients with RAP develop CP, with a higher risk of disease progression among smokers, alcoholics, and men [2] [3] [9]. These findings attest to the understanding of CP as a continuous disease process evolving from AP, over RAP, to early and end-stage CP as also outlined in a recent international consensus draft on a mechanistic definition of CP [1]. Importantly, this understanding provides a theoretical framework for studying the fibro-inflammatory processes involved in the development of CP by prospectively assessing patients admitted with AP and RAP during their course of disease towards early and end-stage CP. Hence, prospective assessment based on state-of-the-art imaging modalities as well as biochemical markers of inflammation, fibrosis and oxidative stress may provide a better understanding of the underlying pathological processes and help identifying novel biomarkers of disease with the ultimate goal of early diagnosis, intervention and prevention of progression. Along these lines, a holistic framework for prevention of pancreatitis has recently been introduced and outlined preventive measures at both primary, secondary and tertiary levels based on current knowledge on the natural course of disease progression [3].

Current standards and challenges in assessment of pancreatitis

Imaging

Various imaging techniques can be used to characterize pancreatic morphology. CT is in most cases used for the initial work-up and is particularly useful for detection of advanced disease characteristics of CP including parenchymal and intraductal calcifications, gross duct pathology and parenchymal atrophy [10]. Furthermore, CT is key in ruling out relevant differential diagnosis including pancreatic cancer. Despite CT being the imaging modality of choice for initial investigation, it cannot exclude a diagnosis of CP, nor can it be used to exclusively diagnose ‘early CP’.

MRI based techniques including magnetic resonance cholangiopancreatography (MRCP) are more accurate for detection of pancreatic duct abnormalities (dilatations and strictures) and subtle changes of the pancreatic parenchyma and side branches, which can be attributed to early signs of CP [11]. The MR based techniques can be further refined by assessment of pancreatic gland volume and administration of secretin whereby a combined quantitative morphological and functional assessment of the pancreatic gland can be obtained. This includes characterization of subtle ductal abnormalities and pancreatic secretion. In addition, diffusion weighted imaging (DWI) can provide a proxy of fibrotic changes and emerging methods like the DIXON technique can be used to assess fat signal fraction (FSF) for evaluation of fatty infiltration of the pancreatic parenchyma.

The gold standard for establishing a diagnosis of early CP imaging is currently endoscopic ultrasound (EUS). Diagnostic criteria related to the parenchyma and the pancreatic duct have been established, and in 2009 a weighted scoring system was proposed by a panel of experts, the Rosemont score [12]. Emerging endoscopic methods with assessment of pancreatic blood flow and tissue stiffness by elastography may further improve the diagnostic utility of EUS [13].

Taken together, a multimodal approach combining advanced MRI and EUS techniques will likely provide novel and complementary information on pancreatic morphology and function that may reflect unique aspects of the pathology underlying progressive pancreatitis disorders (AP → RAP → CP) that have not previously been identified using conventional imaging modalities.

Circulating biomarkers of inflammation, fibrosis and oxidative stress

Most of our knowledge about physiological and pathophysiological mechanisms in healthy and diseased pancreas comes from experimental studies. Due to the anatomy, complexity and risk associated with direct sampling from pancreatic tissue, most of our information on human pancreas arises from circulating marker of pancreatic physiology or injury.

Inflammation: The inflammatory profile characterising early stages of pancreatitis may reflect the trajectory of disease and subsequent development of fibrosis. Hence, patients with CP have increased serum levels of interleukin (IL)-6, IL-4, IL-18 [14][15][16], which are

implicated in the wound healing response and the progression of fibrotic disease. Also, tumour necrosis factor (TNF)-α may be implicated in disease progression [17].

Fibrosis: Patients with CP have elevated plasma levels of fibro genesis markers such as transforming growth factor beta-1 (TGF-β1), soluble fractalkine (s-FR) and monocyte chemoattractant protein 1 (MCP-1) [18][19][20]. Decreasing MCP-1 and TGF-β1 levels with anti-inflammatory drugs was shown to reduce the severity of CP in a caerulein-induced CP mouse model [21]. Matrix metalloproteinases (MMPs) are a group of enzymes involved in the degradation of collagen, proteoglycans, elastin and fibronectin, all of which play an important role in the remodelling of the extracellular matrix. The role of MMPs in the development of progressive fibrosis and cirrhosis is well established [22] and emerging evidence suggests that MMPs may have a similar role in CP [23]. Elevated serum MMP-9 levels have been found in patients with both AP and CP, and active form of MMP-9 could be involved in the development of diabetes mellitus associated with CP [24].

Oxidative stress is a result of imbalance between reactive oxygen species and their neutralizing mechanisms, which may result in cell or tissue damage. Increased oxidative stress with the resulting formation of free radicals has been implicated in the pathophysiology of CP [25] and treatment with antioxidants has been shown to reduce disease and pain severity in randomized controlled trials [26,27], although the effects may be related to the underlying aetiology [28,29]. Recent studies have shown that oxidative stress may also be implicated in RAP [30]. Free radicals do not only exert local effect in pancreas but are also released in systemic circulation and can thus be quantified.

Pain processing and quantitative sensory testing

The processing of pain in central pain pathways undergoes changes in a large proportion of patients with painful CP. These include sensitization of central pain pathways, impaired capacity of pain modulation as well as structural and functional changes in the brain [31]. Although the presence of these changes is well documented in numerous cross-sectional studies, the temporal aspects of changes in pain processing during disease progression have not previously been investigated in the context of pancreatitis. Quantitative sensory testing (QST) can be used to map the pain system; the technique is based on the rationale that different neural pathways and networks can be explored using standardized stimulation

with simultaneous recording of the evoked pain response by psychophysical and/or objective methods [32]. Due to spinal convergence between visceral afferents from the pancreas and somatic afferents from upper abdominal skin dermatomes, somatic QST can be used to assess if the pain system is sensitized by nociceptive input from the pancreas [33]. Together with specific test paradigms (temporal summation and assessment of pain modulation) QST can be used to characterize the state of the pain system and to document if patients have abnormal central pain processing.

Nutrition

The risk factors associated with underweight and malnutrition in patients with CP are complex and likely multifactorial with the most frequently reported being pancreatic exocrine insufficiency. However, many patients lose weight early in their disease course and before evolution of pancreatic exocrine insufficiency. For example, postprandial pain, which is seen in many patients, may also limit food intake and lead to underweight and malnutrition. There is a paucity of data on this important topic, and continuous characterization of nutritional status in patients going through phases of AP, to RAP to CP may provide us with essential knowledge on metabolic processes related to inflammation and fibrosis. A combined use of bioelectrical impedance, hand-grip strength (HGS), timed up and go test (TUG), assessment of endocrine- (Hb1AC) and exocrine insufficiency (FE), and biochemical tests enables qualitative and quantitative assessment of nutritional status.

Hypotheses

Current knowledge on the progression of CP is mostly based on cross-sectional studies including patients with different stages of RAP and CP. By prospectively following well characterised cohorts of patients with pancreatitis at different stages (AP → RAP → CP) with serial imaging (advanced MRI and EUS), profiling of fibro-inflammatory pathways using circulating biomarkers and assessment of nutritional status and pain processing using QST, this study will help elucidate the underlying pathophysiology of pancreatitis.

The hypotheses of this study are:

- Prospective assessment of patients progressing from AP to CP, using advanced imaging techniques and circulating biomarkers of inflammation, fibrosis and oxidative stress, can provide new mechanistic insight to the underlying fibro-inflammatory

process. This knowledge may be used to design future clinical studies focusing on prevention of inflammation and fibrosis in the pancreas and other organ systems.

- Changes in pain processing over time are poorly understood in patients progressing from AP to CP. This information may be used to identify patients at risk for developing a chronic pain syndrome with irreversible neuroplastic changes in the central nervous system.
- Changes in nutritional state over time are poorly understood in patients progressing from AP to CP. This information may be used to identify patients at risk for developing malnutrition.

Aims

Primary aim: To prospectively investigate the fibro-inflammatory process involved in progression from acute to chronic pancreatitis using state-of-the-art diagnostic imaging modalities (contrast enhanced EUS, EUS guided elastography and advanced MRI) and circulating biomarkers of inflammation, fibrosis and oxidative stress.

Secondary aim: To prospectively characterize pain processing and nutritional status in patients progressing from acute to chronic pancreatitis.

Methods

Study design

Prospective cohort study including adult patients with RAP, probable or definitive CP recruited from outpatient clinics at the three participating sites (Hvidovre, Bispebjerg, and Aalborg University Hospitals). The study is approved by the Danish Data Protection Agency (VD-2018-298; I-suite no.: 6542) and the Regional Committee on Health Research Ethics (Journal-no.: H-18017705). All study participants will provide written informed consent and the study will be conducted according to the Declaration of Helsinki. The study is non-interventional; all participants will be treated and monitored according to the current best clinical evidence-based practice. The patient inclusion began in February 2019. We anticipate to complete the inclusion of all cohorts during a two years period. We will follow the patient cohorts for 15 years, and the anticipated completion date is February 1st, 2035.

Inclusion criteria

- Age 18-75 years
- **Cohort 1 (definitive CP)**: Patients with definitive CP (N=60) of any aetiology except gallstone induced CP. The M-ANNHEIM diagnostic criteria will be used for the definition of definitive CP. Hence, patients will be included in this cohort if they fulfil one or more of the following criteria: pancreatic calcifications, moderate or marked pancreatic duct changes (Cambridge III or IV) or typical histology of CP [34].
- **Cohort 2 (suspected CP):** Patients with RAP except gallstone induced RAP and patients with probable CP according to the M-ANNHEIM criteria (N=60). RAP is defined as two or more cases of AP as diagnosed by the revised Atlanta Criteria [35]. Probable CP is defined as patients with a typical history of CP (persistent abdominal pain and/or a history of a single AP episode) in combination with any of the following: mild pancreatic duct changes (Cambridge I or II), recurrent or persistent pseudocysts, abnormal exocrine pancreatic function test or post pancreatitis diabetes mellitus (elevated glycated haemoglobin >3 months after diagnosis of pancreatitis) [3] [34].
- **Cohort 3 (control subjects):** Subjects without any previous history of pancreatic or gastrointestinal diseases will be included in the control cohort (n=40).

Exclusion criteria

Patients with the following conditions will be excluded from participation in the study:

a. Pregnant or lactating patients
b. Patients in whom MRI is contra-indicated (metallic implants, pacemaker, implantable cardioverter defibrillator, claustrophobia)
c. Patients with chronic liver disease, chronic renal failure, malignancy, chronic inflammatory bowel syndrome, chronic obstructive lung disease, pulmonary fibrosis. If the patient develops any of the listed diseases during the study period they will continue their participation in the study.
d. Patients treated with anti-inflammatory drugs of any kind at the time of inclusion: local or systemic corticosteroids, NSAID´s, salazopyrins or other. However, once included, they will continue their participation even though they receive treatment with anti-inflammatory drugs.

Sample size, time schedule and follow up

The present study is observational. No previous long-term prospective studies have evaluated the fibro-inflammatory process associated with the development of CP and we were therefore unable to undertake a valid sample size calculation. Consequently, the sample size was set to 60 participants in the patient cohorts, assuming that 36% of participants in cohort 2 will progress to definitive CP [3][36][37]. Similar sample size estimates apply for a recently published study protocol from the Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer (CPDPC) that is also based on MRI assessment parameters [38]. We expected that we will be able to reach our sample size during an inclusion period of two years, based on experience from previous cohort studies from our departments. We anticipate that all three centres will include approximately equal numbers of participants.

Included patients will be followed up prospectively for 15 years. An interim analysis, assessing both clinical, functional, imaging and biochemical parameters will be performed after 4 years [39]. Patient cohorts will follow the same time schedule and plan for follow up as outlined in Table 1.

Included patients will be evaluated during a quiescent phase of disease; consequently, an acute attack of pancreatitis or exacerbation in CP requiring hospital admission will lead to a quarantine period of 4 weeks before evaluation by the protocol can be performed. Resolution of pancreatitis will be documented by assessment of plasma amylase and CRP.

On inclusion, review of medical history including medication, physical examination will be performed. The following patient characteristics will be recorded: age at inclusion; gender; date the patient was diagnosed with AP/RAP/CP; aetiology of pancreatitis; previous and current patterns of tobacco and alcohol consumption; presence of diabetes mellitus and other comorbidities; medications. Advanced MRI and contrast enhanced EUS with elastography will be used to characterize pancreatic morphology [40][41] (Table 2). Endocrine and exocrine pancreatic function will be characterized by Hb1AC and faecal elastase and routine biochemical tests including white blood count, haemoglobin, platelets, sodium, potassium, urea, creatinine, C-reactive protein, albumin, ALT, amylase, alkaline

phosphatase, bilirubin, INR, calcium, phosphate, magnesium, cobalamin, vitamin-D, PTH will be obtained. In addition to routine biochemical tests, blood samples (see later) will be deposited in a biobank for future evaluation of biomarkers of inflammation, fibrosis, and oxidative stress (Table 3). Nutritional assessment and evaluation of pain processing by QST will be performed, and the measurement variables reported in Table 4 will be recorded.

On subsequent yearly visits review of medical history including medication, patterns of smoking and alcohol consumption during the past year and physical examination will be performed. Endocrine and exocrine pancreatic function will be reassessed together with the routine biochemical tests outlined above. Blood samples will be deposited in a biobank for future evaluation of circulating biomarkers and nutritional assessment and evaluation of pain processing by QST will be performed as described for the inclusion visit. In addition to the yearly assessments, patients will undergo advanced MRI and contrast enhanced EUS as described at the inclusion visit every second year.

During the study period, patients will be counselled and treated according to best clinical practice, including advice on alcohol and smoking cessation.

Biobank

Ninety ml of blood (processed as plasma, serum, buffy coat and cells) will be deposited in a biobank on the day of inclusion, and subsequently on a yearly basis for 15 years. The purpose of the biobank is to allow analyses of potential biomarkers for disease progression. The analyses will be conducted before January 1th, 2040 (the study termination date). Any remaining blood will be destroyed. The deposited blood material will also enable us to perform genetic studies if deemed relevant. The genetic analyses will only be performed following approval from the Regional Committee on Health Research Ethic.

Patient and Public Involvement

Patients and public were not involved in the design and recruitment of the present study. As the study is designed as a part of a clinical setup and monitoring, the results of the study will be continuously discussed with the participating patients.

Study procedures:

Magnetic resonance imaging

MRI is performed in collaboration with the radiological departments of the participating institutions. A common MRI protocol is used at all participating sites; the protocol has been described in detail previously [8,42].

Endoscopic ultrasound

EUS is performed under conscious sedation with fasting (min 6 hours) patient lying on the left side, and under continuous monitoring of pulse, blood pressure and oxygenation. EUS B-mode is applied to calculate Rosemont score (performed before further examination). Ten seconds sequences from the body and head of the pancreas are recorded. From stomach, elastography is applied on pancreatic body: 10 seconds loop will be recorded with three images and a histogram. Contrast enhanced EUS is performed by a bolus administration of 2,4 ml SonoVue®. A film sequence of 90 seconds is recorded.

Circulating biomarkers

Venous blood samples are drawn from an antecubital vein. Following this procedure, the blood is processed as plasma, serum, buffy coat and cells, and stored at -80° C until subsequent analysis (biobank).

Quantitative sensory testing

Pressure algometry: The pressure pain detection threshold (pPDT) and pressure pain tolerance threshold (pPTT) are determined on six different sites, corresponding to the somatic dermatomes above the clavicle (C5), middle of the anterior axillary fold (T4), dorsum (T10(D)), spina illiaca anterior superior (L1), rectus femoris (L4), and the abdominal pancreatic area (T10(P)). Pressure algometry is performed using an electronic pressure algometer (Somedic AB, Stockholm, Sweden), with a surface area of 1 cm^2. Pressure is increased at a rate of 30 kPa/sec until pPDT and pPTT is reached, and subjects are instructed to press a button at this point, which stops the stimulation, and the corresponding pressure (kPa) is recorded.

Conditioned pain modulation (CPM): To induce CPM, a cold pressor test is applied as the conditioning stimulus and pressure stimulations are used as test stimuli. Pressure

stimulation is applied at the on the non-dominant rectus femoris until subjects reach the PTT using the equipment and procedure described above. The cold pressor test is performed by asking the subject to immerse their dominant hand in cooled water (2 °C) for 2 minutes, or less if the evoked pain is considered to be intolerable. The CPM effect is assessed as the absolute and relative change in PTT before and after the cold pressor test.

Temporal summation: In this test, the perceived intensity of a single pinprick stimulus of 256 mN, is tested over the epigastric area (T10) and the dominant forearm and compared with that of a series of 10 repetitive pinprick stimuli of the same physical intensity (1/s applied within an area of 1 cm^2). The subject is asked to give a pain rating representing the single stimulus, and the estimated mean over the whole series of 10 stimuli using a '0–10' numerical rating scale (NRS). The temporal summation ratio is calculated as the absolute and relative change in pain scores to single and repetitive pinprick stimulations. Pin pricks are applied using a modified von Frey hairs (Optihair2-Set, Marstock Nervtest, Germany).

Nutritional assessment

Body composition: Bioelectrical impedance analysis enables a fast and accurate measurement of body compartments. For the purpose of this study, bioelectrical impedance will be assessed by the seca medical Body Composition Analyzer 514/515 (seca gmbh & co. kg, Hamburg) or BioScan 920-II (Maltron, Essex, UK). The analyser consists of a platform with an integrated scale, a handrail system, and a display and operation unit. The device uses four pairs of electrodes that are positioned at each hand and foot, with one electrode in each pair through which the electrical current enters the limb and the other electrode detects the voltage drop. Analysis time is 20-30 seconds.

Hand grip strength: Muscle strength is determined by hand grip strength measured to the nearest kilogram using a hydraulic hand dynamometer (NC70142, North Coast Medical, Arcata, CA, USA or Jamar® Smart Hand Dynamometer, Patterson medical, Warrenville, Il, USA). The dynamometer is held in the second handle position and the patient is instructed to sit on a chair with the shoulder neutrally rotated, holding the elbow bend 90º and the wrist in neutral position. Hand grip strength is measured 3 times for each hand; assessments are separated by intervals of approximately 10 seconds. The highest value for each hand is recorded and the mean value is calculated.

Timed up-and-go test: Muscle function is characterized by the timed up-and-go test (TUG). This test is performed and reported (seconds) as the time it takes a patient to get up from sitting position on a chair, walk three meters, turn around, walk back to the chair, and sit down.

Questionnaires

Quality of life: The EORCT QLQ-C30 questionnaire is used to document life quality, physical function, and a number of other health-related parameters [43]. The questionnaire has been validated specifically for assessment of patients with CP and is composed of single-item measures and multi-item scales with scores ranging from 0 to 100 after linear transformation of the raw score [44]. A high score for a functional scale represents a high level of functioning, as does a high score for the global health status, while a high score for the symptom items represents a high level of symptomatology.

Pain symptoms: The brief pain inventory short form is used to document the patients pain symptoms and its interference with daily activities (interference score) [45].

Ethics and dissemination

This study will be conducted according to the Danish national legislation on health. Permissions from the Regional Science Ethics committee and the Regional Data Protection Agency are obtained. The project will lead to better understanding of fibrogenic process that leads to CP and better understanding of changes in pain perception and nutritional aspects during the course of the disease. The combination of diagnostic imaging and biochemical parameters will enable improved identification of those patients who are at particular risk of developing early chronic pancreatitis. The improvement in identification is vital, as there are emerging inhibitors of fibrosis, which potentially could impair the ongoing fibrogenesis of pancreas. Hence, the project will lead to new strategies for patient management. Socio-economically, the results of this study may lead to reduced costs for hospitalizations and accidents related to CP. The techniques developed in the project period may also be used to understand and evaluate the fibrogenic processes in other organs, primarily the liver.

The investigations and examinations that are planned in present protocol are all a part of existing routine work-up. The major change associated with the participation in the study is

that the examinations are scheduled at regular time intervals. Blood for the purpose of biobank will be drawn simultaneously with the routine blood tests. Faecal elastase, bioelectrical impedance, hand-grip test, timed-up-and-go test, quantitative sensory testing and MRI are not associated with any risks. The endoscopic ultrasound can be associated with discomfort and in very rare cases with serious complications such as perforation of esophagus, stomach or duodenum.

The study may contain several combinations of data that may be written as papers in peer reviewed international journals. We will publish the study results regardless of whether the results are positive, negative or inconclusive.

Discussion

Recently the US based Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer (CPDPC) has published their study protocol for the Prospective Evaluation of Chronic Pancreatitis for Epidemiologic and Translational Studies (PROCEED) [39]. Although the PROCEED study is mainly based on CT based imaging parameters, as opposed to the MRI based approach in our study, the design of the PROCEED study is in many ways comparable to ours. Hence, both studies focus on prospective assessment of disease progression parameters in well-defined cohorts of patients with CP at different disease stages using cross-sectional imaging and circulating biomarkers. The patient cohorts have been characterised using comparatively similar criteria, which will allow comparison across studies.

A unique characteristic of our study, compared to the PROCEED study, is the annual assessment of pain processing and modulation based on a QST protocol specifically designed for pancreatic pain. In addition, detailed nutritional assessment, using bioelectrical impedance and muscle function tests, distinct our protocol from that of the PROCEED study [39]. Finally, our study population comprises of a very homogenous population, both genetically, socially and economically, which provides us with an opportunity to study the natural course of CP with reduced bias from diverse patient characteristics.

Author contributions

All authors made substantial contributions to the design of the study. **SN**, **LLG** and **SSO** contributed to drafting the protocol and revising it critically for important intellectual content. **ABJ**, **JBF** and **FKJ** contributed with important aspects on diagnostic imaging. **DK**, **PNS** and **EFH** contributed with important aspects on endoscopic techniques. **MW**, **AB**, **CN**, **MBH**, **LNJ** and **AMD** contributed critical revisions to the draft for important intellectual content. All authors reviewed and approved the final version submitted for publication.

Conflicts of interest

None to declare.

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References

[1] Whitcomb DC, Frulloni L, Garg P, Greer JB, Schneider A, Yadav D, et al. Chronic pancreatitis: An international draft consensus proposal for a new mechanistic definition. Pancreatology 2016;16:218–24. doi:10.1016/j.pan.2016.02.001.

[2] Xiao AY, Tan ML, Wu LM, Asrani VM, Windsor JA, Yadav D, Petrov MS. Global incidence and mortality of pancreatic diseases: a systematic review, meta-analysis, and meta-regression of population-based cohort studies. Lancet Gastroenterol Hepatol. 2016 Sep;1(1):45-55. doi:10.1016/S2468-1253(16)30004-8.

[3] Petrov MS, Yadav D. Global epidemiology and holistic prevention of pancreatitis. Nat Rev Gastroenterol Hepatol. 2019 Mar;16(3):175-184. doi: 10.1038/s41575-018-0087-5.

[4] Jupp J, Fine D, Johnson CD. The epidemiology and socioeconomic impact of chronic pancreatitis. Best Pract Res Clin Gastroenterol 2010;24:219–31. doi:10.1016/j.bpg.2010.03.005.

[5] Nøjgaard C, Bendtsen F, Becker U, Andersen JR, Holst C, Matzen P. Danish patients with chronic pancreatitis have a four-fold higher mortality rate than the Danish population. Clin Gastroenterol Hepatol 2010;8:384–90. doi:10.1016/j.cgh.2009.12.016.

[6] Bang UC, Benfield T, Hyldstrup L, Bendtsen F, Beck Jensen JE. Mortality, cancer, and comorbidities associated with chronic pancreatitis: A Danish nationwide matched-cohort study. Gastroenterology 2014;146:989–94. doi:10.1053/j.gastro.2013.12.033.

[7] Etemad B, Whitcomb DC. Chronic pancreatitis: Diagnosis, classification, and new genetic developments. Gastroenterology 2001;120:682–707. doi:10.1053/gast.2001.22586.

[8] Madzak A, Olesen SS, Wathle GK, Haldorsen IS, Drewes AM, Frøkjær JB. Secretin-Stimulated Magnetic Resonance Imaging Assessment of the Benign Pancreatic Disorders: Systematic Review and Proposal for a Standardized Protocol. Pancreas 2016;45:1092–103. doi:10.1097/MPA.0000000000000606.

[9] Ahmed Ali U, Issa Y, Hagenaars JC, Bakker OJ, van Goor H, Nieuwenhuijs VB, et al. Risk of Recurrent Pancreatitis and Progression to Chronic Pancreatitis After a

First Episode of Acute Pancreatitis. Clin Gastroenterol Hepatol 2016;14:738–46. doi:10.1016/j.cgh.2015.12.040.

[10] Löhr JM, Dominguez-Munoz E, Rosendahl J, Besselink M, Mayerle J, Lerch MM, et al. United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). United Eur Gastroenterol J 2017;5:153–99. doi:10.1177/2050640616684695.

[11] Robinson PJA, Sheridan MB. Abdominal radiology Review article Pancreatitis : computed tomography and magnetic resonance imaging 2000;408:401–8.

[12] Catalano MF, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W, et al. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. Gastrointest Endosc 2009;69:1251–61. doi:10.1016/j.gie.2008.07.043.

[13] Kawada N, Tanaka S. Elastography for the pancreas: Current status and future perspective. World J Gastroenterol 2016;22:3712–24. doi:10.3748/wjg.v22.i14.3712.

[14] Pedersen N, Larsen S, Seidelin JB, Nielsen OH. Alcohol modulates circulating levels of interleukin-6 and monocyte chemoattractant protein-1 in chronic pancreatitis. Scand J Gastroenterol 2004;39:277–82. doi:10.1080/00365520310008296.

[15] Sandström A, Andersson R, Segersvärd R, Löhr M, Borrebaeck CAK, Wingren C. Serum proteome profiling of pancreatitis using recombinant antibody microarrays reveals disease-associated biomarker signatures. Proteomics - Clin Appl 2012;6:486–96. doi:10.1002/prca.201200051.

[16] Schneider A, Haas SL, Hildenbrand R, Siegmund S, Reinhard I, Nakovics H, et al. Enhanced expression of interleukin-18 in serum and pancreas of patients with chronic pancreatitis. World J Gastroenterol 2006;12:6507–14.

[17] Sri Manjari K, Jyothy A, Shravan Kumar P, Prabhakar B, Uma Devi M, Ramanna M, et al. A single-nucleotide polymorphism in tumor necrosis factor-α (-308 G/A) as a biomarker in chronic pancreatitis. Gene 2014;539:186–9. doi:10.1016/j.gene.2014.02.014.

[18] Kamath MG, Pai CG, Kamath A, Kurien A. Monocyte chemoattractant protein-1, transforming growth factor-β1, nerve growth factor, resistin and hyaluronic acid as serum markers: comparison between recurrent acute and chronic pancreatitis. Hepatobiliary Pancreat Dis Int 2016;15:209–15. doi:10.1016/S1499-3872(15)60029-7.

[19] Ceyhan GO, Deucker S, Demir IE, Erkan M, Schmelz M, Bergmann F, et al. Neural fractalkine expression is closely linked to pain and pancreatic neuritis in human chronic pancreatitis. Lab Investig 2009;89:347–61. doi:10.1038/labinvest.2008.170.

[20] Ito T. Can measurement of chemokines become useful biological and functional markers of early-stage chronic pancreatitis? J Gastroenterol 2007;42:72–7. doi:10.1007/s00535-006-1929-4.

[21] Bai H, Chen X, Zhang L, Dou X. The effect of sulindac , a non-steroidal anti-inflammatory drug , attenuates inflammation and fibrosis in a mouse model of chronic pancreatitis 2012.

[22] Roeb E. Matrix metalloproteinases and liver fibrosis (translational aspects). Matrix Biol 2017. doi:10.1016/j.matbio.2017.12.012.

[23] Kurzepa J, M A, Czechowska G, Kurzepa J, Celiński K, Kazmierak W, et al. Role of MMP-2 and MMP-9 and their natural inhibitors in liver fibrosis, chronic pancreatitis and non-specific inflammatory bowel diseases. Hepatobiliary Pancreat Dis Int 2014;13:570–9. doi:10.1016/S1499-3872(14)60261-7.

[24] Descamps FJ, Van den Steen PE, Martens E, Ballaux F, Geboes K, Opdenakker G. Gelatinase B is diabetogenic in acute and chronic pancreatitis by cleaving insulin. FASEB J 2003;17:887–9. doi:10.1096/fj.02-0725fje.

[25] Tandon RK, Garg PK. Oxidative Stress in Chronic Pancreatitis: Pathophysiological Relevance and Management. Antioxid Redox Signal 2011;15:2757–66. doi:10.1089/ars.2011.4115.

[26] Uden S, Bilton D, Nathan L, Hunt LP, Main C, Braganza JM. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. Aliment Pharmacol Ther 1990;4:357–71.

[27] Bhardwaj P, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. Gastroenterology 2009;136:149–159.e2.

[28] Forsmark CE, Liddle RA. The challenging task of treating painful chronic pancreatitis. Gastroenterology 2012;143:533–5.

[29] Siriwardena AK, Mason JM, Sheen AJ, Makin AJ, Shah NS. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study. Gastroenterology 2012;143:655–63.e1. doi:10.1053/j.gastro.2012.05.046.

[30] Bopanna S, Nayak B, Prakash S, Shalimar, Mahapatra SJ, Garg PK. Increased oxidative stress and deficient antioxidant levels may be involved in the pathogenesis of idiopathic recurrent acute pancreatitis. Pancreatology 2017;17:529–33. doi:10.1016/j.pan.2017.06.009.

[31] Poulsen JL, Olesen SS, Malver LP, Frokjaer JB, Drewes AM. Pain and chronic pancreatitis: a complex interplay of multiple mechanisms. World J Gastroenterol 2013;19:7282–91. doi:10.3748/wjg.v19.i42.7282.

[32] Bouwense SA, de Vries M, Schreuder LT, Olesen SS, Frokjaer JB, Drewes AM, et al. Systematic mechanism-orientated approach to chronic pancreatitis pain. World J Gastroenterol 2015;21:47–59. doi:10.3748/wjg.v21.i1.47.

[33] Olesen SS, Graversen C, Bouwense SAW, van Goor H, Wilder-Smith OHG, Drewes AM. Quantitative sensory testing predicts pregabalin efficacy in painful chronic pancreatitis. PLoS One 2013;8:e57963. doi:10.1371/journal.pone.0057963.

[34] Schneider A, Löhr JM, Singer M V. The M-ANNHEIM classification of chronic pancreatitis: Introduction of a unifying classification system based on a review of previous classifications of the disease. J Gastroenterol 2007;42:101–19. doi:10.1007/s00535-006-1945-4.

[35] Banks P a, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013;62:102–11. doi:10.1136/gutjnl-2012-302779.

[36] Kalaria R, Abraham P, Desai DC, Joshi A, Gupta T, Abraham P. Rate of recurrence in Indian patients presenting with acute pancreatitis and identification of chronicity on follow up : Possible risk factors for progression 2018:10–5.

[37] Sankaran SJ, Xiao AY, Wu LM, Windsor JA, Forsmark CE, Petrov MS. Frequency of Progression from Acute to Chronic Pancreatitis and Risk Factors: A Meta-analysis. Gastroenterology 2015;149:1490–1500.e1. doi:10.1053/j.gastro.2015.07.066.

[38] Tirkes T, Shah ZK, Takahashi N, Grajo JR, Chang ST, Venkatesh SK, et al; Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer. Reporting Standards for Chronic Pancreatitis by Using CT, MRI, and MR Cholangiopancreatography. Radiology. 2019 Jan;290(1):207-215. doi: 10.1148/radiol.2018181353.

[39] Yadav D, Park WG, Fogel EL, Li L, Chari ST, Feng Z, et al;Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC). PROspective Evaluation of Chronic Pancreatitis for EpidEmiologic and Translational StuDies: Rationale and Study Design for PROCEED From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer. Pancreas. 2018 Nov/Dec;47(10):1229-1238. doi: 10.1097/MPA.0000000000001170.

[40] Schreyer AG, Jung M, Riemann JF, Niessen C, Pregler B, Grenacher L, et al. S3 guideline for chronic pancreatitis - Diagnosis, classification and therapy for the radiologist. RoFo Fortschritte Auf Dem Gebiet Der Rontgenstrahlen Und Der Bildgeb Verfahren 2004;186:1002–8. doi:10.1055/s-0034-1385005.

[41] Kuwahara T, Hirooka Y, Kawashima H, Ohno E, Ishikawa T, Kawai M, et al. Quantitative diagnosis of chronic pancreatitis using EUS elastography. J Gastroenterol 2017;52:868–74. doi:10.1007/s00535-016-1296-8.

[42] Madzak A, Olesen SS, Lykke Poulsen J, Bolvig Mark E, Mohr Drewes A, Frøkjær JB. MRI assessed pancreatic morphology and exocrine function are associated with disease burden in chronic pancreatitis. Eur J Gastroenterol Hepatol 2017;29. doi:10.1097/MEG.0000000000000955.

[43] Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull A, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst 1993;85:365–76.

[44] Fitzsimmons D, Kahl S, Butturini G, Van Wyk M, Bornman P, Bassi C, et al. Symptoms and quality of life in chronic pancreatitis assessed by structured interview and the EORTC QLQ-C30 and QLQ-PAN26. Am J Gastroenterol 2005;100:918–26. doi:10.1111/j.1572-0241.2005.40859.x.

[45] Mendoza T, Mayne T, Rublee D, Cleeland C. Reliability and validity of a modified Brief Pain Inventory short form in patients with osteoarthritis. Eur J Pain 2006;10:353–61. doi:10.1016/j.ejpain.2005.06.002.

Tables

Table 1. Schedule of Events.

		On inclusion	Yearly	Every second year
Informed consent and assessment of eligibility criteria		X		
Review of medical history including medication		X	X	
Physical examination	Anthropometrics	X	X	
	Vital Signs	X	X	
Clinical Lab and Biobank		X	X	
F-elastase-1 and HbA_1C		X	X	
Diagnostic Imaging	Advanced MRI	X		X
	Contrast enhanced EUS and elastography	X		X
Pain Processing	Quantitative Sensory Testing	X	X	
Questionnaires		X	X	
Body composition: Bioelectrical impedance		X	X	
Muscle strength and function: Hand grip strength and timed up and go test		X	X	

Table 2. Imaging assessment parameters.

Method	Modality	Measurement variables
MRI	T1, T2, MRCP	Cambridge classification with modification for MRCP [40]
		Main pancreatic duct diameter
		Pancreatic dimensions and volume
	DWI	Apparent diffusion coefficient (ADC)
	DIXON	Fat signal faction (FSF)
EUS	EUS B-mode	Rosemont score [12]
	Contrast enhanced	90 sec film recorded from stomach of pancreatic body will be recorded for later analysis
	Elastography	Histogram analysis and measurement of strain ratio [41]

Table 3. Circulating biomarker assessment parameters.

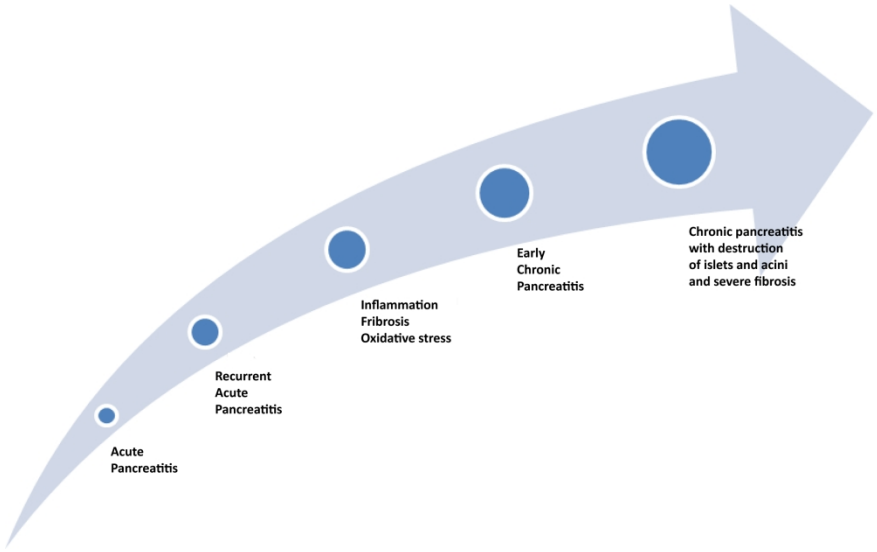
	Measurement variable
Inflammation	Interelukin(IL)-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-alpha
Fibrosis	Transforming growth factor beta-1 (TGF-β1), Soluble fractalkine (s-Fr), Monocyte chemoattractant protein 1 (MCP-1), Matrix metalloproteinases (MMPs)
Oxidative stress	Glutathione peroxidase, Vitamin C, Ferric reducing ability of plasma, Malondialdehyde, 4-hydroxynonenal, superoxide dismutase, nitric oxide

Table 4. Nutritional and QST assessment parameters

	Method	Measurement variable
Nutritional assessment	Muscle strength: Hand held dynamometer	Muscle strength (kg)
	Muscle function: Timed up and go test	Seconds
	Body composition: Bioelectrical impedance	Various bioelectrical impedance parameters
Assessment of pain processing	Pressure algometry	Pressure pain and tolerance thresholds (kPa)
	Temporal summation	Absolute and relative change in pain scores (NRS) to single and repetitive pinprick stimulations
	Conditioned pain modulation	Absolute and relative change in pressure pain tolerance thresholds (kPa) before and after cold pressor test

Figure legend

Figure 1. A large proportion of patients develop CP after an episode of acute pancreatitis. The development of CP is associated with continued or recurrent inflammation and progressive development of fibrosis.


Acute
Pancreatitis
Recurrent
Acute
Pancreatitis
Inflammation
Fribrosis
Oxidative stress
Early
Chronic
Pancreatitis
Chronic pancreatitis
with destruction
of islets and acini
and severe fibrosis

Figure