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BY
ADNAN MADZAK

DISSERTATION SUBMITTED 2017

AALBORG UNIVERSITY
DENMARK

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Adnan Madzak

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PhD supervisor: Prof. Jens Brøndum Frøkjær, MD, PhD
Aalborg University Hospital
Aalborg University, Denmark

Assistant PhD supervisor: Prof. Asbjørn Mohr Drewes, MD, PhD, DMSc
Aalborg University Hospital
Aalborg University, Denmark

Associate Prof. Søren Schou Olesen, MD, PhD
Aalborg University Hospital
Aalborg University, Denmark

PhD committee: Clinical Associate Professor, Soeren Hagstroem (chair.)
Aalborg University Hospital
Aalborg University, Denmark

Associate Professor, Fatih Akisik
Indiana University School of Medicine, USA

Staff Specialist, Srdan Novovic
Hvidovre Hospital, Copenhagen, Denmark

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CURRICULUM VITAE

Personal data

Adnan Madzak

Born march 28th, 1984 in Trebinje, Bosnia and Hercegovina

Education and clinical work

2006-2011	MD from University of Southern Denmark, Odense, DK
2011-2012	Internship (rotation: internal medicine and GP), Viborg Region Hospital, DK
2012-2013	First Year Resident (introduction position) Department of Radiology, Aalborg University Hospital, DK
2013-2017	PhD student, Department of Radiology, Mech-Sense, Aalborg University Hospital

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Juel J, Brock C, Olesen SS, **Madzak A**, Farmer AD, Aziz Q, Frøkjær JB, Drewes AM. Accentuation of vagal tone has no effect on pain or gastrointestinal motility in patients with chronic pancreatitis. *Submitted to Journal of Pain Research February 2017*

Andersen PL, **Madzak A,** Olesen SS, Drewes AM, Frøkjær JB. Quantification of pancreatic calcifications in chronic pancreatitis: association to parenchymal atrophy, duct irregularities and clinical parameters. *In preparation*

LIST OF PAPERS

This thesis was based on following papers:

I. **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 Sep;45(8):1092-103.*

II. **Madzak A**, Engjom T, Wathle GK, Olesen SS, Tjora E, Njølstad PR, Lærum BN, Drewes AM, Dimcevski G, Frøkjær JB, Haldorsen IS. Secretin-stimulated MRI assessment of exocrine pancreatic function in patients with cystic fibrosis and healthy controls. *Abdominal Radiology (NY). 2016 in press*

III. **Madzak A**, Olesen SS, Haldorsen IS, Drewes AM, Frøkjær JB. Secretin-stimulated MRI characterization of pancreatic morphology and function in patients with chronic pancreatitis. *Pancreatology. 2017 in press*

IV. **Madzak A**, Poulsen JL, Olesen SS, Haldorsen IS, Drewes AM, Frøkjær JB. MRI assessed pancreatic morphology and exocrine function are associated with disease burden in chronic pancreatitis. Submitted to Clinical Gastroenterology and Hepatology March 2017

ABBREVIATIONS

CP	Chronic pancreatitis
CT	Computed tomography
MRI	Magnetic resonance imaging
EPI	Exocrine pancreatic insufficiency
CF	Cystic fibrosis
MPD	Main pancreatic duct
s-MRCP	Secretin-stimulated magnetic cholangiopancreatography
DWI	Diffusion weighted imaging
ADC	Apparent diffusion coefficient
FSF	Fat signal fraction
s-MRI	Secretin-stimulated magnetic resonance imaging
HC	Healthy controls
EST	Endoscopic secretin test
QOL	Quality of life
SI	Signal intensity
ROI	Region of interest

ENGLISH SUMMARY

Chronic pancreatitis has an annual incidence between 5-10 per. 100.000 which has quadrupled through the last 30 years. The pathological changes of the pancreas in chronic pancreatitis are characterized by chronic inflammation, fibrosis, dilatation/stenosis of the main pancreatic duct and cystic changes. These changes are accompanied by loss of both exocrine and endocrine pancreatic parenchyma. The advanced stages of chronic pancreatitis are characterized by typical clinical and imaging appearances which results in easy diagnosis of the disease. However, the challenge remains to identify the disease in the early subclinical stages when the morphological changes are mild. Chronic pain is the hallmark symptom of chronic pancreatitis but within 5 years of the diagnosis more than 50 % of the patients have developed both endocrine and exocrine insufficiency. Chronic pancreatitis is a disabling disease with dramatic social and economic consequences for the patients.

The objective of this PhD thesis was to develop and apply magnetic resonance imaging (MRI) techniques for a detailed characterization of pancreatic morphology and function in patients with chronic pancreatitis. Furthermore, we hypothesized that these new MRI parameters could be used for characterization of disease mechanisms, monitoring disease progression and aid in treatment of patients with chronic pancreatitis.

Data from two studies were collected for this thesis. In study I, our new secretin-stimulated MRI (s-MRI) protocol and the new semi-automatic application for analyses of pancreatic exocrine function were validated in patients with cystic fibrosis. In study II, pancreatic morphology and function was characterized in 82 patients with chronic pancreatitis. The morphological parameters included pancreatic gland volume, main pancreatic duct diameter, Cambridge classification, diffusion properties in the pancreatic parenchyma as a measure for fibrosis and measurement of fatty infiltrations in the parenchyma. The functional parameters were exocrine pancreatic function characterized by quantification of pancreatic secretion and changes in diffusion properties after secretion-stimulation.

The main results showed that patients with chronic pancreatitis had a smaller pancreatic gland volume, increased fibrosis and fatty infiltrations in the pancreatic parenchyma compared to healthy controls. The exocrine pancreatic function (ability to produce pancreatic juice after secretin-stimulation) was significantly reduced in patients with chronic pancreatitis. Additionally, patients with more advanced stages of chronic pancreatitis had smaller pancreatic gland volumes and reduced pancreatic exocrine function was associated with reduced quality of life.

In conclusion, the advanced s-MRI parameters could differentiate between healthy controls and patients with chronic pancreatitis. Additionally, some s-MRI parameters

were of clinical relevance for severity and disease burden of chronic pancreatitis. S-MRI is a safe, non-invasive method for characterization of pancreatic morphology and function, and could in the future aid in understanding the complex disease mechanism, improve diagnosis and treatment of patients with chronic pancreatitis.

DANSK RESUME

Kronisk pankreatitis (bugspytkirtelbetændelse) har en årlig incidens på 5-10 per 100.000 indbyggere, hvilket er firdoblet igennem de sidste 30 år. De patologiske forandringer i pankreas ved kronisk pankreatitis er kendetegnet ved kronisk inflammation, fibrose, forsnævring/dilatation af udførselsgangen samt cystedannelse. Dette er ledsaget af tab af både eksokrint og endokrint væv. Fremskreden sygdom er karakteriseret ved typiske kliniske og billedmæssige fund, som gør den nem at diagnosticere. Den største udfordring er at identificere sygdommen i den tidlige subkliniske fase hvor de morfologiske forandringer er minimale. Kroniske smerter er det dominerende symptom men indenfor 5 år efter diagnosen har over 50 % udviklet både eksokrin og endokrin insufficiens med fejlernæring og diabetes. Det er en meget invaliderende sygdom med store sociale og økonomiske konsekvenser for patienterne.

Formålet med dette ph.d. projekt var at udvikle og anvende magnetisk resonans billeddannelse (MRI) teknikker til detaljeret kortlægning af pankreas morfologi og funktion hos patienter med kronisk pankreatitis. Målet var at disse nye MRI parametre kunne bruges til kortlægning af sygdomsmekanismer, monitorering af sygdomsudviklingen og forbedring af behandlingen af patienter med kronisk pankreatitis.

Resultater fra to studier blev inkluderet i dette projekt. I første studie blev vores nye sekretin-stimulerede MRI (s-MRI) protokol og nye semi-automatisk program til analyse af pankreas eksokrine funktion valideret i patienter med cystisk fibrose. I andet studie blev pankreas morfologi og funktion kortlagt med s-MRI hos 82 patienter med kronisk pankreatitis. De morfologiske parametre var pankreas kirtel-volumen, gang-diameter, Cambridge klassificering, diffusionsegenskaber i parenkymet som udtryk for fibrosegrad samt måling af fedt infiltration i parenkymet. De funktionelle parametre var eksokrine funktion karakteriseret ved kvantificering af pankreas sekretionen samt ændringer i diffusionsegenskaber i parenkymet efter secretin-stimulation.

Hovedresultaterne viste at patienter med kronisk pankreatitis havde en mindre kirtel volumen, øget fibrose og fedtinfiltrering i pankreas parenkymet sammenholdt med de raske kontroller. Den eksokrine funktion (evnen til at danne pankres saft efter sekretin-stimulering) var også betydeligt nedsat hos patienter med kronisk pankreatitis. Vi viste også at patienter med mere fremskredne stadier af kronisk pankreatitis havde en mindre kirtel, og at reduceret eksokrin funktion havde en sammenhæng med nedsat livskvalitet.

Det var således muligt for de avancerede s-MRI parametre at skelne mellem raske kontroller og kronisk pankreatitis patienter. Ydermere, var enkelte s-MRI parametre af klinisk relevans for særligt sværhedsgraden og sygdomsbyrden af kronisk

pankreatitis. S-MRI er en sikker, non-invasiv metode til at karakterisere morfologiske og funktionelle forandringer i pankreas og kan i fremtiden være med til at forstå sygdomsmekanismerne, sikre en forbedret udredning og behandling af patienter med kronisk pankreatitis.

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INTRODUCTION

The pancreas is a retroperitoneal gland placed behind the stomach and surrounded by small intestine, liver, and spleen. Anatomically, it is divided in to the pancreatic head, body and tail and has both endocrine and exocrine functions (1). Histologically, the endocrine functions are managed by the islet cells that compromise only 2% of the normal pancreatic parenchyma, while the exocrine function is handled by ductal and acinar cells with secretion of pancreatic juice to the duodenum. Major diseases of the pancreas include pancreatic cancer, diabetes mellitus, cystic fibrosis, and pancreatitis. Despite significant progress in tools for detecting pancreatic pathology over the past decades, a detailed, non-invasive test for combined morphological and functional characterization of pancreas is still highly warranted.

1.1. CHRONIC PANCREATITIS

Chronic pancreatitis (CP) is a fibro-inflammatory disease characterized by irreversible morphological and functional changes in the pancreas (2). The most common cause for developing CP is long-term excessive alcohol intake which accounts for about 50 % of all CP cases in the Western world (3). Other causes include smoking, genetic (SPINK1, SPSS1 and CFTR mutations), autoimmune (type 1 and type 2), environmental and idiopathic factors (4), Figure 1-1. It affects men more often than women, and the mean age of 48.9 (± 15) years for onset of CP was reported in one large North American survey (5).

"Common features of established and advanced CP include pancreatic atrophy, fibrosis, pain syndrome, duct distortion and strictures, calcifications, pancreatic exocrine and endocrine dysfunction, and dysplasia", as defined by Whitcomb et al. 2016 (2), Figure 1-1. It was also stated that not all features are necessary present in the individual patient. Additionally, the underlying pathophysiological mechanisms for developing these common features of CP are complex and not well understood. Identifying the common features is done through clinical assessment (alcohol abuse, abdominal pain, steatorrhea), morphological changes based on imaging (computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound), and testing for exocrine and endocrine insufficiency (6). However, a great challenge in the current clinical practice is that these disease characteristics are of relevant for mainly well-established or advanced CP. This implies that we are currently not able to make an accurate diagnosis of early stages of CP. Furthermore, there is no well-established diagnostic criteria or disease markers for the early stages of CP which furthermore makes the diagnosis of early CP complicated. In clinical practice, CP is mainly diagnosed from clinical context (e.g. recurrent acute pancreatitis in heavy alcoholics) and relevant symptoms, and confirmed several years later by abnormal duct imaging or pancreatic calcifications (7). New non-invasive biomarkers characterizing the early

features of CP are highly wanted to identify patients in risk of developing CP, monitor disease progressions, develop and evaluate new treatment approaches.

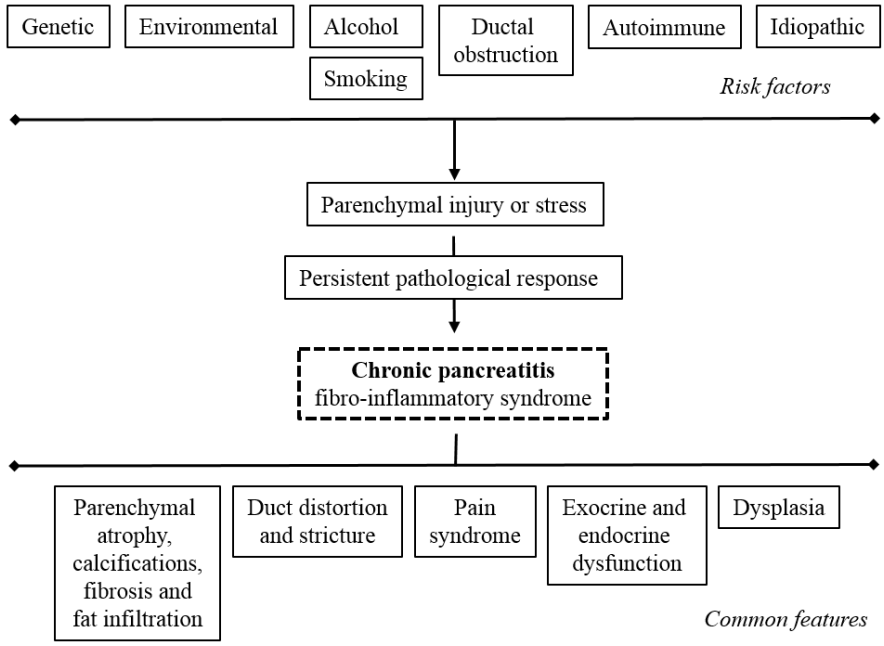

Figure 1-1: Overview of risk factors and common features in chronic pancreatitis

1.1.1. DISEASE BURDEN IN CHRONIC PANCREATITIS

The three hallmark clinical features of CP are upper abdominal pain, exocrine pancreatic insufficiency, and type 3 diabetes mellitus. Pain is the most dominating symptom and present in the 80-90 % of patients during their disease course and its treatment remains a major clinical challenge (8). Additionally, pain in CP is associated with reduced quality of life and it is the primary cause of hospitalization in most patients (9). In USA, rates for ambulatory care visits and hospitalizations for acute and chronic pancreatitis combined have increased nearly 62% between 1988 and 2004 (10). In USA, the estimated health care costs in 2004 was 3.7 billion $, additionally the retail cost for prescriptions was roughly 88.6 million $. The pancreatic enzyme replacement therapy constituted 84.5% of all the prescriptions, the remaining drug were analgesic and antiemetic agents. In addition, these number do not include visits at the general practitioners who handle much of the long-term care. CP is further complicated by psychiatric comorbidity including anxiety and depression (11). The heterogeneous character of the disease affects all aspects of patients' life: work, leisure, travel and relationship (12). Thus, the cumulative disease burden in CP is high

and associated with reduced quality of life and increased health resource utilization (5).

1.1.2. EXOCRINE PANCREATIC FUNCTION

The clinical symptoms of mild exocrine pancreatic insufficiency (EPI) are vague, including abdominal bloating and cramping, whereas steatorrhea, weight loss and malnutrition develops with more severe EPI (13). The diagnosis of EPI requires the documentation of maldigestion, along with evidence that reduced exocrine pancreatic function is the cause of maldigestion.

Direct pancreatic function tests are considered the golden standard for diagnosis of EPI (6,14). These tests require intravenous administration of secretin to stimulate secretion of pancreatic juice. *Secretin* is a hormone that under normal psychological circumstances is secreted from upper intestinal mucosa after ingestion of a mixed meal, and its major effect is secretion of bicarbonate rich fluid from the pancreatic ductal cells (15). After secretin stimulation, duodenal intubation is performed to collect the pancreatic juice and concentrations of digestive enzymes and bicarbonate are measured. In the modern approach, the secretin function test has been combined with endoscopic ultrasound where pancreatic juice is collected endoscopically (16,17). This also allows a morphological assessment of the pancreas. Unfortunately, these invasive test are expensive and cumbersome for the patients, and therefore only performed at few specialized centers (18).

Indirect pancreatic function tests do not require direct hormonal stimulation of the pancreas, and include 72-h fecal fat and fecal elastase test. Although 72-h fecal fat test is accurate for EPI, it is not feasible in the outpatient setting nor with hospitalized patients in pain. It includes 5 days of strict diet, 3 days of cumulative stool collection and additional laboratory personal (19). In clinical practice, fecal elastase is the simplest non-invasive test for assessing EPI (20). It should be performed on a solid stool sample to avoid falsely positive results during e.g. diarrhea. However, the test is of little help in diagnosing mild to moderate EPI (21). Both 72-h fecal fat test nor fecal elastase cannot independently diagnose CP. Newer methods like ^{13}C-MTG triglyceride breath test have reported promising results in diagnosing EPI but are still only performed at specialized centers (22). Additionally, none of the indirect pancreatic function test can identify patients with mild EPI or in risk for developing EPI.

In summary, a novel, non-invasive and accurate test for EPI with the possibility to detect early CP is still highly warranted.

1.2. CYSTIC FIBROSIS

Cystic fibrosis (CF) is caused by a mutation in the gene encoding the CF transmembrane conductance regulator (23). This mutation leads to abnormal fluid secretion from epithelial cells, causing dysfunction in organ systems including the lung, gastrointestinal tract, liver, male reproductive tract and pancreas, which is one of the earliest affected organs (24). Dysfunction of pancreatic ductal cells results in altered composition of pancreatic juice leading to obstruction of small duct and acini. With progression, it is accompanied with inflammation, fibrosis and fatty infiltration ending with EPI (25). In CF, 85-90% of the patients have EPI which can be present at birth or evolves mainly over the first year of life, although some develop EPI later in life (26). Development of severe EPI at young age with established marked reduction in pancreatic secretion makes patients with CF an optimal cohort for evaluating new methods for assessment of exocrine function and volume output failure in EPI (27). Additionally, MRI has gained more interest in CF and could potentially provide biomarkers for monitoring EPI and identifying CF patients in risk of developing EPI (28).

1.3. MAGNETIC RESONANCE IMAGING

The signal measured in MRI arises from the water protons in the body, whether located in the intracellular or extracellular space. Since its clinical introduction in the 1980s, the development of MRI has been fast and its range of clinical applications has constantly broadened. Currently, conventional T1- and T2-weighted MRI provides a detailed depiction of pancreatic anatomy and structure and has impact on diagnosis and treatment of various pancreatic diseases such as pancreatic cancer and acute or chronic pancreatitis (29).

In 1991 Wallner et al. introduced a pancreatic MRI method in which the biliary tracts were visualized and the method was named “MR cholangiography” (30). Over the following years, the resolution of the images increased which was paralleled by reduced acquisition time. This also allowed visualization of the main pancreatic duct (MPD), and this improved imaging method was named MRCP (30–34). However, visualization of the entire MPD and visualization of side branches or subtle changes such as minor strictures of the ducts was still problematic. In 1995 secretin-stimulated MRCP (s-MRCP) was described by a Japanese group (35) and in 1997 Matos et al. published a paper presenting a method for combined morphological and functional evaluation of the pancreas (36). The great value of s-MRCP in evaluating MPD is reported in numerous publications and s-MRCP is increasingly employed as the first-choice examination for detection of detailed MPD pathology (37–40). Additionally, various secretin-stimulated MRI approaches for detection of EPI (i.e., bicarbonate secretion) have been published and shown promising results when compared to direct (e.g. endoscopic secretin tests) and indirect (e.g. the fecal elastase test) pancreatic function tests (36,41–44).

Besides imaging of simple anatomy and the main pancreatic duct, the technical MRI development continuously provides us with new techniques for evaluation of the pancreatic parenchyma. Diffusion weighted imaging (DWI) is a well-established MRI technique that assess the random diffusion/motion of water protons in the body (45). DWI measures microscopic diffusion of intracellular, extracellular and vascular water protons, and is quantified by the term apparent diffusion coefficient (ADC) (46). Several studies have reported that fibrosis causes diffusion restriction in both liver and pancreas, meaning lower ADC values in affected organs compared to healthy (47,48). Additionally, dynamic DWI before and after secretion stimulation can be used for assessment of pancreatic secretory capacity (48,49).

Additionally, the Dixon imaging technique for separation of water and fat signal in tissue was introduced in 1984 (50). Significant improvement of the original technique through the last 2 decades has enabled quantitative measurement of diffuse parenchymal fat infiltration in abdominal solid organs (51). Studies in the liver have reported that Dixon imaging has good diagnostic accuracy for both mild and moderate/severe steatosis compared to histological assessment from liver biopsies (52). A recent study in pancreas reported positive correlation with fat signal fraction (FSF) assessed with Dixon imaging and degree of pancreatic steatosis in specimens after pancreatomy (53).

In addition to development within MRI, new applications for quantitative image analyses are constantly evolving. This has enabled quantitative measurements of pancreatic secretin and pancreatic gland volume. However, current applications for image analyses are still dependent on time consuming manual segmentation and not clinically feasible. Functional MRI with new more automated applications for image analyses have the potential revolutionize the field of radiology. In summary, there are currently numerous advanced MRI techniques that can characterize pancreatic morphology and function. However, relatively little is known about their ability to detect CP, their internal relationship and the association to disease burden and other clinical features in patients with CP.

HYPOTHESIS & AIM

The *overall aim* was to investigate pancreatic morphology and function with an advanced secretin-stimulated MRI (s-MRI) protocol and provide numerous differentiated imaging parameters that could represent important pathological features in patients with chronic pancreatitis. We hypothesized that a combination of such morphological and functional imaging parameters could provide a non-invasive tool for monitoring disease progression and aid the treatment of patients with CP. An overview of aims and papers are illustrated in Figure 2-1.

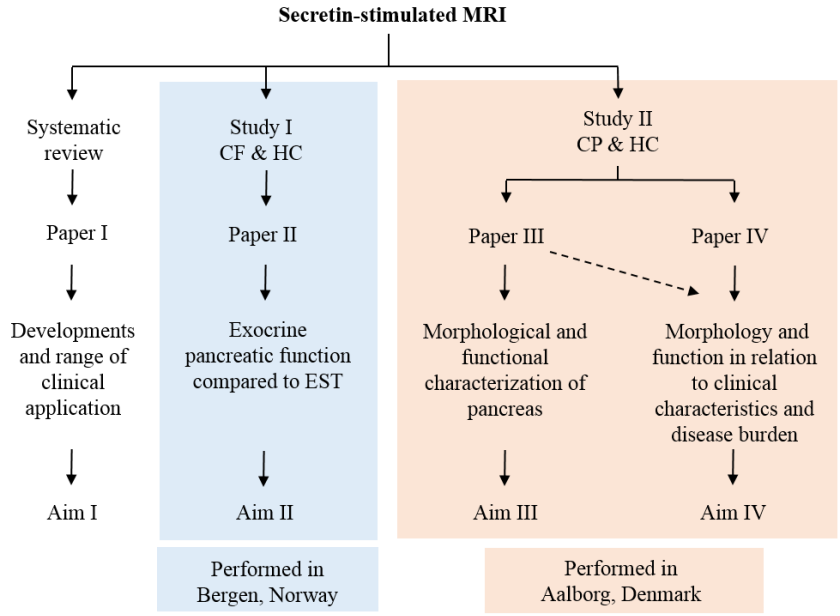

Figure 0-1: Overview of the aims from study I (Cystic fibrosis (CF) patients and healthy controls (HC)) and study II (Chronic pancreatitis (CP) patients and HC). MRI: magnetic resonance imaging; EST: endoscopic secretin test.

Subsequently, to fulfill the overall aim, this thesis contains 4 aims:

I. *To investigate latest developments in s-MRI and the range of clinical application in benign pancreatic disorders (paper I).*

- The hypothesis was by reviewing the latest literature that an advanced s-MRI protocol can be applied in a clinical setting and provide relevant information regarding pancreatic morphology and especially exocrine function.

II. *To evaluate a s-MRI protocol including a novel semi-automatic application for assessment of exocrine pancreatic function against a "gold standard" endoscopic secretin test in patients with cystic fibrosis compared to healthy controls (paper II).*

- The hypothesis was that s-MRI with a new semi-automatic application for quantification of pancreatic secretion could be a valid, non-invasive alternative for detection of exocrine pancreatic dysfunction when compared to the "gold standard" endoscopic secretin test.

III. *To investigate pancreatic morphology and exocrine function with the novel s-MRI protocol in patients with chronic pancreatitis compared to healthy controls (paper III).*

- The hypothesis was that CP patients had morphological changes and impaired exocrine pancreatic function compared to HCs, which could reflect different aspects of the CP pathophysiology.

IV. *To explore the association between pancreatic morphological and functional s-MRI parameters, clinical characteristics, and disease burden in patients with chronic pancreatitis (paper III + IV).*

- The hypothesis was that the changes in pancreatic morphology and exocrine function identified by s-MRI could be used in prognostication and monitoring of patients with CP.

MATERIAL & METHODS

1.4. MATERIALS

Two MRI studies contributed to this thesis. The studies were approved by the local Ethics Committees (approval number: REK: 2010/2857-7 (study I) and N-20130040 & N-20130059 (study II)) and conducted according to the Declaration of Helsinki. Informed consent was obtained from both patients and healthy controls (HC) after both oral and written information was given.

1.4.1. STUDY I

Study I was conducted at the Cystic Fibrosis Clinic, Haukeland University Hospital, Bergen, Norway. Nineteenth patients with CF and 10 HC were included. The diagnostic criteria for CF was based on the CF foundation consensus report (54). Information on CF transmembrane conductance regulator mutation and sweat test values were extracted from patient journal as documentation for CF. Healthy controls had no previous history of gastrointestinal disease. Secretin-stimulated MRI and endoscopic secretin test (EST) was performed on all subjects included in the study. Median time between EST and s-MRI was 28 (range 24-38) months for CF patients and 24 (range 12-51) months for HC.

1.4.2. STUDY II

Study II was conducted from at Centre for Pancreatic Disease, Department of Radiology and Department of Gastroenterology & Hepatology, Aalborg University Hospital, Denmark. Eighty-two patients with CP and 22 HC were included in the study. The diagnosis of CP was based on the Lüneburg criteria and CP was defined as a score $\geq$ 4 points (55). Patients with suspected acute exacerbation of CP were excluded. All healthy subjects were screened to exclude any history of pancreas-related disease or gastrointestinal disease. From November 2013 – February 2015 s-MRI was performed on all CP patients and HC. From the date of the patients individual s-MRI examination to August 2016, data on all CP related hospitalizations was collected prospectively. Additionally, data on quality of life (QOL) and pain was collected from questionnaires filled out at the outpatient clinic annual exam closest to the individual date for s-MRI.

1.5. METHODS

Study I and II were performed at two different hospitals using two different brands of MRI scanners (Siemens and General Electric Healthcare) for acquiring the data. However, the MRI scanners had the same magnetic field (1.5T) and great efforts were made to make the s-MRI protocol sequences as similar as possible. The methods including the s-MRI protocols are described in the following section and Figure 3-1 shows an overview of the two studies.

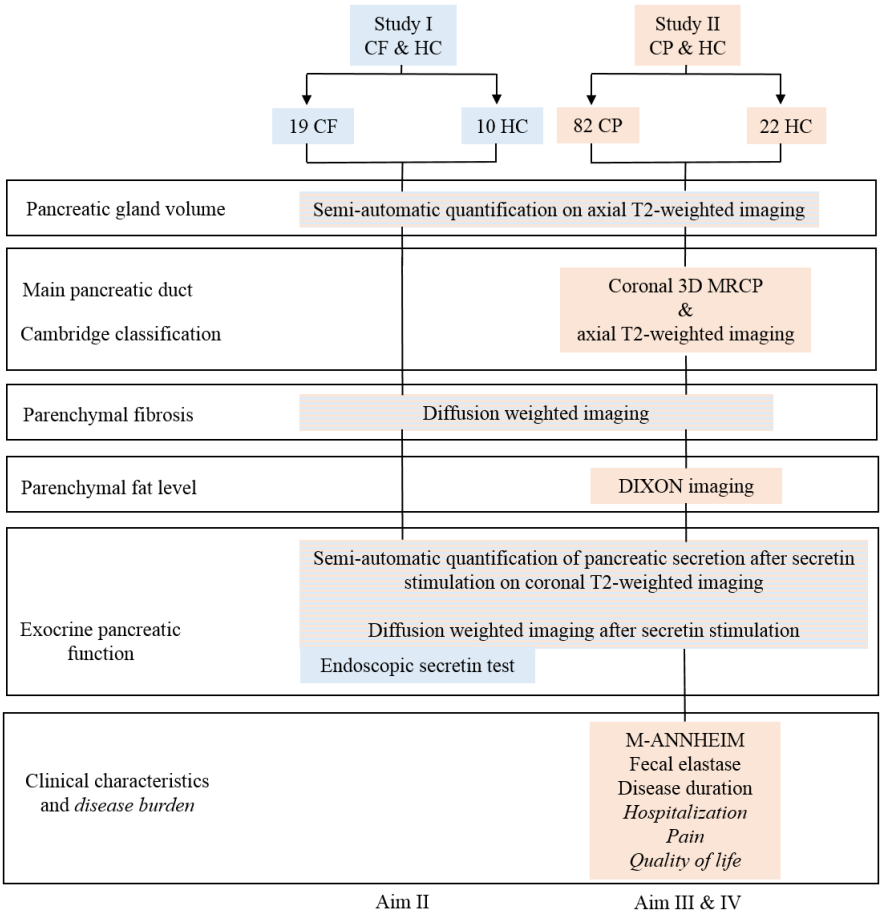

Figure 3-1: Overview of the study I and study II. Abbreviations: CP: chronic pancreatitis; CF: cystic fibrosis; HC: healthy controls; 3D MRCP: 3-dimensional magnetic cholangiopancreatography; M-ANNHEIM: M-ANNHEIM clinical staging of chronic pancreatitis.

1.5.1. SECRETIN-STIMULATED MRI PROTOCOL

S-MRI was performed on all subjects in supine position after minimum 4 hours of fasting. Intravenous administered of secretin (1 CU/kg, maximum dose of 70 CU, Secrelux®, Sanochemia Diagnostics, Neuss, Germany) was performed over a period of 3 minutes.

For study I, an 1.5T MR scanner (Siemens Healthcare, Erlangen, Germany) was used with a 24-channel spine matrix coil and a six-channel body coil. The imaging protocol is provided in Table 3-1.

Imaging sequence	Plane	TR/TE (ms)	Slice thickness (mm)	Matrix	Acquisition
FS T1 VIBE	axial	5.4/2.4	2.5	320x221	BH
T2 TrueFISP	axial	2.79/1.17	4	512x205	BH
DWI	axial	1400/70	6.0	192x144	FB
T2 HASTE	coronal	3500/113	10	256x238	BH

Table 3-1: Secretin-stimulated MRI protocol for study I. All sequences were performed before secretin. T2 HASTE and DWI sequences were performed 10 and 4 minutes before and repeated 1, 5, 9 and 13 minutes after secretin stimulation. Axial DWI was performed with b-values 50 and 800 s/mm^2. Abbreviations: TR/TE: repetition time/echo time; FA VIBE: Fat saturated volume interpolated examination; TrueFISP: Balanced steady-state free precession: HASTE: Half-fourier acquisition single-shot turbo spin echo; DWI: diffusion weighted imaging; BH: Breath hold; FB RTs: Free breathing with respiratory triggering.

For study II, an 1.5T MR scanner (Signa HDxt, version 23, General Electric Healthcare, Milwaukee, Wisconsin, USA) was used with an 8-channel body coil. The imaging protocol is provided in Table 3-2.

Imaging sequence	Plane	TR/TE (ms)	Slice thickness (mm)	Matrix	Acquisition
FIESTA	axial	3.5/1.5	4.0	256x192	BH
3D MRCP	coronal	3500/630	2.6	320x256	FB RTs
LAVA-Flex	axial	7/4	2.6	320x192	BH
DWI	axial	4000/70	6.0	160x192	FB
T2 SSFSE	coronal	2500/120	10.0	256x224	BH

Table 3-2: Secretin-stimulated MRI protocol for study II. All sequences were performed before secretin. T2 SSFSE and DWI sequences were performed 10 and 4 minutes before and repeated 1, 5, 9 and 13 minutes after secretin stimulation. Axial DWI was performed with b-values 50, 400 and 800 s/mm^2. Abbreviations: TR/TE: repetition time/echo time; FIESTA: fast imaging employed steady-state acquisition; SSFSE FS: Single shot fast spin echo fat saturated: 3D MRCP: 3-dimensional magnetic resonance cholangiopancreatography; LAVA-flex: liver acquisition with volume acceleration flex; DWI: diffusion weighted imaging; BH: Breath hold; FB RTs: Free breathing with respiratory triggering.

1.5.2. PANCREATIC GLAND VOLUME

For study I, pancreatic gland volume was measured on axial FS T1 VIBE in the commercially available software NordicICE 2.3.12 (NordicNeuroLab, Bergen, Norway). Contour of the pancreas was manually segmented on every slice, considering each area to represent a volume of 2.5 mm thickness. The areas were added to calculate the pancreatic volume. For study II, gland volume was measured on axial FIESTA images in a customized semi-automatic application developed in Matlab 2013b (Mathworks, Natick, Massachusetts, USA). The contour of the pancreas was also manually segmented on each slice excluding main pancreatic duct segments over 3 mm and all cystic lesions, see Figure 3-2A. The application then automatically calculated pancreatic gland volume.

1.5.3. MAIN PANCREATIC DUCT (ONLY STUDY II)

The maximum diameter of the main pancreatic duct, pathological side branches and potential cystic lesions were measured on 3-dimensional MRCP obtained before secretin administration using commercially available software (EazyViz v. 7.2.6, Karos Health A/S, Valby, Denmark), see Figure 3-2B.

1.5.4. CAMBRIDGE CLASSIFICATION (ONLY STUDY II)

The original Cambridge classification was limited to duct visualization via endoscopic retrograde pancreatography with an expansion to include CT and ultrasound (56). For our studies we used a MRI adaption of the Cambridge classification to accommodate the additional information about pancreatic duct and parenchyma in classification of patients with CP (57).

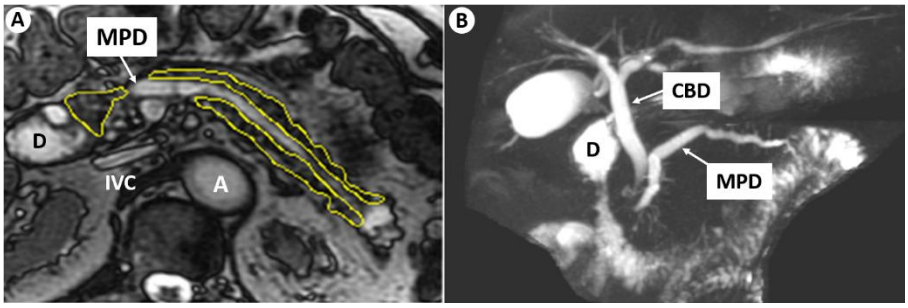

Figure 3-2: Measurements of the pancreatic gland volume (A) and main pancreatic duct (B) in a patient with chronic pancreatitis. Abbreviations: MPD: main pancreatic duct; CBD: common bile duct; D: duodenum; A: aorta; IVC: inferior vena cava.

1.5.5. FIBROSIS IN PANCREATIC PARENCHYMA

Diffusion weighted imaging (DWI) was obtained before and 1, 5, 9 and 13 minutes after secretin stimulation. For study I, pancreatic apparent diffusion coefficient (ADC) maps were generated by calculating the slope of the logarithmic decay curve for signal intensity against b-value (50 and 800 sec/mm^2) with use of MRI software (Syngo, Siemens). For study II, the ADC maps were based on three b values (50, 400 and 800 sec/mm^2) and generated by commercially available software (AW server 2.0, General Electric, Milwaukee, Wisconsin, USA). Regions of interest (ROIs) were in both studies manually placed in the pancreatic head, body, and tail for measurement of ADC.

1.5.6. FAT LEVEL IN PANCREATIC PARENCHYMA (ONLY STUDY II)

Measurements of pancreatic fat signal fraction (FSF) was performed on LAVA-flex images (Dixon) obtained prior to secretin. Analyses was done in EazyViz, placing ROIs in the pancreatic head, body, and tail on both water-only and fat-only images. Same positions were used as in the DWI analysis. Mean signal intensity (SI) in the ROI's was used to calculate FSF by the formula: FSF= *SI fat only / (SI water only + SI fat only).*

Additionally, for study II the analyses of ADC and FSF was only performed in subjects with pancreatic gland volume larger than 25 ml. The decision was made to accommodate enough pancreatic parenchyma for a ROI of 8 mm in diameter. The main pancreatic duct and cystic lesions were avoided when placing the ROIs, see Figure 3-3.

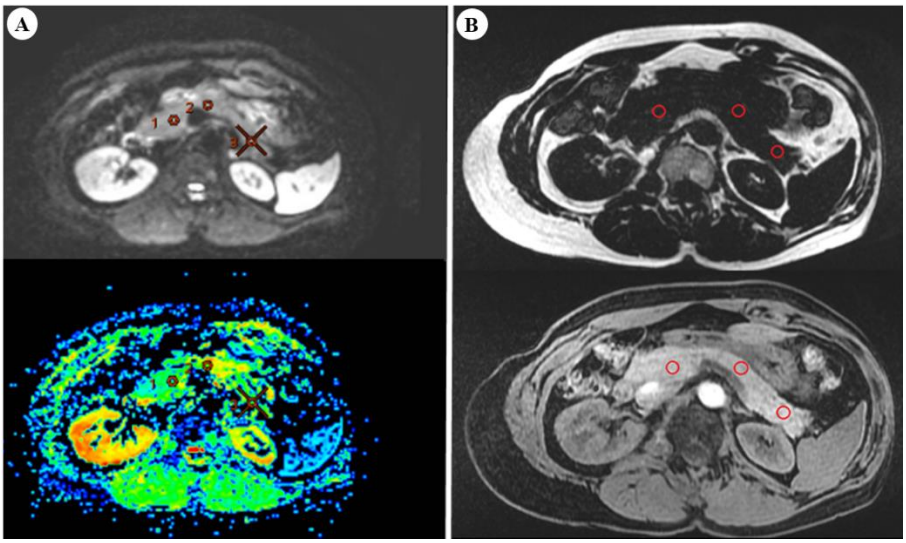

Figure 3-3: Placement of regions of interest (red circle) for measurements of apparent diffusion coefficient (A) and fat signal fraction (B).

1.5.7. EXOCRINE PANCREATIC FUNCTION

Evaluation of exocrine pancreatic function with s-MRI was performed by analyzing coronal T2-weighted imaging (slice thickness 10 mm) obtained 10 and 4 minutes before and 1, 5, 9 and 13 minutes after secretin stimulation. As in previous studies, changes in signal intensity on T2-weighted images were used as a measure of changes in small bowel fluid volumes (40,44,58). Analyses was performed in the new semi-automatic application developed in Matlab 2013b (Mathworks, Natick, MA, USA) for more automated (i.e., faster, more objective and quantitative) calculation of pancreatic fluid output, see Figure 1 in PhD paper II (59). The raw DICOM data was uploaded to the application and a rough ROI was manually drawn around duodenum and upper small bowel. The application automatically adjusted the ROI to only include regions with high signal intensity (high fluid content) within the small bowel and provides a fluid volume for each slice. By adding all slices the application automatically calculates the small bowel fluid volume for each time point. Pancreatic secretion for each time point is calculated by subtracting the fluid volume 4 minutes before secretin. Additionally, changes in pancreatic ADC 1, 5, 9 and 13 minutes after secretin stimulation were used as an indirect evaluation of exocrine pancreatic function.

Changes in ADC for each time point were calculated by subtracting the ADC obtained 4 minutes before secretin administration.

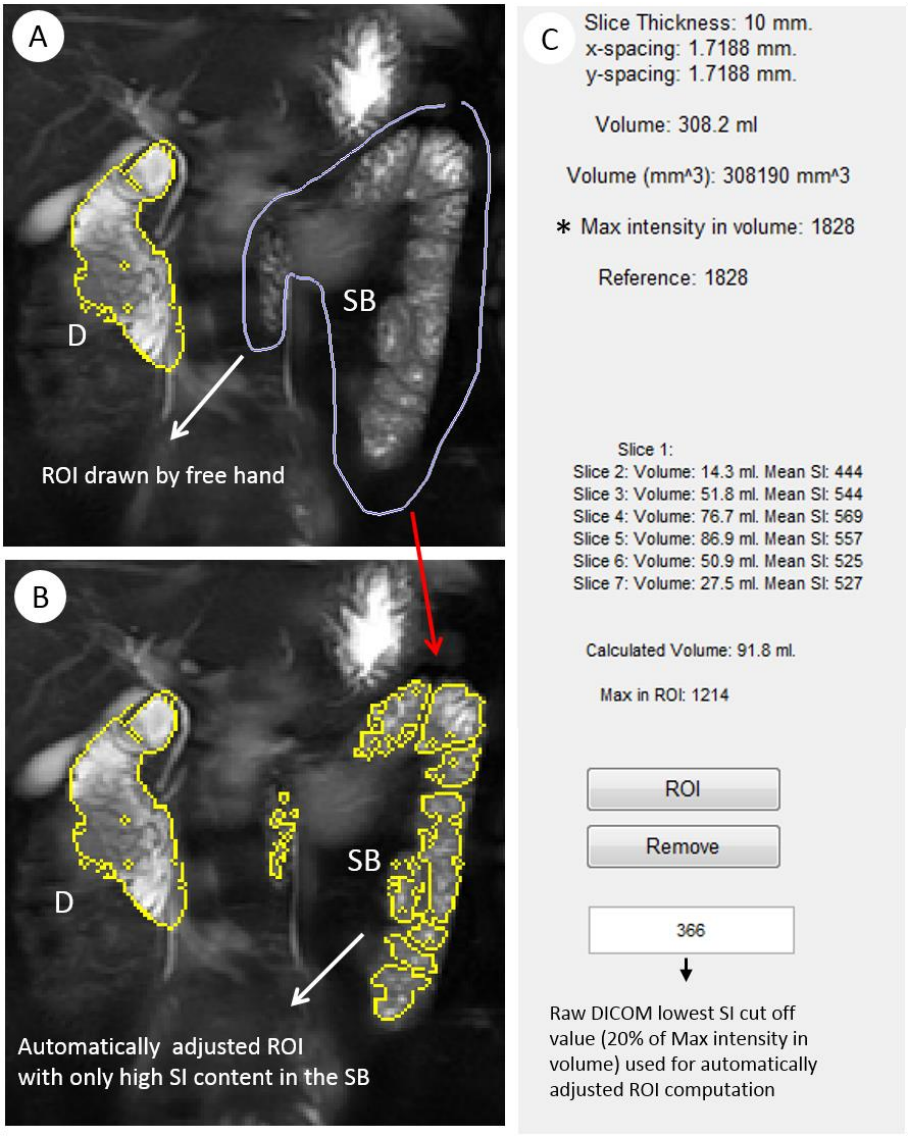

Figure 3-4: Segmentation of small bowel fluid volume in the new semi-automatic application for calculation of pancreatic secretion. Abbreviations: D: duodenum; SB: small bowel; ROI: regions of interest; SI: signal intensity

Fecal elastase was measured in all subject as this is the most routinely and clinically performed measure of exocrine pancreatic function at most centers worldwide. Commercially available kits were used in both study I (ScheBo Biotech, Giessen, Germany) and in study II (Pancreatic Elastase, ELISA, BIOSERV diagnostics GmbH, Rostock, Germany). Exocrine pancreatic insufficiency was defined as fecal elastase below 200 µg/g (60).

In study I, the short endoscopic secretin test (EST) was used as golden standard for exocrine pancreatic function (27). The subject fasted for 8 hours and received both pharyngeal lidocaine spray (Xylocaine; AstraZeneca AB, Sweden) and conscious sedation with intravenous midazolam (Midazolam; Actavis Group HF, Iceland) 2-5 mg before the procedure. Twenty-five minutes after intravenous secretin administration a duodenoscope was positioned in the stomach, and after luminal examination all gastric fluids all gastric fluids were aspirated and discarded. Thirty minutes after secretin stimulation the tip of the duodenoscope was positioned distally to the papilla of Vater and three samples of duodenal juice were collected, each sampling lasting for 5 minutes. The details of duodenal juice handling and analyses are thoroughly described in previous publications (17,27,29,61). EPI was defined as peak bicarbonate concentration below 80 mEq/L (14).

1.5.8. CLINICAL CHARACTERISTICS IN CP

Fecal elastase was measured by a commercially available kit and EPI was defined as fecal elastase below 200 µg/g (paper III) (62). When attending the annual follow-up in the outpatient setting, CP patients were classified according to M-ANNHEIM clinical staging (M-ANNHEIM 0: Stage of subclinical CP; M-ANNHEIM I: CP without pancreatic insufficiency; M-ANNHEIM II: CP with partial pancreatic insufficiency; M-ANNHEIM III: Painful CP with complete pancreatic insufficiency; M-ANNHEIM IV: Secondary painless CP (burnout)) (63). Duration of CP was from the date for the initial diagnosis till the date for the s-MRI scan.

1.5.9. DISEASE BURDEN IN CP

For study II, disease burden was characterized by *hospitalization, quality of life (QOL) score and pain score*. Patients with CP were prospectively followed during the observation period, which started from the date of the individual s-MRI examination (index date) and ended in august 2016. The time to first hospitalization or death from the index date, total number of hospitalizations and duration of hospitalizations was noted. When attending the annual follow-up examinations at the Centre for Pancreatic Disease, patients filled out questionnaires for evaluation of quality of life (QOL) and pain symptoms. The European organization for research and treatment of cancer quality of life questionnaire (EORTC QLQ-C30) was used to evaluate QOL (64,65). Pain scores were measured using the modified Brief Pain Inventory short form (m-BPI-sf) (66).

1.5.10. STATISTICAL ANALYSES

Different analyses approaches were used in study I and study II depending on the aims and the data construction. The statistical analyses of the studies are reported in details in paper II-IV. In general, the difference between continues variables were calculated using student's t-test or two-sample Wilcoxon rank-sum test. Analyses of variances (ANOVA) was used to analyze differences in total secreted volumes and ADC values, with different time points (secreted volume 1, 5, 9 and 13 minutes after secretin) as a within-subject factor, and group (CP vs. HC and CF vs. HC) as a between subject factor. The potential confounding effects of age on s-MRI parameters were explored using linear regression models. Correlation analysis were performed using Pearson or Spearman´s correlation coefficients (r) as appropriate. P values <0.05 was considered statistically significant. The software package STATA version 14.2 (StataCorp LP, College Station, Texas) was used.

RESULTS

The key results from the studies are presented in this chapter. More detailed results are reported in paper I-IV. An overview of the main results from paper II-IV is illustrated in Figure 4.1.

1.6. AIM I

Aim: To investigate latest developments in s-MRI and the range of clinical application in the benign pancreatic disorders (paper I).

Key results:

- The literature review concludes that S-MRI is feasible in most benign pancreatic clinical scenarios and has the potential to aid in the identification of early main pancreatic duct changes and exocrine pancreatic dysfunction.
- There is a great variation in the applied imaging protocols, image interpretation strategies, and consequently, the information that may be extracted from s-MRI.

Interpretation: There is a need for standardization and evaluation of s-MRI protocols to exploit the full spectrum of morphological and functional changes that can be measured with s-MRI.

1.7. AIM II

Aim: To evaluate our s-MRI protocol including a novel, semi-automatic application for assessment of exocrine pancreatic function against a "gold standard" endoscopic secretin test in patients with cystic fibrosis compared to healthy controls (paper II).

Key results:

- Pancreatic secretion measured at 9 and 13 minutes after secretin stimulation was reduced in CF patients with EPI compared to CF patients without EPI and HC ($P = 0.035$).
- Pancreatic secretion at 13 minutes had an AUROC of 0.93 for prediction of EPI when compared to the endoscopic secretin test. It was the best performing s-MRI parameter and outperformed the fecal elastase test (AUROC of 0.84).
- CF patients with EPI had lower baseline ADC values than patients without EPI and HC in the pancreatic head ($P = 0.02$) and body ($P = 0.016$). No

significant changes in ADC values were seen after secretin administration for any of the subgroups.

Interpretation: Semi-automatic quantification of pancreatic secretion at 13 minutes post-secretin has high diagnostic accuracy for EPI in patients with CF.

1.8. AIM III

Aim: To investigate pancreatic morphology and exocrine function with the novel s-MRI protocol in patients with chronic pancreatitis compared to healthy controls (paper III).

Key results:

- Pancreatic gland volume was lower in CP patients as compared to HC ($P < 0.001$).
- Main pancreatic duct diameter was larger in CP patients as compared to HC ($P < 0.001$).
- Patient with CP had lower baseline ADC values than the HC in the pancreatic head ($P < 0.001$) and body ($P < 0.001$).
- CP patients had significantly higher FSF in the pancreatic body compared to HC ($P = 0.002$).
- Following secretin stimulation, patients with CP had a significantly lower increase in ADC values from baseline to 1 min after secretin (ΔADC) compared to HC (head $P < 0.001$; body $P < 0.001$).
- Pancreatic secretion at 9 minutes ($P = 0.017$) and 13 minutes ($P = 0.003$) after secretin administration was significantly lower in CP patients compared to HC.
- Pancreatic gland volume had negative correlation with Cambridge classification ($r = -0.26$, $P = 0.02$) and baseline ADC ($r = -0.35$, $P = 0.027$), and positive correlation with ΔADC ($r = 0.38$, $P = 0.015$).

Interpretation: Morphological and functional s-MRI parameters provide non-invasive, detailed, and complementary information about the pathological processes involved in CP. However, the morphological and functional parameters are not well correlated, reflecting the complex nature of the disease.

1.9. AIM IV

Aim: To explore association between pancreatic morphological and functional s-MRI parameters, clinical characteristics, and disease burden in patients with chronic pancreatitis (paper III + IV).

Key results:

- The morphological and functional s-MRI parameters were comparable between patients with alcoholic and non-alcoholic etiology of CP (all *P*-values > 0.12).
- The disease duration of CP was negatively correlated to pancreatic gland volume ($P < 0.001$) and fecal elastase ($P = 0.05$).
- Pancreatic gland volume was higher in M-ANNHEIM clinical stage I compared to II ($P = 0.001$) as well as in M-ANNHEIM clinical stage I compared to stage III & IV (polled together) ($P < 0.001$).
- Large pancreatic gland volume was associated with high fecal elastase values (r=0.66, $P = 0.0016$).
- The Cambridge classification was associated with M-ANNHEIM clinical staging ($P = 0.033$).
- A main pancreatic duct diameter below 5 mm was associated with reduced time to first hospitalization (HR 2.06, $P = 0.043$).
- Pancreatic secretion at 13 minutes after secretin administration was positively correlated with QOL ($P = 0.0072$) and negatively correlated with pain interference score ($P = 0.032$).
- Morphological and functional s-MRI parameters were not related to pain intensity score (all $P > 0.07$).

Interpretation: Morphological and functional information derived from s-MRI are relevant clinical biomarkers that in the future could aid in understanding the mechanisms underlying symptoms and the different stages in the development of CP, in monitoring disease progress, and optimizing treatment of CP.

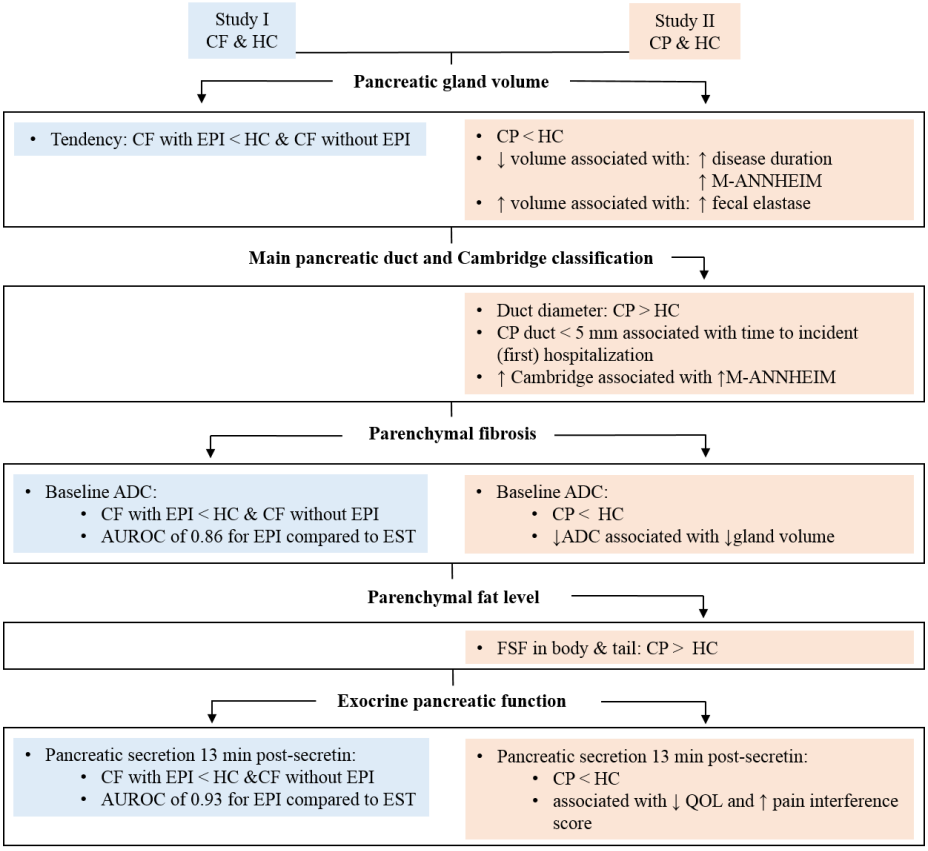

Figure 4-1: Overview of the main results from paper II-IV. Abbreviations: CP: chronic pancreatitis; CF: cystic fibrosis; HC: healthy controls; EPI: exocrine pancreatic insufficiency; ADC: apparent diffusion coefficient; FSF: fat signal fraction; QOL: quality of life; AUROC: area under receiver operating characteristics curve; >/<: larger/lower; ↑: high/increased; ↓: low/decreased

DISCUSSION

Advanced MRI with secretin-stimulation was used to characterize pancreatic morphology and exocrine function in patients with CP. The discussion will focus on s-MRI parameters ability to differentiate between patients with CP and HC, the relation to clinical characteristics and disease burden in CP, and technical considerations of the methods used.

1.10. PANCREATIC GLAND VOLUME

Evaluation of pancreatic gland volume has been performed on CT since 1980 with measurements of anterior-posterior diameter in the pancreatic head, body and tail (67). Several studies have reported age and sex-related decrease in pancreatic size in adults without known pancreatic disease (see Paper I) (67,68). When adjusting for age, we found that patients with CP had a significantly lower pancreatic gland volume compared to sex-matched HC (see Paper III). Pancreatic atrophy is a widely accepted common feature of CP, however the Cambridge classification categorizes enlargement of pancreas as a diagnostic criteria for CP (2,57). Additionally, recent study of pancreatic size with US based on anterior-posterior diameter reported that CP patients had a marginally larger gland compared to HC but they conclude that the value in diagnosis of CP is questionable (69). There are several differences compared to our study; the diagnostic criteria for CP, duration of the disease among CP patients was larger in our study and we excluded all patients with suspected acute exacerbation of CP. Furthermore, pancreatic gland volume in our study was the volume of the pancreatic parenchyma as we excluded main pancreatic duct and all cystic lesions. Time needed for analyses was reduced using a new, semi-automatic application. Additionally, we found that duration of CP was negatively correlated to gland volume (see Paper III). Our study confirms that pancreatic atrophy is a feature of CP that is associated to duration of the disease. More studies with volume segmentation in patients with suspected/mild CP are needed to evaluate the value of gland volume measurements in diagnosis of CP.

1.11. MAIN PANCREATIC DUCT & CAMBRIDGE CLASSIFICATION

Main pancreatic duct distortion in CP and the classification according to Cambridge system has been well-established since 1984 (56). Studies have also confirmed that MPD size varies according to age, sex and location in the pancreas (head, body and tail) (see Paper I) (70). Thus, adjusting for age in a sex-matched study population, we could report that patients with CP had a significantly wider maximal main pancreatic duct diameter compared to HC (see Paper III, table 2). Measurements were performed on 3D MRCP prior to secretin-stimulation. In clinical settings, especially in patients

with suspected or mild CP, dynamic MRCP after secretin-stimulation is preferred as it increases visibility and enables assessment of duct dynamics (see Paper I, Table 1). However, with our explorative study design we focused on evaluation of exocrine pancreatic function after secretin-stimulation leaving no time for dynamic MRCP for duct evaluation.

Main pancreatic duct diameter was not associated with any of the other s-MRI parameters. However, higher Cambridge classification score was associated with reduced pancreatic gland volume (see Paper III, table 3). These findings suggest that change in pancreatic duct caliber alone seems insufficient as a diagnostic criterion in CP, and that the Cambridge classification, which includes other pathological features i.e. cysts, irregular parenchymal structure, and calcification alongside dilatation of main duct dilatation and side branches, could be a more valuable parameter for the diagnosis of CP (see Paper III).

1.12. PARENCHYMAL FIBROSIS

In line with several other studies, patients with CP had lower ADC values (before secretin stimulation) compared to HC reflecting fibrosis in the pancreatic parenchyma (49,71). When adjusting for age, the fibrotic changes were only present in the pancreatic head and body (see Paper III, table 2) (72). It is important to measure ADC in the different anatomical regions of pancreas as studies in healthy have confirmed that ADC differs significantly from the pancreatic head, body and tail (73). Additionally, the ADC is an artificial parameter, without any true intrinsic relevance or histological correlate, that combines both diffusion and perfusion characteristics (74). The impact of diffusion and perfusion properties on the ADC value of the tissue is determined by the b values; the higher b values have stronger diffusion weighting and weaker perfusion weighting, whereas the lower b values have stronger perfusion weighting and lower diffusion weighting (see Paper I). It has been reported that alcoholic chronic pancreatitis is associated with both tissue fibrosis and reduction of blood vessel density on histological examination (48,75). Multiple b values (50, 400 and 800) used in our study enable a more precise calculation of ADC with less perfusion contamination (76). Additionally, several papers have reported good correlation between DWI imaging and fibrosis in histological specimens in both pancreas and liver (53,77). Our study supports that DWI is a valuable, non-invasive method for quantification of what is considered to be fibrotic changes of the pancreas in CP. Furthermore, baseline ADC values were negatively correlated with pancreatic gland volumes suggesting that parenchymal fibrotic changes accompany the gradual pancreatic atrophy. However, there was no association between parenchymal fibrosis, EPI and Cambridge classification as previously reported (78).

1.13. PARENCHYMAL FAT LEVEL

Mean FSF values in the pancreatic body were significantly higher in patients with CP compared to HC (see Paper III, table II). Surprisingly, the changes were not present in the pancreatic head and tail. A recent study from Yoon et al. in 2016 reported a moderate correlation with estimated fat fraction on Dixon imaging compared to fat amount in histological specimens after pancreatectomy (53). In addition, Tirkes et al. in 2016 reported that mean FSF in patients with mild CP was significantly higher compared to HC (79). Our results provide supporting evidence for the rising number of studies on quantification of pancreatic fatty infiltrations with advanced MRI (80,81). In our study, FSF in the pancreatic body did no correlate to any other s-MRI parameter, clinical characteristics, or disease burden. Especially our non-existing correlation between FSF and ADC indicates that development of fibrosis and fatty infiltration might be two separate pathological processes of the CP disease. Currently, the role of fatty infiltration in development and progression of CP is still largely unknown and more studies are needed.

1.14. EXOCRINE PANCREATIC FUNCTION

Mild to moderate EPI, with vague clinical symptoms, is present in several diseases and could be a relevant marker for early (asymptomatic) stages of CP but remains challenging to diagnose. Matos et al. 1997 proposed a s-MRI based semi-quantitative visual assessment of pancreatic secretion (see Figure 5-1) and several publications followed proposing different quantitative techniques for estimating pancreatic output in milliliters as a quantitative volume (36,58,82,83). They were all based on the assumption that changes in signal intensity on T2-weighted imaging can be used to assess changes in liquid containing volumes in a region of interest (see Paper I) (83). We evaluated pancreatic secretion with a new, semi-automatic application using coronal T2-weighted images, 7-8 slices with thickness of 10 mm, that had shown good correlation with EST in characterizing pancreatic exocrine function in healthy controls (44). In contrast, most studies use dynamic 2D MRCP for semi-quantitative visual and quantitative assessment of EPI as it also allows more dynamic assessment of the main pancreatic duct (see Discussion, subsection Main pancreatic duct & Cambridge classification) (40,58).

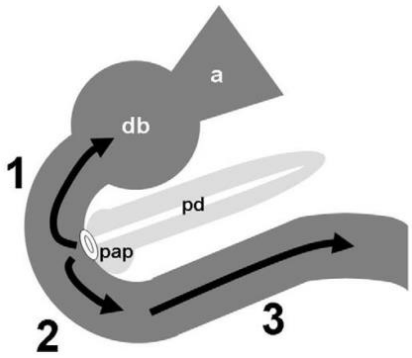

Figure 5-1: Semi-quantitative visual assessment of pancreatic exocrine function after secretin-stimulation, figure from Schneider et al. 2006 (84). *a: antrum ventriculi; db: duodenal bulb; pd: main pancreatic duct; pap: papilla of Vater; Grade 0-3.*

In patients with CF (Study I, Paper II), we could demonstrate that pancreatic secretion 13 minutes after secretin-stimulation had a high diagnostic accuracy for EPI when compared with the "gold standard" EST. Furthermore, pancreatic secretion performed better than the fecal elastase test (Paper II, Table 4). This supports the assumption that our protocol settings and the new semi-automatic application for calculation of pancreatic secretion are valid and a precise tool for assessment of EPI. In CF, a small portion of patients develops EPI later in life and need regular follow up. For these patients, s-MRI could provide a precise and non-invasive test comparable with EST for diagnosing EPI. However, the majority of CF patients develop EPI early in life and fecal elastase is a well-described and reliable tool for diagnosis and follow-up of EPI (85,86).

In patients with CP (Study II, Paper III), pancreatic secretin at 9 and 13 minutes after secretin-stimulation was significantly lower when compared to HC. Thus, pancreatic secretion at 1 and 5 minutes after secretin was similar. Mensel et al. performed a large s-SMI study in 819 healthy subjects and could report that the highest pancreatic flow output (11.7 ml/min) was observed 9 min after secretin. Our results indicate that patient with CP have a decline in their secretory capacity after 5 min compared to HC which could reflect the loss of pancreatic parenchyma. However, pancreatic secretion was not associated with pancreatic gland volume or any of the other s-MRI parameters (Paper III, Table 3). Another potential explanation could be that secretin also stimulated biliary secretin of water and bicarbonate alongside gastric secretion in duodenum that blurs the initial "true pancreatic secretin"(15). Currently, we are not able to fully explain the secretory profile in patients with CP but our study confirms that pancreatic secretion is significantly hampered compared to the HC. However, pancreatic secretion is a complex parameter that seems unaffected by pancreatic atrophy, duct distortion, fibrotic changes, and fatty infiltrations. Our results are obtained from a population of CP patients where the majority had morphologically

marked CP changes (54% of CP patient classified with Cambridge score IV, see Paper III, Table 2). This contradicts the general assumption that pancreatic morphology and function are more related in the advanced stages of CP. In conclusion, increasing number of papers have reported that s-MRI can be used to diagnose EPI. The challenge remains to provide a more standardized s-MRI protocol so that larger studies can be performed in CP which could help in reporting some cut-off values for normal vs. pathological pancreatic secretion, and thereby potentially help detecting mild/early CP.

Additionally, CP patients had a significantly lower increase in ADC from baseline to 1 min after secretin stimulation compared to HC. This indicates that measuring ADC could detect CP and maybe EPI after secretin stimulation. However, previous studies in post-secretin ADC have reported inconclusive results. Erturk et al. reported that patients with CP had a later peak (median 7 min) in ADC compared to HC (median 2 min) (48). We did not observe any such peak in ADC in patients with CP, baseline ADC and post-secretin ADC at all timepoint were similar (Figure 2, Paper III). Akisik et al. reported that mean pre-secretin and maximum post-secretin ADC was higher in HC compared to CP, but the percentage increase ADC and time to peak ADC did not differ between the groups (87). However, their healthy population also had the highest increase in ADC between baseline and 2 minutes after secretin stimulation. Different imaging protocols and definition of CP preclude direct comparison of the results. However, HC did have a steep increase in ADC in all three studies approximately between baseline and 2 min after secretin, the reports on CP are somewhat inconsistent. If we assume that pancreatic secretion is the same at 1 min after secretin, then the reason for increase in ADC in HC could be due to increased blood flow that is hampered in patients with CP. However, our choice of multiple b-values in calculation of ADC reduces the impact of perfusion. Therefore, it can be argued that the increase in ADC is due to higher ductal and acinar secretion in the pancreatic parenchyma in HC. Currently, the role of ADC measurements after secretin stimulation remains unclear and further studies in larger populations are required.

1.15. S-MRI & CLINICAL CHARACTERISTICS

More severe M-ANNHEIM clinical stage and low fecal elastase were associated with loss of pancreatic parenchyma (pancreatic gland volume) and higher Cambridge classification score, see Figure 5-2. M-ANNHEIM clinical stage and fecal elastase were mutually correlated, however as EPI is included in M-ANNHEIM classification this result was expected. Additionally, low pancreatic gland volume was associated with longer disease duration.

The clinical value of measuring pancreatic gland volume has, to our best knowledge, not previously been reported. Our findings indicate that presence of multiple common features in CP (exocrine and endocrine dysfunction, pain, duct distortion) and longer disease duration is accompanied with loss of pancreatic parenchyma (Figure 5-2). A

potential explanation for the importance of pancreatic volume loss could be that multiple parallel pathological processes co-exists (main duct distortion, side branch pathology, cystic lesions, parenchymal fibrosis, and fatty infiltration) which over time leads to destruction and loss of pancreatic parenchyma as the ultimate end result, and during the process results in functional insufficiency. This is further supported with the association of M-ANNHEIM clinical stage, gland volume and the modified Cambridge classification. Consequently, our results indicate that monitoring pancreatic volume and/or Cambridge classification in CP could provide valuable information about disease progression. The Cambridge scoring is performed on MRI without the need for secretion stimulation, expensive software programs and could be easily integrated in daily radiological routine.

Interestingly, fecal elastase and pancreatic secretion were not correlated as reported in previous studies (18,88). This suggest that functional loss of ductal cell (reflected in reduced pancreatic secretion) and acinar cell (reflected in low fecal elastase) does not necessarily coexist, and may occur at different stages of the disease. This highlights the complexity of EPI in patients with CP and that pancreatic fluid secretion only uncovers some aspects of the exocrine problem. MRI is not able measure concentrations of enzymes used for digestion as possible with EST, however diagnostic accuracy for EPI is similar as reported in patients with CF. Thus, s-MRI for evaluation of EPI is a valid tool and could be of great value in patients with suspected EPI where fecal elastase has a documented low sensibility.

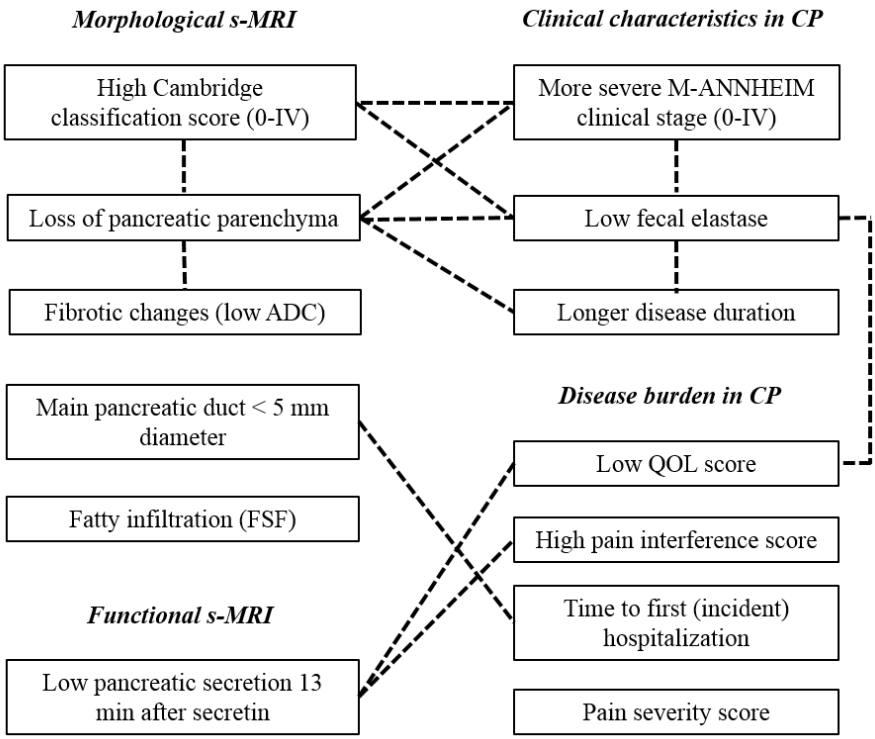

Figure 5-2: Overview of associations between s-MRI parameters, clinical characteristics, and disease burden in chronic pancreatitis (CP) (study II). Dashed lines represent a significant (P<0.05) association.

1.16. S-MRI & DISEASE BURDEN IN CP

A main pancreatic duct diameter < 5 mm was associated with a shorter time to first CP related hospitalization, see Figure 5-2. None of the other s-MRI parameters were associated with hospitalization frequency and duration. This indicates that patients with less severe duct dilatation are more prone to earlier hospitalization compared to patients with more severe duct distortion. In a recent study from Olesen et al., pain was identified as the most frequent cause for hospitalization followed by common bile duct stenosis, symptomatic pseudocysts and acute in chronic pancreatitis (9). The reason for hospitalization is not identified in our study, but patients with smaller duct diameter could have more preserved parenchyma and hence be more prone to developing painful clinical situations like acute inflammation, pseudocysts, or significant duct obstruction. However, main pancreatic duct diameter was not associated with pancreatic gland volume nor with pain intensity score. Nevertheless, this is an interesting finding and suggests that MRI could be used in identifying patients at risk.

Low pancreatic secretion and low fecal elastase were associated with reduced QOL, which could be explained by that patients with preserved pancreatic function may have lower risk of malnutrition and associated complications such as sarcopenia and osteoporosis, which all lead to higher QOL. Previous papers have reported that low body weight in patients with CP was associated with poor health status (89). However, Olesen et al. have previously reported that exocrine insufficiency was not related to QOL in a multivariate analysis including pain symptoms, diabetes and different pancreatic and extrapancreatic complications (90). Patients with CP have a decreased QOL compared to general population and especially pain has been identified as the main cause for this (5,90). However, our results show that QOL in context of CP could be determined by numerous factors and their intricate interaction in the individual patient. Additionally, reduced pancreatic secretion was associated with higher pain interference score, which is calculated by parameters such as activity, mood, working capacity and sleep. Consequently, pain interference could also be affected by exocrine malfunction and malnutrition rather than pain itself.

None of the morphological and functional s-MRI parameters were associated with pain intensity score. Our results are in line with the emerging evidence suggesting that inflammation and fibrotic changes of pancreatic parenchyma leads to alternations in function and morphology of intrapancreatic nerves, which subsequently results in development of persistent pain in CP with sensitization of the central nervous system (i.e. neurogenic pain) (91,92). Hence, pancreatic morphology and function has no direct relation to persistent pain in chronic pancreatitis patients with more established/long-lasting disease (71)

1.17. STUDY LIMITATIONS

There are study limitations in both study I and study II.

The study population in study I was relatively small which could affect the power of statistical analyses. Additionally, time interval between s-MRI and EST was no predefined and varied from subject to subject, see Table 1, Paper II. In study II, the ADC and FSF analyses was performed only in patients with pancreatic gland larger than 25 ml to accommodate a ROI of 8 mm in diameter. Though, this resulted in a much smaller sample size (only 38-44 of 83 patients) for these parameters. Furthermore, MRI is not able detect calcifications in the pancreatic parenchyma meaning that ROIs could contain different amounts of calcifications. Theoretically, this would result in lower ADC values and probably higher FSF values in patients with CP. Currently, we are not able to estimate if this had an impact on our ADC and FSF results. Furthermore, our CP patients had quite severe disease stages of CP, hence, extrapolation of our results to early disease and identifying early disease markers is difficult.

The s-MRI protocol and the new semi-automatic application for quantification of pancreatic secretion were validated against the "gold standard" EST in patients with CF for estimation of EPI. After displaying high diagnostic accuracy for EPI, the same concept was used in patients with CP. However, the genetics and pathogenesis of CF are well described and similar in most patients resulting in a very homogenous population. In contrast, the pathogenesis of CP is more complex and yet not fully understood resulting in more heterogenous population. This means that patients with CP probably have more complicated pathways for developing EPI compared to CF. The consequence could be that the very high diagnostic accuracy of our models for EPI in CF cannot be expected in patients with CP.

1.18. CLINICAL & FUTURE PERSPECTIVES

MRI is a currently the preferred imaging method for more advanced assessment of the pancreatic gland in benign disorders. However, the lack of systematic approach and little use of quantitative measures in reporting radiological findings leave much room for improvement. Several morphological and functional s-MRI parameters presented in this thesis could be integrated in the daily routines to aid the clinicians in the diagnosis and follow-up in patients with CP. The staging according to the modified Cambridge classification is after some practice easily performed without any need for expensive software programs. Evaluation of the pancreatic gland volume and pancreatic secretion require additional software and time for post-processing and analyses. But as our Study I and Study II demonstrate, pancreatic secretion as an estimate for EPI with high diagnostic accuracy only require of two image series (before and 13 minutes after secretin-stimulation). This significantly reduces the time needed for post-analyses, and will also enable to perform a regular MRCP with dynamic assessment of the main pancreatic duct after secretin stimulation. Measurement of fibrosis (ADC) and fatty infiltrations are promising parenchymal parameters, but further exploration is needed to assess their implications in natural history and progression of CP. In general, more and faster automatization of the analyses is the key for quicker implementation of the s-MRI parameters in the clinical daily routine. Furthermore, as presented in Paper I, there is also a need for harmonization and standardization of s-MRI protocols to enable more reliable comparison of the results from different studies, and to pave the road for initiation of multicenter studies. Optimization of the s-MRI protocol and the obtained results could also be of value in other diseases like acute pancreatitis, pancreatic cystic neoplasm and sphincter of Oddi dysfunction (Paper I).

Several other MRI methods for assessing pancreatic parenchyma are currently gaining more interest. Tirkes et al. reported that T1 relaxation time in the pancreatic parenchyma (referred to as the T1-mapping method) was significantly increased in patients with mild CP compared to the control group (79). T1 mapping is also showing promising results in detailed tissue characterization in cardiac MRI (including identification of myocardial scaring) so further improvement within this area can be

expected. Sugita et al. reported that optimized arterial spin-labeling techniques can be used for directs visualization of pancreatic juice flow (93), and is hence a potential method for exocrine secretory assessment without the use of secretin stimulation. MRI elastography for measurement of changes in mechanical properties (tissue stiffness) associated with fibrosis and more advanced DWI techniques like Intra-Voxel Incoherent Motion diffusion imaging (assessment of both the perfusion and diffusion component of DWI) are currently being tested in liver (94,95). A potential breakthrough in these areas could also be used for characterization of the pancreatic parenchyma. Magnetic resonance spectroscopy has also been used in liver imaging, and, with improvement of the scanner technique, it could lead to assessment of metabolites of the pancreas(96). The ability to provide a morphological and functional assessment of a specific organ in one examination is the future of MRI. Therefore, as the optimal goal, we aim to provide an advanced MRI protocol for detailed characterization of morphological and functional pancreatic parameters to improve diagnosis, follow-up and treatment of patients with CP.

CONCLUSION

The literature review identified numerous advanced MRI parameters for characterization of the pancreatic gland. In this thesis, morphological s-MRI parameters included pancreatic atrophy (gland volume), main pancreatic duct diameter, fibrosis (ADC), fatty infiltrations (FSF) and the modified Cambridge classifications score. The exocrine pancreatic function was estimated with assessment of pancreatic secretory volumes and changes in ADC before and after secretin-stimulation.

The pancreatic secretion 13 minutes after secretin had high diagnostic accuracy for EPI when compared to the "gold standard" EST in patients with CF. Thus, the method and the new semi-automatic application are considered valid, non-invasive tools for assessment of EPI. All the morphological and functional s-MRI parameters could differentiate between patients with CF and HC. In patients with CP, lower pancreatic gland volume was associated with higher Cambridge classification score and lower ADC reflecting increased fibrotic changes. These results are in line with the assumption that s-MRI can provide a detailed, non-invasive characterization of the pancreatic parenchyma which can be used to detect pathological features in patients with CP. In addition, a low association between morphological and functional features in CP was demonstrated. Pancreatic gland volume was lower and the Cambridge classification score higher with more severe M-ANNHEIM clinical staging. Additionally, higher pancreatic secretion 13 minutes after secretin was associated with QOL and large pancreatic gland was associated with high fecal elastase test. These results indicate that several pathophysiological processes likely are involved at the same time during the complex CP disease course, and that our s-MRI parameters provide information on the different disease features that can be used as relevant clinical biomarkers in diagnosing and grading disease severity in patients with CP.

The overall results in this thesis show that several common pathological features of CP can be detected with present advanced MRI techniques. Thus, s-MRI parameters can be used for diagnosis, monitoring disease progress and in the future likely be used for more efficient follow-up and treatment of patients with CP.

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APPENDIX: PAPER I-IV

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