A short endoscopic Secretin test for the diagnosis of chronic pancreatitis

Friedemann Georg Erchinger

Avhandling for graden philosophiae doctor (ph.d.)
Universitetet i Bergen
2018

UNIVERSITETET I BERGEN

A short endoscopic Secretin test for the diagnosis of chronic pancreatitis

Friedemann Georg Erchinger

Avhandling for graden philosophiae doctor (ph.d.)
ved Universitetet i Bergen

2018

Dato for disputas: 12.06.2018

År: 2018

Tittel: A short endoscopic Secretin test for the diagnosis of chronic pancreatitis

Navn: Friedemann Georg Erchinger

Trykk: Skipnes Kommunikasjon / Universitetet i Bergen

A short endoscopic Secretin test for the diagnosis of chronic pancreatitis

Friedemann Georg Erchinger

Dissertation for the degree philosophiae doctor (PhD)

At the University of Bergen

20.01.2018

Scientific environment

The Pancreas group is connected to Section of Gastro and Nutrition and the Bergen Research Group for Ultrasound in Gastroenterology (BRUSE) at Department of Clinical Medicine, University of Bergen. This research milieu has served as a stimulating environment for this dissertation. BRUSE is working to develop and validate new methods for clinical ultrasonography, with the goal of improving patient care and treatment. The Gastro Section is continuously working to improve basic and clinical methods for pancreatic diseases. BRUSE is internationally recognized as pioneers in both transabdominal and endoscopic ultrasound and has a broad international collaboration. Altogether 19 PhD dissertations plus 10 ongoing, 6 books and over 300 research publications have emerged from this research group in Bergen.

National Centre for Ultrasound in Gastroenterology (NCUG) was established at Haukeland University Hospital by the National Health Authorities in 2001 as a national service of excellent competence. NCUG is a leading international ultrasound centre and was in 2014 accredited as a European Learning Centre.

The research has been performed in cooperation with Medical Department, Voss Hospital and the Paediatric Department. Regular and valuable cooperation has also been performed with the Pancreas group at Aalborg University, Denmark.

Nasjonalt Senter for
Gastroenterologisk Ultrasonografi
National Centre for Ultrasound in Gastroenterology
Haukeland University Hospital, Bergen, Norway

,

Acknowledgements

It was an honour to be elected as the first partner from the very beginning to a continuously growing scientific environment, “The Bergen Pancreatic Club”. The enormous engagement of my main supervisor motivated me to study pancreas function testing in all its facets in our clinic, a hallmark in updated diagnostic procedures in chronic pancreatitis.

First, I want to thank my main supervisor Professor PhD MD Georg Dimcevski for extraordinary help. Planning, organizing and performing all projects were not possible without his enormous energy and authority. There was no obstacle, which could not be eluded. His humour, experience and pragmatism were irreplaceable in futile situations. Without my co-supervisor Professor PhD MD Odd Helge Gilja, it was not possible to be accepted as a non-funded PhD candidate at the University of Bergen and simultaneously working as external clinician. His overall competence and experience in leadership was the guarantee to perform this work. Especially help in establishing ultrasound in our project is his merit. The other co-supervisor Professor Trygve Hausken encouraged me in 2004 to reintroduce scientific work, 14 years after a German doctor degree.

MD Trond Engjom is a central person in this work. As we shared aspects of the same task, articles were prepared very closely, cooperation in project planning and interpretation of data was perfect. His critical review augmented the quality of presentations, posters and articles fundamentally.

PhD, MD Erling Tjora also participated in the pancreas project at an early stage. He contributed essentially with the measurement of enzymes in duodenal juice and broadened the knowledge of Diabetes type III in our group.

Professor Lage Aksnes provided generously his laboratory facilities and helped to establish enzyme analyses in duodenal juice. Sadly, he died suddenly and left behind an irrecoverable gap.

PhD Oddrun Gudbrandsen became interested in pancreas function testing when we cooperated as supervisors for master students in nutrition. With her, we could establish automation of measuring bicarbonate, Amylase and Lipase in duodenal juice in the clinical repertoire.

It was generous of my former teacher and mentor Professor Arnold Berstad to reveal the secrets of measuring faecal fat after the method of van de Kamer. As this method was performed modified over decades at our clinic, we studied together its accuracy. In this project, I was lucky to meet senior bioengineer Aud Sissel Hjartholm-Eriksen. She performed back titration of bicarbonate in duodenal juice with high accuracy and worked extraordinary exactly.

Liv Aasmul, senior bioengineer was irreplaceable from the first endoscopic secretin test. She prepared duodenal juice in the endoscopic unit before analyses. Her help with maintenance of the patient database was magnificent.

PhD MD Roald Flesland Havre performed EUS of the pancreas virtuously. He established the routine of Rosemont scoring in patients with symptoms suspicious for chronic pancreatitis; if necessary interventions were performed immediately.

Advanced diagnostic and therapeutic endoscopic interventions were not possible without high skilled endoscopist Khanh Do-Cong Pham.

Great thanks to the staff at the endoscopic unit with their positive and open mind and helpfulness.

I was happy that the CEO of Voss Hospital Rolf Abrahamsen, and the leader of Medical Department Anita Spikkeland provided for the first time the possibility to work additionally as a PhD candidate. The flexibility and support of my working colleagues was remarkable.

The importance of a family cannot be underestimated. My wife Esther and my children Vera, Rebecca and Noah tolerated my life as commuter well and patiently. When at home, they answered my absence of mind with a knowing smile. Thus, we

experienced the time left together much more intensively and the social cohesion was strengthened.

Abbreviations

CP:	Chronic pancreatitis
EUS:	Endosonographic Ultrasonography
CT:	Computed Tomography
MRI:	Magnetic Resonance Imaging
ERCP:	Endoscopic Retrograde Cholangiography
MRCP:	Magnetic Resonance Cholangio-Pancreaticography
EST:	Endoscopic Secretin Test
FE1:	Faecal Elastase 1
MOPS:	3-(*N*-morpholino) propanesulfonic acid
BSA:	Bovine serum albumin
EPI:	Exocrine Pancreatic Insufficiency
CCK:	Cholecystokinin
PRSS1:	Protease, Serine 1
SPINK:	Serine Protease Inhibitor Kazal-type 1
CFTR:	Cystic fibrosis transmembrane conductance regulator
CTRC:	Chymotrypsin C
CEL-MODY:	Carboxyl-ester lipase-maturity-onset diabetes of the young

Preface

The diagnoses of chronic pancreatitis (CP) may range from a simple and straight forward task to a tremendously challenging and intensive investigative process. The most important exogenous risk factors are alcohol consumption and smoking. Genetics, anatomical variants and biliary diseases are also important. One risk factor alone or in combination with others gives different morphological and functional changes. Prediction of clinical outcome is dubious, and the range is broad, from asymptomatic via mild to severe disease manifestations.

Pancreatic function is of importance, as it may be key for early diagnoses of CP. Therapy of pancreatic failure at an early state can prevent complications as weight loss, deleterious nutritional state and increased mortality.

Direct pancreas function testing is worldwide rarely used in clinical practice as it is cumbersome, not standardized, and needs a broad clinical infrastructure.

In our opinion, with this work, we have substantially simplified the endoscopic procedure, analyses of bicarbonate and enzymes in duodenal juice. Thus, the practical performance of direct pancreas function testing is possible in any clinical unit with basic endoscopic and laboratory services.

This thesis describes a clinical approach to simplify diagnostics of CP with emphasizes on exocrine pancreatic insufficiency.

Abstract

Background:

The diagnosis of CP is not yet clearly defined. Many national guidelines exist, but - as in many other not clearly defined diseases - there is no worldwide consensus. In CP, evaluation of exocrine pancreatic function is crucial because symptoms are often diffuse and overlooked by the doctors. Additionally, early diagnosis of exocrine pancreatic failure is important as its consequence, malnutrition and commonly abdominal pain, leads to serious complications and reduced life expectancy. Direct pancreas function testing with analyses of enzymes in duodenal juice may give this information.

Aims:

The main aim was to develop and establish a multimodal algorithm for the diagnoses of CP, accurate and easy to handle in clinical practice. Secondary, we wanted to simplify direct pancreas function testing including a): the performance of a short endoscopic test (article I) and b): the analyses of ingredients in duodenal juice by automation: bicarbonate (article II), Amylase (article III) and Lipase (article IV).

Materials and Methods:

We examined consecutively healthy controls and patients referred to our outpatient clinic due to symptoms suspicious of CP. We assessed patients with a modified Layer (Mayo) score, which includes imaging, pancreas function testing and medical history. We established a short endoscopic secretin test and analysed bicarbonate, Amylase and Lipase in duodenal juice as markers for ductal and acinar exocrine pancreatic function. In article I, we determined sensitivity, specificity and accuracy of bicarbonate and faecal-elastase, using our modified secretin-stimulated upper endoscopy (short endoscopic secretin test, or EST). In article II, III, IV, we describe correlation between automation of analyses of Bicarbonate, Amylase and Lipase in duodenal juice to labour-intensive manual methods.

Results:

I. Short endoscopic secretin test: Fifty-two patients aged 19 to 67 years and 25 healthy controls aged 19 to 64 years were included. Twenty-four patients fulfilled the modified Layer score for CP or non-CP. The overall accuracy of the EST versus FE1 test was 85%/71%, with positive and negative predictive values of 100%/79% and 80%/69%, respectively.

II. Automation of bicarbonate measurement: 177 samples from 71 patients were analysed. Correlation coefficient of all measurements was $r = 0.98$ ($p < 0.001$). Correlation coefficient of fresh versus frozen samples conducted with automatic spectrophotometry ($n = 25$): $r = 0.96$ ($p < 0.001$).

III. Automation of amylase measurement: We analysed 52 samples for assay of amylase in pairs. Correlation between measurements with the two methods was $r = 0.99$ ($p<0.001$).

IV. Automation of lipase measurement: We tested stability of 54 samples from 21 patients. Diluting samples with MOPS buffer, added BSA gave stable results, and was superior to diluting samples in saline. We compared the two assays in 50 samples from 20 patients and found a good correlation between the two assays ($r=0.91$, $p<0.001$).

Conclusions:

I: Short EST is rapid and easy to perform and can be incorporated in daily routine in every clinical endoscopic unit. EST is superior to FE1 in the assessment of pancreatic insufficiency, leading to earlier diagnosis of moderate and early or mild CP.

II: The measurement of bicarbonate in fresh and thawed samples by automatic spectrophotometric analysis correlates excellent with the back-titration gold standard.

III and IV: Quantification of duodenal amylase and lipase activity with automated spectrophotometry has excellent correlation to measurements made by the manual methods.

Overall, Endoscopic secretin test is easy to perform, and can be incorporated in a diagnostic endoscopic examination. Automated measurement of bicarbonate, lipase

and amylase in duodenal juice simplifies the analytical methods and shortens time from test to result substantially. Standardized, centre-independent analyses of duodenal juice with quantification of ductal and acinar function in any unit with basic endoscopic and laboratory services is within reach.

List of Publications

Erchinger F, Engjom T, Tjora E, Hoem D, Hausken T, Gilja OH, Dimcevski G. "Quantification of pancreatic function using a clinically feasible short endoscopic secretin test." Pancreas. 2013 Oct; 42(7):1101-6.

Erchinger F, Engjom T, Gudbrandsen OA, Tjora E, Gilja OH, Dimcevski G. "Automated spectrophotometric bicarbonate analysis in duodenal juice compared to the back titration method." Pancreatology. 2016 Mar-Apr; 16(2):231-7.

Erchinger F, Engjom T, Tjora E, Aksnes L, Dimcevski G, Gudbrandsen OA. "Analysis of amylase in duodenal juice - Automated kinetic spectrophotometric analysis versus manual colorimetric endpoint assay" Pancreatology. 2017 Mar - Apr;17(2):182-187.

Tjora E, **Erchinger F**, Engjom T, Aksnes L, Dimcevski G, Gudbrandsen OA. "Analysis of Lipase in duodenal juice Automated kinetic spectrophotometric analysis versus manual colorimetric endpoint assay." Submitted to PLOS ONE. 2017

Contents

1. Introduction

Medical history

In the western world the pancreas was first described by the Greek anatomist and surgeon Herophilus of Chalcedon (*b.* 336 B.C.)(1). The first documentation of the pancreas accepted as anatomical term was in 100 A.D. (Ruphus of Ephesus, Greek anatomist and surgeon)(2;3). It consists of two words: Pan = all and Kreas = flesh, meaning an organ without bone or cartilage. In the 17th century Johan Georg Wirsung described the main pancreatic duct as an anatomical structure(4). More than 100 years later, Thomas Sömmering was the first describing its function by using the word abdominal salivary gland (Bauchspeicheldrüse)(5).

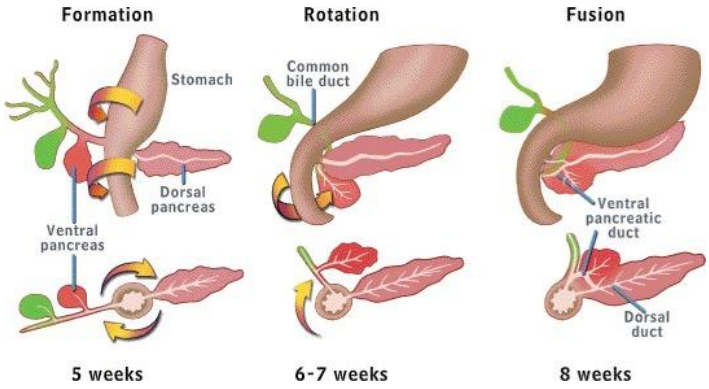
Samuel Thomas von Sömmerring. Portrait by Karl Thelott (1793–1830) - Dr. Senckenbergische Stiftung, Nibelungenallee 37 – 41, Frankfurt am Main, Germany; open source

In 1812 Johann Friedrich Meckel recognized the connection between embryology and pancreas divisum, including the accessory duct and sphincter discovered by Santorini(6;7).

Fundamentals of embryology

One month after gestation, a ventral and dorsal bud is present. After rotation of the ventral pancreas and the bile duct the two parts fusion, the duodenum gets in ventral

position. The ventral pancreas gets the ventrale Anlage (dorsal part of the caput) and uncinate process. The dorsale Anlage forms the ventral part of the caput, the corpus and the cauda(8).



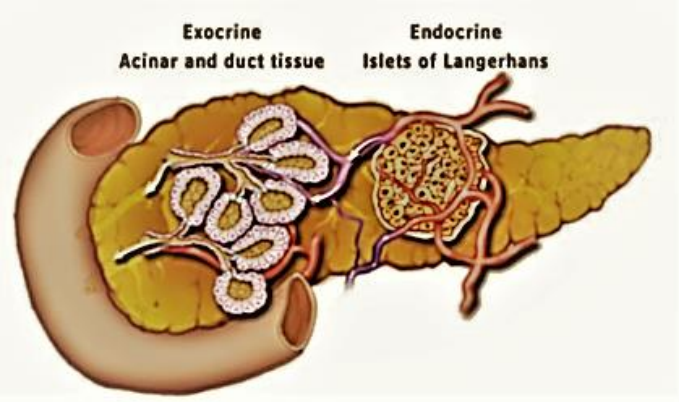
From: Pancreatic Embryology and Development; The Exocrine Pancreas. Pandol SJ. San Rafael (CA): Morgan & Claypool Life Sciences; 2010; open source

If the two parts of the pancreas do not fusion, various variants of the duct system exist. This is called pancreas divisum (9). Mostly, it is an incidental finding in asymptomatic subjects, but pancreas divisum can also be the source of relapsing acute pancreatitis especially in combination with chromosomal aberrations.

Anatomy

The pancreas is the most central organ in our body; it is half way in the longitudinal, sagittal and transversal plain. The neck is an important landmark as the superior and inferior mesenteric vein fusion to the portal vein at this place. Measures have a wide range, from 80-100 g in weight, 14 to 18 cm in length, 2 to 9 cm width and 2 to 3 cm thickness. Head, neck, body and tail form the whole organ. It mainly consists of water (71%), protein (13%) and fat (3-20%).The pancreas is also the most veiled organ because of its retroperitoneal position and neighbourhood to many other organs: Stomach, duodenum, liver, gallbladder, biliary tree, aorta and celiac trunk, vena cava, colon, kidneys and spleen. This may be the reason of complex arterial, venous and lymph supply with many anastomoses. Veins from the enteral drainage supply also the pancreas and complete the enteropancreatic circle. Pancreatic ducts drain

pancreatic juice to the duodenum, and the main pancreatic duct joins with the main biliary duct proximal the papilla of Vater. The small glandular parts are forming the exocrine and endocrine pancreas. The exocrine part consists of the ducts and the acini. Both subdivisions have its own functions: the ducts transport enzymes and proenzymes (zymogenes), to nutrition parts in the duodenum. The centro acinar cells produce bicarbonate, water, sodium, potassium and calcium, the acinar cells lipase, amylase, nucleases, trypsinogen, chymotrypsinogen, proelastase and propeptidase A/B(10). The endocrine pancreas transports hormones in the blood vessels. It consists of Langerhans islets. Its cells produce Insulin (beta cells; 68%), glucagon (alpha cells; 20%), somatostatin (delta cells, 10%) and pancreatic polypeptide (pp cells; 2%). Both parts of the autonomous nerve system, the sympathetic and parasympathetic nerves, but also visceral afferent nerves affect the pancreas.



From: Pandol, Stephen J. (2015). Normal Pancreatic Function. Pancreapedia: Exocrine Pancreas Knowledge Base. Open source

The acini are surrounded by nerve bundles and each single acinus is innervated by its own fibre(11). In this setting the muscarinic nerve system is of importance considering pancreatic enzyme secretion(12;13).

Physiology

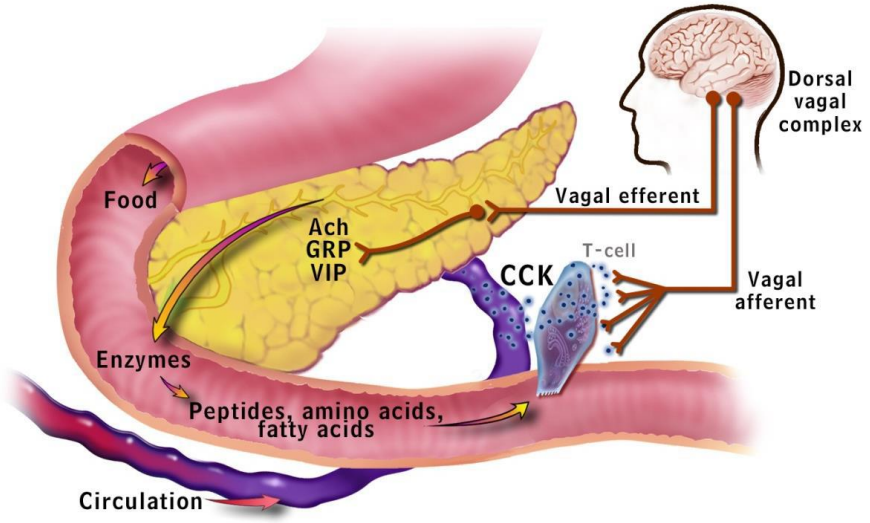
The pancreas is important for metabolism and produces hormones and digestive juice. The interplay between, nerves and hormones with other organs, structures and cells is

multifarious and complex(14). Importantly, most basic physiology is based on studies with animals and not humans(15). Therefore, cautious interpretation of former basic research is recommended. For example, rats have no gallbladder. Thus, bile acids and salts are not as concentrated in the duodenum as in humans. This may have consequences to fat absorption(16).

Pavlov saw that acid chyme entering the duodenum provoked secretion of pancreatic juice; additionally he demonstrated that different food composition caused differences in metabolic capabilities of pancreatic juice, peptic when feeding with meat, lipolytic when feeding with fat and amylolytic when feeding with carbohydrates(17).

Starling described in 1902 the first hormone secretin, a very potent stimulator of pancreatic secretion(17). He concluded that the role of the autonomous nerve system is not as strong as the hormonal mechanism; the afferent vagus nerve delivers olfactory, gustatory, gastric and intestinal signals to the dorsal motor nucleus of the vagus nerve which stimulates exocrine pancreatic function(18).

The sympathetic nerve in the paraganglia inhibits pancreatic exocrine secretion indirectly by reducing blood flow. After ingestion of fat and protein, secretion of cholecystokinin begins. It provokes contraction of the gall bladder, relaxation of sphincter of Oddi, delayed gastric emptying and satiety. The role as a secretagogue of pancreatic juice is marginal (19-21). This is controversial to old dogmas, which described CCK as only strong hormonal stimulator for acinar cells. Later literature describes the strong effect of CCK to enzyme delivery out of the acini as a paracrine effect, as it provokes vago-vagal enteropancreatic reflexes(22). Interestingly chemical structure of CCK is similar to gastrin and both hormones have similar characteristics. Secretin and CCK complement each other by using two different intracellular pathways: Secretin the adenylate cyclase, CCK the phospholipase C system. As consequence, after being stimulated by a meal, secretin and CCK potentiate their effect on acinar enzyme output(23;24).



CCK Stimulates Pancreatic Enzyme Secretion by Both Neural and Hormonal Pathways. From: Pandol, Stephen J. (2015). Normal Pancreatic Function.; Open source

Historically, three phases of pancreatic secretion are important. The cephalic phase induced by olfactory, gustatory, optical and commemoration factors leading to efferent vagal stimulation. The gastric phase has its origin in gastric distention leading to a gastro pancreatic reflex. The intestinal phase is most important. Gastric Chyme in the small bowel provokes nerve and hormonal response. Maximal output of stimulated pancreas juice is 20-50% of total output in the cephalic phase, 10% in the gastric phase and 50-100% in the intestinal phase(25). Other authors describe a fourth phase, the absorbed nutrient phase(14). Bicarbonate output is strongly associated with entering of gastric chyme in the duodenum, S-cells there may give maximal secretin output if the pH is <four.(26;27)

A portal system links the islet cells together with the acinar cells. Via this exocrine – endocrine axes insulin interplays with centro-acinar and acinar cells. Increase of bicarbonate, water and electrolytes and amylase output is the consequence(28;29).

Enzymes and zymogens in pancreatic juice ferment the chyme to molecules, which are able to cross the intestinal brush border into the portal venous system.

Enzymes

Already the cephalic phase stimulates enzyme output via vagal activity(30). Meals stimulate secretion of enzymes, followed by synthesis of enzymes in fasting periods(11). Acini in the pancreas are in distance to the location of digestive activity making secretion via the pancreatic ducts into the duodenum necessary. To prohibit pancreatic auto digestion, the acini deliver not enzymes, but inactive precursors. Only with sufficient bicarbonate production, pH optimum of pancreatic enzymes can be reached (trypsin 7.5-8.5, chymotrypsin 7.8-8.0, elastase 8.5, lipase 7.0-8,0, amylase 7.0). The interplay of enzymes and substrate and velocity of cleavage is equivalent to the term enzyme kinetics. It can be described as mathematic equation(31). Enzyme output may be selectively, as shown in the fourth phase of pancreatic stimulation, the nutritional phase. After duodenal infusion with amino acids, output of Proteases is increased, but not output of Amylase and Lipase(32). Loss of enzyme activity is different from enzyme to enzyme. From duodenum to ileum activity of lipase is reduced 99 %, of amylase 26% and of trypsin 78 % (33).

An intensive discussion about the destiny of enzymes is ongoing. Three mechanisms are important: Activation and inactivation of enzymes, cleavage of enzymes and recycling via the enteropancreatic circulation(34).

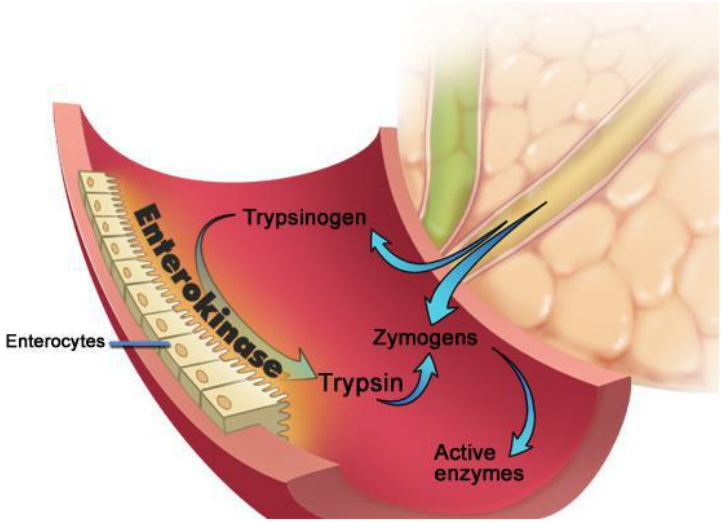
Proteases (zymogens)

80 % of the mass in pancreatic enzymes are proteases, but proteins stand only for 10 % of calorie intake in western diet(15). Peptides in the stomach have a pH optimum of 1.8 to 3.5 and are an important precondition for further cleavage by pancreatic proteases in the small intestine. Peptidases from the small intestine as aminopeptidases, 2 carboxypeptidases, 2 endopeptidases and γ -glutamic trans peptidase cleave the products of gastric and pancreatic proteases in smaller molecules(35).

Trypsin

In 1876, Kühne described the enzyme and its maximum action in alkaline milieu. He recognized that it cleaves proteins in chyme(36). Today we know that it preferentially hydrolyses peptides at the site of basic amino acids (lysine and arginine). Trypsin represents 19% of protein in pancreatic juice(15).

The precursor Trypsinogen produced in the acinar cells occur in three isoforms: cationic (PRSS1), anionic (PRSS2) meso trypsinogen (PRSS3)(37). Enteropeptidase from the small bowel mucosa converts trypsinogen to trypsin. Once activated, a process of auto activation begins.



From: Pandol, Stephen J. (2015). Normal Pancreatic Function. Pancreapedia: Exocrine Pancreas Knowledge Base; Open source

Trypsin also activates other pancreatic zymogens, like chymotrypsinogen, proelastase, procarboxypeptidase and prolipase once they are secreted in the duodenum(38). Pepsin provokes degradation of trypsin. Pancreatic secretory trypsin inhibitor is located in the same zymogen granule as trypsinogen. A small amount of activation of trypsinogen to trypsin in acinar cells makes the presence of trypsin inhibitor in acinar cells mandatory. In genetic failures (mutations of PRSS1, SPINK) impaired inhibition process leads to pancreatitis. The same process is coming up if

there is an overstimulation or overproduction of trypsinogen compared to trypsin inhibitor(39). Additionally, calcium overload in acinar cells triggers trypsinogen activation(39) . In CFTR mutations lack of flushing water in pancreatic ducts delays trypsinogen output and activated trypsin remains in the ductal part of the pancreas(40). Trypsin and trypsin inhibitor seem to influence pancreatic secretion by a negative feedback mechanism: trypsin in duodenum is limiting pancreatic secretion, trypsin inhibitor stimulates output of pancreatic juice (41). Remarkably, these basic processes, forming the underlying theoretical pathological mechanisms in pancreatic physiology are mostly evaluated in rats, and may not be equal in humans.

Chymotrypsin

Preferential cleavage: hydrolyses peptides involving aromatic amino acids (phenylalanine, tyrosine, tryptophan)(15). It is about 9% of protein in pancreatic juice(42).

The synthesis of chymotrypsinogen takes place in acinar pancreatic cells and trypsin in duodenal chyme activates it. Beside the proteolytic activity it is also an esterase and amidase(42). Like trypsin and elastase, it hydrolyses polypeptide chains. Beside its proteolytic activity, it is involved in control of blood pressure and blood clotting. It is one of the most studied enzymes because it's stable and can be easily obtained(43). Four isoforms exist: B1 (CTRB1), chymotrypsinogen B2 (CTRB2), chymotrypsinogen C (CTRC), and chymotrypsin-like enzyme-1 precursor (CTRL1)(37).

Elastase

This enzyme hydrolysis proteins, including elastin. Elastase splits the protein backbone at bonds at uncharged small amino acids (such as alanine, glycine, and serine)(15).

In 1950 it was shown that the proteolytic activity of trypsin and chymotrypsin was not as effective in cleavage of elastic fibres as a factor X in pancreatic juice, called elastase(44). This was the discovery of the third serine protease in pancreatic juice. In

zymogen granules of the acinar cells, it is stored as a precursor called proelastase. Trypsin activates it in the duodenum. The isoforms proelastase 2A (ELA2A), proelastase 3A (ELA3A) and proelastase 3B (ELA3B), are identified as active proteases once activated by trypsin in duodenal juice (45).

Carbopeptidase1

Carboxypeptidases exist as isoforms and are as the other proteases stored in the acinar cells as precursors, procarboxypeptidase A1 (CPA1), procarboxypeptidase A2 (CPA2) and procarboxypeptidase B1 (CPB)(46).

Carboxypeptidase-A attacks the last amino acid of a target peptide chain when it is aromatic, neutral, or acidic, while carboxypeptidase-B attacks basic amino acids(15).

Lipases

As early as in 1849 Claude Bernard described the hydrolysis of triacylglycerol in human duodenal juice(47) Pancreatic lipase cleaves dietary fat as follows: triacylglycerol + H_2O <=> diacylglycerol + a carboxylate.

Long chain triglycerides stand for 92-96% of fat content in western diet which is at least 100g total fat / day(48). Dietary fat stands for 42% of calorie intake in the western world(49).Typical American food calorie content is divided in 35% fat, 15% proteins and 50% sugar(50). Worldwide food intake differs considerably(51).

The pancreatic enzyme acts only on an ester-water interface; the outer ester links are preferentially hydrolysed(52;53). Bile salts for emulsification and colipase for linking the active enzyme to the water interface are needed(52). Additionally colipase compensates for the inhibitory potential of bile salts, phospholipids, cholesterol esters, dietary proteins and dietary carbohydrates(54).

In moderate to severe exocrine pancreatic insufficiency, absorption of up to 70% of triglycerides in the small bowels is possible. A possible explanation is the existence of human gastric lipase. Human gastric lipase does not only cleave

triglycerides in the stomach, its activation in the duodenum by bile salts plays an important role(55).

Carboxyl ester lipase CEL

This enzyme hydrolyses triglycerides, cholesterol esters, phospholipids, lysophospholipids, ceramides, vitamin esters and galactolipids(56). 4% of protein mass in pancreatic juice is CEL(57). Newer studies elucidated the importance of this enzyme (58-60).

Amylase

The enzyme works by Endo hydrolysis of (1->4)-alpha-D-glucoside linkages in polysaccharides containing three or more (1->4)-alpha-linked D-glucose units.

Pancreatic α-amylase catalyses breakup of starch into malto-oligosaccharides in the gut. Cleavage of them by gut wall α-glucosidases split oligosaccharides to glucose(61). About 40 to 50% of Western diet consists of Carbohydrates(62). 5-6% of pancreatic juice consists of this enzyme. Unlike proteases, it is not stored as an inactive precursor in acinar cells and is the only glycogen cleaving enzyme in pancreatic juice(63). Even without pancreatic amylase about 80 % of complex carbohydrates can be absorbed(64). Probably other amylases can compensate this deficiency. Salivary Amylase can partly compensate(65).

Chronic pancreatitis

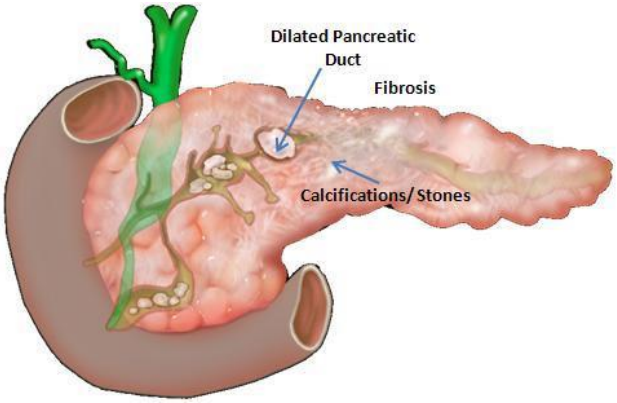
Today, widespread guidelines for the diagnosis and management of CP exist. They all reflect historical development of understanding physiology, pathophysiology and epidemiology, with consecutive approach to diagnosis and treatment (66-68).

However, a new way of thinking with a mechanistic definition of a multifactorial syndrome may change our understanding and clinical approach to CP (69).

CP affects the pancreas by different mechanisms. It is not one entity or clearly defined disease but rather a conglomerate of pathophysiological processes leading to

various symptoms as abdominal pain and discomfort, nausea, loose stools, malnutrition and weight loss (70;71). Not all symptoms occur in every patient. Often the course begins with acute pancreatitis leading to recurrent acute pancreatitis and at the end CP with loss of pancreatic function (70-73). Not all patients are symptomatic and diagnoses appears accidentally by hyperamylasaemia or hyperlipasemia, severe maldigestion in end-stage CP or even in post mortem examination (74-76). Frequently, not only one, but also several risk factors linked together will cause CP (77).

Morphologic changes are the consequence of chronic inflammation(78). Calcifications, duct irregularities, pseudocysts, atrophy are the main findings(79). Subtle changes have been described by advanced imaging methods(80).



From: Aghani, Elham.(2015). Introduction to Pancreatic Disease: Chronic pancreatitis. Pancreapedia: Exocrine Pancreas Knowledge Base; Open source.

Chronic inflammation involves exocrine pancreas with progressive loss of pancreatic function by damaging ductal and acinar cells (81). Destruction of stellate cells causes fibrosis (82;83). In end stage of CP, Langerhans islets are also involved, with impairment or loss of endocrine function and diabetes type 3c occurs (84). At any stages of CP, pancreas cancer can occur (85;86).

Alcohol consumption is the most frequent cause of CP; however, the pathophysiological mechanism is still not clear. Not all patients with high intake of alcohol get CP, but also patients' consuming relatively low quantities of alcohol are

in risk for development of CP. Most studies based on epidemiology classify alcohol as risk factor rather than a pathophysiological entity(87). Possibly alcohol and its metabolic products sensitize the pancreas for necroinflammatory processes (88). Other co-factors may lead to the deleterious process called acute or CP. Newer studies postulate minor CFTR activity, and direct toxic effects to acinar and stellate cells, partly due to augmented mucosal permeability in the small bowels(89).

Smoking is also an important risk factor often linked to alcohol; epidemiological studies in the last years have confirmed its importance (90-92). Nicotine and nicotine derived nitrosamine ketone trigger acinar cells to zymogen output involving CCK and preganglionic nicotine receptors(93). Nitric oxide may be the cause of microvascular changes, and nicotine may impair CFTR function (94).

Gallstones can block the main pancreatic duct if they are located proximally the papilla of Vater(95). In unrecognized coincidental other risk factors, they can lead to CP. Prompt endoscopic or surgical radical treatment in acute gallstone related pancreatitis is the key to prevent mortality in acute pancreatitis, recurrent acute pancreatitis, and consequently chronic pancreatitis(96).

Anatomical variants as annular pancreas or pancreas divisum can lead to pancreatitis. Mostly they are asymptomatic (97). Only in case of obstruction, these anatomic variants trigger inflammation processes (98;99). Coincidental efferent obstruction and genetic mutations of PRSS1, SPINK or CTRC can induce activation of zymogens (97).

Genetic mutations have drawn attention in the last years (100;101). They are the key to intrapancreatic activation of serine proteases especially trypsin which activates most other pancreatic enzymes(102). If once activated, auto digestion process is ongoing. Referral of young patients with idiopathic pancreatitis to genetic assessment should be mandatory. Consequent advice to avoid known risk factors may prevent repeated attacks of pancreatitis with impaired exocrine function over time and risk for development of pancreatic cancer could be minimized in a patient group with augmented frequency of pancreatic malignancy(103). Combination of diabetes with

exocrine pancreatic insufficiency is suspicious for CEL-MODY if several members of a family over generations are involved (60). Mutations of CFTR gene can lead to cystic fibrosis with pancreatic phenotype. Sticky, mucinous pancreas juice reaches duodenum marginally, auto digestion with mild progressive pancreatitis, loss of function and fibroses in young age are the consequences (104;105).

In tropical pancreatitis, rapid calcifying pancreatitis with intraductal stones and diabetes, beginning in young age are the main characteristics. In traditional theories, malnutrition and overconsume of cassava are the causal factors. Recently, SPINK1 mutations were found in up to 64% in patients with tropical pancreatitis, 50% homozygote and 14 % heterozygote(106). CFTR mutations alone or in combination may also occur. Thus, some authors point out that malnutrition is not cause but a consequence of tropical CP (107).

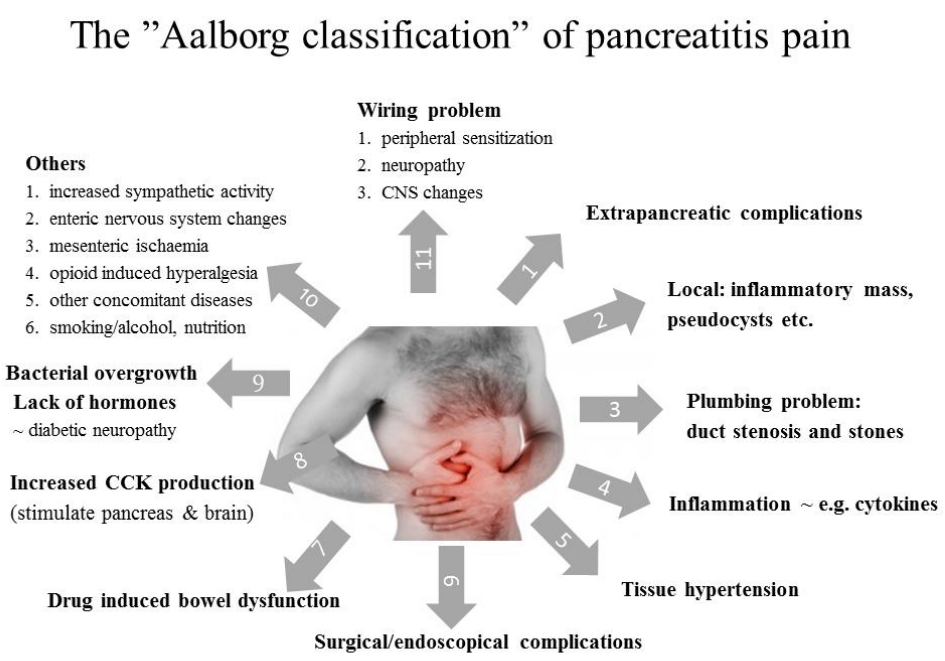
Hypertriglyceridemia alone or in combination with other risk factors can trigger pancreatitis. Hypertriglyceridemia often reflects the fact that pancreatitis is a multifactorial disease with different risk factors at the same time. Alcohol and biliary obstruction are the most important examples(108). As single factor, triglycerides have to be as high as over 20 mmol/L to induce pancreatitis(109). Still, hypertriglyceridemia type IV with high triglyceride levels causes up to 20% pancreatitis in some materials. Chylomicrons my cause microvascular ischemia. Combination of long standing hypertriglyceridemia with mutations are not rare, lipoprotein lipase deficiency is an example(110).

Hypercalcemia often due to hyperparathyroidism activates zymogens intrapancreatic, inducing an inflammatory cascade(111). Calcium plays an important role in pancreatitis, as calcifications are a diagnostic hallmark of CP. In principle, calcifications are the result of saponification with fatty acids and precipitate as salts(112). Older studies describe unbalance between Calcium and bicarbonate leading to precipitation of Calcium carbonate stones(113). A human stone protein plays also a role in the pathogenesis of pancreatic stones(114).

Autoimmune pancreatitis is an own entity first described by Yoshida in 1995(115). Emerging knowledge has classified two types; an IgG4 mediated autoimmune disease with obstructive jaundice and the idiopathic duct centric pancreatitis mimicking AIP, partly associated with inflammatory bowel disease(116). Confounding AIP with pancreatic cancer is a serious obstacle. Inadequate action with irreversible mutilations as a Whipple`s operation instead of immunosuppressive treatment are not rare.

Pain in chronic pancreatitis

Severe chronic abdominal pain is a serious and the dominant complaint in CP patients, regardless to aetiology. Insufficient nutrition, work disability, drug addiction and lack of participation in social life is the consequence (117).



With permission from Olesen, Søren Schou. Tieftrunk, Elke. Ceyhan, Güralp O. Drewes, Asbjørn Mohr. (2015). Pathogenesis and Treatment of Pain in Chronic pancreatitis. Pancreapedia

The decades-long assumption that the causes of pain in CP are efferent factors like ductal compression or obstruction is fading. Chronic pain in pancreatitis is more

complex than assumed before. Neuropathic pain seems to play a key role in CP. Chronic inflammation and fibrosis cause hypertrophy and increased density of pancreatic nerves (118). Growing evidence explains the pathophysiological part of chronic pain by sensitization of afferent nociceptive fibres. Hyperalgesia related to food intake is seen as an inadequate response of the spinal cord and pain brain centres. Somatic and visceral impulses from other organs, peripheral muscles or skin may be misinterpreted as pain signals due to excitation of afferent nerves in the spinal cord altering the sensory processing in the brain. In other words, spontaneous axon firing leads to impulsive firing of the dorsal horns neurons. The result is stimulus independent pain i.e. firing of the c-fibre even though it is not stimulated. Over time, the spinal cord becomes independent from signals with origin in the pancreas leading to cortical reorganization. This may explain chronic opioid dependent pain after total pancreatectomy(119-124).

Despite better understanding of pathophysiology of pain, medical therapy remains a unsolved challenge(125).

Classification of chronic pancreatitis

Most classification systems are based on etiologic, morphologic, functional and clinical information(126).

The *Marseille classification* from 1963 consists of clinical information and histology. It differentiates between four types of pancreatitis: Acute pancreatitis, acute relapsing pancreatitis, chronic relapsing pancreatitis and asymptomatic CP. Acute pancreatitis was seen as a severe disease which could been cured meanwhile CP was seen as a progressive process which could not be stopped(127;128).

In 1983, the *Cambridge-classification* pointed out morphological features as imaging studies gave crucial new insights. It is an ERCP based duct focused grading, which was later adapted by CT and MR(CP) with the advantage that also organ size and parenchymal features could be included(129).

Interestingly, in 1983, *Lankisch et al*. recognized the importance of pancreatic exocrine insufficiency and proposed a three step loss of pancreatic function with direct pancreas function testing and faecal fat output: pancreatic enzymes in duodenal juice were indicative for all grades of exocrine pancreatic insufficiency (EPI); meanwhile bicarbonate output was impaired in moderate and severe, and faecal fat only in severe PEI(130).

The *TIGAR-O classification* (2001) is describing main etiologic factors of CP: Toxic-metabolic, idiopathic, genetic, autoimmune, recurrent acute pancreatitis and obstructive causes. This classification is of importance because of it emphasizes that CP is a multifactorial disease with sometimes more than one triggering factor(131).

In 1994, *Layer* described a clinical scoring system to diagnose CP, later called MAYO diagnostic scoring system for CP. It also has three parts: a) Morphology: pancreatic calcifications, histology, pancreatic duct abnormalities after the Cambridge classification; b) Exocrine pancreatic function: steatorrhea or lipase output, diabetes (type 3c); c) classical clinic appearance: upper abdominal pain or weight loss over 10 kg in 12 months (74;132). The scoring system of the Japanese pancreas society is similar to this score and separates definite from probable CP(133).

The *M-ANNHEIM classification* system published in 2007 is a multimodal synopsis of former classification systems(134).
Module 1 describes etiological factors analogue to the TIGAR-O classification. It emphasises the importance of multiple risk factors (M Pancreatitis). Then the most frequent causes of CP in Europe are listed: Alcohol (A) graded in three quantity categories and smoking (N) expressed by pack years. Nutritional (N), hereditary (H), Efferent duct (E), immunological (I), and miscellaneous factors (M):

The M-ANNHEIM multiple risk factor classification of chronic pancreatitis

M Pancreatitis with **M**ultiple risk factors

A	**A**lcohol consumption Excessive consumption (>80 g/day) Increased consumption (20–80 g/day)

	Moderate consumption (<20 g/day)
N	**N**icotine consumption
	(In cigarette smokers: description of nicotine consumption by pack-years)
N	**N**utritional factors
	Nutrition (e.g., high caloric proportion of fat and protein)
	Hyperlipidaemia
H	**H**ereditary factors
	Hereditary pancreatitis (defined according to Whitcomb(135))
	Familial pancreatitis (defined according to Whitcomb(135))
	Early-onset idiopathic pancreatitis
	Late-onset idiopathic pancreatitis
	Tropical pancreatitis
	(possible mutations in the *PRSS1*, *CFTR*, or *SPINK1* genes)
E	**E**fferent duct factors
	Pancreas divisum
	Annular pancreas and other congenital abnormalities of the pancreas
	Pancreatic duct obstruction (e.g., tumors)
	Posttraumatic pancreatic duct scars
	Sphincter of Oddi dysfunction
I	**I**mmunological Factors
	Autoimmune pancreatitis
	Sjögren syndrome-associated chronic pancreatitis
	Inflammatory bowel disease-associated chronic pancreatitis
	Chronic pancreatitis with autoimmune diseases
	(e.g., primary sclerosing cholangitis, primary biliary cirrhosis)
M	**M**iscellaneous and rare metabolic factors
	Hypercalcemia and hyperparathyroidism
	Chronic renal failure
	Drugs
	Toxins

Adopted from A. Schneider et al.: M-ANNHEIM classification

Another module describes clinical staging in four steps, with subdivision in up to three categories: zero (asymptomatic), I symptomatic, without EPI, II symptomatic and partial EPI, III symptomatic with pain and complete EPI, IV symptomatic without pain, but total EPI:

M-ANNHEIM clinical staging of chronic pancreatitis

Asymptomatic chronic pancreatitis

0 Stage of subclinical chronic pancreatitis

a Period without symptoms (determination by chance, e.g., autopsy)
b Acute pancreatitis -single episode (possible onset of chronic pancreatitis)

c Acute pancreatitis with severe complications

Symptomatic chronic pancreatitis

I Stage without pancreatic insufficiency

a (Recurrent) acute pancreatitis (no pain between episodes of acute pancreatitis) *
b Recurrent or chronic abdominal pain (including pain between episodes of acute pancreatitis)
c I a/b with severe complications

II Stage of partial pancreatic insufficiency

a Isolated exocrine (or endocrine) pancreatic insufficiency (without pain)
b Isolated exocrine (or endocrine) pancreatic insufficiency (with pain)
c II a/b with severe complications

III Stage of painful complete pancreatic insufficiency

a Exocrine and endocrine insufficiency (with pain, e.g., requiring pain medication)
b III a with severe complications

IV Stage of secondary painless disease (burnout)

a Exocrine and endocrine insufficiency without pain and without severe complications
b Exocrine and endocrine insufficiency without pain and with severe complications

Adopted from A. Schneider et al.: M-ANNHEIM classification

Module 3 defines the diagnostic criteria of CP. Classical clinical features are required. According to the Zurich workshop, it classifies into definite and probable CP(136). The M-ANNHEIM classification system adds a borderline category. It includes patients with classical symptoms but no morphological and functional features. Additionally, it emphasises the importance of alcohol consumption. Classical histology, calcifications, persistent EPI or moderate marked duct changes after the Cambridge classification specify definite CP. Mild duct alterations, pseudocysts, endocrine insufficiency, or pathological EPI test define probable CP.

M-ANNHEIM diagnostic criteria of chronic pancreatitis

The diagnosis of chronic pancreatitis requires a typical clinical history of chronic pancreatitis (such as recurrent pancreatitis or abdominal pain, except for primary painless pancreatitis).

Definite chronic pancreatitis is established by one or more of the following additional criteria:
1. Pancreatic calcifications
2. Moderate or marked ductal lesions (according to the Cambridge classification)
3. Marked and persistent exocrine insufficiency defined as pancreatic steatorrhea markedly reduced by enzyme supplementation

4. Typical histology of an adequate histological specimen

Probable chronic pancreatitis is established by one or more of the following additional criteria:
1. Mild ductal alterations (according to the Cambridge classification)
2. Recurrent or persistent pseudocysts
3. Pathological test of pancreatic exocrine function (such as faecal elastase-1 test, secretin test, secretin-pancreozymin test)
4. Endocrine insufficiency (i.e., abnormal glucose tolerance test)

Borderline chronic pancreatitis is already established and is defined by a typical clinical history of the disease but without any of the additional criteria required for definite or probable CP. This form is also established as a first episode of acute pancreatitis with or without a family history of pancreatic disease (i.e., other family members with acute pancreatitis or pancreatic cancer) or the presence of M-ANNHEIM risk factors.

Pancreatitis associated with alcohol consumption requires in addition to the above-mentioned criteria for definite, probable, or borderline chronic pancreatitis one of the following features:
1. History of *excessive* alcohol intake (>80 g/day for some years in men, smaller amounts in women) or
2. History of *increased* alcohol intake (20–80 g/day for some years) or
3. History of *moderate* alcohol intake (<20 g/day for some years)

Adopted from A. Schneider et al.: M-ANNHEIM classification

The imaging module complies the Cambridge classification and integrates transabdominal and endoscopic ultrasound, CT, MR and MRCP:

M-ANNHEIM pancreatic imaging criteria for US, CT, MRI/MRCP, and EUS based on imaging features as defined by the Cambridge classification

Cambridge grading	CT, US, MRI/MRCP	EUS
Normal	Quality study depicting whole gland without abnormal features	
Equivocal	One abnormal feature	
Mild changes	Two or more abnormal features, but normal main pancreatic duct	Four or fewer abnormal features (no differentiation between equivocal and mild)
Moderate changes	Two or more abnormal features, including minor main pancreatic duct abnormalities (either enlargement between 2 and 4 mm or increased echogenicity of the duct wall)	Five or more abnormal features (no differentiation between moderate and marked)

Marked changes	As above with one or more of the required features of marked changes (4 points)

Adopted from A. Schneider et al.: M-ANNHEIM classification

Another module deals with grading of clinical features as pain, pain control, surgical interventions, exocrine and endocrine insufficiency, morphological status on pancreatic imaging and severity of organ complications:

M-ANNHEIM scoring system for the grading of clinical features of chronic pancreatitis

Clinical features		Points
Patient report of pain		
No pain without therapy	Patient reports requiring no pain medication	0
Recurrent acute pancreatitis	Recurrent acute pancreatitis (patient reports freedom from pain between attacks of acute pancreatitis)	1
No pain with therapy	No pain with therapy (patient reports freedom from pain with pain medication or endoscopic intervention)	2
Intermittent pain	Intermittent pain (patient reports intermittent pain-free episodes, either with or without therapy; possibly additional attacks of acute pancreatitis)	3
Continuous pain	Continuous pain (patient reports absence of pain-free episodes, either with or without therapy; possibly additional attacks of acute pancreatitis)	4
Pain control		
No medication		0
Use of no opioid drugs or use of mild opioids (WHO step 1 or 2)		1
Use of potent opioids (WHO step 3) or endoscopic intervention		2
Surgical intervention		
Pancreatic surgical intervention for any reason		1
Exocrine insufficiency		
Absence of exocrine insufficiency		0
Presence of mild, moderate, or unproven exocrine insufficiency not requiring enzyme supplementation (including patient reports of intermittent diarrhoea)		1
Presence of proven exocrine insufficiency (according to exocrine function tests) or presence of marked exocrine insufficiency defined as steatorrhea (>7 g fat/24 h), normalized or markedly reduced by enzyme supplementation		2
Endocrine insufficiency		
Absence of diabetes mellitus		0
Presence of diabetes mellitus		1

Morphologic status on pancreatic imaging (according to the Cambridge classification)	
Normal	0
Equivocal	1
Mild	2
Moderate	3
Marked	4
Severe organ complications (not included in the Cambridge classification)	
Absence of complications	0
Presence of possibly reversible complications	1
Presence of irreversible complications	2

Adopted from A. Schneider et al.: M-ANNHEIM classification

The sum of points of these modules create a severity index in five categories.

M-ANNHEIM severity index of chronic pancreatitis

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

Adopted from A. Schneider et al.: M-ANNHEIM classification

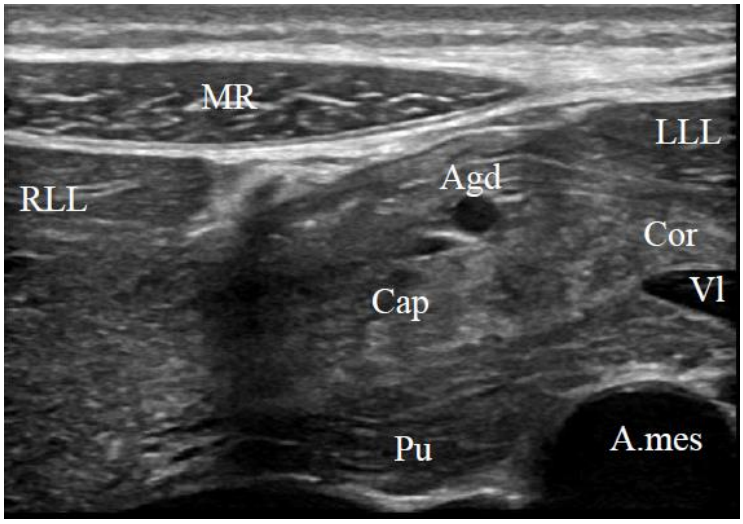
Imaging in chronic pancreatitis

Traditionally imaging describes morphological changes, but as early as in the seventies of the last century ultrasound of the pancreas after secretin stimulation crossed the border to functional imaging.

Until upcoming alternative methods, x-ray without intraluminal contrast agents or invasive techniques gave only vague information(137). Only calcifications in CP gave substantial information.

Development of B-mode sonography paved the way for transabdominal ultrasonography. It showed its potential in pancreas imaging early in the seventies of the last century(138). Compared to endoscopic retrograde pancreaticography ultrasonography was equal in ductal pathology, additionally it could visualize parenchymal findings and was considerably safer and easy to repeat(139). In the last

decades, substantial technical development in ultrasonography enhanced the ultrasound imaging quality; in given conditions even above other imaging methods(140). Furthermore, ultrasonography used for estimation of the pancreatic exocrine function after secretin stimulation enriches clinical routine by adding a functional parameter to the morphological features (141;142). Specificity and sensitivity of the method is excellent under good ultrasound conditions(143). Transabdominal ultrasound is a non-invasive, harmless, widely available, inexpensive diagnostic modality. In our opinion, it should be the first- line method for patients submitted for both new diagnosis and follow-up of diseases of the pancreas.



Head of pancreas with processus uncinatus scanned with a 15 MHZ transducer. Details shown with high resolution. MR: Musculus rectus abdominis; RLL: Right liver lobe; LLL: Left liver lobe; Cap: Caput pancreatis; Cor: Corpus pancreatis; Pu: Processus uncinatus; A. mes: Arteria mesenterica superior; Vl: Vena lienalis; Agd: Arteria gastroduodenale
From Erchinger, Transabdominal ultrasonography of the pancreas: basic and new aspects

In 1972 and in 1975, after the invention of the side view duodenoscope, Cotton and Koch published their work about ERCP, a method, revolutionizing pancreas duct diagnostics and therapy (144;145). With the Cambridge classification, it classified morphologically CP as early as in 1973(129). Because of its invasive nature, complication rate and operator dependent success, non-invasive techniques, mainly MRCP, but also s-MRCP, CT and EUS have, with modifications, replaced diagnostic

ERCP (146-148). Today, the significance of ERCP is based on its therapeutic potential(149).

CT of the pancreas was established in the 70^{ths} of the last century(150;151). It was cost intensive and artefacts made it comparable to transabdominal ultrasound(152). Recent technical development, including the introduction of contrast agents, thus obtaining an excellent imaging quality in the majority of the subjects, operator independence and availability have made CT one of the most used imaging methods(153). Detection of calcifications strengthen this method in CP diagnosis(154). However, micro calcifications can mislead to the diagnosis of CP as they can represent arteriosclerosis in intrapancreatic vessels(155). Visualization of pancreatic ducts is not optimal, but CT pancreas is in this sentence also adapted into the Cambridge classification(146).

From the beginning of MRI imaging of the pancreas until today, continuous work with elimination of artefacts and development of contrast agents have made it to an extraordinary method(156). It shows small details, can discriminate from parenchymal and ductal malignancy, and can characterize cysts. Only in detection of calcifications it is not suitable(157). High cost and thus low availability have limited the method in some degree.

Today, MRCP has in countries with broad access replaced ERCP in diagnoses of pancreatic duct abnormalities and can easily be combined with MRI of the pancreas(158). It can classify pancreatitis adapted to the Cambridge criteria and can find intraductal processes(159). One of the important advantages of MRCP is that there is no need for contrast agents. MR contrast agents are not without risk in patients with renal insufficiency and can lead to fatal complications as nephrogenic systemic sclerosis(160).

Secretin stimulated MRCP (sMRCP) augments the diagnostic value of MRCP additionally. By measuring the volume output of pancreatic juice in the small bowels, this method also represents a pancreatic function test (161). However, it cannot replace direct pancreas function testing completely as it gives only information about

volume of juice in the small bowels, but not its content (bicarbonate concentration, enzymes and electrolytes)(162).

Endoscopic ultrasonography of the pancreas has had an tremendous technological development since it was established in the early 80^{ths} of the last century(163). Milestones are the invention of radial and linear scanning, implementation of Doppler and duplex technique, elastography and use of contrast agents(164). With these tools, the method is superior to all other procedures in diagnosing microstructures (165;166). In addition, it allows invasive diagnostic (fine-needle-aspiration) and therapy (trans- gastral drainage of cysts and necrosectomy in acute pancreatitis).(167-170). The Rosemont classification has broadened the repertoire of imaging in the diagnosis of CP remarkably(171). It gives more information than the Cambridge classification as the latter bases only on pathological features of the pancreatic ducts; I contrast, the Rosemont classification visualises morphological details unique. However, the method is invasive and labour intensive. Especially, operator dependence makes inter observer agreement to a challenge(172).

In conclusion, a variety of methods is used in pancreatic imaging. Availability of diagnostic tools and operators experience in different clinical units make it difficult to recommend the ideal diagnostic approach. In general, it is wise to begin with an imaging method, which is not cost intensive, but gives a good overview. In many cases, transabdominal ultrasound can give this information. If not, CT is the next step as it is broadly available. If more detailed diagnostic is necessary or if the first imaging methods do not solve clinical challenges, MR/MRCP/S-MRCP or EUS are appropriate.

Pancreas function testing

Diagnosis of EPI is important as its consequences have severe clinical impact and may shorten live expectancy (173;174). Consecutive, malabsorption results in inadequate uptake of calories, vitamins and micronutrients (175). The most common cause is CP(176). Pancreatic cancer, pancreatic surgery, diabetes or inborn errors as cystic fibrosis, Schwachman-Diamond syndrome can also lead to EPI (177). Enteral

malabsorption in celiac disease, diabetes mellitus, Crohn's disease, after gastric surgery, short bowel syndrome, and Zollinger–Ellison syndrome can mimic EPI i.e. secondary EPI (178).

Direct invasive pancreas function testing

Direct pancreas function testing is the historical gold standard but its use in clinical routine is rare(179). Thus, most of our understanding of pancreatic physiology bases on studies performed in the last centuries (180-182).

The principle of these tests is stimulation of the pancreas by hormones, pharmaceuticals or meals. After stimulation of pancreatic secretion and sampling of duodenal juice, analyses of bicarbonate, electrolytes and enzymes is possible. The tests are invasive, formerly performed with a double lumen tube placed by x-ray control. The distal end in the duodenum, the other end in the stomach. These tests are time-consuming, work intensive and uncomfortable for the patients, but they are able to provide information about volume of duodenal juice in the measured time period and concentration of its ingredients(183).

The test, closest to physiological conditions, seems to be the Lundh test as it bases on a standardized test meal(184).

Stimulation with secretin and cholecystokinin or its analogue cerulein test both ductal and acinar pancreatic function. This may be the reason that all variants of secretin-CCK tests are been referred as "gold standard" of direct pancreas function testing. Compared to the Lundh test pollution of duodenal juice by a test meal is not expected (185;186). However, CCK or cerulein, probably due to induction of pancreatitis, can have more, partly serious side effects compared to secretin (187;188). CCK induced gallbladder contraction can especially in patients with gallstones provoke biliary colic(189). Nausea, vomiting and abdominal pain after injection of ceruletide are described(190).

Stimulation with only secretin combined with an upper endoscopy is coming up the last years(191). Most articles focus on easy feasibility, performed as a short

endoscopic secretin test (192-194). The intention is to shorten the test i.e. make the test more “patient friendly” and reduce the whole workload around the test. As it stimulates bicarbonate secretion, it – in theory - only measures the ductal function and importance for information about acinar function is not yet clear in practice. Some authors discuss the potential of diagnosing acinar function with only secretin stimulation(195). The role of this short endoscopic test in relation to the classical Dreiling tube test and EUS is still under continuous discussion. Especially, when performing the short endoscopic secretin test, the cut off of bicarbonate concentration in duodenal juice is a topic of interest(196). However, in our mind, there is no doubt that the old gold standard test, which requires 2-3 hours sampling time, is not feasible any more.

Bombesin, a peptide from frog skin, stimulates pancreatic acinar function, but also detachment of gastrin and acetylcholine. Combination with administration of Secretin is possible(197).

Indirect non-invasive pancreas function tests

In the NBT-PABA (*N*-benzoyl-L-tyrosyl-p-amino benzoic acid of bentiromide) test, pancreatic chymotrypsin breaks N-PABA down to N benzoyl l-Tyrosine and PABA. After intestinal absorption three metabolites are excreted in the urine one of them is additionally glucuronated in the liver(198). This test is not in clinical use any more and is not valuable in renal insufficiency(199). The serum PABA test may be easier to handle and is possibly more accurate(199).

The Pancreolauryltest bases on metabolism of Fluorescin dilaurate by pancreatic specific esterase; the product is absorbed in the small intestine, partly glucuronated an excreted in the urine after a sampling period of 10 hours. It is suitable for moderate to severe EPI (199;200). In addition, this test is not in clinical use any more. As serum test it may be a good alternative in diagnosing mild to moderate EPI and helpful in following EPI in patients with CP(201).

^{13}C mixed triglyceride breath tests can also diagnose pancreatic insufficiency and are in use in the follow-up of patients with diseases leading to progressive EPI, mostly CP. Also patient compliance regarding enzyme supplement treatment can be checked(202). This is important to rule out other causes of malnutrition and weight gain as e.g. cancer. However it is important to take the following pitfalls of the test into consideration: physical activity, gastric emptying velocity, lipolytic capacity in liver diseases(195). Other disadvantages are long test duration and lack of standardization and cost of agents. Additionally, there is need for specialized personal and equipment. Therefore, tertiary centres stand for its performance(203).

Faecal elastase 1 (FE1) is - performed as a spot sample – the most used test to diagnose exocrine pancreatic insufficiency in clinical practice. It is a stable molecule as there is no cleavage on the way through the bowels. The patients do not have to interrupt enzyme substitution, as the pharmaceuticals are the product of porcine pancreas and do not interfere with the human elastase test (204). It is of importance to advice patients not to collect samples in periods with watery stools. Dilution gives false positive results and leads to unnecessary, partly invasive and cost intensive diagnostic. Unfortunately, this test can only rule out moderate to severe EPI and is not eligible to diagnose early, mild EPI. Sensitivity in detecting mild, moderate and severe EPI was described to be superior to the PABA, Pancreolauryl test and faecal Chymotrypsin test(205). The most used test in clinical routine is the monoclonal FE11 test. Testing all Elastase isomers with polyclonal analyses has not yet been accepted, possibly because of additional information is marginal and not of importance.

Chymotrypsin in stool is a good alternative to FE1as it can be used to test patients' compliance to enzyme supplementation(206). However, it is not any more in clinical use as FE11 test is dominating clinical routine(207).

Most authors and textbooks describe faecal fat quantification as the gold standard in the diagnoses of steatorrhea, the leading symptom of severe EPI in end stage CP(178;208-210). Steatorrhea occurs before protein malabsorption and carbohydrate

absorption(211). However, for clinical routine, this test is not in use in most countries as compliance to diet containing 100 g fat/day, stool sampling and storing over three days is questionable and homogenization of faeces is inconvenient, analyses after the protocol of van de Kamer is cumbersome and chemicals are toxic thus requiring safety protocols(212). Inconvenience of the test can be reduced. In a small series of patients we demonstrated that minimizing of chemicals to the tenth of the original protocol is possible and standardized diet and coefficient of fat absorption are not necessary(213). Additionally, we were also able to show that healthy controls pass the cut off 7g fat/day in the stool up to 9 g fat/day(214). Steatorrhea occurs not only in pancreatic malabsorption, but also in infectious diarrhoea, celiac disease or inflammatory bowel syndrome as secondary intestinal malabsorption. Some authors grade steatorrhea in normal fat output to 7 g/d, steatorrhea of various reasons with fat output between 7 and 14 g/d and pancreatic steatorrhea with faecal fat output >14g/d(209;215). In conclusion, faecal fat is a cumbersome method in reserve for special cases with voluminous stools of unknown origin where other diagnostic tools could not lead to diagnoses.

Acid steatocrit was seen to be more practical than faecal fat quantification, but its use is limited to few centres as FE1is superior in the evaluation of pancreatic function(216).

2. Aims of this work

Article I

Short endoscopic Secretin test

We investigated if our short variant of the endoscopic pancreas function test, as a part of routine upper endoscopy, could improve clinical diagnostics of CP when using Layer (Mayo) score.

We tested the following study hypothesis: our short endoscopic secretin test has acceptable diagnostic accuracy for the diagnosis of CP.

Article II

Automation of bicarbonate measurement

We aimed to evaluate an automated spectrophotometric method in samples spanning the effective range of bicarbonate concentrations in duodenal juice.

Study hypotheses:
A) Accuracy of an automated spectrophotometric method is equal to back titration.
B) Freezing of samples before analyses would not affect its results.

Article 3

Automation of Amylase measurement in duodenal juice

We aimed to compare an automated kinetic spectrophotometric method for pancreatic amylase measurement in duodenal juice with a standardized colorimetric end-point assay.

Study hypothesis:

The two methods have good correlation.

Article 4

Automation of Lipase measurement

We sought to develop a feasible and time-effective method for measuring lipase activity in duodenal juice, using a commercial kit and standardized automated method widely available. Secondly, we wanted to evaluate this method by comparing the results from a microplate fluorometric, kinetic assay for lipase activity. Additionally, we wanted to find chemical supplements, which stabilize Lipase activity over a time for sufficient analyses. These reagents should not influence the measurements.

Study hypotheses:
A) The two methods have good correlation.
B) MOPS buffer and Bovine serum albumin give stable results over time.

The combined aim of the project was to integrate direct pancreas function testing in a multimodal diagnostic algorithm in the diagnosis of CP.

This multimodal approach generated many other works, not included in this thesis. We evaluated pancreatic enzymes, faecal fat and FE1 (193;213;214;217;218). Our research group also compared and validated pancreatic imaging with MRI techniques, transabdominal and endoscopic ultrasonography (141;217;219;220). Perfusion studies of the pancreas in patients with cystic fibrosis and CP should also be mentioned (221;222). Pancreas function testing and imaging studies of patients with different kinds of diabetes type III were performed (193;218). Genetic studies revealed innovative knowledge (59;223;224). Data from our local CP database were integrated in a Nordic Baltic database, to characterize epidemiology, diagnostic, treatment and follow-up in the different Nordic countries (225).

We limited this thesis to establish direct pancreas function testing as a diagnostic tool in clinical routine. By simplifying the method as much as possible, we intended to implement it in a routine gastroscopy without exceeding the time schedule in a busy endoscopic unit. Standardized handling and automatizing the process of analysing duodenal juice markers opens the possibility to implement it in routine laboratory activity.

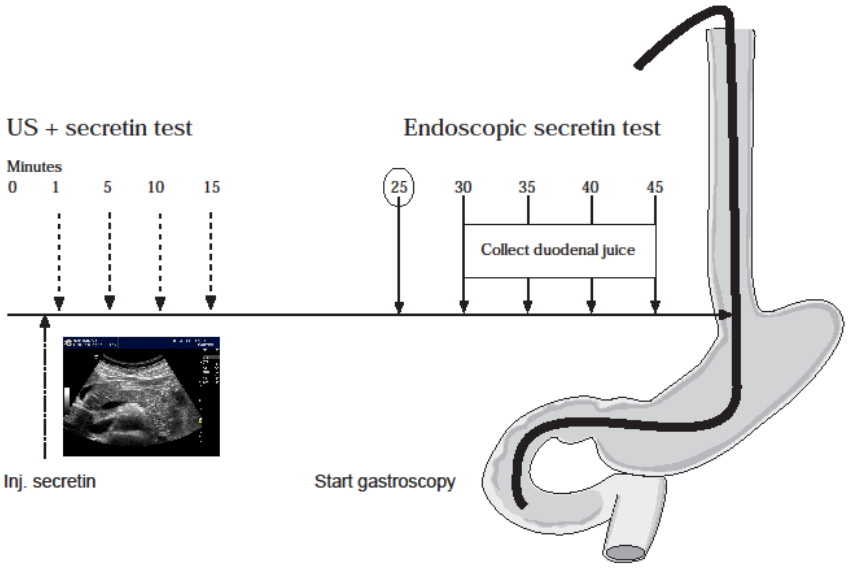
3. Methodological Aspects

Patients referred to the outpatient clinic with symptoms suspicious for CP were offered to participate. All subjects were included after an informed and signed written consent. The studies were approved by the regional ethical committee, REK vest. Approval numbers: 2011/590 and 2010/2857-7. The studies were performed according to the Helsinki declaration(226). Heathy controls were called attention to the project by board notes in our clinic.

To separate patients with CP from those without, we chose the Layer (Mayo) score, with ≥ 4 points as cut off. The following points were summed up: typical histology or pancreatic calcifications: 4 points, typical imaging characteristics: 3 points, pathological pancreas function test: 2 points, classical abdominal pain lasting at least 6 month or proven acute pancreatitis in patient's history: 2 points, Diabetes: 1 point(74).

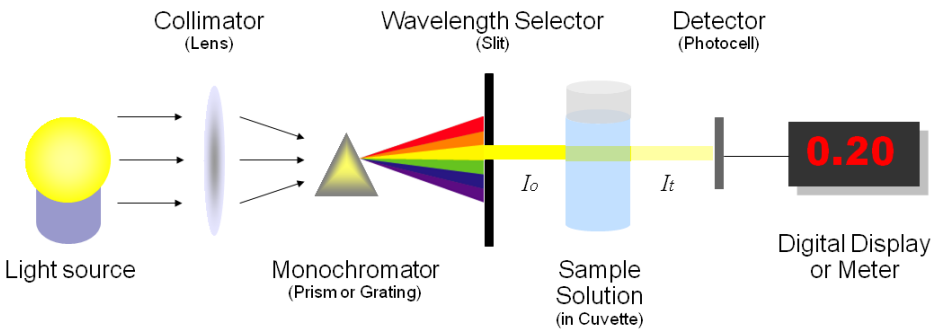
In the original score, FE1 and faecal fat were used for the evaluation of exocrine pancreatic function. We used FE1 and bicarbonate concentration in duodenal juice from short EST and compared sensitivity, specificity, pos. and neg. predictive values for each of the tests. When scoring with bicarbonate concentration was different to scoring with FE1, we used the highest sum of points and called it peak score.

Our short endoscopic secretin test is displayed in the following figure:



Short endoscopic pancreas function test; the procedure in detail: 25 minutes after injection of secretin (1 CU/kg bodyweight, maximum), upper endoscopy was started. During the first 5 minutes, we performed diagnostic gastroduodenoscopy and evacuated all juice from stomach and duodenum. Thereafter, the tip of the endoscope was placed distal the papilla of Vater for 15 minutes; duodenal juice was sampled in three 5-minute portions. The whole procedure lasted 20 minutes.

Automation of bicarbonate, Amylase and Lipase measurement was performed with enzyme-based, spectrophotometric methods. The principle of spectrophotometry: White light is bundled up by a lens and send to a monochromator (prism) which splits the light in different wavelengths. Only light with a predefined wavelength can pass a filter. The filtered light then passes through a sample solution; a part of it is absorbed. The difference between “light out” (l1) and “light in” (lo) can be measured and is proportional to enzyme activity(227).



Basic structure of spectrophotometers (illustrated by Heesung Shim); in open source https://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry

The principle of enzyme based bicarbonate, amylase and lipase measurement with an autoanalyzer usually used in routine diagnostics is explained as follows:

In bicarbonate measurement, the enzyme added to the sample solution (duodenal juice) is phosphoenolpyruvate carboxylase; finally, NADH is losing a Hydrogen, which leads to absorption of filtered light with a predefined wavelength.

In amylase measurement, α-glukosidase is the enzyme used in chemical reactions leading finally to nitrophenol. Its absorption is direct proportional to Amylase activity.

In Lipase measurement, the final product is Methylresorufin; its colour intensity is direct proportional to Lipase activity. The chemical reaction needs colipase and cholates from the biliary system.

Calibration of the autoanalyzer is necessary and performed with a commercial available Multi-Kit with known bicarbonate concentration or enzyme activity.

The older standard measurement of bicarbonate concentration is back-titration(228). The principle is to measure pH before strong acid HCl is added; after evacuation of Carbondioxide by stirring leading to evaporation, a strong chemical base, NaOH, is added to titrate back to the pH measured before the procedure. By a formula, the

amount of evaporated bicarbonate is calculated. We analysed in parallels and always with a solution of known bicarbonate concentration (control).

The older enzyme-activity semi-automated measurement methods for amylase used in this work is finally also a spectrophotometric method. Lipase is measured by a fluorometric method. The preparation of the sample solutions is a complicated, manual multistep procedure. It is important to mention that these methods are always linked to a standard curve with blanks as baseline; a common approach is to measure in Triplets; a ≤ 10% coefficient of variance was obligatory, otherwise the result was not used because of its inaccuracy.

The semi-automated old measurement of amylase-activity is based on an analyses procedure used over decades, the Phadebas method(229). Starch with blue dye is cleaved exclusively by alpha amylase; its colour intensity is measured over a defined time and is proportional to Amylase activity.

The older lipase measurement needs colipase as additive and a fluorescent, linked to a chemical bond with glycerol, which is the central reagent. The increase of fluorescence activity is directly proportional to Lipase activity (230;231).

4. Main Results

4.1. Short endoscopic secretin test

We demonstrated that the short endoscopic Secretin test, implemented in the clinical Layer score, showed better sensitivity and specificity than FE1. We could diagnose patients with symptoms suspicious for CP more precisely. In cases with inconclusive basic diagnostic, the test was helpful to diagnose pancreatic insufficiency early. Thus, we strongly believe that this test, performed in a multimodal sense (Layer score) could be useful in diagnosing early and mild CP (67;232). Furthermore, we used the time between secretin injection and maximal bicarbonate output after 30 minutes to perform secretin stimulated ultrasonography of the pancreas and duodenum. That gave us instant knowledge of the pancreatic structure i.e. diagnostic imaging of the pancreas. Secondary, grade of early dilatation of the pancreatic duct followed by liquid filling of the duodenum gave an estimate of the pancreatic secretory function, before the endoscopy. Finally, the endoscopy provided us with pancreatic juice and the possibility to analyse the content (bicarbonate and enzymes). Low bicarbonate level and low enzyme concentration can lead to a proper diagnosis, even when clinical symptoms and imaging is inconclusive. We conclude that EST is rapid and easy to perform and incorporation in daily routines to diagnose CP is possible.

4.2. Bicarbonate in duodenal juice

We could show that automated measurement of bicarbonate in duodenal juice was equal to back titration in the range of interest. Strongest correlation was at the cut off (80 mmol/L). With automation, bicarbonate measurement is much easier to perform. Freezing in nitrogen and thawing did not influence the results.

4.3. Amylase in duodenal juice

Fluorometric, semi-automated analyses showed good correlation to automated measurement. Simplification of amylase measurement succeeded.

4.4. Lipase in duodenal juice

We could establish automated measurement of Lipase in duodenal juice and shorten time of analyses considerably. Duodenal juice samples diluted in MOPS buffer added BSA have excellent stability at room temperature within normal analysis time. Correlation to an older semi-automated measurement was good.

5. Discussion

5.1. Short endoscopic secretin test

Direct pancreas function testing is the gold standard in the diagnoses of exocrine pancreas insufficiency (179;180;233). The old standard tests with a test meal or direct hormonal stimulation with secretin and/or Cholecystokinin base on measurement of volume and concentration of different ingredients of pancreatic juice. They are time consuming (about 2 hours), cost intensive and inconvenient for the patient (179;234;235). The old methods of bicarbonate, enzyme and electrolyte measurement require laboratory staff with special competence and specific equipment. All factors described above make direct pancreas function testing too troublesome to establish in today's clinical routine.

Even if the short endoscopic secretin test is concentration and not volume based, it gives reliable results and is more precise than FE1, the only pancreas function test available for all, from the general practitioner to the high-specialized pancreatologist.

A generally accepted cut off 80 mmol/l bicarbonate concentration after secretin stimulation must be interpreted with caution when using the short endoscopic secretin test. Contamination with gastric juice, regurgitation of juice of the small bowels when vomiting, different secretion of mucosal glands, delayed or early plateau of bicarbonate concentration weaken the cut-off. We could minimize these pitfalls by using three aliquots of five minutes between 30 and 45 minutes after secretin injection. In the diagnoses of exocrine pancreatic function, it is essential to find values below the cut-off. Consecutively we chose the highest measurement result of the three aliquots ("peak-bicarbonate").

In proven pancreatic malabsorption, there is no indication for the endoscopic secretin test. In most cases, a multimodal approach gives enough information using the synopsis of clinical, laboratory, imaging parameters and FE1or more rarely faecal fat analyses. However, in doubt, the test is of high value, especially in early CP with possible exocrine pancreatic insufficiency(232). The test can also differentiate

between enteral and pancreatic malabsorption, as FE1is commonly false positive in watery diarrhoea and pathological results for faecal fat tell us about fatty malabsorption without its cause.

In addition, by collecting duodenal juice after Secretin Stimulation, analyses of not only bicarbonate and enzymes, but also electrolytes, salts and genetic material from the pancreatic tissue is possible(224;236). This may open for diagnosis of early EPI, selective diagnosis of impaired acinar or ductal cell function and patients in risk for fast accelerating pancreatitis with loss of pancreatic function and cancer.

The main advantage of the test is the ease in performance. One can implement it in a routine gastroscopy, abolishing the need for highly specialized operators or equipment. Compared to the old tests, the shortness of collection time minimizes volatility of bicarbonate and degradation of enzymes in the time from collection of duodenal juice to pre-analytical preparation of the samples. Additionally, as shorter the length of a procedure, as more easily it can be tolerated by the patient

However, there are several limitations:

CP is complex and an easy to use clinical score cannot reflect all the facets of aetiology, pathogenesis, and morphological, functional and clinical aspects. Furthermore, the definition of CP is under ongoing discussion (69). Especially around the cut off 4 points in the CP score after Layer, the diagnostic value remains a challenge.

The only way to overcome the “twilight zones” around the cut off 4 points in the Layer (Mayo) score is a structural follow-up of patients in the outpatient clinic and repetition of diagnostic procedures. The foundation of the Scandinavian-Baltic pancreas database in 2015 as a structured, multimodal tool, gives the possibility to harmonize diagnosis, follow-up and therapeutic options over the involved countries. A challenge of this proceeding in follow-up is to decide when invasive procedures like EST or EUS, a radiological method (CT) or a cost intensive method (MRI) are indicated again, as they cannot be repeated too often. Thus - the old clinical “gut

instinct" practiced over several millenniums in medical professions remains as the only solution with all its disadvantages and inaccuracies. In our clinic, we have incorporated transabdominal ultrasound in the structured follow-up of CP patients or patients with symptoms, strongly suspicious for CP, but negative score. In good conditions for performance of ultrasonography, this non-invasive, cost-effective and repeatable method can evaluate pancreatic morphology partly after the Rosemont criteria and help to overcome the obstacles described above to a certain degree (217).

The cut off 80 mmol/L bicarbonate concentration when performing the endoscopic secretin test is not absolute. This may give false results when practicing the clinical Layer score. A smart approach to avoid this pitfall may be adaption of the result to clinical indication. Our calculations showed a range of bicarbonate concentration between 60 and 90 mmol/L to be an intermediate zone. Stevens T. et al. discussed the same phenomenon and calculated the best sensitivity and specificity to be at bicarbonate concentration of 78 mmol/L(237). Kothari et al. highlight the same topic with a poorer concordance of secretin-stimulated endoscopy, EUS and the old Dreiling tube test(238). Even if the design of the latter study was suboptimal (e.g. only one sample for bicarbonate analyses, consecutively no use of peak bicarbonate), the importance of using a multimodal score is emphasized.

The number of patients and controls is low; thus, our results should be interpreted with caution and optimally confirmed by other study groups. However, although the sample size was relatively small in each individual study, the result was significant. Furthermore, our results do not deviate from others' results in different patient groups, confirming the usefulness of the short endoscopic test.

We also measured the volumes aspirated from EST. Mean volume was 2.6 mL with a range from 0 to 6 ml (not published). Our experience after performance of more than 350 EST indicates that volume after Secretin stimulated transabdominal ultrasound or MRI may agree with bicarbonate, enzyme concentration and FE1. In our group, there is ongoing work to correlate volume of collected duodenal juice to volume estimation in ultrasound and bicarbonate or enzyme concentration.

5.2. Bicarbonate in duodenal juice

Bicarbonate in duodenal juice reflects the ductal function of the exocrine pancreas. CP causes damage of ductal cells and their membrane, leading to impaired function of the cystic fibrosis transmembrane conductance regulator which minimizes the output of bicarbonate, water and electrolytes. A wide professional milieu accepts bicarbonate concentration in duodenal juice after secretin stimulation as a reliable parameter for pancreatic function (191;239). Back titration as the gold standard for bicarbonate measurement in duodenal remains juice still remains(234).

However, immediate analyses are of importance, as we know after the Henderson–Hasselbalch equation, bicarbonate acts as buffer and can also change to H_2O and CO_2. The latter is highly volatile and thus generates false positive results. Prompt analyses by automated measurement could overcome this problem. Handling of this technique is superior to back titration, which needs special equipment and specialized laboratory staff. Furthermore, the samples can be stored (snap freezing) for later analyses, without influencing the results.

Weakness of automated measuring is, compared to back titration, lower accuracy in very high and very low bicarbonate concentration. As an enzyme based method, it is dependent on the pH optimum of the enzymes in use. In bicarbonate analysis, the automated measurement is most precise in the range of interest between 60 and 90 mmol/L, including the cut-off 80 mmol/L. Thus – inaccuracy in extreme values is in clinical practice of minor importance.

5.3. Amylase in duodenal juice

Pancreas amylase activity can be compensated by other amylases. Its deficiency is thereby less important in a clinical setting. However, in direct pancreas function testing, it may give purer information about exocrine pancreatic insufficiency as it is not a proenzyme before it is an active enzyme in the duodenum. Additionally, it is stable. Thus, measuring decrease in activity of a pure enzyme without the existence of a preliminary state is possibly more precise than the Lipase enzyme with its short

activity period or the mixture of proteases and their zymogens. Previously used, older, manual multistep methods with boiling are difficult to repeat accurately, time-consuming and work intensive. Rapid automated measurement overcomes these obstacles.

5.4. Lipase in duodenal juice

An ongoing intense discussion considers pancreatic lipase as a marker in the diagnoses for early exocrine pancreatic insufficiency (240). We chose therefore to include pancreatic lipase in our diagnostic repertoire. It is common knowledge that lipase activity decreases fast without auxiliary supplies. Consecutively the nihilists in direct pancreas function testing disbelieve the postulate that pancreas lipase is an early marker for exocrine pancreatic insufficiency. They dispute that reason for early decrease of lipase activity is the result of its instability. Nevertheless, a fast analysis procedure is less vulnerable. Therefore, we established rapid analyses at our unit. As fatty malabsorption plays an important role in the diagnoses of exocrine pancreatic insufficiency, we think it is wise to have the possibility to measure the labile enzyme.

A serious limitation of automation of measurement of bicarbonate concentration and enzyme activity in duodenal juice is the fact that no commercially available spectrophotometric auto analyser has certification for analyses in duodenal juice. However, our approach to compare two methods is the gold standard used in laboratory chemistry, when certifying measurements of parameters in blood, plasma, serum and urine for certification of auto analysers. We argue, the same methodology is appropriate for evaluating duodenal juice.

Another weakness is the need for standard curves in the old methods for enzyme activity analyses. Technically speaking, when using the old methods, results are dependent on the actual standard solution with its standard curve. When storage time is over, production of a new standard solution with a new standard curve is necessary. Different standard curves can never be the same. The consequence is that comparison of the series using new standard solutions with the previous is not exactly possible. We think therefore that the use of automated measurement overcomes this serious

obstacle and taking that in account, our work, comparing the two methods was long-overdue.

6. Conclusions

We have shown that simplification of direct pancreas function testing is possible. Shortening the invasive procedure to a total of 20 min gastroscopy makes it less uncomfortable for patient. Additionally, it does not exceed consultation time to a large extent in a busy endoscopic unit, leading to balance between economic aspects and extraordinary information for the clinician. We demonstrate that bicarbonate concentration in duodenal juice after secretin stimulation is a more precise parameter for exocrine pancreatic function than FE1, an indirect pancreas function test. Consecutively, identification of patients with mild CP succeeds better by measuring bicarbonate than with FE1when using a multimodal score including clinical information, imaging and pancreas function testing, the Layer (Mayo) score. By replacing the manual, time consuming multistep analysing procedures with automatic measurement with the same excellent accuracy, abolishes the need for specialized laboratory personal and can be performed in any basic laboratory.

We have demonstrated that a three-step approach, with simplification of patient related procedure, pre-analytic preparation and automatized measurement of ingredients in duodenal juice, paves the way for implementation of direct pancreas function testing in clinical routine. The thought to use it at every hospital-based units with basic endoscopy and laboratory services is seductive. Most tertiary centres perform all studies on exocrine pancreatic function, CP and its aetiology. Therefore, generalizing of results may be a pitfall as non-tertiary centre units may underestimate exocrine pancreatic insufficiency and CP. Simplification of direct pancreas function tests suitable for primary and secondary hospitals could overcome this problem.

7. Future Perspectives

By optimizing the short endoscopic secretin test, we intend to unravel the secret of early exocrine pancreatic insufficiency. For this purpose, especially automation of lipase measurement is an important tool, as some authors regard the decrease in secretion of this enzyme earlier than decrease of amylase and proteases(241). A multimodal approach with analyses of all enzymes and other ingredients exploring genome, proteome and microbiome in duodenal juice could fill the gap.

The clinical milieu at our location can offer a broad spectre in the diagnoses of pancreatitis, its causes and consequences. Formalized collaboration of different specialties could give a synergistic effect in diagnosing, treatment and follow-up of patients:

1. Paediatric gastroenterologists and specialists in diabetes and genetics, diagnose patients with inborn errors, diabetes type III, and new genetic variants like MODY 5 and 8 and CEL-MODY diabetes beside mutations in PRSS1, SPINK, CTRC and CFTR.

2. Radiologists contribute substantially in the diagnostic evaluation of CP by performing morphologic and functional imaging with CT, MRI, MRCP and sMRCP.

3. Gastroenterologists offer transabdominal and endoscopic ultrasonography with therapeutic options and direct pancreas function testing. Structured long-time follow-up in the outpatient clinic is the guarantee to identify patients in risk for complications. Thus – early intervention is possible.

4. Broad collaboration with surgeons and especially early diagnosis in case of malignancy can lead to a curative treatment of pancreatic cancer. Additionally, early operative treatment and ERCP related interventions minimize irreversible disease.

Good clinical work-up is one of premises for good clinical science. With formalized a closer, interdisciplinary collaboration between the milieus as described above, clinical research could give essential impact.

We would like that different national or international centres could evaluate our short endoscopic secretin test. To our knowledge, a direct pancreas function test is not practiced in any other Scandinavian country. To date there is no common direct function test worldwide. Thus, our short endoscopic Secretin test may open for multicentre studies. Duodenal juice after Secretin stimulation could be analysed for more than the known markers for exocrine pancreatic insufficiency. Proteomic, genetics or cancer specific markers may be of interest. If stored in a biobank, analyses of to date not known markers can be possible in the future.

Faecal fat is not only interesting in a diagnostic setting, but also in the follow-up of patients, especially, when enzyme supplementation is not as effective as expected. An alternative test for quantification of faecal fat without collecting faeces and chemical analyses could make the procedure repeatable. MRI may be a possibility. Since Dixon found a method to differentiate fat and water, quantification of fat of all anatomic structures in the body got possible(242). Tremendous evolution of MRI techniques modified this method and today MR spectroscopy can differentiate between different types of fat (242;243).

Transabdominal ultrasonography is inexpensive, convenient for the patient and can be repeated many times without complications, compared to all other imaging modalities often with radiation.

As classification of CP in all its facets is only sufficient with a multimodal approach, we established a patient database. It enables structured and individualized follow-up with detection of CP complications in an early state. To date, we have included more than 120 patients with CP. Thus – in a few patients - diagnosis of malignant processes at an early state was possible and subsequently radical curative therapy led to survival. Furthermore, nutritional state, elimination of reversible risk factors and patient compliance improved in many patients. Our follow-up registration was integrated with a broader network, leading to the founding of the Scandinavian Baltic Database for CP, which is based on a multimodal diagnostic approach, the M-ANNHEIM score.

The Scandinavian Baltic database also opens for research in epidemiology. Furthermore, the database reveals different clinical approaches in the participating countries and may thus lead to common and improved standards in diagnosing and treating patients with CP.

Reference List

(1) Tsuchiya R, Fujisawa N. On the etymology of "pancreas". Int J Pancreatol 1997 Jun;21(3):269-72.

(2) Tando Y, Yanagimachi M, Matsuhashi Y, Nakamura T, Kamisawa T. A brief outline of the history of the pancreatic anatomy. Dig Surg 2010;27(2):84-6.

(3) Navarro S. [A brief history of the anatomy and physiology of a mysterious and hidden gland called the pancreas]. Gastroenterol Hepatol 2014 Nov;37(9):527-34.

(4) Ceranowicz P, Cieszkowski J, Warzecha Z, Kusnierz-Cabala B, Dembinski A. The Beginnings of Pancreatology as a Field of Experimental and Clinical Medicine. Biomed Res Int 2015;2015:128095.

(5) John Malone Howard, Walter Hess. History of the Pancreas: Mysteries of a Hidden Organ. Springer; 2002.

(6) Flati G, Andren-Sandberg A. Wirsung and Santorini: the men behind the ducts. Pancreatology 2002;2(1):4-11.

(7) Stern CD. A historical perspective on the discovery of the accessory duct of the pancreas, the ampulla 'of Vater' and pancreas divisum. Gut 1986 Feb;27(2):203-12.

(8) Pandol SJ. The Exocrine Pancreas. 2010. San Rafael (CA), Morgan & Claypool Life Sciences. Colloquium Series on Integrated Systems Physiology: From Molecule to Function to Disease. Ref Type: Serial (Book,Monograph)

(9) Agha FP, Williams KD. Pancreas divisum: incidence, detection, and clinical significance. Am J Gastroenterol 1987 Apr;82(4):315-20.

(10) Dale E.Bockman. Anatomy of the Pancreas. In: Vay Liang W.Go, editor. The Pancreas: Biology, Pathobiology and Disease. 2 ed. New York: Raven Press, Ltd; 1993. p. 1-8.

(11) Morisset J. Seventy years of pancreatic physiology: take a look back. Pancreas 2014 Nov;43(8):1172-84.

(12) Valenzuela JE, Weiner K, Saad C. Cholinergic stimulation of human pancreatic secretion. Dig Dis Sci 1986 Jun;31(6):615-9.

(13) Morisset J. Control of pancreatic secretion in humans. Adv Med Sci 2010;55(1):1-15.

(14) Rashmi Chandra, Rodger A.Liddle. Regulation of Pancreatic Secretion . Pancreapedia Exocrine Pancreas Knowledge base 2015(1). Online Source

(15) Whitcomb DC, Lowe ME. Human pancreatic digestive enzymes. Dig Dis Sci 2007 Jan;52(1):1-17.

(16) McMaster PD. Do species lacking a fall bladder possess its functional equivalent? J Exp Med 1922 Jan 31;35(2):127-40.

(17) Bayliss WM, Starling EH. The mechanism of pancreatic secretion. J Physiol 1902 Sep 12;28(5):325-53.

(18) Love JA, Yi E, Smith TG. Autonomic pathways regulating pancreatic exocrine secretion. Auton Neurosci 2007 Apr 30;133(1):19-34.

(19) Rodger A Liddle. Physiology of cholecystokinin. uptodate 2017Available from: URL: https://www.uptodate.com/contents/physiology-of-cholecystokinin

(20) Ji B, Bi Y, Simeone D, Mortensen RM, Logsdon CD. Human pancreatic acinar cells do not respond to cholecystokinin. Pharmacol Toxicol 2002 Dec;91(6):327-32.

(21) Ji B, Bi Y, Simeone D, Mortensen RM, Logsdon CD. Human pancreatic acinar cells lack functional responses to cholecystokinin and gastrin. Gastroenterology 2001 Dec;121(6):1380-90.

(22) Singer MV, Niebergall-Roth E. Secretion from acinar cells of the exocrine pancreas: role of enteropancreatic reflexes and cholecystokinin. Cell Biol Int 2009 Jan;33(1):1-9.

(23) Gardner JD, Jensen RT. Characterization of receptors for gastrointestinal peptides. Scand J Gastroenterol Suppl 1983;82:7-18.

(24) Trimble ER, Bruzzone R, Meehan CJ, Biden TJ. Rapid increases in inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate and cytosolic free Ca2+ in agonist-stimulated pancreatic acini of the rat. Effect of carbachol, caerulein and secretin. Biochem J 1987 Feb 15;242(1):289-92.

(25) Singer MV. Pancreatic secretory response to intestinal stimulants: a review. Scand J Gastroenterol Suppl 1987;139:1-13.

(26) Dembinski A, Konturek SJ, Thor P. Gastric and pancreatic responses to meals varying in pH. The Journal of Physiology 1974;243(1):115-28.

(27) Meyer JH, Way LW, Grossman MI. Pancreatic bicarbonate response to various acids in duodenum of the dog. Am J Physiol 1970 Oct;219(4):964-70.

(28) Chey WY, Chang TM. Secretin: historical perspective and current status. Pancreas 2014 Mar;43(2):162-82.

(29) Morisset J, Julien S, Laine J. Localization of cholecystokinin receptor subtypes in the endocine pancreas. J Histochem Cytochem 2003 Nov;51(11):1501-13.

(30) Anagnostides A, Chadwick VS, Selden AC, Maton PN. Sham feeding and pancreatic secretion. Evidence for direct vagal stimulation of enzyme output. Gastroenterology 1984 Jul;87(1):109-14.

(31) Michaelis L, Menten ML, Johnson KA, Goody RS. The original Michaelis constant: translation of the 1913 Michaelis-Menten paper. Biochemistry 2011 Oct 4;50(39):8264-9.

(32) Go VL, Hofmann AF, Summerskill WH. Pancreozymin bioassay in man based on pancreatic enzyme secretion: potency of specific amino acids and other digestive products. J Clin Invest 1970 Aug;49(8):1558-64.

(33) Layer P, Groger G. Fate of pancreatic enzymes in the human intestinal lumen in health and pancreatic insufficiency. Digestion 1993;54 Suppl 2:10-4.

(34) Rothman S, Liebow C, Isenman L. Conservation of digestive enzymes. Physiol Rev 2002 Jan;82(1):1-18.

(35) Schmitz J. Maldigestion and Malabsorption. In: Walker WA, editor. Payhophysiology, Diagnosis, Management. 4 ed. New York: Mosby; 2004. p. 8-20.

(36) Kuehne W. Über das Verhalten verschiedener organisirter und sog. ungeformter Fermente. Verhandlungen des naturhistorisch-medicinischen Vereins zu Heidelberg.Heidelberg: 1877. p. 190-3.

(37) Sahin-Toth M, Szabo A. Expression, purification and activity measurements of pancreatic proteases. Pancreapedia: Exocrine Pancreas Knowledge Base 2011.

(38) Kunitz M, Northrop JH. Isolation from beef pancreas of cristalline trypsinogen, trypsin and a trypsin inhibitor and an inhibitor-trypin compound. J Gen Physiol 1936 Jul 20;19(6):991-1007.

(39) Hirota M, Ohmuraya M, Baba H. The role of trypsin, trypsin inhibitor, and trypsin receptor in the onset and aggravation of pancreatitis. J Gastroenterol 2006 Sep;41(9):832-6.

(40) Whitcomb DC. Value of genetic testing in the management of pancreatitis. Gut 2004 Nov;53(11):1710-7.

(41) Green GM, Lyman RL. Feedback regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor-induced hypersecretion in rats. Proc Soc Exp Biol Med 1972 May;140(1):6-12.

(42) Carrere J, Figarella C, Guy O, Thouvenot JP. Human pancreatic chymotrypsinogen A: a non-competitive enzyme immunoassay, and molecular forms in serum and amniotic fluid. Biochim Biophys Acta 1986 Aug 6;883(1):46-53.

(43) Kumar A, Venkatesu P. Overview of the stability of alpha-chymotrypsin in different solvent media. Chem Rev 2012 Jul 11;112(7):4283-307.

(44) Balo J, Banga I. The elastolytic activity of pancreatic extracts. Biochem J 1950 Apr;46(4):384-7.

(45) Toth AZ, Szabo A, Hegyi E, Hegyi P, Sahin-Toth M. Detection of human elastase isoforms by the ScheBo Pancreatic Elastase 1 Test. Am J Physiol Gastrointest Liver Physiol 2017 Jun 1;312(6):G606-G614.

(46) Jurij Dolenšek, Viljem Pohorec, Marjan Slak Rupnik, Andraž Stožer. In: Andrada Seicean, editor. Pancreas Physiology. 1 ed. Rijeka: IN TECH d.o.o; 2017. p. 34-5.

(47) Bernard C. Du suc pancréatique, et de son role dans les phénomènes de la digestion . Paris: Rignoux, Imprimeur de la Faculté de Médecine; 1849.

(48) Carey MC, Hernell O. Digestion and absorption of fat. Semin Gastrointest Dis, 3, 189-208. Semin Gastrointest Dis 1992;3:189-208.

(49) Wang TY, Liu M, Portincasa P, Wang DQ-H. New insights into the molecular mechanism of intestinal fatty acid absorption. European journal of clinical investigation 2013;43(11):1203-23.

(50) Last AR, Wilson SA. Low-carbohydrate diets. Am Fam Physician 2006 Jun 1;73(11):1942-8.

(51) What the world eats; Compare Diet and Consumption Patterns Over Time. 6-12-2014. National Geographic Magazine. Ref Type: Online Source

(52) Chapus C, Rovery M, Sarda L, Verger R. Minireview on pancreatic lipase and colipase. Biochimie 1988 Sep;70(9):1223-34.

(53) Bezzine S, Ferrato F, Ivanova MG, Lopez V, Verger R, Carriere F. Human pancreatic lipase: colipase dependence and interfacial binding of lid domain mutants. Biochemistry 1999 Apr 27;38(17):5499-510.

(54) Iqbal J, Hussain MM. Intestinal lipid absorption. Am J Physiol Endocrinol Metab 2009 Jun;296(6):E1183-E1194.

(55) Gargouri Y, Pieroni G, Lowe PA, Sarda L, Verger R. Human gastric lipase. The effect of amphiphiles. Eur J Biochem 1986 Apr 15;156(2):305-10.

(56) Andersson L, Carriere F, Lowe ME, Nilsson A, Verger R. Pancreatic lipase-related protein 2 but not classical pancreatic lipase hydrolyzes galactolipids. Biochim Biophys Acta 1996 Aug 16;1302(3):236-40.

(57) Andersson L, Bratt C, Arnoldsson KC, Herslof B, Olsson NU, Sternby B, et al. Hydrolysis of galactolipids by human pancreatic lipolytic enzymes and duodenal contents. J Lipid Res 1995 Jun;36(6):1392-400.

(58) Johansson BB, Torsvik J, Bjorkhaug L, Vesterhus M, Ragvin A, Tjora E, et al. Diabetes and pancreatic exocrine dysfunction due to mutations in the carboxyl ester lipase gene-maturity onset diabetes of the young (CEL-MODY): a protein misfolding disease. J Biol Chem 2011 Oct 7;286(40):34593-605.

(59) Molven A, Njolstad PR, Weiss FU. Lipase gene fusion: a new route to chronic pancreatitis. Oncotarget 2015 Oct 13;6(31):30443-4.

(60) Xiao X, Jones G, Sevilla WA, Stolz DB, Magee KE, Haughney M, et al. A Carboxyl Ester Lipase (CEL) Mutant Causes Chronic Pancreatitis by Forming Intracellular Aggregates That Activate Apoptosis. J Biol Chem 2016 Oct 28;291(44):23224-36.

(61) Zhang X, Caner S, Kwan E, Li C, Brayer GD, Withers SG. Evaluation of the Significance of Starch Surface Binding Sites on Human Pancreatic alpha-Amylase. Biochemistry 2016 Nov 1;55(43):6000-9.

(62) Caspary WF. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr 1992 Jan;55(1 Suppl):299S-308S.

(63) Stiefel DJ, Keller PJ. Preparation and some properties of human pancreatic amylase including a comparison with human parotid amylase. Biochim Biophys Acta 1973 Apr 12;302(2):345-61.

(64) Layer P, Zinsmeister AR, DiMagno EP. Effects of decreasing intraluminal amylase activity on starch digestion and postprandial gastrointestinal function in humans. Gastroenterology 1986 Jul;91(1):41-8.

(65) Lankisch PG, Otto J. Salivary isoamylase in duodenal aspirates. Dig Dis Sci 1986 Dec;31(12):1299-302.

(66) Ito T, Ishiguro H, Ohara H, Kamisawa T, Sakagami J, Sata N, et al. Evidence-based clinical practice guidelines for chronic pancreatitis 2015. J Gastroenterol 2016 Feb;51(2):85-92.

(67) Conwell DL, Lee LS, Yadav D, Longnecker DS, Miller FH, Mortele KJ, et al. American Pancreatic Association Practice Guidelines in Chronic Pancreatitis: evidence-based report on diagnostic guidelines. Pancreas 2014 Nov;43(8):1143-62.

(68) Lohr 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 European Gastroenterol J 2017 Mar;5(2):153-99.

(69) 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 Mar;16(2):218-24.

(70) 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 Nov;149(6):1490-500.

(71) Ahmed AU, Issa Y, Hagenaars JC, Bakker OJ, van GH, Nieuwenhuijs VB, et al. Risk of Recurrent Pancreatitis and Progression to Chronic Pancreatitis After a First Episode of Acute Pancreatitis. Clin Gastroenterol Hepatol 2016 May;14(5):738-46.

(72) Sajith KG, Chacko A, Dutta AK. Recurrent acute pancreatitis: clinical profile and an approach to diagnosis. Dig Dis Sci 2010 Dec;55(12):3610-6.

(73) Machicado JD, Yadav D. Epidemiology of Recurrent Acute and Chronic Pancreatitis: Similarities and Differences. Dig Dis Sci 2017 Jul;62(7):1683-91.

(74) Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. Gastroenterology 1994 Nov;107(5):1481-7.

(75) Antonini F, Belfiori V, Pagano N, Buscarini E, De MS, Lo CM, et al. Endoscopic Ultrasonography May Select Subjects Having Asymptomatic Chronic Pancreatic Hyperenzymemia Who Require a Stricter Follow-up. Pancreas 2017 Apr;46(4):524-7.

(76) Donati F, Boraschi P, Gigoni R, Salemi S, Faggioni L, Bertucci C, et al. Secretin-stimulated MR cholangio-pancreatography in the evaluation of asymptomatic patients with non-specific pancreatic hyperenzymemia. Eur J Radiol 2010 Aug;75(2):e38-e44.

(77) Conwell DL, Banks PA, Sandhu BS, Sherman S, Al-Kaade S, Gardner TB, et al. Validation of Demographics, Etiology, and Risk Factors for Chronic Pancreatitis in the USA: A Report of the North American Pancreas Study (NAPS) Group. Dig Dis Sci 2017 Aug;62(8):2133-40.

(78) Brock C, Nielsen LM, Lelic D, Drewes AM. Pathophysiology of chronic pancreatitis. World J Gastroenterol 2013 Nov 14;19(42):7231-40.

(79) Kloppel G. Chronic pancreatitis, pseudotumors and other tumor-like lesions. Mod Pathol 2007 Feb;20 Suppl 1:S113-S131.

(80) Rana SS, Vilmann P. Endoscopic ultrasound features of chronic pancreatitis: A pictorial review. Endosc Ultrasound 2015 Jan;4(1):10-4.

(81) Braganza JM, Lee SH, McCloy RF, McMahon MJ. Chronic pancreatitis. Lancet 2011 Apr 2;377(9772):1184-97.

(82) Omary MB, Lugea A, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest 2007 Jan;117(1):50-9.

(83) Pang TCY, Wilson JS, Apte MV. Pancreatic stellate cells: what's new? Curr Opin Gastroenterol 2017 Jun 5.

(84) Hart PA, Bellin MD, Andersen DK, Bradley D, Cruz-Monserrate Z, Forsmark CE, et al. Type 3c (pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer. Lancet Gastroenterol Hepatol 2016 Nov;1(3):226-37.

(85) Yabar CS, Winter JM. Pancreatic Cancer: A Review. Gastroenterol Clin North Am 2016 Sep;45(3):429-45.

(86) Pinho AV, Chantrill L, Rooman I. Chronic pancreatitis: a path to pancreatic cancer. Cancer Lett 2014 Apr 10;345(2):203-9.

(87) Witt H, Apte MV, Keim V, Wilson JS. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. Gastroenterology 2007 Apr;132(4):1557-73.

(88) Clemens DL, Schneider KJ, Arkfeld CK, Grode JR, Wells MA, Singh S. Alcoholic pancreatitis: New insights into the pathogenesis and treatment. World J Gastrointest Pathophysiol 2016 Feb 15;7(1):48-58.

(89) Minotti V.Apte, Romano C.Pirola, Jeremy S.Wilson. Alcohol and the Pancreas . Pancreapedia; Exocrine Pancrea Knowledge Base . 23-1-2017. American Pancreatic Association. Ref Type: Online Source

(90) Mouad Edderkaoui, Edwin Thrower. Smoking induced pancreatitis and pancreatic cancer . Pancreapedia: Exocrine Pancreas Knowledge Base . 23-1-2017. American Pancreatic Association. Ref Type: Online Source

(91) Sadr-Azodi O, Andren-Sandberg A, Orsini N, Wolk A. Cigarette smoking, smoking cessation and acute pancreatitis: a prospective population-based study. Gut 2012 Feb;61(2):262-7.

(92) Barreto SG. How does cigarette smoking cause acute pancreatitis? Pancreatology 2016 Mar;16(2):157-63.

(93) Edderkaoui M, Thrower E. Smoking and Pancreatic Disease. J Cancer Ther 2013 Nov 1;4(10A):34-40.

(94) Hegyi P, Wilschanski M, Muallem S, Lukacs GL, Sahin-Toth M, Uc A, et al. CFTR: A New Horizon in the Pathomechanism and Treatment of Pancreatitis. Rev Physiol Biochem Pharmacol 2016;170:37-66.

(95) Fogel EL, Sherman S. ERCP for gallstone pancreatitis. N Engl J Med 2014 Jan 9;370(2):150-7.

(96) Machicado JD, Yadav D. Epidemiology of Recurrent Acute and Chronic Pancreatitis: Similarities and Differences. Dig Dis Sci 2017 Jul;62(7):1683-91.

(97) Hafezi M, Mayschak B, Probst P, Buchler MW, Hackert T, Mehrabi A. A systematic review and quantitative analysis of different therapies for pancreas divisum. Am J Surg 2017 Jan 3.

(98) Kiernan PD, ReMine SG, Kiernan PC, ReMine WH. Annular pancreas: May Clinic experience from 1957 to 1976 with review of the literature. Arch Surg 1980 Jan;115(1):46-50.

(99) Etienne D, John A, Menias CO, Ward R, Tubbs RS, Loukas M. Annular pancreas: a review of its molecular embryology, genetic basis and clinical considerations. Ann Anat 2012 Sep;194(5):422-8.

(100) Rebours V, Levy P, Ruszniewski P. An overview of hereditary pancreatitis. Dig Liver Dis 2012 Jan;44(1):8-15.

(101) LaRusch J, Whitcomb DC. Genetics of pancreatitis. Curr Opin Gastroenterol 2011 Sep;27(5):467-74.

(102) Hegyi E, Sahin-Toth M. Genetic Risk in Chronic Pancreatitis: The Trypsin-Dependent Pathway. Dig Dis Sci 2017 Jul;62(7):1692-701.

(103) Weiss FU. Pancreatic cancer risk in hereditary pancreatitis. Front Physiol 2014;5:70.

(104) Kessler L, Abely M. [Pancreatic infringement exocrine and endocrine in cystic fibrosis]. Arch Pediatr 2016 Dec;23(12S):12S21-32.

(105) Walkowiak J, Sands D, Nowakowska A, Piotrowski R, Zybert K, Herzig KH, et al. Early decline of pancreatic function in cystic fibrosis patients with class 1 or 2 CFTR mutations. J Pediatr Gastroenterol Nutr 2005 Feb;40(2):199-201.

(106) Bhatia E, Choudhuri G, Sikora SS, Landt O, Kage A, Becker M, et al. Tropical calcific pancreatitis: strong association with SPINK1 trypsin inhibitor mutations. Gastroenterology 2002 Oct;123(4):1020-5.

(107) Garg PK. Chronic pancreatitis in India and Asia. Curr Gastroenterol Rep 2012 Apr;14(2):118-24.

(108) Gan SI, Edwards AL, Symonds CJ, Beck PL. Hypertriglyceridemia-induced pancreatitis: A case-based review. World J Gastroenterol 2006 Nov 28;12(44):7197-202.

(109) Fortson MR, Freedman SN, Webster PD, III. Clinical assessment of hyperlipidemic pancreatitis. Am J Gastroenterol 1995 Dec;90(12):2134-9.

(110) Burnett JR, Hooper AJ, Hegele RA. Familial Lipoprotein Lipase Deficiency. In: Pagon RA, Adam MP, Ardinger HH, editors. GeneReviews® [Internet].Seattle (WA): University of Washington, Seattle; 1999.

(111) Kota SK, Krishna SV, Lakhtakia S, Modi KD. Metabolic pancreatitis: Etiopathogenesis and management. Indian J Endocrinol Metab 2013 Sep;17(5):799-805.

(112) Wells HG. Pathological Calcification. J Med Res 1906 Apr;14(3):491-525.

(113) Moore EW, Verine HJ. Pancreatic calcification and stone formation: a thermodynamic model of calcium in pancreatic juice. Am J Physiol 1987 May;252(5 Pt 1):G707-G718.

(114) De CA, Multigner L, Dagorn JC, Sarles H. The human pancreatic stone protein. Biochimie 1988 Sep;70(9):1209-14.

(115) Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. Dig Dis Sci 1995 Jul;40(7):1561-8.

(116) Majumder S, Takahashi N, Chari ST. Autoimmune Pancreatitis. Dig Dis Sci 2017 Jul;62(7):1762-9.

(117) Machicado JD, Amann ST, Anderson MA, Abberbock J, Sherman S, Conwell DL, et al. Quality of Life in Chronic Pancreatitis is Determined by Constant Pain, Disability/Unemployment, Current Smoking, and Associated Co-Morbidities. Am J Gastroenterol 2017 Apr;112(4):633-42.

(118) Ceyhan GO, Bergmann F, Kadihasanoglu M, Altintas B, Demir IE, Hinz U, et al. Pancreatic neuropathy and neuropathic pain--a comprehensive pathomorphological study of 546 cases. Gastroenterology 2009 Jan;136(1):177-86.

(119) Olesen SS, Frokjaer JB, Lelic D, Valeriani M, Drewes AM. Pain-associated adaptive cortical reorganisation in chronic pancreatitis. Pancreatology 2010;10(6):742-51.

(120) Dimcevski G, Sami SA, Funch-Jensen P, Le PD, Valeriani M, Arendt-Nielsen L, et al. Pain in chronic pancreatitis: the role of reorganization in the central nervous system. Gastroenterology 2007 Apr;132(4):1546-56.

(121) Buscher HC, Wilder-Smith OH, van GH. Chronic pancreatitis patients show hyperalgesia of central origin: a pilot study. Eur J Pain 2006 May;10(4):363-70.

(122) Drewes AM, Krarup AL, Detlefsen S, Malmstrom ML, Dimcevski G, Funch-Jensen P. Pain in chronic pancreatitis: the role of neuropathic pain mechanisms. Gut 2008 Nov;57(11):1616-27.

(123) Bouwense SA, de VM, Schreuder LT, Olesen SS, Frokjaer JB, Drewes AM, et al. Systematic mechanism-orientated approach to chronic pancreatitis pain. World J Gastroenterol 2015 Jan 7;21(1):47-59.

(124) Pasricha PJ. Unraveling the mystery of pain in chronic pancreatitis. Nat Rev Gastroenterol Hepatol 2012 Jan 24;9(3):140-51.

(125) Goulden MR. The pain of chronic pancreatitis: a persistent clinical challenge. Br J Pain 2013 Feb;7(1):8-22.

(126) Chronic Pancreatitis; Diagnostic criteria. 11-11-2016. BMJ Publishing Group Limited 2017. Ref Type: Online Source

(127) Sarles H. Definitions and classifications of pancreatitis. Pancreas 1991 Jul;6(4):470-4.

(128) Perrier CV. Symposium of the etiology and pathological anatomi of chronic pancreatics. Marseilles. Am J Dig Dis 1964 May;9(5):371-6.

(129) Axon AT, Classen M, Cotton PB, Cremer M, Freeny PC, Lees WR. Pancreatography in chronic pancreatitis: international definitions. Gut 1984 Oct;25(10):1107-12.

(130) Lankisch PG, Schreiber A, Otto J. Pancreolauryl test. Evaluation of a tubeless pancreatic function test in comparison with other indirect and direct tests for exocrine pancreatic function. Dig Dis Sci 1983 Jun;28(6):490-3.

(131) Etemad B, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. Gastroenterology 2001 Feb;120(3):682-707.

(132) Raimondo M, Imoto M, DiMagno EP. Rapid endoscopic secretin stimulation test and discrimination of chronic pancreatitis and pancreatic cancer from disease controls. Clin Gastroenterol Hepatol 2003 Sep;1(5):397-403.

(133) Homma T, Harada H, Koizumi M. Diagnostic criteria for chronic pancreatitis by the Japan Pancreas Society. Pancreas 1997 Jul;15(1):14-5.

(134) Schneider A, Lohr JM, Singer MV. 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 Feb;42(2):101-19.

(135) Whitcomb DC. Hereditary diseases of the pancreas. In: YamadaT ADKNLLOCPD, editor. Textbook of Gastroenterology. 4 ed. Philadelphia: Lippincott Williams & Wilkins; 2003. p. 2147-65.

(136) Ammann RW. A clinically based classification system for alcoholic chronic pancreatitis: summary of an international workshop on chronic pancreatitis. Pancreas 1997 Apr;14(3):215-21.

(137) Harper RA. Some observations on radiology of the pancreas. Proc R Soc Med 1946 Jul;39:534-7.

(138) Schierl W, Otte M, Kremer H, Blechschmidt M, Forell M. [Diagnostic value of sonography and pancreatic function tests in chronic pancreatitis]. Verh Dtsch Ges Inn Med 1978;(84):1025-8.

(139) Lutz H, Petzoldt R, Hofmann KP, Rosch W. [Efficacy of ultrasonography in pancreatic disorders compared to endoscopic retrograde pancreaticography (author's transl)]. Klin Wochenschr 1975 May 1;53(9):419-24.

(140) Erchinger FG, Dimcevski G, E, jom T, ilja OH. Transabdominal ultrasonography of the pancreas: basic and new aspects. Imaging in Medicine 2011 Aug 1;3(4):411-22.

(141) Engjom T, Erchinger F, Tjora E, Laerum BN, Georg D, Gilja OH. Diagnostic accuracy of secretin-stimulated ultrasonography of the pancreas assessing exocrine pancreatic failure in cystic fibrosis and chronic pancreatitis. Scand J Gastroenterol 2015 May;50(5):601-10.

(142) Nojgaard C, Olesen SS, Frokjaer JB, Drewes AM. Update of exocrine functional diagnostics in chronic pancreatitis. Clin Physiol Funct Imaging 2013 May;33(3):167-72.

(143) Issa Y, Kempeneers MA, van Santvoort HC, Bollen TL, Bipat S, Boermeester MA. Diagnostic performance of imaging modalities in chronic pancreatitis: a systematic review and meta-analysis. Eur Radiol 2017 Jan 27.

(144) Koch H, Classen M, Schaffner O, Demling L. Endoscopic papillotomy. Experimental studies and initial clinical experience. Scand J Gastroenterol 1975;10(4):441-4.

(145) Cotton PB. Cannulation of the papilla of Vater by endoscopy and retrograde cholangiopancreatography (ERCP). Gut 1972 Dec;13(12):1014-25.

(146) Choueiri NE, Balci NC, Alkaade S, Burton FR. Advanced imaging of chronic pancreatitis. Curr Gastroenterol Rep 2010 Apr;12(2):114-20.

(147) Sai JK, Suyama M, Kubokawa Y, Watanabe S. Diagnosis of mild chronic pancreatitis (Cambridge classification): comparative study using secretin injection-magnetic resonance cholangiopancreatography and endoscopic retrograde pancreatography. World J Gastroenterol 2008 Feb 28;14(8):1218-21.

(148) Mariani A, Arcidiacono PG, Curioni S, Giussani A, Testoni PA. Diagnostic yield of ERCP and secretin-enhanced MRCP and EUS in patients with acute recurrent pancreatitis of unknown aetiology. Dig Liver Dis 2009 Oct;41(10):753-8.

(149) Kolodziejczyk E, Jurkiewicz E, Pertkiewicz J, Wejnarska K, Dadalski M, Kierkus J, et al. MRCP Versus ERCP in the Evaluation of Chronic Pancreatitis in Children: Which Is the Better Choice? Pancreas 2016 Sep;45(8):1115-9.

(150) Weyman PJ, Stanley RJ, Levitt RG. Computed tomography in evaluation of the pancreas. Semin Roentgenol 1981 Oct;16(4):301-11.

(151) Haaga JR, Alfidi RJ, Zelch MG, Meany TF, Boller M, Gonzalez L, et al. Computed tomography of the pancreas. Radiology 1976 Sep;120(3):589-95.

(152) Lackner K, Frommhold H, Grauthoff H, Modder U, Heuser L, Braun G, et al. [The value of computer tomography and sonography in the investigation of the pancreas (author's transl)]. Rofo 1980 May;132(5):509-13.

(153) Ginde AA, Foianini A, Renner DM, Valley M, Camargo CA, Jr. Availability and quality of computed tomography and magnetic resonance imaging equipment in U.S. emergency departments. Acad Emerg Med 2008 Aug;15(8):780-3.

(154) Anaizi A, Hart PA, Conwell DL. Diagnosing Chronic Pancreatitis. Dig Dis Sci 2017 Jul;62(7):1713-20.

(155) Lesniak RJ, Hohenwalter MD, Taylor AJ. Spectrum of causes of pancreatic calcifications. AJR Am J Roentgenol 2002 Jan;178(1):79-86.

(156) Smith FW, Reid A, Hutchison JM, Mallard JR. Nuclear magnetic resonance imaging of the pancreas. Radiology 1982 Mar;142(3):677-80.

(157) O'Neill E, Hammond N, Miller FH. MR imaging of the pancreas. Radiol Clin North Am 2014 Jul;52(4):757-77.

(158) Tamura R, Ishibashi T, Takahashi S. Chronic pancreatitis: MRCP versus ERCP for quantitative caliber measurement and qualitative evaluation. Radiology 2006 Mar;238(3):920-8.

(159) Balci C. MRI assessment of chronic pancreatitis. Diagn Interv Radiol 2011 Sep;17(3):249-54.

(160) Fraum TJ, Ludwig DR, Bashir MR, Fowler KJ. Gadolinium-based contrast agents: A comprehensive risk assessment. J Magn Reson Imaging 2017 Aug;46(2):338-53.

(161) Boraschi P, Donati F, Cervelli R, Pacciardi F. Secretin-stimulated MR cholangiopancreatography: spectrum of findings in pancreatic diseases. Insights Imaging 2016 Dec;7(6):819-29.

(162) Alkaade S, Cem BN, Momtahen AJ, Burton F. Normal pancreatic exocrine function does not exclude MRI/MRCP chronic pancreatitis findings. J Clin Gastroenterol 2008 Sep;42(8):950-5.

(163) Lees WR. Endoscopic ultrasonography of chronic pancreatitis and pancreatic pseudocysts. Scand J Gastroenterol Suppl 1986;123:123-9.

(164) Seicean A. Endoscopic ultrasound in chronic pancreatitis: where are we now? World J Gastroenterol 2010 Sep 14;16(34):4253-63.

(165) Xu MM, Sethi A. Imaging of the Pancreas. Gastroenterol Clin North Am 2016 Mar;45(1):101-16.

(166) Fusaroli P, Kypraios D, Caletti G, Eloubeidi MA. Pancreatico-biliary endoscopic ultrasound: a systematic review of the levels of evidence, performance and outcomes. World J Gastroenterol 2012 Aug 28;18(32):4243-56.

(167) Wang J, Zhao S, Chen Y, Jia R, Zhang X. Endoscopic ultrasound guided fine needle aspiration versus endoscopic ultrasound guided fine needle biopsy in sampling pancreatic masses: A meta-analysis. Medicine (Baltimore) 2017 Jul;96(28):e7452.

(168) Khan MA, Grimm IS, Ali B, Nollan R, Tombazzi C, Ismail MK, et al. A meta-analysis of endoscopic ultrasound-fine-needle aspiration compared to endoscopic ultrasound-fine-needle biopsy: diagnostic yield and the value of onsite cytopathological assessment. Endosc Int Open 2017 May;5(5):E363-E375.

(169) Baron TH. Endoscopic pancreatic necrosectomy. Gastroenterol Hepatol (N Y) 2008 Sep;4(9):617-20.

(170) Law R, Baron TH. Endoscopic management of pancreatic pseudocysts and necrosis. Expert Rev Gastroenterol Hepatol 2015 Feb;9(2):167-75.

(171) 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 Jun;69(7):1251-61.

(172) Kalmin B, Hoffman B, Hawes R, Romagnuolo J. Conventional versus Rosemont endoscopic ultrasound criteria for chronic pancreatitis: comparing interobserver reliability and intertest agreement. Can J Gastroenterol 2011 May;25(5):261-4.

(173) Montalto G, Soresi M, Carroccio A, Scafidi E, Barbagallo CM, Ippolito S, et al. Lipoproteins and chronic pancreatitis. Pancreas 1994 Jan;9(1):137-8.

(174) Nojgaard 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 Apr;8(4):384-90.

(175) Lindkvist B, Dominguez-Munoz JE, Luaces-Regueira M, Castineiras-Alvarino M, Nieto-Garcia L, Iglesias-Garcia J. Serum nutritional markers for prediction of pancreatic exocrine insufficiency in chronic pancreatitis. Pancreatology 2012 Jul;12(4):305-10.

(176) Keller J, Layer P. Human pancreatic exocrine response to nutrients in health and disease. Gut 2005 Jul;54 Suppl 6:vi1-28.

(177) Piciucchi M, Capurso G, Archibugi L, Delle Fave MM, Capasso M, Delle FG. Exocrine pancreatic insufficiency in diabetic patients: prevalence, mechanisms, and treatment. Int J Endocrinol 2015;2015:595649.

(178) Pezzilli R, Andriulli A, Bassi C, Balzano G, Cantore M, Delle FG, et al. Exocrine pancreatic insufficiency in adults: a shared position statement of the Italian Association for the Study of the Pancreas. World J Gastroenterol 2013 Nov 28;19(44):7930-46.

(179) Pollack BJ, Grendell JH. Where have all the dreiling tubes gone? Am J Gastroenterol 2006 Feb;101(2):356-9.

(180) Boeck WG, Adler G, Gress TM. Pancreatic function tests: when to choose, what to use. Curr Gastroenterol Rep 2001 Apr;3(2):95-100.

(181) Dreiling DA, Hollander F. Studies in pancreatic function; preliminary series of clinical studies with the secretin test. Gastroenterology 1948 Nov;11(5):714-29.

(182) Agren G, Lagerlof H. The pancreatic secretion in man after intravenous administration of secretin. Acta Medica Scandinavica 1936;90:1-29.

(183) Chowdhury RS, Forsmark CE. Review article: Pancreatic function testing. Aliment Pharmacol Ther 2003 Mar 15;17(6):733-50.

(184) Lundh G. Pancreatic exocrine function in neoplastic and inflammatory disease; a simple and reliable new test. Gastroenterology 1962 Mar;42:275-80.

(185) Gullo L, Costa PL, Fontana G, Labo G. Investigation of exocrine pancreatic function by continuous infusion of caerulein and secretin in normal subjects and in chronic pancreatitis. Digestion 1976;14(2):97-107.

(186) Lieb JG, Draganov PV. Pancreatic function testing: here to stay for the 21st century. World J Gastroenterol 2008 May 28;14(20):3149-58.

(187) Gorelick FS, Lerch MM. Do Animal Models of Acute Pancreatitis Reproduce Human Disease? Cell Mol Gastroenterol Hepatol 2017 Sep;4(2):251-62.

(188) Mensel B, Messner P, Mayerle J, Fluhr G, Volzke H, Lerch MM, et al. Secretin-stimulated MRCP in volunteers: assessment of safety, duct visualization, and pancreatic exocrine function. AJR Am J Roentgenol 2014 Jan;202(1):102-8.

(189) Zhu J, Han TQ, Chen S, Jiang Y, Zhang SD. Gallbladder motor function, plasma cholecystokinin and cholecystokinin receptor of gallbladder in cholesterol stone patients. World J Gastroenterol 2005 Mar 21;11(11):1685-9.

(190) Morewood DJ, Whitehouse GH. Assessment of gallbladder function using ceruletide in oral cholecystography. Gastrointest Radiol 1985;10(2):97-105.

(191) Stevens T, Parsi MA. Update on endoscopic pancreatic function testing. World J Gastroenterol 2011 Sep 21;17(35):3957-61.

(192) Engjom T, Erchinger F, Laerum BN, Tjora E, Aksnes L, Gilja OH, et al. Diagnostic Accuracy of a Short Endoscopic Secretin Test in Patients With Cystic Fibrosis. Pancreas 2015 Nov;44(8):1266-72.

(193) Tjora E, Wathle G, Engjom T, Erchinger F, Molven A, Aksnes L, et al. Severe pancreatic dysfunction but compensated nutritional status in monogenic pancreatic disease caused by carboxyl-ester lipase mutations. Pancreas 2013 Oct;42(7):1078-84.

(194) Jensen NM, Larsen S. A rapid, endoscopic exocrine pancreatic function test and the Lundh test: a comparative study. Pancreatology 2008;8(6):617-24.

(195) Keller J, Bruckel S, Jahr C, Layer P. A modified (1)(3)C-mixed triglyceride breath test detects moderate pancreatic exocrine insufficiency. Pancreas 2011 Nov;40(8):1201-5.

(196) Kothari D, Ketwaroo G, Sawhney MS, Freedman SD, Sheth SG. Comparison of Combined Endoscopic Ultrasonography and Endoscopic Secretin Testing With the Traditional Secretin Pancreatic Function Test in Patients With Suspected Chronic Pancreatitis: A Prospective Crossover Study. Pancreas 2017 Jun 22.

(197) Basso N, Giri S, Improta G, Lezoche E, Melchiorri P, Percoco M, et al. External pancreatic secretion after bombesin infusion in man. Gut 1975 Dec;16(12):994-8.

(198) Imondi AR, Stradley RP, Wolgemuth R. Synthetic peptides in the diagnosis of exocrine pancreatic insufficiency in animals. Gut 1972 Sep;13(9):726-31.

(199) Scharpe S, Iliano L. Two indirect tests of exocrine pancreatic function evaluated. Clin Chem 1987 Jan;33(1):5-12.

(200) Kaffarnik H, Meyer-Bertenrath JG. Zur Methodik und klinischen Bedeutung eines neuen Pankreaslipase-Tests mit Fluoresceindilaurinsäureester. Klin Wochenschr 2017;(47):221.

(201) Dominguez-Munoz JE, Malfertheiner P. Optimized serum pancreolauryl test for differentiating patients with and without chronic pancreatitis. Clin Chem 1998 Apr;44(4):869-75.

(202) Laterza L, Scaldaferri F, Bruno G, Agnes A, Boskoski I, Ianiro G, et al. Pancreatic function assessment. Eur Rev Med Pharmacol Sci 2013;17 Suppl 2:65-71.

(203) Braden B. (13)C breath tests for the assessment of exocrine pancreatic function. Pancreas 2010 Oct;39(7):955-9.

(204) Dominguez-Munoz JE, Hardt D, Lerch MM, Lohr MJ. Potential for Screening for Pancreatic Exocrine Insufficiency Using the Fecal Elastase-1 Test. Dig Dis Sci 2017 May;62(5):1119-30.

(205) Gullo L, Ventrucci M, Tomassetti P, Migliori M, Pezzilli R. Fecal elastase 1 determination in chronic pancreatitis. Dig Dis Sci 1999 Jan;44(1):210-3.

(206) Keller J, Layer P. Pancreatic Enzyme Supplementation Therapy. Curr Treat Options Gastroenterol 2003 Oct;6(5):369-74.

(207) Dominguez Munoz JE. Diagnosis of chronic pancreatitis: Functional testing. Best Pract Res Clin Gastroenterol 2010 Jun;24(3):233-41.

(208) Hart PA, Conwell DL. Diagnosis of Exocrine Pancreatic Insufficiency. Curr Treat Options Gastroenterol 2015 Sep;13(3):347-53.

(209) Corinaldesi R, Stanghellini V, Barbara G, Tomassetti P, De GR. Clinical approach to diarrhea. Intern Emerg Med 2012 Oct;7 Suppl 3:S255-S262.

(210) Rosenberg IH, Sitrin MD. Screening for fat malabsorption. Ann Intern Med 1981 Dec;95(6):776-7.

(211) DiMagno EP, Go VL, Summerskill WH. Relations between pancreatic enzyme outputs and malabsorption in severe pancreatic insufficiency. N Engl J Med 1973 Apr 19;288(16):813-5.

(212) Holmes GK, Hill PG. Do we still need to measure faecal fat? Br Med J (Clin Res Ed) 1988 Jun 4;296(6636):1552-3.

(213) Berstad A, Erchinger F, Hjartholm AS. Fecal fat determination with a modified titration method. Scand J Gastroenterol 2010 May;45(5):603-7.

(214) Erchinger F, Engjom T, Jurmy P, Tjora E, Gilja OH, Dimcevski G. Fecal Fat Analyses in Chronic Pancreatitis Importance of Fat Ingestion before Stool Collection. PLoS One 2017;12(1):e0169993.

(215) Fine KD, Fordtran JS. The effect of diarrhea on fecal fat excretion. Gastroenterology 1992 Jun;102(6):1936-9.

(216) Kamath MG, Pai CG, Kamath A, Kurien A. Comparing acid steatocrit and faecal elastase estimations for use in M-ANNHEIM staging for pancreatitis. World J Gastroenterol 2017 Mar 28;23(12):2217-22.

(217) Engjom T, Sangnes DA, Havre RF, Erchinger F, Pham KD, Haldorsen IS, et al. Diagnostic Accuracy of Transabdominal Ultrasound in Chronic Pancreatitis. Ultrasound Med Biol 2017 Apr;43(4):735-43.

(218) Tjora E, Wathle G, Erchinger F, Engjom T, Molven A, Aksnes L, et al. Exocrine pancreatic function in hepatocyte nuclear factor 1beta-maturity-onset diabetes of the young (HNF1B-MODY) is only moderately reduced: compensatory hypersecretion from a hypoplastic pancreas. Diabet Med 2013 Aug;30(8):946-55.

(219) Engjom T, Tjora E, Wathle G, Erchinger F, Laerum BN, Gilja OH, et al. Secretin-stimulated ultrasound estimation of pancreatic secretion in cystic fibrosis validated by magnetic resonance imaging. Eur Radiol 2017 Nov 13.

(220) Madzak A, Engjom T, Wathle GK, Olesen SS, Tjora E, Njolstad PR, et al. Secretin-stimulated MRI assessment of exocrine pancreatic function in patients with cystic fibrosis and healthy controls. Abdom Radiol (NY) 2017 Mar;42(3):890-9.

(221) Stangeland M, Engjom T, Mezl M, Jirik R, Gilja OH, Dimcevski G, et al. Interobserver Variation of the Bolus-and-Burst Method for Pancreatic Perfusion with Dynamic - Contrast-Enhanced Ultrasound. Ultrasound Int Open 2017 Jun;3(3):E99-E106.

(222) Engjom T, Erchinger F, Laerum BN, Tjora E, Gilja OH, Dimcevski G. Ultrasound echo-intensity predicts severe pancreatic affection in cystic fibrosis patients. PLoS One 2015;10(3):e0121121.

(223) Raeder H, Johansson S, Holm PI, Haldorsen IS, Mas E, Sbarra V, et al. Mutations in the CEL VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. Nat Genet 2006 Jan;38(1):54-62.

(224) Bjorlykke Y, Vethe H, Vaudel M, Barsnes H, Berven FS, Tjora E, et al. Carboxyl-ester lipase maturity-onset diabetes of the young disease protein biomarkers in secretin-stimulated duodenal juice. J Proteome Res 2015 Jan 2;14(1):521-30.

(225) Olesen SS, Poulsen JL, Drewes AM, Frokjaer JB, Laukkarinen J, Parhiala M, et al. The Scandinavian baltic pancreatic club (SBPC) database: design, rationale and characterisation of the study cohort. Scand J Gastroenterol 2017 Aug;52(8):909-15.

(226) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013 Nov 27;310(20):2191-4.

(227) Muelling RJ, Jr. Basic spectrophotometry. Am J Clin Pathol 1957 Jan;27(1):97-107.

(228) Kenkel John. Back Titrations. In: Kenkel J, editor. Analytical chemistry for technicians. 3rd ed. Boca Raton Florida: Lewis; 2003. p. 108-9.

(229) Ceska M, Birath K, Brown B. A new and rapid method for the clinical determination of alpha-amylase activities in human serum and urine. Optimal conditions. Clin Chim Acta 1969 Dec;26(3):437-44.

(230) Zandonella G, Haalck L, Spener F, Faber K, Paltauf F, Hermetter A. Inversion of lipase stereospecificity for fluorogenic alkyldiacyl glycerols. Effect of substrate solubilization. Eur J Biochem 1995 Jul 1;231(1):50-5.

(231) Duque M, Graupner M, Stutz H, Wicher I, Zechner R, Paltauf F, et al. New fluorogenic triacylglycerol analogs as substrates for the determination and chiral discrimination of lipase activities. J Lipid Res 1996 Apr;37(4):868-76.

(232) Park WG. Clinical chronic pancreatitis. Curr Opin Gastroenterol 2016 Jun 22.

(233) Stevens T, Conwell DL, Zuccaro G, Van LF, Khandwala F, Purich E, et al. Electrolyte composition of endoscopically collected duodenal drainage fluid after synthetic porcine secretin stimulation in healthy subjects. Gastrointest Endosc 2004 Sep;60(3):351-5.

(234) Lagerlof H. Chapter 2 Methods. Acta Medica Scandinavica 109[S128], 16. 1942. Ref Type: Journal (Full)

(235) Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency. World J Gastroenterol 2013 Nov 14;19(42):7258-66.

(236) Eshleman JR, Norris AL, Sadakari Y, Debeljak M, Borges M, Harrington C, et al. KRAS and guanine nucleotide-binding protein mutations in pancreatic juice collected from the duodenum of patients at high risk for neoplasia undergoing endoscopic ultrasound. Clin Gastroenterol Hepatol 2015 May;13(5):963-9.

(237) Stevens T, Conwell DL, Zuccaro G, Jr., Van LF, Purich E, Khandwala F, et al. A randomized crossover study of secretin-stimulated endoscopic and dreiling tube pancreatic function test methods in healthy subjects. Am J Gastroenterol 2006 Feb;101(2):351-5.

(238) Kothari D, Ketwaroo G, Sawhney MS, Freedman SD, Sheth SG. Comparison of Combined Endoscopic Ultrasonography and Endoscopic Secretin Testing With the Traditional Secretin Pancreatic Function Test in Patients With Suspected Chronic Pancreatitis: A Prospective Crossover Study. Pancreas 2017 Jun 22.

(239) Forsmark C, Adams PC. Pancreatic function testing--valuable but underused. Can J Gastroenterol 2009 Aug;23(8):529-30.

(240) Keller J, Aghdassi AA, Lerch MM, Mayerle JV, Layer P. Tests of pancreatic exocrine function - clinical significance in pancreatic and non-pancreatic disorders. Best Pract Res Clin Gastroenterol 2009;23(3):425-39.

(241) DiMagno EP, Malagelada JR, Go VL. Relationship between alcoholism and pancreatic insufficiency. Ann N Y Acad Sci 1975 Apr 25;252:200-7.

(242) Dixon WT. Simple proton spectroscopic imaging. Radiology 1984 Oct;153(1):189-94.

(243) Hu HH. Magnetic Resonance Techniques for Fat Quantification in Obesity.: IEEE Conference Publications; 2012 p. 1-10.

II

Pancreatology 16 (2016) 231–237

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Original article

Automated spectrophotometric bicarbonate analysis in duodenal juice compared to the back titration method☆

Friedemann Erchinger [a, b, *], Trond Engjom [b, c, 1], Oddrun Anita Gudbrandsen [b, 1], Erling Tjora [d, e], Odd H. Gilja [b, f], Georg Dimcevski [b, c]

[a] Department of Medicine, Voss Hospital, Haukeland University Hospital, Bergen, Norway
[b] Department of Clinical Medicine, University of Bergen, Bergen, Norway
[c] Department of Medicine, Haukeland University Hospital, Bergen, Norway
[d] Pediatric Department, Haukeland University Hospital, Bergen, Norway
[e] Department of Clinical Science, University of Bergen, Bergen, Norway
[f] National Centre for Ultrasound in Gastroenterology, Haukeland University Hospital, Bergen, Norway

ARTICLE INFO

Article history:
Available online 3 February 2016

Keywords:
Pancreatic disorders
Pancreatic function test
Pancreatic juice
Secretin
Short endoscopic secretin test
Bicarbonate

ABSTRACT

Objectives: We have recently evaluated a short endoscopic secretin test for exocrine pancreatic function. Bicarbonate concentration in duodenal juice is an important parameter in this test. Measurement of bicarbonate by back titration as the gold standard method is time consuming, expensive and technically difficult, thus a simplified method is warranted. We aimed to evaluate an automated spectrophotometric method in samples spanning the effective range of bicarbonate concentrations in duodenal juice. We also evaluated if freezing of samples before analyses would affect its results.

Methods: Patients routinely examined with short endoscopic secretin test suspected to have decreased pancreatic function of various reasons were included. Bicarbonate in duodenal juice was quantified by back titration and automatic spectrophotometry. Both fresh and thawed samples were analysed spectrophotometrically.

Results: 177 samples from 71 patients were analysed. Correlation coefficient of all measurements was $r = 0.98$ ($p < 0.001$). Correlation coefficient of fresh versus frozen samples conducted with automatic spectrophotometry ($n = 25$): $r = 0.96$ ($p < 0.001$)

Conclusions: The measurement of bicarbonate in fresh and thawed samples by automatic spectrophotometrical analysis correlates excellent with the back titration gold standard. This is a major simplification of direct pancreas function testing, and allows a wider distribution of bicarbonate testing in duodenal juice. Extreme values for Bicarbonate concentration achieved by the autoanalyser method have to be interpreted with caution.

Introduction

Direct pancreas function testing (DPFT) is invasive and thereby challenging. Used on correct indication it adds useful information in exocrine pancreatic function testing. The test probably serves best as a second line test in situations where primary tests, as faecal elastase 1, are insufficient. DPFT can discriminate primary from secondary pancreatic dysfunction [1,2]. Furthermore, direct tests may prove useful in detecting early exocrine dysfunction, before development of clinical obvious pancreatic exocrine insufficiency. In our short endoscopic secretin test (short EST), duodenal juice aspiration is performed in the period from 30 to 45 min after secretin stimulation, in the plateau phase of duodenal bicarbonate concentration. The whole endoscopic procedure, including a diagnostic gastroscopy, lasts normally not longer than 20 min [1–3], hence overcoming some of the disadvantages of the time-

Abbreviations: DPFT, direct pancreas function testing; MDH, malatdehydrogenase; PEPC, phosphoenolpyruvate carboxylase.

☆ The work was conducted at Haukeland University Hospital in Bergen, Norway.

* Corresponding author. Voss Hospital, Medical Department, 5700 Voss, Norway. Tel.: +47 56533500; fax: +47 56513072.
E-mail address: friedemann.erchinger@helse-bergen.no (F. Erchinger).

[1] Engjom and Gudbrandsen have contributed equally to this work.

http://dx.doi.org/10.1016/j.pan.2016.01.010

consuming and cumbersome tube based tests [3–7]. To distinguish pancreatic exocrine failure from sufficient pancreas function a cut off of 80 mmol/L bicarbonate concentration in duodenal juice is generally accepted. Beneficial information about pancreatic exocrine insufficiency (PEI) can be obtained in a variety of patients with chronic pancreatitis, diabetes, cystic fibrosis, celiac disease. We have used our short EST to evaluate exocrine pancreatic function in patients with chronic pancreatitis, cystic fibrosis and diabetes [3,8–13].

Back titration has long been considered the gold standard for duodenal bicarbonate measurements [14,15]. This analysis is time consuming (minimum 2 h) and technically difficult, hence being expensive and vulnerable in routine diagnostics. As a consequence, only a few specialised or research centres perform this test today. Furthermore, back titration requires minimum 0.5 mL of duodenal juice. Such volumes are sometimes difficult to obtain from patients with severe ductal failure. In contrast, the autoanalyser used in this study requires only a few microlitres of duodenal juice and a short analysing time of 7 min. At present, autoanalysers are certified to quantify ingredients in blood or urine but not in duodenal juice. However, some earlier small studies have demonstrated a good correlation between autoanalysers and the back titration method [16,17] Automation of bicarbonate analyses is required to simplify short EST, but the method still needs further validation to replace back titration. Daily routine in a busy medical institution makes immediate analyses of duodenal juice to a challenge, and instant freezing of samples could be an option for institutions sending samples for analyses elsewhere.

In this study, we aimed to demonstrate the accuracy of an automated spectrophotometric method compared to back titration when analysing bicarbonate in duodenal juice. Additionally, we studied if freezing of samples affected bicarbonate concentrations.

Materials and methods

Patients

The use of samples from short EST in the following projects was approved by the local ethical committee: Chronic pancreatitis or other causes of abdominal pain (approval no. 3.2008.2516), cystic fibrosis (approval no.: 2010/2857-7) and celiac disease (approval no. 2011/1592). Short EST of these patients was performed between September 2012 and October 2014. Samples were chosen at random for comparison of back titration and automated spectrometry. Three consecutive aliquots of duodenal juice with different bicarbonate concentrations are collected in 15 min during the short EST, hence 1 to 3 samples per patient could be analysed.

Short endoscopic secretin test

Secretin was administered intravenous at a dose of 1 CU per kg bodyweight, maximum 70 CU. Gastroscopy started 25 min after secretin administration. A diagnostic gastroscopy was initially performed to identify or exclude other pathological findings. All gastric juice was aspirated and discharged. After 30 min the tip of the endoscope was placed distal to the papilla Vateri. Duodenal juice was aspirated in three 5 min sequences. The procedure is illustrated in Fig. 1 and described in detail elsewhere [3].

Handling of duodenal juice before analysis

The pH and volume of each sample was measured. Duodenal juice with pH < 6 was discarded due to probable pollution from gastric juice. One aliquot of duodenal juice from each sampling period was immediately placed on ice and bicarbonate concentration was immediately analysed using back titration and automated analysis. Otherwise samples were frozen to −196 °C. In the experiment

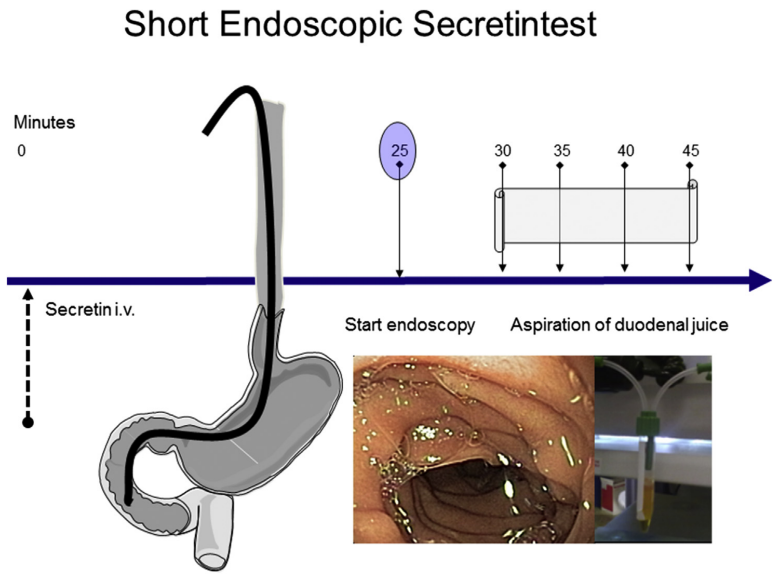


Fig. 1. Short endoscopic pancreas function testing (EST): 25 min after injection of secretin (1 CU/kg bodyweight, max 70 CU) an upper endoscopy was started. During the first 5 min a diagnostic gastroscopy was carried out. All juice from stomach and duodenum was discharged. Thereafter the tip of the endoscope was placed below the papilla for 15 min; duodenal juice was collected in three aliquots of 5 min. The intervention requires only 20 min.

comparing fresh and frozen samples, storage temperature was −80 °C before analyses with automated spectrophotometry.

Analysis of bicarbonate in duodenal juice by back titration

The principles of back titration are described elsewhere [14]. The back titration method for bicarbonate analyses in detail:

Before analyses, pH was measured. Ideally 1 mL of duodenal juice was needed to perform analyses but down to 0.5 mL was accepted. If the volume of duodenal juice was <1 mL, the sample was diluted to 1 mL using 0.9% NaCl. First, the pH of the sample was analysed. To acidify the sample, 1.5 mL of 0.1 N HCl was added, and the reaction between bicarbonate and HCl produced NaCl, H_2O and CO_2. The CO_2 (g) was evacuated by stirring the sample until pH was stable. Next, the solution was titrated ("back titrated") with 0.1 N NaOH until the original pH value was achieved. Based on the volume of NaOH used, the bicarbonate concentration in the duodenal juice was calculated using the formula.

(1000 × (0.1 mol/L HCl × 1.5 mL 0.1 mol/L HCl − 0.1 mol/L NaOH × Y mL 0.1 mol/L NaOH))/1 mL sample volume. Samples were analysed in parallels.

Automated measurement (spectrophotometry)

For automated analyses, the spectrophotometer COBAS® c111 (Roche Diagnostics GmbH; D-68298 Mannheim; Germany; www.roche.com) and the appropriate kit for measuring bicarbonate (CO2-L Bicarbonate liquid, Roche Diagnostics AG, CH-6343 Rotkreuz, Switzerland; www.roche-diagnostics.ch) were used. Analyses were performed using 15 μL of duodenal juice diluted with 30 μL 0.9% NaCl in water before it is pipetted by the instrument and added the start reagent containing phosphoenolpyruvate (≥40 mmol/L), NADH analogue (≥2.0 mmol/L), porcine malatdehydrogenase (≥314.3 ukat/L) and phosphoenolpyruvate carboxylase (≥30.8 ukat/L).

The principle of the test is as follows: Bicarbonate in the samples reacts with phosphoenolpyruvate in the presence of phosphoenolpyruvate carboxylase (PEPC), to produce oxaloacetate and phosphate. One hydrogen from a NADH analogue is transferred to oxaloacetate using porcine malatdehydrogenase (MDH) to produce malate and an NAD^+ analogue. The consumption of NADH results in a decreased absorbance at 409 nm, proportional to the bicarbonate concentration in the sample.

Statistical methods

Difference of groups was tested by t-test. Normality was tested by the Shapiro–Wilk test, and when it failed a Mann–Whitney rank sum test was run. Correlation was calculated with Pearson test, agreement with Bland Altman plot [18,19]. Adjustment was done by linear regression. ROC curves were drawn to evaluate sensitivity and specificity of spectrophotometry compared to gold standard back titration. A 5% level of statistical significance was used. Softwares used were ACCESS, EXCEL (Microsoft Office, Redmond WA, USA), and SigmaPlot 12 (Systat Software Inc., San Jose, CA, USA).

Results

177 randomly chosen samples analysed by back titration and automated spectrophotometry

In the time period of the study short EST was performed in 94 patients and 257 samples of duodenal juice were obtained. 177 samples from 71 patients were chosen arbitrary and analysed by both back titration and automated spectrophotometry. Pearson test showed an excellent correlation of all measurements with a coefficient of $r = 0.98$, $p < 0.001$ (Fig. 2a). We performed supplementary a Bland Altman plot to further explore the agreement over the whole range: Mean difference (bias) was −4.4 with a 95% CI of −5.5 to −3.2. Agreement was excellent around the cut-off of 80 mmol/L but weak in extreme values: Compared to back titration as gold standard, the results from the autoanalyser showed higher levels for very low concentrations of bicarbonate, and lower levels for very high levels of bicarbonate (Fig. 2b). In a clinical setting, bicarbonate concentration of 80 mmol/L is used to differentiate between normal and insufficient ductal pancreatic function. Using back titration as gold standard, sensitivity in spectrophotometry was 97%, specificity 92% (AUC 0.99, $p < 0.001$). This implies that automated spectrophotometry can discriminate between PEI and non-PEI compared to back titration (Fig. 2c). Due to lack of agreement in the range of high and low values of bicarbonate concentration we calculated the following correction factor from linear regression: [HCO^3] back titration = −7172 + (1137*[HCO^3] autoanalyser) (mmol/L). When we recalculated the results using this correlation factor we got the following results: Correlation coefficient of all measurements was $r = 0.98$, $p < 0.001$. Linear regression did not change the excellent correlation (Fig. 2d). Bland Altman plot: Mean difference was −0.03 with a 95% CI −1 to 1. Adjustment by linear regression produced better agreement in extreme values (Fig. 2e). Sensitivity and specificity with a cut-off of 80 mmol/L using back titration as gold standard: sensitivity in spectrophotometry was 94%, specificity 94% (AUC 0.99, $p < 0.001$). ROC analyses show no difference in AUC after mathematical correction in the identification of PEI.

25 pairs of fresh and frozen samples analysed by automated spectrophotometry

Twenty-five samples from 9 patients were randomly chosen. In all measurements correlation after Pearson was excellent with a coefficient of $r = 0.96$ with $p < 0.001$ (Fig. 3). The agreement evaluated by the Bland Altman Plot was very good: Mean difference was −1.3 with a 95% CI of − 5.1 to 2.6. There was no bias in extreme values. Using the analysis of fresh samples as gold standard a ROC curve for the measurement of frozen samples for a cut-off of 80 mmol/L bicarbonate was drawn. Sensitivity and specificity was 100% (AUC 1; $p < 0.001$). Storing samples at −80 °C does not influence differentiation between PEI and non-PEI. Correlation and agreement between the pairs of fresh and frozen samples are excellent.

Standard solutions measured by back titration and spectrophotometric autoanalyser

A series of 7 standard solutions with bicarbonate concentrations of 20, 40, 60, 80, 100, 120, 140 mmol/L were analysed by back titration and spectrophotometric autoanalyser. Pearson test in back titration showed ideal correlation with a coefficient of 1 $p < 0.001$ (Fig. 4a). Bland Altman Plot pointed out nearly ideal agreement: Mean difference was −0.4 with a 95% CI of −0.6 to −0.3. Also with the spectrophotometric autoanalyser method an ideal correlation factor of 1 with $p < 0.001$ could be reached (Fig. 4b). Evaluation using the Bland Altman method revealed a bias analogue to experiment I: In higher bicarbonate concentrations the difference between standard solution and results by spectrophotometric autoanalyser enlarges. Mean difference was −3.2 with a 95% CI of −5.5 to −0.9 (Fig. 4c).

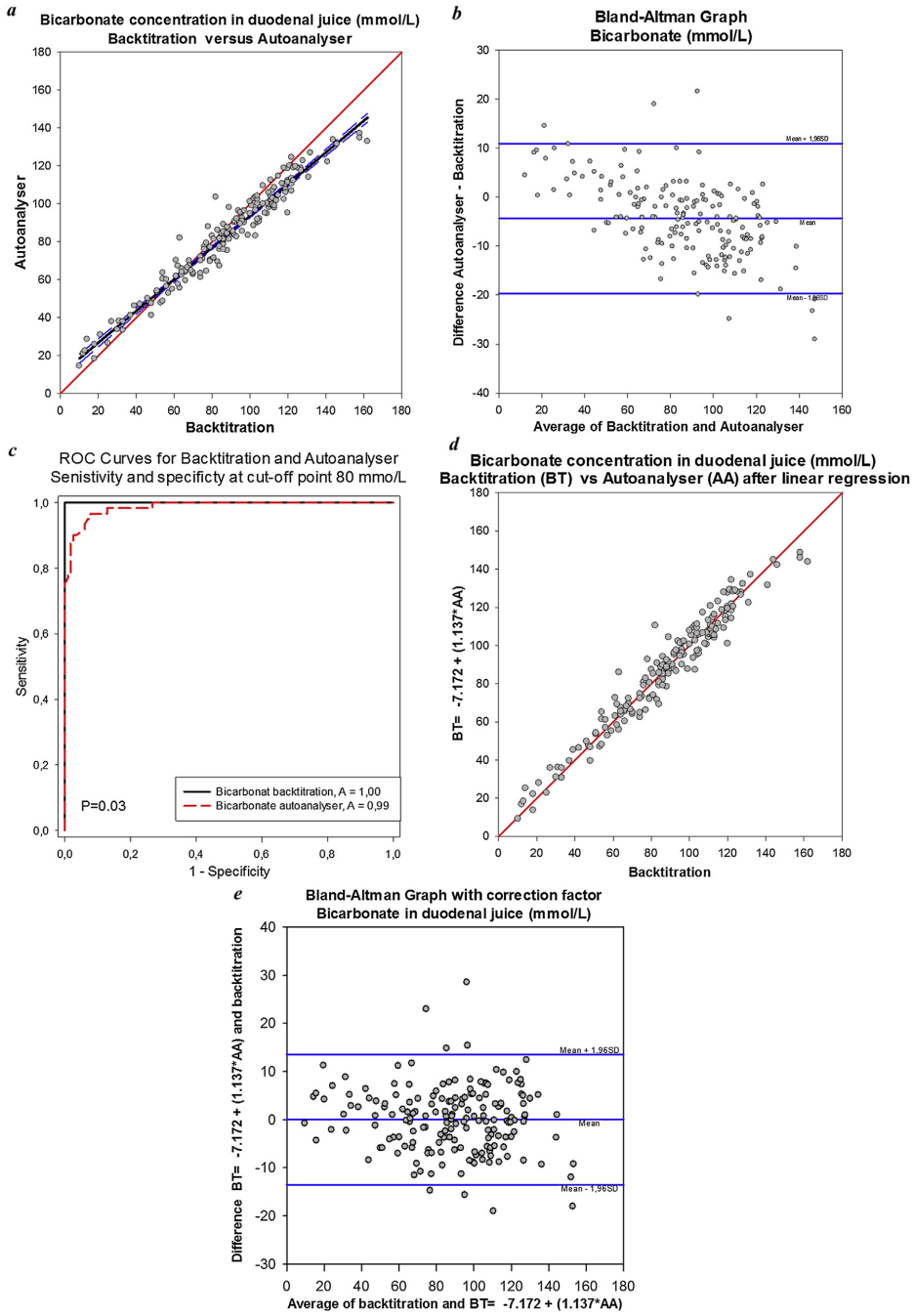


Fig. 2. Correlation and agreement evaluated by measuring Bicarbonate in duodenal juice with back titration (BT) or autoanalyser (AA). Sensitivity and specificity of spectrophotometry at a cut-off point of 80 mmol/L. Back titration is the gold standard. A cut-off point of 80 mmol/L is generally accepted to discriminate insufficient from sufficient ductal pancreatic function. N = 177. a: Excellent correlation between the two methods ($r = 0.98$; $p < 0.001$). b: Agreement between the two methods, excellent around the cut-off point of 80 mmol/L but weak in extreme values: In relation to back titration, autoanalyser measures in very low bicarbonate concentrations for high and in very high bicarbonate concentrations for low. We used therefore linear regression for correction: BT = −7.172 + (1.137*AA). c: Evaluation of the cut-off point of 80 mmol/L: Using ROC analyses with back

Discussion

In this study, we demonstrate that automated spectrophotometric measurement of bicarbonate in duodenal juice correlates excellent to analysis by the gold standard back titration method. Although the back titration method on standard solutions performed better than the spectrophotometric method, automated measurement discriminated sufficient from insufficient pancreatic function with good accuracy. Secondly, we also demonstrated that freezing and thawing did not influence the spectrophotometric result.

Changing from the gold standard back titration method to automated spectrophotometry presents an important simplification of the duodenal juice measurement. The excellent correlation and agreement around the generally accepted cut-off value for pancreatic exocrine failure makes it possible to replace the expensive and cumbersome back titration method by an automated test which can be run in common, basic laboratory services.

Our results are comparable to earlier small studies using an autoanalyser [16,17,20]. The present study comprises a larger number of samples over the whole physiological and pathophysiological range of bicarbonate concentration, thus adding information on reduced agreement in the lower and the higher concentration ranges. One possible explanation is that the high and low range measurements are a result of false measurements. However the same effect demonstrated in the standard solutions argue against this possibility. Over the range to higher bicarbonate concentrations the difference between standard solution and results by spectrophotometric autoanalyser enlarges. Back titration did not show this phenomenon. A possible explanation is the enzyme dependence in the autoanalyser method. Optimum pH of Phosphoenolpyruvate carboxylase (PEPC) is 8–9 [21] and optimum pH for malatdehydrogenase (MDH) is 7.4 (NADH —> NAD) [22]. We assume that pH in extreme bicarbonate concentration is outside the range of optimum pH of malatdehydrogenase. This would influence the consumption of NADH analogue and consecutively the absorbance of light at 409 nm leading to false measures in extreme bicarbonate concentrations.

Additionally, in both back titration and autoanalyser methods, dilution with NaCl 0.9% is substantial. Producers of NaCl 0.9% describe a range of pH between 4 and 7 at 20 °C (106404 | Sodium chloride, Merck Millipore Corporation. Merck KGaA, Darmstadt, Germany). Our measures on different batch numbers revealed a pH between 5.35 and 5.93. For this reason we analysed a small series of duodenal juice with autoanalyser and diluted with either buffer or NaCl; no difference was found. This indicates that results are not influenced by NaCl 0.9%, or buffer. Also other studies have demonstrated inferior agreement in bicarbonate values over 100 mmol/L. In these studies deionised water was used for dilution [17]. A solution to minimise bias in extreme low or high levels of bicarbonate concentration could be different dilution procedures. Explanations by chemistry are not sufficient and lead to new questions without answers. The size of our study using 177 samples with values distributed over the effective range, from very low to high bicarbonate concentrations made it possible to calculate a correction factor with the linear regression. In practice very low or very high concentrations of bicarbonate are not as interesting as the range between 60 and 100 mmol/L. Regardless of the demonstrated weaknesses in the agreement in the lower and the higher range, we demonstrate that this does not has substantial influence on the overall accuracy of the test. The use of a correlation factor is probably not necessary in clinical practice, but it implies that clinicians are aware of this fact.

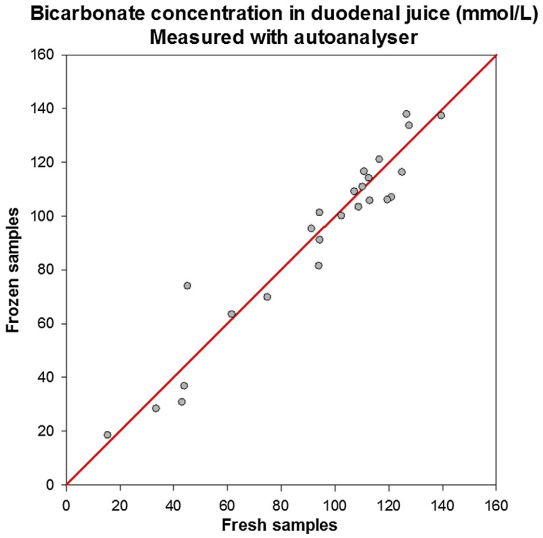


Fig. 3. Correlation and agreement of 25 samples analysed by autoanalyser under two conditions: 1) as fresh sample immediately after DPFT, 2) as frozen and thawed samples: excellent correlation ($R = 0.96$; $p < 0.001$).

The size of the samples over the whole effective range may explain that we can show the bias in the higher and lower values. This may explain that the bias in the lower values is not shown by other authors [16,17].

The amount of duodenal juice in a 5 min aliquot varies between 0 mL (dry tap) and 10 mL. In average we suctioned 2–5 mL per aliquot. In severe pancreatic exocrine insufficiency (PEI) the amount of duodenal juice is very poor. Bicarbonate analysis with back titration needs minimum 0.5 mL of duodenal juice. Consequently smaller amounts cannot be analysed with this method. In contrast, we performed the spectrophotometric method with only 15 μl. If necessary this method allows measuring a lower quantum of juice. The smaller volume requirement in the spectrophotometric analysis increases the possibility to analyse duodenal bicarbonate in all patients.

Limitations of the study

Most limitations of the study can be found in the handling and contents of the specimen. Samples were not centrifuged; consecutively pollution from debris or blood might affect the results of the analyses. On the other hand, we excluded obviously polluted samples and both methods will equally be affected.

For practical reasons in the sampling of juice from a large number of patients, frozen samples were stored up to 197 days at −196 °C before analysis. There are no data about long-term storage of samples at −196 °C and its possible influence on bicarbonate results. We evaluated bicarbonate concentrations in cohorts

titration as gold standard. Automated spectrophotometric method is nearly as good as back titration. d: After the linear regression there was no change of correlation after correction ($r = 0.98$; $p < 0.001$), but the regression line is very close to the equality line. e: Bias is corrected; no deviation of high and low values is shown. Good agreement over the whole range was reached.

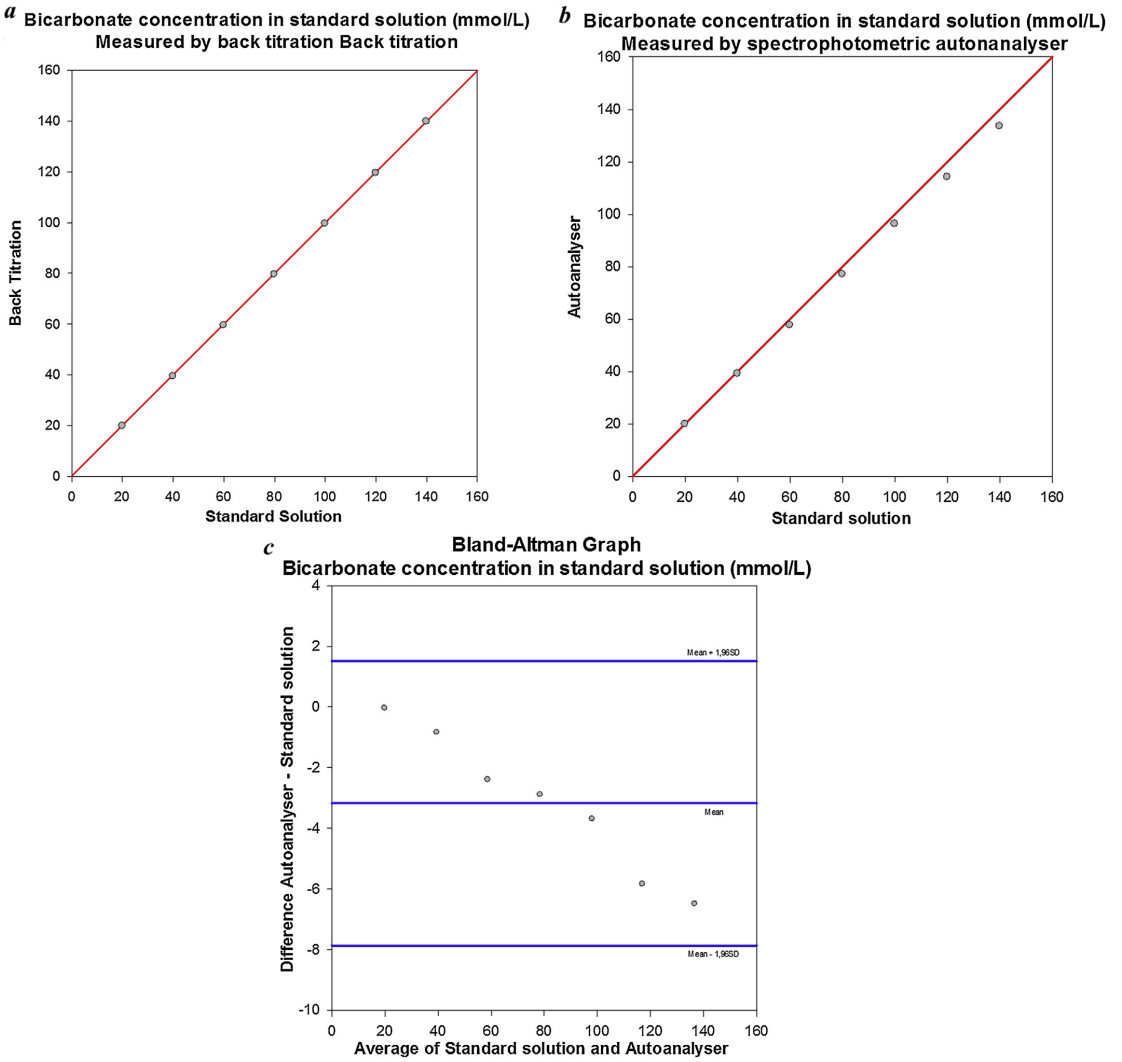


Fig. 4. 7 samples of standard solutions with bicarbonate concentrations (mmol/L) of 20, 40, 60, 80, 100, 120, 140 measured by back titration and spectrophotometric autoanalyser. a) Equality of standard solution and back titration ($r = 1$, $p < 0.001$). b) Equality of standard solution and autoanalyser ($r = 1$, $p < 0.001$). c) Bland Altman Plot unmasks a proportional bias: over the range to higher bicarbonate concentrations the difference between standard solution and results by spectrophotometric autoanalyser enlarges.

differentiated by storage time. No significant differences in correlation were shown. There was acceptable agreement up to 120 days. In experiment II, samples were frozen at minus 80 °C. As shown there was excellent correlation and agreement with fresh samples. This opens for storing duodenal juice in institutions without possibility of snap freezing to −196 °C.

Differentiations in physiological contents of the aspirated specimens might influence the results. The dye of aspirates of duodenal juice varied from colourless to all grades of yellow suggesting different contamination with biliary juice. Biliary acids may influence activity of enzymes used in the spectrophotometric method. However we think that this is negligible because back titration is a chemical method without enzymes and has excellent correlation and agreement to the spectrophotometric method in the range of interest.

Conclusion

Endoscopic secretin-stimulated pancreas function testing has not reached widespread distribution due to the invasive nature and the complexity of the analysing procedures. Easy accessible, simple and cost-effective indirect tests like faecal elastase 1 will probably still be the first line or screening tests. Nevertheless, invasive direct tests have a place in the second line testing due to the well-known pitfalls in the simpler tests. Back titration as old gold standard is time consuming and cumbersome. The validation of an automated

spectrophotometric analysis of bicarbonate in duodenal juice represents a significant simplification of the DPFT. We argue that simplifications of testing and analysis make standardisation of DPFT within reach in the years to come. It will be possible to perform DPFT globally in any health care institution with an endoscopy unit and basic laboratory services.

Acknowledgements

We thank the supporting lab technicians Aud Sissel Hjartholm and Liv Aasmul for preserving and running analyses on duodenal juice. The authors have received travelling grants and scholarships from the Norwegian Gastroenterological Association, Bergen Medical Research Foundation and MedViz.

References

[1] Lankisch PG. Function tests in the diagnosis of chronic pancreatitis. Critical evaluation. Int J Pancreatol 1993;14:9–20.
[2] Keller J, Aghdassi AA, Lerch MM, Mayerle JV, Layer P. Tests of pancreatic exocrine function – clinical significance in pancreatic and non-pancreatic disorders. Best Pract Res Clin Gastroenterol 2009;23:425–39.
[3] Erchinger F, Engjom T, Tjora E, Hoem D, Hausken T, Gilja OH, et al. Quantification of pancreatic function using a clinically feasible short endoscopic secretin test. Pancreas 2013 Oct;42(7):1101–6. http://dx.doi.org/10.1097/MPA.0b013e3182847a86. PMID: 23921960.
[4] Stevens T, Conwell DL, Zuccaro Jr G, Van Lente F, Purich E, Khandwala F, et al. A randomized crossover study of secretin-stimulated endoscopic and dreiling tube pancreatic function test methods in healthy subjects. Am J Gastroenterol 2006;101:351–5.
[5] Stevens T, Conwell DL, Zuccaro Jr G, Van LF, Lopez R, Purich E, et al. A prospective crossover study comparing secretin-stimulated endoscopic and Dreiling tube pancreatic function testing in patients evaluated for chronic pancreatitis. Gastrointest Endosc 2008;67:458–66.
[6] Stevens T, Conwell DL, Zuccaro Jr G, Lewis SA, Love TE. The efficiency of endoscopic pancreatic function testing is optimized using duodenal aspirates at 30 and 45 minutes after intravenous secretin. Am J Gastroenterol 2007;102:297–301.
[7] Wu B, Conwell DL. The endoscopic pancreatic function test. Am J Gastroenterol 2009;104:2381–3.
[8] Raeder H, McAllister FE, Tjora E, Bhatt S, Haldorsen I, Hu J, et al. Carboxyl-ester lipase maturity-onset diabetes of the young is associated with development of pancreatic cysts and upregulated MAPK signaling in secretin-stimulated duodenal fluid. Diabetes 2014;63:259–69.
[9] Bjorlykke Y, Vethe H, Vaudel M, Barsnes H, Berven FS, Tjora E, et al. Carboxyl-ester lipase maturity-onset diabetes of the young disease protein biomarkers in secretin-stimulated duodenal juice. J Proteome Res 2015 Jan 2;14(1):521–30. http://dx.doi.org/10.1021/pr500750z. Epub 2014 Nov 4. PMID: 25369532.
[10] Engjom T, Erchinger F, Tjora E, Laerum BN, Georg D, Gilja OH. Diagnostic accuracy of secretin-stimulated ultrasonography of the pancreas assessing exocrine pancreatic failure in cystic fibrosis and chronic pancreatitis. Scand J Gastroenterol 2015:1–10.
[11] Tjora E, Wathle G, Erchinger F, Engjom T, Molven A, Aksnes L, et al. Exocrine pancreatic function in hepatocyte nuclear factor 1beta-maturity-onset diabetes of the young (HNF1B-MODY) is only moderately reduced: compensatory hypersecretion from a hypoplastic pancreas. Diabet Med 2013 Aug;30(8):946–55. http://dx.doi.org/10.1111/dme.12190. Epub 2013 Apr 19. PMID: 23600988.
[12] Tjora E, Wathle G, Engjom T, Erchinger F, Molven A, Aksnes L, et al. Severe pancreatic dysfunction but compensated nutritional status in monogenic pancreatic disease caused by carboxyl-ester lipase mutations. Pancreas 2013 Oct;42(7):1078–84. http://dx.doi.org/10.1097/MPA.0b013e3182920e9c. PMID: 23770712.
[13] Engjom T, Erchinger F, Lærum BN, Tjora E, Aksnes L, Gilja OH, et al. Diagnostic accuracy of a short endoscopic secretin test in patients with cystic fibrosis patients. Pancreas 2015 Nov;44(8):1266–72. http://dx.doi.org/10.1097/MPA.0000000000000425. PMID: 26348465.
[14] Kenkel John. Back titrations. In: Kenkel J, editor. Analytical chemistry for technicians. Boca Raton, Florida: Lewis; 2003. p. 108–9.
[15] Kenkel J. Applications of titrimetric analysis; analytical chemistry for technicians. CRC Press; 2002. p. 98–147.
[16] Xiao Z, Lopez R, Parsi MA, Dodig M, Stevens T. Comparison of autoanalyzer and back titration for measurement of bicarbonate concentration in endoscopically collected pancreatic fluid. Pancreas 2011;40:237–41.
[17] Zhong N, Saenger AK, Topazian M, Gleeson FC, Chari ST, Clain JE, et al. An automated analyzer provides clinically concordant results to manual back titration for quantitation of bicarbonate in pancreatic juice. Pancreas 2011;40:422–5.
[18] Bilic-Zulle L. Comparison of methods: Passing and Bablok regression. Biochem Med Zagreb 2011;21:49–52.
[19] Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet 1995;346:1085–7.
[20] Ceryak S, Steinberg WM, Marks ZH, Ruiz A. Feasibility of an endoscopic secretin test: preliminary results. Pancreas 2001;23:216–8.
[21] Phosphoenolpyruvate carboxylase. In: Worthington V, editor. Worthington enzyme manual: enzymes, enzyme reagents, related biochemicals. 730 Vassar Ave., Lakewood, NJ 08701: Worthington Biochemical Corporation; 2015.
[22] Malate dehydrogenase. In: Worthington V, editor. Worthington enzyme manual: enzymes, enzyme reagents, related biochemicals. 730 Vassar Ave., Lakewood, NJ 08701: Worthington Biochemical Corporation; 2015.

III

Pancreatology 17 (2017) 182–187

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Analysis of amylase in duodenal juice - Automated kinetic spectrophotometric analysis versus manual colorimetric endpoint assay

Friedemann Erchinger [a, b, *], Trond Engjom [b, c], Erling Tjora [d, e], Lage Aksnes [d, e], Georg Dimcevskir [b, c], Oddrun Anita Gudbrandsen [b]

[a] Department of Medicine, Haukeland University Hospital, Voss, Norway
[b] Department of Clinical Medicine, University of Bergen, Bergen, Norway
[c] Department of Medicine, Haukeland University Hospital, Bergen, Norway
[d] Department of Pediatrics, Haukeland University Hospital, Bergen, Norway
[e] Department of Clinical Science, University of Bergen, Bergen, Norway

ARTICLE INFO

Article history:
Received 28 October 2016
Accepted 1 February 2017
Available online 4 February 2017

Keywords:
Amylase
Duodenal juice
Enzymes
Secretin test
Pancreatic function

ABSTRACT

Objective: The measurement of duodenal amylase by a colorimetric end-point assay has been the most used method for amylase activity analyses. The method is manual, time consuming and dependent on specialized equipment. In this study, we compare an automated kinetic spectrophotometric method for pancreatic amylase measurement in duodenal juice with a standardized colorimetric end-point assay.
Methods: We used specimen of duodenal juice at random from a biobank obtained by short endoscopic secretin test in patients with suspected exocrine pancreatic failure of different reasons. Duodenal juice was tested for amylase activity with a conservative manual colorimetric endpoint assay (Phadebas Amylase test, Magle AB) and an automated enzymatic kinetic spectrophotometric method using standard reagents for pancreatic amylase activity for Cobas c111 (Roche Diagnostics).
Results: 52 samples for assay of amylase were analyzed in pairs. Correlation between measurements with the two methods was $r = 0.99$ ($p < 0.001$), linear regression 0.99 ($p < 0.001$).
Conclusion: Quantification of duodenal amylase activity with automated spectrophotometry has excellent correlation to measurements made by the manual method. This allows for standardized, center independent analyses of duodenal amylase for the assessment of acinar pancreatic function.

1. Introduction

Direct pancreas function testing (dpft) is still the gold standard in the assessment of exocrine pancreatic function (PEI) [1–3]. The short endoscopic secretin tests (EST) have simplified the old dpft procedures thus far that they now easily can be implemented in a routine gastroscopy [4]. We have recently shown the potential of the EST to determine pancreatic function in different patient groups [5–9]. Furthermore, we have simplified the practicability of the test by automation of the bicarbonate analysis in duodenal juice [10]. A simplified EST has the potential for a broader distribution in general hospitals with basic endoscopic and laboratory services [11].

Enzymes such as amylase (1,4-a-D-Glucan 4-Glucanohydrolase, EC 3.2.1.1) reflect the acinar function and may be the key for the diagnosis of PEI in an early stage. The methods for analyzing enzymes in duodenal juice have not been standardized. These tests are often multifarious and cumbersome. Challenges in preservation and analysis of the enzymes have been the main obstacle to a widespread direct test for the acinar axis. By automation and increased reliability of enzyme measurement, we intend to bring the EST a further step in the direction of a reliable and clinically applicable, complete dpft.

In this study, we aimed to investigate the agreement between an

Abbreviations: dpft, direct pancreas function testing; PEI, exocrine pancreatic function; EST, short endoscopic secretin test; IMEP, International Measurement Evaluation Programme; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine.

* Corresponding author. Haukeland University Hospital, Voss, Medical Department, Sjukehusvegen 16, 5704 Voss, Norway.
E-mail address: friedemann.erchinger@helse-bergen.no (F. Erchinger).

http://dx.doi.org/10.1016/j.pan.2017.02.001
1424-3903/

automated kinetic spectrophotometric methods with a standardized manual colorimetric end-point assay for analyses of amylase in duodenal juice.

2. Materials and methods

2.1. Patients

Samples of duodenal juice were chosen at random from a biobank (registration number 2010/198) obtained by short EST in patients with suspected exocrine pancreatic failure due to various causes. The samples were collected between March 2014 and January 2015. The two different assays for amylase were performed simultaneously in two sequences in March 2016.

The study was conducted in accordance to the Helsinki II Declaration and approved by the local ethics committee (registration numbers 3.2008.2516; 2010/2857-7). Study subjects were included after signing written consent.

2.2. Short endoscopic secretin test

In the period 30–45 min after intravenous administration of secretin (1 Clinical Unit/kg bodyweight, max. 70 CU. 1 CU = 2.9 μg Secretin pentachloride), duodenal juice was suctioned through an endoscope with the tip placed in the descending duodenum distal to the papilla vateri. The juice was collected in 3 aliquots of 5 min. This procedure is illustrated in Fig. 1 and described in detail elsewhere [12].

2.3. Handling of duodenal juice before analysis

The pH and amount of each sample was measured. Duodenal juice with pH < 6 was discarded due to possible pollution from gastric juice. The samples were added a mixture of protease inhibitors (cOmplete, Roche Diagnostics, Mannheim, Germany; 1 tablet dissolved in 1.5 ml water, 0.2 ml of this solution added per 1 ml duodenal juice), and were snap-frozen within five minutes after collection and stored on liquid nitrogen until analysis.

2.4. Analysis of amylase in duodenal juice

2.4.1. Semi-automated assay

Alpha-amylase activity was determined with the colorimetric end-point assay Phadebas Amylase test (Magle AB, Lund, Sweden). We used α-amylase from Bacillus sp. (Sigma-Aldrich, St. Louis, MO, USA) as standard, and physiological saline added 0.2% BSA (bovine serum albumin) and 20 mmol calcium chloride as assay solvent. Duodenal juice was diluted 1:100 with this solvent [8,13].

The analyses of catalytic activity of amylase were performed on microplate assays, using a Tecan Infinite M200 Pro microplate reader with iControl™ Tecan software (Tecan group Ltd, Männedorf, Switzerland). Samples were thawed on ice before

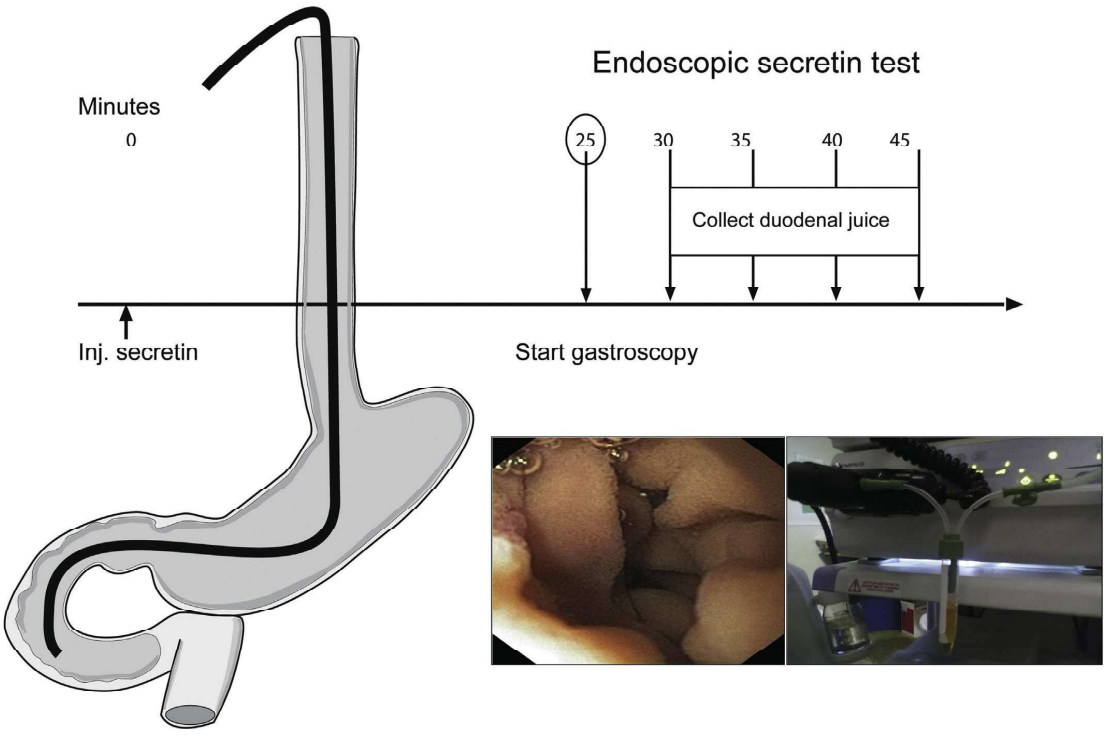


Fig. 1. Short endoscopic function test. Short endoscopic pancreas function testing: We started the procedure 25 min after injection of secretin (1 Clinical Unit/kg bodyweight, max. 70 CU. 1 CU = 2.9 μg Secretin pentachloride). During the first five minutes, all juice from stomach and duodenum was removed in combination with a diagnostic gastroscopy. Subsequently the tip of the endoscope was placed below the papilla for 15 min; from 30 to 45 min after secretin injection duodenal juice was sampled in three portions of 5 min. The endoscopic procedure lasted 20 min.

analyses. The samples were analyzed in triplicates, and related to four standards in duplicates (0.1, 0.5, 1 and 4 U/ml) and blanks. Baseline was determined from pairs of buffer and substrate (blank measurement samples). The limit for acceptance of the triplets' coefficient of variance (CV) was set at 10%.

2.4.2. Automated assay

Samples were diluted 1:300 with saline water before analyses with the COBAS c111 spectrophotometer (Roche Diagnostics; Mannheim; Germany) using the α-amylase EPS Pancreatic kit (Roche Diagnostics).

The kinetic method is based on inhibition of the activity of human salivary α-amylase by two different monoclonal antibodies, and cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)- α.

followed by hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100% chromophore liberation) [14]. The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity, and its absorbance is measured at 409 nm. PreciControl ClinChem Multi 1 and 2 (Roche Diagnostics) were used as controls.

2.4.3. Statistical methods

Values are expressed as median and interquartile range (IQR). We used a 5% level of significance, under this presumption a statistical power of >0.8 was acceptable. Student's *t*-test was used for simple comparisons between the two methods. Normality was checked by the Shapiro Wilk test. We calculated Spearman correlation, linear regression and Passing Bablok regression with Cusum test [15]. Bland Altman plot [16] was performed to test correlation and agreement between methods.

Measurands were expressed by the SI unit μkatal/l (μkat/l) [17]. Calculations were performed in ACCESS, EXCEL (Microsoft Office, Redmond WA, USA), and SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA). For Passing Bablok regression with Cusum test we used software from ACOMED statistics (Leipzig, Germany).

3. Results

We analyzed 54 samples from 19 patients. One sample was excluded because the coefficient of variance of one triplet in the Phadebas method was over the predefined limit of 10% and one sample was too viscous to be analyzed with the COBAS method (Fig. 2). Patients' demography is displayed in Table 1. The two different assays were performed at the same day and with samples from the same test tubes. Median time between EST and enzyme assays was 530 (range 463/642) days. Spearman Correlation between the Phadebas method and the COBAS method was 0.99 ($p < 0.001$). Linear regression showed $r = 0.99$ ($p < 0.001$). Passing Bablok regression revealed systematic and proportional differences between the two methods: Slope was 0.96 (95% CI 0.93/1), Intercept was 44.6 (95% CI 7.8/85.0). Cusum test indicated applicability of the Passing Bablok model ($p > 0.2$); (Fig. 3). Bland Altman plot unmasked a proportional BIAS in both sequences, where values > 3750 μkat/l tended to have weak accuracy (Fig. 4).

4. Discussion

Enzyme measurements are complex, and there are currently no standardized methods for direct molecular quantification. All present methods deal with catalytic enzyme activity under different predefined conditions. When introducing new and automated methods for amylase activity in duodenal juice, a defined standard method for calculation of correlation and agreement does not exist. To complicate the matters further, the amylase standards are derived from plants or animals, not humans. Every enzyme has its own catalytic activity characteristics. Presently more than 200 different methods exist for the measurement of α-amylase [18].

In this study, we demonstrate that automated measures of amylase in duodenal juice correlate excellent with the older and cumbersome manual method.

To our knowledge, this is the first study, which compares enzyme measurement in duodenal juice using the Phadebas versus the automated colorimetric method using Ethylidene as substrate and α-glucosidase as auxiliary enzyme in a COBAS c111 analyzer. The automated assay used in the COBAS c111 has been validated by comparing two assays for alpha amylase in blood and urine but not in duodenal juice [19]. This study from Winn-Deen et al. was technically well performed and highlighted different biochemical aspects. Statistical analyses for agreement are limited in this study, as Bland Altman plots were not used.

In 1983 Hafkenscheid compared the Phadebas method with another spectrophotometric method [20], using turbid duodenal

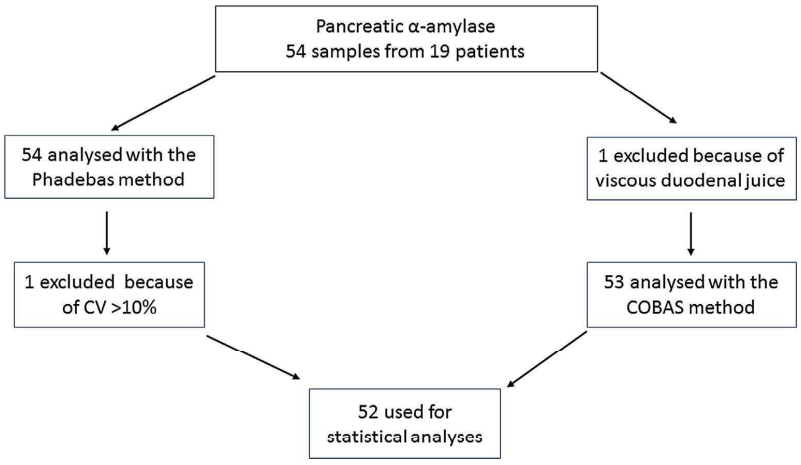


Fig. 2. Flowchart of samples for analyses of pancreatic α-amylase. Two were excluded before statistical calculations.

Table 1
Composition of patient groups: Difference of numbers between women and men ($p = 0.016$) and related test tubes ($p < 0.001$), difference in age is not statistically significant ($p = 0.116$).

Numbers (n)	Patients (Test Tubes)	Age (Range)	CP (Test Tubes)	Recurrent AP (Test Tubes)	Early CP (Test Tubes)	IBS (Test Tubes)	Other (Test Tubes)
All	19 (52)	46 (39/69)	4 (12)	3 (8)	1 (3)	5 (13)	6 (16)
Female	12 (32)	45 (39/68)	3 (9)	1 (3)	1 (3)	4 (10)	3 (7)
Male	7 (20)	58 (42/71)	1 (3)	3 (5)	0	1 (3)	3 (9)

CP = chronic pancreatitis; recurrent AP = recurrent acute pancreatitis; early CP = early chronic pancreatitis; IBS = irritable bowel syndrome; other: cystic fibroses; chologene diarrhea; abdominal pain; diabetic gastroparesis; acute pancreatitis (in patient history) with morphologic sequela; bacterial overgrowth with enteropathy and diarrhea.

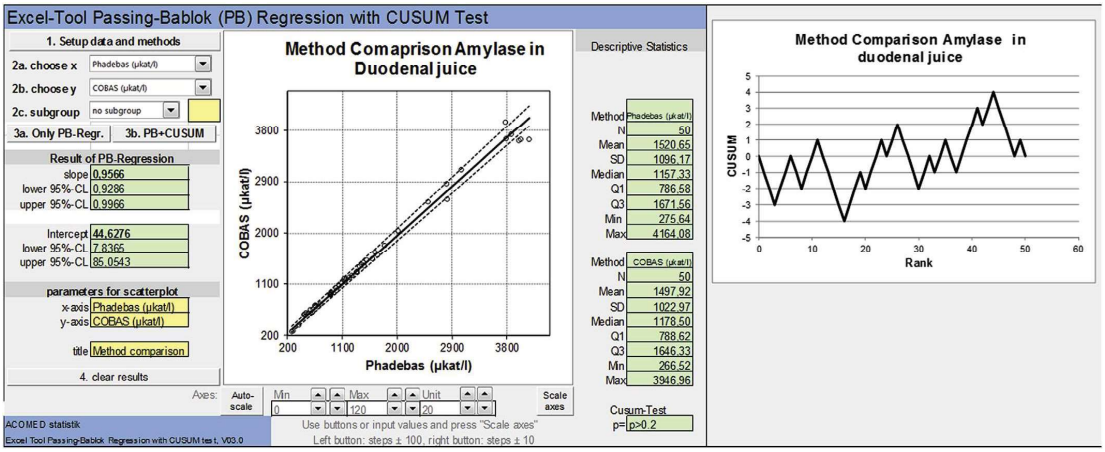


Fig. 3. Results Passing Bablok regression. A slope of 0.956 is confirming the excellent correlation as calculated in Spearman correlation and linear regression. Cusum test with $p > 0.2$ confirms linearity in the range of measurements. The 95% CI of the slope demasks a proportional BIAS.

juice (Lundh test), frozen to minus 20° Celsius without adding a protease inhibitor. We argue that use of protease inhibitor and snap freezing is of importance to prevent proteolytic degradation or inactivation of the enzyme. However, Hafkenscheid et al. demonstrated good agreement between the two methods. The sampling period takes much longer time in the Lund test compared to the short EST. A long sampling period may infer increased influence from proteolytic enzymes on the amylase activity.

In another study where Amylase was measured in duodenal juice from pigs, the Phadebas method was compared with an older modified method using potato starch as substrate. Correlation was good, but tests for agreement after Bland Altman were not performed [21].

In our study, units of α-amylase from bacillus sp. used in the Phadebas method are defined as follows: One unit will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. The assay used in COBAS c111 uses EPS as substrate and α-glucosidase as auxiliary enzyme, the method includes porcine amylase for calibration and controls with pooled human pancreatic amylase (PreciControl, Roche). One unit is the amount of amylase that cleaves ethylidene-pNP-G7 to generate 1.0 µmole of *p*-nitrophenol per minute at 25 °C [22]. The Phadebas method is a colorimetric endpoint assay, meanwhile the method used in the COBAS c111 is a kinetic method. Consecutively comparison between the Phadebas method and the assay used in COBAS c111 is difficult. In 1998 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) published an approved recommendation for the measurement of catalytic concentration of a-Amylase (1,4-a-D-Glucan 4-Glucanohydrolase, EC 3.2.1.1) [18]. Because of easy handling the method, using EPS as substrate and α-Glucosidase as in the COBASc111 got popular. Total human amylase is stable from pH 5 to 10,5 and its pH optimum is defined at pH 7 [23]. It is not clear if different pH in duodenal juice and blood has consequences for measurement in the automated method. It can be assumed that use of buffer overcomes this problem.

The International Measurement Evaluation Programme IMEP-17 in 2013 from the European commission showed considerable differences in measuring predefined concentration of amylase in blood. Especially the Phadebas method is mentioned as an outlier [24]. Our study displays near perfect correlation of the two different measurement methods of amylase in duodenal juice.

The Phadebas method did not include the use of quality controls, but every measurement series was related to activity of a predefined standard. There are no commercially available controls in the Phadebas method. We tried to use the controls of the COBAS method without success. This can, maybe be explained by another substrate used in the COBAS method and its low concentration and amount not suitable for the Phadebas method.

However, we claim that lack of controls in the Phadebas method and the differences of the two methods described above do not have serious consequences in practice as there was no difference between the grouped results ($p = 0.997$).

Testing agreement after Bland Altman requires measurands with the same units. Chemical substrate, reaction and standard in the two methods are different. This makes comparison of units challenging and international standardization to SI units is nearly impossible. Resolution 12 of the 21st CGPM (1999) defined katal as SI unit [17,25], where1 U/l = 0.0167 µkat/l = 0.0167 µmol/(s*l). This

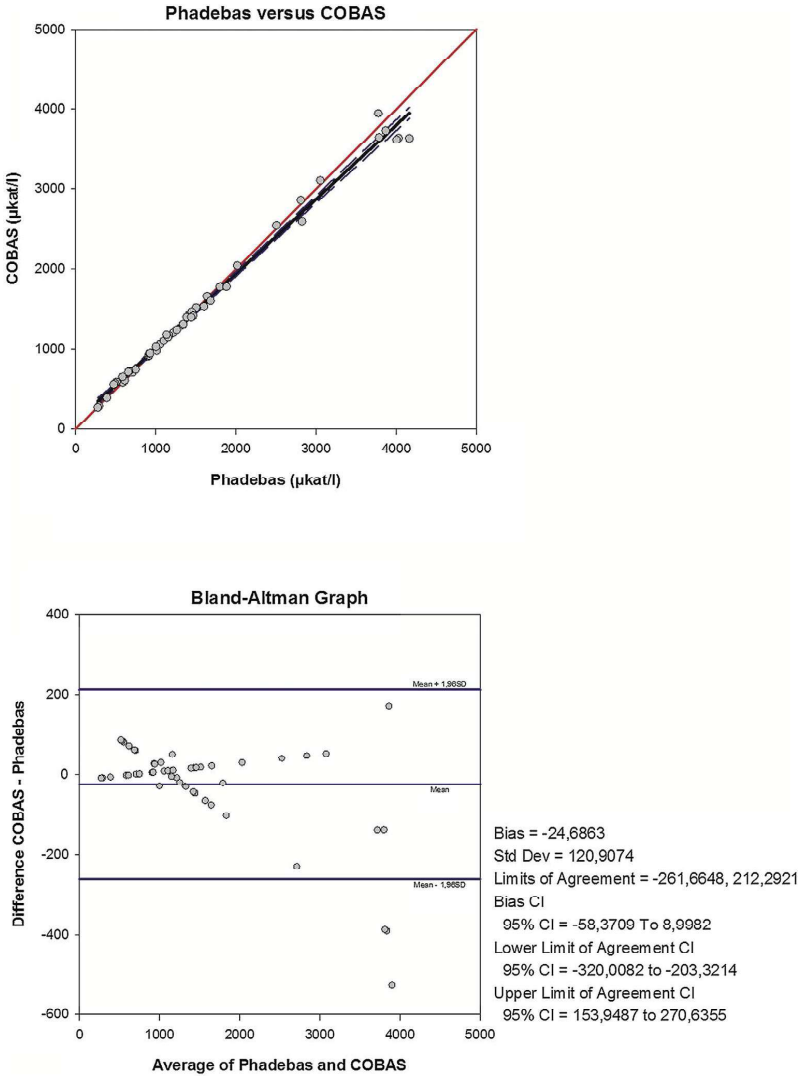


Fig. 4. Results Amylase Bland Altman Plot. Regression analyses confirmed the results of the Spearman correlation, linear-, and Passing Bablok regression. Values nearly fit the equality curve. The BIAS uncovered by absent normality in the linear regression and the two-sided 95% confidence limit of the slope in Passing Bablok regression is unmasked most clearly with the Bland Altman Plot. Values are expressed in µkat/l.

calculation is also valid for the two methods used in this study; (confirmed by Magle (Phadebas) and Roche (COBAS) by E-mail).

In our study, we also found concentrations of amylase >3750 µkat/l have to be considered with caution. Using pancreatic amylase in duodenal juice is to verify acinar exocrine pancreatic insufficiency. Only low values are of interest. Inaccuracy in high values does not have clinical implications.

4.1. Limitations

Time between EST and analyses of samples was 463–642 days. Although protease inhibitor was added to the samples and the storage was carried out in liquid nitrogen, enzyme degradation may have been a problem over this time-period. A study from Jung et al. describes higher amylase activity in human serum that have been frozen at −196 °C up to 10 months [26]. Other authors point out the velocity of freezing and thawing of purified amylase as an important factor [27]. Lorentz (IFCC) found that amylase is stable and single freezing of serum to −75 °C over 5 years did not change catalytic activity. On the other hand, the same author advices against freezing of urine and recommends analyses within 12 h at 20 °C or 5 days at 5 °C degrees' storage temperature. Duodenal juice is not mentioned in the IFCC recommendations indicating that optimal pre-analytic and analytic conditions remain unclear [18].

One study had an observation time of 12 months. Amylase in duodenal juice frozen to −80 °C or liquid nitrogen remained stable [28]. To our knowledge there is no study which illuminates the stability of pancreatic α-Amylase in duodenal juice after storing it in liquid nitrogen over the time we used in our study. However, we argue that any potential change in the amylase activity over this time period will not affect our results as our tests were performed at the same day and with samples from the same test tubes.

As we could not find any controls suitable for the Phadebas method, every measure sequence had to be adjusted to the standard in use. This problem could be unveiled with the Bland Altman plot; however, difference in sequences was not unacceptable.

4.2. Clinical implication

The old and manual Phadebas amylase assay in duodenal juice is time consuming, complex and expensive. Automation of analyses of ductal and acinar markers in duodenal juice is a substantial simplification, and opens for a standardized and widely distributed direct pancreas function test. Further standardization of the automated method is needed for comparison of the results between centers. Indirect, simpler test like fecal Elastase will still be first line for testing pancreatic function. Supplementary testing is needed in cases where the pitfalls of the indirect tests create uncertainty [29]. Shortened ESTs with a simplified analysis of the juice parameters in the ductal and acinar axis may be the solution. Some authors indicate that acinar function parameters are the most reliable in the assessment of early stage exocrine pancreatic failure [30–32]. Further studies are warranted to evaluate EST performance analyzing different pancreatic enzymes.

In this study, we conclude that automated measurement of α-amylase in duodenal juice can be performed in an easy and reliable way comparable to the manual methods.

Sources of financial support (i.e. grants) that require acknowledgment

Erchinger, Engjom and Dimcevski have received travelling grants and scholarships from the Norwegian Gastroenterological Association.

Acknowledgements

We want to thank especially to the supporting lab technicians Liv Aasmul and Aud Sissel Hjartholm for preserving and running Phadebas analyses of amylase in duodenal juice.

References

[1] Conwell DL, Zuccaro Jr G, Vargo JJ, Dumot JA, Vanlente F, Khandwala F, et al. Comparison of the secretin stimulated endoscopic pancreatic function test to retrograde pancreatogram. Dig Dis Sci 2007;52:1076–81.
[2] Chowdhury R, Bhutani MS, Mishra G, Toskes PP, Forsmark CE. Comparative analysis of direct pancreatic function testing versus morphological assessment by endoscopic ultrasonography for the evaluation of chronic unexplained abdominal pain of presumed pancreatic origin. Pancreas 2005;31:63–8.
[3] Keller J, Aghdassi AA, Lerch MM, Mayerle JV, Layer P. Tests of pancreatic exocrine function - clinical significance in pancreatic and non-pancreatic disorders. Best Pract Res Clin Gastroenterol 2009;23:425–39.
[4] Stevens T, Conwell DL, Zuccaro Jr G, Lewis SA, Love TE. The efficiency of endoscopic pancreatic function testing is optimized using duodenal aspirates at 30 and 45 minutes after intravenous secretin. Am J Gastroenterol 2007;102:297–301.
[5] Engjom T, Erchinger F, Tjora E, Laerum BN, Georg D, Gilja OH. Diagnostic accuracy of secretin-stimulated ultrasonography of the pancreas assessing exocrine pancreatic failure in cystic fibrosis and chronic pancreatitis. Scand J Gastroenterol 2015:1–10.
[6] Engjom T, Erchinger F, Laerum BN, Tjora E, Aksnes L, Gilja OH, et al. Diagnostic accuracy of a short endoscopic secretin test in patients with cystic fibrosis. Pancreas 2015;44:1266–72.
[7] Erchinger F, Engjom T, Tjora E, Hoem D, Hausken T, Gilja OH, et al. Quantification of pancreatic function using a clinically feasible short endoscopic secretin test. Pancreas 2013;42:1101–6.
[8] Tjora E, Wathle G, Engjom T, Erchinger F, Molven A, Aksnes L, et al. Severe pancreatic dysfunction but compensated nutritional status in monogenic pancreatic disease caused by carboxyl-ester lipase mutations. Pancreas 2013;7:1078–84.
[9] Tjora E, Wathle G, Erchinger F, Engjom T, Molven A, Aksnes L, et al. Exocrine pancreatic function in hepatocyte nuclear factor 1beta-maturity-onset diabetes of the young (HNF1B-MODY) is only moderately reduced: compensatory hypersecretion from a hypoplastic pancreas. Diabet Med 2013;30:946–55.
[10] Erchinger F, Engjom T, Gudbrandsen OA, Tjora E, Gilja OH, Dimcevski G. Automated spectrophotometric bicarbonate analysis in duodenal juice compared to the back titration method. Pancreatology 2016;16:231–7.
[11] Conwell DL, Lee LS, Yadav D, Longnecker DS, Miller FH, Mortele KJ, et al. American pancreatic association practice guidelines in chronic pancreatitis: evidence-based report on diagnostic guidelines. Pancreas 2014;43:1143–62.
[12] Erchinger F, Engjom T, Tjora E, Hoem D, Hausken T, Gilja OH, et al. Quantification of pancreatic function using a clinically feasible short endoscopic secretin test. Pancreas 2013;7:1101–6.
[13] Tjora E, Wathle G, Erchinger F, Engjom T, Molven A, Aksnes L, et al. Exocrine pancreatic function in hepatocyte nuclear factor 1beta-maturity-onset diabetes of the young (HNF1B-MODY) is only moderately reduced: compensatory hypersecretion from a hypoplastic pancreas. Diabet Med 2013;8:946–55.
[14] Pancreatic α-amylase: Roche diagnostics GmbH sandhofer straße 116 68305 Mannheim Germany. 2011.
[15] Passing H, Bablok. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, part I. J Clin Chem Clin Biochem 1983;21:709–20.
[16] Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet 1995;346:1085–7.
[17] Resolution 12 of the 21st CGPM (1999); Special name for the SI derived unit mole per second, the katal, for the expression of catalytic activity; The 21st Conférence Générale des Poids et Mesures: 1999.
[18] Lorentz K. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 9. IFCC method for alpha-amylase (1,4-alpha-D-glucan 4-glucanohydrolase, EC 3.2.1.1). Int Fed Clin Chem Laboratory Med (IFCC) Comm Enzym Clin Chem Lab Med 1998;36:185–203.
[19] Winn-Deen ES, David H, Sigler G, Chavez R. Development of a direct assay for alpha-amylase. Clin Chem 1988;34:2005–8.
[20] Hafkenscheid JC, Hessels M, van der Hoek EW. Determination of alpha-amylase, trypsin and lipase in duodenal fluid: comparison of methods. J Clin Chem Clin Biochem 1983;21:167–74.
[21] Gabert VM, Jensen MS. A comparison of two methods to measure amylase, lipase, trypsin, and chymotrypsin activity and the effect of freezing and thawing on enzyme activities in pancreatic juice. Pancreas 1997;15:183–90.
[22] Rauscher E, Bülow S, Hägele E, Neumann U, Schaich E. Ethylidene protected substrate for the assay of human a-amylase. Fresenius' Z für Anal Chem 1986;32:304–5.
[23] Sky-Peck HH, Thuvasethakul P. Human pancreatic alpha-amylase. II. Effects of pH, substrate and ions on the activity of the enzyme. Ann Clin Lab Sci 1977;7:310–7.
[24] Van Nevel L, Örnemark U, Smeyers P, Harper C, Taylor PDP. IMEP-17 trace and minor constituents in human serum. EUR 20657 EN, report to participants. Eorpean Commission; Directorate General JRC; Joint Research Centre; IRMM; Institute for Reference Materials and Measurements; 2003.
[25] Dybkaer R. The tortuous road to the adoption of katal for the expression of catalytic activity by the general conference on weights and measures. Clin Chem 2002;48:586–90.
[26] Jung K, Bader K, Grutzmann KD. Long-term stability of enzymes in human serum stored in liquid nitrogen. Enzyme 1984;31:209–16.
[27] Whittam JH, Rosano HL. Effects of the freeze-thaw process on alpha amylase. Cryobiology 1973;10:240–3.
[28] Ulleberg EK, Comi I, Holm H, Herud EB, Jacobsen M, Vegarud GE. Human gastrointestinal juices intended for use in in vitro digestion models. Food Dig 2011;2:52–61.
[29] Lankisch PG. Now that fecal elastase is available in the United States, should clinicians start using it? Curr Gastroenterol Rep 2004;6:126–31.
[30] Mitchell RM, Byrne MF, Baillie J. Pancreat Lancet 2003;361:1447–55.
[31] Lankisch PG. Natural course of chronic pancreatitis. Pancreatology 2001;1:3–14.
[32] Jensen NM, Larsen S. A rapid, endoscopic exocrine pancreatic function test and the Lundh test: a comparative study. Pancreatology 2008;8:617–24.

Grafisk design: Kommunikasjonsavdelingen, UiB / Trykk: Skipnes Kommunikasjon AS

uib.no

ISBN: 978-82-308-3744-3