



HANNA PELLI

Recurrence of Acute Alcoholic Pancreatitis

Rate, Characteristics and Risk Factors



ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the Small Auditorium of Building B,
Medical School of the University of Tampere,
Medisiinarinkatu 3, Tampere, on May 30th, 2008, at 12 o'clock.

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION

University of Tampere, Medical School

Tampere University Hospital, Department of Gastroenterology and Alimentary Tract Surgery

National Graduate School of Clinical Investigation (CLIGS)

Finland

Supervised by

Docent Isto Nordback

University of Tampere

Docent Juhani Sand

University of Tampere

Reviewed by

Docent Esko Kemppainen

University of Helsinki

Docent Pekka Miettinen

University of Kuopio

Distribution

Bookshop TAJU

P.O. Box 617

33014 University of Tampere

Finland

Tel. +358 3 3551 6055

Fax +358 3 3551 7685

taju@uta.fi

www.uta.fi/taju

http://granum.uta.fi

Cover design by

Juha Siro

Acta Universitatis Tamperensis 1312

ISBN 978-951-44-7306-7 (print)

ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 720

ISBN 978-951-44-7307-4 (pdf)

ISSN 1456-954X

http://acta.uta.fi

Tampereen Yliopistopaino Oy – Juvenes Print

Tampere 2008

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TABLE OF CONTENTS		page
LIST OF ORIGINAL COMMUNICATIONS		5
ABBREVIATIONS		6
DEFINITIONS		8
ABSTRACT		9
TIIVISTELMÄ		11
INTRODUCTION		13
REVIEW OF THE LITERATURE		15
1	Epidemiology of pancreatitis	15
2	Aetiology of pancreatitis	15
3	Pathophysiology of pancreatitis	16
4	Pathogenesis of alcoholic pancreatitis	17
4.1	Flow-reflux hypothesis of acute pancreatitis	18
4.1.1	Duodeno-pancreatic reflux	18
4.1.2	Biliary-pancreatic reflux	18
4.2	Ductal protein plug hypothesis of chronic pancreatitis	18
4.3	Toxic-metabolic hypothesis	19
4.4	Hypertriglyceridemia	20
4.5	Cholecystokinin and its regulators	20
5	From acute to chronic alcoholic pancreatitis	21
5.1	Necrosis-fibrosis hypothesis	21
5.2	Sentinel acute pancreatitis event hypothesis	22
5.3	Genetic disorders	22
5.4	Tobacco	23
6	Diagnosis for pancreatitis	23
6.1	Symptoms and signs	23
6.1.1	Acute pancreatitis	23
6.1.2	Chronic pancreatitis	23
6.2	Laboratory tests	24
6.2.1	Acute pancreatitis	24
6.2.2	Chronic pancreatitis	25
6.3	Radiology	26
6.3.1	Secretin stimulated magnetic resonance cholangio pancreatography	27
6.3.2	Radiology in clinical practice	27
6.4	Differentiating between acute and chronic alcoholic pancreatitis	28
7	Identification of the alcoholic aetiology	28
8	Severity classifications for acute pancreatitis	28
9	Classifications for chronic pancreatitis	29
10	Recurrent alcoholic pancreatitis	30

AIMS OF THE STUDY	31
PATIENTS AND METHODS	32
1 Study I - Retrospective analysis of recurrent alcoholic pancreatitis	32
2 Study II - Prospective analysis of risk factors for recurrent alcoholic pancreatitis	35
2.1 Follow-up	36
3 Study III – Pancreatic damage after the first acute alcoholic pancreatitis and its association with the later recurrences	42
3.1 Follow-up	42
4 Study IV - Do fasting cholecystokinin and a potential cholecystokinin releasing factor (DBI) expression associate with the recurrence of alcoholic pancreatitis?	44
4.1 Assay procedures	45
4.1.1 Plasma cholecystokinin analysis	45
4.1.2 RNA isolation and cDNA synthesis	45
4.1.3 Real-time quantitative PCR	45
5 The statistical analysis	47
6 Ethical aspects	47
RESULTS	48
1 Study I - Retrospective analysis of recurrent alcoholic pancreatitis	48
2 Study II - Prospective analysis of risk factors for recurrent alcoholic pancreatitis	51
2.1 Follow-up	52
3 Study III – Pancreatic damage after the first acute alcoholic pancreatitis and its association with the later recurrences	56
3.1 Endocrine pancreatic function	56
3.1.1 Patients with pre-existing diabetes	56
3.1.2 Patients with new diabetes or glucose metabolism impairment developed by the two-year visit	56
3.1.3 Diabetes control	57
3.2 Exocrine pancreatic function	57
3.3 Pancreatic morphology	58
4 Study IV - Do fasting cholecystokinin and a potential cholecystokinin releasing factor (DBI) expression associate with the recurrence of alcoholic pancreatitis?	62
DISCUSSION	64
1 Recurrence rate of acute alcoholic pancreatitis (I, II)	64
2 Risk factors associated with recurrent alcoholic pancreatitis (I, II, IV)	65
3 Follow-up after the first acute alcoholic pancreatitis and associations with recurrence (II, III)	67
SUMMARY AND CONCLUSIONS	73
ACKNOWLEDGEMENTS	75
REFERENCES	77
ORIGINAL COMMUNICATIONS	88

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following publications:

- I** **Pelli H**, Sand J, Laippala P, Nordback I. Long-term follow-up after the first episode of acute alcoholic pancreatitis: time course and risk factors for recurrence. *Scand J Gastroenterol* 2000; 35: 552 – 5.
- II** **Pelli H**, Lappalainen-Lehto R, Piironen A, Sand J, Nordback I. Risk factors for recurrent acute alcohol-associated pancreatitis: A prospective analysis. *Scand J Gastroenterol* 2008; 43: 614 – 21.
- III** **Pelli H**, Lappalainen-Lehto R, Piironen A, Sand J, Nordback I. Pancreatic damage after the first episode of acute alcoholic pancreatitis and its association with the later recurrence rate. (Submitted)
- IV** **Pelli H**, Herzig KH, Uotila S, Rätty S, Laine S, Sand J, Nordback I. Duodenal diazepam-binding protein expression and plasma cholecystokinin after alcoholic pancreatitis. *Scand J Clin Lab Invest* 2006; 66: 677 – 83.

ABBREVIATIONS

AAP	acute alcoholic pancreatitis
APACHE II	acute physiology and chronic health evaluation II score
AUDIT	alcohol use disorders identification test
BMI	body mass index
CCK	cholecystokinin
cDNA	complementary deoxyribonucleic acid
CT	computed tomography
DBI	diazepam binding protein
DM	diabetes mellitus
DNA	deoxyribonucleic acid
ERCP	endoscopic retrograde cholangio pancreatography
EUS	endoscopic ultrasonography
GHbA1C	glycosylated haemoglobin
GMI	glucose metabolism impairment
HR	hazard ratio
MRCP	magnetic resonance cholangio pancreatography
mRNA	messenger ribonucleic acid
N	number
OGTT	oral glucose tolerance test
OR	odds ratio
PaO ₂	arterial oxygen tension
PCR	polymerase chain reaction
RNA	ribonucleic acid
SADD	short alcohol dependence data
SEM	standard error of mean

SMRP	secretin stimulated magnetic resonance pancreatography
SOFA	sequential organ failure assessment
SPINK1	serine protease inhibitor, Kazal type 1
UNR	upper normal range
US	ultrasonography
WHO	World health organization

DEFINITIONS:

Acute pancreatitis is characterized by an acute inflammatory disease of pancreas with wide clinical variation from mild abdominal complaint to life threatening organ failure and infectious condition (Bradley 1993, Bollen 2008).

Chronic pancreatitis is a disease which is often characterized by chronic inflammatory lesions and by the destruction of exocrine pancreatic parenchyma, fibrosis and, at least in the late stages, the destruction of endocrine parenchyma which may lead to functional insufficiency (Sarles et al. 1989).

Recurrent pancreatitis diagnosis is given when patients have more than one clinical episode of acute pancreatitis (Levy and Geenen 2001).

ABSTRACT

The incidence of acute alcoholic pancreatitis in Finland is one of the highest in the world, the hospitalizations for acute pancreatitis increasing from 47 to 102 / 100 000 / year during last 30 years. There has also been an increase of annual alcohol consumption from 4.2 to 9.0 liters / inhabitant respectively. In Finland 68 % of the patients with pancreatitis were heavy alcohol consumers. During the holiday seasons both the alcohol consumption and the incidence of alcoholic pancreatitis are at their highest. The aim of this study was to investigate the recurrence rate, risk factors and behaviors associated with recurrent acute pancreatitis.

The recurrence rate and pattern were analyzed retrospectively in 1972 – 1991 when altogether 562 patients out of 1555 alcoholic pancreatitis patients had their first episode of acute alcoholic pancreatitis in Tampere University Hospital. Overall 46% developed recurrent disease. Younger patients were at the highest risk for recurrence. Patients, whose first episode of alcoholic pancreatitis was mild, were at a higher risk in developing multi recurring pancreatitis.

The association of the risk factors in patients with their first episode of acute alcoholic pancreatitis was analyzed prospectively for recurrences in 68 patients, who survived the first acute episode in January 2001 - January 2004. Abstinence from alcohol protected from recurrent pancreatitis during a 2 – 5 years follow-up. Patients who developed recurrent acute pancreatitis had increased dependency on alcohol as measured during the first episode of acute alcoholic pancreatitis and in two years compared to those who did not develop recurrence during the follow-up. These findings should direct the therapy against heavy alcohol consumption, preferring abstinence, after the first episode of acute alcoholic pancreatitis.

Pancreatic endocrine and exocrine function, morphology and their association to the recurrent acute pancreatitis were prospectively studied early and at two years after the first episode of acute alcoholic pancreatitis in 54 patients. Thirty five per cent of the patients

developed new impairment of glucose metabolism after the first episode. The severity of the first episode was not associated with either pancreatic endocrine or exocrine function and the latter considerably recovered within two years after the first episode of acute alcoholic pancreatitis. The number of patients with chronic changes in secretin stimulated magnetic resonance pancreatography (SMRP) increased independent on alcohol consumption. Chronic pseudocyst seen in SMRP at two years was a significant risk factor for recurrent pancreatitis later on, which is why their therapy needs to be studied even in asymptomatic patients.

The possible association between pancreatic stimulating hormone plasma cholecystokinin (CCK) concentration and its releasing factor diazepam binding protein (DBI) expression levels in duodenum in patients with the acute alcoholic pancreatitis or its recurrence were compared to healthy controls and people with heavy alcohol consumption but no pancreatitis in their history. There were no changes in the fasting CCK plasma concentrations or DBI expression levels. This may suggest that they do not play a major role as risk factors for acute alcoholic pancreatitis or its recurrence.

Preventing acute alcoholic pancreatitis from recurring has not been effective partly due to the lack of recognizing the patients that are at highest risk. In this study it has been demonstrated that the patients who have high dependency on alcohol or develop a pseudocyst after the first acute alcoholic pancreatitis are in the highest risk of developing the recurrent pancreatitis. Thus this study warrants for further studies for helping the patients, especially those of young age, during the first episode of acute alcoholic pancreatitis to reduce their alcohol dependency and if complications such as pseudocysts develop, even asymptomatic, for managing them in more invasive treatments.

TIIVISTELMÄ

Akuutin alkoholihaimatulehduksen ilmaantuvuus on Suomessa korkeimpia maailmassa. Sairaalahoitajaksojen määrä on lisääntynyt 47 - 102 / 100 000 / vuosi viimeisten 30 vuoden aikana. Haimatulehdusten sairaalahoitajaksojen lisääntymisen ja alkoholin kulutuksen lisääntymisen (4.2 - 9.0 litraa / asukas / vuosi) välillä on ollut positiivinen yhteys näinä vuosina. Suomessa 68 % haimatulehdukseen sairastuneista on alkoholin suurkuluttajia. Lomakuukausina sekä alkoholin kulutus että haimatulehdusten sairaalahoitajaksojen määrä ovat korkeimmillaan. Tämän tutkimuksen tavoitteena oli selvittää alkoholin aiheuttaman äkillisen haimatulehduksen uusiutumisen yleisyys ja siihen yhteydessä olevat riskitekijät.

Uusimisen yleisyyttä ja riskitekijöitä arvioitiin takautuvasti vuosilta 1972 - 1991. Yhteensä 1555 äkilliseen alkoholihaimatulehdukseen sairastuneista 562 potilaalla oli kyseessä ensimmäinen haimatulehdus. Yhteensä 46 %:lle heistä kehittyi uusi äkillinen haimatulehdus. Nuoremmilla potilailla oli suurin riski kehittää uusintatulehdus. Jos ensimmäinen äkillinen alkoholin aiheuttama haimatulehdus oli lievä, olivat nämä potilaat suurimmassa riskissä kehittää useita uusia äkillisiä haimatulehduksia.

Ensimmäisestä äkillisestä alkoholin aiheuttamasta haimatulehduksesta tammikuu 2001 – tammikuu 2004 selvinneitä 68 potilasta seurattiin 2 – 5 vuoden ajan ja heidän sairastumisensa uudelleen rekisteröitiin. Pidättäytyminen alkoholin käytöstä kokonaan ensimmäisen haimatulehduksen jälkeen suojasi sairastumasta uudestaan. Potilaat, joille kehittyi uusinta haimatulehdus, olivat voimakkaammin riippuvaisia alkoholinkäytöstä eikä tämä riippuvuus vähentynyt seurannan aikana. Näiden löydösten tulisi ohjata hoitoa ensimmäisen äkillisen alkoholin aiheuttaman haimatulehduksen jälkeen.

Haiman sisä- ja ulkoeritteisen toiminnan muutosten ja kuvantamislöydösten yhteyttä äkillisen haimatulehduksen uusiutumiseen arvioitiin ensimmäisen äkillisen alkoholin aiheuttaman haimatulehduksen yhteydessä ja kahden vuoden kuluttua sairastumisesta 54 potilaalla. Näistä 35 %:lla todettiin kahden vuoden kohdalla heikentynyt glukoosi-

aineenvaihdunta. Sisä- ja ulkoeritteiseen toimintaan ensimmäisen äkillisen haimatulehduksen vaikeudella ei ollut yhteyttä. Magneettikuvauksessa todettujen kroonisten haimatulehduslöydösten määrä lisääntyi kahdessa vuodessa riippumatta alkoholin käytöstä. Jos magneettikuvauksessa todettiin krooninen haiman pseudokysta kahden vuoden kuluttua, niin tämä oli riskitekijä uusivalle äkilliselle haimatulehdukselle myöhemmin. Tämä ohjaa arvioimaan haimatulehduksen jälkeisen pseudokystan hoitoa oireettomillakin potilailla.

Haiman asinus-soluja stimuloivan hormonin kolekystokiniinin (CCK) pitoisuuden ja sen vapautumista säätelevän diatsepaamia sitovan proteiinin (DBI) geenin ekspressiota pohjukaissuolessa välistä yhteyttä selvitettiin äkillisen alkoholin aiheuttaman haimatulehduksen uusiutumiseen 44 tutkimushenkilöllä. Tällaista yhteyttä ei todettu, joten tämä saattaa osoittaa, etteivät nämä ole merkittäviä riskitekijöitä äkilliselle alkoholin aiheuttamalle haimatulehdukselle.

Hyviä keinoja äkillisen alkoholin aiheuttaman haimatulehduksen uusiman ehkäisemiseksi ei ole, osin koska uusimisen riskitekijöitä ei ole todettu. Tässä tutkimuksessa todettiin, että ne potilaat, jotka olivat voimakkaimmin riippuvaisia alkoholin käytöstä ja potilaat, joilla todettiin haiman pseudokysta ensimmäisen äkillisen alkoholin aiheuttaman haimatulehduksen jälkeen, olivat suurimmassa riskissä sairastua uusimaan. Alkoholiriippuvuuden hoidon ja oireettomienkin potilaiden haiman pseudokystien hoidon arviointi äkillisen uusiutuvan haimatulehduksen ehkäisyksi on syytä jatkossa selvittää.

INTRODUCTION

Acute and chronic alcoholic pancreatitis are conditions which may have serious effects on the individual, and the treatment involves time, labour and financial resources (Pezzilli et al. 2006, Lilja et al. 2008). The mortality rate in severe acute pancreatitis is still high varying from 16 – 42 % (Fu et al. 2007, Harrison et al. 2007 and Lilja et al. 2008). A part of the patients will develop recurrent acute pancreatitis after the first episode of acute alcoholic pancreatitis, but the patients in the highest risk for the recurrence have not been identified (Gullo et al. 2002a and 2002b, Gislason et al. 2004).

In Finland the incidence of acute pancreatitis is high and has been increasing during the past 30 years from 47 to 102 pancreatitis episodes / 100 000 inhabitants / year (Jaakkola and Nordback 1993, Sand et al. 2006). Alcohol is the aetiology in about 70 % of the cases (Jaakkola et al. 1993). The changes in the nationwide alcohol consumption correlate with the number of the pancreatitis episodes (Jaakkola and Nordback 1993, Sand et al. 2006).

The role of alcohol use before the first attack as a risk factor for recurrent attacks or the association of continuing alcohol consumption after the first pancreatitis with the recurrent pancreatitis have not been studied. Besides alcohol, there may also be other possible risk factors for the recurrent pancreatitis. The risk factors may include e.g. smoking, obesity, endocrine and exocrine pancreatic malfunction, later development of gall stones or variability in regulative hormones to pancreatic secretion, such as cholecystokinin (CCK) (Herzig et al. 1996, Herzig 1998, Talamini et al. 2000, Lindkvist et al. 2008). Furthermore, acute pancreatitis may result in morphologic changes such as ductal strictures, pseudocysts and inflammatory masses, the role of which as a risk factor for recurrent pancreatitis has not been established (Maejima et al. 1996).

One should recognise the pattern of relapse and the patients at the greatest risk in getting recurrent acute pancreatitis already when released from the hospital after the first

episode of acute alcoholic pancreatitis. This might also help in the attempts to understand the pathogenesis of recurrent disease as well as in preventing relapses.

REVIEW OF THE LITERATURE

1 Epidemiology of pancreatitis

There is a great variety in epidemiology of acute pancreatitis in different countries. The recent studies vary in incidence between 15 and 44 / 100 000 outside of Finland (Appelroos and Borgström 1999, Gullo et al. 2002a and 2002b, Andersson et al. 2004, Gislason et al. 2004, Lund et al. 2006). For chronic pancreatitis the incidence varies from 3.5 to 10 / 100 000 (Witt et al. 2007). Thus it seems that the incidence of pancreatitis in Finland is one of the highest in the world, the hospitalizations for acute pancreatitis increasing from 47 to 102 / 100 000 / year in 1989 – 2001, and the incidence of chronic pancreatitis has increased in 1977 – 89 from 10 to 13 pancreatitis episodes / 100 000 inhabitants / year (Jaakkola and Nordback 1993, Sand et al. 2007a).

There has been a positive correlation in the incidence of pancreatitis in Finland from 47 to 102 pancreatitis episodes / 100 000 inhabitants / year (1970 – 2001) and the increase of annual alcohol consumption from 4.2 to 9.0 litres / inhabitant respectively during the last 30 years (Jaakkola et al. 1993, Sand et al. 2006). In Finland 68 % of the patients with acute pancreatitis were heavy alcohol consumers (Jaakkola et al. 1993). Also during the holiday seasons both the alcohol consumption and the incidence of alcoholic pancreatitis are at their highest (Räty et al. 2003).

2 Aetiology of pancreatitis

Many different conditions, such as gallstones, tumour, pancreas divisum, hypertriglyceridemia, hypercalcemia, heredity, trauma, medication, viral infection etc. can be aetiological factors for pancreatitis and these factors may also co-exist with heavy alcohol consumption (Nordback et al. 2007). A classification system for the aetiological risk factors of chronic pancreatitis including toxic-metabolic (including heavy alcohol consumption),

idiopathic, genetic, autoimmune, recurrent and severe acute pancreatitis or obstructive conditions (TIGAR-O) has been developed (Chari et al. 1994).

Defining alcohol as the aetiology of the pancreatitis has not been easy. A consensus statement was made in the international European Pancreatic Club meeting in 2006 where it was accepted to use three categories for alcoholic aetiology. The aetiology of alcohol for the pancreatitis can be probable (with heavy alcohol consumption), possible (with less alcohol consumption) and non-alcoholic (with negligible alcohol consumption) (Nordback et al. 2007). In previous literature preceding the consensus meeting the definition for alcoholic pancreatitis may thus vary.

3 Pathophysiology of pancreatitis

In physiologic situation the digestive enzymes that the pancreas produces are located in pro-enzyme form in zymogen granules. Pro-enzymes are transported to duodenum according to recent studies where enterokinase starts the cascade for their activation (Raraty et al. 2005, van Acker et al. 2006). The zymogens inside the acinar cells are bound to calcium ions. Calcium concentration is lower in cytosol than in extracellular space or inside the endoplasmic reticulum. When hyperstimulation or other triggering elements occur, calcium is released from the cellular structure into cytosol. In calcium overload ATP production by the mitochondria is decreased and thus the ability to buffer the excess calcium is reduced (Pandol et al. 2007, Mukherjee et al. 2008). A formation of vacuoles appears containing zymogens and lysosomal enzymes. In the vacuoles there are lysosomal hydrolases such as cathepsin B which can activate trypsinogen into trypsin during this co-localization (Raraty et al 2005, van Acker et al. 2006). Active trypsinogen is normally inhibited inside the cell both by specific inhibitor mechanism (pancreatic secretory trypsin inhibitor gene (the serine protease inhibitor, Kazal type 1 (SPINK1)) and increasing autoactivation by trypsin cleavage. In a co-localization situation these mechanisms are inadequate in relation to active trypsin, which further activates

other zymogens resulting in cell injury, autodigestion and pancreatitis (Raraty et al. 2005 and van Acker et al. 2006).

The active digestive enzymes in the pancreas stimulate the immune system response by attracting leucocytes in response to e.g. Toll like receptors recognizing danger signals and inducing the formation of pro-inflammatory cytokines (tumour necrosis factor, interleukin 1 etc.). Cytokines are then released into the circulation where the higher concentration is associated with the severity of the pancreatitis and circulating cytokines are affecting the systemic inflammatory response. They also may participate in the pancreatic injury (Saluja and Steer 1999, Raraty et al. 2005, Szabo et al. 2007).

4 Pathogenesis of alcoholic pancreatitis

Although the association between the amount of alcohol consumption and the development of pancreatitis is clear in the population level, it is less clear in the individual level. Altogether only 5 % of the people with heavy alcohol consumption will develop pancreatitis and it is still an unsolved question who are the people in the highest risk for pancreatitis (Dreiling and Koller 1985). It has been shown, that the heavier the alcohol consumption had been before the first episode of acute alcoholic pancreatitis the more severe the pancreatitis was (Jaakkola et al. 1994b). Also, the heavier the consumption has been the more rapid is the appearance of symptoms leading to the diagnosis of acute pancreatitis after the cessation of alcohol (Nordback et al. 2005).

There are several different theories to explain the pathogenesis of acute alcoholic pancreatitis. In different studies there has been an attempt to create an animal model of the alcoholic pancreatitis. Continuous alcohol feeding produced acinar cell vacuolization and lipid droplets in acinar cells but no pancreatitis was found (Norton et al. 1998, He et al. 2001). When alcohol administration was combined with e.g. cerulein injections in animal studies

alcohol sensitised the pancreas to cerulein induced pancreatitis (Pandol et al. 1999, Werner et al. 2002, Perides et al. 2005).

4.1 Flow-reflux hypothesis of acute pancreatitis

4.1.1 Duodeno-pancreatic reflux

In this hypothesis it was assumed that ethanol could reduce the pressure level of sphincter Oddi and thus allow the duodenal content to reflux into the pancreatic duct. In the duct enterokinase would activate the pancreatic proenzymes and cause pancreatitis. This hypothesis was criticised for an unphysiological model used to support it. Also the relaxation caused by alcohol was not considered to be effective enough to allow reflux and this hypothesis was soon rejected (Pfeffer et al. 1957, Wisniewski et al. 1963).

4.1.2 Biliary-pancreatic reflux

The other approach to the effects of ethanol is that it would produce a spasm into the sphincter of Oddi and due to the common channel the bile would reflux to the pancreatic duct causing a pancreatitis (Opie 1901, Wilson et al. 1989). It was shown that when bile was injected into a rat pancreatic duct it would cause pancreatitis (Jalovaara and Apaja 1978). There has also been a lot of criticism to this hypothesis, since all patients do not have a common channel in the papilla and the hypothesis has been related more with the acute biliary pancreatitis than acute alcoholic pancreatitis (Lerch et al. 1992).

4.2 Ductal protein plug hypothesis of chronic pancreatitis

According to this hypothesis the secretion of the protein rich pancreatic fluid is increased and it would clot the small pancreatic ductules leading to obstruction (even stones) and cause inflammation and pancreatitis (Allan and White 1974, Renner et al. 1978). It might be more of a pathogenesis hypothesis for a chronic pancreatitis, since healthy individuals

showed protein plugs in 4 %, alcoholics in 21 % and chronic pancreatitis patients in 59 % of the cases (Guy et al. 1983). The primary plug theory has been later criticized and nowadays a more belief is that the plug formation would only happen in the late course of chronic pancreatitis (Pitchumoni 2001).

4.3 Toxic-metabolic hypothesis

Ethanol or its metabolites might also have a direct toxic effect on the pancreatic cells causing the pancreatitis changes (Noronha et al. 1981). Ethanol metabolism occurs also in pancreas. Through the oxidative pathway one of the metabolites is acetaldehyde and through the non-oxidative pathway ethyl esters are formed. Acetaldehyde formation induces the formation of active xanthine oxidase with production of free radicals that are thought to increase the oxidative stress in the pancreas and thus cause pancreatitis (Nordback et al. 2001, Braganza et al. 1995). With pre-treatment of the free radical scavengers (superoxide dismutase and catalase) the experimental pancreatitis induced by acetaldehyde could be ameliorated (Nordback et al. 2001). In the diet of the alcoholic people there are not many antioxidants to overcome the oxidative stress for the alcohol metabolism (Lieber 2003). However, in an in vivo model the accumulation of acetaldehyde in ethanol fed rat pancreas did not cause pancreatitis changes (He et al. 2001).

Fatty acid ethyl esters are formed in the pancreas as a result of non-oxidative ethanol metabolism. Fatty acid ethyl esters are formed in the acinar cells in a rate that can induce cell damage and pancreatitis (Haber et al. 2004). Especially fatty diet and heavy alcohol consumption seem to accelerate pancreatic hyper stimulation yet there has not been shown any increased risk for pancreatitis in human or animal studies (Pitchumoni et al. 1980, Wilson et al. 1985, Cronholm et al. 1988). When the test animals were on alcohol diet they developed more cholesteryl ester in the cellular cytoplasm and calcium regulation was lost (Wilson et al. 1988). This seemed to cause pancreatic hyper stimulation (Cronholm et al. 1988). Ethanol diet

has decreased the trypsin inhibition mechanism and increased the concentration of the proteolytic and lysosomal enzymes. This has been thought to lead to acinar cell injury both in human and in animal models (Rinderknecht et al. 1979, Singh et al. 1982).

Like the acetaldehyde hypothesis also the fatty acid ethyl ester hypothesis fails to explain why only about 5 % of the people with heavy alcohol consumption will develop pancreatitis (Dreiling and Koller 1985).

4.4 Hypertriglyceridemia

It has been noticed by Dickson et al. that 4.5 % of the patients with alcoholic pancreatitis patients had hypertriglyceridemia (Dickson et al. 1984). It has been speculated whether the hypertriglyceridemia was the cause for or consequence of pancreatitis (Saharia et al. 1977). It was shown that if the use of alcohol was ceased the lipid balance improved (Dickson et al. 1984). Chronic alcohol consumption itself already can cause disturbances in pancreatic lipid metabolism (Simsek and Singh 1990). Hypertriglyceridemia however, is probably seldom able to function alone as the mediator of acute alcoholic pancreatitis.

4.5 Cholecystinin and its regulators

Cholecystinin is secreted from the duodenum. It induces the gallbladder contraction, the sphincter of Oddi relaxation and the stimulation of pancreatic secretion (Liddle 1997, Laugier et al. 1998). CCK stimulates human pancreas through vagal neural pathways but direct humoral effect on acinar cells may be lacking. It is believed that ethanol sensitises acinar cells to physiological concentrations of CCK (Pandol et al. 1999). CCK mediated acinar cell injury was present in rats that were chronically ethanol fed and injected with supraphysiological doses of CCK-8 but not in rats with no ethanol in diet (Pandol et al. 1999). This injury was mediated by mobilizing intracellular calcium and also by activating protein kinase C pathway

which then mediated the nuclear regulatory factor κ B activation, a transcription factor of inflammation (Pandol et al. 2007).

CCK agonist, caerulein, has been used to induce acute pancreatitis in animals (Nordback et al. 1991). After endoscopic retrograde cholangio pancreaticography (ERCP) plasma CCK concentration increased significantly within the first 24 hours in the patients who developed post ERCP pancreatitis, compared to those who did not develop acute pancreatitis, and then plasma CCK concentration declined to immeasurable levels which has been detected in other aetiologies (Räty et al. 1999 and 2000).

There are three possible CCK releasing peptides that are secreted from the proximal small intestine (Herzig 1998). The sequence information of diazepam binding inhibitor (DBI) is known. DBI expression can be evaluated in the duodenal epithelial cells by RNA isolation and cDNA synthesis. The sequence information of luminal CCK releasing factor is not known. Monitor peptide has been suggested not to play a role in pancreatitis (Herzig et al. 1996, Li and Owayang 1996). The role of CCK and its regulators is, however, far from clear in the pathogenesis of acute pancreatitis in a human being. It is not known, for example, whether there are differences in the regulation of CCK release in people with heavy alcohol consumption with or without alcoholic pancreatitis.

5 From acute to chronic alcoholic pancreatitis

5.1 Necrosis-fibrosis hypothesis

Repeated acute alcoholic pancreatitis episodes may lead to acinar cell necrosis and fat necrosis and the healing process eventually leads to fibrosis in the pancreas presenting progressive development of chronic pancreatitis (Comfort et al. 1946, Klöppel and Maillet 1991). This hypothesis is supported by a study where the autopsies of 247 patients who had died of acute alcoholic pancreatitis and even though chronic pancreatitis changes were found in

47 % of the patients, 53 % of them showed no histological evidence of that (Renner et al. 1985).

5.2 Sentinel acute pancreatitis event hypothesis

The lack of evidence of necrosis in patients with alcoholic pancreatitis as well as in patients with hereditary pancreatitis was a reason for this hypothesis to be developed (Whitcomb 1999). It is based on the course of hereditary pancreatitis that is then compared to the alcoholic chronic pancreatitis and tries to explain why only about 10 % of the patients with acute alcoholic pancreatitis develop into symptomatic chronic pancreatitis (Klöppel 1999, Whitcomb 1999). It is suggested that in these cases there is such a severe acute event in the pancreas which initiates a cascade. During the acute pancreatitis monocytes and macrophages are recruited, cytokine release triggers the activation of the pancreatic stellate cells and fibrosis formation thus leading to the development of chronic pancreatitis as found also in animal models (Whitcomb 1999, Apte and Wilson 2003 in rats). Stellate cells can also be activated directly by ethanol through acetaldehyde and oxidative stress (Apte and Wilson 2003).

5.3 Genetic disorders

The genetic polymorphisms that have most usually been connected with pancreatitis are variations in the cationic trypsinogen gene and SPINK1 gene that both function as part of the trypsin inhibitory mechanism and also the cystic fibrosis transmembrane conductance regulator gene that normally regulates ductal bicarbonate secretion (Whitcomb 1999). Repeated acute pancreatitis episodes eventually lead to chronic pancreatitis (Etemad and Withcomb 2001).

In Finland 9 % of the patients with acute alcoholic pancreatitis had polymorphism in SPINK1 gene, in locus N34S (Tukiainen et al. 2005). In chronic alcoholic pancreatitis patients in Finland the polymorphism was 10 % whereas the percentage of the healthy controls was 3 % (Lempinen et al. 2005). These studies, however, do not have a control group with alcoholic

pancreatitis to patients with heavy alcohol consumption but no pancreatitis. Furthermore, at most these changes in SPINK1 gene suggest a mechanism only in few patients with alcoholic pancreatitis.

5.4 Tobacco

Out of patients with acute alcoholic pancreatitis 78 % and of the patients with chronic alcoholic pancreatitis, 92 % smoke cigarettes (Talamini et al. 2000). Smoking has been shown to increase the formation of the free radicals and contributing to ischemia in the pancreas. There is evidence of smoking being a risk factor for chronic pancreatitis, and it might be associated with acute pancreatitis as well (Pitchumoni 2000, Hartwig et al. 2000, Lindkvist et al. 2008).

6 Diagnosis for pancreatitis

6.1 Symptoms and signs

6.1.1 Acute pancreatitis

The clinical picture of acute pancreatitis typically includes upper abdominal pain that can radiate to the back, causing nausea, vomiting, fever, ileus, abdominal tenderness and jaundice. Ecchymosis in body wall, on the flank or umbilical area (Grey–Turner or Cullen sign) may occur in severe acute pancreatitis (Bradley 1993). The symptoms and signs are indicative at their best and the diagnosis for acute pancreatitis cannot be clearly assessed without further tests (Bradley 1993, Steinberg and Tenner 1994).

6.1.2 Chronic pancreatitis

Typically patients with chronic pancreatitis have epigastric pain especially postprandially, but about 10 % of the chronic pancreatitis patients do not experience abdominal pain (Schneider and Singer 2005). There can be alterations in the intensity of pain and

especially during acute bursts of chronic pancreatitis. Pain can be very intense requiring opioids for pain relief (Ammann 2006). Steatorrhea and weight loss are usually present in the end stage of chronic pancreatitis (Lankisch 1999, Etemad and Withcomb 2001, Ammann 2006).

6.2 Laboratory tests

6.2.1 Acute pancreatitis

In clinical use serum or urine amylase activity has become a widely used diagnostic marker for acute pancreatitis. Amylase levels arise within one or two days and return back to normal within three to five days (Nordback 1985, Matull et al. 2006). The sensitivity of amylase testing is decreased in patients with alcohol abuse and also other intra-abdominal inflammatory conditions can increase the serum amylase levels (Kemppainen et al. 1998, Yadav et al. 2002). In a mild pancreatitis the sensitivity of serum amylase measurement can also be decreased and thus lower increase may support the acute pancreatitis diagnosis (Clavien et al. 1989). Widely accepted serum amylase level supporting the diagnosis for acute pancreatitis is at least three times the upper normal range (UNR) which would provide sensitivity of 61 - 90 % and specificity of 95 % (Lin et al. 1989, Yadav et al. 2002, Matull et al. 2006).

Serum lipase activity has been shown to stay increased for longer even till 8-14 days after the onset of the disease (Frank and Gottlieb 1999). Other intra-abdominal conditions can also increase serum lipase activity. The sensitivity and the specificity of lipase seem to be similar to amylase 55 – 100 % and 95 % respectively (Kylänpää-Bäck et al. 2002, Matull et al. 2006).

Serum or urine trypsinogen-2 levels increase during acute pancreatitis within a few hours and decrease gradually reaching the upper reference concentration (90 µg/l) in 30 days depending on severity of pancreatitis and over nine days in mild pancreatitis (Kemppainen et

al. 2000). The sensitivity of the urine trypsinogen-2 strip test at 50 µg/l was 94 % and specificity 95 % (Kemppainen et al. 1997).

Serum or plasma amylase activity is most commonly used in Finnish hospitals. There needs to be awareness for the somewhat low sensitivity, when the cut off point three times UNR is used, as well as the decrease of the activity level within a few days after the onset of the disease. Understanding these conditions serum amylase activity provides a good and economic tool for diagnosing acute pancreatitis.

6.2.2 Chronic pancreatitis

Serum amylase activity does not differ between healthy individuals and chronic pancreatitis patients with or without functional insufficiency. Serum lipase activity was significantly lower in patients with chronic pancreatitis and functional insufficiency when compared to healthy individuals or chronic pancreatitis patients without functional disorders giving sensitivity of 92 % and specificity of 100 % (Lesi et al. 1985).

Exocrine function has been measured either with direct (pancreatic juice secretion into the duodenum) or indirect tests (pancreatic enzymes or enzyme metabolism products in stool, urine or serum). The secretin-pancreozymin test is considered “a golden standard” of the direct tests. Pancreatic juice is collected after secretin and cholecystokinin-pancreozymin stimulation by endoscopy and volume, pH, bicarbonates, calcium, lipase, trypsin, chymotrypsin, elastase-1 and amylase can be measured. This test is invasive and time consuming which reduces its use (Lüth et al. 2001, Lankisch 2003). Of the indirect exocrine pancreatic function tests the use of faecal elastase-1 has increased. Human elastase-1 is measured by enzyme linked immunosorbent assay from stool and correlates well with the secretin-pancreozymin test in moderate or severe chronic pancreatitis (Lüth et al. 2001, Lankisch 2003). In case of severe maldigestion and steatorrhea deficiency in fat soluble vitamins (e.g. vitamin A and E) may occur (Meier and Beglinger 2006).

Endocrine function tests such as fasting glucose and glucose tolerance test often show abnormalities in more advanced chronic pancreatitis (Etemad and Withcomb 2001 and Ammann 2006). Insulin production can be evaluated by cleavage product C-peptide, which is often reduced in moderate or severe chronic pancreatitis (Angelopoulos et al. 2005). Glycosylated haemoglobin has also been used as a marker for diabetic control in longer term (Jeffcoate 2003).

6.3 Radiology

The abdominal ultrasound (US), magnetic resonance cholangio pancreatography (MRCP) or endoscopic US (EUS) are used primarily to evaluate the aetiology of the pancreatitis especially to detect the gallstones and biliary obstruction. EUS is also very useful in detecting chronic pancreatitis and has high sensitivity when compared to ERCP (Sahai et al. 1998) which also carries a risk of mortality (0.3 %) and relatively high morbidity (6 %) such as pancreatitis, infections, bleeding, perforations etc. (Andriulli et al. 2007).

Computed tomography (CT) is better than US in differentiating acute pancreatitis from other abdominal inflammatory conditions and also with contrast enhancing also the complications such as pancreatic necrosis and abscesses are detected in high accuracy (Kivisaari et al. 1983, Clavien et al. 1988). In chronic pancreatitis parenchymal calcification, atrophy and sometimes also dilated pancreatic duct can be detected (Etemad and Withcomb 2001) Contrast enhanced CT has contraindications (renal failure and allergy towards the contrast medium) which can prevent the examination in acute pancreatitis patients in 11 % of cases (Piironen 2001, Arvanitakis et al. 2004). In such instances native CT also has a value both in the diagnosis and severity assessment of acute pancreatitis (Schröder et al. 1985, Lankisch et al. 2003).

6.3.1 Secretin stimulated magnetic resonance cholangio pancreatography

When differentiating the aetiology of pancreatitis MRCP is very useful. Secretin stimulated MRCP may give more information on the pancreatic ductal side branches than ERCP thus enhancing also the diagnosis of chronic pancreatitis and being a non invasive examination (Manfredi et al. 2000). In comparison to ERCP, secretin stimulated MRCP reaches to 76 – 100 % sensitivity and 94 – 100 % specificity detecting chronic pancreatitis in general (Tamura et al. 2006). When EUS and secretin stimulated MRCP were compared in detecting the chronic pancreatitis changes, EUS had higher sensitivity, though the different methods were more complementary when used together (Pungpapong et al. 2007).

6.3.2 Radiology in clinical practice

In acute pancreatitis patient admission to hospital, abdominal US is performed as a first line radiological examination to evaluate the aetiology. Usually when there is a question of the diagnosis or suspicion of severe pancreatitis with necrosis or local complications the contrast enhanced CT provides the second line radiological examination, but active recognition of the contraindications for contrast medium (renal failure, allergy) needs to be emphasized. If the aetiology still remains unclear or there are contraindications to the contrast media the MRCP is a good option, whereas EUS demands an experienced performer and this limits its usability in everyday clinical practice in Finnish hospitals when treating patients with acute pancreatitis.

In chronic pancreatitis abdominal US is usually the first line examination that can possibly reveal information also on the aetiology. Often the evaluation of the stage of chronic pancreatitis by either CT or MRCP is performed and ERCP may be needed especially for the treatment of complications (e.g. biliary obstruction). EUS has the same limitations in Finnish hospitals, yet the non-urgency of chronic pancreatitis may allow more room in the use of this modality.

6.4 Differentiating between acute and chronic alcoholic pancreatitis

In the early stage of chronic pancreatitis reaching the diagnosis is more difficult. This is a controversial area where it is disputed whether there are chronic changes behind an acute pancreatitis attack or whether the chronic changes start to develop to the acute lesions in the pancreas which could be detected in histological specimens (Lankisch 2003, Sand et al. 2007b). This debate continues because there are no means that are sensitive enough and non-invasive to detect early changes. For example the sensitivity of the faecal elastase-1 test is only 68 % in mild chronic pancreatitis (Lüth et al. 2001).

7 Identification of the alcoholic aetiology

Diagnosing heavy alcohol consumption can be difficult even though there are several ways to detect it. The history of the alcohol consumption can be estimated by the patients themselves or by the family members, but this has turned out to be a fairly unreliable method. Additional tools for detecting heavy alcohol consumption or dependence on alcohol include Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al. 1993, Reinert and Allen 2002), CAGE (Cut down, Annoyed, Guilty, Eye opener) (Aertgeers et al. 2001, Coulton et al. 2006) and a Short Alcohol Dependence Data (SADD) (Monteiro and Masur 1987). Increases in laboratory markers such as mean red blood cell corpuscular volume, glutamyl transferase activity and carbohydrate deficient transferrin activity can also be used in detecting heavy alcohol consumption. Their usefulness for screening has been shown quite limited (Jaakkola et al. 1994a, Aertgeers et al. 2001, Methuen et al. 2007).

8 Severity classifications for acute pancreatitis

Acute pancreatitis is considered severe when it leads to either death, intensive care treatment, long hospitalization, acute complications such as organ failure, necrosis, abscess or pseudocyst, diabetes or exocrine pancreatic insufficiency (Bradley 1993). This widely used

Atlanta classification has been under revision for differences in application (Bollen et al. 2008).

For predicting the severe acute pancreatitis there are several different classifications available such as Ranson and Glasgow scoring systems. These reach the sensitivity of 77 – 89 % and specificity of 64 – 80 % (Ranson and Pasternack 1977, Leese and Shaw 1988, Neoptolemos et al. 2000). Acute Physiology and Chronic Health Evaluation II Score (APACHE II) (Larvin and McMahon 1989) and Sequential Organ Failure Assessment (SOFA) were developed for characterization of severe pancreatitis in the intensive care unit conditions of measuring and can be evaluated any time during the course of the pancreatitis (Bradley 1993, Halonen et al. 2002).

Also some single laboratory tests can be used in predicting the severity of pancreatitis. Serum C-reactive protein concentration higher than 150 – 280 mg/l has proven to be an excellent method (Puolakkainen et al. 1987, Neoptolemos et al. 2000). Predictive laboratory tests on the admission to the hospital are also trypsinogen (Kylänpää-Bäck et al. 2001a, Lempinen et al. 2001), procalcitonin (Kylänpää-Bäck et al. 2001a and 2001b) and combination of plasma interleukin 10 and serum calcium measurements (Mentula et al. 2005) but they are not yet widely used in clinical practice.

9 Classifications for chronic pancreatitis

In addition to the risk factor classification system TIGAR-O there are several classifications for the degree of chronic pancreatitis. Marseille-Rome classification (Sarles et al. 1989) and Mayo Clinic score (Layer et al. 1994) emphasize the morphologic findings, Cambridge classification is often used to classify the ductal changes (Sarnar and Cotton 1984). Lüneburg score recognizes morphologic, functional and imaging findings (Lankisch 1999). Manchester classification notifies the clinical condition including symptoms, function and imaging (Bagul and Siriwardena 2006).

10 Recurrent alcoholic pancreatitis

Acute alcoholic pancreatitis recurs in almost half of the patients, more often than e.g. biliary pancreatitis (Appelros and Borgström 1999, Gullo et al. 2002a and 2002b, Gislason et al. 2004). That again raises the question of the first episode of acute alcoholic pancreatitis as an early stage of chronic pancreatitis. This has led to two different hypothesis of the pathogenesis of alcoholic pancreatitis. In 1878 Friedrich et al. recognized the condition which they called “the drunkard’s pancreas” where they found signs of chronic interstitial pancreatitis findings (Schneider and Singer 2005). Then in 1946 Comfort et al. noticed that patients with recurrent acute pancreatitis episodes will lead to chronic pancreatitis (Comfort et al. 1946). In 1965 in Marseille consensus meeting the conclusion was that there are microscopic chronic changes which probably have developed before the first acute attack (Lankisch 1999, Etemad and Withcomb 2001, Ammann 2006). Yet in 1991 Klöppel and Maillet looked back to the Comfort et al. findings and developed a “necrosis-fibrosis” hypothesis where recurrent acute (clinical or subclinical) pancreatitis attacks will develop into chronic pancreatitis (Klöppel and Maillet 1991). There has been histological evidence supporting both hypotheses. The study of pancreatic specimens maximum of two years after the first acute pancreatitis of 45 patients by Gullo et al. supported the view of early chronic pancreatitis whereas Renner et al. reported an autopsy series of 247 patients who had died in acute alcoholic pancreatitis and about half of them showing no signs of chronic pancreatitis (Renner et al. 1985, Gullo et al. 2006). Several clinical studies have supported the hypothesis of series of acute pancreatitis episodes producing chronic pancreatitis (Schneider and Singer 2005). However, 10 – 38 % of the patients with recurrent acute alcoholic pancreatitis seem to develop chronic pancreatitis (Klöppel 1999, Lankisch et al. 2007). So, the relation with recurrent acute alcoholic pancreatitis as well as early chronic pancreatitis still remains unclear.

AIMS OF THE STUDY

The aims of the present study were to investigate the recurrence rate of acute alcoholic pancreatitis and risk factors for the recurrence.

The specific aims were to study:

1. The pattern of recurrence after the first acute alcoholic pancreatitis (studies I, II).
2. Clinical factors and behaviours associated to predict the recurrence for the acute pancreatitis after the first episode of acute alcoholic pancreatitis (studies I, II, III).
3. The development of changes in the pancreatic morphology endocrine and exocrine function and their association with the development of later recurrences (study III).
4. The possible association between CCK plasma concentration and DBI expression levels in patients with the acute alcoholic pancreatitis or its recurrence (study IV).

PATIENTS AND METHODS

1 Study I - Retrospective analysis of recurrent alcoholic pancreatitis

In Tampere University Hospital there were altogether 2678 consecutive acute pancreatitis episodes in 1972 – 92. These episodes were recorded retrospectively. Of these 2678 episodes 1555 (58 %) were alcohol induced. Alcohol was considered to be the aetiology when the history of heavy alcohol consumption had been received from the patient or the family, and other aetiological factors like gallstones, hypercalcemia, hypertriglyceridemia or tumour had not been detected in laboratory tests or abdominal imaging. In 591 of the 1555 alcoholic pancreatitis episodes (38 %) it was the first acute pancreatitis ever experienced by the patient. The patient inclusion is shown in Figure 1.

The diagnostic criteria for acute pancreatitis were serum or urine amylase activity more than double the UNR (300 U/l for serum and 2000 for urine) and the clinical presentation consistent with acute pancreatitis. In the most cases the diagnosis had been confirmed by US, CT or laparotomy. Twenty-nine patients (5 %) who died during their first acute alcoholic pancreatitis episode were excluded. Of the remaining 562 acute alcoholic pancreatitis patients with the first attack there were 59 females and 503 males, with the mean age of 41 years (range 18 - 85 yr.) without a difference between the females and males. There were 151 (42 %) patients who were admitted in 1972-81 and 321 (58 %) in 1982-91. Altogether 478 patients were followed up at least for four years.

The follow-up was done till the end of the year 1992 to detect recurrent acute pancreatitis episodes by checking the patients' country-wide hospital discharge data collected by the ministry of health and medical records. Some patients developed recurrent acute pancreatitis episodes and some had been diagnosed with chronic pancreatitis. The criteria of chronic pancreatitis included e.g. the demonstration of complications of chronic pancreatitis, chronic pseudocyst in imaging tests or pancreatic exocrine insufficiency in function tests. The

patients had been screened for chronic pancreatitis during the follow-up only if they had repeated epigastric pain, diarrhoea or other symptoms indicating possible chronic pancreatitis.

Factors analyzed as possible risk factor for recurrence were oedematous or necrotic first episode of acute alcoholic pancreatitis, hospital stay, intensive care unit stay, number of the Glasgow criteria (Table 1) (Leese and Shaw 1988), arterial oxygen tension, blood leukocyte count, serum urea or serum creatinine, serum lactic dehydrogenase, serum calcium, blood glucose, serum alanine amino transferase and serum C-reactive protein as single factors. The most pathologic laboratory test values were used for the analysis.

Figure 1. Flow chart of the patient inclusion.

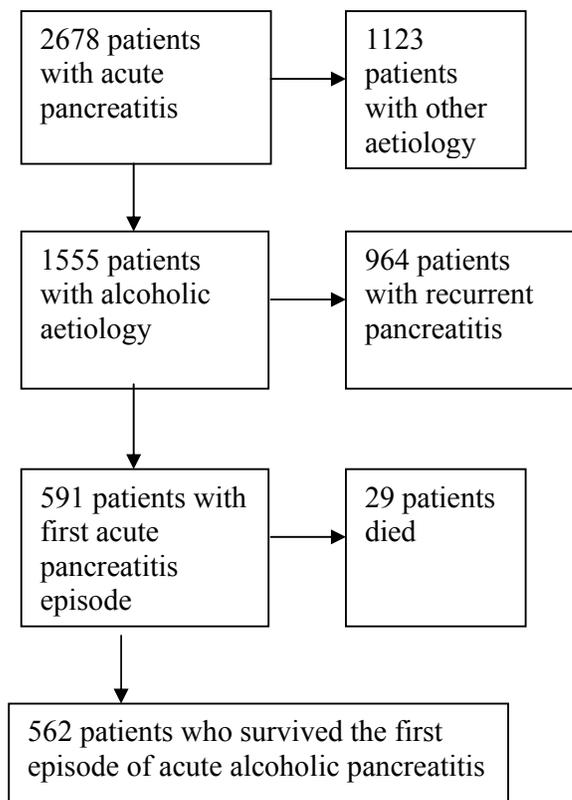


Table 1. The Glasgow prognostic multifactor score (Imrie score) (Leese and Shaw 1988).

Age (years)	> 55
Serum transaminase (units/l)	> 100
White cell count ($\times 10^9/l$)	> 15
Blood glucose (mmol/l)	>10
Serum urea (mmol/l)	> 16
Arterial oxygen saturation (kPa)	< 8
Serum calcium (mmol/l)	< 2
Serum albumin (g/l)	< 32
Serum lactate dehydrogenase (units/l)	> 600

0-2 adverse factors predict mild and 3 or more severe acute pancreatitis.

2 Study II - Prospective analysis of risk factors for recurrent alcoholic pancreatitis

To study the risk factors for recurrent pancreatitis after the first acute alcoholic pancreatitis the patients who volunteered and survived their first episode of acute alcoholic pancreatitis from January 1st 2001 till January 31st 2004 were included in this prospective study. Primarily 86 patients were recruited during the early hospitalization. Patient's co-operation was needed attending to the study. The diagnostic criteria for the acute pancreatitis were epigastric pain, plasma amylase activity at least three times over the UNR, increased inflammatory markers (blood leukocyte count and serum C-reactive protein concentration) and either pancreatic or peripancreatic edema or pancreatic necrosis in abdominal imaging (ultrasound or computed tomography). The flow chart of the patients attending the study is presented in Figure 2.

There were 28 patients who declined attending the study, 20 (71 %) males and 8 (29 %) females. Their mean age was 47 (range 23 - 76) years. These patients did not differ from the study population in gender or age.

Other aetiologies were excluded by patient history, laboratory tests and imaging. Heavy alcohol consumption was detected by an interview. The amount of alcohol consumed during the last two months before the first pancreatitis was evaluated. It has been previously experienced that it is extremely difficult to satisfactorily assess the actual amount of alcohol consumed. That is why the AUDIT and the SADD questionnaires were also used to obtain more reliable data (Davidson and Raistrick 1986, Monteiro and Masur 1987, Reinert and Allen 2002). Laboratory markers that are commonly used for heavy alcohol consumption (mean red blood cell corpuscular volume, serum glutamyl transferase activity and serum disialotransferrin concentration) were also measured (Jaakkola 1994a, Methuen et al. 2007).

The patient history of previous illnesses (especially diabetes) and the use of health care services (especially against heavy alcohol consumption) within the previous year were recorded. Smoking, social condition, marital status, working situation, degree of education,

subjective evaluation of the current relationships, housing condition and subjective evaluation of financial situation were also evaluated.

Pancreatic exocrine and endocrine functions were studied early after the admission (median four days). Faecal elastase-1 concentration and plasma fat soluble vitamin A and E concentrations with plasma glycosylated haemoglobin and fasting plasma glucose and plasma c-peptide concentrations were analyzed. Serum lipase, trypsin and amylase activities were analyzed on admission.

All the patients went through an intervention against heavy alcohol use during the hospitalization at a recovery phase. They were informed about the risks of continuous heavy alcohol consumption with the aim that the patient would recognize the need for change. The decision to reduce or stop the drinking of alcohol was supported and the patient was referred to local affiliations for treatment.

Twenty-one out of 86 initially recruited patients had severe pancreatitis according to the Atlanta criteria (Bradley 1993). Seventeen patients had pancreatic necrosis, six were treated in the intensive care unit for other organ dysfunctions and none were treated operatively. All the initially recruited patients survived the primary hospitalization. The median hospital stay among the 86 initially recruited patients was 7 (range 2 - 41) days. The patients' pre-illness data is presented in Table 2.

2.1 Follow-up

Fifty-four patients underwent SMRP median three months after the admission to the hospital to detect the early morphologic abnormalities. Fourteen patients refused secretin stimulated MRCP examination. ERCP was considered having higher risk for complications and possibly the patient compliance would have been lower than for SMRP. Examinations were performed at 1.5 T (Signa Horizon, GE Medical Systems, Milwaukee, Wis) with a phased-array torso coil. Fat saturated T2-weighted Fast Spin Echo (twenty 8mm thick slices

with a 2mm gap, TR 4650 ms, TE 96 ms) and T1-weighted Spin Echo (twenty 8/2 mm slices, TR 360-440, TE 10 ms) sequences were first obtained in the axial plane to assess the position and morphology of the pancreas, followed by the heavily T2-weighted fat saturated Single Shot Fast Spin Echo (SSFSE) MRCP sequence (nine 20/0 mm slices, TR 22750 ms, TE 959 ms, acquired during an 23-sec breath-hold) in the coronal plane obtained radially with 15 degree intervals, without and repeatedly at 1-minute interval up to 9 minutes from the injection of 100 IU secretinpentahydrochloride (Secrelux, Sanochemia Diagnostics, Neuss, Germany).

All the patients were scheduled for a two-year follow-up visit. Of the 86 patients, four patients (5 %) died before the two-year visit on causes unrelated to pancreatitis. From the surviving 82 patients 54 came to the follow-up visit. In addition 14 patients could be reached and interviewed for alcohol consumption, but they did not undergo laboratory testing. These 68 patients comprised the study population. Of the 68 patients 59 (87 %) were males and nine (13 %) were females. Median age was 46 (range 25 – 71) years. Eighteen (26 %) patients had severe pancreatitis according to the Atlanta criteria. The hospital data is presented in Table 3 in the study II.

At the two-year time point the 68 patients were re-interviewed for the alcohol consumption and smoking during the past two years. The AUDIT and SADD questionnaires were also filled to evaluate the heavy alcohol consumption and dependence on alcohol. The laboratory markers of alcohol consumption (mean red blood cell corpuscular volume, serum glutamyl transferase activity and disialotransferrin concentration) were analysed again. The alcohol consumption was also evaluated after the two year visit by the same methods as mentioned above at three year time point.

Pancreatic exocrine and endocrine functions were also studied at two years. Faecal elastase-1 concentration and plasma fat soluble vitamin A and E concentrations with plasma glycosylated haemoglobin, fasting plasma glucose concentrations and glucose tolerance test were analyzed.

By the end of January 2006, the median overall follow-up time was 38 (range 1 - 61) months. The diagnostic criteria for recurrent acute pancreatitis were the same as for the primary attack. For a suspected or very likely recurrent acute pancreatitis they were the same except the serum amylase level at least two times the UNR. If the patient had been hospitalized elsewhere, the data was obtained from there. The descriptions of the patients' follow-up data are expressed in Table 4 of the study II.

Figure 2. Flow chart of the patients attending the study.

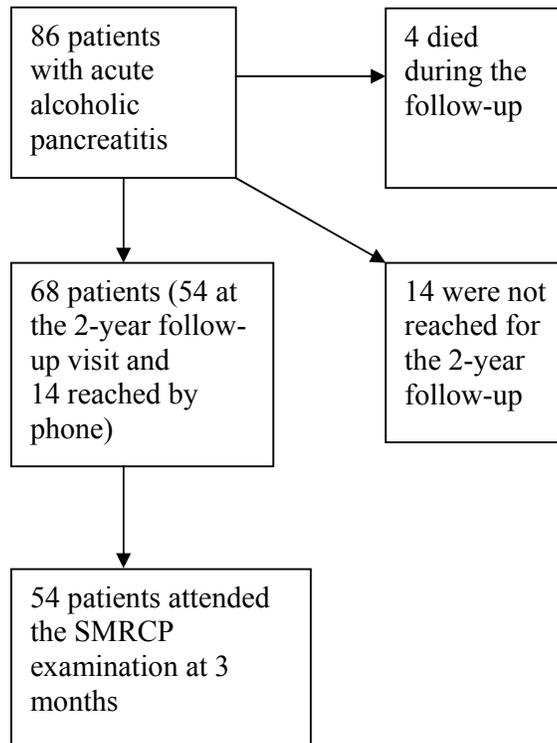


Table 2. The descriptions of the 68 patients' pre-illness data (study II).

Variable	All patients	Normal value
PRE-ILLNESS INFORMATION		
Age: years, median (range)	46 (25 – 71)	
Sex: male/female (%)	59 (87) / 9 (13)	
Healthy: N (%)	22 (32)	
Diabetic: N (%)	10 (15)	
Contacts with local doctor during past six months: N (%)	16 (24)	
Psychiatric treatments during past year: N (%)	17 (25)	
Detoxication treatment during past year: N (%)	21 (31)	
Sleeping disorders: N (%)	17 (25)	
Unemployed: N (%)	17 (25)	
Financial problems subjectively: N (%)	24 (35)	
Housing problems subjectively: N (%)	5 (7)	
Relational problems subjectively: N (%)	23 (34)	
Problems at work or studies: N (%)	23 (34)	
Occupational education: N (%)	59 (87)	
Body mass index (mean \pm SEM)	27.6 \pm 0.4	20 - 25 kg/m ²
Weight loss during the past six months (N)	7 (10)	
Alcohol consumption prior the first acute alcoholic pancreatitis		
Grams / 2 months: mean \pm SEM	4015 \pm 385	
AUDIT: mean points \pm SEM	21 \pm 1	< 8 points*
SADD: mean points \pm SEM	14 \pm 1	0 points [#]
The last drinking period: days \pm SEM	27 \pm 13	
Use of other sedatives: N (%)	12 (18)	
Smoking: N (%), cigarettes/day, mean \pm SEM	41 (60), 12 \pm 1	
Laboratory markers, mean the 4th day in the hospital		
Mean red blood cell corpuscular volume: mean \pm SEM	93 \pm 1.8	82 - 98 fl
Glutamyl transferase: mean \pm SEM	216 \pm 31	10 - 115 U/l
Disialotransferrin: mean \pm SEM	5.7 \pm 0.8	< 1.8 %

Relational problems subjectively = patients' subjective evaluation of possible problems in their relationships

Occupational education = the degree of education, additional training after compulsory education, providing skills for an occupation

* 8 points or more refers to heavy consumption

[#] 1 - 9 mild, 10 – 19 moderate, > 20 severe alcohol dependency

Table 3. The descriptions of the data collected during the hospital stay (study II).

Variable	All patients	Normal value
DURING HOSPITAL STAY		
Acute laboratory markers (plasma), on admission or mean on the 4th day in the hospital		
Blood haemoglobin	118 ± 2	117 - 167 g/l
Thrombocytes: mean ± SEM	187 ± 12	150 - 360 x 10 ⁹ /l
Leukocyte count: mean ± SEM	14.0 ± 0.7	3.4 - 8.2 x 10 ⁹ /l
Sodium: mean ± SEM	134 ± 0.5	137 - 144 mmol/l
Potassium: mean ± SEM	3.4 ± 0.05	3.3 - 4.8 mmol/l
Creatinine: mean ± SEM	90 ± 10	50 - 100 µmol/l
Amylase: mean ± SEM	1339 ± 164	< 300 U/l
Lipase: mean ± SEM	265 ± 50	< 60 U/l
Trypsin: mean ± SEM	2295 ± 366	110 - 600 µg/l
Alanineaminotransferase: mean ± SEM	79 ± 9	10 - 70 U/l
Alkaline phosphatase: mean ± SEM	241 ± 22	35 - 105 U/l
Bilirubin: mean ± SEM	48 ± 8	5 - 25 µmol/l
Severity markers		
C-reactive protein: peak value, mean ± SEM	225 ± 16	< 10 mg/l
Pancreatic necrosis in CT# scan (%)	14 (21)	
Pseudocyst or abscess in CT# scan (%)	2 (3)	
Severe pancreatitis by Atlanta criteria (%)	18 (26)	
Active calcium: mean ± SEM	1.19 ± 0.01	1.20 - 1.35 mmol/l
Arterial blood oxidation concentration: mean ± SEM	10.4 ± 0.5	10.0 - 14.7 kPa
Intensive care unit stay: N (%)	5 (7)	
Hospital stay: days, mean ± SEM	9.0 ± 0.9	
Pancreatic function tests		
Fasting blood glucose: mean ± SEM	6.8 ± 0.4	4.0 - 6.1 mmol/l
Glycosylated haemoglobin: mean ± SEM	5.6 ± 0.1	4.0 - 6.5 %
C-peptide: mean ± SEM	1.08 ± 0.09	0.20 - 1.20 nmol/l
Vitamin E: mean ± SEM	25.98 ± 1.77	> 12 µmol/l
Vitamin A: mean ± SEM	1.39 ± 0.09	1.0 - 3.0 µmol/l
Faecal elastase-1: mean ± SEM	278 ± 27	> 200 µg/g

Table 4. The descriptions of the 68 patients' follow-up data (study II).

Variable	All patients	Normal value
FOLLOW-UP		
Secretin stimulated magnetic resonance cholangio pancreaticography at median three months after the first attack		
Pseudocysts: N (%)	5 (7)	
Ductal changes: N (%)	12 (18)	
Parenchymal changes: N (%)	13 (19)	
Laboratory markers		
Mean red blood cell corpuscular volume: mean \pm SEM	92 \pm 1.2	82 - 98 fl
Glutamyl transferase: mean \pm SEM	155 \pm 62	10 - 115 U/l
Disialotransferrin: mean \pm SEM	2.0 \pm 0.5	< 1.8 %
Faecal elastase-1: mean \pm SEM	452 \pm 26	> 200 μ g/g

3 Study III – Pancreatic damage after the first acute alcoholic pancreatitis and its association with the later recurrences

The study was based on the same initial population as Study II, except the recruitment period was from January 1st 2001 till February 28th 2004, adding seven more patients to the 86 already recruited to the study II, comprising altogether 93 patients admitted to Tampere University Hospital for their first episode of acute alcoholic pancreatitis. These patients were scheduled to have additional tests compared to study II of pancreatic function and SMRP at two years. The diagnostic criteria for acute pancreatitis, the detection of heavy alcohol consumption and also the exclusion of other aetiologies were performed with the same methods as in study II. The flow chart of the patient inclusion is presented in Figure 3.

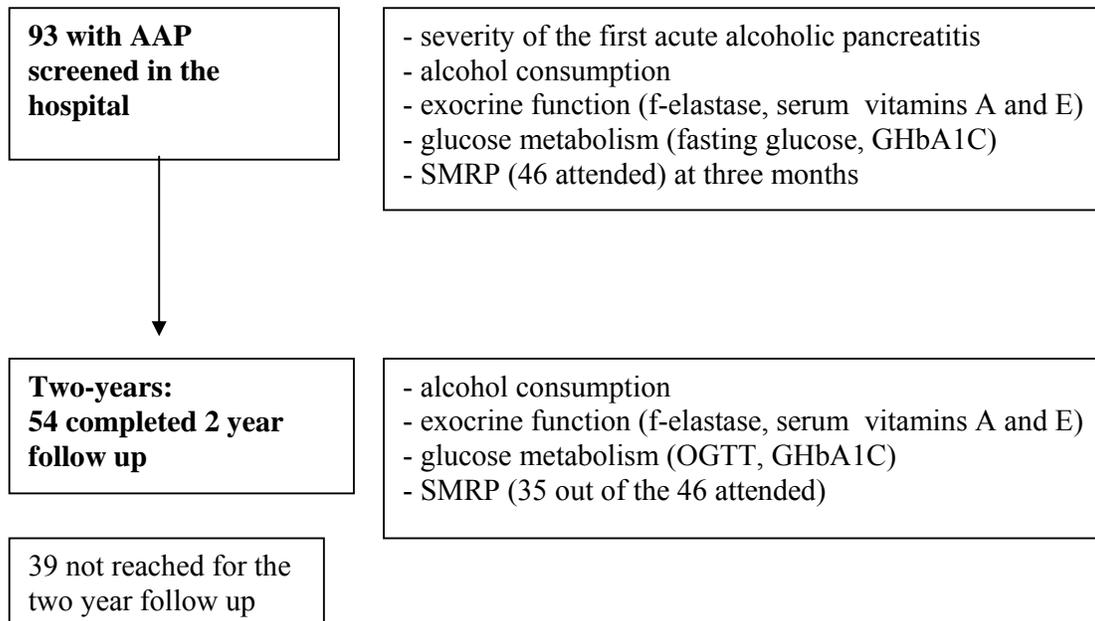
3.1 Follow-up

The patients were recruited for a follow-up study where the pancreatic function and morphology were evaluated at three months and at two year time point. The endocrine function testing consisted of fasting blood glucose and plasma glycosylated haemoglobin during hospitalization and at two years, added with two-hour oral glucose tolerance test (OGTT) when the patient was not overtly diabetic. The exocrine function testing consisted of faecal elastase-1 concentration and plasma fat soluble vitamins A and E concentrations. Pancreatic morphology was evaluated with SMRP. The pancreatic parenchyma changes (atrophy, calcification when detectable), ductal changes and pseudocysts were analyzed both at three months and at two years after the first pancreatitis attack. SMRP was performed as in study II.

There were 54 patients who attended to the two year follow-up visit for the functional testing; 47 (87 %) males and seven (13 %) females. The median age of the patients was 49 (range 25 – 71) years. When the follow-up ended on June 30th, 2006 it was median 47 (range 24 – 66) months.

There were 39 patients who were not reached for the two-year follow-up visit, of them 9 were females and 30 males, median age of 44 (range 18 - 73) years. Nine patients (23 %) had had a severe first episode of acute alcoholic pancreatitis according to the Atlanta criteria. Prior to the first acute pancreatitis episode their alcohol consumption was a mean of 3 967 (SEM 585) g / 2 months, the AUDIT points were a median of 22 (range 7 – 38) and the SADD points were a median of 12 (range 1 – 29). None of them had died during the follow up. The lost patients did not differ from the final study group.

Figure 3. Flow chart of the patient inclusion and the different tests applied (study III).



4 Study IV – Do fasting cholecystokinin and a potential cholecystokinin releasing factor (DBI) expression associate with the recurrence of alcoholic pancreatitis?

Because previous studies have suggested some role of CCK in the initiation of pancreatitis (Räty et al. 1999 and 2000) four study groups were investigated to search for possible differences in CCK in those with heavy alcohol consumption and who had developed pancreatitis compared to the control groups. Group A consisted of patients who had recently suffered from their first (1 – 4 weeks after) episode of acute alcoholic pancreatitis episode (n = 9). Group B consisted of patients who had had at least three episodes of the acute alcoholic pancreatitis, the last one having taken place recently (n = 11). The patients of groups A and B were recruited during the hospitalization for an acute or recurrent alcoholic pancreatitis and were examined after the recovery stage. Group C consisted of people with heavy and long-term alcohol consumption but with no history of pancreatitis (n = 11). They were recruited from a detoxification center. All these subjects in groups A, B and C had had severe alcohol abuse for decades. Group D consisted of healthy volunteers without heavy alcohol consumption (n = 13). Description of the patient groups is presented in the Table 5.

The diagnostic criteria for the acute pancreatitis were epigastric pain, plasma amylase activity at least three times over the UNR, increased inflammatory markers (blood leukocyte count and serum C-reactive protein concentration) and either pancreatic or peripancreatic edema or pancreatic necrosis in abdominal imaging (ultrasound or computed tomography). None of the study objects had had their gall bladder previously removed. The study subjects had neither symptoms of chronic pancreatitis nor had they signs of chronic pancreatitis in the abdominal imaging. All patients had a six-hour fast before the blood sampling. After the blood sample was drawn they underwent an oesophago-gastro-duodenoscopy. Biopsies were taken for histology from the gastric body, antrum and duodenum. Additional duodenal biopsies were immediately frozen in liquid nitrogen for further investigation. The *Helicobacter pylori* infection was examined and classified according to the Sidney system (Price 1991).

4.1 Assay procedures

4.1.1 Plasma cholecystokinin analysis

Plasma CCK was measured with a commercial kit (Euro-Diagnostica, Malmö, Sweden). The normal fasting level of CCK indicated by the manufacturer was $\leq 1,12$ pmol/L and the lowest detectable concentration was 0.3 pmol/L (Cantor 1986, Rehfeld 1998).

4.1.2 RNA isolation and cDNA synthesis

The total ribonucleic acid (RNA) extraction from the snap frozen biopsies was done by using RNeasy (QIAGEN) to detect DBI expression level in the duodenal epithelial cells. The deoxyribonucleic acid (DNA) was digested by a thorough treatment with Dnase I (QIAGEN). A reverse transcription was done by using oligo(dt)₁₅ -primers with “Your prime First strand cDNA synthesis kit”® (Amersham Biosciences, Freiburg, Germany).

4.1.3 Real-time quantitative PCR

Specific primers for human DBI were 5' GAA GCG CCT CAA GAC TCA GC3' (forward) and TTC AGC TTG TTC CAC GAG TCC3' (reverse) and for human β -actin 5'CTG GAA CGG TGA AGG TGA C3' (forward) and 5'AAG GGA CTT CCT GTA ACA ATG3'(reverse). DBI primers were ordered from TAG Copenhagen and β -actin primers were synthesized in the A.I. Virtanen Institute in Kuopio, Finland. The polymerase chain reactions (PCR) were performed with the ABI-PRISM 7700 sequence detection system (Applied Biosystems), in total volume of 30 μ l. The reactions contained 5 ng of cDNA, 15 μ l of SYBR Green master mix (Applied Biosystems) and 2.5 pmol of DBI or β -actin primers. All samples were made into duplicates in following conditions: 2 min at 50°C and 10 min at 95°C followed by 42 cycles of 15 s at 95°C and 1 min at 60°C. Each assay included a relative standard curve of three serial dilutions of human cDNA and No Template Controls. Results were calculated as instructed in ABI PRISM 7700 Sequence Detection System User Bulletin #2.

Table 5. The description of the groups (study IV).

	Group A	Group B	Group C	Group D
N	9	11	11	13
Female	2	0	1	6 ¹
Male	7	11	10	7
Age in years / median (range)	43 (21-58)	45 (33-54)	42 (30-59)	39 (26-66)
Days since last alcohol intake/ median (range)	9 (2-300)	6 (3-60)	9 (1-15)	8 (1-21)
Pure alcohol consumption / week, grams/mean (SEM)	837 (209)	1384 (377)	1680 (337)	26 (6) ²

Group D had more women (¹p = 0.03, Fisher exact test) and less alcohol consumption (²p < 0.01, one way ANOVA) than other groups (alcohol because of the study design).

5 The statistical analysis

The pancreatitis recurrence time (study I) and recurrence rate in univariate analysis (study II) were studied using the Kaplan Maier (log rank test) procedure or with time-dependent Cox regression analysis. The prevalences were compared with chi-square (or Fisher's exact test). Mann-Whitney U test was used to compare medians when the distribution was not normal (study III). The values in studies II and IV are expressed as a mean (SEM) with a normal distribution and a median (range) when the distribution was not normal. Logistic forward stepwise regression analysis or Cox's proportional hazard's method were used for the multivariate analysis (studies II and IV). P-value was considered significant in lower than 0.05 values. Results are reported with p-values and odds ratios (OR) or hazard ratios (HR), supported by 95 % confidence intervals (95 % CI) (studies I – IV). A statistician was also consulted.

6 Ethical aspects

The retrospective study I was performed by reviewing hospital records after the permit of the Chief Executive Medical Officer of the hospital. The prospective studies II - IV were performed according to the Helsinki declaration and were approved by the Ethical Committee of Tampere University Hospital. All the patients gave a written informed consent (studies II – IV).

RESULTS

1 Study I - Retrospective analysis of recurrent alcoholic pancreatitis

There were 260 patients (46 %) out of 562 who had a recurrent acute pancreatitis after the first acute alcoholic pancreatitis. Out of the 260 patients with recurrent acute pancreatitis, 133 (51 %) had one recurrence, and the rest had more or had been diagnosed as having chronic pancreatitis. There were 478 patients who completed a four year follow-up, during which period 80 % (208/260 patients) of the recurrences occurred. The recurrence rate has not changed from 1970's to 1980's (Figure 4).

The patient age under 45 years during the course of the first episode of acute alcoholic pancreatitis was the only significant factor to predict overall later recurrence of acute pancreatitis (OR = 2.42, 95 % CI (1.66 - 3.35), $p < 0.0001$).

Age under 45 years, arterial oxygen tension over 60 mercury millimetres (the lowest value during the hospitalization) and fewer than three positive Glasgow criteria significantly predicted the development of several relapses (Table 6). If the patient did not develop pulmonary complications during the hospital stay there was some tendency for recurrence (Table 6). There was no significant difference between sexes, operative versus conservative treatment or the type of the operation (including biliary surgery, lavation, necrosectomy, pancreas resection). The occurrence of other hospital complications, except for the lack of the pulmonary complications (ileus, sepsis, kidney failure, abscess, cyst, diabetes etc.) had no significant association on the relapse. Later complications such as diabetes, pseudocyst etc. did not associate with the development of the relapses either.

Of those 208 patients who developed their recurrent acute pancreatitis within four years, risk study showed that in addition to the age under 45 years and arterial oxygen tension over 60 mercury millimetres, also serum calcium concentration higher than 2.0 mmol/l (normal values 2.15 - 2.51 mmol/l) significantly associated with the recurrence (OR = 2.25, 95% CI (1.11 - 4.57), $p < 0.022$). Some tendency for recurrence was again found if the patient had not

experienced any acute pulmonary complications (OR = 5.00, 95 % CI (0.63 - 39.5), $p = 0.027$) or sepsis (OR = 5.00, 95 % CI (0.63 - 39.5), $p = 0.027$) during the first episode.

When the risk factors during the second episode of acute alcoholic pancreatitis were studied to predict further recurrences, it was found out that only the hospital stay of 6 - 8 days significantly increased the risk compared to those with longer stay (OR = 2.71, 95 % CI (1.39 - 4.27), $p = 0.004$).

Figure 4. Development of recurrent pancreatitis after the first episode of alcoholic acute pancreatitis according to Kaplan-Maier analysis (study I). There were 151 (42 %) patients admitted in 1972-81 and 321 (58 %) in 1982-91.

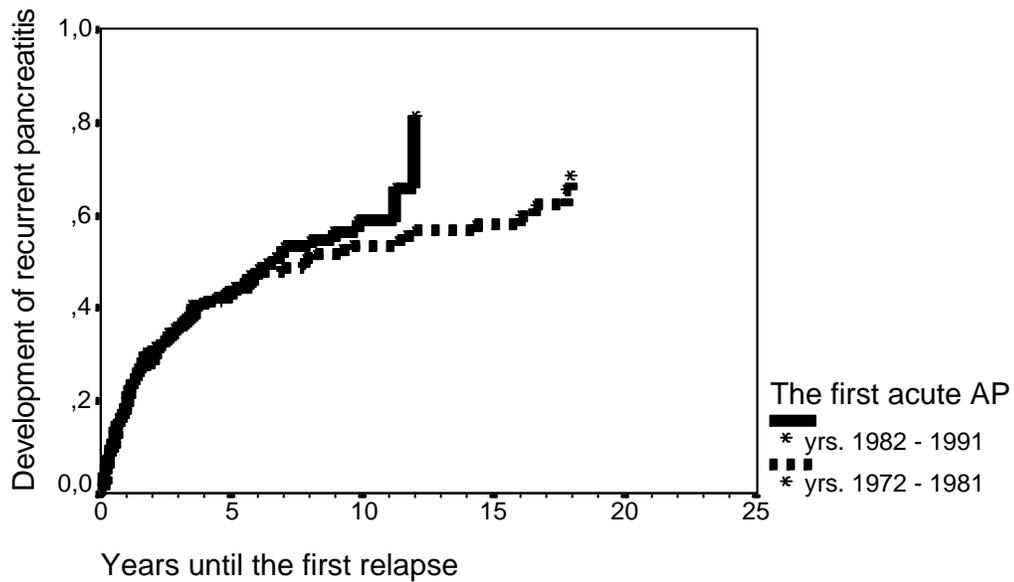


Table 6. Risk factors predicting multi recurrent pancreatitis (Study I).

	Odds ratio	95% confidential interval	p-value
Age < 45 years	2.42	1.66 - 3.35	< 0.0001
PaO₂ > 60 mmHg	9.90	1.32 - 74.5	0.02
0 -2 Glasgow positive criteria	2.45	1.16 - 5.19	0.02
No pulmonary complication	10.92	0.64 - 185.8*	0.02

* 0.5 added to each cell because of a zero frequency

2 Study II - Prospective analysis of risk factors for recurrent alcoholic pancreatitis

Of the 68 patients who had had their first episode of acute alcoholic pancreatitis 17 (25 %) developed recurrent pancreatitis during the 2 - 5 years follow-up. Fifteen fulfilled all of the criteria for recurrent acute pancreatitis and two other patients had very likely acute pancreatitis episodes. The recurrence occurred median 10 (range 1 - 47) months after the first episode of acute alcoholic pancreatitis. Seven (10 %) patients had at least two recurrent pancreatitis episodes. None of the patients had a clinical diagnosis of chronic pancreatitis. However six (9 %) patients had severe pancreatic insufficiency according to faecal elastase-1 examination at the two-year follow-up testing.

The heavy alcohol consumption or dependence before the first episode in grams of pure alcohol as expressed with AUDIT or SADD points were not associated with a greater risk for overall recurrence. The chemical markers of heavy alcohol consumption (mean red blood cell corpuscular volume, serum glutamyl transferase or disialotransferrin value) were not associated with the recurrent pancreatitis either.

Twelve (18 %) patients used other sedatives (e.g. benzodiazepines) with alcohol before the first pancreatitis episode. Seven (58 %) of them had a recurrent pancreatitis during the 1 – 61 months follow-up compared to 10 (of 56 patients, 18 %) who did not use other sedatives ($p = 0.001$). The cumulative ratio of recurrent acute pancreatitis patients is presented on Figure 5. Patient's age, sex, other diseases or social situation did not associate with the recurrence. Body mass index (BMI) or possible recent weight loss before the first pancreatitis did not associate with recurrent pancreatitis.

None of the several laboratory markers, including the pancreatic function tests, evaluated during the hospital stay showed significant association with the development of recurrent pancreatitis. The severity of the first pancreatitis episode (Atlanta criteria, pancreatic necrosis, local complications, intensive care unit stay and overall hospital stay) did not associate with the recurrence.

The changes in the SMRP (pseudocysts, impaired ductal response to secretin and parenchymal edema) early after the first episode of acute pancreatitis episode did not associate with the recurrent acute pancreatitis.

Of the 14 patients who were not reached for the two year follow-up there were 4 females and 10 males, median age 45 (range 18 - 57) years. Among them three patients (21 %) had had a severe first acute alcoholic pancreatitis according to the Atlanta criteria. Before the first acute pancreatitis episode their alcohol consumption was mean 4 343 (SEM 114) g / 2 months, the AUDIT points were median 20 (range 7 – 36) and the SADD points were median 12 (range 3 – 27). Thus the lost patients did not differ in these respects from the final study group.

2.1 Follow-up

The patients with recurrent acute pancreatitis had significantly higher points in the AUDIT and SADD questionnaires at two year follow-up with median 15 (range 4 – 35) vs. 6 (range 0 – 33), $p = 0.034$, in AUDIT and 12 (range 0 – 28) vs. 2 (range 0 – 25), $p = 0.032$, in SADD than those without recurrent pancreatitis. Change in the alcohol consumption was evaluated with a change in AUDIT points during the two years, and there was a reduction with median 10 (range -3 – 37) points in patients with no recurrent pancreatitis compared to no reduction, median 5 (range -13 – 31) points in patients with recurrent pancreatitis ($p = 0.040$). Change in the dependency on alcohol during the two years was evaluated with a change in SADD points. There was a reduction with median 6 (range -3 – 35) points in patients with no recurrent pancreatitis compared to no reduction, median 0 (range -19 – 28) points in patients with recurrent pancreatitis ($p = 0.001$).

There were altogether 13 (19 %) patients who reported at the two year follow-up visit that they had stayed totally abstinent from drinking any alcohol. None of them had developed a recurrent pancreatitis during the 1 – 61 month follow-up ($p = 0.020$), this is presented in Figure

6. Abstinence for one year after the first acute alcoholic pancreatitis attack was more common among the patients with no recurrences (15/51 patients, 29 %) compared to those with recurrence (0/17 patients, 0 %, $p = 0.019$).

The chemical markers for heavy alcohol consumption, the reported cigarette smoking or changes in that by the two years follow-up did not associate with recurrent acute pancreatitis.

Cox regression analysis was performed to evaluate the independent risk factors for the recurrent pancreatitis after the first acute alcoholic pancreatitis. The use of sedatives (e.g. benzodiazepines) before the first pancreatitis attack (HR = 6.95, 95 % CI 2.45 – 19.72, $p = 0.001$) was the only significant factor associated with the recurrence.

Figure 5. Cumulative ratio of recurrent acute pancreatitis patients who used other sedatives with alcohol before the first episode of acute alcoholic pancreatitis and those who did not ($p = 0.001$) (study II).

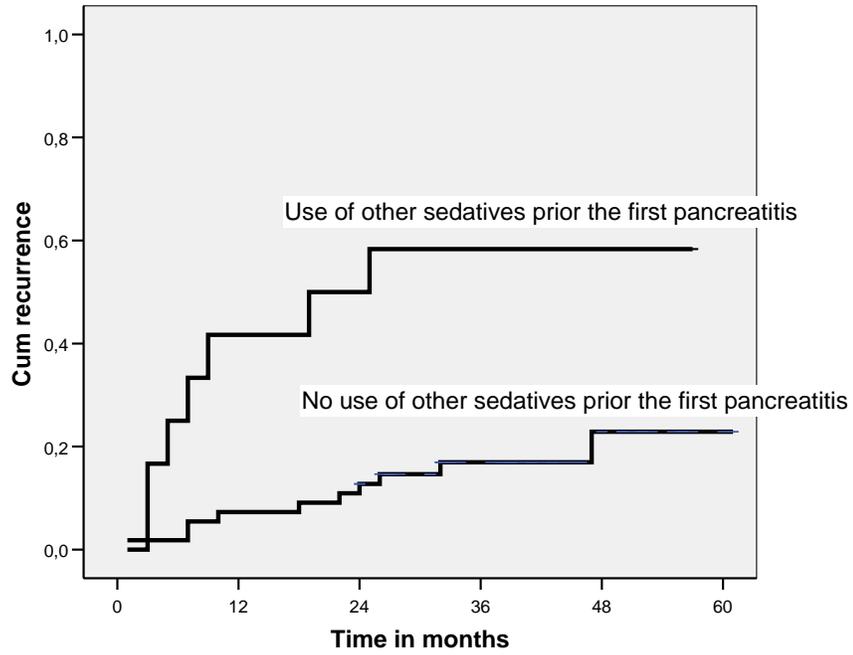
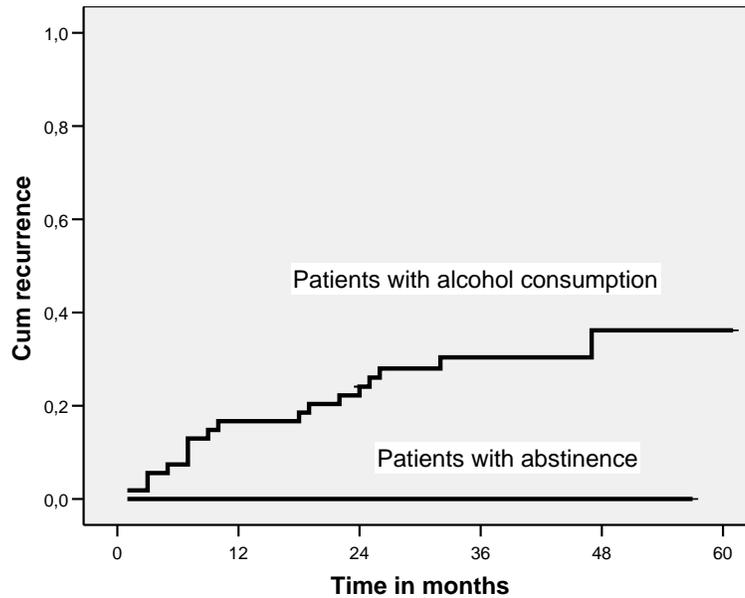


Figure 6. The cumulative ratio of recurrent acute pancreatitis patients who stayed abstinent from alcohol after the first acute alcoholic pancreatitis and who did not ($p = 0.020$) (study II).



3 Study III – Pancreatic damage after the first acute alcoholic pancreatitis and its association with the later recurrences

3.1 Endocrine pancreatic function

3.1.1 Patients with pre-existing diabetes

There were eight patients who had diabetes diagnosed previously before the first episode of acute alcoholic pancreatitis. The severity of the first pancreatitis episode, BMI, recurrence rate or alcohol consumption did not differ between the diabetic and non-diabetic patients.

3.1.2 Patients with new diabetes or glucose metabolism impairment developed by the two-year visit

Within the two years follow up five more patients developed new diabetes in addition to the eight patients with previously diagnosed diabetes (before the first acute pancreatitis episode). Seven more patients had elevated fasting glucose levels and five additional patients exhibited impaired glucose tolerance according to the WHO criteria (Herman and Wareham 1999). Altogether 17 patients out of 46 (37 %), when the patients with previous diabetes were excluded, had newly developed impairment of glucose metabolism after the first episode of acute alcoholic pancreatitis.

The patients' BMI at baseline was mean 27 (SEM 0.6) in patients who stayed non-diabetic and 28 (SEM 1.1) in patients with new diabetes or impaired glucose metabolism. Changes in BMI during the two year follow up did not significantly correlate with newly developed impairment of glucose metabolism.

The newly developed diabetes or glucose metabolism impairment did not differ at two years between the patients who had suffered from severe pancreatitis, as assessed by the Atlanta criteria, or mild pancreatitis. The patients who developed recurrent acute pancreatitis

during the two-year follow-up did not differ in glucose metabolism compared to those who had not developed recurrent pancreatitis.

The patients who developed new diabetes or impaired glucose metabolism (the eight patients with diabetes diagnosed previously were excluded) had higher dependency on alcohol at two years as measured by the SADD questionnaire (mean 6.9 SEM 1.9 points) than the patients who did not have disorders in glucose metabolism (mean 3.9 SEM 2.4 points, $p = 0.03$). There was no significant difference when alcohol consumption was evaluated either by AUDIT questionnaire or by self-reported grams of alcohol.

3.1.3 Diabetes control

Of the patients with severe pancreatitis according to the Atlanta criteria, four patients (31 %) had elevated glycosylated haemoglobin concentration compared to the three patients (7 %) with mild pancreatitis ($p = 0.05$, Odds Ratio = 5.48, 95 % confidence interval: 1.04 – 29.0) (Table 7). When patients with previous diabetes were excluded the difference subsided.

3.2 Exocrine pancreatic function

During the hospital stay, 21 patients (39 %) had faecal elastase-1 concentrations below 200 $\mu\text{g/g}$ whereas at two years only five patients (9 %) continued to maintain concentrations below $< 200 \mu\text{g/g}$. Vitamin A concentration was low ($< 1.0 \mu\text{mol/l}$) during the hospital stay in 13 patients (24 %), but normalized by two years in all patients. Vitamin E concentration was low ($< 12 \mu\text{mol/l}$) during the hospital stay in four patients (7 %), but had normalized by two years in all patients. Faecal elastase-1 concentration did not differ in those who had suffered from severe pancreatitis according to Atlanta criteria and those with mild pancreatitis at two years (Table 7). The patients, who developed recurrent pancreatitis before the two-year follow-up visit or during the 23 months after that, did not differ in pancreatic exocrine function compared to those who had not developed recurrent pancreatitis.

3.3 Pancreatic morphology in SMRP at baseline and at 2 years

There were 35 patients who underwent SMRP examination both in three months and in two years after the first episode of acute alcoholic pancreatitis. In the first SMRP examination at three months, 18 patients had normal findings, 10 patients still had acute changes in the pancreas (oedema) and seven patients had chronic changes such as pseudocysts, ductal changes (strictures, reduced response to secretin), atrophy of the gland or calcifications. In the second SMRP examination at two years acute changes had disappeared. Seventeen patients had normal findings and 18 patients had chronic changes. The changes in the SMRP morphology during the two year time period are illustrated in Figure 7. While the acute changes disappeared the total number of chronic changes increased. These changes developed without significant association with continuous alcohol consumption, as measured at two years, or with the length of the abstinence period after the first episode of acute alcoholic pancreatitis. There were three patients who maintained total abstinence from alcohol after the first episode of acute alcoholic pancreatitis, but were still found to have chronic pancreatitis changes at two years that were not observed in the first SMRP examination. The changes seen in the first SMRP at three months did not associate with pancreatic function at two years (Table 8). Neither did the changes in pancreatic function at two years associate with the morphological changes on SMRP at two years (Table 9).

Of the individual morphologic parameters detected by SMRP, chronic pseudocyst at two years was significantly associated with recurrent pancreatitis after this time point (4 [80 %] vs. 5 [17 %], $p = 0.01$, OR = 20.0, 95 % CI: 1.83 – 219). In two of the patients who developed recurrent acute pancreatitis the pseudocyst had been visible already at the three-month examination. Of the nine patients who had a pseudocyst detected in the second two year SMRP examination three had already a pre-existing pseudocyst in the first SMRP, the rest six formed pseudocysts during the two year follow-up. Other individual changes, such as ductal changes

or parenchymal changes in SMRP, were not associated with recurrent pancreatitis after two years.

The patients who had normal findings or chronic findings in the two year SMRP did not differ in alcohol consumption measured with AUDIT and SADD questionnaires after two year time point. Neither did the patients who had or had not a pseudocyst detected in SMRP differ in their alcohol consumption.

High consumption and dependence on alcohol at two years as measured by AUDIT and SADD questionnaires did not differ between the patients with recurrent pancreatitis and patients with no recurrence (median 7 [1-21] vs. 6 [0-37] points and 2 [0-12] vs. 1 [0-37] points) after this time point.

Table 7. The pancreatic function two years after the first episode of acute alcoholic pancreatitis in patients who had severe or mild pancreatitis according to the Atlanta criteria (study III).

Atlanta criteria	New DM or GMI	Glycosylated haemo-globin > 6.5 mmol/l	Faecal elastase-1 < 200 µg/g
Severe pancreatitis (n = 13)	2 (15 %)	4 (31 %)*	1 (8 %)
Mild pancreatitis (n = 41)	15 (37 %)	3 (7 %)*	4 (10 %)

GMI includes elevated fasting glucose concentration [≥ 6.1 mmol/l] an elevated glucose concentration in oral glucose tolerance test at two hours [≥ 7.8 mmol/l] according to WHO (15). * p = 0.05, Odds Ratio = 5.48, 95 % confidence interval: 1.04 – 29.0

Table 8. The changes in SMRP at three months, (n = 46 patients) and their association with the pancreatic function in two years (study III).

SMRP findings at three months	New DM or GMI	Glycosylated haemoglobin > 6.5 mmol/l	Faecal elastase-1 < 200 µg/g
Acute (n = 10)	3 (30 %)	1 (10 %)	1 (10 %)
Chronic (n = 7)	3 (43 %)	2 (29 %)	2 (29 %)
Normal (n = 18)	7 (39 %)	2 (11 %)	0 (0 %)

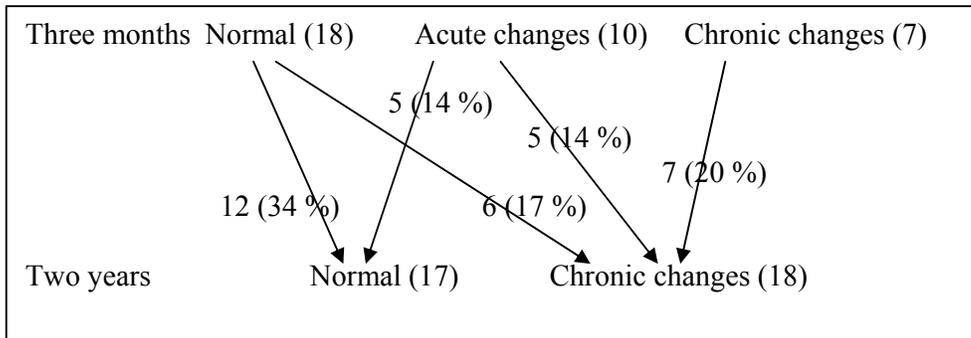
GMI includes elevated fasting glucose concentration [≥ 6.1 mmol/l] and elevated glucose concentration in oral glucose tolerance test at two hours [≥ 7.8 mmol/l] according to WHO (15). Chronic changes = persisting pseudocysts, atrophy, calcifications, reduced response to secretin. There were no significant differences between the groups.

Table 9. The changes in SMRP at two years (n = 35 patients) and their association with the pancreatic function in two years (study III).

SMRP findings at three months	New DM or GMI	Glycosylated haemoglobin > 6.5 mmol/l	Faecal elastase-1 < 200 µg/g
Chronic (n = 18)	6 (18 %)	3 (17 %)	3 (17 %)
Normal (n = 17)	7 (41 %)	2 (12 %)	0 (0 %)

GMI includes elevated fasting glucose concentration [≥ 6.1 mmol/l] and elevated glucose concentration in oral glucose tolerance test at two hours [≥ 7.8 mmol/l] according to WHO (15). Chronic changes = persisting pseudocysts, atrophy, calcifications, reduced response to secretin. There were no significant differences between the groups.

Figure 7. The development of the changes in the SMRP at three months and at two years. The number of the patients in the parenthesis (study III).



Acute changes = oedema in the pancreas, new fluid collections (not seen in the admission imaging, but were detected at three months). Chronic changes = persisting pseudocysts, atrophy, calcifications, reduced response to secretin. Changes were considered chronic at three months when also found already at the computed tomography or ultrasonography on admission. (%) per cent of all the patients who went through the both SMRP examinations. There were no significant differences between the changes.

4 Study IV - The role of fasting cholecystokinin and a potential cholecystokinin releasing factor (DBI) expression after alcoholic pancreatitis

In 40 out of 44 studied subjects DBI messenger RNA (mRNA) could be isolated from the duodenal samples, and measured by real-time quantitative PCR. DBI expression in the duodenum and the circulating plasma CCK in the four study groups were not different from each other (Table 10). Nor did the CCK/DBI ratio differ between the groups.

The endoscopic and histological findings in the different groups are displayed in Table 11. The study subjects in Group C (heavy alcohol consumers without pancreatitis) had more often *Helicobacter pylori* infection than the study subjects in other groups (Table 11). The other histological findings did not differ between the groups. When both the Groups A and B (pancreatitis) were combined as one group and compared to Groups C and D (no pancreatitis) other significant differences were not found. Similarly, when Groups A – C (heavy alcohol consumption) were combined as one Group and were compared to Group D (without alcohol consumption), no differences were found.

There were no differences in DBI mRNA levels in the duodenal samples in the study subjects with bulbar duodenitis (bulbitis) compared with those who had a normal mucosa. There was only one study subject with duodenitis who also had *Helicobacter pylori* infection.

Table 10. The DBI expression levels and plasma CCK concentrations in different study groups (study IV).

	Group A 1 AAP n=9	Group B ≥3 AAP n=11	Group C Alcohol + no AAP n=11	Group D Healthy Controls n=13
Relative DBI expression level in duodenum, mean (mRNA DBI/mRNA18S)	0.60 ± 0.07	0.57 ± 0.06	0.52 ± 0.42	0.57 ± 0.05
Plasma CCK (pmol/l)	0.15 ± 0.10	0.28 ± 0.23	0.20 ± 0.20	0.14 ± 0.14

There were no significant differences between the groups.

Table 11. The number of endoscopic and histological findings in the different study groups (study IV).

	Group A 1 AAP n=9	Group B ≥ 3 AAP n=11	Group C Alcohol + no AAP n=11	Group D Healthy Controls n=13
Ulcer (endoscopy)	2	0	3	1
Duodenitis (histology)	2	1	1	0
Gastritis (histology)	8	10	7	5
Helicobacter pylori infection (histology)	1	2	5 ¹	0

¹ p < 0.01 in the Fisher exact test.

DISCUSSION

1 Recurrence rate of acute alcoholic pancreatitis (studies I, II)

The recurrence rate of acute alcoholic pancreatitis in Scandinavian countries has been reported to be 36 - 48 % (Appelroos and Borgström 1999, Andersson et al. 2004, Gislason et al. 2004, Lund et al. 2006). In the retrospective study I the recurrence rate of acute alcoholic pancreatitis was 46 % being very similar to the other Scandinavian experiences. These rates seem to be somewhat higher than those reported from the continental European countries (Italy, Hungary, France, Greece and Germany) though, there the recurrence rate for acute alcoholic pancreatitis has been 37 % (Gullo et al. 2002a and 2002b). In our study the recurrence in those who ever experienced the recurrent acute pancreatitis episode occurred already within four years in 80 % of the patients with recurrence. This has not been reported from other countries possibly due to the lack of extensive follow-up. In the prospective analysis (study II) the recurrence rate of the acute alcoholic pancreatitis at three and a half year follow-up was 25 % of all the patients, which is less than what was found during the same follow-up time in the retrospective study I of all the patients (35 %). One possible reason for a lower recurrence frequency in the prospective study may be that only part of the patients were motivated enough to participate, which is why they represent a part that might also have been better motivated in attempting to reduce the alcohol consumption resulting in fewer recurrences than what might be found in an unselected population.

The priority criteria for the first acute pancreatitis were epigastric pain, the serum or plasma amylase activity at least three times the UNR and elevated inflammation markers (plasma CRP concentration and blood leukocyte count). Clinical signs and abdominal imaging were also required to be consistent with acute pancreatitis. Slightly lower increase of serum amylase was considered to support suspected / likely pancreatitis diagnosis (study II), because especially in recurrent pancreatitis the sensitivity of serum amylase measurement is decreased, since these patients often have milder pancreatitis and may come later to the hospital (Clavien

et al. 1989). Amylase activity may also be lower in acute alcoholic pancreatitis than e.g. in biliary pancreatitis (Hiatt et al. 1987). In study I serum amylase activity two times the UNR was also accepted to support the diagnosis of the first acute pancreatitis episode. This may reduce the specificity of the diagnosis, but it was retrospectively based on the whole hospital stay, not only on admission diagnosis.

There were 28 patients who were not willing to attend the study (II, III). These patients did not differ from the study population in age or gender. Attending the study a good co-operation was needed. The fact that these patients were not recruited did not affect the number of patients collected for the study population.

2 Risk factors associated with recurrent alcoholic pancreatitis (studies I, II, IV)

There are no previously published studies on the risk factors for recurring acute alcoholic pancreatitis. The present retrospective analysis (study I) indicated that the younger age and a mild first acute alcoholic pancreatitis were risk factors for multi-recurring acute pancreatitis. Because of the retrospective set up of the study it was not possible to adequately differentiate chronic pancreatitis with acute pain relapses from multi-recurring acute pancreatitis with all characteristics of acute inflammation. There was also variation in the laboratory testing between patients especially from different decades. This consequently missing information in some patients made the multivariate analysis unable to show the single independent factors. Importantly, the alcohol consumption could not be retrospectively assessed either.

In the prospective study II with 1 – 61 months follow-up, age and severity were not risk factors for recurrent disease in general. There were only seven patients (10 %) who had developed more than one recurrent acute pancreatitis episodes, and none had been set up the diagnosis of chronic pancreatitis. Therefore the risk factors for multi-recurring acute

pancreatitis or chronic pancreatitis were not possible to assess due to the small number of patients.

Previously it has been shown that the more alcohol was consumed prior the first episode of acute alcoholic pancreatitis the more severe the first attack then was (Jaakkola et al. 1994b). Since the retrospective study I suggested mild pancreatitis to be a risk for multiple recurrences the association of alcohol consumption with recurrences was considered important to study. Again the retrospective nature of the first study hindered us from obtaining the information of the actual amounts of alcohol consumed prior to the first episode. In the prospective setting when this information was obtained it did not associate with recurrence rate. Yet the patients who used sedatives in addition to alcohol before the first episode of acute alcoholic pancreatitis more likely developed recurrent pancreatitis. This might be related to the dependency problem rather than the sedative medication itself. It is supported by the finding that the patients with combined sedative use in this prospective study had higher dependence on SADD questionnaire at the beginning than the patients without prior sedative use. These patients with the use of sedatives had also more often used psychiatric treatments or treatments for dependency problems in other health care units.

Smoking has been found to be a risk factor for chronic pancreatitis and there are some indications that it could be associated with acute pancreatitis too (Pitchumoni 2000, Hartwig et al. 2000, Lindkvist et al. 2008). In the prospective study there was no association found between smoking and recurrent acute pancreatitis after the first episode of acute alcoholic pancreatitis. Since it is in contradiction with the previous findings the association between smoking and recurrent pancreatitis still warrants for further investigation.

In symptomatic chronic pancreatitis there are usually clear findings in the SMRP imaging, yet in early stages of chronic pancreatitis and especially overlaying with acute changes the diagnosis might not be that clear (Tamura et al. 2006, Pungpapong et al. 2007). In our study, when the SMRP was performed within three months after the first episode of acute

alcoholic pancreatitis, ductal or parenchymal changes in the secretin stimulated MRCP were not associated with the risk of recurrent acute pancreatitis. Some of the changes observed were reversible after the first attack thus not showing to be precursors of chronic pancreatitis.

The pancreatic function during the acute alcoholic pancreatitis and its association with recurrences has not been studied before. In the prospective study II the early pancreatic function was not associated with the later risk for recurrence. It is understandable because both the endocrine and exocrine pancreatic function tests were mainly within the normal range.

The reason why only about 5 % of the people with heavy alcohol consumption develop pancreatitis has remained obscure (Dreiling and Koller 1985). One suggestion has been the differences of CCK and its regulation (Räty et al. 1999 and 2000). It is believed that ethanol sensitises acinar cells to physiological concentrations of CCK and its agonist caerulein has been shown to cause pancreatitis in animals (Nordback et al. 1991, Pandol et al. 1999). The CCK releasing factors could thus be associated with a higher risk of pancreatitis in patients with heavy alcohol consumption. In study IV there were no differences in the circulating plasma CCK concentration or in the expression of potential CCK releasing factor DBI mRNA in the duodenal mucosa between the patients with acute alcoholic pancreatitis or recurring acute alcoholic pancreatitis and alcoholics without pancreatitis and healthy individuals. This suggests that persisting high levels of circulating CCK and its stimulus with DBI are not involved in the pathogenesis of the acute or recurrent alcoholic pancreatitis.

3 Follow-up after the first episode of acute alcoholic pancreatitis and associations with recurrence (studies II, III)

After a severe acute alcoholic pancreatitis two thirds of the patients reduce their heavy drinking at least to a moderate level and only one third continue heavy alcohol consumption (Davidson and Raistrick 1986, Frossard et al. 2000). Unfortunately in our retrospective study I the alcohol consumption could not be analysed quantitatively. In the prospective studies (II –

IV) it was found out that the measurement of continuous alcohol consumption is extremely difficult. These patients do not keep diaries of their alcohol consumption and it is very difficult for them to remember the amounts of alcohol they have consumed. The attempt was to catch an estimate of the continuous alcohol consumption by scheduling a visit to each patient and not interviewing only those suffering from the recurrent attack receiving a rough estimate of the continuing behaviour with alcohol consumption added again with SADD and AUDIT questionnaires (Davidson and Raistrick 1986, Monteiro and Masur 1987, Reinert and Allen 2002) which enabled the evaluation of the association with the recurrence during the follow-up. All the patients in the prospective study were motivated in participating which is why they may have been more active in reducing the alcohol consumption. This reduction may have resulted in fewer recurrences in the study population than what could be found in an unselected population. Increasing the population might still have left a similar portion of patients not being reached for the follow-up. Extending the follow-up would not have added many more recurrent acute attacks since 70 % occur during the first three years (study I) covered by this study.

Continuous alcohol consumption has been previously found important in the progression of chronic alcoholic pancreatitis but the role of alcohol consumption in the recurring acute pancreatitis has not been defined (Lankisch 1999). In the prospective study II it was found out that the continuous alcohol consumption after the first episode of acute alcoholic pancreatitis and the lack of reduction in the dependence on alcohol (SADD) during the two year time period were associated with recurrent pancreatitis. The consumption prior to the first attack was not associated with an increased incidence of recurrent pancreatitis. The use of other sedatives in addition to alcohol before the first episode of acute alcoholic pancreatitis was independently associated with the recurrent pancreatitis. The laboratory markers of alcohol consumption at two years did not associate with the development of recurrent pancreatitis. The patients diminished their alcohol consumption before the scheduled visit and that may have

affected the accuracy of the chemical markers reflecting more recent use of alcohol. The patients reported their daily alcohol consumption being lower than the heavy consumption level during the past two months prior the two-year visit, whereas half of them were still heavy consumers or dependent on alcohol according to the AUDIT or the SADD questionnaire. Alcohol consumption after two years did not associate with recurrent pancreatitis episodes after two years in the study III. This may be due to the small numbers of recurrences and would warrant for further studies.

The incidence of acute pancreatitis is increasing and thus the number of late consequences such as pancreatic function impairments, morphological changes and recurrent pancreatitis may be increasingly expected. There are few follow-up studies concentrating on pancreatic function after the acute pancreatitis, whereas less attention has been paid on the recurring disease and its association with previous damage on pancreas (Ammann 1994).

In previous studies it has been shown that the incidence of diabetes after acute pancreatitis has ranged from 25 – 100 % depending on the severity of the disease (Nordback and Auvinen 1985, Doepel et al. 1993, Halonen et al. 2003, Andersson and Andersson 2004). In our study 35 % of the patients without known diabetes before the first episode of acute pancreatitis developed impaired glucose metabolism by the two-year meeting. In most of the patients with the first episode of acute alcoholic pancreatitis BMI was over 25 kg/m². Therefore also type II diabetes is possible with insulin resistance. The pancreatic insulin secretion was not measured in these patients leaving the question unanswered in this population. Yet the onset of diabetes soon after the pancreatitis without concomitant changes in BMI supports an association with acute pancreatitis.

The pancreatic exocrine function has been reported decreased often times early on after the acute pancreatitis. Yet the recovery of the function is prominent (Andersson and Andersson 2004). When the exocrine pancreatic function was measured by the faecal elastase-1 test, it was found declined during the acute phase but recovered by the two year follow-up visit. Mild

exocrine insufficiency may not have been detected due to low sensitivity of elastase-1 test (Lüth et al. 2001). Vitamins A and E concentrations were normalized in all patients by the two-year follow-up. Both of these vitamins have been demonstrated to function as antioxidants during the acute phase. Therefore low concentrations during the acute pancreatitis may not necessarily indicate exocrine pancreatic insufficiency but either low intake of nutrients prior to the first pancreatitis or high consumption during the disease (Curran et al. 2000, Lieber 2003). It can be concluded that the decline in exocrine pancreatic function during the first episode of acute alcoholic pancreatitis recovers within two years.

In the study III the severity (assessed by the Atlanta criteria) was not associated with the incidence of impaired glucose metabolism, as previously suggested, when it was evaluated with fasting blood glucose or glucose tolerance test, but instead high glycosylated haemoglobin concentrations were more frequent at two years after severe pancreatitis. Yet the difference subsided when the patients with diabetes prior to the first episode of acute alcoholic pancreatitis were excluded. Glycosylated haemoglobin has excellent specificity in detecting the diabetic control (Jeffcoate 2003). The current patients with mainly oedematous pancreatitis showed abnormal glycosylated haemoglobin in one out of six patients in two years after the first pancreatitis. Thus while exocrine function improves after the first acute alcoholic pancreatitis, glucose metabolism deteriorates.

Secretin stimulated MRCP has 76 – 100 % sensitivity and 94 – 100 % specificity in detecting chronic pancreatitis (Tamura et al. 2006). This is comparable with ERCP and EUS (Tamura et al. 2006, Pungpapong et al. 2007). However, questions have been raised on the sensitivity of this modality in detecting early chronic pancreatitis changes (Pungpapong et al. 2007). Of the 35 patients who underwent both SMRPs, seven (20 %) had changes of chronic pancreatitis already in the three month examination. All these changes persisted on the second SMRP at two years. On the other hand there were 10 patients (29 %) who had changes of acute pancreatitis in the three month examination, half of resolving and the rest developing into

chronic pancreatitis changes. It remains obscure whether there were early underlying chronic changes not detectable in SMRP in these patients already in the three month examination. Half of the patients showed changes attributable to chronic pancreatitis at two years SMRP compared to 20 % in the three month examination. The chronic changes developed independent on the development of recurrent pancreatitis. This supports the current understanding that chronic inflammatory changes increase in time without further attacks of diagnosed acute pancreatitis episodes (Schneider and Singer 2005).

It has been estimated that pancreatic function impairment happens only when a great amount of pancreatic tissue is damaged (Pungpapong et al. 2007). Changes in the three month SMRP examination were not associated with later changes of pancreatic exocrine function or the development of diabetes during the next two years in this study indicating recovery of the pancreas. In these patients the necrotizing process was limited, and none had an infection necessitating operative treatment and thus most of the pancreatic tissue was preserved. A routine SMRP examination in three months after the first episode of acute alcoholic pancreatitis is thus not needed for evaluation of the degree of pancreatic tissue damage.

The impaired pancreatic exocrine function at two years did not predict the later (after 2 years) recurrence of acute pancreatitis. This may be because the tests of exocrine function are too insensitive to detect minor changes in function. A chronic pseudocyst detected on the second SMRP at two years was a significant risk factor for the later recurrence. A down stream duct obstruction may prevent pseudocyst resolution and can serve thus as a trigger of recurrent pancreatitis (Pungpapong et al. 2007). In only one out of nine patients with pseudocyst a stricture of the main duct was visible in the SMRP, but it is possible that the rest of the patients had strictures in the secondary or tertiary pancreatic ductules not visible in SMRP. Continuous alcohol consumption after the first episode of acute alcoholic pancreatitis was associated with recurrent pancreatitis in study II. A pseudocyst detected at two years was a risk factor for a recurrent acute pancreatitis even when the heavy alcohol consumption and dependency on

alcohol were taken into consideration. The number of patients with recurrences after two years was still very small and especially the evaluation of whether a treatment of these pseudocysts (using either endoscopic or percutaneous approach) could prevent a recurrent attack warrants for further investigation. As considered asymptomatic, they were not treated in the current patients.

SUMMARY AND CONCLUSIONS

In the present study the recurrence rate and associated factors for recurrent alcoholic pancreatitis were studied in human alcoholic pancreatitis patients.

The major findings and conclusions were:

- 1 Nearly half of the patients with the first episode of acute alcoholic pancreatitis develop a recurrent disease in the long term whereas the other half will never experience acute pancreatitis again.
- 2 Younger age of the patient at the time of the first acute alcoholic pancreatitis and a mild first pancreatitis episode were risk factors for multi-recurring pancreatitis in the retrospective analysis. The patients with highest risk for recurrent acute pancreatitis, prospectively analyzed, are the ones who used other sedatives in addition to alcohol prior to the first acute alcoholic pancreatitis and who continued alcohol consumption after the first acute alcoholic pancreatitis failing to reduce the dependency on alcohol as measured with the SADD questionnaire. Total abstinence from alcohol seemed to be a good protector from recurrences.
- 3 Changes of acute pancreatitis in pancreatic morphology vanish during two years as measured with SMRP. These changes were not associated with pancreatic function. The number of patients with chronic changes increased from one out of five to half of the patients in two years. Chronic pseudocysts seen in SMRP at two-year time point were associated with more frequent recurrent pancreatitis later on. The treatment of such pseudocysts needs to be studied in order to reduce recurrent acute pancreatitis episodes.

The clinical severity of the first episode of acute alcoholic pancreatitis was associated with reduced diabetic control at two years. Such an association was not found between the severity of pancreatitis and the pancreatic exocrine function, as measured with faecal elastase-1 assay at two years when the post acute changes have subsided.

- 4 There were no significant differences found in the fasting plasma CCK levels or the respective duodenal DBI expression between the patients who had had one episode or a recurrent alcoholic pancreatitis and the heavy alcohol consumers without pancreatitis or healthy individuals.

ACKNOWLEDGEMENTS

The present study was carried out at the Department of Gastroenterology and Alimentary Tract Surgery in Tampere University Hospital, the Medical Faculty of University of Tampere and The National Graduate School of Clinical Investigation.

I wish to express my profound gratitude to Professor Markku Järvinen M.D. who as being a professor in surgery facilitated excellent working facilities both inside the faculty and other institutes during the early phases of this work. My deepest gratitude also belongs to Professor Teuvo Tammela M.D. who after the organizational change in the hospital, has facilitated the completion of the current work by giving his active support, advice and as a professor in urology taken care of the connections to the faculty when lacking the professorship in gastrointestinal surgery.

My warmest thanks belong to my supervisor and the Director of the Division of Surgery, Gastroenterology and Oncology Docent Isto Nordback M.D. who has introduced me to the clinical scientific work and has placed the research facilities to conduct the study. His dedication and wide knowledge in the pancreatic research field as well as is constantly encouraging guidance have made it possible to perform this work.

I am deeply grateful to my co-supervisor and the Chairman of the Department of Gastroenterology and Alimentary Tract Surgery Docent Juhani Sand M.D., whose expertise and insight to the clinical and scientific field have been irreplaceable by advising in different steps during this work.

I am also deeply grateful Professor James DeGregori for facilitating my last moments of work towards this thesis while working in his lab in the University of Colorado at Denver Health Sciences Center.

I would like to present my warmest thanks to Docent Martti Matikainen M.D. whose role as an expert in the field, and facilitating the clinical conduct of this work have been valuable for this study.

I wish to thank Docent Esko Kempainen M.D. and Docent Pekka Miettinen M.D. for revisions and constructive criticism, which helped me to accomplish the final preparation of this manuscript.

My sincere thanks are also owned to my co-workers Riitta Lappalainen-Lehto MPH, Anneli Piironen M.D., Ph.D, Docent Sari Rätty M.D., Satu Järvinen RN, Professor Karl-Heinz Herzig M.D., Sanna Uotila M.Sc, Seppo Laine M.Sc and late Professor Pekka Laippala and late Mika Jaakkola M.D, Ph.D for pleasant collaboration.

Special thanks are due to Taina Ahlgren and Maarit Mäki for their multiple practical advice and help. I wish also to thank the laboratory personnel, especially Marjo Leponiemi and Tuula Kuningas. Anna-Maija Koivisto B.Sc. and Ville Autio B.Sc are thanked for their help in the statistics. I am also thankful to all the members of the pancreato-biliary study group for the excited atmosphere towards the current work. Tiina Välimäki M.A. is acknowledged for careful revision of the English language.

I wish to express my deepest gratitude to all the colleagues and nurses in the Department of Gastroenterology and Alimentary Tract Surgery of Tampere University Hospital for their positive attitude. I also owe my respectful gratitude to my earlier teachers and colleagues in Tampere City Hospital and Valkeakoski District Hospital towards the study.

My warmest thanks go to my family, my father Hannu Pelli, mother Raija Pelli and brother Pekka Pelli who have always supported me in all endeavours to pursue scientific and clinical work. Special thanks are owed for my friends for their encouraging attitude.

This study was financially supported by grants from Medical Research Fund of Tampere University Hospital, The National Graduate School of Clinical Investigation, Mary and Georg C Ehrnrooth foundation, Research Foundation of AstraZeneca Corporation, Research Foundation of Orion Corporation and Yrjö Jahnsson Foundation.

Tampere, May 2008

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