Metabolic and Lifestyle related risk factors for pancreatic cancer

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2010

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Akademisk avhandling

Som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds Universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i CRC föreläsningssalen i Malmö, fredagen den 16/4 2010 kl 13.15

Fakultetsopponent:
Prof. Leif Bergkvist, Centrum för klinisk forskning,
Kirurgiska kliniken, Centrallasarettet Västerås

LUND UNIVERSITY
Faculty of Medicine

Lund 2010
Dept of Clinical Sciences, Surgery, Malmö, Lund University

Organization
LUND UNIVERSITY
Department of Clinical Sciences, Surgery,
Malmö University Hospital

Document name
DOCTORAL DISSERTATION

Date of issue
March 8, 2010

Sponsoring organization

Author(s)
Dorthe Johansen

Title and subtitle
METABOLIC AND LIFESTYLE RELATED RISK FACTORS FOR PANCREATIC CANCER

Abstract

Background and aims: In spite of the fact that pancreatic cancer is a relatively infrequent disease, it ranks 8th in the worldwide ranking of cancer death due to the poor prognosis. The mortality rate is almost as high as the incidence with a M/I ratio of 98%, indicating an extremely dismal clinical course. This makes it imperative to try to develop new therapeutic strategies and to try to identify risk factors in order to intensify preventive efforts. The most important risk factor for pancreatic cancer is tobacco smoking, but there are other putative environmental risk factors and some pre-existing diseases that have been linked to pancreatic cancer. The aim of this thesis is to evaluate different epidemiological aspects in relation to pancreatic cancer; in more specific terms to investigate the relation between alcohol and pancreatic cancer, between trypsinogen, pancreatic secretory trypsin inhibitor (PSTI) and pancreatic cancer, between Helicobacter pylori infection and pancreatic cancer and to investigate if the metabolic syndrome is associated with the risk of pancreatic cancer.

Results and conclusion: High alcohol intake, estimated using both a questionnaire on attitude towards alcohol and a laboratory marker in the form of γ-GT is associated with a subsequent high risk of developing pancreatic cancer. The previously established association between smoking and pancreatic cancer is confirmed. The hypothesis that pancreatic cancer is related to an imbalance between the trypsinogen isoforms is in line with the finding concerning the ratio of human anionic trypsinogen and human cationic trypsinogen (HAT/HCT). There is no overall association between H.pylori infection and the risk of pancreatic cancer, but H.pylori infection may increase the risk of pancreatic cancer in never smokers and in low alcohol consumers. High mid-blood pressure, high fasting glucose and the metabolic syndrome as an entity are associated with an increased risk of pancreatic cancer in women. In men, high mid-blood pressure is associated with the risk of pancreatic cancer and there is an indication of an association between high glucose levels and the risk of pancreatic cancer. Growing evidence have consistently shown that obesity, diabetes, metabolic factors, smoking and alcohol are associated with a high risk of pancreatic cancer.

Key words:
Keywords: Pancreatic cancer, epidemiology, smoking, alcohol consumption, body mass index, trypsinogen, pancreatic secretory trypsin inhibitor, Helicobacter pylori, metabolic syndrome.

Classification system and/or index terms (if any):

Supplementary bibliographical information:

Language
English

ISSN and key title: 1652-8220

ISBN 978-91-86443-44-3

Recipient's notes

Number of pages 130

Price

Security classification

Livet förstås baklänges – men måste levas framlänges

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Layout & typography: Maria Näslund/Formfaktorn
Printed by Media-Tryck, Lund 2010

ISBN 978-91-86443-44-3
ISSN 1652-8220

Contents

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I Johansen, D; Borgström, A; Lindkvist, B; Manjer, J
Different Markers of Alcohol Consumption, Smoking and Body Mass Index in Relation to Risk of Pancreatic Cancer
Pancreatology, 2009. 9(5): p. 677-686.

II Johansen, D; Manjer, J; Regnèr S; Lindkvist B
Pre-Diagnostic Levels of Anionic Trypsinogen, Cationic Trypsinogen, and Pancreatic Secretory Trypsin Inhibitor in Relation to Pancreatic Cancer Risk
Pancreatology, 2010;00:00-00, in press

III Lindkvist, B; Johansen, D; Borgström A,: Manjer, J
A Prospective Study of Helicobacter Pylori in Relation to Risk of Pancreatic Cancer
BMC Cancer 2008, 8;321

IV Johansen, D; Stocks, T; Jonsson, H; Lindkvist, B; Björge, T; Concin, H; Almquist, M; Häggström, C; Engeland, A; Ulmer, H; Hallmans, G; Selmer, R; Nagel, G; Tretli, S; Stattin, P; Manjer, J
Metabolic Factors and the Risk of Pancreatic Cancer: A Prospective analysis of almost 600,000 men and women in the Me-Can Project
Submitted

Abbreviations

WHO	Worlds Health Organisation
IPMN	Intraductal papillary mucinous neoplasms
PanIN	Pancreatic intraepithelial neoplasias
PET	Pancreatic endocrine tumour
VIP	Vasoactive intestinal polypeptide-secreting tumour
NFκB	Nuclear factor κB
COX-2	Cyclooxygenase-2
PSTI	Pancreatic secretory trypsin inhibitor
HCT	Human cationic trypsinogen
HAT	Human anionic trypsinogen
TATI	Tumour-associated trypsin inhibitor
PAR	Proteinase acrivated receptor
FAMMM	Familial Atypical Multiple Mole Melanoma
FAP	Familial Adenomatous Polyposis
GWAS	Whole genome association study
A, T, C, G	Adenine, Tyrosine, Cytosine, Guanine
SNP	Single polymorphism
ROS	Reactive Oxygen Species
NIDDM	Non-insulin Dependent Diabetes Mellitus
IGF-I	Insulin-like Growth Factor
IDDM	Insulin-dependent Diabetes Mellitus
GLUT	Glucose Transporter Protein
Hp	Helicobacter pylori
BMI	Body Mass Index
TNF-α	Tumour Necrosis Factor-α
CRP	C Reactive Protein
FFA	Free Fatty Acid
CVD	Cardiovascular Disease

MetS	Metabolic Syndrome
NCEP	National Cholesterol Education Programme
IR	Insulin Resistence
MPP	Malmö Preventive Project
Me-Can	The Metabolic Syndrome and Cancer project
NCS	Norwegian Counties Study
CONOR	The Cohort of Norway
40-y	The age 40 programme
VHM&PP	The Vorarlberg Health Monitoring and Prevention Programme
VIP	Västerbotten Intervention Project
γ-GT	Serum γ-glutamyl transferase
Mm-MAST	Malmö Modification of the brief Michigan Alcohol Screening Test
CV	The interassay Coefficient of Variance
ELISA	Enzyme linked Immunosorbent assay
RR	Relative Risk
CI	Confidence Interval
OR	Odds Ratio
BP	Blood Pressure
SD	Standard Deviation
RDR	Regression Dilution Ratio
RC	Regression Coefficient
COHb	Carboxyhaemoglobin

1. Background

1.1 Introduction

To inform a patient about a diagnosis of pancreatic cancer is devastating. In most other cancer forms, unless there is widespread disease, it is possible to offer some form of curative treatment. That does not apply to pancreatic cancer. Even with an early diagnosis, the results after surgical removal of the pancreas are extremely bleak. This is reflected in the fact that, at diagnosis only less than 10% of the cases presents with a disease locally confined to the pancreas and therefore accessible for surgery. Of these cancer patients for whom surgery is an option, the 5-year survival rate is only between 10 and 15% [1].

According to the World health organisation (WHO) pancreas cancer ranks as the 13th most common cancer worldwide [2]. Nevertheless, because of its high mortality rate, pancreatic cancer ranks 8th in the worldwide ranking of cancer death, causing about a quarter of a million deaths each year. This poor outcome makes it imperative to intensify molecular research and clinical studies to understand tumour biology and hopefully thereby be able to develop new therapeutic strategies. In addition, preventive efforts, aimed at risk factors, are mandatory to reduce the high incidence and mortality of pancreatic cancer and identifying risk factors ought to be a strong motivator for epidemiological research.

1.2 Function of the pancreas

The pancreas is a dual function organ, with both endocrine and exocrine functions. The endocrine part is made up of cell clusters, the islets of Langerhans, which produce hormones mainly for regulation of blood glucose [3]. The islets of Langerhans, which constitute approximately 2% of the pancreas, are scattered in clusters within the exocrine tissue. The islets are made up by hormone-producing cells; α cells secreting glucagon, β cells secreting insulin, δ cells secretes somatostatin and PP cells secreting pancreatic polypeptide. The endocrine cells are in direct contact with capillaries and a rapid exchange is ensured between the blood stream and the islets due to a higher perfusion in comparison to exocrine pancreatic tissue. The exocrine part of the pancreas, which constitutes the bigger part of the organ (80%), produces digestive enzymes and bicarbonate, and excretes them via the main duct to the duodenum, in response to lipids and proteins in food products. It is intriguing that the local concentrations of pancreatic islets cell products is a magnitude higher in the pancreatic milieu than in the systemic circulation and that the anatomic proximity of pancreatic islets cells and pancreatic ductal cells is unique [4, 5].

The exocrine pancreas is composed of acinar functional units, which synthesize and secrete enzymes and epithelial cells lining the small pancreatic ducts which secrete bicarbonate (fig.1) [3]. Depending on nutrient intake, the pancreas secretes about 3 l of juice per day. There are three major groups of enzymes; proteases (trypsin and chymotrypsin), pancreatic lipase and amylase. Proteases are dangerous enzymes to harbour in cells and are therefore synthesized as proenzymes (zymogens) without activity, and are packed into condensing vacuoles, maturing into granules before exocytosis from the apical side of the acinar cell into the ducts. An additional safeguard is that trypsin inhibitors (PSTI) are synthesized and released together with the zymogens (fig.1). The inactive zymogens, containing trypsinogens, are converted to trypsin in the duodenum by enterokinase. In the duodenum, trypsin acts to hydrolyse proteins/peptides into amino acids that can be absorbed in the ileum.

Both pancreatic lipase and amylase are secreted in their active form into the pancreatic ducts. In the duodenum, pancreatic lipase breaks apart bile coated fat droplets and amylase breaks down starch into di- and trisaccha-

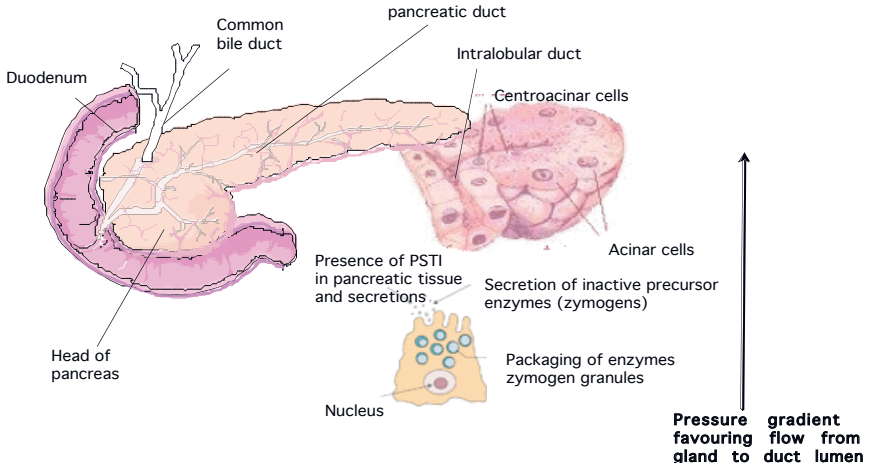

Fig 1. *Anatomy of the pancreas and a model of the trypsin release system.*

rides, which are converted by other enzymes to glucose. The high bicarbonate content of pancreatic juice causes its alkaline pH, which serves to protect enzymes from acidic denaturation and increases hydrolytic activity of pancreatic enzymes within the intestinal lumen (*ibid.*).

1.3 Neoplasms of the exocrine pancreas

Exocrine neoplasms of the pancreas are far more common than endocrine pancreatic cancers and can be subdivided into those that are cystic and those that are solid [6]. The most common cystic lesions are serous cystadenomas, they arise mostly in women (70%) and mean age at diagnosis is 65 years. They are well-demarcated lesions, which do not communicate with the ducts. The vast majority of serous cystadenomas are entirely benign. Mucinous cystic neoplasms arise mainly in women (95%) and mean age at diagnosis is 45 years. Like serous cystadenomas, they do not communicate with the ducts. Non-invasive mucinous neoplasms can be categorized into low-grade, moderate and high-grade dysplasia (carcinoma in situ). One-third of all mucinous cystic neoplasms are associated with an invasive component, usually ductal adenocarcinoma. It is notable that the survival rate is significantly better for patients with a mucinous cystic neoplasm associated with invasive adenocarcinoma than for patients with an invasive adenocarcinoma not associated with a mucinous cystic neoplasm. Intraductal papillary mucinous neoplasms (IPMNs) grow predominantly in the main ducts and are slightly more common in men than in women (ratio 60:40). Mean age at diagnosis is 63 years. Non-invasive IPMNs display various degrees of dysplasia and one-third have an associated invasive carcinoma, which can be a typical ductal adenocarcinoma or in half the cases a colloid adenocarcinoma. The latter appears to have a better prognosis than is the case for patients with an IPMN with an associated ductal adenocarcinoma. Solid-pseudopapillary neoplasms are low-grade neoplasms that predominantly arise in young women (90% and at a mean age at diagnosis of 28 years). It is rare for the patients to die of their disease (*ibid.*).

The solids are pancreatic ductal adenocarcinoma, the most common malignancy of the pancreas and highly aggressive, with a mortality rate almost as high as the incidence (M/I ratio is 98%) [7]. It arises in the epithelial lining of the ducts, from histological well-defined non-invasive precursor lesions called PanINs (pancreatic intraepithelial neoplasias) [8]. There are three grades of PanIN, reflecting varying degrees of architectural and nuclear atypia, and for each grade the risk of developing pancreatic cancer increases. How these lesions come about is not known, but high-grade PanINs have the same genetic profile as infiltrating pancreatic cancer, so it is most likely they share the same risk factors [9]. There are several variants of adenocarcinoma including adenosquamous carcinoma, colloid carcinoma, heptoid carcinoma, medullary carcinoma, signet ring cell carcinoma and undifferentiated carcinoma. Another solid neoplasm is the acinar cell carcinoma, which is highly aggressive. As the name states, it arises in the acinar cell, producing excess quantities of exocrine enzyme. Mean age at diagnosis is 58 years, but the disease can occur in children. The male to female ratio is 3:1. Finally, there are the very rare pancreatoblastomas, which mainly occur in children (mean age 9.8 years). Half of the patients with this form will die from the disease [6].

1.4 Neoplasms of the endocrine pancreas

Even though this thesis is concerned with the exocrine pancreas, a brief summary of the endocrine neoplasms of the pancreas will be given. Pancreatic endocrine tumours (PETs) represent about 2–3% of pancreatic neoplasms [3]. PETs can be divided into non-functioning or functioning, depending on whether, as the name indicates, the neoplasm is associated with a clinical syndrome caused by excess hormone production. Approximately 45% of functioning PETs are insulinomas, 20% are gastrinomas, 15% glucagonomas, 10% vasoactive intestinal polypeptide-secreting tumours (VIPs) and 5% somatostatinomas. Non-functioning neoplasms typically present later in the course of the disease, when the tumour begins to cause symptoms related to the mass effect. Small-cell carcinomas are extremely rare and are mainly metastases from a lung primary, but small-cell malignancies primary to the pancreas do occur. These are highly malignant and usually disseminated at diagnosis (*ibid.*).

1.5 Pathophysiology

Like many other malignant diseases, pancreatic cancer results from the accumulation of acquired or inherited mutations and these are believed to occur in a predictable time course [10]. Several genetic alterations have been identified recently, including inactivation of tumour-suppressor genes, genomic maintenance genes and activation of oncogenes. The p16/CDKN2A tumour-suppressor gene is found to be inactivated in 95% of pancreatic cancers and <30% in low grade PanIN, 55% in PanIN -2 lesions and 70% in grade 3. The p16 protein plays an important role in the control of cell division. The second most frequently inactivated tumour-suppressor gene is p53, which appears to be a relatively late event in the development of pancreatic cancer, as it is seen predominantly in high-grade PanINs. The p53 function is to control cell cycle and apoptosis. K-ras oncogene was the first mutation to be identified and more than 85% of pancreatic cancers have a point mutation in this gene. K-ras mutations are found in early stage lesions with progressive accumulation of defects including p16 and p53 inactivation, as well as alterations in other cancer-associated genes such as BRCA2. The K-ras gene mediates a number of important cellular functions, including promotion of cell proliferation, invasion, metastasis and tumour angionesis. DNA repair genes, like BRCA2, are

only inactivated in <5% of pancreatic cancers, but when inactivated in the germline, they can be associated with familial aggregation of the disease [11, 12].

The molecular mechanism that links the genetic changes to pancreatic cancer is poorly understood. A potential pathway is that genetic mutations in oncogenes and/or suppressor genes activate transcription factors, thereby stimulating numerous growth factors and inflammatory cytokines, presumably in the early stage of the disease (Fig.2). Later on, it is thought that important stress factors, such as hypoxia and acidosis further up-regulate activation of many transcription factors, causing uncontrolled tumour angiogenesis growth and metastasis [10]. NFκB (nuclear factor κB) is a family of transcription factors which activates expression of genes involved in tumourigenesis, metastasis, differentiation, embryonic development, apoptosis and inflammation. Activation of NFκB has been observed in human pancreatic tissue and is thought to promote pancreatic cell growth via inhibition of apoptosis. An alternative mechanism is the NFκB ability to increase the angiogenic potential of pancreatic cancer cells via vascular endothelial growth factor and interleukin-8 [13].

NFκB activation leads to cyclooxygenase 2 (COX-2) expressions. COX is the rate-limiting enzyme in prostaglandin synthesis and the isoform COX-2 is the inducible form, whose synthesis can be up-regulated by several cytokines, growth factors and tumour promoters. Prostaglandins promote cell proliferation, inflammatory cytokine synthesis and suppress immune surveillance. Several studies have demonstrated that COX-2 is up-regulated in

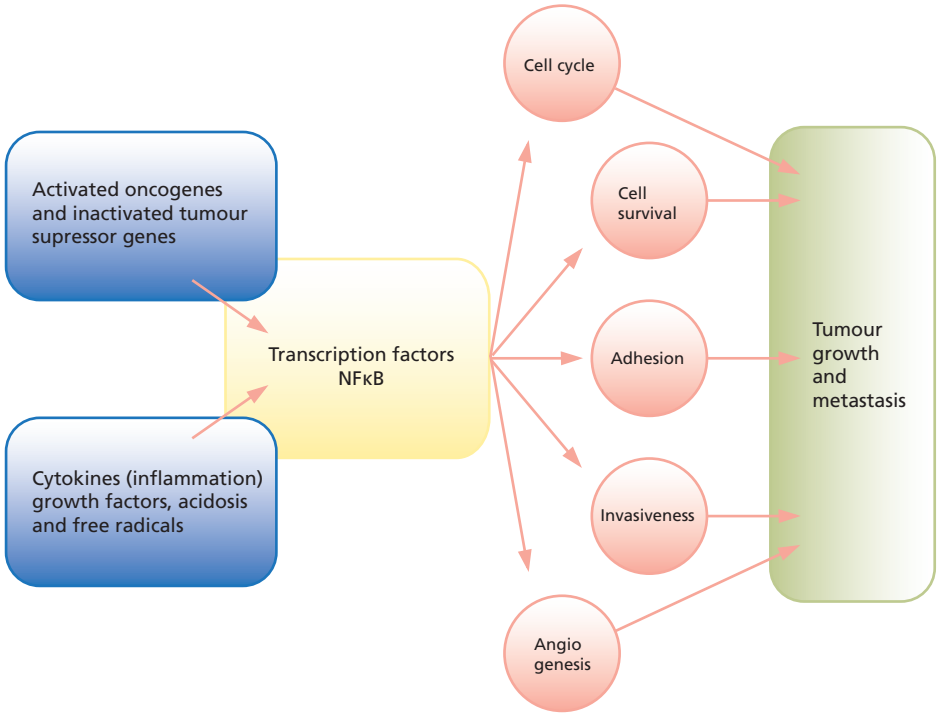

Fig. 2. Molecular biology of pancreatic cancer growth and metastasis.

pancreatic cancer and it has been shown that COX-2 expression is up-regulated in PanIN lesions [14]. Normally, an inflammatory response is immediately followed by the production of anti-inflammatory cytokines. It is not known why this system fails. However, chronic low grade inflammation induces cell division and increases the concentration of free radicals, which can lead to DNA damage [15]. Repeated DNA damage causes a progressive accumulation of genetic defects, resulting in pre-cancerous changes such as PanIN.

2. Biochemistry and clinical role of trypsinogens and PSTI

2.1 Trypsinogens, PSTI and pancreatic cancer

Trypsinogens are part of the serine protease family widely expressed in various tissues and cancer cells. It was one of the first enzymes, together with its active form trypsin, to be isolated and characterized from the human pancreas [16]. Several trypsinogen isoforms have up until now been identified. Trypsinogens were first thought to be solely involved in the digestive process, but in 1986 Bohe *et al.* [17] detected trypsinogen immunoreactivity in the Paneth cells of the mucosa of the small intestine. Since then, trypsinogen expression has been detected in epithelial cells of a variety of organs and in vascular endothelial cells [18]. In the early 1980s, LaBombardi *et al.* [19] identified a trypsin-like protease in the membrane of carcinoma cells. Since then, trypsinogen expression has been demonstrated in several cancer forms [20].

Pancreatic secretory trypsin inhibitor (PSTI) has a well established function as an inhibitor of trypsin [21, 22]. The main physiological role is thought to be protection of the pancreas from destruction by inadvertently activated trypsin [21, 23]. PSTI was originally thought to be solely synthesized by the pancreatic acinar cells, but in 1985 Halila *et al.* [24] detected normal levels of PSTI in pancreatectomized patients, indicating extra-pancreatic production. Nowadays we know, that PSTI, like trypsinogen, is widely expressed in the gastrointestinal tract, the kidney, the acinar cells of the breast and in the urothelium [18], as well as in various tumours. At least three different mechanisms can increase the release of PSTI into circulation, apart from production in the normal pancreas, namely; production by tumours, leakage from a diseased pancreas, and reaction against tissue destruction [25].

2.2 Nomenclature

To clarify the nomenclature, which differs between various investigators, the isoform encoded by the T4 gene is called human cationic trypsinogen (HCT) or trypsinogen-1. The isoform encoded by the T8 gene is called human anionic trypsinogen (HAT) or trypsinogen-2. PSTI encoded by the serine protease inhibitor Kazal type 1 gene (SPINK1) is identical to the tumour-associated trypsin inhibitor (TATI). The name PSTI is generally used for pancreatic inhibitor while TATI has been used to emphasize that the inhibitor originates from a tumour. In this thesis the terms HCT, HAT and PSTI are used [20].

2.3 Possible routes for cancer development

Apart from their normal biological function, serine proteases seem to be of crucial importance in numerous pathological processes and trypsins are no exception. Trypsins activate other proteases and thereby indirectly contribute to the degradation of the extracellular matrix and modulate cell behaviour. This, in turn, is thought to facilitate cell migration and

tumour invasion. Trypsins are able to modulate the functions of cell surface receptors, such as integrins and PARs (proteinase activated receptors) and it seems they can act as a potent growth factor [20]. By PAR-2 activation, trypsin has been shown to mediate inflammation in several cell types [18] and to mediate a hormone-like action, not only via PARs receptors, but also by other signalling mechanisms. Interestingly, trypsin has been shown to mimic the action of insulin to promote glycogen formation, stimulate glucose oxidation and inhibit lipolysis, i.e. growth promoting factors [26, 27].

Even PSTI seems to possess growth factor activity in vitro, but the physiological role is not known [28]. Haglund *et al.* [29] found in a study of patients diagnosed with pancreas cancer, as compared to healthy controls, that PSTI was elevated in 75–90% of the patients, but such an elevation has been seen almost as often in patients with benign pancreatic- and/or biliary disease [25].

3. Epidemiology of pancreatic cancer

3.1 Introduction

Pancreatic cancer remains an oncologic challenge because of its resistance to treatment and because of the location of the pancreas deep within the abdomen; factors, which make the diagnosis of this cancer more difficult than for other gastrointestinal cancers. Moreover, the symptoms that the patient presents are often discrete and uncharacteristic until advanced disease. In fact most patients present with jaundice because of obstruction of the common bile duct, and at that time most tumours are already inoperable.

As stated in section 1.5 pancreatic cancer is fundamentally a disease caused by damage to the DNA and thereby the creation of mutations. These mutations can be inherited (germline mutations) or acquired (somatic) either by chance or as a result of ones behaviour [10]. Age is the strongest predictor of pancreatic cancer as well as most other cancers and there are well-recognized racial differences [30]. Incidence rates are higher in men than in women until later in life, when incidence rates become nearly equivalent [2]. The most important risk factor for pancreatic cancer is tobacco smoking, but there are other putative environmental risk factors, such as alcohol, obesity, nutritional factors and physical activity. Some pre-existing benign diseases have been linked to pancreatic cancer including chronic pancreatitis, type II diabetes and infection [31]. The epidemiology of these risk factors followed by the known and/or putative pathophysiology will be described in section 4.

3.2 Time trends

In most developed countries, there has been a steep increase in the age-standardized mortality rates for pancreatic cancer from the sixties and up until the eighties, when rates levelled and in some cases, as in the USA a slight decrease occurred [32]. This is true even for the Nordic countries (fig. 3) where age-standardized mortality rates have continued to increase until 1988, but in the past decade have levelled. The question is whether the number of patients diagnosed with pancreatic cancer will continue to rise over the next decades? Cancer rates increase rapidly with age (fig.5), so based upon the expected increase in the number of individuals over 65 years in most countries, we can anticipate a measurable age-related increase in the global burden of pancreatic cancer.

Fig. 3 shows the age-standardised incidence in the Nordic countries and indicates that age is not the only explanation for the increase in incidence over time. Smoking is the other major factor influencing the frequency of pancreatic cancer and in populations where smoking has increased, such as

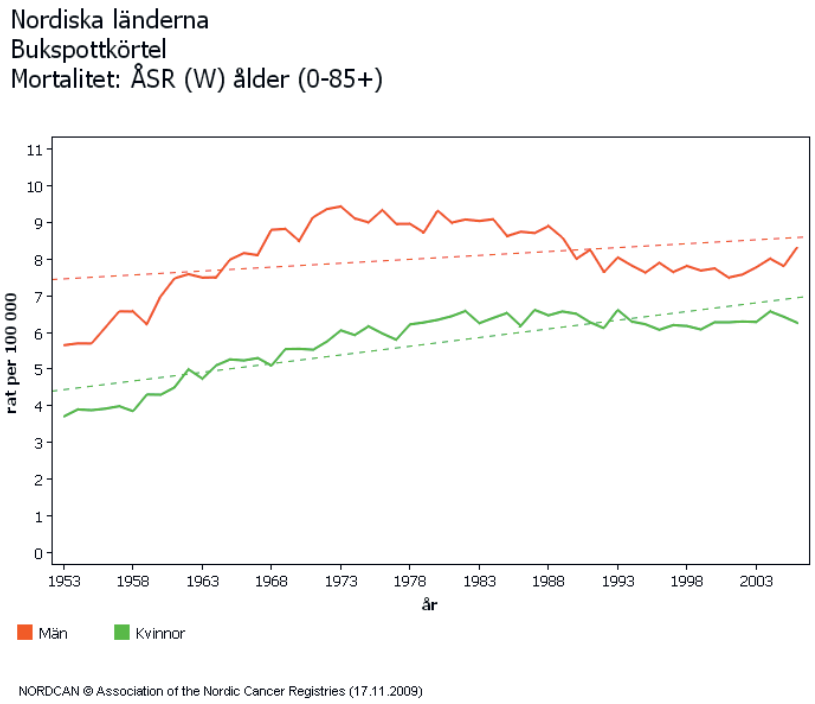

Fig.3. *Age-standardized incidence in the Nordic countries [33].*

Japan, the frequency of pancreatic cancer has also increased [34]. Smoking is considered to be the reason behind the decline in the USA, because the decline follows the decreasing smoking rates in white males and this could even be the explanation behind the levelling in the Nordic countries.

3.3 A global perspective

Pancreatic cancer has an uneven distribution in the world, with a generally higher incidence and mortality rates in the developed industrialized countries, and lower rates in developing countries. Incidences are generally high in the Americas, Europe, Australia and Japan, and low in India, Africa, Southeast Asia and parts of the Middle East, as shown in fig.4.

More specifically, incidence and mortality rates are highest in African-American men, New Zealand Maoris (particularly women), Korean Americans and the male population in Kazakhstan, whereas the lowest incidence and mortality rates are found in India [2]. These differences may well originate at the molecular level; it has been shown that Chinese pancreatic cancer patients may have different K-ras and p53 expressions than in other populations [35]. Moreover, Longnecker *et al.* have shown that there might be a racial difference in survival patterns, and that this might be attributable to differences in the aggressiveness of the tumour type [30].

3.4 Age

As mentioned above, age is one of the main determinants of pancreatic cancer as well as most other cancers. As can be observed in Figure 5, the relationship between age and incidence in

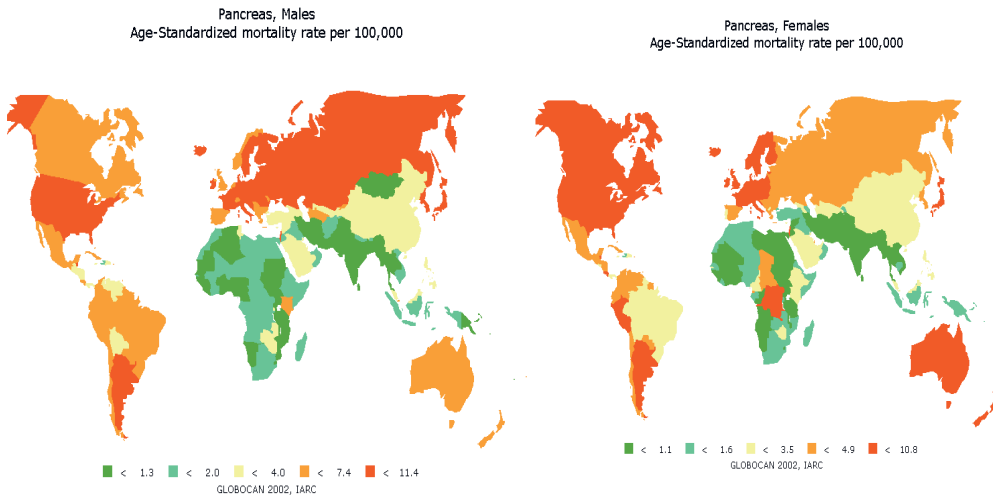

Fig. 4. *Age standardized mortality rate across the world according to Globocan 2002*

the Nordic countries, displays a steep increase after the age of 65 [33], the figure also shows that about 10% of patients develop the disease before the age of 50. The aetiology of early onset cancer is unknown, but patients who are exposed to multiple risk factors, such as the hereditary form of pancreatitis, are candidates. Lowenfels *et al.* have shown that if these patients smoke, pancreatic cancer develops two decades sooner than in non-smokers [36]. This finding suggests an interaction between one or more risk factors and that genetic factors are likely to play an important role.

3.5 Gender

Pancreatic cancer is somewhat more common in males than in females although, as stated earlier, the incidence differs in younger ages but becomes nearly equivalent later in life [37].The reason for the difference is not known. It has been speculated that it may be due to different lifestyle factors i.e. smoking was, at least in earlier times, much more frequent among men than among women. However this cannot explain all the differences, considering tobacco smoking is found to account for only about 25% of the cases and not all [38]. The presence of oestrogen and androgen receptors in pancreatic cancer has been shown [39], but a review of the literature performed by Wahi *et al.* [40] of ten case-control studies and five cohort studies, did not reveal any strong support for the hypotheses that early menarche and late menopause, more pregnancies and/or use of oral contraceptives/ hormone replacement therapy – all of which result in an increased exposure to oestrogen, were associated with a decreased risk of pancreatic cancer in women.

4. Risk factors and biological pathways for Pancreas cancer

4.1 Genetic factors

Mutated genes in pancreatic cancer are divided into three distinct functional groups; oncogenes, tumour-suppressor genes and genome-

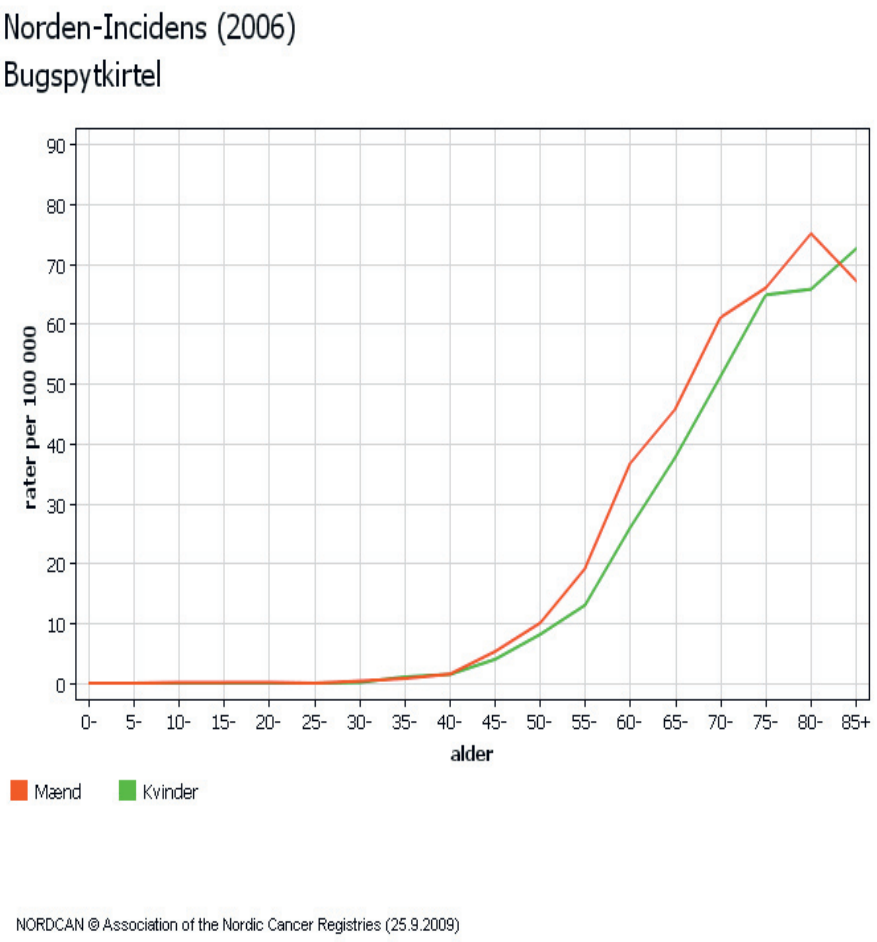

Fig.5. *Incidence per age per 100,000 persons of PDAC in the Nordic countries in 2006 [33].*

maintenance genes. Oncogenes and tumour-suppressor genes are involved predominantly in growth-controlling pathways, whereas genome-maintenance genes are involved in DNA repair [3]. Interestingly, histologically examined pancreas species removed surgically from patients with a strong family history of pancreatic cancer have show that PanINs develop in many patients.

Approximately 5% to 10% of pancreatic cancer patients report a history of pancreatic cancer in a close family member, but the genetic basis is largely unknown, except for a small portion, of which the best known is the germline mutation in BRCA1 and BRCA2 [3, 41]. This is an autosomal dominantly inherited disease characterized by early-onset breast and/or ovarian cancer, which increases the risk of pancreatic cancer 3.5–10 fold. Another known syndrome is hereditary pancreatitis caused by either germline mutations in the cationic trypsinogen gene (autosomal dominant) or germline mutations in SPINK1 (autosomal recessive), which increases the risk

of developing pancreatic cancer 53 fold. Other known syndromes causing pancreatic cancer are FAMMM (Familial Atypical Multiple Mole Melanoma) and FAP (Familial Adenomatous Polyposis). FAMMM is due to mutations in the suppressor gene p16 and is characterized by a large number of melanocytic nevi (50 to 100) in one or more first- or second-degree relatives. The cumulative risks of developing malign melanomas are approximately 50% by 50 years of age and there is a 17% cumulative risk of developing pancreatic cancer by 75 years of age. FAP is an autosomal dominant disease, due to mutations in a tumour suppressor gene (APC), causing numerous polyps in the large intestine, with a much increased risk of developing colon cancer. Cases of pancreatoblastoma as well as pancreatic cancer have been described in FAP patients, but the risk of developing pancreatic cancer has not yet been established (*ibid*).

4.1.1 Whole Genome Association Studies (GWAS)

Virtually all diseases have a hereditary component, transmitted from parent to child through the 3 billion base pairs of DNA letters (Adenine, Tyrosine, Cytosine and Guanine) that make up the human genome [42]. The Human Genome Project completed the final analysis in April 2003 and confirmed that the base pairs in humans were 99.9 percent identical in every person on the planet, which meant that on average 0.1 percent differ genetically from every other person. This variant is based on the order of the base pair, which can shift. An A, for example, may become a C or a G. This kind of variation is called a single polymorphism, or SNP. Most are of no importance but sometimes they cause a slight change and sometimes a complete change of function and thereby, for instance, make a person more susceptible to disease. There are some 10 million SNPs in the human genome and it has been shown that these variants cluster into local neighbourhoods called haplotypes, reducing SNPs to as few as 300,000. This is the basis for the genome-wide association studies. Researchers compare 100,000`s of SNPs between individuals with an illness to unaffected individuals (case-control studies) in order to identify differences, even when the genetic differences are subtle (*ibid*).

During the fifties and sixties, epidemiologists reported an association between ABO blood type and gastrointestinal cancers, most strongly for gastric cancers, but also for pancreatic cancer. They showed an increased risk of these cancers for individuals of A and B blood groups, as compared to blood group O [43, 44]. Recently, this finding has been supported by a genome-wide association study (GWAS) of pancreatic cancer, which has identified the contribution of genetic variation in the ABO locus to pancreatic carcinogenesis [45]. The research group have recently performed further investigations in this exciting new field in order to try to identify additional loci associated with pancreatic cancer. The result of their investigation has yielded three new genomic regions associated with the risk of pancreatic cancer, besides the ABO locus [46]. A case-control study has furthermore been conducted in order to evaluate whether a subjects ABO blood group alleles provide additional risk information on pancreatic cancer carcinogenesis [47]. The researchers found an increased risk for pancreatic cancer among subjects with blood group alleles A and B compared with those with blood group O, and an increased risk were noted with addition of each non-O allele, with the largest risk in subjects with blood type BB. Considering that chronic inflammation is an important factor in pancreatic carcinogenesis, it is very interesting that recent GWAS have suggested that ABO blood group antigens may affect the systemic inflammatory state, [48].

4.2 Pre-existing diseases and pancreatic cancer

4.2.1 Chronic pancreatitis

The aetiology of chronic pancreatitis is probably a multifactorial phenomenon with genetic variations of central influence. The disorder is complex and may involve the interaction of several environmental and genetic factors. Traditionally, the aetiology has been divided into three groups, namely alcohol consumption, idiopathic, and "other". Another classification is the TIGAR-O, which organises the aetiologies by prevalence [49]. "T" categorizes the risk factors as toxic–metabolic (50–80%). "I" is idiopathic (10–30%), tropical pancreatitis is considered to be an idiopathic disorder; it was described by Sarles *et al.* in 1979 [50] as an early-onset chronic pancreatitis in patients who were malnourished in childhood, with a low fat, low protein diet, and who were not alcoholics. "G" is hereditary pancreatitis, "A" is autoimmune pancreatitis, R" is recurrent severe acute pancreatitis and "O" describes obstructive mechanisms.

All types of chronic pancreatitis, i.e. alcoholic, non-alcoholic, hereditary and tropical, have been linked to subsequent development of pancreatic cancer. A multi-centre cohort survey performed by Lowenfels *et al.* [51] in 1993, found a cumulative risk of pancreatic cancer in subjects with chronic pancreatitis for 10 and 20 years of 1.8% and 4% respectively and the association between chronic pancreatitis and cancer has been confirmed by subsequent studies [52–56].

The extensive and prolonged inflammation in chronic pancreatitis is thought to be the factor behind cancer development. Pancreatic inflammation is associated with reactive oxygen production (ROS), release of cytokines and up-regulation of pro-inflammatory transcription factors, which can induce genetic damage, cell proliferation and inhibition of apoptosis [57], all of which create a microenvironment where carcinogenesis is favoured. The progressive accumulation of genetic defects, for example k-ras mutations, has been found in up to 42% of patients with chronic pancreatitis [13] and might lead to pancreatic intra-epithelial neoplasms (PanIN), as described in section 1.3. Another possible route is a transcription factor (NFκB) that plays an important role in genes associated with cancer development [58].

4.2.2 Diabetes

Numerous studies have tried to determine if diabetes increases the risk of pancreatic cancer [59–69] and it has been established that non-insulin dependent diabetes mellitus (NIDDM) or Type II diabetes occur more frequently in patients with pancreatic cancer than in the general population, but the question remains of whether diabetes is the cause or the effect of pancreatic cancer? This question cannot be answered unless a randomized prospective trial is performed, and thus may never be fully resolved, although a well-designed prospective cohort study probably could give useful indications. Supportive of reverse causality is that Chari et al estimated a 40% prevalence of Type II diabetes in pancreatic cancer patients and showed a close temporal association with the diagnosis of pancreatic cancer, suggesting the notion that pancreatic cancer causes diabetes [70 1232]. This hypothesis is further supported by a small clinical study in which resection of the tumour has been shown to improve glucose intolerance and reverse the metabolic defect [71]. However, epidemiological data suggests that Type II diabetes is linked to pancreatic cancer, and most studies detected a two-fold increased risk of pancreatic cancer in patients with Type II diabetes [72, 73]. This may be an exaggeration of the true strength of the relationship, but a review performed by Huxley *et al.* [64] of a total of 17 case-control and 19 cohort or nested case-control studies, showed a 50% risk increase for

pancreatic cancer in individuals with Type II diabetes of more than five years, and thereby supported a modest but causal relationship.

Type II diabetes is characterized by high levels of glucose caused by insulin resistance in fat, muscle and liver cells. Hyperglycaemia induces elevation of insulin and insulin-like growth factor-I (IGF-I) to try to overcome the problem, resulting in a hyperinsulinemic state [62]. The disease differs fundamentally from Type I or insulin-dependent diabetes mellitus (IDDM), which is characterized by a loss of insulin-producing β-cells in the Langerhans islets of the pancreas, either immune-mediated or idiopathic. Sensitivity and responsiveness to insulin in fat, muscle and liver cells are normal and the IDDM has not been linked to pancreatic cancer.

Insulin has been shown to have a direct dose-dependent growth-promoting effect on pancreatic cancer cell lines in vitro, and hyperinsulinemia is thought to allow increased levels of insulin to pass through pancreatic exocrine cells, bind to insulin receptors and trigger mitotic activity [4, 63]. Studies have shown that pancreatic cancer cell lines possess high-affinity receptors for insulin [74]. High concentrations of insulin are able to bind and activate IGF-I, which has a growth-promoting effect, and can modulate cell cycle progression. Excess insulin can down-regulate insulin-like growth factor binding protein-I, thereby increasing the bioavailability of IGF-I, which has been shown to stimulate cell proliferation in vitro [75, 76].

Besides the effect of insulin, glucose may itself have a direct tumour promoting effect, as it is used as an energy substrate in tumour cells, particularly fast-growing, highly proliferative tumour cells [77]. It has been shown that the production of a glucose transporter protein (GLUT) is increased in many tumours in order to meet the enhanced need of energy. It has also been shown that enzymes involved in glycolysis have increased activity and/or expression in cancer cells, in order to meet the increased requirement for energy and ATP production [78]. That excess energy favours cancer development has been shown in animal models subjected to energy restriction. The restriction inhibited cancer progression in the models [79]. Excess glucose also promotes the formation of ROS, which can damage DNA in genes that are important in cell proliferation (such as ras) and/or cell survival (such as p53), which in turn can trigger cancer progression [80]. ROS are molecules formed by incomplete reduction of oxygen, including O_2^-, superoxides, peroxides, hydroxyl radical and hypochlorous acid. These species have been shown to contribute to the activity of phagocytes, regulation of signal transduction and gene expression, and induce oxidative damage to nucleic acid, proteins end lipids. Recent studies in vitro have revealed that ROS not only plays a role in cancer promotion, but also seems to play a role in cancer suppression and the relevance of ROS in these events remains to be elucidated in vivo [81].

4.3.3 Infectious disease

Infectious diseases are a major cause of cancer throughout the world and are aetiological agents for tumours in liver cancer, cervical cancer and gastric cancer. Helicobacter pylori (Hp) infection is a known carcinogen related to gastric cancer [82], but it is unclear if there is a link between Hp infection and pancreatic cancer. To date only a few studies have been performed and the results diverge [83–85], so further investigation is needed to confirm whether or not there is a link.

There are three main hypotheses by which Hp infection is proposed to be associated with pancreatic cancer. The first concerns antral colonization by Hp, which has been associated with increased gastric acid output, which in turn leads to increased secretin release from the duodenum. Secretin stimulation has been proven to accelerate the development and frequency of pancreatic cancer tumours induced

by nitrosamines in a hamster model [86], either per se or by acting as a co-carcinogen [87]. Opposite to antral colonization, Hp infection in the corpus area of the stomach is associated with a loss of parietal cells and a decrease in gastric output [87]. The second hypothesis has been derived from this model. Hypoacidity can lead to bacterial overgrowth and increased production of N-nitroso compounds which can be activated in the ductal epithelium after transportation to the pancreas by the circulation. This hypothesis is supported by the observation that pernicious anaemia is associated with pancreatic cancer [88, 89] and in a recently published register, Luo *et al.* demonstrated an increased risk of pancreatic cancer in patients with gastric ulcers, but not with duodenal ulcers suggesting that if there is an association between Hp and pancreatic cancer, the second hypothesis is the possible mechanism [90].

The third model concerns the possibility of intra-pancreatic infestation by Helicobacter species. Hp infection has been investigated in resection specimens from pancreatic cancer patients, patients having chronic pancreatitis and normal pancreas tissue [91]. Helicobacter DNA could be detected in 30 out of 40 patients with pancreatic cancer, in 3 out of 5 with chronic pancreatitis and none of 7 samples from normal pancreas tissue. Increased secretion of vascular endothelial growth factor and interleukin 8 has been observed in vitro after incubation of pancreatic cancer cell lines with Hp, providing a possible way for Hp to increase the malignant potential if intra-pancreatic infection occurred [92]. The relevance of these findings remains to be elucidated.

4.3 Lifestyle factors

4.3.1 Smoking

Smoking is the most well-established risk factor for pancreatic cancer worldwide besides age [93–100]. Most studies have found a two-fold risk increase and confirm a dose response pattern, with higher rates of pancreatic cancer in heavier smokers. It has also been shown that an excess risk seems to persist in former smokers for more than 10 years [101]. In Western countries there has been an increasing awareness of the possible hazards of smoking, and anti-tobacco use legislation has been increasingly intensified, with varying results in different countries. An example of this difference can be estimated by calculating the population attributable risk or proportion of pancreatic cancer caused by smoking using the formula: P(RR-1)/(P(RR-1)+1) where P is the prevalence of smoking in a population and RR the risk rate. According to the World Health Organization, the prevalence of smoking in 2002–2005 was 47% in Austria, one of the highest rates in Europe, whereas Sweden had a prevalence of 16%, which in contrast was one of the lowest rates. Assuming a risk rate of 2, calculations reveals that 32% of pancreatic cancer cases in Austria and 14% in Sweden can be attributed to smoking, i.e. the amount of pancreatic cancer cases that could be reduced in a population if all gave up smoking, assuming complete causality and immediate benefits of giving up smoking. The latter of course would not occur, but a computer simulation performed in 2002 estimated that with immediate smoking cessation pancreatic cancer incidence could be reduced by 15% by the year 2015 in Europe [102]. It is noteworthy to mention that smoking is an independent risk factor for chronic pancreatitis and the development of Type II diabetes in pancreatitis [31, 103–105], both of which are considered risk factors for pancreatic cancer.

Cigarette smoke contains more than 4,000 chemicals, about 60 components of which are proven carcinogenic and some of the components are known to have additional toxic effects [103]. Nicotine is not by itself carcinogenic, but in humans nicotine and its metabolites affect the composition of pancreatic secretion [106], leading to a decreased vol-

ume and output of bicarbonate in healthy men [107] and increases pancreatic enzyme secretion [108]. Nicotine has been shown to cause acute inflammatory changes in experimental models, but without chronic inflammatory changes, indicating that other components in tobacco smoke are more likely to induce of the chronic inflammatory response which is thought to be the pathway to pancreatic cancer development [109].

4.3.2 Alcohol

Excess alcohol consumption is associated with malignancies of the upper digestive tract, liver, colon and breast [110]. The relationship with pancreatic cancer has been far more controversial, as previous studies have been conflicting. Most case-control studies have found a null association [94, 97, 104, 111, 112], whereas studies that have shown a positive association were mostly cohort studies [56, 93, 96, 113]. A problem in studies on alcohol is how to measure the consumption. Self-reported consumption may have low validity, due to difficulties establishing previous drinking habits, and retrospective and cross-sectional studies may be subject to recall bias or changed alcohol consumption due to subclinical disease. Another possible problem in the study on alcohol and pancreatic cancer is controlling for potential interacting factors, such as smoking and obesity, which might have differed between studies. It is possible that alcohol is not a direct risk factor for pancreatic cancer, but works through other "agents". However, it is metabolized to acetaldehyde, a known carcinogenic agent, causing inflammation in the pancreatic tissue. Alcohol leads to chronic pancreatitis and diabetes mellitus [114] which are risk factors for pancreatic cancer and alcohol seems to work in synergy with smoking, considering the high prevalence of k-ras mutation in drinkers and smokers [115].

Alcohol (i.e. ethanol) is not known to be a carcinogen, but might function as a promoter or co-carcinogen. Ethanol is metabolized in the pancreas acinar cells into acetaldehyde (via alcohol dehydrogenase), free radicals (cytochrome P450) and fatty acid ethyl esters (non-oxidative pathway) [116]. Acetaldehyde is a known carcinogen that can mediate inflammation and fibrosis through different pathways, either by injuring the pancreatic tissue directly and/or through its genotoxicity damaging DNA [105]. Metabolism of ethanol by cytochrome P450 generates reactive oxygen species (ROS) leading to cell injury and lipid peroxidation. ROS initiates tissue injury through activation of NFκB and thereby increases transcription of pro-inflammatory cytokines [54]. Furthermore, synergistic effects between the metabolism of ethanol and the activation of nitrosamines via cytochrome P450 have been reported [54,117]. The non-oxidative pathway of ethanol metabolism results in formation of free fatty acids. Free fatty acids can increase the fragility of pancreatic zymogens converting trypsinogen to trypsin prematurely and they can act to induce pancreatic calcium toxicity, resulting in predisposition to autodigestive injuries and pancreatitis [116].

4.4.3 Obesity

Overweight and obesity is rapidly becoming a major health problem in both industrialized and developing countries and it seems to be affecting all ages, including childhood [118]. There are several well-known adverse health consequences of elevated body weight, including type II diabetes, hypertension, coronary heart disease and some cancer forms [119, 120]. Regarding pancreatic cancer and obesity, there has been inconsistent evidence on whether or not they are associated. A meta-analysis of 14 studies on obesity and pancreatic cancer from 2003 provided some evidence that the risk of pancreatic cancer may increase slightly with increasing body mass index (BMI) [121]. LLi *et al.* recently published a paper in which they showed that overweight

or obesity during early adulthood was associated with a greater risk of pancreatic cancer and a younger age at disease onset. Obesity in an older age did not seem to be associated with an increased risk of pancreatic cancer, but tended to be associated with a lower overall survival [122]. It is possible that obesity at a younger age has a more profound effect on risk and onset of pancreatic cancer than obesity at older age, and that this is the explanation for why the results has been so inconsistent.

Adipose tissue contains adipocytes, preadipocytes (not yet loaded with lipids), endothelial cells and macrophages. It was long regarded mainly as a reservoir for storage and release of fatty acids i.e. energy storage, but this view has been progressively challenged and a series of novel discoveries have shown that adipose tissue acts as a complex endocrine organ, releasing a number of signalling factors, including tumour necrosis factor-α (TNF-α), adiponectin and other hormonal factors [123]. Besides that, it has been discovered that the infiltration of macrophages in obese adipose tissue results in secretion of various inflammatory cytokines and macrophage-inhibiting factor [124]. It is worth noting, that adiponectin is an adipokine with anti-inflammatory, anti-diabetic, anti-atherogenic and anti-angiogenic properties, which generally decrease with increased adipocity, and rise after weight loss [123].

Through the metabolic effects of adipose tissue, obesity has been shown to generate a condition of low-grade inflammation, characterized by abnormal cytokine production, increased synthesis of acute-phase reactants, such as C-reactive protein (CRP), and the activation of pro-inflammatory signalling pathways [125]. A high concentration of adipocytokines and a low concentration of adiponectin have been shown to have a deleterious effect on glucose homeostasis and pancreatic β-cell function, sustaining insulin resistance and hyperglycaemia [126]. There is increasing evidence that pro-inflammatory factors play an important role in the progression from normal to impaired glucose tolerance, and eventually to type II diabetes [127].

Another main action of adipose tissue is to regulate free fatty acid (FFA). FFA is stored as triglycerides in adipose tissue and released into circulation through lipolysis, in accordance with its function as an energy-providing fuel for skeletal muscle [128]. Prolonged periods of excess energy intake and enhanced fat stores cause chronically elevated plasma FFA. Elevated FFA has been reported to correlate with insulin resistance [129] and FFA elevation has been shown to impair hepatic glycolysis, contributing to hyperglycaemia [130]. FFA can also cause insulin resistance through production of ROS, which can activate protein kinase and NF-κB, resulting in decreased GLUT activity and decreased glucose uptake [131]. In addition, it has been proposed that excessive cytotolic triglyceride accumulation in non-adipose tissue such as liver and muscle, enhances ROS produced by the mitochondria, which are cell power houses producing energy in the form of ATP and ROS [132]. ROS can react with FFA, forming fatty acid peroxidation products that are highly reactive and that decompose to aldehyde, which has been reported to cause mutation of the p53 gene in human hepatocarcinoma and to upregulate COX-2 [133].

4.5.4 Nutrition and physical activity

Numerous studies (nearly 500) have been performed on the relationship between dietary intake and pancreatic cancer [69]. Because of the difficulties in ascertaining accurate dietary information from pancreatic cancer patients the relationship remains unclear. Most of the research involves case-control studies, with the possibility of recall bias and reverse causality, which may explain the contradictory results. It has been suggested that a diet in fruits and vegetables is a way of reducing the risk of can-

cer. Michaud *et al.* conducted a report, based on the combined data from two large cohort studies comprising nearly 125,000 persons, where a consumption of a diet rich in fruit and vegetables was compared to a diet high in meat and fat, but showed no reduction in the risk of pancreatic cancer [134]. Nevertheless, the World Cancer Research Fund concluded in their second report, that elevated energy density foods are associated with increased risk of overall cancer, whereas low energy dense foods are associated with a reduced risk [135].

Regular moderate-intensity physical activity is associated with a reduced risk of several cancer types, and a dose-response relationship has been found, for example engaging in longer exercise sessions, or exercising with greater intensity for more years, produces greater reductions in cancer risk [136]. Most epidemiologic findings are inconsistent regarding the association between physical activity and pancreatic cancer. In a review from 2008, Bao *et al.* performed a meta-analysis of 16 prospective cohort studies, one nested case-control study and two retrospective case-control studies, and found overall no association between physical activity and pancreatic cancer [137], but there are several methodological problems in studies on physical activity and pancreatic cancer. Physical activity encompasses a variety of types, including occupational, leisure time and transport, and characteristics, such as frequency, intensity and duration. Moreover, in most studies the statistical power was inadequate because of the limited number of pancreatic cancer cases.

A "Western" lifestyle characterized by low levels of physical activity and a diet rich in energy-dense food is the cause of an increasing prevalence of obesity and it has been speculated that the association between nutrition/physical activity and cancer is the result of excess body weight and obesity [138]. Physical activity though, has been shown to improve insulin sensitivity and to reduce inflammation and affecting circulating levels of IGF-I, without affecting body composition [139]. The coherence between diet and cancer is much more elusive, and at an even more basic level, so is the coherence between diet and obesity. This problem is very clearly reflected in all the different dietary patterns recommended for weight loss and cardiovascular risk reduction, among which are high-fat/low-carbohydrate diets, the high-protein dietary approach, high-carbohydrate, high-fibre, low-glycemic index and low-fat diets [140].

5. Metabolic syndrome (MetS)

5.1 Introduction and definition

In 1988 Reaven gave a Banting lecture to the American Diabetes association, in which he described insulin resistance as a fundamental feature of several conditions associated with cardiovascular disease (CVD) [141]. Over the years, a general consensus regarding the main components of the syndrome, termed "metabolic syndrome", has been reached. These components include obesity, hyperglycaemia, hypertension and dyslipidemia [142]. Unfortunately, no clear-cut definition of the MetS has been reached. Most definitions are based on "opinions" rather than prospective studies and it remains uncertain whether the components really are characteristic of the MetS and if the thresholds at which each component is present or absent are optimal. This problem confuses the interpretation of epidemiological studies and it has been shown how the prevalence of the MetS can vary with definition and criteria modifications. A German study calculated a prevalence ranging from 19% to 31% using different definitions [143] and by modifying the criteria, the prevalence of the MetS changed from 26% to 32% for men and from 23% to 28% in women in the National

Cholesterol Education program (NCEP) 2001 as compared to the revised 2004 version. Only about 30% of people appear to be diagnosable by most definitions, and only 35–40% of those diagnosed with the MetS are eligible for such a classification using one definition [144].

It is true that insulin resistance is often associated with the proposed components of the syndrome, but for the MetS to be termed a syndrome, the level of the risks of the individual components should exceed all of the MetS considered simultaneous, or as Franks says; "the whole should exceed the sum of its part" [142]. Nevertheless, it might be justified to use the MetS as a tool to indicate the more frequent coexistence of certain characteristics than would be expected by chance, as most epidemiological studies tend to use the cluster of these risk factors [145]. Moreover, a series of prospective studies have shown that the presence of the MetS using different definitions is associated with a significantly increased risk of total mortality and CVD and mortality [146].

5.2 The MetS and cancer risk

Epidemiological evidence linking the MetS to cancer is sparse, but it is interesting to note that most of the single components of the syndrome have individually been linked to cancer, mainly diabetes and obesity [133]. Most studies on hypertension and cancer have failed to demonstrate a statistically significant association when BMI was taken into account. A meta-analysis performed by Grossman *et al.* revealed that systolic hypertension, in particular, was associated with a general increased cancer mortality [147], but there is insufficient evidence to indicate that hypertension per se increases cancer risk. The effects of cholesterol and triglycerides on cancer risk are controversial because there are studies that support and studies that refute this finding [133] and for cholesterol there has been some evidence of reverse causation – that preclinical cancer leads to a drop in cholesterol [148]. Only two studies have indicated an association between the MetS as a cluster of components and the risk of a specific cancer, namely colorectal cancer [149, 150]. Studies investigating the incidence of pancreatic cancer in patients with the MetS are generally lacking, Russo *et al.* [151] used a modified classification as they used subjects who were simultaneously prescribed with antihypertensive, lipid lowering and anti-diabetic drugs. In spite of the pharmacological control, they found a statistically significant increase in the risk of pancreatic cancer in men.

5.3 Pathophysiology

The pathophysiology behind the MetS and cancer development is not clearly understood; it is possible that insulin resistance (IR) holds the potential to explain most of the components of the MetS and thereby cancer development [133]. Clinically the term IR implies that a higher level of insulin is required to maintain normoglycemia, but the mechanism behind the pathophysiology is poorly understood. Insulin is released from the β-cells of the islets of Langerhans post-prandially in order to ensure normal utilization of glucose by insulin target tissues, such as muscle- and adipose tissue, and thereby to ensure euglycemia. In other words, insulin facilitates the transport over the cell membrane into the cell. Concomitantly, insulin stimulates intracellular utilization of glucose by many other tissues as well, i.e. it facilitates glucose as an energy substrate. Finally, insulin maintains euglycemia by suppressing glucose production by the liver. If either of these aspects is compromised, IR develops at the level of skeletal muscle and fat or at the hepatic level [152]. In recent years a number of circulating factors that modulate insulin action have been identified and most of these factors are secreted by adipose tissue. In fact adipose tissue dysfunction associated with obesity is now believed to be the main underlying defect in the development of IR accompanying MetS [131].

The sedentary lifestyle that is becoming the norm in most Western countries, with a high energy diet and lack of physical activity, creating increasing numbers of overweight/obese individuals is, as mentioned above, thought to be the main reason for developing insulin resistance and compensatory hyperinsulinemia. Adipose tissue not only stores fat but is also an active endocrine organ, as described in section 4.4.3. Adipose tissue is a source of metabolically active substances, including free fatty acids that affect the insulin-signalling pathways in the liver, skeletal muscle and blood vessels, causing hyperglycaemia and endothelial dysfunction. This leads to increased gluconeogenesis, decreased glucose uptake in skeletal muscle, loss of vasodilatation, platelet aggregation and increased oxidative stress and affects the production of inflammatory cytokines. Obesity leads to visceral fat, and fat deposits in the liver are associated with dyslipidemia with elevated triglycerides and low cholesterol levels. The atherogenic dyslipidemia, impaired glucose tolerance and insulin resistance promotes the development of hypertension, at least in some individuals [153].

5.4 The MetS and pancreatic cancer risk

To summarize, there are a growing number of studies on the Mets and cancer, but most studies have investigated the relationship between single components of the MetS and cancer. A problem is the lack of consensus, which makes it virtually impossible to compare different studies. Moreover, a better understanding of the underlying mechanisms for the association between the MetS and cancer is needed. It has been shown that many of the components of the MetS may promote pancreatic cancer development by generating ROS and providing an energy-rich environment, which in turn promotes cell transformation, angiogenesis, migration, proliferation and apoptosis. All of these have been linked to obesity, insulin resistance, hyperglycaemia and elevated free fatty acids. However, the mechanisms linking cholesterol and hypertension to pancreatic cancer remain unclear and need further study.

6. Study aims

The aim of this thesis is to evaluate different epidemiological aspects in relation to pancreatic ductal adenocarcinoma. In more specific terms the objectives are:

- To investigate if different pre-diagnostic measurements of alcohol consumption are associated with the risk of developing pancreatic cancer.
- To investigate if pre-diagnostic levels of HAT, HCT and PSTI and the ratio between these parameters are associated with the risk of pancreatic cancer
- To investigate the association between H.pylori infection and the risk of pancreatic cancer in relation to smoking and drinking habits.
- To investigate if the metabolic syndrome or its individual components is associated with the risk of pancreatic cancer.

7. Material and Methods

7.1 Cohorts

7.1.2 The Malmö Preventive Project (MPP)

In 1974 The Department of Medicine, Malmö, Sweden, set up a primary preventive unit, the Malmö Preventive Project (MPP) [154], in order to screen a middle-aged population for risk factors related to cardiovascular disease and alcoholism. Malmö University Hospital, which is situated in the city of Malmö, in the southern region of Sweden, is the only hospital in the city. Malmö had, at

the time, a fairly stable population of about 230,000 inhabitants and the possibility of record linkage to relevant demographic, social and medical registries. It also had a very high autopsy rate, and thus provided favourable conditions for a population-based epidemiological investigation [155].

Between 1974 and 1992, invited subjects attended a baseline examination, comprising a self-administered questionnaire consisting of 200 questions on lifestyle and medical history, a physical examination and a panel of laboratory tests. Complete birth-year cohorts of registered residents in Malmö were invited by letter to participate, and all men born in 1921, 1926–1942, 1944, 1946 and in 1948–1949 and all women born in 1926, 1928, 1930–36, 1938, 1941–1942 and 1949 were invited. Mean age at baseline was 44 years in men and 50 years in women. The attendance rate was on average 71% over the years and when recruitment ended, a total of 33,346 men and women had participated in the baseline screening. Apart from 5,722 men (born 1926–38) and 387 women (born in 1931), who participated in a second screening, none of the examinations were repeated.

7.1.2 The Me-Can study

The Metabolic syndrome and Cancer project (Me-Can) was initiated in 2006 in order to create a large pooled cohort to investigate components of the metabolic syndrome on the association with overall- and site specific cancer risk [156]. The large data set made it possible to study a large numbers of cancers, including rare forms, and to study the risk of incident cancer and the association with cancer death. Me-Can consists of seven cohorts: four from Norway; the Oslo study I (Oslo) [157], the Norwegian Counties Study (NCS) [158], the Cohort of Norway (CONOR) [159] and the Age 40 programme (40-y) [160], from Austria; The Vorarlberg Health Monitoring and Prevention Programme (VHM&PP) [161] and two cohorts from Sweden; the Västerbotten Intervention Project (VIP) [162] and the Malmö Preventive Project (MPP) [155]. The coordination centre of the project is at the Department of Surgical and Perioperative sciences, Urology and Andrology, Umeå University, Sweden.

All cohorts are population-based and include data from one or more health examinations, to which men and women of a predefined age and sex were invited during a period of years as summarized in table 1. In all cohorts, except for VHM&PP, participants were asked to fill in a questionnaire concerning lifestyle and medical history. In the VHM&PP, the examining physician asked about these issues and recorded the answers.

The Me-Can study population includes 940,060 subjects with data from 1,600,296 health examinations. In order to reduce the possibility of reverse causation, the Me-Can study group decided that prevalent cancers should not be included, that follow-up should always start one year after baseline examination, and considering that BMI was the main possible confounder, the group decided that data on BMI was required. Exclusions were therefore made for observations with a cancer diagnosis before the date of baseline examination, for observations with less than one year of follow-up and for missing data on height and weight. Furthermore, exclusions were made for data missing on glucose or fasting time and for observations in the 40-y cohort from 1993, for which glucose levels were considered unrealistically low. Extreme values for exposure factors, such as; height < 100 cm or > 250 cm, Weight < 35 kg or > 250 kg, BMI < 15 or > 60 kg/m², systolic blood pressure < 75 mmHg, diastolic blood pressure < 40 mmHg, systolic blood pressure < diastolic, glucose < 1 mmol/l, cholesterol < 0.5 or > 20 mmol/l and triglycerides < 0.05 or > 30 mmol/l, were excluded.

Of the remaining 611,459 subjects with 1,025,940 observations eligible for the study, the first observation for each subject was

Table 1. Description of Me-Can cohorts. Table reproduced from Stocks [163]

Cohort	Purpose	Participants	Year	Att. rate	N subj/ obs in raw files
Oslo	To study risk factors and to prevent CVD	Men in Oslo. Aged 40–49 years and a subset of men aged 20–39 years	1972– 1973	60%	17.973/17.973[a]
NCS	To prevent CVD	Men and women in the counties Finnmark. Sogn og Fjordane and Oppland. Aged 35–49 years and in 1974–1978. a subset of subjects aged 20–34 years	1974– 1978 1977– 1983 1985– 1988	78–90%	91.847/188.536[a]
CONOR	To collect data for research on the aetiology of various diseases	Men and women in different regions all over Norway within different age–groups	1994– 2003	Average 56%. range 30–76% in surveys	169.355/176.464[a]
40-y	To study risk factors and to prevent CVD	Men and women aged 40–42 years. in all Norwegian counties	1985– 1999	69%	415.045/426.768[a]
VHM&PP	To prevent chronic diseases. particularly CVD and cancer	Men and women of 19 years or more in the Vorarlberg province	1985– 2005	66%	175.618/638.906
VIP	To prevent diabetes and CVD	Men and women aged 30 (before 1996). 40. 50 and 60 years in the Västerbotten county	1985– ongoing	60%	85.692/112.300
MPP	To prevent CVD and alcohol abuse	Men and women in Malmö. born in 1921– 1949.	1974– 1992 and a subset in 1981– 1989	71%	33.344/39.453

Att. rate. attendance rate; subj. subjects; obs. observations; CVD. cardiovascular disease.

[a] There was an overlap of subjects between the Norwegian subjects; the total number of subjects was 645.406 and the total number of observations was 809.637.

selected. If data from a fasting state and data on smoking status were available, the first of these observations was selected. A policy imposed by the Norwegian Institute of Public Health states that the proportion of Norwegian subjects in Me-Can studies must not exceed approximately 50% (56% after the above selection). As a result, a further 1,868 subjects in the Norwegian cohorts without data on smoking status were excluded.

7.2 Endpoint retrieval and study population

7.2.1 The MPP cohort study (paper I)

The MPP was linked to the Swedish Cancer Registry and the Regional Tumour Registry of Southern Sweden and cases of pancreatic cancer were identified using the ICD 7 code 157, and ICD 10 code C25. End of follow-up was the 31 of December 2004. Vital status was established using The Swedish Cause-of-Death Registry. The record linkage yielded 187 cases of incident pancreatic cancer among the participants in The Malmö Preventive Project. There were no prevalent cases at baseline. The records for all incident cases were reviewed using clinical notes, radiological - and pathological findings, i.e.; biopsies, specimens obtained during surgery and autopsy reports.

After reviewing all cases, four cases were found to have had pancreatic cancer other than adenocarcinoma, according to their histopathology report (two islets cell tumours, one endocrine and one anaplastic malignancy) and were excluded. In 70 cases the diagnosis was verified by autopsy, 19 cases had undergone surgery and had a clear histopathological diagnosis. Another 82 cases had the diagnosis based on tissue biopsy consistent with adenocarcinoma of the pancreas, their clinical presentation and radiological findings. A further 7 cases were verified by the combination of clinical notes, radiological examination and biopsies that showed unspecified adenocarcinoma, findings that taken together stated a high probability for cancer of the pancreas. Finally, 5 cases were accepted by their clinical and radiological findings, although no biopsies had been taken. Thus, 183 subjects remained in the study as incident pancreatic cancer. This group consisted of 128 men, with a mean age at diagnosis of 63 years and 55 women, with a mean age at diagnosis of 65 years.

7.2.2 The MPP case/control study (paper II and III)

By record linkage to the Swedish Cancer Registry and the Regional Tumour Registry of Southern Sweden, using ICD 7 diagnostic code for pancreatic cancer (157), cases that occurred up until 31 December 1999 were identified and included in these studies. The record linkage yielded 117 subjects registered with the diagnosis of incident pancreatic cancer within the Malmö Preventive Project. Clinical and pathology records were reviewed in all subjects: The diagnosis of pancreatic cancer could be verified in 113 cases. Four cases were found to have been erroneously registered as pancreatic cancer (two islet cell tumours, one endocrine and one anaplastic malignancy) and were therefore excluded from further analysis.

All 113 cases were matched to three controls by sex, age and time of baseline investigation, resulting in a set of 452 subjects. A large proportion of all subjects examined during the first year (1974–1975) had no available biological material. It was therefore decided that only subjects examined from 1 January 1976 should be included in the set intended for laboratory analyses; a total of 104 cases and 311 controls. Following sample retrieval and aliquoting, 87 cases had the necessary amounts of biological material. Considering the relatively large number of subjects with missing biological material, the matched analysis was abandoned at this point. The laboratory analyses were finally performed in 87 cases with

three controls for each case. Following analysis of another three controls, no more cases were available and the laboratory analyses were concluded. In all, 351 subjects were included in the analyses; 87 cases and 264 controls.

In paper II there were three cases diagnosed within the first two years from baseline. One of these cases had extremely high values of both anionic and cationic trypsinogens and PSTI. The records concerning the three early cancers were re-validated. Two had no co-morbidities prior to the pancreatic cancer diagnosis, and one had insulin-dependent diabetes with nephropathies. All three cases underwent autopsy and were found to suffer from widespread disease. We therefore assumed that they may all have had pancreatic cancer at the time of baseline investigation, and that their levels of trypsinogens and PSTI may have been seriously affected by the disease. Following this these three cases were excluded from further analysis, leaving 84 cases and 264 controls included in the analyses. In paper III, one analyzed control subject was excluded because of failure in the H.pylori serology analysis, leaving 87 cases and 263 controls, excluding 102 of the 452 subjects initially intended for analysis.

7.2.3 The Me-Can cohort study (paper IV)

Cohorts in the Me-Can study were linked to the respective countries National registers for a) cancer diagnosis, b) migration, c) vital status and d) cause of death [156]. The end of follow-up results for each cohort was as follows: The Austrian cohort a) 2003, b) no information available, c–d) 2003; the Norwegian cohorts a–c) 2005, d) 2004; and the Swedish cohorts a–c) 2006, d) 2004. As the Eurostat European shortlist for cause of death [164] had been used in the Norwegian cohorts, the same categorization of cause of death was used in the remaining cohorts. Incident pancreatic cancer was identified through linkage to the National Cancer registries, using the International Classification of Diseases, seventh edition (ICD-7) code 157. After matching to date of diagnosis, migration, vital status and end of follow-up, a further 1,385 subjects with a follow-up of less than a year , a total of 288,339 women and 288,976 men (577,315 subjects), including 862 cases of pancreatic cancer, 315 in women and 547 in men, were eligible for the present study.

8. Assessment of potential risk factors

8.1 The MPP cohorts

8.1.1 Laboratory measurements

All examinations and laboratory measurements were performed in a fasting state by a trained nurse. Weight and height were measured and blood-pressure was measured with a mercury sphygmomanometer after 10 minutes` rest in a supine position, with no shoes and in light clothes. Selected biochemical analyses were performed and the remaining biological material was stored in a biological specimen bank at –20°C.

8.1.2 Alcohol

Two independent methods were used to estimate alcohol consumption in papers I–III. The first method was the use of a biochemical marker, serum γ-glutamyl transferase (γ-GT). Virtually all participants in the MPP had γ-GT analysed (99.7%) as a part of the baseline examination. γ-GT is an enzyme involved in the transfer of certain amino acids across the cell membrane and in leukotriene metabolism (part of the immune system that contributes to inflammation seen for instance in asthma). The main source is the hepatobiliary system, but it is found in endothelial cell membranes in various organs as well. Serum

γ-GT activity is quite steady without any substantial circadian or day-to-day difference in a given person [165]. Serum levels of γ-GT may be affected by several conditions, such as hepatic or biliary diseases, obesity and insulin resistance. Multiple drugs can increase serum γ-GT and increased levels have been found in smokers [165]. As a screening marker for heavy drinking, γ-GT therefore poses a problem. Ideally a good screening marker should have high sensitivity (probability of a positive test among individuals with excessive alcohol intake) and high specificity (probability of a negative test among individuals without excessive alcohol intake). Unfortunately, the sensitivity of γ-GT for detecting heavy drinkers is rather poor (30–50%), and the specificity varies greatly in different settings (40%–90%) [166]. In spite of this, γ-GT is a useful adjunct for identifying and management of excessive drinkers and has been proven to be a useful determinant for the risk of alcohol-related comorbidities [167–169].

The second method was a scoring system based on a modified version of the Michigan Alcoholism Screening Test [170], referred to as the Malmö modification of the brief MAST (Mm-MAST) [167]. The notion of these tests is to detect alcohol addiction, using questions on attitudes and customs, rather than questions on actual amounts of ingested alcohol. The Mm-MAST consists of seven questions concerning drinking habits (table 2). These questions were not introduced into the questionnaire until December 1976 and this is why there are no registered answers for the first 2,142 subjects attending the MPP. A scoring system was established whereby a "yes" gave one point and a "no" gave no points, except for the question "are you a teetotaller?" where the scoring was reversed. Alcohol consumption was classified as "low" for subjects with a scoring of 0–1, "intermediate" for a scoring of 2–3 and "high" for subjects with a scoring of 4 or more. Alcohol consumption was registered as "missing" for individuals with one or more

Table 2. Malmö modification of the brief MAST (Mm-MAST)

Questions	No. of "yes" answers	Percent*
1. Are you a teetotaller?	2.590	8.5
2. Do you take a drink before going to a party?	5.440	17.9
3. Do you usually drink a bottle of wine or corresponding amounts of alcohol during the weekend?	10.093	33.1
4. Do you drink a couple of drinks (or beers) a day to relax?	1.176	3.9
5. Do you tolerate more alcohol now than you did ten years ago?	2.605	8.6
6. Do you fall asleep after moderate drinking. not knowing how you got to bed?	2.359	7.7
7. Do you have a bad conscience after drinking?	2.264	7.4

* Total number of subjects: 30.451

missing answers, comprising 753 subjects.

Recognizing that alcohol-related disorders pose a problem in somatic and psychiatric medical care has made it necessary to develop tests aimed at detecting alcoholism. Questionnaires such as Mm-MAST have been developed for this purpose and have been found to be simple, rapid and convenient instruments to administer. The original MAST questionnaire has been validated and proven to be a relatively sensitive and specific instrument [171]. In the Malmö modification of the brief MAST, the researchers chose questions on attitude and customs rather than serious symptoms, in order to avoid upsetting the respondents. The main purpose was to make use of the Mm-MAST as a supplement in screening heavy alcohol users by γ-GT and the Mm-MAST was shown to be a valid tool for identifying heavy drinkers and alcoholism, with a sensitivity of 73% and a specificity of approximately 95% [167].

8.1.3 Smoking Habits

The questionnaire at baseline examination in the MPP consisted of questions regarding daily tobacco dose and time since cessation. The questions "do you smoke?" and "have you ever smoked on a daily basis for a period of at least six months?" were used to classify smoking status as never, current and former smokers in papers I–III. If the answer was negative for both questions, the subject was classified as a never smoker, if the first question ("do you smoke?") was answered positively, the subject was classified as a current smoker. A respondent who did not currently smoke but who confirmed a previous habit of daily smoking that lasted at least 6 months was regarded as a former smoker. Missing and inconsistent answers could be identified and resolved using the other questions on smoking habits, such as the questions regarding tobacco dose and time since cessation. These questions were further used to define amount of tobacco used on a daily basis and the time since cessation, but the number of cases was too small in these subgroups to allow for separate analysis.

8.1.4 Body Mass Index and weight gain

Weight and height were measured without shoes and in light indoor clothes by a trained nurse. Body mass index (BMI) was calculated as weight in kg divided by the squared height in meter (kg/m^2). BMI was classified as follows; underweight <20, normal weight 20–≥25, overweight 25–≥30 and obesity >30, but in papers I and II underweight were added to the normal weight category in the subgroup analysis because of the small number of cases.

In paper I the question "have you gained more than 10 kg since the age of 30 years?" with the possible answers "yes" or "no", was used to define weight gain.

8.1.5 H.pylori, HAT, HCT, PSTI (paper II–III)

In the MPP, blood samples were drawn following an overnight fast, as mentioned in section 8.1.1. The samples were separated and several routine tests were performed immediately. Remaining serum and plasma samples were entered into a bio-bank and stored at –20°C, until thawed in December 1999. The median storage time, meaning the time that had elapsed from baseline investigation until analysis, was 25 years on average.

HAT and HCT were analysed using two specific in-house solid-phase-double antibody enzyme-linked immunosorbent assays (ELISAs), described by Kimland *et al.* [172]. The interassay coefficient of variance (CV) was 3.5% for HAT and 3.1% for HCT. PSTI was measured by a specific monoclonal antibody against human PSTI, produced by Bohe *et al.* in 1992 [173]. This antibody was used in an ELISA to measure PSTI. The interassay CV was 2.1%.

IgG antibodies against H.pylori were measured by an in-house enzyme-linked immunosorbent assay (ELISA) at the department of microbiology, Malmö University Hospital. Absorbance > 0.70 was regarded as a positive test. The validity of this assay has previously been investigated in a similar setting on stored blood samples from the same cohort. In that study immunoreactivity was found to be stable over time and a bimodal distribution in the absorbance level was demonstrated with two distinct populations well separated by the cut-off level of 0.70 [174].

The prolonged storage time may have affected the antigen immunoreactivity i.e. the antigen may have undergone some degradation over time or fluid may have evaporated due to insufficiently tightened caps, causing either a decrease or an increase in concentrations. There was no significant association between IgG antibody levels and storage time regarding H.pylori [174]. The serum values of HAT, HCT and PSTI were similarly plotted against storage time. All showed a slight decline over time. However, for HAT and HCT, the linear correlation coefficient for both controls and cases was close to zero and not statistically significant. For PSTI the decline was slightly stronger and statistically significant, with a β coefficient for cases –0.12 (95% CI –0.20–(–0.05)) and β coefficient for controls –0.06 (95% CI –0.10–(–0.01)), i.e. there were no great differences between cases and controls. In order to adjust for changes related to storage time, the multivariable analyses were adjusted for time from baseline until analysis, section 9.2 below.

8.2 The Me-Can study (paper IV)

Data on height, weight, blood pressure and blood/ plasma/ serum levels of glucose, total cholesterol and triglycerides were available in all cohorts. Height and weight were measured in a similar fashion in all seven cohorts; without shoes and wearing light clothes. Blood pressure was measured with a mercury sphygmomanometer except in CONOR and 40-y, where an automatic device was used. Resting time before measurement varied between 2–10 minutes and was performed in a sitting position in all cohorts, except in the VIP and MPP where a supine position was the standard procedure. Participants in the Norwegian cohort were not required to fast before examination, neither were participants in the VIP until 1992 when all were asked to fast for at least eight hours before examination. Participants in the MPP were asked to fast overnight, as was done in the VHM&PP. Glucose was measured in serum in the Norwegian cohorts, in plasma in the VHM&PP and the VIP, and in whole blood in the MPP. Cholesterol and triglycerides levels were measured in serum in all cohorts. Determination of glucose and lipid levels was performed by enzymatic methods in all cohorts, except in the Oslo cohort and NCS, where non-enzymatic methods were used, except for lipids, for which an enzymatic method was used after 1980. In the Norwegian cohorts, glucose levels measured with the non-enzymatic method yielded 0.8–1.1 mmol/l higher levels as compared to levels measured with the enzymatic method [175]. For cholesterol and triglycerides, levels from the non-enzymatic method were compared with the enzymatic method, they were transformed according to the formulas: [$\text{cholesterol}_{\text{enzymatic}} = 0.92 \times \text{cholesterol}_{\text{non-enzymatic}} + 0.03$] and [$\text{triglyceride}_{\text{senzymatic}} = 0.90 \times \text{triglyceride}_{\text{non-enzymatic}} - 0.11$] [176]. A more extensive description can be found in a recently published paper by Stocks *et al.* [156].

9. Statistical analyses

A p-value of less than 0.05 was considered statistically significant in all analyses corresponding to a confidence interval (CI) of 95%. All tests were two-sided.

All statistical calculations were performed using the software SPSS 14.0, 15.0 or 17.0.

9.1 The MPP cohort study (paper I)

All participants in the MPP were followed from baseline until a diagnosis of pancreatic cancer, death or end of follow-up 31 Dec. 2004. Mean follow-up was 22.1 years and the total number of person-years was 739,612.73. The incidence of pancreatic cancer was calculated per 100,000 person-years in different categories of studied exposures. Cox's proportional hazards analysis was used to estimate relative risks (RR) with a 95% CI. In the adjusted analysis, age at diagnosis was entered as a continuous factor, and sex, smoking status, alcohol consumption category (Mm-MAST), γ-GT, BMI and weight gain were entered as categorical variables. To adjust for alcohol consumption the Mm-MAST score was chosen, since it may be a more specific marker of alcohol consumption than γ-GT.

The RR for pancreatic cancer related to smoking and alcohol intake was analyzed in different strata of smoking, alcohol consumption, BMI and weight gain in order to detect modifying effects. Combining different levels of smoking and alcohol consumption required comparisons of groups with a limited number of cases, and some of these analyses used a dichotomized variable on alcohol consumption and γ-GT. That is, high/intermediate vs. low according to Mm-MAST, and γ-GT-quartile 4 vs. γ-GT-quartile 1–3. Interaction between smoking, alcohol and BMI was analyzed by entering one covariate multiplied by the other as an interaction term.

9.2 The MPP case/control study (paper II and III)

In both papers, median age and time from baseline to analysis and the distribution of baseline characteristics between cases and controls were analysed for the examined parameters. In paper II the baseline characteristics were compared between included and non-included cases/controls in order to discover if the two groups differed. In paper III, the distribution of baseline characteristics was compared between subjects with a positive and a negative H.pylori serology. To assess the risk of pancreatic cancer in relation to analyzed measurements, unconditional logistic regression analysis was used to estimate crude and adjusted odds ratio (OR) with a 95% CI. Conditional logistic regression analyses were considered inappropriate since the case-control matching was abandoned due to missing blood samples for several cases and controls.

Medians were calculated for HAT, HCT and PSTI, for the sum of the trypsinogens (HAT+HCT), and for the ratios HAT/HCT and HAT+HCT divided by PSTI. The calculations were performed for all subjects and repeated stratified for sex. In order to analyse whether there were any differences between cases and controls, a Mann-Whitney U-test was used. In paper III, the difference was tested not only between cases/controls, but also between negative vs. positive H.pylori serology, using a Mann-Whitney U-test for continuous variables and a Chi-square test for categorical variables. The unpaired Mann-Whitney test was considered appropriate as the case-control matching had been abandoned.

Adjusted OR was obtained by including age, time from baseline to analysis, and BMI as continuous factors, and sex, alcohol consumption according to Mm-MAST, smoking status and H.pylori serology as categorical factors, in the logistic regression model. In paper III, simultaneous adjustment for Mm-MAST and γ-GT tertiles was considered inappropriate since both are used as surrogate markers for the same exposure, i.e. alcohol consumption. However, in paper III all calculations were repeated with γ-GT tertiles for comparison. To facilitate interpretation of the OR, the levels of HAT, HCT and PSTI were analyzed in mul-

tiples of 10 and for the ratios HAT/HCT and (HAT+HCT)/PSTI in multiples of 0.1.

OR for pancreatic cancer in relation to trypsinogen and PSTI were analyzed in different strata of sex, smoking status, alcohol consumption and BMI and the OR for pancreatic cancer in relation to H.pylori serology in separate strata of smoking status, MmMAST category, γ-GT tertiles and BMI. Due to a small number of cases in each stratum of smoking status and alcohol consumption, the number of covariates included at the same time had to be reduced and were therefore entered one at a time in order to determine factors with a significant impact on the association between analysed parameters and pancreatic cancer. To compare whether or not the statistical models were stable, in spite of the unduly large number of entered covariates, the OR adjusted for all covariates at the same time were calculated.

9.3 The Me-Can cohort study (Paper IV)

The association with pancreatic cancer risk was assessed for BMI, mid-blood pressure [mid BP = $(BP_{systolic} + BP_{diastolic})/2$] and for levels of glucose, cholesterol and triglycerides. The RR was studied in quintile levels and for the Z score for these parameters. Quintile cut-offs were calculated separately within each cohort and sex, and for glucose, cholesterol and triglycerides, also in categories of fasting time. Fasting time was defined as less than four hours, from four to eight hours and more than eight hours. The Z score was derived by standardising the parameters within the same group as was done for quintile cut-off calculation (cohort, sex and fasting time), by [(exposure level – mean)/SD], resulting in a Z score of the examined parameters with a mean of zero and a standard deviation (SD) of one. Glucose and triglycerides were log-transformed before standardization, as they were skewed and had several outliers. A MetS Z score was calculated by summing the five individual Z score values and the sum was standardized within cohort, sex and fasting time. This was done in order to create the same distribution as the single parameters and thereby enable a direct comparison of the collective exposures with the risk of pancreatic cancer.

A Cox proportional hazards analysis was used and stratified by cohort and by categories of birth-year: before 1923, 1923–1930, 1931–1938, 1939–1946, 1947–1954, 1955 and later. Attained age was used as the time scale and the model included adjustment for age at baseline as a continuous variable, and smoking status as a categorical variable. The quintile analyses of all the parameters, except BMI, were further adjusted for BMI. Analyses of the individual Z scores were performed crudely and with the inclusion of all parameters (all adjusted for all) in the adjusted model. In the analysis of the MetS, all estimates were adjusted for age at baseline and smoking status. The Z score was furthermore examined in strata of smoking status and sex in the same way. The p-value for trend over quintiles refers to the Wald test of a linear risk estimate. All analyses were performed separately for men and women. Interaction between metabolic factors, sex and smoking was analyzed by entering one covariate multiplied by the other as an interaction term.

9.3.1 Regression dilution error

Risk estimates were adjusted for random error in exposure measurements. These calculations were based on repeated health examinations in 133,820 subjects, including 406,364 observations in the full Me-Can database. The database was cleared of measurements preceded by a cancer diagnosis, of repeated measurements from a different cohort and of measurements with a different fasting time as compared to baseline measurements. An exception to this was made pairwise for the Oslo and the NCS cohorts and for the CONOR and 40-y

Table 3. Estimated RDR correction values;

	BMI	Mid BP	Glucose (log)	Cholesterol	Triglycerides (log)	MetS
Men	0.899	0.528	0.283	0.644	0.512	0.667
Women	0.897	0.555	0.271	0.660	0.504	0.692

cohorts. That is, if baseline measurement was done in the Oslo study a repeated measurement performed in the NCS was accepted, but not from CONOR or the 40-y cohort and visa versa. Finally, exclusions were made if there was missing data on any of the parameters included in the MetS, fasting time or smoking status.

The combined effect of measurement errors of the different parameters (BMI, mid-blood pressure, glucose, cholesterol and triglycerides) and long-term fluctuations within the individuals may lead to a regression dilution bias. In order to correct for potential regression dilution bias in the analysis based on quintiles, the regression dilution ratio (RDR) was used [177]. This was performed as a linear mixed model, which included the actual exposure (repeated measurement as dependent and baseline measurement as independent variable), age at baseline, birth year, fasting time, smoking status and time from baseline as fixed effects and cohort as random effect. Correction of the RRs were obtained directly by dividing the regression coefficient in the Cox model by the estimated regression dilution ratio (RDR) of the exposures [exp (log (RR)/ RDR)], using a gender specific RDR (table 3). All exposures, except BMI, had a substantial random error [178–180].

The correction by regression dilution ratio was not suitable in models using more than one variable measured with error. In such situations the RRs was corrected indirectly by replacing each original Z score in the Cox model with its conditional expected value, i.e. regression calibration (RC) [181]. With this method, the exposure measured with error (the observed measurement) was replaced with a predicted value calculated from a regression model, similar as described above, but also including the other metabolic factors. The corrected measurement was then used in the risk model estimation [177, 181]. In order to obtain a "usual value" of the examined parameters, RDR and RC were predicted for the time point at approximately half the follow-up time (≈ six years) [178].

10. Main results

10.1 Alcohol and pancreatic cancer (paper I)

Several potential risk factors were included in this paper and the characteristics of the studied subjects are shown in Table 4. Mean age at baseline was slightly higher between cases than in the rest of the cohort and the mean years of follow-up differed by almost seven years between cases and the rest of the cohort.

The co-variation of the potential risk factors at baseline were analysed and the correlation between Mm-MAST and γ-GT was found to be fairly good, even though only 8% of the cohort were classified as high alcohol consumers (high Mm-MAST category). The low percentage was reflected in the fact that only 12% of subjects with a high γ-GT (≥ 0.63 μkat/L), also reported a high alcohol

Table 4. Baseline characteristics of studied subjects in paper I (Malmö Preventive Project MPP)

Factor	Category	Cases	Rest of cohort
Subjects		183	33.163
Age at baseline. mean (SD)		48.3 (6.0)	45.7 (7.4)
Follow up. mean (SD)		15.3 (6.9)	22.2 (5.5)
BMI. mean (SD)		25.1 (3.7)	24.6 (3.6)
Smoking status (%)	Never	38 (20.8)	12.397 (37.4)
	Current	107 (58.5)	14.743 (44.5)
	Former	38 (20.8)	5.972 (18.0)
	Missing	0	51 (0.2)
Alcohol consumption Mm-MAST (%)	Low	71 (38.8)	16.021 (48.2)
	Intermediate	78 (42.6)	11.993 (36.2)
	High	14 (7.7)	2.274 (6.9)
	Missing	20 (10.9)	2.875 (8.7)
γ-GT quartile (μkat/L) (%)	<0.29	32 (17.5)	8978 (27.2)
	0.29–0.41	43 (23.5	7654 (23.2)
	0.41–0.63	40 (21.9)	8263 (25.0)
	≥0.63	68 (37.1)	8161 (24.6)
	Missing	0	107 (0.3)

consumption (fig.6). However, in subjects reporting a high score in Mm-MAST almost 44% were found to have a high γ-GT. A high percentage of subjects that reported intermediate/high alcohol consumption or were in the highest γ-GT quartile were current smokers. Furthermore, subjects in the highest γ-GT quartile were more often obese, although on the other hand there was no large difference between Mm-MAST categories with regard to BMI. The correlation between self-reported weight gain and BMI was very good; 79% had a BMI > 25 among subjects reporting a previous weight gain, as compared to 34% among subjects who reported no weight gain.

Both the high Mm-MAST category and the top quartile of γ-GT were positively associated with an increased risk of pancreatic cancer, although the risk associated with the highest Mm-MAST category did not reach statistical significance (Table 5). To further strengthen the definition of "high alcohol consumption" a composite variable was created by combining the fourth quartile of γ-GT with high alcohol consumption, defined as intermediate/high. This group had a RR of pancreatic cancer of 2.41 (1.51;3.82) as compared to subjects with a low consumption (low Mm-MAST category combined with the first three quartiles of γ-GT).

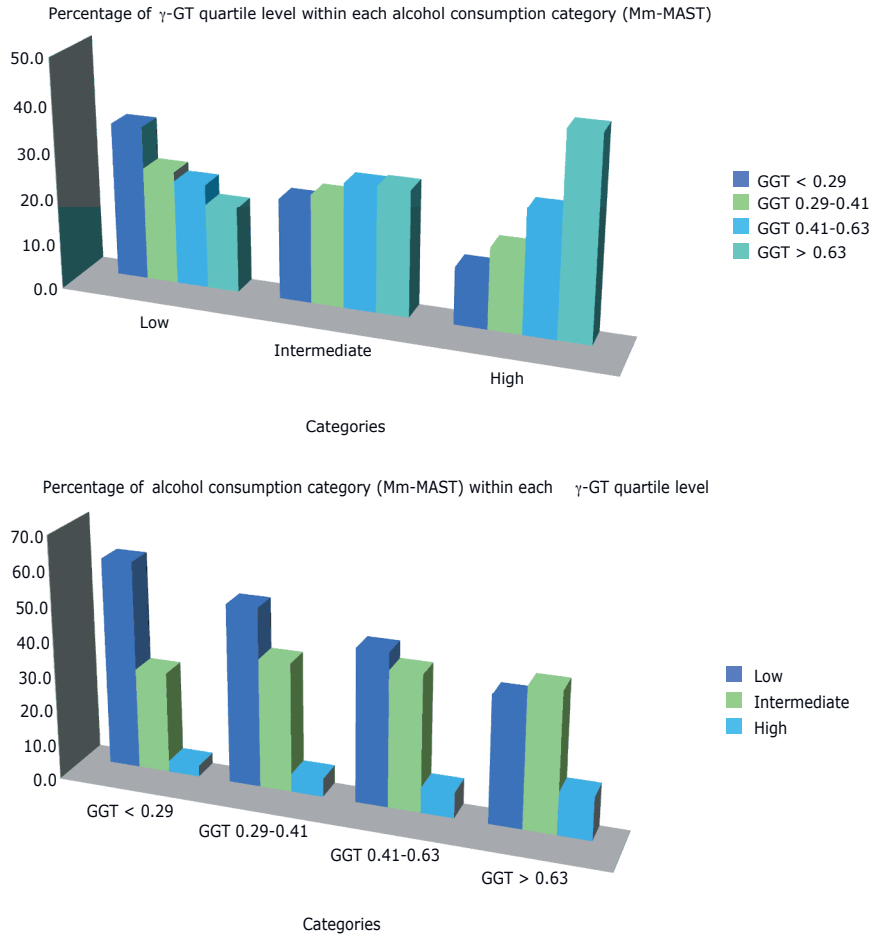

Fig. 6. *Co-variation between the two different measurements of alcohol consumption*

The alcohol consumption category and γ-GT was furthermore explored in the strata of BMI category, weight gain and smoking status. A statistically significant association with the risk of pancreatic cancer was found in γ-GT quartile 2; RR 1.83 (1.04;3.21) and 4; RR 2.22 (1.23;4.00) in subjects with a BMI < 25. Contrary to this finding, subjects that reported a previous weight gain had a risk of pancreatic cancer for the γ-GT quartile 4 of 3.61 (1.29;10.09), as compared to quartile 1. Apart from this, no large differences were seen in relation to different BMI and weight gain categories. A high alcohol consumption category (Mm-MAST) was associated with a high risk in former smokers, RR 2.13(1.05;4.32) and high γ-GT(≥ 0.63 μkat/l) was associated with a high risk in current smokers, RR 2.01(1.34;3.02). Several of the stratified analyses included only a few cases and the corresponding confidence intervals were wide. No statistically significant interactions were found between alcohol and BMI, alcohol and weight gain, or between alcohol and smoking.

Current smoking was statistically significantly associated with pancreatic cancer and

Table 5. Incidence and relative risk of pancreatic cancer in different exposure categories

Factor	Category	Incidence/ 100 000 person-years	Relative Risk ‡ (95% confidence interval)
Smoking status. tobacco dose and former smokers abstinence time	Never	13.7	Reference
	All Current	32.7	2.34 (1.60–3.43)
	<20 cigarettes per day	31.4	2.25 (1.45–3.50)
	>20 cigarettes per day	34.8	2.56 (1.60–4.09)
	Missing dose	32.2	2.31 (1.37–3.89)
	Former	28.4	1.61 (1.02–2.55)
	Abstinence <5 yrs	20.3	1.23 (0.57–2.67)
	Abstinence >5 yrs	36.7	2.00 (1.21–3.29)
	Missing	0	–
	Missing status	0	–
Alcohol consumption (Mm-MAST-category) †	Low	20.1	Reference
	Intermediate	28.7	1.50 (1.07–2.08)
	High	27.9	1.58 (0.88–2.86)
	Missing	31.2	1.06 (0.62–1.79)
	Trend (low–high)		0.05
γ-GT*-quartile (μkat/L)	1 (<0.29)	16.8	Reference
	2 (0.29–0.41)	24.7	1.52 (0.95–2.45)
	3 (0.41–0.63)	20.9	1.24 (0.75–2.03)
	4 (0.63)	37.4	2.15 (1.34–3.44)
	Missing	0	–
	Trend (multiples of 0.1)		1.01 (1.01–1.02)
Body mass index (kg/m^2)	<20	19.1	0.84 (0.44–1.61)
	20–25	25.4	Reference
	25–30	22.6	0.83 (0.60–1.16)
	>30	36.1	1.38 (0.83–2.28)
	Missing	0	–
	Trend (continuous)		0.29
Have you gained >10 kg since the age of 30	No	27.7	Reference
	Yes	32.2	1.07 (0.77–1.48)
	Missing	8.6	0.65 (0.34–1.27)

* γ-GT; γ-Glutamyl transferase.
† Mm-MAST; Malmö modification of the brief Michigan Alcoholism Screening Test.
‡ Adjusted for age. sex. smoking status. Mm-MAST category for all categories except γ-GT). and body mass index for all categories except weight gain.

there was a tendency towards a dose-response relationship (Table 5). A positive association was furthermore seen between previous smoking and the risk of pancreatic cancer. Adjusting the analyses for γ-GT instead of Mm-MAST did not change the estimates. Current smoking was associated with pancreatic cancer in each stratum of BMI, alcohol consumption and γ-GT. In the group of obese (BMI > 30) an even higher risk, associated with current smoking, was found of RR 7.45(1.65;33.64). Contrary to this an increased risk of pancreatic cancer for current smoking was seen in subjects who reported no previous weight gain. In former smokers, there was an especially high risk of pancreatic cancer in the overweight (BMI 25–30) subjects, as compared to participants with a BMI < 25, RR 2.20(1.04;4.67). No statistically significant interactions were found between smoking and any of the other exposures.

10.2 Trypsinogens, PSTI, H.Pylori and pancreatic cancer (paper II and III)

Baseline characteristics, regarding matching factors in cases and controls in papers II and III, showed a highly similar pattern even though the case-control matching was partially disrupted (table 6). There was a higher proportion of current smokers among cases as compared to controls and a slightly higher proportion of smokers among H.pylori positive subjects as compared to H.pylori negative subjects. Alcohol consumption (Mm-MAST) and BMI were similar in cases and controls and in H.pylori positive and negative subjects. The median of the two isoforms of trypsinogen, the sum and the ratios of these and PSTI did not show any differences between cases and controls. The difference related to the ratio HAT/HCT among women, was statistically significantly higher in cases than in controls (p value 0.03).

There was a positive association for the ratio HAT/HCT and the risk of pancreatic cancer in women (table 7). Apart from this there were no strong associations for HAT, HCT, (HAT+HCT), HAT/HCT, PSTI and (HAT+HCT)/PSTI in relation to pancreatic cancer. In the stratified analysis the sizes of the subgroups were small and possible covariates were therefore entered one at a time. HAT and HAT/HCT was positively associated with pancreatic cancer in the intermediate/high alcohol consumption group and among subjects with a BMI < 25. The sum of HAT and HCT and PSTI showed a similar pattern, but was only borderline significant in the intermediate/high alcohol consumption group.

In paper III there were no association between H.pylori seropositivity and pancreatic cancer in the overall analysis (table 8). In the stratified analysis the sizes of the subgroups were small and possible covariates were therefore entered one at a time. BMI and alcohol consumption measured by Mm-MAST were the two covariates that had the most important impact on the association between positive H.pylori serology and pancreatic cancer in never smokers. When both these covariates were entered in the analysis, the OR for pancreatic cancer in H.pylori positive vs. negative subjects was 4.45 (1.19;16.69).

In the subgroup reporting a low Mm-MAST score, the unadjusted model was positively associated with pancreatic cancer for positive H.pylori serology. This association remained statistically significant when adjusted for covariates entered one at a time, except for smoking status and when all covariates were entered at the same time (table 8). In the small subgroup, who reported low Mm-MAST category and who had never smoked, the crude OR for pancreatic cancer was 13.20 (2.31;75.31).

Table 6. Baseline characteristics of included cases and control subjects as compared to non-included cases and controls

		Included in analysis		Excluded from analysis	
Factor	Category	Cases (87)	Controls (264)	Cases (26)	Controls (75)
Age (years)		47.9 (37.7–60.6)	47.5 (37.3–60.6)	48.5 (38.9–55.2)	48.7 (38.5–60.6)
Time from baseline investigation to analysis (years)		24.8 (14.3–28.8)	25.1 (18.1–30.3)	Not analyzed	Not analyzed
Sex	Female	29 (31.3%)	76 (28.9%)	4 (13.8%)	14 (18.7%)
	Male	58 (66.7%)	188 (71.2%)	25 (86.2%)	61 (81.3%)
Body mass index (kilo/meter²)		24.9 (18–41)	24.6 (18–34)	25.4 (21–33)	25.6 (18–40)
Alcohol Consumption (Mm-MAST category)*	Low	35 (40.2%)	129 (48.9%)	6 (20.7%)	25 (33.3%)
	Intermediate	42 (48.3%)	114 (43.2%)	8 (27.6%)	13 (17.3%)
	High	7 (8.0%)	14 (5.3%)	2 (6.9%)	0
	Missing	3 (3.4%9	7 (2.7%)	13 (44.8%)	37 (49.3%)
Smoking status	Never	13 (15.5%)	88 (33.3%)	2 (6.9%9	20 (26.7%)
	Current	54 (64.3%)	116 (43.9%)	18 (62.1%)	35 (46.7%)
	Former	17 (20.2%)	60 (22.7%)	9 (31.0%)	20 (26.7%)
Helicobacter pylori serology	Negative	48 (55.2%)	163 (61.7%)	Not analyzed	Not analyzed
	Positive	39 (44.8%)	100 (37.9%)	Not analyzed	Not analyzed
	Missing	0	1 (0.3%)		

Table 7. Pancreatic cancer risk in relation to the ratio HAT/HCT

Factor	Model	All	Women	Men
HAT/HCT	Cases/controls	81/249	24/73	56/176
(multiples of 0.1)	Crude OR	1.10 (1.00–1.22)	1.35 (1.02–1.79)	1.07 (0.96–1.19)
	Age	1.10 (0.99–1.21)	1.31 (0.98–1.76)	1.07 (0.96–1.19)
	Sex	1.11 (1.00–1.22)	–	–
	Time to analysis	1.10 (0.99–1.21)	1.34 (1.00–1.78)	1.07 (0.96–1.19)
	BMI	1.11 (1.00–1.22)	1.34 (1.02–1.80)	1.07 (0.96–1.19)
	Mm-MAST	1.09 (0.98–1.20)	1.37 (1.01–1.87)	1.05 (0.95–1.18)
	Smoking status	1.08 (0.98–1.19)	1.22 (0.90–1.66)	1.07 (0.96–1.91)
	Hp serology	1.11 (1.00–1.22)	1.34 (1.02–1.78)	1.08 (0.97–1.20)
	All covariates	1.05 (0.95–1.17)	1.15 (0.81–1.62)	1.06 (0.94–1.19)

Table 8. Crude and adjusted odds ratio (OR) for pancreatic cancer in relation to Helicobacter pylori serology. smoking status and alcohol consumption

		Positive H.pyori serology vs. negative	
Factor	Status		OR (95 % CI)
H. pylori serology		Adj. for age. sex. BMI and time to analysis. smoking status and Mm-MAST*	1.25 (0.75–2.09)
Stratified for smoking	Never smoker	Adj. for BMI	3.77 (1.05–13.48)
		Adj. for alcohol**	3.81 (1.06–13.63)
		Adj. for all covariates	4.97 (1.23–20.10)
Stratified for alcohol consumption (Mm-MAST category)*	Low	crude	2.33 (1.09–4.97)
		Adj. for age	2.31 (1.08–4.97)
		Adj. for sex	2.33 (1.09–4.98)
		Adj. for time to analysis	2.32 (1.08–4.96)
		Adj. for BMI	2.33 (1.09–4.97)

*Mm-MAST; Malmö modification of the brief Michigan Alcoholism Screening Test.

Table 9. Baseline characteristics of the Me-Can cohorts

	Men				Women			
	Cases		Rest of cohort		Cases		Rest of cohort	
Subjects. n	547		288.429		315		288.024	
Age at baseline. mean (SD)	49.3	(9.6)	43.9	(11.1)	52.8	(10.6)	44.1	(12.3)
Cohort (%)								
Oslo	119	(21.8)	16.596	(5.8)	0	(0)	0	(0)
NCS	98	(17.9)	25.781	(8.9)	80	(25.4)	24.971	(8.7)
CONOR	35	(6.4)	51.890	(18.0)	22	(7.0)	57.492	(20.0)
40-y	19	(3.5)	60.585	(21.0)	15	(4.8)	68.135	(23.7)
VHM&PP	94	(17.2)	72.843	(25.3)	83	(26.3)	86.420	(30.0)
VIP	49	(9.0)	38.697	(13.4)	52	(16.5)	40.562	(14.1)
MPP	133	(24.3)	22.034	(7.6)	63	(20.0)	10.444	(3.6)
Fasting time (%)								
<4 hrs	223	(40.8)	119.951	(41.6)	103	(32.7)	122.016	(42.4)
4–8 hrs	42	(7.7)	30.627	(10.6)	23	(7.3)	26.727	(9.3)
>8 hrs	282	(51.6)	137.851	(47.8)	189	(60.0)	139.281	(48.4)
BMI. kg/m2 mean (SD)	25.3	(3.5)	25.7	(3.5)	25.8	(4.3)	24.9	(4.4)
Mid BP. mmHg mean (SD)	110.7	(13.7)	108.2	(35.9)	116.4	(72.3)	101.8	(14.2)
Missing (%)	0	(0)	411	(0.1)	2	(0.6)	485	(0.2)
Glucose. mmol/l median (IQR)	5.3	(1.4)	5.2	(1.3)	5.3	(2.2)	5.0	(1.2)
Missing (%)	2	(0.4)	414	(0.1)	2	(0.6)	355	(0.1)
Cholesterol. mmol/l mean (SD)	5.9	(1.1)	5.7	(1.2)	6.2	(1.2)	5.5	(1.2)
Missing (%)	2	(0.4)	590	(0.2)	1	(0.3)	775	(0.3)
Triglycerides. mmol/l median (IQR)	1.5	(1.1)	1.5	(1.3)	1.3	(1.0)	1.1	(0.8)
Missing (%)	16	(2.9)	7.738	(2.7)	9	(2.9)	4.514	(1.6)
Smoking status. n (%)								
Never	141	(25.8)	113.046	(39.2)	155	(49.2)	144.384	(50.1)
Former	127	(23.2)	85.747	(29.7)	42	(13.5)	72.464	(25.2)
Current	277	(50.6)	88.777	(30.8)	115	(36.9)	70.484	(24.5)
Missing	2	(0.4)	859	(0.3)	3	(1.0)	692	(0.2)

SD. standard deviation; IQR. interquartile range; BMI. body mass index; Mid BP. mid blood pressure; all percentages are column
Z-score analysis of single factors and the combined MetS score (Reproduced from paper IV)

10.3 Metabolic syndrome and pancreatic cancer (paper IV)

The majority of the subjects in the Me-Can study group were aged between 30–59 years, and the mean age at baseline was somewhat higher in both the male and female case group as compared to the rest of the cohort. Follow-up was 12.8 years (SD 8.5) among men and 11.3 years (SD 6.9) among women, and there were no great differences in follow-up between cases and rest of the cohort (table 9). Except for BMI, absolute levels of exposure factors were not readilly comparable between the Me-Can cohorts, as different measurement methods had been used. In women, the means/medians for mid BP, glucose and cholesterol were higher in cases, as compared to the rest of cohort. Analyses of repeated measurements revealed that random error for measurements of BMI was low (high RDR), but was high for all other exposure factors.

Absolute risks were calculated in quintiles separately for men and women, and a low risk in low quintiles was found among women. For high quintiles the risk became nearly equal, though it was generally lower in women. The 5th quintile of the adjusted and RDR corrected mid-blood pressure was positively associated with pancreatic cancer in men and a linear positive association was found for mid-blood pressure and glucose. Among women, a positive risk association was found for the 5th quintile level of BMI adjusted and RDR-corrected, for the 5th quintile of mid-blood pressure crude, adjusted and RDR-corrected, and for the 4th and 5th quintile of glucose crude, adjusted and RDR-corrected. The linear analysis of the continuous values was statistically significantly associated with pancreatic cancer for mid-blood pressure and glucose in women.

In the continuous Z score analysis, increased risks for pancreatic cancer were found among both men and women for glucose and mid-blood pressure. For men though, only the adjusted Z score reached statistical significance (table 10). Furthermore, in women a statistically significantly positive association was found for the MetS score both crude, adjusted and calibrated and the risk of pancreatic cancer. Following regression calibration (RC) most point estimates were slightly stronger and CIs were wider. Significant effect modification was found towards a stronger association between glucose and pancreatic cancer in women, as compared to men (p for interaction = 0.02).

In order to explore the possible interactions with smoking status, the continuous Z score was examined in different strata of smoking habits (Table 11). Among those who had never smoked the Z score of glucose, both adjusted and calibrated was positively associated with pancreatic cancer in men. In women mid-blood pressure, glucose and the MetS were associated with pancreatic cancer in the crude, the adjusted and calibrated analysis. In former smoking women a crude association was found for BMI, glucose, triglycerides and the MetS, but in the adjusted and calibrated analysis only the MetS continued to be positively associated with pancreatic cancer. Finally, in current smoking men, the continuous Z score for mid BP crude, adjusted and calibrated was associated with the risk of pancreatic cancer. In women this was found for glucose and for the MetS. Regression calibration did not change the results to any great extent. A statistically significant interaction was found in men between former smokers and cholesterol with a larger effect in those who had never smoked, and between current smokers and mid-blood pressure and current smokers and triglycerides, with a larger effect in current smokers. In women, a statistically significant interaction was found between glucose and never and current smokers, between cholesterol and current smokers and between the MetS and former smokers. For the MetS and current smokers the relationship was inverted i.e. with a larger effect in those who had never smoked.

Table 10. Risk of pancreatic cancer in the Me-Can cohort in relation to metabolic factors.

	Men (*n*= 545)			Women (*n*= 315)			Interaction[5] *p*-value
Exposure	z-score. crude[1]	z-score. adjusted[2]	z-score. calibrated[3]	Z score. crude[1]	Z score. adjusted[2]	z score. calibrated[3]	
BMI	0.98 (0.90–1.07)	0.97 (0.88–1.07)	0.90 (0.80–1.02)	1.07 (0.96–1.20)	1.04 (0.92–1.17)	0.92 (0.79–1.07)	0.45
Mid blood pressure	1.07 (0.98–1.16)	1.10 (1.01–1.20)	1.15 (0.97–1.35)	1.19 (1.07–1.32)	1.22 (1.09–1.36)	1.34 (1.08–1.66)	0.06
Glucose	1.08 (1.00–1.17)	1.09 (1.00–1.18)	1.37 (1.01–1.85)	1.23 (1.14–1.34)	1.20 (1.10–1.32)	1.98 (1.41–2.76)	0.02
Cholesterol	0.92 (0.84–1.00)	0.87 (0.79–0.96)	0.81 (0.69–0.95)	1.10 (0.99–1.23)	1.09 (0.96–1.22)	1.16 (0.96–1.41)	0.08
Triglycerides	1.05 (0.96–1.14)	1.04 (0.94–1.15)	1.04 (0.84–1.29)	1.16 (1.04–1.29)	1.00 (0.88–1.22)	0.91 (0.69–1.96)	0.22
MetS[4]	1.04 (0.95–1.14)	1.13 (0.90–1.41)	1.07 (0.94–1.22)	1.32 (1.18–1.47)	1.36 (1.22–1.53)	1.58 (1.34–1.87)	0.18

MetS; metabolic syndrome. BMI; body mass index.

[1] Relative risk calculated from Cox regression models. with attained age as time scale. stratified by cohort and categories of birth year.

[2] Adjusted for age at baseline. smoking status and for the z-score of analyzed factors i.e. BMI. mid BP. glucose. cholesterol and triglycerides. The MetS adjusted for age at baseline and smoking status

[3] Regression calibration adjusted as for z-score adjusted

[4] Z score for MetS is adj. for age at baseline and smoking status.

[5] P-value for interaction between sex and exposure. Adjusted as in z score adjusted[4].

Table 11. Risk of pancreatic cancer in the Me-Can cohort in relation to metabolic factors. Z-score analysis single and combined MetS score. stratified for smoking status and sex. (Reproduced from paper IV)

		Men				Women			
Smoking status	Exposure	z-score. crude[1]	z-score. adjusted[2]	z-score. calibrated[3]	Interaction[4] p-value	z-score. crude[1]	z-score. adjusted[2]	z-score. calibrated[3]	Interaction[4] p-value
Never smoker	BMI	1.03 (0.87–1.22)	1.04 (0.86–1.27)	1.05 (0.85–1.30)		1.12 (0.95–1.30)	1.01 (0.85–1.20)	1.01 (083–1.23)	
	MidBP	1.03 (0.87–1.21)	1.02 (0.85–1.22)	1.04 (0.74–1.46)		1.35 (1.17–1.56)	1.35 (1.15–1.57)	1.72 (1.29–2.25)	
	Glucose	1.12 (0.97–1.29)	1.18 (1.02–1.36)	1.79 (1.07–2.96)		1.21 (1.07–1.35)	1.15 (1.00–1.31)	1.67 (1.00–2.71)	
	Cholesterol	0.90 (0.75–1.08)	0.91 (0.75–1.11)	0.86 (0.64–1.18)		1.06 (0.90–1.24)	1.04 (0.88–1.24)	1.06 (0.82–1.39)	
	Triglycerides	0.94 (0.78–1.13)	0.92 (0.75–1.13)	0.85 (0.57–1.27)		1.13 (0.96–1.33)	1.04 (0.86–1.24)	1.08 (0.74–1.53)	
	Mets	1.02 (0.85–1.23)	1.04 (0.87–1.25)	1.06 (0.81–1.39)		1.34 (1.41–1.57)	1.39 (1.18–1.63)	1.61 (1.27–2.03)	
Former smoker	BMI	0.99 (0.82–1.19)	0.97 (0.79–1.19)	0.99 (0.77–1.21)	0.37	1.42 (1.11–1.81)	1.30 (0.99–1.72)	1.34 (0.99–1.83)	0.13
	MidBP	1.02 (0.86–1.21)	1.05 (0.88–1.27)	1.10 (0.78–1.57)	0.91	1.22 (0.91–1.63)	1.06 (0.77–1.46)	1.11 (0.62–1.98)	0.43
	Glucose	1.12 (0.96–1.31)	1.14 (0.97–1.34)	1.59 (0.90–2.81)	0.21	1.31 (1.07–1.60)	1.22 (0.99–1.52)	2.08 (0.96–4.69)	0.03
	Cholesterol	0.89 (0.74–1.08)	0.84 (0.68–1.03)	0.76 (0.55–1.05)	0.05	1.12 (0.83–1.51)	1.03 (0.75–1.43)	1.05 (0.65–1.72)	0.67
	Triglycerides	1.02 (0.85–1.22)	1.04 (0.85–1.28)	1.08 (0.73–1.62)	0.84	1.42 (1.06–1.90)	1.18 (0.84–1.65)	1.39 (0.71–2.70)	0.17
	MetS	1.02 (0.84–1.24)	1.03 (0.85–1.25)	1.04 (0.79–1.39)	0.83	1.59 (1.21–2.10)	1.64 (1.25–2.15)	2.04 (1.38–3.02)	<0.01

Table 11 cont.

Smoking status	Exposure	Men				Women			
		z-score. crude[1]	z-score. adjusted[2]	z-score. calibrated[3]	Interaction[4] p-value	z-score. crude[1]	z-score. adjusted[2]	z-score. calibrated[3]	Interaction[4] p-value
Current smoker	BMI	1.01 (0.89–1.13)	0.95 (0.83–1.09)	0.94 (0.81–1.10)	0.64	0.93 (0.76–1.14)	0.91 (0.73–1.34)	0.90 (0.70–1.39)	0.15
	MidBP	1.14 (1.02–1.28)	1.16 (1.03–1.31)	1.32 (1.06–1.67)	0.01	1.06 (0.88–1.28)	1.11 (0.91–1.35)	1.21 (0.84–1.72)	0.37
	Glucose	1.05 (0.94–1.81)	1.02 (0.91–1.16)	1.07 (0.72–1.23)	0.45	1.26 (1.10–1.46)	1.29 (1.12–1.49)	2.55 (1.52–4.36)	<0.01
	Cholesterol	0.91 (0.80–1.03)	0.87 (0.76–0.99)	0.81 (0.65–0.98)	0.22	1.11 (0.93–1.33)	1.18 (0.98–1.43)	1.29 (0.97–1.72)	0.01
	Triglycerides	1.08 (0.95–1.21)	1.10 (0.96–1.27)	0.66 (0.92–1.59)	0.05	1.00 (0.83–1.21)	0.89 (0.72–1.10)	0.79 (0.52–1.21)	0.70
	MetS	1.06 (0.94–1.21)	1.07 (0.94–1.21)	1.11 (0.91–1.33)	0.16	1.20 (0.99–1.45)	1.23 (1.01–1.49)	1.35 (1.01–1.78)	<0.01

RR. relative risk; MetS. metabolic syndrome; BMI. body mass index; Mid BP. mid blood pressure

[1] Relative risk estimate with attained age as time scale and stratified within the model for cohort. sex and categories of birth year.

[2] Adjusted for age at baseline and all exposures BMI. mid BP. glucose. cholesterol and triglycerides. Except MetS which are adjusted for age at baseline

[3] Regression calibrated z-score adjusted as for z-score adjusted.

[4] P-value for interaction between smoking status and exposure. Adjusted as for z score adjusted[3].

11. General discussion

The dismal clinical course of pancreatic cancer and an aging population, even in developing countries, makes it important to find new ways to prevent the disease. Epidemiological observations and experimental work have made valuable contributions to the understanding of some of the risk factors, but we still have a long way to go. The main aim of this thesis was to investigate known and putative risk factors for pancreatic cancer in the quest for a better understanding of the disease.

11.1 Methodological issues

11.1.1 Representivity – a potential selection bias

In any cohort or incidence study, the definition or selection of the population at risk and case retrieval are crucial tasks. The MPP is a prospective cohort study based on birth years cohorts confined to a geographical area (Malmö). One problem in such a study is that not all those who are invited participate. In the MPP approximately 71% who received an invitation actually attended. Do non-participants differ from participants? From a number of studies, we know that non-participants are inclined to differ in basic levels of motivation and attitudes towards health, as well as risk factor status [182]. The effect of the difference between attendees and non-attendees is relevant to the generalizability of the study results.

In the MPP Berglund *et al.* [183] compared attendees to non-attendees using national registries on socio-demographic factors and mortality. The social and demographic characteristics of the non-attendees were unfavourable as compared to the attendees regarding marital status, educational level, socio-economic index, housing and being foreign born. Both male and female non-attendees had a higher total mortality and cause-specific mortality as compared to attendees. This may well be true even for the other six cohorts included in the Me-Can study group, in which the attendance rate varies from 56% to 90%. However, the internal comparison (internal validity) and the calculations of relative risks are considered less sensitive to a potential selection bias. Moreover, the possibility to apply these relative risks to the background population (external validity) is probably good unless non-attendees are for example both heavy smokers and, independent of their smoking, at increased risk of developing pancreatic cancer.

11.1.2 Validity of endpoint information – a potential misclassification bias

In all study cohorts, case retrieval was performed by linking the cohorts to the respective countries National Cancer Registries. These registries have been validated and found to have an almost complete coverage of cancer cases in Norway [184] and in Sweden [185]. The Austrian registry is also of good quality and has been shown to have a high coverage [186]. The probability of getting the diagnosis pancreatic cancer in truly affected cases is probably not prone to be affected by a potential detection bias to any great extent, due to the high mortality rate characteristic of pancreatic cancer. Under-reporting was probably at random and not related to any of the studied exposures. For the MPP, all cases were validated and 2% were found to have been erroneously classified as pancreatic cancer, indicating a high degree of correctness in the registry and it is unlikely that such a small misclassification of unaffected subjects as cases of pancreatic adenocarcinomas would have affected the estimates to any great extent.

All cohorts were linked to the National registers for vitalstatus, migration and cause of death and as stated earlier, these registers exhibit a high degree of correctness, whereas completeness may be somewhat lower. The registries has been analysed and completeness

for the Norwegian registry was estimated to be 3%, about 4% for the Swedish register, and for the Austrian registry it was about 5% [185–187], indicating an overall high completeness for all three countries, and for most uses in epidemiological surveillance the underreporting will have no major impact.

11.1.3 Validity of exposure – a potential misclassification bias

Misclassification with regard to exposure is another problem, and it is highly unlikely that all exposed and non-exposed are correctly classified as such. This poses a problem in any cohort study, but the magnitude depends upon whether the misclassification is independent of the study events. There is no reason to believe that misclassification is not at random i.e. increases the similarity between exposed and non-exposed, causing any true association between risk factor and pancreatic cancer to be diluted or underestimated and thus obscuring the true relationship. Such a random misclassification cannot be responsible for causing an association if it does not truly exists.

An important limitation is that the examined exposures used in the studies were baseline values, except for the repeat measurements used to calculate measurements error. Individuals could have changed their lifestyle during follow-up. In the MPP cohort, smoking habits according to the questionnaire have been compared to plasma levels of COHb (carboxyhaemoglobin) in a cross-sectional population study [188]. The COHb concentrations in plasma showed good agreement between never and former smokers and was increased with daily tobacco consumption, indicating a good validity of self-reported smoking habits. However, this does not tell anything about smoking habits later in life. Even though those who had never smoked probably did not start smoking during follow-up (it is highly unlikely to start smoking after the age of 30), it is not unlikely that some of the current smokers would have given up smoking. This would have resulted in an underestimation of the true risk associated with smoking in current smokers.

How alcohol habits vary over time and by age is difficult to assess. According to The Global Status Report on Alcohol 2004 [118] Austria has had a fairly constant adult per capita consumption over the last 20 years (1980–2001) of approximately 13 litres of pure alcohol (Austria records the highest lever cirrhosis mortality in Europe), Norway has gone from 5 to 6 litres of pure alcohol and Sweden has stayed rather constantly at around 7 litres. However, several problems are related to the assessment of alcohol consumption; i.e. illegally produced or imported alcohol will not be detected by official statistics on quantities of sold alcohol. Indeed, studies using questionnaires to assess alcohol consumption in Sweden has strongly indicated an increasing consumption [189, 190].This indicates that if anything has happened to alcohol consumption, it has been increasing them (fig.7). This may have resulted in an underestimation of the true risk associated with high alcohol intake, as some individuals classified as low consumers at baseline may have increased their alcohol intake during follow-up.

All cohorts had data available on BMI. As for smoking and alcohol consumption these measurements were made at one point in time. In paper IV, correction of random error could be made based on repeat measurements and the calculated RDR; for BMI a value of 0.90 was calculated, indicating a small random error of the intra-individual variation for this exposure. This does not tell anything about how weight varies over time. Moreover, as mentioned in section 4.3.3, it is wellknown that most people gain weight with age and, that overweight/obesity is an increasing problem worldwide. If there is a true positive association between obesity and pancreatic cancer, this association may have been attenuated by misclassification of subjects that had normal weight at baseline, but gained weight during follow-up.

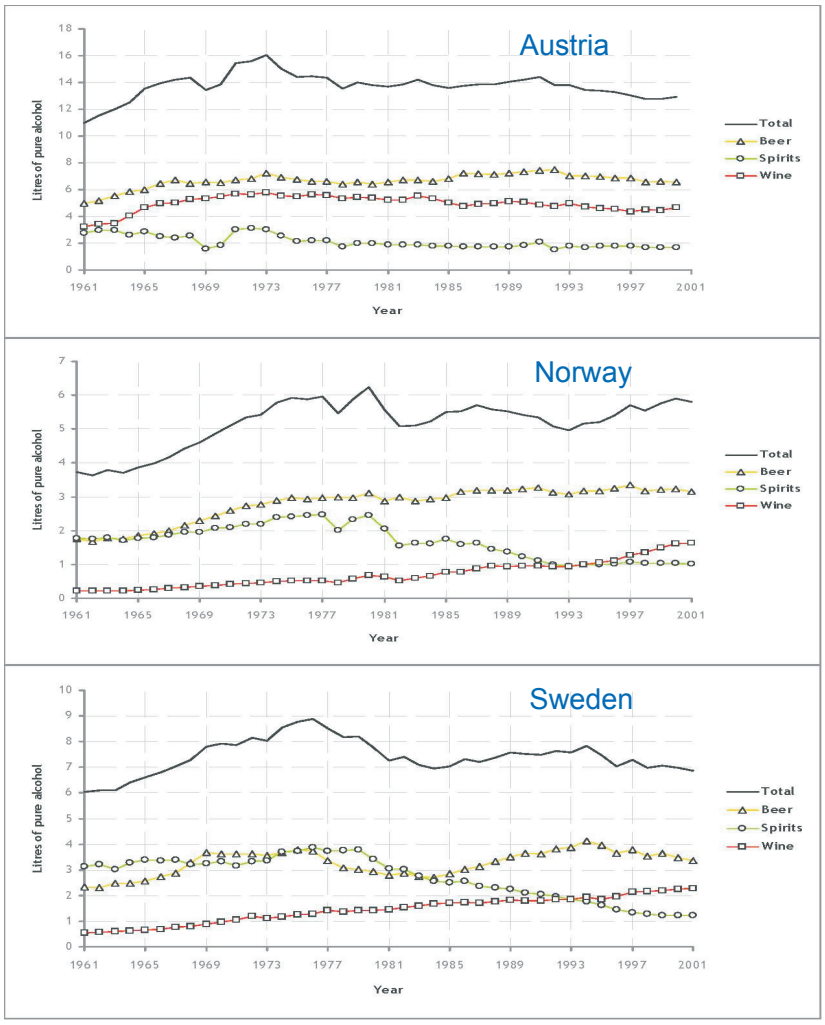

Fig. 7. Recorded adult per capita consumption (age 15+) Sources: FAO (Food and Agriculture Organization of the United States), World drink Trends 2003 [118]

11.2 Findings

11.2.1 Alcohol and pancreatic cancer (paper I)

Findings in paper I add further information on whether or not alcohol consumption is related to an increased risk of pancreatic cancer. In this study a positive association between both Mm-MAST and γ-GT was found and the risk appeared to be higher in subjects reporting a previous weight gain. As stated earlier in this thesis, the question regarding alcohol consumption and pancreatic cancer is controversial, with contradictory results in previous studies. Most population-based studies have found a modest positive association [56, 93, 96, 113], whilst most case-control studies have

not [94, 97, 104, 111, 112]. A problem in many cohort studies is the low number of cases included in these studies. In this population-based prospective cohort study based on the MPP, 183 cases of incident pancreatic cancers, equivalent to an internal incidence of 24.7 per 100,000 person-years, were studied. Another major strength of the study is that it includes two different measurements on alcohol consumption. The measurements were collected years before subjects were taken ill (the mean follow-up was 15 years (SD 6.9)), and thereby avoided the problem of recall bias, which otherwise causes difficulties in studies on alcohol. It is possible that the divergent result between studies on alcohol and pancreatic cancer is that self-reported consumption in case-control studies may have a low validity, due to recall bias and/or changed habits of alcohol consumption because of subclinical disease. Another problem in studies on alcohol and pancreatic cancer is the lack of information on chronic pancreatitis. Alcohol is the most common explanation for chronic pancreatitis [191] and according to Lowenfels *et al.* [51] alcoholic chronic pancreatitis increases the risk of pancreatic cancer several-fold. However, according to Otsuki and Tashiro [192] chronic pancreatitis may be on the causal pathway between alcohol and pancreatic cancer and thus should not be considered a confounder. While confounding by obesity and smoking was controlled for in the analysis, information on other factors, such as pre-existing disease, dietary and nutritional factors and genetic factors, were not available, which is a limitation of the present study.

Although BMI and weight gain covaried, the highest risk associated with alcohol consumption was seen among subjects that had reported a previous weight gain, and contrary to this an increased risk of pancreatic cancer was seen among individuals with a BMI <25 as compared to BMI >30. It is difficult to draw any conclusions from this contradiction, considering the small subgroups, wide confidence intervals and limited statistical power of these sub-analyses. The relationship between obesity and pancreatic cancer remains controversial [121] and that this paper did not find any statistically significant increased risk for pancreatic cancer in obese individuals as compared to those with a normal body weight, suggest that there is no strong association between obesity and risk of pancreatic cancer. However, the present study suggest that obesity may affect the association between smoking and pancreatic cancer in a synergistic way, considerably increasing the risk in current smoking obese subjects, as compared to never smoking individuals (RR 7.45: 1.65;33.64).

Smoking has consistently been associated with an increased risk of pancreatic cancer and the risk is proportional to the duration and intensity of smoking [93–100, 193]. Paper I confirm these findings, with a positive association between current smoking and pancreatic cancer and a tendency towards an increasing risk in subjects who smoked the highest number of cigarettes per day and an increased risk that, to some extent, persisted even after more than 5 years of abstinence.

11.2.2 Trypsinogens, PSTI, H.pylori and pancreatic cancer (paper II and III)

As described in section 2.3 experimental studies have suggested that trypsinogens may enhance tumour progression [20], but to date there have been no prospective studies using blood samples on HAT, HCT and PSTI collected years before subjects developed pancreatic cancer. In an earlier study performed by Borgström *et al.* [194] an almost three-fold increase in the HAT/HCT ratio was found in patients with pancreatic cancer and chronic pancreatitis. Moreover, Haglund *et al.* [29] showed in a study on pancreatic cancer patients that PSTI was elevated in 75–95% of patients as compared to healthy controls, though such an elevation was seen almost as often in patients with benign pancreatic and

biliary disease. From these findings it is possible to hypothesise that an imbalance between protease activity and PSTI may promote tumour invasion. Some earlier studies have in fact suggested that the ratio of trypsinogens to PSTI is disturbed in patients with ovarian cancer and renal cell carcinoma [18, 195, 196], indicating an insufficient inhibitory effect. The findings in paper II on the HAT/HCT ratio are in line with this hypothesis, even though the effect was mainly an effect of a high risk in women. In the overall analysis no statistically significantly association for HAT, HCT, PSTI or (HAT+HCT)/PSTI in relation to pancreatic cancer was found.

The results described in paper III could not confirm any overall association between H.pylori seropositivity and the risk for pancreatic cancer. However, the risk associated with H.pylori seropositivity was increased in never smokers and in subjects reporting low alcohol consumption. The finding, that there is no overall association, is in line with a recent nested case-control study including 104 cases and 262 controls performed by de Martel *et al.* [83]. There are only three previous studies on the association between pancreatic cancer and H.pylori seropositivity and the two other reports did indeed find a positive association [84, 85]. However, it may be difficult to compare our results with the Finnish study [85] since the latter only included middle-aged smoking men.

The prevalence of H.pylori seropositivity increases with age [197]. This is thought to be a birth cohort effect, i.e. the higher prevalence of seropositivity among elderly people reflects a higher childhood infection rate at the time when they were children rather than acquisition during adult life [198,199]. It is therefore reasonable to assume that most subjects with a negative H.pylori serology at baseline investigation remained uninfected until follow-up. In H.pylori seropositive subjects it is possible that some were eradicated after baseline investigation, something that would have attenuated a potential true association. However, since baseline investigation was performed in middle-aged subjects, it is reasonable to assume that they, even if they were eradicated, would have had a fairly long life-time exposure to H.pylori infection.

The lack of a statistically significant association between HAT, HCT, PSTI and (HAT+HCT)/PSTI and the risk of pancreatic cancer and between H.pylori serology and risk of pancreatic cancer in the overall analyses could be due to a Type II error. That is, we might have missed a true difference due to poor statistical power. Several comparisons were made in paper II and the possibility that some of the observed associations were caused by chance, i.e. a Type I error, has to be considered. However, the hypothesis predicted an increased risk of pancreatic cancer related to an imbalance in trypsin activity and/or trypsin inhibitor capacity and our findings supports the interpretation that the statistically significant findings were not simply due to chance.

The association between H.pylori seropositivity and pancreatic cancer in both never smokers and low risk consumers of alcohol, and the association of HAT and HAT/HCT with pancreatic cancer in the intermediate/high alcohol consumption group, could be a potentially important observation. However, these are all subanalyses and have to be interpreted cautiously; all the subgroups are small and the consequence is that it was difficult to adjust for all the potential confounders on the same time in the statistical model. Nevertheless, the statistical model remained quite stabile by including the variables one at a time and did not change considerably when all factors were included at the same time. Confounding due to these factors ought therefore to have been a minor problem. A limitation in these studies is that we had no information on other confounders such as diabetes and chronic pancreatitis.

11.2.3 Metabolic syndrome and pancreatic cancer (paper IV)

As mentioned in section 5.2 the evidence of the putative association between MetS and pancreatic cancer is generally not supported, except in a small study performed by Russo *et al.* [151], who found a positive association between the MetS and pancreatic cancer in men. This finding was not confirmed in the present study, but there was a statistically significant association between the MetS and pancreatic cancer in women.

Epidemiological data supports a modest, but significant association between glucose and pancreatic cancer (section 4.2.2) [62–64, 200], which was confirmed in paper IV, with the strongest association among women. Besides this finding, a positive association between mid-blood pressure and the risk of pancreatic cancer were seen, again with a stronger association in women, than in men. Most studies on hypertension and the risk of pancreatic cancer have not reported such an association [201, 202]. It is unclear why these risks seem to be higher in women and at present there is no support for this finding in the literature. The calculation of absolute risks indicated a lower risk in women in low quintiles, but this difference disappeared at higher exposure levels. Androgens and estrogens are known to have tumour promoting effects in for instance prostate and breast cancer and the presence of oestrogen and androgen receptors in pancreatic cancer has been shown [39]. Whether or not sex hormones affect the development of pancreatic cancer, or if these hormones could modify other risk factors and thereby explain different risk factor profiles in men and women is currently not known. A review performed by Wahi *et al.* [40] focusing on reproductive factors in relation to risk of pancreatic cancer did not reveal any such associations.

As mentioned earlier in this thesis (section 5.2), the effects of cholesterol and triglycerides on cancer risk are controversial [133] and most studies on cholesterol and the risk of pancreatic cancer have not shown any association [202]. Moreover, for cholesterol there has been some evidence of reverse causation, meaning that preclinical cancer leads to a drop in cholesterol [148 1271]. In paper IV, cholesterol was negatively associated with the risk of pancreatic cancer in men, but considering the poor survival, indicative of a rapidly progressive disease, compatible with a short subclinical phase and that exclusions were made for cases diagnosed within one year of health check-up, reverse causation is considered highly unlikely.

In a newly published report by LLi *et al.* [122] it was suggested that obesity at a younger age has a more profound effect on the risk of pancreatic cancer, than obesity at an older age. Obesity as a risk factor for pancreatic cancer has otherwise been controversial, although a meta-analysis of 14 studies performed by Berrington de Gonzalez [121] did find that obesity increased the risk of pancreatic cancer slightly. In paper IV, a positive association was only seen among women in the highest quintile vs. the lowest. Apart from this, no significant association was found. It is possible, that the findings by LLi [122] explain the lack of consistency in epidemiological findings regarding obesity and pancreatic cancer.

The interpretation of the findings in the stratified analysis of smoking habits and investigated exposures is not clear. Smoking is known to be a strong risk factor for pancreatic cancer (see section 4.3.1), but in this analysis no consistent pattern for the relative risks was found, except for glucose in women and mid BP in men. It is possible the result is due to chance, but it has been shown that in some hormone-dependent tumours, cancer incidence increases after smoking cessation [203]. To what extent pancreatic cancer risk may be related to metabolic effects associated with smoking cessation remains to be elucidated.

The main strength of this study is the large sample size from seven population-based cohorts in Europe and its ability to perform record linkage with national cancer registries. Another major strength is the repeated health examinations, which allowed risk estimates to be adjusted for intra-individual variation of the analysed exposures and thereby decreased the risk of misclassification bias related to measured exposures, a potential regression dilution bias.

All cohorts had data available on BMI and smoking status, which allowed adjustment for these potential risk factors. A limitation in paper IV is the lack of data on covariates such as genetic risk factors, alcohol consumption, chronic pancreatitis and physical activity. As far as is known there is no known association between genetic factors associated with pancreatic cancer and metabolic factors. Hence, confounding by genetic factors ought to have been a minor problem. Alcohol consumption and physical activity have both been related to pancreatic cancer [56, 93, 96, 113, 204]. Alcohol is thought to exert its carcinogenic effect on the same pathway as the components of the MetS (via ROS) [54, 133]. If this is true, it would have been problematic to include alcohol in this multivariate analysis. The same might be applicable to physical activity. Michaud *et al.* [204] have shown that physical activity is inversely related to pancreatic cancer in obese people and it has been shown that physical activity can actually lower plasma glucose levels [205]. Confounding by chronic pancreatitis is another concern. However, according to Otsuki and Tashiro [192] chronic pancreatitis may be the link between high alcohol consumption and pancreatic cancer, and should therefore not be considered a confounder. Still, it is a limitation of paper IV that no information on these factors was available.

To overcome the problems concerning the geographical differences between the cohorts and the differences in methods of measurements of investigated exposures, quintile classification and the Z scores were stratified for the individual cohorts. Calculations were furthermore repeated without cohort stratification in the multivariate model and did not reveal any substantial changes in the risk estimates.

Several comparisons were made and the risk of Type I error has to be considered. The results showed a clear pattern when different statistical models were used, and the significant findings were in line with the à priori hypothesis, which supports the view that the results were not simply due to chance. An exception may be the results concerning cholesterol, which was negatively associated with the risk of pancreatic cancer, i.e. opposite to the à priori hypothesis. Confidence intervals were generally narrow, which indicates good statistical power and a low risk of Type II errors.

11.2.4 Biological considerations

Pancreatic tumour growth and metastasis is thought to be stimulated on a molecular basis via activated oncogenes and inactivated tumour-suppressor genes, either as a result of inherited or acquired mutations, and via inflammatory cytokines, growth factor and ROS. The risk factors investigated in this thesis can all be connected to this model (fig.8).

Smoking is thought to exert its carcinogenic effect indirectly via the bloodstream or via the duodenal contents or bile. A potential mechanism through which tobacco carcinogens can act is by activation and progression of an inflammatory response in the pancreas. Observations supporting this are that smoking is an independent risk factor for chronic pancreatitis and the development of diabetes mellitus Type II; two conditions that have been suggested as risk factors for pancreatic cancer [31, 103, 104, 206]. Chronic pancreatitis is thought to exert its carcinogenic effect via much the same system as smoking, namely by

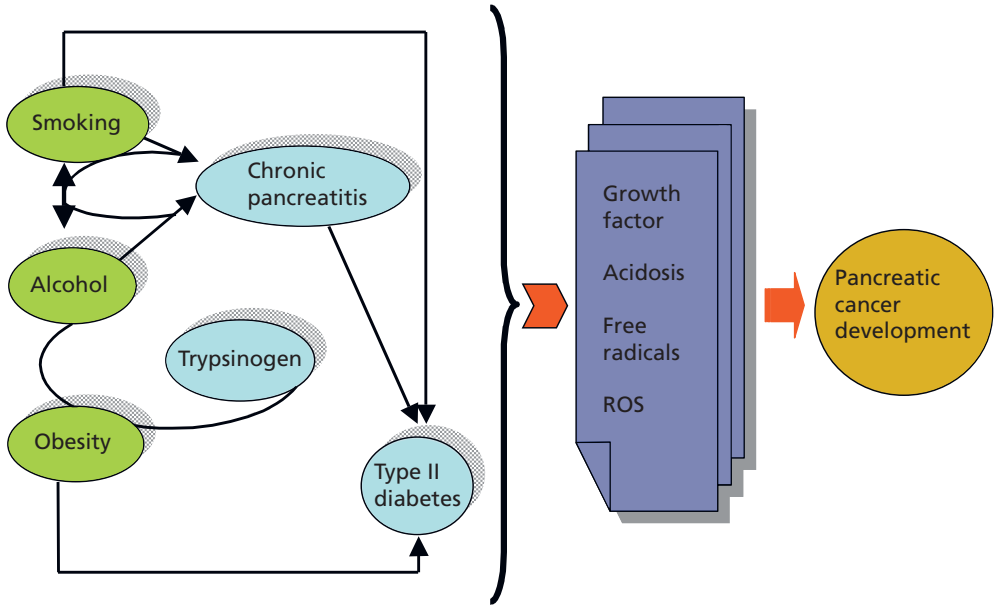

Fig. 8 *Biological pathways, describing the different risk factors interaction with each other in promoting carcinogenesis in the pancreas*

ROS and the release of cytokines [57], whilst the hyperinsulinemia characteristic of Type II diabetes has been shown to have a growth-promoting effect on cancer cell lines in vitro [4, 63]. Moreover, excess glucose is an energy substrate in fast-growing, highly proliferative tumour cells [77].

Alcohol, as stated in section 4.3.2, is not a known carcinogen, but might function as a promoter or co-carcinogen. The metabolism of ethanol generates ROS, which stimulates transcription of pro-inflammatory cytokines [54], and synergistic effects between alcohol and nitrosamines (smoking) have been reported [54, 117]. The non-oxidative pathway of ethanol metabolism resulting in the formation of free fatty acids has been shown to stimulate the conversion of trypsinogen to trypsin prematurely, leading to predisposition to pancreatic inflammation [116]. It is possible that an altered HAT/HCT ratio is a marker of inflammation [207]. Apart from mediating inflammation [18], trypsin has been shown to mimic the action of insulin and thereby stimulate growth promoting factors [26]. Both functions which are thought to enhance cancer development in the pancreas.

Free fatty acids (FFA) are regulated by adipose tissue and prolonged periods of excess energy intake increases the levels of FFA in plasma, causing ROS, which can induce insulin resistance in Type II diabetes [129] and elevated FFA can contribute to hyperglycaemia [130]. The metabolic effect of adipose tissue is not only an effect of FFA, the high concentration of adipocytokines and low concentration of adiponectin generates cytokines and sustains insulin resistance and hyperglycaemia [124, 126]. In general, obesity leads to glucose intolerance and thereby hyperinsulinemia and both smoking and alcohol may affect glucose metabolism and insulin sensitivity [104, 114, 206] and this may be a possible link between obesity and risk of pancreatic cancer.

The potential link between H.pylori infection and pancreatic cancer remains unclear. The present study did not reveal any overall association, but there was an indication that subjects, who were not exposed to smoking or alcohol, had an increased risk of pancreatic cancer associated with H.pylori infection. It is indeed possible that H.pylori is a risk factor, but that it has a weak effect and that this would be apparent only in subjects, who are not exposed to stronger risk factors, i.e. smoking and high alcohol consumption. There are several potential biological mechanisms, which could explain an association. Pancreatic secretion is under hormonal control mainly by cholecystokinin, which stimulate enzyme production and by secretin which induces bicarbonate secretion [3]. Antral colonization by H.pylori has been associated with increased gastric acid output, which in turn stimulates secretin release and secretin stimulation has been proven to accelerate the development and frequency of pancreatic tumours in a hamster model [86]. Opposite to antral colonization, H.pylori infection in the corpus area of the stomach is associated with a loss in parietal cells and a decrease in gastric acid output. The resulting hypoacidity can lead to bacterial overgrowth and the increased production of N-nitroso compounds may be responsible for carcinogesis in the pancreas [87].

For all the investigated risk factors, inflammation is one of the main pathways on the road to cancer development. In addition to inflammation stimulation of growth factors, acidosis, free radicals and ROS are all characteristics of the studied factors. All the risk factors seem to be interconnected; alcoholics are more often smokers and both alcohol and smoking can induce pancreatitis. Smoking furthermore induces Type II diabetes, which in turn can be a result of adiposity. Alcohol can increase the levels of FFA and thereby have an effect on glucose metabolism and trypsinogen. Hypertension as a result of adiposity and/or diabetes, may add to the risk, but it might only be a marker of other, more important metabolic factors.

12. Conclusions and future perspectives

I. High alcohol intake, estimated using both a questionnaire on attitude towards alcohol and a laboratory marker in the form of γ-GT is associated with a subsequent high risk of developing pancreatic cancer. The previously established association between smoking and pancreatic cancer is confirmed.

II. The hypothesis that pancreatic cancer is related to an imbalance between trypsinogen isoforms is in line with findings concerning the ratio of HAT/HCT.

III. There are no overall association between H. pylori infection and the risk of pancreatic cancer. H. pylori infection may increase the risk of pancreatic cancer in never smokers and in low alcohol consumers.

IV. Mid BP, glucose and the MetS are associated with an increased risk of pancreatic cancer in women. In men, mid BP is associated with the risk of pancreatic cancer and there is an indication of an association between high glucose levels and the risk of pancreatic cancer.

Causality is hard to establish in epidemiological science. Randomised controlled trials are the gold standard, as they reduce the effects of possible and, more importantly, unknown confounders. However, conducting such a trials is expensive, time consuming and often not possible because of ethical concerns. Besides, factors of interest (e.g. diabetes) cannot possibly be externally allocated to the study population. Indeed, there are many circumstances were observational studies are the only option.

In many cases, prospective cohort studies offer the strongest study design. In spite of the costs and the long follow-up time, there are a growing number of such studies and in order to gain high statistical power, several large collaborative projects have been launched. The Me-Can study group is such a collaboration that allows investigation of a variety of aspects and these initiatives are likely to yield more conclusive evidence on various relations in cancer epidemiology.

Growing evidence have consistently shown that obesity, diabetes, metabolic factors, smoking and alcohol are, apart from cardiovascular disease, related to several cancer forms, including pancreatic cancer. However, in a field characterized by as much uncertainty as epidemiology, it is rare for the evidence on the presence of a cause-effect relationship to be "without any doubt". This do not allow us to postpone acting upon the observations, even though we know that our findings are liable to be upset or modified by advancing knowledge. This thesis adds to existing knowledge, it is important to act upon the growing burden of obesity and diabetes in the world and continuously lower the use of tobacco and excess alcohol consumption. To change lifestyle habits is extremely difficult, but that does not permit us not to try.

The genome wide association studies (GWAS) are an exciting new way to investigate genetic factors, and the first GWAS on pancreatic cancer was published late in 2009. This technical milestone allows us to identify individuals who are at a genetically determined high risk of developing pancreatic cancer and individuals who may be more susceptible to different environmental exposures. In future studies, these genetic factors can be combined with known and potential environmental exposures, such as for example alcohol consumption, and examined in relation to the risk of pancreatic cancer. This design could be a very useful tool to new insights. In time this could bring us closer to both improved prevention and a better treatment of this devastating disease.

13. General summary in Swedish – Sammanfattning på svenska

Bukspottkörtelcancer, pankreascancer, är en sjukdom med mycket hög dödlighet även vid tidig upptäckt och behandling. För patienten är diagnosen förödande. Mindre än 10% av fallen kan opereras och endast 10–15% av patienterna i denna kategori lever längre än 5 år efter diagnos. För övriga finns i stort sett ingen verksam behandling. Enligt världshälsoorganisationen (WHO) är bukspottkörtelcancer den 13:e vanligaste cancerformen i världen samtidigt som det är den 8:e vanligaste orsaken till att patienter dör i cancer. Totalt dör i hela världen ca 250 000 människor årligen i denna cancerform. Ökad forskning är därför mycket välmotiverat för att, om möjligt, kunna förbättra behandlingen och därmed minska antalet människor som dör av sin sjukdom. Samtidigt är det viktigt att identifiera riskfaktorerna för sjukdomen så det förebyggande arbetet kan förbättras och på så sätt minska insjuknandet och dödligheten.

Bukspottkörteln, pankreas, har två huvudfunktioner. Den ena är produktion av hormon till blodomloppet (endokrin funktion), den andra huvudfunktionen är att utsöndra bukspott till tarmen (exokrin funktion). Hormonproducerande celler, de s.k. Langerhanska öarna, tillhör den endokrina delen och utgör ca 2% av bukspottkörteln. De producerar ett antal olika hormoner vars huvudsakliga funktion är att reglera blodsockerhalten. Cellerna är i direktkontakt med blodkärlen i körteln och detta ger ett snabbt utbyte med blodomloppet. Totalt utgör ca 80% av bukspottkörteln den exokrina delen och den producerar matsmältningsenzym och bikarbonat. Koncentrationen av proteiner och fettsyror i magtarmkanalen reglerar mängden bukspott som utsöndras direkt till tolvfingertarmen och beroende på kostens sammansättning kan upp till 3 liter bukspott utsöndras per dygn.

Tumörer i den exokrina delen av bukspottkörteln svarar för 97–98% av alla tumörer i bukspottkörteln. Ett flertal olika cancerformer förekommer i denna del av bukspottkörteln med varierande aggressivitet och åldersmässig spridning. Vanligast förekommande är ductalt adenocarcinom, en ytterst aggressiv form med mycket hög dödlighet. Bukspottkörtelcancer beror på mutationer orsakade av skador i DNA. Dessa kan vara ärvda eller orsakade av livsstilsfaktorer. Risken att insjukna ökar med ökande ålder och män drabbas i högre utsträckning än kvinnor. Skillnaden mellan könen jämnas dock ut med ökande ålder. Forskning har även visat på skillnader mellan olika folkgrupper och amerikanska svarta män är den grupp som har den högsta sjukligheten.

När det gäller bukspottkörtelcancer är den störste riskfaktorn rökning. Troligen har även andra faktorer som alkoholintag, övervikt, diet samt låg fysisk aktivitet betydelse för risken att drabbas. Där utöver har tidigare forskning pekat på att patienter med andra sjukdomar, som t.ex. inflammation i bukspottkörteln, åldersdiabetes eller olika typer av infektioner kan ha en ökad risk för att utveckla sjukdomen. Likaså verkar ärftliga faktorer i kombination med vissa riskfaktorer öka risken för att insjukna eller påverka tidpunkten för när sjukdomen debuterar. Exempelvis har man visat på att patienter med ärftlig bukspottkörtelinflammation i snitt insjuknar 20 år tidigare om de röker, jämfört med om de aldrig rökt.

Syftet med denna avhandling har varit att studera hur olika livsstilsrelaterade och metabola faktorer påverkar risken att drapas av bukspottkörtelcancer. Underlaget för delarbete I–III hämtades från ett stort sjukdomsförebyggande projekt (Malmö Förebyggande Medicin, MPP) som startades 1974 vid Universitetssjukhuset MAS i Malmö. Huvudsyftet var att kartlägga olika riskfaktorer för hjärt- och kärlsjukdomar och alkoholism. Mellan åren 1974 och 1992 inbjöds samtliga invånare i vissa avgränsade årskullar till projektet. De fick svara på ca 200 olika frågor rörande

livsstil, tidigare sjukdomar och symptom. Alla vägdes och mättes, samt lämnade blodprov. Vissa analyser gjordes direkt på blodproverna, medan resterande blod frystes i en biobank. Totalt deltog 33 346 personer vilket var 71% av de inbjudna. Delarbete IV är baserat på ett samarbete mellan Österrike, Norge och Sverige, (Me-Can projektet) där bl. a. MPP studien ingår. Syftet var att skapa ett stort material av människor (en stor kohort) för att sedan kunna undersöka sambandet mellan metabola faktorer och cancerrisk. Studien omfattar 1 600 296 undersökningar av totalt 940 060 personer. Efter en första bedömning visade sig 577 315 individer vara möjliga att ha med i delarbete IV.

I delarbete I undersöktes huruvida olika mått för alkoholkonsumtion var förknippade med en ökad risk för att insjukna i bukspottkörtelcancer. Alkoholkonsumtion mättes på två sätt, dels med hjälp av ett frågeformulär, där attityder till och bruk av alkohol värderades, och dels med hjälp av ett blodprov där en speciell ”markör” mättes (γ-GT). Efter samkörning av MPP mot det Svenska Cancerregistret och det Regionala Tumörregistret för södra Sverige, fram till och med 31 december, 2004, kunde 187 fall av bukspottkörtelcancer identifieras. Alla fallens journaler, röntgenundersökningar och resultat från olika vävnadsprover, genomgicks och fyra visade sig vara felregistrerade. De resterande 183 fallen kunde sedan jämföras med övriga deltagare i MPP med avseende på kön, ålder, rökning, alkoholkonsumtion och övervikt. Resultaten av undersökningen visade på en ökad risk för bukspottkörtelcancer vid måttlig till hög alkoholkonsumtion. Vidare kunde det sedan tidigare kända sambandet mellan rökning och en ökad risk för bukspottkörtelcancer bekräftas.

Trypsin finns i flera olika varianter, det är ett bukspottkörtelenzym som bryter ner det protein man får i sig via maten. Eftersom trypsin är skadligt för bukspottkörtelns celler utsöndras det som ett s.k. pro-enzym. Först efter utsöndringen till tolvfingertarmen omvandlas pro-enzymenet (trypsinogenet) till det aktiva trypsin vars koncentration kontrolleras av trypsinhämmaren PSTI. Hypotesen inför delarbete II var att obalans mellan trypsinaktivitet och trypsinhämmarkapacitet skulle kunna öka risken för bukspottkörtelcancer. Delarbete II var en s.k. fall-kontroll studie baserad på MPP. Urvalskriterierna var som i studie I, men slutdatum för uppföljningen var 31 december, 1999. Inledningsvis identifierades 117 patienter med bukspottkörtelcancer. På grund av bl.a. avsaknad av blodprover minskades gruppen av patienter till 84 personer. En kontrollgrupp bestående av 264 personer valdes ut som jämförelse till fallen och slutligen omfattade studien 348 individer. I delstudie II påvisades ett samband mellan en hög kvot av anodalt och katodalt trypsinogen (HAT/HCT) och en ökad risk för bukspottkörtelcancer.

Syftet med delarbete III var att undersöka om Helicobacter pylori infektion (”magsårsbakterier”) ökar risken för bukspottkörtelcancer och om en eventuell riskökning efter H.pylori infektion skulle kunna påverkas av samtidig rökning och/eller alkoholkonsumtion. I delarbete III användes samma studiegrupp som i delarbete II, men här var det möjligt att använda ett större antal patienter i analyserna; totalt ingick därför 87 patienter och 263 kontroller i gruppen. Två tidigare studier har visat att H.pylori infektion ökar risken för bukspottkörtelcancer, men detta kunde inte bekräftas i delstudie III. Intressant nog visade studien att H.pylori infektion ökade risken för bukspottkörtelcancer hos dem som aldrig rökt och hos individer med låg alkoholkonsumtion.

I delarbete IV samkördes 7 olika kohortstudier från Norge, Sverige och Österrike (Me-Can projektet) med de nationella cancerregisterna. Totalt kunde 862 fall av bukspottkörtelcancer identifieras. I Me-Can databasen finns information om individernas vikt, längd, blodtryck och nivåerna av blodsocker (glu-

kos) och blodfetter (kolesterol och triglycerider). Studien syftade till att kartlägga eventuella samband mellan dessa faktorer, enskilt och/eller tillsammans och risken för att insjukna i bukspottkörtelcancer. Analysen visade att högt blodtryck, höga halter av glukos och alla faktorer sammanslagna i en variabel (”det metabola syndromet”) ökade risken för bukspottkörtelcancer hos kvinnor. Hos män fanns ett samband mellan högt blodtryck och bukspottkörtelcancer och en antydan till ett samband mellan högt glukos och en hög risk för att utveckla bukspottkörtelcancer.

Stora kohortstudier är mycket värdefulla när man önskar undersöka cancersjukdomar. Dessa studier är ofta dyra och tar lång tid att sätta igång, men när de är genomförda kan man analysera ett stort batteri av olika faktorer relaterad till den sjukdom man är intresserad av. I denna avhandling analyseras en rad kända och misstänkta riskfaktorers relation till bukspottkörtelcancer. För de flesta av dessa riskfaktorer finns en biologisk förklaringsmodell som går via en inflammatorisk reaktion i bukspottkörteln. Denna reaktion kan sedan i sin tur ge upphov till skador i pankreascellernas DNA och så småningom kan detta utvecklas till cancer. På sätt och vis tycks alla dessa riskfaktorer vara relaterade till varandra; alkoholister är ofta också rökare och båda dessa faktorer kan ge kronisk inflammation i bukspottkörteln. Rökning kan dessutom öka risken för åldersdiabetes, som i sin tur även kan vara ett resultat av fetma. Alkohol kan öka halterna av blodfetter i blodet, vilket har effekt på glukos och trypsinogenomsättningen. För högt blodtryck kan vara ett resultat av fetma och/eller diabetes och därvid utgöra antigen en riskfaktor eller en riskmarkör.

Fetma, diabetes, rökning och alkoholmissbruk är alla livsstilsfaktorer, som ökar explosivt över hela världen. Det är av yttersta vikt att utveckla metoder för att vända denna trend. Det är mycket svårt att påverka livsstilen hos människor, men för rökning verkar det som om man lyckats vända trenden i västvärlden, vilket antyder att det är möjligt att genomföra effektiva preventiva åtgärder på samhällsnivå. Tyvärr tycks det nu som om problemet flyttats till utvecklingsländerna, där i dessa år ser en massiv ökning av andelen rökare. För att lägga sten på bördan ökar också fetma och diabetes i dessa länder i takt med att välståndet ökar. Dessa livsstilsrelaterade problem kommer att belasta alla länders sjukvårdsresurser till bristningsgränsen, om vi inte lyckas genomföra effektiva preventiva insatser. I slutändan skulle sådana insatser med all sannolikhet bidra till en minskning av den globala sjukdomsbördan av bukspottkörtelcancer.

14. Acknowledgements – Tack

It has been a long time, since I started this journey together with Professor Anders Borgström, a good friend and collegue, who past away in the autumn of 2007. The loss of such a learned collegue, is difficult to replace and he will always be remembered with great respect and gratitude.

To the many persons who, one way or the other, has been involved in this thesis, I especially want to express my deepest gratitude to:

Jonas Manjer, my principal supervisor. Thank you Jonas, for the warm and caring person you are and for letting me take part of your enormous knowlegde in epidemiologically science. For picking me up when everything looked very dark and cloudy and for constantly taking the time for discussions and forcing the "little grey cells" to understand what I was doing. During the years and especially during these past month, you have been a solid rock of knowlegde, support, pickiness (all the red dots in the text) and cheerfulness, on which I have "built" this thesis. A great many thanks to your family, who have been seeing less of you, because of me.

Björn Lindkvist, my assistant supervisor. You are a very intelligent young man, I have often relied on your abillity to explain difficult matters. Thank you for sharing your thoughts and ideas and even though with a delay (especially when you dissappeared in a bucket full of daipers) you always return with an answer full of wisdom and insight.

Sara Regnèr, for co-authorship and, in spite of a great personal loss, always cheerful and stimulating to exchange ideas with. Thank you for trying to make me understand the fine art of laboratoty measurements.

Professor **Bengt Jeppsson** for support and for fixing the time free of clinically duties.
Collaborators in the **Me-Can**, even though the constant picking in my manuscript, sometimes made me furious.

My collegues, especially to the **Laparoscopic team**, including our fantastic "**sisters**", you are all a constant source of energy and encouragement.

PeO, my beloved husband. Where would I be without you? You are a constant inspiration.

Niclas & Lina, my son and daughter-in-law. You are both wonderful, you enrich my life. Thanks for creating the apple of my eye, my beautiful granddaughter **Mille**.

Bent & Birtha Johansen, my parents. For wanting your children to educate themselves to possibilities you never had and for the constant support and love.

I would furthermore like to express my gratitude to:

S. Karger AG, Medical and scientific Publishers for allowing the reprint of paper I and of paper II, which has not yet been published.

NORDCAN, **Gerda Engholm** for allowing the reprint of figure 3. Age-standardized incidence in the Nordic countries and figure 5. Incidence per age per 100,000 persons of PDAC in the Nordic countries in 2006.

Globocan 2002, for allowing the reprints of figure 4. Age standardized mortality rate across the world according to Globocan 2002.

FAO, for reprints of figure 6. Recorded adult per capita consumption (age 15+).

To **Wordpress**, for allowing me to re-use the picture of Mr. Pavarotti.

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Paper I

Original Paper

Pancreatology

Pancreatology 2009;9:677–686
DOI: 10.1159/000212088

Received: December 17, 2007
Accepted after revision: July 2, 2008
Published online: August 14, 2009

Different Markers of Alcohol Consumption, Smoking and Body Mass Index in Relation to Risk of Pancreatic Cancer

A Prospective Cohort Study within the Malmö Preventive Project

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Key Words
Pancreatic cancer · Epidemiology · Smoking · Alcohol consumption · Body mass index · Weight gain

Abstract
Background/Aim: The association between alcohol consumption and pancreatic cancer is not clear. This study investigates different prediagnostic measurements of alcohol consumption, a laboratory marker (γ-glutamyltransferase; γ-GT), and a score measuring alcohol addiction (Mm-MAST), in relation to the risk of pancreatic cancer. Furthermore, the study investigated whether smoking and alcohol consumption interact with each other, or if the risk of pancreatic cancer associated with these factors is modified by obesity or weight gain. ***Methods:*** A cohort of 33,346 subjects provided prediagnostic information on the above factors. During a mean follow-up of 22.1 years, 183 cases of pancreatic cancer occurred. Cox's analysis yielded relative risks (RR) with 95% confidence intervals (CI). ***Results:*** The highest γ-GT quartile was associated with a high risk of pancreatic cancer (RR = 2.15, 95% CI = 1.34–3.44), and this association was even stronger in subjects that reported a previous weight gain (RR = 3.61, 95% CI = 1.29–10.09). A high Mm-MAST score was also associated with pancreatic cancer (p = 0.02). Current smoking was associated with pancreatic cancer (RR = 2.34, 95% CI = 1.60–3.43), and obese smokers had an even higher risk (RR = 7.45, 95% CI = 1.65–33.64). ***Conclusion:*** High alcohol intake is associated with subsequent risk of pancreatic cancer and this risk may be higher following weight gain. The risk associated with smoking may be even higher in obese subjects.

Introduction

There is a well-established association between smoking and pancreatic cancer, which explains about 25% of all cases of the disease [1]. In addition, a previous pilot study from Malmö has shown that weight gain may modify this relation [2]. The association between alcohol consumption and pancreatic cancer has been less clear, and previous studies have reported inconsistent results [3–15]. Some have shown a positive association [5–7, 11, 14, 15], most of which were cohort studies [5, 7, 14, 15], whereas others have not shown any association [3, 4, 8–13], and these were mainly case-control studies [3, 4, 11–13].

A problem in studies on alcohol is that self-reported consumption may have low validity. It is difficult to es-

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tablish previous drinking habits, and retrospective and cross-sectional studies may be subject to recall bias or changed alcohol consumption due to subclinical disease. Another possible reason for inconsistent results in previous studies on alcohol and pancreatic cancer is that the prevalence of potential interacting factors such as smoking and obesity might have differed between studies.

In 1974, the Department of Medicine, Malmö, Sweden, set up a primary preventive project. By 1992, a total of 33,346 individuals had participated in the baseline examination, which included a physical examination measuring weight and height, laboratory analyses and a questionnaire that assessed smoking and alcohol consumption [16]. In this cohort, 183 cases of incident pancreatic carcinomas were diagnosed to December 31, 2004.

The aim of this study was to investigate whether different prediagnostic measurements of alcohol consumption, laboratory markers and a score measuring alcohol addiction are associated with the risk of developing pancreatic cancer. An additional aim was to investigate the potential interaction between smoking and alcohol consumption in relation to the risk of pancreatic cancer, and to establish if the association of these factors with pancreatic cancer is modified by body mass index (BMI) or weight gain.

Patients and Methods

The Malmö Preventive Project

The Malmö Preventive Project was set up in 1974 as an integrated institute within the Department of Medicine at Malmö University Hospital, Sweden. The main purpose of this institute was to screen a middle-aged population for risk factors such as cardiovascular diseases and alcoholism, and thereby develop methods – on an individual patient basis – for early detection, health education and prevention of a number of diseases and risk factors [16]. Complete birth-year cohorts of registered residents in Malmö were invited by letter to participate. All men born in 1921, 1926–1942, 1944, 1946 and in 1948–1949, and all women born in 1926, 1928, 1930–1938, 1941 and 1949 received an invitation. The attendance rate was high (71%), and when the recruitment ended in 1992, a total of 33,346 individuals (22,444 men and 10,902 women) had participated. Mean age at baseline was 50 years for men and 44 years for women. In baseline examinations subjects responded to a self-administered questionnaire consisting of about 200 questions concerning lifestyle and medical history. Weight and height were measured by a trained nurse. Selected biochemical analyses were performed, and the remaining biological material was stored in a biological specimen bank. Except for about 6,000 men, none of the examinations were repeated following the baseline examinations.

Ethical clearance for the present study was obtained from the Ethical Committee at Lund University, LU-828-02.

Registration of Endpoints

Information on cancer diagnosis was retrieved by record linkage to the Swedish Cancer Registry and the Regional Tumor Registry of Southern Sweden. All cases of pancreatic cancer were identified using the ICD-7 code 157, and ICD-10 code C25. Cause and date of death were established using the Swedish Cause-of-Death Registry. The end of the follow-up period was December 31, 2004. The record linkage yielded 187 cases of incident pancreatic cancer among the participants in the Malmö Preventive Project. There were no prevalent cases at baseline. The records for all incident cases were reviewed using clinical notes, radiological and pathological findings (i.e. biopsies, specimens obtained during surgery and autopsy reports).

After reviewing these cases, 183 subjects could be confirmed to have adenocarcinoma of the pancreas. In 70 cases the diagnosis was verified by autopsy, while 19 cases had undergone surgery and had a clear histopathological diagnosis. In another 82 cases the diagnosis was based on their clinical presentation, radiological findings and tissue biopsy consistent with adenocarcinoma of the pancreas. A further 7 cases were verified by the combination of clinical notes, radiological examination and biopsies that showed unspecified adenocarcinoma, findings that, taken together, stated a high probability for cancer of the pancreas. Finally, 5 cases were accepted because of their clinical and radiological findings, although no biopsies had been taken. Four cases were found to have had pancreatic cancer other than adenocarcinoma, according to their histopathology report (2 islet cell tumors, 1 endocrine and 1 anaplastic malignancy), and were hence excluded. Thus, 183 subjects remained in the present study as incident pancreatic cancers. This group consisted of 128 men and 55 women (mean age at diagnosis, 64 and 65 years, respectively).

Assessment of Potential Risk Factors

Alcohol

Two independent methods were used to estimate alcohol consumption. One method was the use of a biochemical marker, serum γ-glutamyl transferase (γ-GT), and the second method was a scoring system based on a modified version of the Michigan Alcoholism Screening Test [17], referred to in this text as the Malmö modification of the brief MAST (Mm-MAST) [18]. The scoring system consisted of 7 questions regarding drinking habits, and it has been described in detail elsewhere [19]. Every question gave 1 point for a positive answer and no points for a negative answer. Alcohol consumption was regarded as 'low' for subjects with a score of 0–1, 'intermediate' for a score of 2–3, and 'high' for a score of 4 or more. Alcohol consumption was registered as 'missing' for subjects with 1 or more missing answers. Questions on absolute amounts of alcohol intake were not used in the questionnaire. These questions were not introduced into the questionnaire until December 1976, hence there was no information on Mm-MAST for the first 2,142 subjects. Missing answers for 1 or more of the questions were found in 753 subjects. The total number of individuals that could be classified according to this scoring system was 30,551.

Serum γ-Glutamyl Transferase

A standard laboratory method, using γ-glutamyl-*p*-nitroanilin as a substrate, was used by Malmö University Hospital to analyze plasma-γ-GT [20]. In all but 107 individuals, information on

γ-GT levels was available. For further analysis, the cohort was divided into quartiles based on γ-GT at baseline.

Body Mass Index and Weight Gain

At baseline, all subjects underwent measurement of height (cm) and weight (kg). These measurements were used to calculate their BMI. The following definitions were used: underweight was a BMI <20, normal weight was a BMI of 20–25, overweight was a BMI of 25–30 and obese was a BMI >30.

The question 'Have you gained more than 10 kg since the age of 30?', with the possible answers 'Yes' or 'No', was used in order to define weight gain.

Smoking Habits

The question 'Have you ever smoked on a daily basis for a period of at least 6 months?' and 'Do you smoke?' were used to define non-, current and former smokers. If the answer was negative for both questions, the subject was classified as a nonsmoker and if the answer was positive to the question 'Do you smoke?', the subject was classified as a current smoker. A respondent who did not currently smoke but who confirmed a previous habit of daily smoking that lasted at least 6 months was regarded as a former smoker. Missing and inconsistent answers could be identified and resolved using other questions concerning smoking habits (daily tobacco dose and time since cessation). These questions were further used to define the amount of daily smoking in current smokers and the time since smoking cessation in former smokers. The questionnaire consisted of questions concerning tobacco dose and time since cessation, but the number of cases was too small in these subgroups to allow for separate analysis.

Statistical Methods

All participants in the Malmö Preventive Project were followed from baseline until a diagnosis of pancreatic cancer, death or end of follow-up (December 31, 2004). The mean follow-up was 22.1 years and the total number of person-years was 739,612.73. The incidence of pancreatic cancer was calculated per 100,000 person-years in different categories of studied exposures. Cox's proportional hazards analysis was used to estimate relative risks (RR) with a 95% confidence interval (CI). In the adjusted analysis, age at diagnosis was entered as a continuous factor, and sex, smoking status, alcohol consumption category (Mm-MAST), γ-GT, BMI and weight gain were entered as categorical variables. To adjust for alcohol consumption, the Mm-MAST score was chosen, since it may be a more specific marker of alcohol consumption than γ-GT.

Furthermore, the relative risk for pancreatic cancer related to smoking and alcohol intake was analyzed in different strata of smoking, alcohol consumption, BMI and weight gain in order to detect modifying effects. Combining different levels of smoking and alcohol consumption required comparisons of groups with a limited number of cases, and some of these analyses used a dichotomized variable on alcohol consumption and γ-GT. That is, high/intermediate versus low, according to Mm-MAST, and GT-quartile 4 versus GT-quartiles 1–3. Interaction between smoking, alcohol and BMI was analyzed by entering 1 covariate multiplied by the other as an interaction term. $p < 0.05$ was considered to be indicative of a statistically significant interaction. All statistical calculations were performed using the software SPSS 14.0.

Results

Covariation between Potential Risk Factors

A breakdown of mean age, gender and distribution of potential risk factors at baseline is given in table 1. The 2 methods used to estimate drinking habits covaried to a large extent. The highest Mm-MAST category, as compared to the other 2 Mm-MAST categories, had a high proportion of the highest γ-GT category, and vice versa. Current smokers reported high alcohol consumption, measured according to both methods. Subjects in the highest category of γ-GT were more often obese as compared to the lowest γ-GT quartile. Contrary to this, there were no large differences between different Mm-MAST categories with regard to BMI. Current smokers were leaner than non- and former smokers and a previous weight gain was most common among former smokers (table 1). There was a high correlation between self-reported weight gain and overweight/obesity. Among subjects reporting a previous weight gain, 79% had a BMI >25, as compared to 34% among subjects who reported no weight gain (not shown in table).

Alcohol

High alcohol consumption, defined according to both Mm-MAST and γ-GT levels, was positively associated with pancreatic cancer, although the risk associated with the highest Mm-MAST category did not reach statistical significance (table 2).

When the fourth γ-GT quartile was combined with a high alcohol consumption, defined as intermediate/high according to Mm-MAST, in a new covariate, this group had a RR of 2.41 (95% CI 1.51–3.82) as compared to subjects with low alcohol consumption, i.e. low consumption according to Mm-MAST and γ-GT values in quartiles 1, 2 or 3 (data not shown).

The risk of pancreatic cancer was high in the second and the fourth γ-GT quartiles among lean subjects (table 3). Apart from this, no large differences were seen in relation to different BMI categories. High alcohol consumption (measured using both the Mm-MAST and γ-GT) was associated with an increased risk in subjects that reported weight gain. However, several of the stratified analyses included only a few cases and the corresponding confidence intervals were wide.

A high Mm-MAST score was associated with pancreatic cancer in former smokers and a high γ-GT quartile was associated with a high risk in current smokers (table 4). There were no statistically significant interactions

Table 1. Distribution of potential risk factors as measured at baseline examination

Factor	Category	Smoking status, n (%)				Alcohol consumption[1], Mm-MAST category, n (%)				γ-GT quartile, n (%)				
		never	current	former	missing	low	intermediate	high	missing	1	2	3	4	missing
Age, years	mean	45.4	44.8	48.1	52.7	46.4	44.3	42.3	49.9	43.1	45.1	46.5	48.3	46.6
Sex	men	7,391 (59.4)	11,041 (74.4)	4,012 (66.8)	0 (0.0)	9,141 (56.8)	9,092 (75.3)	2,069 (90.4)	2,142 (74.0)	3,143 (34.9)	5,417 (70.4)	6,797 (81.9)	7,045 (85.6)	42 (67.3)
	women	5,044 (40.6)	3,809 (25.6)	1,998 (33.2)	51 (100)	6,951 (43.2)	2,979 (24.7)	219 (9.6)	753 (26.0)	5,867 (65.1)	2,280 (29.6)	1,506 (18.1)	1,184 (14.4)	65 (60.7)
	total	12,435	14,850	6,010	51	16,092	12,071	2,288	2,895	9,010	7,697	8,303	8,229	107
Smoking status	never					7,469 (46.4)	3,640 (30.2)	495 (21.6)	831 (28.7)	4,232 (47.0)	2,927 (38.0)	2,751 (33.1)	2,478 (30.1)	47 (43.9)
	current					5,808 (36.1)	6,147 (50.9)	1,520 (66.4)	1,375 (47.5)	3,138 (34.8)	3,445 (44.8)	3,992 (48.1)	4,242 (51.5)	33 (30.8)
	former					2,815 (17.5)	2,284 (18.9)	273 (11.9)	638 (22.0)	1,617 (17.9)	1,311 (17.0)	1,554 (18.7)	1,502 (18.3)	26 (18.0)
	missing					0	0	0	51 (1.8)	23 (0.3)	14 (0.2)	6 (0.1)	7 (0.1)	1 (0.1)
Alcohol Mm-MAST[1]	low	7,469 (60.1)	5,808 (39.1)	2,815 (46.8)	0 (0)					5,427 (60.2)	3,969 (51.6)	3,661 (44.1)	2,980 (36.2)	55 (51.4)
	intermediate	3,640 (29.3)	6,147 (41.4)	2,284 (38.0)	0 (0)					2,642 (29.3)	2,860 (37.2)	3,254 (39.2)	3,291 (40.0)	24 (22.4)
	high	495 (4.0)	1,520 (10.2)	273 (4.5)	0 (0.0)					282 (3.1)	398 (5.2)	607 (7.3)	996 (12.1)	5 (4.7)
	missing	831 (6.7)	1,375 (9.3)	638 (10.6)	51 (98.1)					659 (7.3)	470 (6.1)	781 (9.4)	962 (11.7)	23 (8.7)
γ-GT quartile, μkat/l	1 (<0.29)	4,232 (34.2)	3,138 (21.2)	1,617 (27.0)	23 (46.0)	5,427 (33.8)	2,642 (21.9)	282 (12.4)	659 (22.9)					
	2 (0.29–0.41)	2,927 (23.6)	3,445 (23.3)	1,311 (21.9)	14 (28.0)	3,969 (24.7)	2,860 (23.7)	398 (17.4)	470 (16.4)					
	3 (0.41–0.63)	2,751 (22.2)	3,992 (26.9)	1,554 (26.0)	6 (12.0)	3,661 (22.8)	3,254 (27.0)	607 (26.6)	771 (27.2)					
	4 (>0.63)	2,478 (20.0)	4,242 (28.6)	1,502 (25.1)	7 (14.0)	2,980 (18.6)	3,291 (27.3)	996 (43.6)	962 (33.5)					
	missing	47 (0.4)	33 (0.2)	26 (0.4)	1 (2.0)	55 (0.3)	24 (0.2)	5 (0.2)	23 (0.8)					
BMI	<20	686 (5.5)	1,375 (9.3)	271 (4.5)	1 (2.0)	1,227 (7.6)	829 (6.9)	137 (6.0)	140 (4.8)	990 (42.6)	5,649 (31.9)	2,005 (18.7)	363 (14.9)	3 (13.0)
	≥20–25	6,553 (52.7)	8,219 (55.3)	2,983 (49.6)	19 (37.3)	8,410 (52.3)	6,677 (55.3)	1,228 (53.7)	1,459 (50.4)	603 (25.9)	4,525 (25.5)	2,145 (20.0)	411 (16.8)	4 (17.4)
	≥25–30	4,122 (33.1)	4,378 (29.5)	2,257 (37.6)	18 (35.3)	5,080 (31.6)	3,875 (32.1)	790 (34.5)	1,030 (35.6)	442 (19.0)	4,275 (24.1)	2,970 (27.7)	593 (24.2)	5 (21.7)
	≥30	1,062 (8.5)	8,71 (5.9)	495 (8.2)	13 (25.5)	1,359 (8.4)	688 (5.7)	133 (5.8)	261 (9.0)	287 (12.3)	3,243 (18.3)	3,593 (33.5)	1,066 (43.6)	5 (21.7)
	missing	12 (0.1)	7 (0.0)	4 (0.1)	0 (0)	16 (0.1)	2 (0.0)	0 (0.0)	5 (0.2)	9 (0.4)	49 (0.3)	32 (0.3)	11 (0.5)	6 (26.1)
Weight gain >10 kg	no	6,564 (52.8)	8,705 (58.6)	3,878 (64.5)	0 (–)	9,821 (61.0)	6,215 (54.0)	927 (40.5)	1,885 (65.1)	6,159 (68.4)	4,402 (57.2)	4,623 (55.7)	3,902 (47.4)	62 (57.4)
	yes	2,685 (21.6)	2,940 (19.8)	2,129 (35.4)	0 (–)	3,809 (23.7)	2,530 (21.0)	478 (20.9)	937 (32.4)	1,695 (18.8)	1,440 (18.7)	1,879 (22.6)	2,702 (32.8)	38 (23.3)
	missing	3,185 (25.6)	3,208 (21.6)	3 (0.0)	51 (100)	2,462 (15.3)	3,026 (25.1)	883 (38.6)	73 (2.5)	1,156 (12.8)	1,855 (24.1)	1,801 (21.7)	1,625 (19.7)	7 (6.5)

[1] Alcohol consumption according to Mm-MAST (Malmö modification of the brief Michigan Alcoholism Screening Test).

Table 2. Incidence and relative risk of pancreatic cancer in different exposure categories

Factor	Category	Individuals n	Cases n	Incidence/ 100,000 person years	RR (95% CI)	RR (95% CI)[2]
Smoking status	never	12,435	38	13.7	1.00 (ref.)	1.00 (ref.)
	all current	14,850	107	32.7	2.37 (1.64–3.44)	2.34 (1.60–3.43)
	≤20 cigarettes/day	6,624	46	31.4	2.27 (1.48–3.49)	2.25 (1.45–3.50)
	>20 cigarettes/day	4,979	37	34.8	2.59 (1.65–4.08)	2.56 (1.60–4.09)
	missing dose	3,247	24	32.2	2.27 (1.36–3.78)	2.31 (1.37–3.89)
	former	6,010	38	28.4	2.05 (1.31–3.22)	1.61 (1.02–2.55)
	abstinence ≤5 years	1,724	8	20.3	1.44 (0.67–3.08)	1.23 (0.57–2.67)
	abstinence >5 years	3,756	30	36.7	2.68 (1.66–4.33)	2.00 (1.21–3.29)
	missing	530	0	0	–	–
	missing	51	0	0	–	–
Alcohol Mm-MAST[1]	low	16,092	71	20.1	1.00 (ref.)	1.00 (ref.)
	intermediate	12,071	78	28.7	1.41 (1.03–1.95)	1.50 (1.07–2.08)
	high	2,288	14	27.9	1.38 (0.78–2.45)	1.58 (0.88–2.86)
	missing	2,895	20	31.2	1.41 (0.83–2.37)	1.06 (0.62–1.79)
	trend (over categories)	30,451	183	24.1	p = 0.050[2]	p = 0.020[2]
γ-GT quartile	1 (<0.29)	9,010	32	16.8	1.00 (ref.)	1.00 (ref.)
	2 (0.29–0.41)	7,697	43	24.7	1.40 (0.88–2.21)	1.52 (0.95–2.45)
	3 (0.41–0.63)	8,303	40	20.9	1.16 (0.73–1.85)	1.24 (0.75–2.03)
	4 (≥0.63)	8,229	68	37.4	2.10 (1.38–3.20)	2.15 (1.34–3.44)
	missing	107	0	0	–	–
	trend (multiples of 0.1)	33,239	183	24.8	1.01 (1.006–1.02)	1.01 (1.005–1.02)
BMI	<20	2,333	10	19.1	0.76 (0.40–1.45)	0.84 (0.44–1.61)
	20 to <25	17,774	101	25.4	1.00 (ref.)	1.00 (ref.)
	25 to <30	10,775	54	22.6	0.89 (0.64–1.23)	0.83 (0.60–1.16)
	≥30	2,423	18	36.1	1.50 (0.91–2.47)	1.38 (0.83–2.28)
	missing	23	0	0	–	–
	trend (continuous)	33,305	183	24.8	1.05 (1.01–1.09)	1.04 (0.995–1.08)
Weight gain >10 kg	no	19,148	118	27.7	1.00 (ref.)	1.00 (ref.)
	yes	7,754	52	32.2	1.21 (0.88–1.68)	1.07 (0.77–1.48)
	missing	6,444	13	8.6	0.30 (0.17–0.53)	0.65 (0.34–1.27)

[1] Alcohol consumption according to Mm-MAST (Malmö modification of the brief Michigan Alcoholism Screening Test).
[2] Adjusted for age, sex, smoking status, Mm-MAST category (Mm-MAST is not adjusted for γ-GT and γ-GT is not adjusted for Mm-MAST) and BMI (weight gain not adjusted for BMI).

between alcohol and BMI, alcohol and weight gain, or between alcohol and smoking (tables 3, 4).

Smoking

Current smoking was associated with pancreatic cancer (table 2). There was a tendency towards an increasing risk in subjects who smoked the highest number of cigarettes per day. When γ-GT was used instead of Mm-MAST category to adjust for alcohol consumption, all results were similar. Current smoking was positively associated with the risk of pancreatic cancer in every strata of BMI and weight gain. In the group of obese subjects (BMI >30), the risk was even higher, with a RR of 7.45 (95% CI 1.65–33.64; table 3).

A positive association was found between previous smoking and the risk of pancreatic cancer, and the risk increased with time since smoking cessation (table 2). Furthermore, the risk of pancreatic cancer in former smokers was especially high in overweight subjects as compared to participants with a BMI <25. Previous

Table 3. Relative risk of pancreatic cancer associated with smoking, alcohol consumption and γ-GT in different categories of BMI and weight gain

Factor	Category	BMI							
		<25		25–30			≥30		
		cases n	RR (95% CI)[2]	cases n	RR (95% CI)[2]	interaction p value[2]	cases n	RR (95% CI)[2]	interaction p value[2]
Smoking status	never	24	1.00 (ref.)	12	1.00 (ref.)		2	1.00 (ref.)	
	current	70	2.05 (1.27–3.29)	24	2.03 (1.00–4.14)	0.79	13	7.45 (1.65–33.64)	0.72
	former	17	1.16 (0.62–2.18)	18	2.20 (1.04–4.67)	0.26	3	3.01 (0.50–18.27)	0.32
	missing status	0	–	0	–		0	–	
Alcohol Mm-MAST[1]	low	44	1.00 (ref.)	20	1.00 (ref.)		7	1.00 (ref.)	
	intermediate	47	1.42 (0.93–2.16)	25	1.74 (0.95–3.21)	0.71	6	1.35 (0.43–4.25)	0.67
	high	10	1.89 (0.93–3.84)	2	0.83 (0.19–3.66)	0.23	2	1.89 (0.36–9.89)	0.57
	missing	10	0.86 (0.41–1.79)	7	1.23 (0.048–3.16)		3	1.93 (0.46–8.10)	
	trend (over categories)	111	p = 0.050[2]	54	p = 0.305[2]		18	p = 0.342[2]	
γ-GT quartile, μ-kat/l	1 (<0.29)	22	1.00 (ref.)	8	1.00 (ref.)		2	1.00 (ref.)	
	2 (0.29–0.41)	33	1.83 (1.04–3.21)	9	1.20 (0.45–3.21)	0.31	1	0.35 (0.03–3.94)	0.24
	3 (0.41–0.63)	25	1.37 (0.75–2.53)	12	1.14 (0.43–2.99)	0.45	3	0.64 (0.09–4.27)	0.65
	4 (>0.63)	31	2.22 (1.23–4.00)	25	2.04 (0.83–4.99)	0.49	12	1.31 (0.24–7.16)	0.90
	missing	0	–	0	–		0	–	
	trend (multiples of 0.1)	111	1.01 (1.005–1.02)	54	1.02 (1.003–1.04)		18	0.98 (0.90–1.06)	

[1] Alcohol consumption according to Mm-MAST (Malmö modification of the brief Michigan Alcoholism Screening Test).
[2] Adjusted for age, sex, smoking status and Mm-MAST category (Mm-MAST is not adjusted for γ-GT and γ-GT is not adjusted for Mm-MAST).

smoking was associated with a slightly higher risk in subjects who had gained weight, as compared to subjects who had not, but this relation did not reach statistical significance (table 3).

Concerning the risk of pancreatic cancer, there were no statistically significant interactions between smoking and any of the other exposures, i.e. alcohol consumption, BMI or weight gain (table 3, 4).

Discussion

An association between different measurements of high alcohol consumption and pancreatic cancer was found in this population-based prospective cohort study. This association may be even higher in subjects reporting a previous weight gain. Moreover, this study confirms previous findings on the positive association between smoking and pancreatic cancer, and indicates that obese current smokers may have a very high risk for pancreatic cancer. However, there are several methodological issues that have to be considered.

Weight gain >10 kg				
no		yes		
case n	RR (95% CI)[2]	case n	RR (95% CI)[2]	interaction p value[2]
26	1.00 (ref.)	10	1.00 (ref.)	
72	2.11 (1.33–3.35)	24	2.13 (0.99–4.55)	0.87
20	1.19 (0.66–2.15)	18	1.96 (0.88–4.33)	0.26
	–	0	–	
50	1.00 (ref.)	18	1.00 (ref.)	
50	1.43 (0.96–2.14)	21	1.63 (0.85–3.12)	0.67
7	1.46 (0.65–3.27)	4	1.61 (0.53–4.89)	0.87
11	0.71 (0.34–1.48)	9	1.82 (0.79–4.17)	
118	p = 0.115[2]	52	p = 0.144[2]	
26	1.00 (ref.)	5	1.00 (ref.)	
33	1.59 (0.93–2.73)	6	1.31 (0.39–4.35)	0.74
28	1.19 (0.67–2.13)	10	1.62 (0.53–4.99)	0.66
31	1.60 (0.90–2.85)	31	3.61 (1.29–10.09)	0.17
0	–	0		
118	1.01 (1.001–1.02)	52	1.02 (1.008–1.02)	

Regarding alcohol consumption, there were no questions on absolute amounts of alcohol intake. The questionnaire was designed to detect alcohol addiction using questions about attitudes and customs, i.e. it focused on behavior rather than quantity. However, other studies have shown that Mm-MAST is a valid tool for identifying both heavy drinking and alcoholism [18]. γ-GT has previously been found to be a useful marker of alcohol consumption [21, 22]. One aspect of γ-GT is that these levels may be affected by several conditions, such as obesity, medications, hepatic or biliary conditions and insulin resistance. Unfortunately, adjustment could be made only for obesity in the present analysis. Nevertheless, γ-GT is considered a useful tool for identifying excessive drinkers and it has been proved to be a useful determinant for alcohol-related comorbidities, as reported by Kristenson and Trell [18]. In the present study we found a strong covariation between Mm-MAST and γ-GT-quartile (table 1), which indicates that γ-GT is a useful tool for identifying heavy drinkers and alcoholics.

Smoking habits according to the questionnaire, mentioned earlier in this paper, have previously been compared to measurements of plasma levels of carboxyhemoglobin, showing a good agreement between the concentration of carboxyhemoglobin in non- and former smokers and an increased concentration with daily tobacco consumption [23].

For the validity of self-reported weight gain, our study showed a high correlation between a positive answer and a high BMI, and between a negative answer and a low BMI. This may indicate a high validity of information on weight gain since the same association has been shown by other authors who have analyzed self-reported information on weight gain as compared to BMI [24].

For all measurements, there is one important limitation: exposure was only measured once, at the baseline examination. The individuals could have changed their lifestyle during the follow-up period. Regarding smoking, the overwhelming majority of nonsmokers probably continued to be nonsmokers, as taking up smoking after the age of 30 is uncommon. However, some current smokers had probably given up smoking, and this would have led to an under-estimation of the risk associated with current smoking, and so the true risk in this group may be even higher than that observed.

How or if alcohol habits vary over time and by age is unclear. According to official statistics and public health reports [25], alcohol consumption has increased in Sweden during recent decades, and in the same period the proportion of strict teetotalers has declined, especially among people over 45 years of age. If these changes can be applied to our cohort, we should expect higher alcohol consumption over time than reflected in the Mm-MAST questionnaire and in the γ-GT values at baseline, and thereby an underestimation of the true risk associated with high alcohol intake.

Approximately 71% of those who were invited to participate in the Malmö primary Preventive Project did attend. It may be difficult to apply observed incidence rates and absolute risks from this study to the general popula-

Table 4. Interaction between smoking, alcohol consumption and γ-GT in relation to relative risk

Factor	Smoking status RR (95% CI)[2]			Alcohol consumption1 Mm-MAST category, RR (95% CI)[2]		γ-GT, μkat/l RR (95% CI)[2]	
	never	current	former	low	intermediate and high	<0.63	>0.63
Cases, n	38	107	38	71	92	115	68
Never smoker				1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Current smoker				2.62 (1.52–4.48)	2.47 (1.32–4.63)	2.06 (1.31–3.24)	3.42 (1.66–7.07)
interaction				–	p = 0.96[2]	–	p = 0.26[2]
Former smoker				1.32 (0.65–2.71)	2.25 (1.11–4.57)	1.71 (1.00–2.93)	1.75 (0.73–4.20)
interaction				–	p = 0.24[2]	–	p = 0.88[2]
Alcohol consumption[1]							
intermediate and high vs. low	1.31 (0.63–2.72)	1.39 (0.91–2.12)	2.13 (1.05–4.32)				
interaction	–	p = 0.96[2]	p = 0.29[2]				
High γ-GT							
≥0.63 vs. <0.63 (μkat/l)	1.32 (0.60–2.90)	2.01 (1.34–3.02)	1.21 (0.59–2.48)				
interaction	–	p = 0.24[2]	p = 0.88[2]				

[1] Alcohol consumption according to Mm-MAST: Malmö modification of the brief Michigan Alcoholism Screening Test.
[2] Adjusted for age, sex, smoking status, BMI, and Mm-MAST category (Mm-MAST is not adjusted for γ-GT and γ-GT is not adjusted for Mm-MAST).

tion. However, we consider that internal comparisons and calculations of relative risks are less sensitive to a potential selection bias.

Studies of pancreatic cancer are probably not prone to being affected by a potential detection bias. The tumor is highly aggressive: most patients who are diagnosed with pancreatic cancer die within a year and the 5-year survival rate is less than 5% [26].

The analysis in this study of single potential risk factors was adjusted – or stratified – for other potential risk factors for pancreatic cancer. Hence, confounding due to these factors was probably a limited problem. Other factors that have previously been associated with pancreatic cancer include race, dietary and nutritional factors, preexisting disease (e.g. diabetes) and genetic factors [1]. Another possible confounder is chronic pancreatitis, which is a well-known risk factor for pancreatic cancer [12, 27, 28]. However, according to Otsuki and Tashiro [28], chronic pancreatitis may be the link between high alcohol consumption and pancreatic cancer, and if this is true, chronic pancreatitis should not be considered as a confounder. Still, a limitation of the present study was that there was no information on these factors.

Several case-control and prospective cohort studies have reported inconsistent results concerning whether or not alcohol is associated with pancreatic cancer. Only a few studies have found a positive association [5–7, 11, 14, 15]. The majority of these studies were cohort studies. Only 2 case-control studies found a positive association, and only in heavy-drinking men [6] and heavy-drinking black people in the USA [11]. The cohort studies generally showed a stronger association between moderate alcohol consumption and the risk for pancreatic cancer [5, 7, 14, 15].

Most previous studies have failed to show any association between obesity and pancreatic cancer. A meta-analysis of 14 studies on obesity and pancreatic cancer from 2003 estimated a 19% increase in risk of pancreatic cancer in obese individuals compared to those with a normal body weight [29]. In this paper we did not show any statistically significant increase, but the number of cases in the obese group (BMI >30) was small. The present study indicates that obesity may affect the association between smoking and pancreatic cancer, considerably increasing the risk in both current and former smokers. For alcohol, the results seemed to be inverse, as high alcohol consumption was associated with an increased risk of pancreatic cancer in lean individuals, but not in obese people. Although BMI and weight gain covaried, the highest risk associated with high alcohol consumption was seen among subjects who reported a previous weight gain. Considering the small subgroups, wide confidence intervals and limited statistical power in these subanalyses, these findings will have to be confirmed in future studies.

Recent reviews are consistent regarding the positive association between smoking and pancreatic cancer [1, 30–32]. Nearly all published reports show that tobacco increases the risk of pancreatic cancer, usually with about a 2-fold increase, as compared to nonsmokers. Furthermore, according to Lowenfels and Maisonneuve [33], the risk persists several years after smoking cessation in former smokers. Our paper confirms these findings, with an increased risk persisting more than 5 years after smoking cessation.

Smoking is thought to exert its carcinogenic effect indirectly via the bloodstream or via the duodenal contents or bile. There are several routes through which tobacco carcinogens can act, and one potential mechanism is that of activation and progression of an inflammatory response in the pancreas. This is supported by the observation that smoking is an independent risk factor for chronic pancreatitis and the development of diabetes mellitus in pancreatitis; 2 conditions which have been suggested as risk factors for the disease [12, 26, 32–34].

Alcohol (i.e. ethanol) is not known to be a carcinogen, but might function as a promoter or cocarcinogen. Ethanol is metabolized into acetaldehyde, free radicals and fatty-acid ethyl esters [32]. Acetaldehyde is a known carcinogen that can mediate inflammation and fibrosis through different pathways. It inhibits DNA repair and is known to directly injure pancreatic tissue [35]. Alcohol metabolism results in reactive oxygen production via P450 2E1, which not only causes cell damage, but also initiates a series of inflammatory cytokines [35]. Furthermore, synergistic effects between the metabolism of ethanol and the activation of nitrosamines via cytochrome P450 2E1 have been reported [35–37]. It has been hypothesized that the metabolic effects of alcohol can enhance proinflammatory and carcinogenic changes in chronic pancreatitis and diabetes mellitus, leading to pancreatic cancer [38].

Although there was only a weak positive association between BMI and pancreatic cancer in this study, there are several potential biological mechanisms that may link obesity and risk of pancreatic cancer. An important one is related to the fact that obesity leads to an abnormal glucose intolerance and hyperinsulinemia, and this has been proposed as the underlying mechanism explaining the positive association between diabetes mellitus and pancreatic cancer [9, 39, 40]. Considering that both smoking and alcohol may affect glucose metabolism and insulin sensitivity [12, 41–43], and given the results of this study, it would be valuable to include information on additional metabolic factors in future studies.

In conclusion, this study reports an association between a high alcohol intake, estimated using both a questionnaire concerning drinking habits and γ-GT, and the risk of developing pancreatic cancer. The risk appears to be higher in subjects reporting a previous weight gain. The previously established associated between smoking and pancreatic cancer could be confirmed. The highest risk of pancreatic cancer related to smoking was found in obese subjects.

Acknowledgements

Financial support was received from the Einar and Inga Nilsson Foundation, the Malmö University Hospital Funds and Donations, the Malmö University Hospital Cancer Foundation, the Crawford Foundation, the Ernhold Lundström Foundation and the Maggie Stephens Foundation.

The authors wish to acknowledge with appreciation the contribution made to this paper by Professor Anders Borgström, who passed away on October 9, 2007.

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Paper II

Original Paper

Pancreatology

Pancreatology 243732
DOI: 10.1159/000243732

Received: March 26, 2009
Accepted after revision: September 21, 2009
Published online: ■■■

Pre-Diagnostic Levels of Anionic Trypsinogen, Cationic Trypsinogen, and Pancreatic Secretory Trypsin Inhibitor in Relation to Pancreatic Cancer Risk

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Key Words
Pancreatic cancer · Trypsinogen · Pancreatic secretory trypsin inhibitor · Smoking · Alcohol · Body mass index · Weight gain

Abstract
Background/Aims: Experimental studies have suggested that trypsinogen may enhance tumor progression and that the ratio between anionic trypsinogen and cationic trypsinogen (HAT/HCT) and between the sum of trypsinogens and pancreatic secretory trypsin inhibitor (PSTI) ((HAT + HCT)/PSTI) are disturbed in patients with pancreatic cancer. The aim of this study was to investigate if pre-diagnostic levels of these parameters are associated with subsequent pancreatic cancer risk. ***Methods:*** A total of 33,346 subjects participated in a health screening programme in Malmö, Sweden. Pancreatic cancer cases (n = 84) were matched to three controls each. HAT, HCT and PSTI were analyzed in pre-diagnostic serum samples. Odds ratios for pancreatic cancer were calculated using logistic regression and were then stratified for other risk factors. ***Results:*** In the main analysis, a statistically significant association between the ratio between HAT/HCT and pancreatic cancer was observed for all, for the crude OR and for the ORs adjusted for sex, BMI or *Helicobacter pylori*. When stratified for sex, statistically significant associations were found for females in the crude OR and for the ORs adjusted for time to analysis, BMI, alcohol consumption or *H. pylori*. There was a positive association between the ratio of HAT/HCT to pancreatic cancer in the intermediate/high alcohol consumption group and subjects with a BMI <25. The sum of trypsinogens showed a similar pattern, but was only of borderline significance in the intermediate/high alcohol consumption group. ***Conclusion:*** Our hypothesis predicted an increased risk for pancreatic cancer related to an imbalance between trypsin activity and trypsin inhibition capacity. The findings concerning the ratio of HAT/HCT are in line with this. The results related to analyses stratified for other risk factors should be considered as mainly explorative.

Introduction

Trypsin is a serine protease responsible for the digestive function of the exocrine pancreas, which, together with other digestive enzymes, acts by degrading dietary

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proteins. Prematurely activated trypsin is inhibited by pancreatic secretory trypsin inhibitor (PSTI) in a 1:1 molar ratio [1, 2]. The nomenclature of the trypsinogen isoforms differs between investigators. The isoform encoded by the T4 gene is called human cationic trypsinogen (HCT) or trypsinogen-1, and the isoform encoded by the T8 gene is called human anionic trypsinogen (HAT) or trypsinogen-2. PSTI encoded by the serine protease inhibitor Kazal type 1 gene (SPINK1) is identical to the tumor-associated trypsin inhibitor (TATI). The name PSTI is generally used for the pancreatic inhibitor while TATI has been used to emphasize that the inhibitor originates from a tumor. In this paper, the terms HCT, HAT and PSTI are used.

Experimental studies have suggested that trypsinogens may enhance tumor progression [3]. In an earlier study, Borgström et al. [4] found high levels of HAT in patients with pancreatic cancer, as compared with healthy controls, whereas HCT levels were normal to low. This resulted in an almost threefold increase in the ratio between serum levels of HAT and HCT in patients with pancreatic cancer, a pattern that was also present in patients with chronic pancreatitis, a condition that has been associated with pancreatic cancer [5].

PSTI has been shown to possess growth factor activity in vitro, but the physiological role is not known [6]. However, in a study of pancreatic cancer patients and healthy controls, Haglund et al. [7] found that PSTI was elevated in 75–95% of patients, but such an elevation has been seen almost as often in patients with benign pancreatic and biliary disease [8].

It is possible to hypothesize that an imbalance between protease activity and PSTI may promote tumor invasion. Indeed, some earlier studies have suggested that the ratio of trypsinogen to PSTI is disturbed in patients with ovarian cancer and renal cell carcinoma [9–11], but to our knowledge, this has not been investigated in patients with pancreatic cancer. To date, there have been no prospective studies using prediagnostic blood samples on HAT, HCT or PSTI in relation to subsequent risk of pancreatic cancer.

The aim of this study was to investigate if prediagnostic levels of HAT, HCT and PSTI and the ratios between these parameters were associated with the risk of developing pancreatic cancer. An additional aim was to study these potential associations in different groups defined by established risk factors for pancreatic cancer, i.e. age, sex, body mass index and smoking, and potential risk factors such as drinking habits and *Helicobacter pylori* infection.

Material and Methods

The Malmö Preventive Project

The Malmö Preventive Project was set up within the regular hospital organization at Malmö University Hospital, Sweden [12]. The main purpose was to screen a middle-aged population for risk factors related to cardiovascular disease and alcoholism. From 1974 to 1992, complete birth-year cohorts of registered residents in Malmö, were invited by letter to participate. All men born in 1921, 1926–1942, 1944, 1946 and in 1948–1949, and all women born in 1926, 1928, 1930–1938, 1941 and in 1949 received an invitation. The attendance rate was high (71%), and when recruitment was ended, a total of 33,346 individuals (22,444 men and 10,902 women) had participated. At baseline examinations subjects responded to a self-administered questionnaire of 200 questions concerning lifestyle and medical history. Weight and height were measured by a trained nurse. Blood samples were drawn and selected biochemical analyses were performed. The remaining serum samples were stored in a biological specimen bank at –20°C. Median age at baseline was 46.6 years (SD 6.3) for men and 52.6 years (SD 4.8) for women.

Ethical clearance for the present study was granted by the Ethical Committee of Lund University, LU-828-02.

Baseline Exposure Assessment

Weight and height were used to calculate body mass index (BMI) as weight (kg) divided by height (m^2). The baseline questionnaire was used to assess smoking habits. The question 'Do you smoke?' was used to define current and nonsmokers. Nonsmokers that answered 'yes' to the question 'Have you ever smoked on a daily basis for more than 6 months?' were classified as former smokers, and those who answered 'no' were regarded as never smokers. The procedure has been described in detail in a previous paper [13].

Alcohol consumption was estimated by a scoring system based on a modified version of the Michigan Alcoholism Screening Test, the 'Malmö modification of the brief MAST' (Mm-MAST) [14]. In short, subjects responded to seven questions concerning drinking habits and the answers were integrated into a scoring system which was used to classify alcohol consumption as low-, intermediate-, or high-risk consumption. The details have been described in a previous publication [15].

Laboratory Investigations

Blood samples were drawn following an overnight fast. The samples were separated and several routine tests were performed immediately. The remaining serum samples were entered into a biobank and stored at –20°C. IgG antibodies against *H. pylori* were measured by an in-house enzyme-linked immunosorbent assay (ELISA) at the Department of Microbiology, Malmö University Hospital. Absorbance >0.70 was regarded as a positive test. The validity of this assay has previously been investigated in a similar setting on stored blood samples from the same cohort [16].

HAT and HCT were analyzed using two specific in-house, solid-phase double-antibody ELISAs described by Kimland et al. [17]. The interassay coefficient of variance (CV) was 3.5% for HAT and 3.1% for HCT. PSTI was measured by a specific monoclonal antibody against human PSTI, produced by Bohe et al. [18] in 1992. This antibody was used in an ELISA to measure PSTI. The interassay CV was 2.1%.

Biological Samples and Potential Effect of Storage

Time to analysis was defined as the time from baseline investigation, when the blood samples were collected, until the time that analysis was carried out. The median time from baseline to analysis was 25 years. The prolonged storage time may have affected the antigen immunoreactivity, i.e. the antigen may have undergone some degradation over time or fluid may have evaporated due to insufficiently tightened caps, causing either a decrease or an increase in concentrations. There was no significant association between IgG antibody levels and storage time regarding *H. pylori* as was also found in a previous study [16]. The serum values of HAT, HCT and PSTI were similarly plotted against storage time. All showed a slight decline over time. However, for HAT and HCT, the linear correlation for both controls and cases was close to zero and not statistically significant. For PSTI the decline was slightly stronger and statistically significant, with a β-coefficient for cases −0.12 (95% CI −0.20 to −0.05) and β-coefficient for controls −0.06 (95% CI −0.10 to −0.01), i.e. there were no great differences between cases and controls.

Identification of Cases and Matching of Control Subjects

By record linkage to the Swedish Cancer Registry and the Regional Tumor Registry of Southern Sweden, using ICD 7 code 157, cases of pancreatic cancer that occurred up until December 31, 1999 were identified and included in the study. The record linkage yielded 117 incident pancreatic cancers in the Malmö Preventive Project. By reviewing all medical records, four were found to be erroneously registered as pancreatic adenocarcinoma (two islet cell tumors, one endocrine and one anaplastic malignancy), and hence 113 could be verified as pancreatic adenocarcinoma. All 113 cases were matched to three controls resulting in a set of 452 subjects. The cases and controls were matched by sex; age was matched as ±180 days and time of baseline investigation as ±180 days. A large proportion of all subjects examined during the first year (1974–1975) had no available biological material. Therefore, only subjects examined from January 1, 1976 were included in the set intended for laboratory analyses; a total of 104 cases and 311 controls. Following sample retrieval and aliquoting, 87 cases had the necessary amounts of biological material. Considering the relatively large number of subjects with missing biological material, the matched analysis was abandoned at this point. The laboratory analyses were finally performed in 87 cases with three controls for each case. Following analysis of another three controls, no more cases were available and the laboratory analyses were concluded. In all, 351 subjects were included in the analyses; 87 cases and 264 controls.

Three cases were diagnosed within the first 2 years from baseline. One of these cases had extremely high values of both anionic and cationic trypsinogens and PSTI. The records concerning the three early cancers were re-validated. Two had no co-morbidities prior to the pancreatic cancer diagnosis, and one had insulin-dependent diabetes with nephropathies. All 3 cases underwent autopsy and were found to suffer from widespread disease. We therefore assumed that they may all have had pancreatic cancer at the time of baseline investigation, and hence excluded them from further analysis, leaving 84 cases and 264 controls included in the analyses.

Statistical Analysis

All statistical calculations were performed using the software SPSS 15.0. Median age and time from baseline to analysis and the distribution of baseline characteristics between cases and controls concerning body mass index, alcohol consumption, smoking status and *H. pylori* serology were examined. Furthermore, the baseline characteristics were compared between included and nonincluded cases/controls in order to discover if the two groups differed.

Medians were calculated for HAT, HCT and PSTI, for the sum of the trypsinogens (HAT + HCT), and for the ratios HAT/HCT and HAT + HCT divided by PSTI. The calculations were first performed for all subjects and then repeated when stratified for sex. In order to analyze whether there were any differences between cases and controls, a Mann-Whitney U test was used and $p < 0.05$ was considered statistically significant.

To assess the risk of pancreatic cancer in relation to analyzed measurements, unconditional logistic regression analysis was used to estimate crude and adjusted odds ratios (OR) with a 95% CI. Conditional logistic regression analyses were considered inappropriate since the case-control matching was abandoned due to missing blood samples for several cases and controls. Adjusted ORs were obtained by including age, time from baseline to analysis, and body mass index as continuous factors, and sex, alcohol consumption according to Mm-MAST, smoking status and *H. pylori* serology as categorical factors. To facilitate interpretation of the ORs, the levels of HAT, HCT and PSTI were analyzed in multiples of 10 and for the ratios HAT/HCT and (HAT + HCT)/PSTI in multiples of 0.1.

The examined parameters were analyzed when stratified for sex, smoking status, alcohol consumption and body mass index. The crude and adjusted OR for pancreatic cancer was calculated. Since some strata had few cases, the number of covariates included at the same time in this multivariate analysis had to be reduced and they were therefore entered one at a time. To compare whether or not the statistical models were stable, in spite of the unduly large number of entered covariates, the OR adjusted for all covariates were calculated.

Results

Baseline characteristics of cases and controls are presented in table 1. In spite of the fact that the case-control matching was partially abandoned, they show very similar features regarding the matching factors of age, sex, and time from baseline investigation to analysis. There were a higher proportion of current smokers among the cases, but alcohol consumption (Mm-MAST), body mass index, and *H. pylori* were similar in the cases and controls.

There were no large differences between the included cases/controls as compared to those not included in the analysis, except for sex and for alcohol consumption, which was expected considering that women were not invited to the Malmö Preventive Project until 1977 and questions on alcohol consumption were not introduced in the questionnaire until 1976.

Table 1. Baseline characteristics of included cases and control subjects as compared to nonincluded cases and controls

Factor	Category	Included in analysis		Not included in analysis	
		cases (n = 84)	controls (n = 264)	cases (n = 29)	controls (n = 75)
Age, years		47.7 (37.7–60.6)	47.5 (37.3–60.6)	48.5 (38.9–55.2)	48.7 (38.5–60.6)
Time from baseline investigation to analysis, years		24.8 (14.3–28.8)	25.1 (18.1–30.3)	not analyzed	not analyzed
Sex	female	26 (31.0)	76 (28.8)	4 (13.8)	14 (18.7)
	male	58 (69.0)	188 (71.2)	25 (86.2)	61 (81.3)
Body mass index	<25	54 (64.3)	161 (61.0)	17 (58.6)	36 (48.0)
	25–30	20 (23.8)	89 (33.7)	9 (31.0)	33 (44.0)
	>30	10 (11.9)	14 (5.3)	3 (10.3)	6 (8.0)
Alcohol consumption (Mm-MAST category)	low	34 (40.5)	129 (48.9)	6 (20.7)	25 (33.3)
	intermediate	41 (48.8)	114 (43.2)	8 (27.6)	13 (17.3)
	high	7 (8.3)	14 (5.3)	2 (6.9)	0
	missing	2 (2.49	7 (2.7)	13 (44.8)	37 (49.3)
Smoking status	never	13 (15.5)	88 (33.3)	2 (6.9)	20 (26.7)
	current	54 (64.3)	116 (43.9)	18 (62.1)	35 (46.7)
	former	17 (20.2)	60 (22.7)	9 (31.0)	20 (26.7)
H. pylori serology	negative	46 (54.8)	163 (61.7)	not analyzed	not analyzed
	positive	38 (45.2)	100 (37.9)	not analyzed	not analyzed
	missing	0	1 (0.3)		

Mm-MAST = Malmö modification of the brief Michigan Alcoholism Screening Test. Values presented as number (percentage) or median (range).

Main Analysis

Table 2 shows the median values of the two isoforms of trypsinogen, and the sum and the ratios of these and PSTI. The median of the ratio HAT/HCT for all subjects was slightly higher in the cases as compared to the controls, but the difference was not statistically significant. When the values were stratified for sex, the female case group showed higher values for HAT, the sum of trypsinogens and the ratio HAT/HCT as compared with the female controls, but only the difference related to the ratio HAT/HCT was statistically significant.

ORs were calculated for all subjects, stratified for sex and adjusted for one variable at a time, as shown in table 3. For the ratio HAT/HCT, there was a statistically significant association for the crude OR and for ORs adjusted for any of the following factors: sex, BMI and *H. pylori* serology. Furthermore, there was a statistically significant association between the ratio HAT/HCT and pancreatic cancer among females concerning the crude OR and ORs adjusted for time to analysis, BMI, alcohol consumption (Mm-MAST) and for *H. pylori* serology. Considering the other measurements, there were no strong associations with the risk of pancreatic cancer.

Stratified Analysis

HAT had a statistically significant association with the risk of pancreatic cancer in the intermediate/high alcohol consumption group and among subjects with a BMI <25, except when adjusted for smoking status and for all covariates entered at the same time (table 4). The sum of trypsinogens showed similar features, with a statistically significant increase in OR related to high levels of HAT + HCT in lean individuals (BMI <25), except when adjusted for smoking status and in the model adjusted for all covariates. In the intermediate/high alcohol consumption group, the ORs were borderline in a statistically significant increase for crude OR and when adjusting for age, sex, time to analysis, BMI, and *H. pylori*, but not for smoking status or for all covariates entered at the same time. PSTI had a statistically significant association with pancreatic cancer in the group of former smokers adjusted for sex or BMI and in the crude OR of the lean group (BMI <25) and when adjusted for sex or alcohol consumption. For HAT/HCT, we found a statistically significant higher crude OR, and ORs adjusted for each variable and for all co-variates entered at the same time, in the intermediate/high alcohol consumption group. In the group with BMI <25, the OR for pancreatic cancer showed

Table 2. Levels in cases vs. controls of anionic trypsinogen (HAT), cationic trypsinogen (HCT), the sum of trypsinogens (HAT + HCT), the ratio HAT/HCT, pancreatic secretory trypsin inhibitor (PSTI) and the ratio (HAT + HCT)/PSTI

Factor	All, median (range)			Female, median (range)			Male, median (range)		
	case	control	p*	case	control	p*	case	control	p*
HAT, µg/l	25.3 (1.40–221) (n = 83)	24.6 (2.60–118) (n = 257)	0.49	29.0 (12.9–73.0) (n = 26)	26.1 (12.6–118) (n = 76)	0.31	25.0 (1.40–221) (n = 57)	24.4 (2.60–91.4) (n = 181)	0.84
HCT, µg/l	37.2 (0.70–141) (n = 81)	37.2 (2.60–111) (n = 254)	0.96	39.9 (17.4–72.6) (n = 24)	39.1 (19.2–95.1) (n = 73)	0.50	36.1 (0.70–141) (n = 57)	36.8 (2.60–111) (n = 181)	0.71
HAT + HCT, µg/l	63.7 (2.10–362) (n = 80)	62.3 (9.00–213) (n = 249)	0.51	71.0 (31.6–146) (n = 24)	64.6 (35.6–213) (n = 73)	0.27	60.0 (2.10–362) (n = 56)	61.5 (9.00–199) (n = 176)	0.89
HAT/HCT	0.76 (0.41–2.00) (n = 80)	0.68 (0.28–2.46) (n = 249)	0.07	0.82 (0.51–1.54) (n = 24)	0.67 (0.36–1.24) (n = 73)	0.03	0.75 (0.41–2.00) (n = 56)	0.69 (0.28–2.46) (n = 176)	0.39
PSTI, µg/l	25.8 (14.5–62.3) (n = 84)	25.7 (13.7–72.3) (n = 264)	0.51	26.8 (16.4–62.3) (n = 26)	25.0 (14.3–53.0) (n = 76)	0.29	25.6 (14.5–44.5) (n = 58)	25.9 (13.7–72.5) (n = 188)	0.91
(HAT + HCT)/PSTI	2.31 (0.06–14.2) (n = 80)	2.37 (0.33–9.53) (n = 249)	0.96	2.47 (1.09–5.09) (n = 24)	2.45 (0.91–6.90) (n = 73)	0.80	2.28 (0.06–14.2) (n = 56)	2.35 (0.33–9.53) (n = 176)	0.91

* Mann-Whitney's U test p value for comparison between cases and control.

a statistically significant increase in the crude OR and in the OR adjusted for sex or *H. pylori* serology. The ratio of (HAT+HCT) and PSTI did not have a statistically significant association with pancreatic cancer in any stratum.

Discussion

In this prospective cohort study, within the Malmö Preventive Project, there was a positive association between the ratio of HAT/HCT and pancreatic cancer risk, but this was mainly an effect of a high risk in women. Considering the other measurements, there were no strong associations with the risk of pancreatic cancer. In the stratified analysis, HAT and HAT/HCT were associated with pancreatic cancer in the intermediate/high alcohol consumption group and among subjects with a BMI <25. The sum of trypsinogen (HAT + HCT) showed a similar pattern, but was only of borderline significance in the intermediate/high alcohol consumption group.

The acinar cells of the exocrine pancreas are responsible for the production of enzymes needed for food digestion. Trypsinogens, together with the other digestive enzymes, are packed into zymogene granules before exocytosis into the pancreatic ducts. PSTIs are synthesized and released together with the zymogens. The main physiological function of PSTI is thought to be protection of the pancreas from destruction by inadvertently activated trypsin [2, 19]. Trypsinogens are activated in the digestive tract by enterokinase to the active form of trypsin.

This study indicates that increased levels of HAT, HAT + HCT and the ratio of HAT/HCT, at least in some subgroups, may increase the risk of pancreatic cancer. Apart from its normal biological function, serine proteases seem to be of crucial importance in numerous pathological processes and trypsins are no exception. Trypsin activates other proteases and thereby directly and indirectly contributes to the degradation of the extracellular matrix and modulates cell behavior, which could facilitate cell migration and tumor invasion [3]. Trypsins modulate the functions of cell surface receptors, such as integrins and PARs (proteinase activated receptors) and it seems as though they can act as a potent growth factor [3]. By PAR-2 activation, trypsin has been shown to mediate inflammation in several cell types [10] and trypsin has been shown to mediate a hormone-like action, not only via PARs receptors, but also by other signaling mechanisms. It has been shown to mimic the action of insulin to promote glycogen formation, stimulate glucose oxidation and inhibit lipolysis [20, 21], i.e. growth-promoting factors. If these functions are of biological importance in cancer development, it is possible to hypothesize that an elevated ratio of trypsinogens and PSTI, indicating an insufficient inhibitory effect, may lead to an increased risk of pancreatic cancer. However, such an effect was not confirmed by the present study.

It is possible that increased trypsin levels, an altered HAT/HCT and a disturbed trypsinogen/PSTI ratio, are markers of inflammation [22]. The relatively high pre-diagnostic levels of HAT, HAT + HCT and HAT/HCT that

Table 3. Pancreatic cancer risk in relation to continuous values of anionic trypsinogen (HAT), cationic trypsinogen (HCT), the sum of trypsinogens (HAT + HCT), the ratio HAT/HCT, pancreatic secretory trypsin inhibitor (PSTI) and the ratio (HAT + HCT)/PSTI: OR with 95% CI in parentheses

Factor	Model	All	Female	Male
HAT	cases/controls	83/257	26/76	57/181
µg/l (multiples of 10)	crude OR	1.11 (0.98–1.26)	1.16 (0.89–1.51)	1.09 (0.95–1.26)
	age	1.11 (0.98–1.26)	1.14 (0.87–1.50)	1.09 (0.95–1.26)
	sex	1.11 (0.97–1.26)	–	–
	time to analysis	1.10 (0.97–1.25)	1.13 (0.87–1.48)	1.09 (0.95–1.26)
	BMI	1.12 (0.99–1.27)	1.19 (0.91–1.56)	1.10 (0.95–1.27)
	Mm-MAST	1.10 (0.96–1.25)	1.13 (0.86–1.50)	1.09 (0.94–1.26)
	smoking status	1.07 (0.94–1.21)	1.05 (0.78–1.39)	1.07 (0.92–1.23)
	Hp serology	1.11 (0.98–1.27)	1.17 (0.89–1.52)	1.10 (0.95–1.27)
	all covariates	1.06 (0.93–1.21)	1.02 (0.75–1.39)	1.08 (0.93–1.25)
HCT, µg/l (multiples of 10)	cases/controls	81/254	24/73	57/181
	crude OR	1.02 (0.87–1.20)	1.10 (0.79–1.54)	0.99 (0.82–1.21)
	age	1.02 (0.87–1.21)	1.08 (0.78–1.50)	1.00 (0.82–1.21)
	sex	1.02 (0.86–1.20)	–	–
	time to analysis	1.01 (0.86–1.20)	1.07 (0.77–1.49)	1.00 (0.83–1.22)
	BMI	1.03 (0.87–1.22)	1.15 (0.81–1.62)	1.00 (0.82–1.21)
	Mm-MAST	1.02 (0.86–1.20)	1.06 (0.75–1.49)	1.00 (0.82–1.22
	smoking status	0.98 (0.83–1.16)	0.99 (0.70–1.40)	0.96 (0.79–1.76)
	Hp serology	1.03 (0.87–1.21)	1.10 (0.79–1.54)	1.00 (0.82–1.22)
	All covariates	0.98 (0.82–1.17)	0.94 (0.64–1.37)	0.98 (0.80–1.21)
HAT + HCT, µg/l (multiples of 10)	cases/controls	79/249	24/73	56/156
	crude OR	1.04 (0.97–1.12)	1.08 (0.93–1.26)	1.03 (0.95–1.12)
	age	1.04 (0.97–1.12)	1.07 (0.92–1.24)	1.03 (0.95–1.12)
	sex	1.04 (0.97–1.12)	–	–
	time to analysis	1.04 (0.97–1.12)	1.07 (0.92–1.25)	1.03 (0.95–1.12)
	BMI	1.05 (0.98–1.13)	1.11 (0.95–1.30)	1.04 (0.95–1.13)
	Mm-MAST	1.04 (0.96–1.12)	1.07 (0.91–1.25)	1.03 (0.95–1.12)
	smoking status	1.02 (0.95–1.10)	1.02 (0.87–1.20)	1.02 (0.93–1.11)
	Hp serology	1.05 (0.97–1.13)	1.08 (0.93–1.26)	1.04 (0.95–1.23)
	all covariates	1.02 (0.94–1.10)	0.99 (0.83–1.18)	1.03 (0.94–1.12)
HAT/HCT (multiples of 0.1)	cases/controls	81/249	24/73	56/176
	crude OR	1.10 (1.00–1.22)	1.35 (1.02–1.79)	1.07 (0.96–1.19)
	age	1.10 (0.99–1.21)	1.31 (0.98–1.76)	1.07 (0.96–1.19)
	sex	1.11 (1.00–1.22)	–	–
	time to analysis	1.10 (0.99–1.21)	1.34 (1.00–1.78)	1.07 (0.96–1.19)
	BMI	1.11 (1.00–1.22)	1.34 (1.02–1.80)	1.07 (0.96–1.19)
	Mm-MAST	1.09 (0.98–1.20)	1.37 (1.01–1.87)	1.05 (0.95–1.18)
	smoking status	1.08 (0.98–1.19)	1.22 (0.90–1.66)	1.07 (0.96–1.91)
	Hp serology	1.11 (1.00–1.22)	1.34 (1.02–1.78)	1.08 (0.97–1.20)
	all covariates	1.05 (0.95–1.17)	1.15 (0.81–1.62)	1.06 (0.94–1.19)
PSTI, µg/l (multiples of 10)	cases/controls	84/264	26/76	58/188
	crude OR	1.15 (0.86–1.55)	1.46 (0.90–2.37)	1.00 (0.68–1.47)
	age	1.14 (0.85–1.54)	1.43 (0.88–2.33)	0.99 (0.67–1.47)
	sex	1.15 (0.86–1.55)	–	–
	time to analysis	1.10 (0.81–1.49)	1.35 (0.82–2.23)	0.96 (0.65–1.43)
	BMI	1.17 (0.87–1.58)	1.52 (0.93–2.50)	1.01 (0.69–1.48)
	Mm-MAST	1.17 (0.87–1.58)	1.53 (0.93–2.53)	1.01 (0.69–1.49)
	smoking status	1.05 (0.77–1.43)	1.24 (0.72–2.12)	0.95 (0.63–1.41)
	Hp serology	1.14 (0.84–1.54)	1.43 (0.88–2.34)	0.99 (0.66–1.46)
	all covariates	0.98 (0.70–1.37)	1.26 (0.69–2.30)	0.90 (0.56–1.39)

Table 3 (continued)

Factor	Model	All	Female	Male
(HAT + HCT)/PSTI (multiples of 0.1)	cases/controls	80/249	24/73	57/181
	crude OR	1.01 (0.99–1.03)	0.99 (0.94–1.05)	1.01 (0.99–1.03)
	age	1.01 (0.99–1.03)	0.99 (0.94–1.05)	1.01 (0.99–1.04)
	sex	1.01 (0.99–1.03)	–	–
	time to analysis	1.01 (0.99–1.03)	0.99 (0.94–1.06)	1.01 (0.99–1.04)
	BMI	1.01 (0.99–1.03)	0.99 (0.94–1.05)	1.01 (0.99–1.04)
	Mm-MAST	1.01 (0.99–1.03)	0.98 (0.92–1.03)	1.01 (0.99–1.03)
	smoking status	1.01 (0.99–1.03)	0.99 (0.94–1.04)	1.01 (0.99–1.04)
	Hp serology	1.01 (0.99–1.03)	0.99 (0.94–1.05)	1.01 (0.99–1.04)
	all covariates	1.01 (0.99–1.03)	0.98 (0.92–1.03)	1.01 (0.99–1.04)

were seen in pancreatic cancer cases in this study are compatible with the hypothesis that inflammation precedes cancer development. The results are not conclusive as these associations were only seen in some strata, but it is indeed possible that the pathophysiology might be modified by other established risk factors.

There are several methodological considerations. The validity of the data collected in baseline investigations invites discussion. In order to minimize systematic differences in the laboratory analysis, all analyses were performed by the same biomedical laboratory assistant. The two isoforms of trypsinogen and PSTI were measured by an in-house antibody ELISA and the interassay variations (CV) were low in all three assays, indicating high reproducibility. Relatively longer storage was associated with a slight decrease in immunoreactivity concerning PSTI, but there were no large differences between cases and controls. Similarly, in a previous study, *H. pylori* immunoreactivity showed no degradation over time [16]. Another possible source of misclassification bias is related to the detection of cases. The Swedish Cancer Registry has been reported to be 98% complete, equivalent to a low risk for missed cases [23]. All case files and pathology reports were reviewed in this study in order to validate the diagnosis of pancreatic adenocarcinoma and ensure correct diagnosis.

Estimates of alcohol consumption were obtained by the Mm-MAST questionnaire. This is a validated tool that is designed to detect alcohol addiction using questions on attitudes and customs, rather than questions on amounts of ingested alcohol. It has been argued that leaving out quantifying questions improves the validity of the tool for detecting individuals with high-risk alcohol consumption [14]. The validity of self-reported smoking habits in this cohort has previously been investigated, comparing measurements of plasma levels of carboxyhemoglobin in nonsmokers and former smokers, showing a good agreement between these measurements [24].

In our study, the median age at diagnosis was 61.1 years, the median age usually reported for pancreatic cancer is 65 years of age [25]. Besides that, approximately 29% of those who were invited to participate in the Malmö Preventive Project did not attend. It is possible that the results are not applicable to pancreatic cancer occurring at a higher age and that non-participants differ from participants regarding risk factor distribution and type or incidence of pancreatic cancer. However, we consider the high attendance rate, the population-based recruitment, and the internal comparisons and calculations of odds ratio in this study to be a considerable strength that has probably limited the potential selection bias.

The analysis in this study of potential risk factors was adjusted or stratified for other known and potential risk factors for pancreatic cancer. Confounding due to these factors was therefore probably a minor problem. A limitation of the present study was that we had no information on several potential risk factors such as race, dietary factors, genetic factors and chronic pancreatitis [26–29].

In the overall analysis, there was a borderline statistically significant association between pancreatic cancer and HAT/HCT but no association between pancreatic cancer and HAT, HCT, PSTI or (HAT + HCT)/PSTI. Considering the relatively small number of cases in our study, we cannot exclude the possibility that the lack of an association between these markers and pancreatic cancer in the overall analysis was due to poor statistical power.

Table 4. Stratified analysis of pancreatic cancer risk in relation to continuous values of anionic trypsinogen (HAT), cationic trypsinogen (HCT), the sum of trypsinogens (HAT + HCT), the ratio HAT/HCT, pancreas secretory trypsin inhibitor (PSTI) and the ratio (HAT + HCT)/PSTI

		Smoking status			Alcohol consumption		Body mass index	
		never	current	former	low	intermediate/high	BMI <25	BMI >25
HAT, µg/l (multiples of 10)	cases/controls	13/87	53/113	17/57	34/129	47/122	53/158	30/99
	crude OR	0.91 (0.51–1.63)	1.08 (0.94–1.24)	1.02 (0.62–1.67)	0.76 (0.52–1.12)	1.18 (1.00–1.40)	1.25 (1.04–1.51)	0.75 (0.52–1.08)
	all covariates†	0.99 (0.50–1.99)	1.09 (0.94–1.26)	0.99 (0.58–1.70)	0.70 (0.46–1.07)	1.17 (0.99–1.39)	1.20 (0.99–1.46)	0.73 (0.51–1.06)
	min.*	0.88 (0.46–1.69)[c]	1.07 (0.93–1.23)[c]	0.98 (0.59–1.61)[e]	0.73 (0.49–1.09)[b]	1.15 (0.98–1.35)[f]	1.19 (0.99–1.44)[f]	0.70 (0.47–1.03)[e]
	max.**	0.97 (0.54–1.75)[e]	1.11 (0.96–1.27)[d]	1.04 (0.63–1.70)[b]	0.80 (0.55–1.18)[g]	1.21 (1.02–1.42)[d]	1.26 (1.03–1.52)[g]	0.78 (0.55–1.12)[a]
HCT, µg/l (multiples of 10)	cases/controls	12/85	52/110	17/59	32/126	47/121	52/153	29/101
	crude OR	0.84 (0.50–1.42)	1.02 (0.85–1.24)	0.87 (0.55–1.37)	0.80 (0.57–1.12)	1.10 (0.91–1.34)	1.17 (0.96–1.43)	0.71 (0.50–1.01)
	all covariates†	0.92 (0.49–1.73)	1.04 (0.85–1.28)	0.88 (0.54–1.41)	0.79 (0.55–1.23)	1.08 (0.88–1.33)	1.91 (0.95–1.50)	0.71 (0.49–1.03)
	min.*	0.80 (0.45–1.42)[c]	1.01 (0.84–1.23)[b, c]	0.81 (0.50–1.28)[e]	0.79 (0.56–1.11)[c]	1.06 (0.87–1.30)[f]	1.11 (0.91–1.36)[f]	0.70 (0.49–1.00)[b, e]
	max.**	0.90 (0.53–1.54)[e]	1.05 (0.86–1.27)[d]	0.89 (0.56–1.42)[d]	0.81 (0.57–1.15)[g]	1.12 (0.92–1.36)[d, g]	1.21 (0.99–1.48)[a]	0.73 (0.52–1.04)[f]
HAT + HCT, µg/l (multiples of 10)	cases/controls	12/85	50/107	17/57	32/126	45/115	49/151	29/97
	crude OR	0.93 (0.69–1.25)	1.04 (0.95–1.12)	0.96 (0.75–1.24)	0.88 (0.73–1.06)	1.08 (0.99–1.19)	1.12 (1.01–1.24)	0.84 (0.70–1.02)
	all covariates†	0.96 (0.68–1.38)	1.04 (0.96–1.14)	0.96 (0.74–1.25)	0.85 (0.69–1.04)	1.08 (0.98–1.18)	1.11 (0.99–1.24)	0.83 (0.68–1.02)
	min.*	0.90 (0.65–1.26)[c]	1.03 (0.95–1.12)[a–c]	0.94 (0.73–1.21)[e]	0.87 (0.71–1.05)[a, e, f]	1.06 (0.97–1.17)[f]	1.09 (0.98–1.20)[f]	0.82 (0.67–1.00)[e]
	max.**	0.97 (0.72–1.31)[e]	1.05 (0.96–1.14)[d]	0.97 (0.75–1.25)[b, d, g]	0.89 (0.73–1.08)[g]	1.09 (0.99–1.19)[g]	1.13 (1.02–1.24)[a]	0.85 (0.71–1.03)[f]
HAT/HCT (multiples of 0.1)	cases/controls	12/85	51/107	17/57	32/126	46/117	51/152	29/97
	crude OR	0.99 (0.76–1.28)	1.07 (0.93–1.22)	1.24 (0.96–1.61)	0.91 (0.72–1.14)	1.16(1.03–1.32)	1.23 (1.00–1.28)	1.07 (0.91–1.26)
	all covariates†	0.94 (0.71–1.25)	1.05 (0.92–1.21)	1.18 (0.88–1.59)	0.78 (0.59–1.03)	1.18(1.03–1.34)	1.05 (0.92–1.20)	1.08 (0.90–1.30)
	min.*	0.94 (0.72–1.22)[a]	1.04 (0.91–1.20)[e]	1.21 (0.94–1.56)[d]	0.84 (0.65–1.08)[a]	1.15(1.02–1.31)[c]	1.10 (0.97–1.24)[f]	1.04 (0.88–1.23)[f]
	max.**	1.03 (0.78–1.32)[b]	1.07 (0.94–1.23)[d]	1.28 (0.98–1.69)[g]	0.93 (0.74–1.18)[g]	1.17(1.03–1.32)[b]	1.13 (1.00–1.28)[b, g]	1.09 (0.92–1.28)[a]
PSTI, µg/l (multiples of 10)	cases/controls	13/89	54/116	17/59	34/130	48/127	54/160	30/104
	crude OR	0.96 (0.41–2.23)	0.84 (0.56–1.27)	1.88 (0.99–3.57)	1.39 (0.90–2.17)	1.03 (0.68–1.56)	1.45 (1.00–2.11)	0.75 (0.41–1.35)
	all covariates†	0.93 (0.35–2.47)	0.79 (0.50–1.25)	1.90 (0.93–3.86)	1.23 (0.73–2.06)	0.90 (0.57–1.43)	1.11 (0.72–1.73)	0.78 (0.41–1.43)
	min.*	0.91 (0.37–2.23)[g]	0.74 (0.47–1.16)[c]	1.87 (0.98–3.56)[g]	1.27 (0.80–2.01)[f]	0.92 (0.59–1.42)[f]	1.35 (0.92–1.98)[c]	0.67 (0.36–1.93)[f]
	max.**	1.05 (0.44–2.52)[b]	0.87 (0.57–1.32)[d]	1.99 (1.03–3.86)[d]	1.40 (0.90–2.19)[d]	1.05 (0.69–1.59)[d]	1.49 (1.02–2.17)[e]	0.71 (0.39–1.28)[a]
(HAT + HCT)/PSTI (multiples of 0.1)	cases/controls	12/87	50/106	17/57	32/126	45/116	50/152	29/97
	crude OR	0.98 (0.90–1.06)	1.02 (0.99–1.04)	0.95 (0.89–1.02)	0.93 (0.87–0.98)	1.02 (0.99–1.05)	1.02 (0.99–1.04)	0.98 (0.94–1.03)
	all covariates†	1.00 (0.90–1.10)	1.02 (0.99–1.04)	0.95 (0.88–1.03)	0.93 (0.88–1.00)	1.02 (0.99–1.05)	1.03 (0.99–1.06)	0.97 (0.92–1.03)
	min.*	0.96 (0.88–1.05)[b]	1.02 (0.99–1.04)	0.94 (0.87–1.01)[e]	0.92 (0.87–0.98)[a–c]	1.02 (0.99–1.05)[a, c–e]	1.02 (0.99–1.05)[a, c, g]	0.97 (0.93–1.02)[e]
	max.**	0.99 (0.90–1.10)[e]	1.02 (0.99–1.04)	0.95 (0.89–1.02)[b–d, g]	0.93 (0.88–1.00)[f]	1.02 (0.99–1.04)[a, f]	1.02 (0.99–1.04)[b, e, f]	0.99 (0.92–1.02)[f]

* Min. is the model with the lowest OR. ** Max. is the model with the highest OR. † Adjusted for [a] age, [b] sex, [c] time from baseline to analysis, [d] body mass index, [e] alcohol consumption measured as the Malmö modified Michigan alcoholism screening test (Mm-MAST), [f] smoking status and [g] Hp serology. Small letters next to OR indicate the covariate with min./max. value. The OR is the same throughout if no letter is given.

Several statistically significant associations were observed in the stratified analysis. Since a high number of comparisons were made at this stage, the possibility that some of these associations were caused by chance is not excluded. However, our hypothesis predicted an increased risk for pancreatic cancer related to an imbalance between trypsin activity and trypsin inhibition capacity. Our findings are in line with this hypothesis, which supports the interpretation that the statistically significant findings in this study were not simply due to type I errors. Nevertheless, the stratified analysis should be considered as mainly explorative and will have to be confirmed in future studies.

Acknowledgments

The authors wish to thank Mrs Anne-Marie Rohrstock for her excellent help with laboratory analyses. Financial support was received from The Einar and Inga Nilsson Foundation. The Ernhold Lundström Foundation. The Malmö University Hospital Cancer Research Fund. The Malmö University Hospital Funds and Donations. The Crafoord Foundation. The Region of Skåne and The Mossfelt Foundation.

The authors wish to acknowledge, with gratitude, the contribution made to this paper by the late professor Anders Borgström, our mentor, colleague and friend.

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Paper III

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A prospective study of *Helicobacter pylori* in relation to the risk for pancreatic cancer

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* Corresponding author †The author's whish, with appreciation, to acknowledge the contribution made to this paper by Professor Anders Borgström, who died on 9th October 2007.

Published: 5 November 2008

BMC Cancer 2008, **8**:321 doi:10.1186/1471-2407-8-321

Received: 25 April 2008
Accepted: 5 November 2008

This article is available from: http://www.biomedcentral.com/1471-2407/8/321

Abstract

Background: The relationship between *Helicobacter pylori* infection and pancreatic cancer has been investigated in three previous studies with contradictory results. The aim of the present study was to investigate the association between *H. pylori* seropositivity and the risk for pancreatic cancer in a nested case-control study within a population based cohort.

Methods: Selected birth-year cohorts (born 1921–1949) of residents in Malmö, Sweden, were invited to a health screening investigation. A total of 33 346 subjects participated. Cases with pancreatic cancer (n = 87) were matched to controls (n = 263) using age, sex and time for baseline investigation as matching variables. *H. pylori* serology was analysed in stored serum samples using an enzyme-linked immunosorbent assay. Odds ratios (OR) for pancreatic cancer were calculated with 95% confidence intervals (CI) using logistic regression.

Results: *H. pylori* seropositivity was not associated with pancreatic cancer in the total cohort (adjusted OR 1.25 (0.75–2.09)). However, a statistically significant association was found in never smokers (OR 3.81 (1.06–13.63) adjusted for alcohol consumption) and a borderline statistically significant association was found in subjects with low alcohol consumption (OR 2.13 (0.97–4.69) adjusted for smoking).

Conclusion: We conclude that no association between *H. pylori* infection and the risk for pancreatic cancer was found in the total cohort. However, in never smokers and in subjects with low risk alcohol consumption, a positive *H. pylori* serology was associated with an increased risk for pancreatic cancer. These findings should be interpreted cautiously due to the limited number of cases in these subgroups.

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Background

Pancreatic cancer is a relatively infrequent form of cancer but due to the poor prognosis associated with the disease, it ranks eight among the leading causes of cancer related deaths worldwide [1]. Smoking is the most well documented risk factors for pancreatic cancer, estimated to account for about 25% of all cases [2]. Alcohol consumption is not an established risk factor for pancreatic cancer, but there is a well known association between alcohol consumption and chronic pancreatitis, and chronic pancreatitis is associated with an increased risk for pancreatic cancer [3].

Helicobacter pylori infection has previously been associated with gastric cancer [4-7] and mucosa-associated lymphoid tissue lymphoma [8,9]. The association between *H. pylori* infection and pancreatic cancer has been investigated in three previous studies. One case-control study and one prospective cohort study among smoking men have both indicated an about doubled risk for pancreatic cancer in *H. pylori* infected individuals [10,11]. However, this association could not be confirmed in a recent nested case-control study performed in a cohort of subscribers to a medical care program in the US [12].

The Malmö Preventive Project was set up in 1974 with the main purpose to screen the middle-aged population for cardiovascular disease risk factors [13]. The cohort consists of 33 346 individuals subjected to a health screening investigation sometime between 1974 and 1992 including physical examination and a self-administered questionnaire. Stored blood samples are available from the baseline investigation.

The objective of the present study was to investigate the association between *H. pylori* infection and the risk of pancreatic adenocarcinoma in relation to smoking and drinking habits in this population based cohort.

Methods

The Malmö Preventive Project Cohort

In 1974, a Department of Preventive Medicine was set up within The Department of Medicine at Malmö University Hospital [13]. The main goal was to screen the middle-aged population for risk factors for cardiovascular diseases, diabetes mellitus and alcoholism. Complete birth-year cohorts of registered residents in Malmö, Sweden, were invited by letter to a health screening investigation from 1974 to 1992. All men born in 1921, 1926–1942, 1944, 1946 and in 1948–49, and all women born in 1926, 1928, 1930–1938, 1941 and in 1949, were included. The attendance rate was high (71%), and when the recruitment ended a total of 33 346 individuals (22 444 men and 10 902 women) had participated. At baseline examination subjects responded to a self-administered questionnaire, weight and height were measured and blood samples were collected. Selected biochemical analyses were performed at baseline and the remaining biological material was stored in a biological specimen bank at -20°C.

Baseline exposure assessment

Weight and height were measured at baseline investigation by a trained nurse. Body mass index (BMI) was calculated as weight (kg) divided by length $(m)^2$. Smoking habits were assessed by questionnaire at baseline investigation. The question "Have you ever been smoking on a daily basis for at least six months?" was used to separate those who had ever smoked ("ever smokers") from those who had never smoked ("never smokers"). Ever smokers were classified as current smokers if they had confirmed current smoking, the remaining were classified as former smokers. This procedure has been described in detail previously [14]. Alcohol consumption was estimated using a scoring system that has been described in a previous publication[14] based on a modified version of the Michigan Alcoholism Screening Test [15], the "Malmö modification of the brief MAST" (Mm-MAST)[16]. In brief, subjects answered 7 yes or no questions regarding drinking habits, these answers were integrated into a scoring system which was used to classify alcohol consumption as "low" "intermediate" or "high" risk consumption [14]. No attempts to quantify alcohol consumption were made. Gamma glutamyl transferase (γ-GT) was measured at the time for baseline investigation using γ-glutamyl-p-nitroanilin as a substrate [17]. Values of γ-GT were available for all but 2 subjects. γ-GT values were used as an alternative marker for alcohol consumption. The cohort was divided into tertiles of γ-GT values, cut-off levels for the different tertiles were identified in cases in order to construct three groups of individuals with an equal number of cases in each group.

Identification of cases and matching of control subjects

Cases of pancreatic cancer were identified by record linkage of the Malmö Preventive Project cohort database to the Swedish Cancer Registry using the ICD 7 diagnostic code for pancreatic cancer (157). Cases that occurred until 31 December 1999 were included in the study. The record linkage yielded 117 subjects registered with the diagnosis of pancreatic cancer in the Malmö Preventive Project. Clinical and pathology records were reviewed in all subjects. The diagnosis of pancreatic adenocarcinoma could be verified in 113 cases. Four cases were found to have been erroneously registered as pancreatic adenocarcinomas (two islet cell tumors, one endocrine tumor and one anaplastic malignancy) and were therefore excluded from further analysis. All 113 cases were matched to 3 controls resulting in a set of 452 subjects. Age was matched as +/- 180 days and time of baseline examination as +/- 180

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days. A large proportion of subjects examined during the first year (Oct. 1974–1975) had no available biological material. It was therefore decided that only subjects examined from 1 Jan. 1976 should be included in analyses, resulting in a data set of 104 cases and 311 controls. Following sample retrieval and aliquoting, 87 cases had the necessary amount of biological material. Considering the relatively large number of subjects with missing biological material, the matched analysis was abandoned at this point. The laboratory analyses were, hence, performed in 87 risk sets ordered as 3 controls and 1 case. Following analysis of another 3 controls, no more cases were available and the laboratory analyses were finished. One analyzed control subject was excluded because of failure in the *H. pylori* serology analysis. In all, 350 subjects were included in the data analyses; 87 cases and 263 controls, excluding 102 of the 452 subjects initially intended for the study. Age, BMI and smoking habits were similar in excluded and included subjects but there was a higher proportion of men and a higher proportion of missing information on alcohol consumption in the excluded group. This difference was expected since mostly men were investigated during the early phase of the study and questions on alcohol were not introduced in the questionnaire until December 1976. Among included cases, evidence for the diagnosis of pancreatic adenocarcinoma was found in pancreatic resection specimens in nine cases, at autopsy in 40 cases and by fine needle biopsy in 36 cases (in 31 cases the biopsy was conclusive for pancreatic adenocarcinoma and in 5 cases the biopsy showed low differentiated adenocarcinoma of unclear origin but radiological findings were indicative for a primary pancreatic tumor). In the remaining two cases the diagnosis was based on the clinical picture and radiological findings without any cytological or histopathological data.

H. pylori *serology*

IgG antibodies against *H. pylori* were measured by an in house enzyme-linked immunosorbent assay (ELISA) at the department of microbiology, Malmö University Hospital, in blood samples from cases and controls stored at baseline investigation [7]. Absorbance > 0.70 was regarded as a positive test. The validity of this assay has previously been investigated in a similar setting on stored blood samples from the same cohort. Immunoreactivity was found to be stable over time in that study. A bimodal distribution in the absorbance level was demonstrated with two distinct populations well separated by the cut-off level of 0.70 [7].

Statistical analysis

All statistical calculations were performed using the software SPSS 15.0. Median age, body mass index and time from baseline investigation to analysis at baseline investigation were calculated. The distribution of baseline characteristics was compared between cases and controls and between subjects with a positive and a negative *H. pylori* serology. Unconditional logistic regression was used to estimate crude and adjusted odds ratios (OR) with a 95% confidence interval (CI). Conditional logistic regression was considered inappropriate since the case-control matching was disrupted due to the fact that blood samples were missing for several cases and controls. Adjusted OR were obtained by including age, sex, body mass index (BMI), smoking, alcohol consumption according to the Mm-MAST test, *H. pylori* serology and time from baseline investigation to analysis, in the logistic regression model. Simultaneous adjustment for Mm-MAST and γ-GT tertiles was considered inappropriate since both are used as surrogate markers for the same parameter, ie alcohol consumption. Mm-MAST score was chosen as the principal marker for alcohol abuse, since this variable was considered to be a more specific marker for alcohol abuse than γ-GT. However, all calculations were repeated replacing Mm-MAST category with γ-GT tertiles for comparison. OR for pancreatic cancer in relation to *H. pylori* serology was further studied in separate strata of smoking habits, Mm-MAST category, γ-GT tertiles and BMI. Due to the small number of cases in each stratum of smoking status or alcohol consumption, the number of covariates included had to be reduced in these analyses. Possible covariates (age, sex, body mass index (BMI), smoking, alcohol consumption measured by Mm-MAST category, and time from baseline investigation to analysis) were therefore included one at a time in order to determine factors with a significant impact on the association between *H. pylori* serology and pancreatic cancer. As a comparison to the overall risk calculations, OR adjusted for all covariates were calculated in order to allow assessment of the stability of the statistical model, although the number of entered covariates in this final analysis was formally unduly large.

Ethical Approval

The Ethical Committee at Lund University approved the current study (LU 828-02; 6 Feb. 2003). In line with the requirements of the local ethical committee, all participants in the Malmö Preventive Project were informed of the present study by advertisements in local newspapers. The possibility to withdraw from the current analysis was explicitly stated.

Results

Baseline characteristics

Baseline characteristics for included cases and controls are presented in table 1. Cases and controls were highly similar regarding matching factors despite the fact that the case-control matching was partially disrupted (due to missing blood samples as stated above). There was a higher proportion of current smokers among cases. Alcohol consumption, as measured by the Mm-MAST test, was

Table 1: Baseline characteristics of case with pancreatic cancer and control subjects

Factor		Cases (n = 87)	Controls (n = 263)	p-value
Age (years)		*47.9 (37.7–60.6)*	*47.5 (37.3–60.6)*	0.53*
Sex	Male	58 (66.7%)	187 (71.1%)	
	Female	29 (33.3%)	76 (28.9%)	
Time from baseline investigation to analysis (years)		*24.8 (14.3–28.8)*	*25.1 (18.1–29.0)*	0.14*
Body mass index (kg/m^2)		*23.8 (18.0–41.0)*	*24.2 (17.6–34.2)*	0.82*
Smoking status	Never smoker	13 (14.9%)	88 (33.5%)	0.002**
	Current smoker	55 (63.2%)	115 (43.7%)	
	Former	19 (21.8%)	60 (22.8%)	
Mm-MAST category†	Low	35 (40.2%)	129 (49.0%)	0.48**
	Intermediate	42 (48.3%)	113 (43.0%)	
	High	7 (8.0%)	14 (5.3%)	
	Missing	3 (3.4%)	7 (2.7%)	
γ-glutamyle transferase-tertiles (μkat/l)	1 (< 0.35)	31 (35.6%)	98 (37.5%)	0.63**
	2 (0.35–0.60)	27 (31.0%)	90 (34.5%)	
	3(> 0.60)	29 (33.3%)	73 (28.0%)	
Helicobacter pyori serology	Negative	48 (55.2%)	163 (62.0%)	0.26**
	Positive	39 (44.8%)	100 (38.0%)	

Numbers represent n with column percent in brackets except for numbers in italics which represent medians with range in brackets.
*Mann-Whitney U-test ** Chi-square test.
†Mm-MAST, Malmö modification of the brief Michigan Alcoholism Screening Test.

similar in cases and controls. The median age for diagnosis among cases was 60.7 years (range 47.6–76.5). Baseline characteristics by *H. pylori* serology are presented in table 2. There was a slightly higher proportion of current smokers among *H. pylori* positive subjects. Groups were highly similar with regards to age, sex distribution and time from baseline investigation to analysis of *H. pylori* serology.

Association between H. pylori *serology and pancreatic cancer in the total cohort*

The risk for pancreatic cancer in different categories of *H. pylori* serology, smoking habits and alcohol consumption are presented in table 3 as crude OR and OR adjusted for age, sex, BMI, *H. pylori* serology, smoking status, Mm-MAST category and time from baseline investigation to analysis. There was no association between *H. pylori* seropositivity and pancreatic cancer in the overall analysis (adjusted OR = 1.25 (0.75–2.09)). Current smoking was associated with a statistically significantly increased OR for pancreatic cancer (adjusted OR = 3.59 (1.79–7.21)), and there was a borderline significant increase in the OR for pancreatic cancer among former smokers (adjusted OR = 2.16 (0.97–4.82)). A tendency towards increased OR's for pancreatic cancer in subjects with intermediate and high risk alcohol consumption was observed but the association was not statistically significant in any of these groups (adjusted OR = 1.33 (0.77–2.32) and 1.84 (0.65–5.23), respectively).

Stratified analyses of the association between H. Pylori *and pancreatic cancer*

The material was then stratified for smoking habits and alcohol consumption in order to study the association between a positive *H. pylori* serology and pancreatic cancer in subgroups defined by these risk factors (table 4). The size of the resulting subgroups was small and possible covariates were therefore entered one at a time. BMI and

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Table 2: Baseline characteristics by *Helicobacter pylori* serology

Factor	Category	*H. pylori* serology		p-value
		Negative	Positive	
Age (years)		*47.6 (37.3–60.6)*	*47.4 (37.7–60.6)*	0.53*
Sex	Male	144 (68.2%)	101 (72.7%)	0.38**
	Female	67 (31.8%)	38 (27.3%)	
Time from baseline investigation to analysis (years)		*25.1 (14.3–28.9)*	*24.8 (14.3–29.0)*	0.14*
Body mass index (kg/m^2)		*24.0 (17.6–37.8)*	*24.2 (18.2–41.0)*	0.82*
Smoking status	Never smoker	69 (32.7%)	32 (23.0%)	0.14**
	Current smoker	96 (45.5%)	74 (53.2%)	
	Former	46 (21.8%)	33 (23.7%)	
Mm-MAST category†	Low	97 (46.0%)	67 (48.2%)	0.81**
	Intermediate	97 (46.0%)	58 (41.7%)	
	High	12 (5.7%)	9 (6.5%)	
	Missing	5 (2.4%)	5 (3.6%)	
γ-glutamyle transferase-tertiles (μkat/l)	1 (< 0.35)	87 (41.2%)	42 (30.7%)	0.14**
	2 (0.35–0.60)	66 (31.3%)	51 (37.2%)	
	3(> 0.60)	58 (27.5%)	44 (32.1%)	

Numbers represent n with column percent in bracket except for numbers in italics which represent medians with range in brackets.
*Mann-Whitney U-test ** Chi-square test.
†Mm-MAST, Malmö modification of the brief Michigan Alcoholism Screening Test.

alcohol consumption measured by the Mm-MAST test were the two cofactors that had the most important impact on the association between positive *H. pylori* serology and pancreatic cancer in never smokers. The OR for pancreatic cancer related to positive *H. pylori* serology was 4.45 (1.19–16.69) when both these cofactors were entered in the analysis (not shown in table). *H. pylori* seropositivity was associated with the risk for pancreatic cancer in subjects with a low risk alcohol consumption in the unadjusted model (2.33 (1.09–4.97)). This association remained statistically significant when adjusting for all entered covariates, except for smoking status which resulted in a borderline significant result (2.13 (0.97–4.69)). In the small subgroup of subjects who reported a low risk alcohol consumption and were never smokers (8 cases and 55 controls), the crude OR for pancreatic cancer related to a positive *H. pylori* serology was 13.20 (2.31–75.31) (not shown in table).

As a complement to the Mm-MAST test, γ-GT-values were used to provide an alternative marker for alcohol consumption. In subjects with a γ-GT value in the lowest tertile, the crude OR for pancreatic cancer for subjects with a positive compared to a negative *H. pylori* serology was 1.72 (0.75–3.96), in the middle γ-GT tertile it was 1.54 (0.65–3.66) and in the upper γ-GT tertile it was 0.90 (0.38–2.16) (not shown in table). In subjects who were both never smokers and presented at baseline investigation with γ-GT values in the lowest tertile, the crude OR for pancreatic cancer was 3.78 (0.79–18.13) for *H. pylori* positive vs. *H. pylori* negative subjects (not shown in table). Stratifying for BMI categories did not reveal any statistically significant association between *H. pylori* positive subjects and pancreatic cancer in any BMI category (data not shown).

Discussion

The association between pre-diagnostic measurements of *H. pylori* serology and pancreatic cancer was investigated in this population-based cohort study including both men and women. *H. pylori* seropositivity was not associated with an increased risk for pancreatic cancer in the

Table 3: Crude and adjusted odds ratios (OR) for pancreatic cancer with 95% confidence intervals (CI) by *Helicobacter pylori* serology, smoking status and alcohol consumption

Factor	Status	Crude OR (95% CI)	OR (95% CI)*
H. pylori serology	Negative	1.00 (reference)	1.00 (reference)
	Positive	1.32 (0.81–2.16)	1.25 (0.75–2.09)
Smoking	Never smoker	1.00 (reference)	1.00 (reference)
	Current smoker	3.24 (1.67–6.30)	3.59 (1.79–7.21)
	Former smoker	2.14 (0.99–4.67)	2.16 (0.97–4.82)
Mm-MAST** category	Low	1.00 (reference)	1.00 (reference)
	Intermediate	1.37 (0.82–2.29)	1.33 (0.77–2.32)
	High	1.84 (0.69–4.92)	1.84 (0.65–5.23)
	Missing	1.58 (0.39–6.43)	1.86 (0.42–8.15)
γ-glutamyle transferase-tertiles (μkat/l)	1 (< 0.35)	1.00 (reference)	1.00 (reference)
	2 (0.35–0.60)	0.95 (0.53–1.71)	0.83 (0.44–1.59)
	3 (> 0.60)	1.27 (0.70–2.27)	1.07 (0.54–2.12)

* Odds ratios for the risk factors *H. pylori* serology, smoking and Mm-MAST category are adjusted for age, sex, body mass index, *H. pylori* serology status, smoking status, time from baseline investigation to analysis and alcohol consumption. Mm-MAST is used as surrogate marker for alcohol consumption, no adjustment is done for γ-glutamyle transferase values. Adjusted OR for γ-glutamyle transferase-tertiles are adjusted for the same cofactors except for Mm-MAST category.
** Mm-MAST, Malmö modification of the brief Michigan Alcoholism Screening Test.

overall analysis. However, in never smokers, there was a statistically significant association between *H. pylori* and pancreatic cancer, and in subjects with low alcohol consumption there were also indications for such an association, although not statistically significant.

The validity of data collected at baseline investigation invites discussion. A previous study in the Malmö Preventive Medicine cohort has determined cut-off level of the ELISA and demonstrated that the immunoreactivity in the stored samples is stable despite the long storage time [7]. The prevalence of *H. pylori* seropositivity increases with age [18]. This is thought to be a birth cohort effect, ie the higher prevalence of seropositivity among elderly people reflects a higher childhood infection rate at the time when they were children rather than acquisition during adult life [19,20]. It is therefore reasonable to assume that most subjects with a negative *H. pylori* serology at baseline investigation probably remained uninfected until end of follow-up. Since controls were matched both for age and

Table 4: Odds ratios for pancreatic cancer with 95% confidence intervals by *Helicobacter pylori* serology stratified for smoking status and alcohol consumption (no reference categories shown in table)

Factors	Smoking status						Alcohol consumption **					
	Never smoker		Current smoker		Former smoker		Low risk		Intermediate risk		High risk	
	Cont	Case*	Cont	Case*	Cont	Case*	Cont	Case*	Cont	Case*	Cont	Case*
H. pylori positive (n)	*25*	*7*	*50*	*24*	*25*	*8*	*47*	*20*	*41*	*17*	*7*	*2*
H. pylori negative (n)	*63*	*6*	*65*	*31*	*35*	*11*	*82*	*15*	*72*	*25*	*7*	*5*
H. pylori (crude)	2.94 (0.90–9.61)		1.01 (0.53–1.92)		1.02 (0.36–2.90)		2.33 (1.09–4.97)		1.19 (0.58–2.47)		0.40 (0.057–2.80)	
H. pylori + age	2.95 (0.89–9.81)		1.01 (0.53–1.92)		1.01 (0.35–2.87)		2.31 (1.08–4.97)		1.20 (0.58–2.48)		0.42 (0.058–3.04)	
H. pylori + sex	3.04 (0.91–10.18)		1.01 (0.53–1.93)		0.99 (0.35–2.86)		2.33 (1.09–4.98)		1.23 (0.59–2.55)		0.34 (0.048–2.46)	
H. pylori + time to analysis	3.40 (0.99–11.72)		1.00 (0.52–1.91)		1.07 (0.37–3.08)		2.32 (1.08–4.96)		1.25 (0.60–2.61)		0.37 (0.052–2.69)	
H. pylori + BMI***	3.77 (1.05–13.48)		1.00 (0.52–1.92)		1.20 (0.41–3.53)		2.33 (1.09–4.97)		1.19 (0.57–2.46)		0.30 (0.035–2.61)	
H. pylori + alcohol**	3.81 (1.06–13.63)		1.03 (0.53–1.99)		1.19 (0.39–3.61)		-		-		-	
H. pylori + smoking status	-		-		-		2.13 (0.97–4.69)		1.29 (0.61–2.73)		0.29 (0.035–2.40)	
H. pylori + all covariates	4.97 (1.23–20.10)		1.01 (0.52–1.97)		1.52 (0.45–5.10)		2.19 (0.98–4.88)		1.38 (0.64–2.98)		0.24 (0.023–2.48)	

*Controls/Cases
**Alcohol consumption estimated by the Malmö modification of the Michigan Alcoholism Screening Test. OR in subjects with missing alcohol consumption data are not reported due to small number of controls (n = 7) and cases (n = 3).
***BMI, body mass index

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time of baseline investigation we do not believe that the changing *H. pylori* prevalence over time and age in this population has introduced any major bias of the results. It is possible that some of the *H. pylori* positive subjects have been eradicated after baseline investigation which may have attenuated a potential association between *H. pylori* infection and pancreatic cancer to some extent. However, since the baseline investigation in this study was performed in middle aged subjects, it is reasonable to assume that even an individual that was eradicated after the screening visit would have had a fairly long life-time exposure for *H. pylori* infection. Alcohol consumption was estimated by two separate means, the Mm-MAST test and γ-GT levels in serum. The Mm-MAST test is a validated questionnaire for detection of high risk alcohol consumption. The questionnaire is directed towards drinking behavior but does not contain questions on amounts of ingested alcohol [16]. It has been argued that leaving out this type of quantifying question would make the test a more valid tool for detecting individuals with a high risk alcohol consumption [16]. The validity of γ-GT as a tool for detection high alcohol consumption has been investigated in numerous previous studies. In the primary health care setting, the reported sensitivity is 20–40% and the specificity around 90% in most studies [21]. Using γ-GT instead of the Mm-MAST test to adjust for alcohol consumption in the logistic regression model gave similar results. The validity of self-reported data on smoking habits has been investigated previously in this cohort. A high agreement between plasma levels of carboxyhaemoglobin and self-reported smoking status was reported indicating that the risk for misclassification bias in this regard is probably low [22].

The ascertainment of cases is another potential source of misclassification bias. The Swedish Cancer registry has previously been reported to be 98% complete, indicating a low risk for missed cases [23]. In this study, all case files and pathology reports were reviewed in order to validate the diagnosis of pancreatic adenocarcinoma. The median age at diagnosis in our study was 60.7 years which is slightly lower than the median of 65 years that is usually reported for pancreatic cancer [2]. This can be explained by the fact that the majority of the cohort has not yet been followed to a very high age. Consequently, the results might not be applicable to pancreatic cancer occurring at higher ages. It is possible that participants and non-participants in the Malmö Preventive Project differed regarding the distribution of risk factors or the type of pancreatic cancer. However, the high attendance rate (71%) and the population-based recruitment are considerable strengths in this study that have probably limited a potential selection bias.

The present study included 87 cases which makes it slightly smaller than the previously published studies from Finland and the US [11,12]. Hence, it is possible that the lack of a statistically significant association between *H. pylori* serology and pancreatic cancer in the overall analysis and among current smokers is due to a type II error. That is, we may have missed a true difference due to poor statistical power. However, it is probable that such a potential association is not very strong, considering that the estimated OR with 95% CI for pancreatic cancer among *H. pylori* positive subjects was 1.25 (0.75–2.09) in the total cohort and 1.01 (0.53–1.92) among current smokers in this study. Furthermore, our results are in accordance with the US study [12].

The association between *H. pylori* seropositivity and pancreatic cancer in both never smokers and low risk consumers of alcohol is a potentially important observation but being a subanalysis it has to be interpreted cautiously. These subgroups were small and, as a consequence, it was difficult to adjust for all potential confounders on the same time in the statistical model. However, the inclusion of these variables one at a time did not influence the results to any major extent, and we consider that confounding due to these factors can only have been a minor problem.

The association between *H. pylori* and pancreatic cancer has been investigated in three previous studies. A case control study was performed in Austria by Raderer *et al* on 92 patients with pancreatic cancer that were compared to a control group consisting of 35 patients with colorectal cancer and 27 healthy volunteers [10]. *H. pylori* seropositivity was associated with an OR of 2.1 (1.1–4.1) for pancreatic cancer in that study. A nested case-control study on the association between *H. pylori* serology and pancreatic cancer in a Finnish cohort was performed by Stolzenberg-Solomon *et al* [11]. In that study the OR for pancreatic cancer was 1.87 (1.05–3.34) adjusted for total time of smoking. Recently, a nested case-control study on the association between *H. pylori* and pancreatic cancer within a US cohort of subscribers to the Kaiser Permanente Medical Care Program, including 104 cases and 262 controls, was published [12]. In contrast to the previous two studies, no association between *H. pylori* infection and pancreatic cancer was observed. Subgroup analysis did not reveal any association in smoking men. The association between *H. pylori* serology and pancreatic cancer among never smokers was not reported. The relatively large sample size and the nested case-control design with analysis of *H. pylori* serology on prospectively collected blood samples are strengths in the latter two studies. However, it may be difficult to compare our results to the Finnish study since it only included smoking middle-aged men. Our findings are in accordance with the US study that was performed in a more heterogeneous cohort including both men and women regardless of smoking habits. A strong point in our study is that it is performed in a population based

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cohort, facilitating generalization of the results to other populations.

Pancreatic secretion is under hormonal control mainly by cholecystokinin which stimulates enzyme production and secretion from acinar cells and by secretin which induces bicarbonate secretion from ductal cells [24]. Secretin is released by cells in the duodenal wall in response to a local fall in pH that occurs when foods mixed with gastric acid enters the duodenum [25]. Several mechanisms have been proposed for the potential association between *H. pylori* infection and pancreatic cancer. Antral colonization by *H. pylori* has been associated with increased gastric acid output which will lead to increased secretin release from the duodenum. Secretin stimulation has been proven to accelerate the development and frequency of pancreatic tumors induced by nitrosamines in a hamster model [26]. One possible hypothesis is that the increased secretin levels associated with antral *H. pylori* infection, either per se or by acting as a cocarcinogen, increase the risk of pancreatic cancer [27]. Opposite to antral colonalization, *H. pylori* infection of the corpus area of the stomach is associated with a loss of parietal cells and a decrease in gastric acid out-put [27]. A second mechanism for an association between *H. pylori* infection and pancreatic cancer has been proposed derived from this model. Hypoacidity can lead to bacterial overgrowth and increased production of N-nitroso compounds which can be activated in the ductal epithelium after transportation to the pancreas by the circulation. This second hypothesis related to hypoacidity is supported by the observation that pernicious anemia is associated with pancreatic cancer [28,29]. An increased risk for pancreatic cancer was demonstrated in patients with gastric, but not duodenal ulcers in a recently published register based Swedish study by Luo *et al* [30]. This observation suggests that if there is an association between *H. pylori* and pancreatic cancer, the second hypothesis with decreased acid out-put due to *H. pylori* infection of the gastric mucosa is the probable mechanism since gastric, but not duodenal, ulcers are associated with this form of infection.

The possibility of intrapancreatic invasion by *Helicobacter* species has been investigated in resections specimens from pancreatic cancer, chronic pancreatitis and normal pancreatic tissue [31]. In that study *Helicobacter* DNA could be detected in 30 out of 40 patients with pancreatic cancer, and in 3 out of 5 patients with chronic pancreatitis. All 7 samples from normal pancreas were *Helicobacter* negative. Increased secretion of vascular endothelial growth factor and interleukin 8 has been observed in vitro after incubation of pancreatic cancer cell-lines with H pylori, providing a possible way for how H pylori could increase the malignant potential if intrapancreatic infection occurred [32]. The relevance of these findings remains to be elucidated.

Gastric acid secretion has been demonstrated to be influenced by smoking [33-35] and consumption of non-distilled alcoholic beverages produced by fermentation has been proven to increase gastric acid out-put and gastrin release [36]. Moreover, smoking is an important source of n-nitroso compound exposure in humans [37], and increased concentrations of tobacco-specific nitrosamines has been demonstrated in the pancreatic juice of smokers [38]. In the present study, there was an association between *H. pylori* seropositivity and pancreatic cancer only in never smokers and in subjects with low risk alcohol consumption. A possible explanation to this is observation could be that the effect of a weak risk factor such as *H. pylori* infection is more important in the absence of stronger risk factors such as smoking and alcohol consumption since all three possible risk factors have been suggested to influence the risk for pancreatic cancer through the same mechanisms, i.e. gastric acid secretion and production of n-nitroso compounds.

Conclusion

In summary, we did not find any statistically significantly increased risk for pancreatic cancer among subjects with a positive *H. pylori* serology in the overall analysis in this nested case-control study. We cannot completely exclude that the lack of an association between H. Pylori seropositivity and pancreatic cancer in our study was due to insufficient statistical power but our results indicate that *H. pylori* infection is probably not a strong risk factor for pancreatic cancer in the general population. However, a positive *H. pylori* serology was associated with an increased risk for pancreatic cancer in subgroups of subjects classified as never smokers or low risk consumers of alcohol. These observations should be interpreted cautiously, bearing in mind the limited number of cases in these subgroups.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BL contributed to the planning of the study, performed the data analyses and drafted the manuscript. DJ contributed to the planning of the study, validated all cases of pancreatic cancer, took part in the interpretation of the results, reviewed the manuscript, and approved the final version. AB was responsible for the planning of the study and obtained funding. JM obtained funding, supervised data analysis, interpretation of the results and manuscript writing and approved the final manuscript.

Note

† The author's wish, with appreciation, to acknowledge the contribution made to this paper by Professor Anders Borgström, who died on 9th October 2007.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council, project number 17-X-8305-09A, The Malmö University Hospital Funds and Donations, The Einar and Inga Nilsson Foundation, The Malmö University Hospital Cancer Foundation, and The Ernhold Lundström Foundation.

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Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/8/321/prepub

Paper IV

Metabolic Factors and the Risk of Pancreatic cancer:

A Prospective analysis of 580,000 men and women in the Me-Can project

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Abstract

Background/Aim: Epidemiological evidence linking the metabolic syndrome (MetS) to cancer is sparse. The aim of this study was to investigate the association between factors in the metabolic syndrome, single and combined, with the risk of pancreatic cancer.

Methods: In the Metabolic Syndrome and Cancer Project (Me-Can) data on body mass index (BMI), blood pressure and blood levels of glucose, cholesterol and triglycerides have been collected. During follow-up 862 individuals were diagnosed with pancreatic cancer. Cox proportional hazards analysis was used to calculate relative risks (RR) with 95% confidence intervals for pancreatic cancer using the above mentioned factors categorized into quintiles and transformed into z-scores. All z-scores were summarized and a second z-transformation creating a composite z-score for the MetS was performed. All risk estimates were calibrated in order to correct for regression dilution bias.

Results: The trend over quintiles was positively associated with the risk of pancreatic cancer for

mid-BP and glucose in men and for BMI, mid-BP and glucose in women. The z-score for the adjusted mid BP, RR 1.10(1.01;1.20) and calibrated z-score for glucose, RR 1.37(1.14;1.34) was positively associated with pancreatic cancer in men. In women, a positively association were found for calibrated z-score for mid BP, RR 1.34(1.08;1.66) as well as for the calibrated z-score for glucose, RR 1.98(1.41;2.76) , and for the composite z-score of the MetS, RR1.58 (1.34;1.87).

Conclusion: Our study adds further evidence to a possible link between abnormal glucose metabolism and risk of pancreatic cancer. We did not observe a synergistic effect between the single metabolic factors and the MetS in relation to risk of pancreatic cancer.

Introduction

Pancreatic cancer is characterized by an extremely dismal clinical course, with an overall 5-year survival of less than 4% [1]. Despite the relatively low incidence, pancreatic cancer ranks eight in the worldwide ranking of cancer mortality due to its high fatality rate. The poor outcome is a strong motivation for epidemiological research aimed at identifying and/or reducing risk factors for pancreatic cancer. Besides age and genetic risk factors, several lifestyle and environmental factors, such as smoking, obesity, low physical activity and alcohol consumption have been reported to be associated with pancreatic cancer [1]. A recent study from Malmö showed that the association between BMI and risk of pancreatic cancer may be modified by exposure to smoking, increasing the risk several-fold in obese smokers [2]. Still, most cases of pancreatic cancer cannot be attributable to established risk factors and as a consequence several other potential risk factors have been suggested, one of these is the metabolic syndrome.

The metabolic syndrome (MetS) was first described by Reaven in 1988 [3]. Insulin resistance was described as a fundamental feature of several risk factors predisposing to cardiovascular morbidity and mortality. One of the main ideas was that the total influence of the MetS should exceed the sum of each component. Today there is a general consensus regarding the main components of the syndrome [4], but no consensus regarding the definition has been reached [5], and the prevalence of the MetS therefore varies widely with the definition used. Regardless of this, a series of prospective studies have shown that the presence of MetS using different definitions is associated with a significantly increased risk of total mortality and CVD [6].

Epidemiological evidence linking MetS to cancer is to date sparse, although most of the components have been associated to the risk of cancer [7]. Only a few prospective studies have indicated that the clustering of the components of the MetS is associated with an increased risk of cancer [8, 9]. The aim of this study was to investigate the association between metabolic syndrome and its individual components in relation to the risk of pancreatic cancer. An additional aim was to examine if a potential association is modified by tobacco smoking.

Material and methods

The Metabolic Syndrome and Cancer project (Me-Can)

The Metabolic Syndrome and Cancer project (Me-Can) was initiated in 2006 in order to create a large pooled cohort to investigate components of the MetS on the association with overall- and site specific cancer risk. A detailed description of the project has recently been published [10].

In brief, Me-Can includes data from seven population-based cohorts in Austria, Norway and Sweden. The Austrian cohort con-

sists of the Vorarlberg Health Monitoring and Prevention Program (VHM&PP) [11], the Norwegian cohorts includes the Oslo study I cohort (Oslo) [12], the Norwegian Counties Study (NCS) [13], the Cohort of Norway (CONOR) [14] and the Age 40-programme (40-y) [15]. The Swedish cohorts are the Västerbotten Intervention Project (VIP) [16] and the Malmö Preventive Project (MPP) [17].

Ethical clearance for the present study was obtained from the three countries` ethical committees.

Baseline examinations

In all Me-Can cohorts, baseline measurements on height and weight were performed in a similarly way; without shoes and wearing light indoor clothes. Body mass index (BMI) was calculated as weight in kg divided by the squared height in meter (kg/m^2). Systolic and diastolic blood pressure was assessed in the supine position in the VIP and MPP cohorts. In the remaining cohorts blood pressure was measured in a sitting position. Blood, plasma or serum levels of glucose, total cholesterol and triglycerides were analyzed.

In the Norwegian cohorts fasting was not required before health examination and fasting time was recorded as less than 1 hour, 1–2, 2–4, 4–8 or more than 8 hours. Fasting time in the VIP was recorded as less than 4 hours, 4–8 hours or more than 8 hours, but from 1992 participants was asked to fast for at least eight hours before the examination. In the MPP and after the initial three years in the VHM&PP a minimum of eight hours of fasting was used as standard procedure.

In the Oslo and the NCS cohorts glucose levels was measured in serum with a non-enzymatic method; in CONOR and the 40-y cohort with a serum/enzymatic method; in the VHM&PP and the VIP with a plasma/enzymatic method; and in MPP with a whole blood/enzymatic method. Cholesterol- and triglyceride levels were measured in serum with a non-enzymatic method in the Oslo and NCS cohorts up until 1980 thereafter with an enzymatic method. In the other cohorts all measurements was obtained by an enzymatic method.

In the Me-Can cohorts, except for VHM &PP, participants were asked to fill in a questionnaire concerning smoking habits. In VHM&PP questions about these issues were asked by the examining physician, and the answers recorded. Smoking status was classified as never-, former- and current smokers.

Study population

The Me-Can study population includes 940,060 subjects with data from 1,600,296 health examinations. Exclusions were made for observations with a cancer diagnosis before the date of baseline examination, for a glucose level lower than 1 mmol/l and for missing data on height and weight. Furthermore, exclusions were made for observations with data missing on glucose or fasting time and for observations in the 40-y cohort from 1993, for which glucose levels were considered unrealistically low. Of the remaining 611,459 subjects with 1,025,940 observations eligible for the study, the first observation for each subject was selected. If data from a fasting state and data on smoking status were available, the first of these observations was selected.

A policy imposed by the Norwegian Institute of Public Health states that the proportion of Norwegian subjects in Me-Can studies must not exceed approximately 50% (56% after the above selection), a further 1,868 subjects in Norway without data on smoking status were excluded, leaving a total of 288,834 women and 289,866 men (578,700 subjects) eligible for the present study. For a more detailed description of inclusions and exclusions, please see Stocks *et al.* [10].

After matching the 578,700 subjects to the date of event, i.e. diagnosis of pancreatic cancer, or until the date of death, migration

or end of follow-up, whichever occurred first, a further 1,385 subjects with a follow-up of less than a year were excluded, leaving a total of 577,315 individuals in the present study population.

Follow-up of cancer diagnosis

The seven cohorts were linked to the respective National registers for a) cancer diagnosis, b) migration, c) vital status and d) cause of death. End of follow-up for each cohort was as follows: The Austrian cohort a) 2003, b) no information available, c–d) 2003; the Norwegian cohorts a–c) 2005, d) 2004; and the Swedish cohorts a–c) 2006, d) 2004. Incident pancreatic cancer was identified through linkage to the National Cancer registries, using the International Classification of Diseases, seventh edition (ICD-7), code 157, resulting in 862 cases of pancreatic cancer, 315 in women and 547 in men.

Statistical analysis

To reduce the probability of reverse causation all statistical analysis was calculated with follow-up starting one year after baseline examination. Quintile cut-offs for the five parameters were calculated separately within each cohort and sex, and for glucose, cholesterol and triglycerides, also in categories of fasting time, as less than four hours, from four to eight hours and more than eight hours. The risk of pancreatic cancer was compared to quintile levels of body mass index (BMI), mid-blood pressure [mid BP = $(BP_{systolic} + BP_{diastolic})/2$] and to quintile levels of glucose, cholesterol and triglycerides. A Cox proportional hazards analysis was used to calculate relative risks (RR) with a 95% confidence interval (CI). Attained age was used as the time scale and the model were stratified by cohort and by categories of birth-year: before 1923, 1923–1930, 1931–1938, 1939–1946, 1947–1954, 1955 and later. The RRs were adjusted for age at baseline as a continuous variable, and for smoking status and quintile levels of BMI (except BMI) as categorical variables. The p-value for trend over quintiles refers to the Wald test of a linear risk estimate.

In order to make the variables comparable on a continuous scale and to create a combined MetS variable, the z-score standardization was used [(exposure level–mean)/SD], resulting in a z-score of the exposures with a mean of 0 and a standard deviation (SD) of 1. Glucose and triglycerides were log-transformed before standardization, as they were skewed and had outliers. BMI and mid-blood pressure were standardized separately in groups defined by subcohort and sex. In addition, log (glucose), cholesterol and log (triglycerides) were standardized based on subcohort, sex and fasting time. The MetS score was calculated by summarizing the five individual z-scores before standardization. Cox proportional hazard regression was used to calculate RRs for the continuous z-score of the exposures with the risk of pancreatic cancer. Again, attained age was used as the time scale and the model was stratified by cohort and birth-year categories. In the analysis of the MetS all estimates were adjusted for age at baseline and smoking status. In the analyses of the separate exposures; BMI, mid-blood pressure, glucose, cholesterol and triglycerides, the adjusted model refers to adjustment for all other single metabolic factors on the same time.

In order to detect modifying effects, all analyses were made separately for men and women, and the z-score analyses were furthermore stratified for smoking status. Interaction between gender and the examined factors and between smoking status and the examined factors was analyzed by entering one covariate multiplied by the other as an interaction term. A p-value of < 0.05 was considered to be indicative of a statistically significant interaction. All statistical analysis were performed using the software SPSS 17.0.

Correction of a random error

The combined effect of measurement errors of the different exposures (BMI, mid-blood pressure, glucose, cholesterol and triglycerides) and long-term fluctuations within the individuals may lead to a regression dilution bias. Corrections were made by calculating the regression dilution ratio (RDR) and by using regression calibration (RC) [18–20] . These calculations were based on repeated health examinations in 133,820 subjects, including 406,364 observations in the full Me-Can database [10]. The database was cleared from measurements preceded by a cancer diagnosis, from repeated measurements from a different cohort and from measurements with a different fasting time as compared to baseline measurements. An exception from this was made pairwise for the Oslo and the NCS cohorts and for the CONOR and 40-y cohorts. That is, if baseline measurement was done in the Oslo study a repeated measurement performed in the NCS was accepted, but not from CONOR or the 40-y cohort and visa versa. Finally, exclusions were made if there was missing data on any of the exposures included in the MetS and fasting time.

In order to correct for potential regression dilution bias in the analysis based on quintiles, a regression coefficient was calculated, the regression dilution ratio (RDR) as described by Wood *et al.* [20]. RDRs were estimated for the mean follow-up time in the full Me-Can database divided by two, i.e. six years and modelled among men and women separately. This was performed as a linear mixed model, which included the actual exposure (repeated measurement as dependent and baseline measurement as independent variable), age at baseline, birth year, fasting time, smoking status and time from baseline as fixed effects and cohort as random effect. Correction of the RRs for RDRs were obtained in a direct way by dividing the estimated parameter with RDR [exp (log (RR)/RDR)], using a gender specific RDR. The estimated RDR correction values for men/women were for BMI 0.90/0.90, mid BP 0.53/0.56, glucose 0.28/0.27, cholesterol 0.64/0.66, triglycerides 0.51/0.50 and the MetS 0.68/0.69. This indicates that all the metabolic factors except BMI have a substantial random error.

The correction by regression dilution ratio was not suitable in models using more than one variable measured with error. In such situations a regression calibration model (RC) was used [18] for the analysis of the z score. With this method, the exposure measured with error (the observed measurement) was replaced with a predicted value calculated from a regression model, similar as described above, but also including the other metabolic factors as adjustment. The corrected measurement was then used in risk model estimation.

Results

Baseline characteristics

Age at baseline among male participants in Me-Can was 43.9 years (SD=11.1) and among female participants 44.1 years (SD =12.3) (Table 1). The majority of participants were aged between 30–59 years. The mean follow-up time was 12.8 years (SD=8.5) among men and 11.3 years (SD=6.9) among women. There were no great differences between follow-up time of cases and rest of the cohort in either group. The prevalence of overweight, i.e. BMI greater than 25 kg/m², was 55 % among men and 41% among women, but there were no great differences in the distribution of the weight categories between cases and the rest of cohort in men or women. The means/medians for mid BP, glucose and cholesterol were somewhat higher in the female case group as compared to the rest of the cohort.

Table 1. Baseline characteristics

	Men				Women			
	Cases		Rest of cohort		Cases		Rest of cohort	
Subjects, *n*	547		288,429		315		288,024	
Age at baseline, mean (SD)	49.3	(9.6)	43.9	(11.1)	52.8	(10.6)	44.1	(12.3)
Cohort (%)								
Oslo	119	(21.8)	16,596	(5.8)	0	(0)	0	(0)
NCS	98	(17.9)	25,781	(8.9)	80	(25.4)	24,971	(8.7)
CONOR	35	(6.4)	51,890	(18.0)	22	(7.0)	57,492	(20.0)
40-y	19	(3.5)	60,585	(21.0)	15	(4.8)	68,135	(23.7)
VHM&PP	94	(17.2)	72,843	(25.3)	83	(26.3)	86,420	(30.0)
VIP	49	(9.0)	38,697	(13.4)	52	(16.5)	40,562	(14.1)
MPP	133	(24.3)	22,034	(7.6)	63	(20.0)	10,444	(3.6)
Fasting time (%)								
<4 hrs	223	(40.8)	119,951	(41.6)	103	(32.7)	122,016	(42.4)
4–8 hrs	42	(7.7)	30,627	(10.6)	23	(7.3)	26,727	(9.3)
>8 hrs	282	(51.6)	137,851	(47.8)	189	(60.0)	139,281	(48.4)
BMI, kg/m2 mean (SD)	25.3	(3.5)	25.7	(3.5)	25.8	(4.3)	24.9	(4.4)
Mid BP, mmHg								
mean (SD)	110.7	(13.7)	108.2	(35.9)	116.4	(72.3)	101.8	(14.2)
Missing (%)	0	(0)	411	(0.1)	2	(0.6)	485	(0.2)
Glucose, mmol/l								
median (IQR)	5.3	(1.4)	5.2	(1.3)	5.3	(2.2)	5.0	(1.2)
Missing (%)	2	(0.4)	414	(0.1)	2	(0.6)	355	(0.1)
Cholesterol, mmol/l								
mean (SD)	5.9	(1.1)	5.7	(1.2)	6.2	(1.2)	5.5	(1.2)
Missing (%)	2	(0.4)	590	(0.2)	1	(0.3)	775	(0.3)
Triglycerides. mmol/l								
median (IQR)	1.5	(1.1)	1.5	(1.3)	1.3	(1.0)	1.1	(0.8)
Missing (%)	16	(2.9)	7,738	(2.7)	9	(2.9)	4,514	(1.6)
Smoking status, n (%)								
Never	141	(25.8)	113,046	(39.2)	155	(49.2)	144,384	(50.1)
Former	127	(23.2)	85,747	(29.7)	42	(13.5)	72,464	(25.2)
Current	277	(50.6)	88,777	(30.8)	115	(36.9)	70,484	(24.5)
missing	2	(0.4)	859	(0.3)	3	(1.0)	692	(0.2)

SD, standard deviation; IQR, interquartile range; BMI, body mass index; Mid BP, mid blood pressure; all percentages are column percent.

Quintile levels of exposures and risk of pancreatic cancer

The risk of pancreatic cancer was examined in quintile levels of BMI, mid-blood pressure, glucose, cholesterol and triglycerides, using the first quintile as the reference category (table 2). Absolute risks were calculated and revealed a lower risk in women, as compared to men in the low quintiles, for high quintiles the risk became nearly equal, though generally lower in women. The only positively statistically significant association among men was for the fifth quintile of the mid-blood pressure and pancreatic cancer and for the trend over the quintiles for the crude and adjusted glucose level. Among women, a statistically significant association were found in the fifth quintile of BMI, in the fifth quintile of mid-blood pressure and in the fourth and fifth quintile of the glucose levels (Table 3). A statistically significant positive association were furthermore found for the crude and adjusted trend for mid BP and glucose and for the crude RR for triglycerides in relation to risk of pancreatic cancer. The RRs corrected for RDR were similar as compared to uncorrected RRs among men, except a somewhat stronger association between mid-blood pressure and pancreatic cancer. Among women, the corrected RR was markedly higher for the 5th glucose quintile.

Z-score of exposures and risk of pancreatic cancer

In the analysis of the continuous z-scores for the five exposures and the exposures combined (MetS); there was a statistically significant association between mid-blood pressure and pancreatic cancer, and between glucose and pancreatic cancer, in both men and women, table 4. Moreover, in women there was a positively statistically significant association between the MetS and the risk of pancreatic cancer. Following regression calibration (RC) most point estimates were slightly stronger and CIs were wider. Significant effect modification was found towards a larger effect among women (p=0.02).

Metabolic factors and risk of pancreatic cancer in relation to smoking

To explore the possible interaction with smoking status, the continuous z-score was analyzed in different strata of never smokers, former smokers and current smokers for men and women separately, table 5. In male never smokers, a positive risk association were found for the adjusted and calibrated z-score for glucose. In current smokers, there was a statistically significant association between pancreatic cancer and the crude, adjusted and calibrated mid-blood pressure. In female never smokers, the risk of pancreatic cancer were positively associated with the crude, adjusted and calibrated mid BP, glucose and for the MetS. In former smoking females, an association were found for the crude BMI, glucose, triglycerides and for the crude, adjusted and calibrated MetS. In current smoking females, a positively significant association were found for the crude, adjusted and calibrated glucose z-score, as well as for the MetS z-score adjusted and calibrated. In men, the risk of pancreatic cancer associated with mid BP in current smokers was statistically significantly higher than the risk associated with mid BP in never smokers. Likewise for triglycerides, but for cholesterol the risk was found to be statistically significantly higher in never smokers as compared to former smokers. In women, the risk associated with glucose was statistically significant higher in former and current smokers, as compared to never smokers. The risk associated with cholesterol in current smokers was statistically significantly higher than the risk in never smokers. For the MetS the risk was higher in former smokers, but the relationship was inverted between current smokers and never smokers i.e. with a larger effect in never smokers.

Table 2. Risk of pancreatic cancer in the Me-Can cohort in relation to metabolic factors. Quintile analysis in men.

Exposures	Quintile level [1]	Mean (SD)	*n*, cases	Incidence/ 100,000 pers.yrs
BMI	1	21.0 (1.3)	101	13.5
(kg/m^2)	2	23.3 (0.7)	105	13.9
	3	24.8 (0.7)	115	v15.4
	4	26.5 (1.0)	101	13.6
	5	30.1 (2.9)	123	17.3
	All		545	14.7
Mid BP	1	92.2 (5.5)	79	10.1
(mmHg)	2	101.0 (3.0)	96	12.3
	3	106.9 (2.8)	112	15.8
	4	112.7 (3.2)	101	13.7
	5	127.2 (10.3)	v157	21.1
	All		545	14.7
Glucose	1	4.2 (0.5)	102	13.2
(mmol/l)	2	4.8 (0.3)	81	10.9
	3	5.1 (0.3)	121	16.1
	4	5.6 (0.3)	101	14.2
	5	6.9 (2.0)	138	19.2
	All		543	14.6
Cholesterol	1	4.5 (0.5)	100	13.6
(mmol/l)	2	5.3 (0.3)	98	13.1
	3	5.8 (0.4)	120	16.2
	4	6.4 (0.4)	117	15.9
	5	7.6 (0.7)	108	14.6
	All		543	14.6
Triglycerides	1	0.8 (0.2)	87	12.0
(mmol/l)	2	1.2 (0.2)	108	14.7
	3	1.5 (0.3)	109	15.1
	4	2.0 (0.3)	111	15.4
	5	3.4 (1.4)	114	16.0
	All		529	14.3

RR, relative risk; SD, standard deviation; Pers.yrs, person years; BMI, body mass index; Mid BP, mid blood pressure; RDR, regression dilution ratio
[1] Quintile levels grouped by cohort and sex and for glucose, cholesterol and triglycerides even for fasting time.

RR crude [2]	RR adjusted [3]	RR RDR corrected [4]
1.00	1.00	1.00
0.92 (0.70–1.20	0.96 (0.73–1.26)	0.96 (0.70–1.29)
0.93 (0.71–1.22)	0.99 (0.76–1.29)	0.99 (0.74–1.33)
0.77 (0.59–1.02)	0.83 (0.63–1.10)	0.81 (0.60–1.11)
0.72 (0.73–1.24)	1.04 (0.79–1.35)	1.04 (0.77–1.40)
P trend; 0.42	P trend; 0.54	
1.00	v1.00	1.00
1.08 (0.81–1.46)	1.12 (0.83–1.51)	1.24 (0.70–2.18)
1.25 (0.93–1.66)	1.32 (0.99–1.76)	1.69 (0.98–2.92)
0.96 (0.72–1.30)	1.04 (0.77–1.41)	1.08 (0.61–1.92)
1.26 (0.95–1.66)	1.39 (1.04–1.85)	1.87 (1.08–3.21)
P trend; 0.16	P trend; 0.06	
1.00	1.00	1.00
0.80 (0.60–1.07)	0.81 (0.60–1.08)	0.49 (0.18–1.29)
1.12 (0.86–1.46)	1.14 (0.88–1.49)	1.55 (0.65–3.81)
0.99 (0.75–1.30)	1.01 (0.76–1.34)	1.03 (0.40–2.67)
1.20 (0.92–1.55)	1.24 (0.95–1.61)	2.05 (0.84–4.94)
P trend; 0.05	P trend; 0.03	
1.00	1.00	1.00
0.79 (0.60–1.04)	0.78 (0.59–1.03)	0.68 (0.44–1.04)
0.90 (0.69–1.17)	0.88 (0.68–1.15)	0.82 (0.55–1.24)
0.81 (0.62–1.06)	0.79 (0.61–1.04)	0.69 (0.46–1.06)
0.73 (0.56–0.97)	0.70 (0.53–0.93)	0.57 (0.37–0.89)
P trend; 0.20	P trend; 0.12	
1.00	1.00	1.00
1.13 (0.85–1.49)	1.10 (0.83–1.47)	1.20 (0.69–2.12)
1.12 (0.84–1.48)	1.09 (0.82–1.44)	1.18 (0.68–2.03)
1.21 (0.85–1.49)	1.08 (0.81–1.44)	1.16 (0.66–2.04)
1.19 (0.90–1.56)	1.13 (0.84–1.52)	1.30 (0.71–2.27)
P trend; 0.82	P trend; 0.94	

[2] RR estimated from Cox regression model with attained age as time scale, stratified by cohort and categories of birth years

[3] Adjusted for quintiles levels of BMI (except BMI) and smoking status as categorical variables and age at baseline as a continuous variable

[4] Corrected RR was obtained by [exp (log (adj.RR)/RDR)].

Table 3. Risk of pancreatic cancer in the Me-Can cohort in relation to metabolic factors. Quintile analysis in women

Exposures	Quintile level [1]	Mean (SD)	*n*, cases	Incidence/ 100,000 pers.yrs
BMI	1	20.0 (1.2)	37	5.7
(kg/m^2)	2	22.3 (0.8)	55	8.4
	3	24.1 (0.8)	59	9.0
	4	26.4 (1.0)	74	11.3
	5	31.7 (3.7)	90	14.1
	All		315	9.7
Mid BP	1	88.7 (4.7)	29	4.6
(mmHg)	2	95.8 (2.2)	37	5.9
	3	101.2 (2.5)	58	8.2
	4	109.2 (3.3)	70	10.7
	5	126.4 (10.7)	119	18.7
	All		313	9.6
Glucose	1	4.1 (0.6)	34	5.1
(mmol/l)	2	4.8 (0.4)	51	7.5
	3	5.0 (0.4)	49	7.8
	4	5.4 (0.4)	73	10.9
	5	7.1 (3.3)	106	17.3
	All		313	9.6
Cholesterol	1	4.4 (0.5)	38	5.9
(mmol/l)	2	5.1 (0.3)	43	6.6
	3	5.7 (0.3)	50	7.8
	4	6.3 (0.3)	73	11.2
	5	7.6 (0.8)	110	16.7
	All		314	9.6
Triglycerides	1	0.6 (0.1)	46	7.0
(mmol/l)	2	0.9 (0.1)	36	5.9
	3	1.1 (0.1)	60	9.4
	4	1.4 (0.2)	65	10.1
	5	2.5 (1.2)	99	15.4
	All		306	9.4

RR, relative risk; SD, standard deviation; Pers.yrs, person years, BMI, body mass index; Mid BP, mid blood pressure; RDR, regression dilution ratio

[1] Quintile levels grouped by cohort and sex and for glucose, cholesterol and triglycerides even fasting time.

RR crude [2]	RR adjusted [3]	RR RDR corrected [4]
1.00	1.00	1.00
1.18 (0.78–1.79)	1.26 (0.83–1.91)	1.29 (0.81–2.06)
1.05 (0.69–1.59)	1.16 (0.77–1.76)	1.18 (0.75–1.88)
1.13 (0.76–1.68)	1.29 (0.86–1.93)	1.33 (0.85–2.08)
1.31 (0.89–1.93)	1.54 (1.04–2.29)	1.62 (1.04–2.52)
P trend; 0.61	P trend; 0.23	
1.00	1.00	1.00
1.11 (0.68–1.81)	1.18 (0.72–1.92)	1.35 (0.55–3.24)
1.31 (0.83–2.05)	1.42 (0.90–2.24)	1.88 (0.83–4.28)
1.17 (0.76–1.83)	1.33 (0.85–2.08)	1.67 (0.75–3.74)
1.68 (1.09–2.56)	1.94 (1.24–3.00)	3.30 (1.47–7.24)
P trend; 0.04	P trend; 0.01	
1.00	1.00	1.00
1.36 (0.88–2.10)	1.36 (0.88–2.09)	2.96 (0.64–13.53)
1.31 (0.85–2.04)	1.32 (0.85–2.05)	2.67 (0.56–12.64)
1.77 (1.18–2.67)	1.79 (1.19–2.70)	7.82 (1.85–33.44)
2.31 (1.57–3.41)	2.39 (1.61–3.54)	21.7 (5.38–87.08)
P trend; < 0.01	P trend;.< 0.01	
1.00	1.00	1.00
0.86 (0.56–1.34)	0.87 (0.56–1.34)	0.81 (0.42–1.56)
0.80 (0.52–1.22)	0.81 (0.53–1.25)	0.73 (0.38–1.40)
0.95 (0.64–1.42)	0.96 (0.64–1.44)	0.94 (0.51–1.74)
1.12 (0.76–1.65)	1.11 (0.75–1.64)	1.17 (0.64-2.12)
P trend; 0.35	P trend; 0.42	
1.00	1.00	1.00
0.72 (0.46-1.11)	0.67 (0.44-1.05)	0.45 (0.20-1.10)
1.01 (0.68-1.48)	0.91 (0.62-1.34)	0.83 (0.39-1.79)
0.99 (0.68-1.46)	v0.86 (0.58-1.27)	0.74 (0.34-1.61)
1.33 (0.93-1.01)	1.09 (0.75-1.59)	1.19 (0.57-2.51)
P trend; 0.03	P trend; 0.16	

[2] RR estimated from Cox regression model with attained age as time scale, stratified by cohort and categories of birth years

[3] Adjusted for quintiles levels of BMI (except BMI) and smoking status as categorical variables and age at baseline as a continuous variable

[4] Corrected RR was obtained by [exp (log (adj.RR)/RDR)

Table 4. Risk of pancreatic cancer in the Me-Can cohort in relation to metabolic factors. Z-score analysis of single factors and the combined MetS score

	Men (*n*=545)		
Exposure	z-score, crude[1]	z-score, adjusted[2]	z-score, calibrated[3]
BMI	0.98 (0.90–1.07)	0.97 (0.88–1.07)	0.90 (0.80–1.02)
Mid blood pressure	1.07 (0.98–1.16)	1.10 (1.01–1.20)	1.15 (0.97–1.35)
Glucose	1.08 (1.00–1.17)	1.09 (1.00–1.18)	1.37 (1.01–1.85)
Cholesterol	0.92 (0.84–1.00)	0.87 (0.79–0.96)	0.81 (0.69–0.95)
Triglycerides	1.05 (0.96–1.14)	1.04 (0.94–1.15)	1.04 (0.84–1.29)
MetS4	1.04 (0.95–1.14)	1.13 (0.90–1.41)	1.07 (0.94–1.22)

MetS, metabolic syndrome; BMI, body mass index;
[1] Relative risk calculated from Cox regression models, with attained age as time scale, stratified by cohort and categories of birth year.
[5] P-value for interaction between sex and exposure. Adjusted as in z score adjusted[4].

Table 5. Risk of pancreatic cancer in the Me-Can cohort in relation to metabolic factors. Z-score analysis single and combined MetS score, stratified for smoking status and sex.

		Men		
Smoking status	Exposure	z-score, crude[1]	z-score, adjusted[2]	z-score, calibrated[3]
Never smoker	BMI	1.03 (0.87–1.22)	1.04 (0.86–1.27)	1.05 (0.85–1.30)
	MidBP	1.03 (0.87–1.21)	1.02 (0.85–1.22)	1.04 (0.74–1.46)
	Glucose	1.12 (0.97–1.29)	1.18 (1.02–1.36)	1.79 (1.07–2.96)
	Cholesterol	0.90 (0.75–1.08)	0.91 (0.75–1.11)	0.86 (0.64–1.18)
	Triglycerides	0.94 (0.78–1.13)	0.92 (0.75–1.13)	0.85 (0.57–1.27)
	Mets	1.02 (0.85–1.23)	1.04 (0.87–1.25)	1.06 (0.81–1.39)
Former smoker	BMI	0.99 (0.82–1.19)	0.97 (0.79–1.19)	0.99 (0.77–1.21)
	MidBP	1.02 (0.86–1.21)	1.05 (0.88–1.27)	1.10 (0.78–1.57)
	Glucose	1.12 (0.96–1.31)	1.14 (0.97–1.34)	1.59 (0.90–2.81)
	Cholesterol	0.89 (0.74–1.08)	0.84 (0.68–1.03)	0.76 (0.55–1.05)
	Triglycerides	1.02 (0.85–1.22)	1.04 (0.85–1.28)	1.08 (0.73–1.62)
	MetS	1.02 (0.84–1.24)	1.03 (0.85–1.25)	1.04 (0.79–1.39)
Current smoker	BMI	1.01 (0.89–1.13)	0.95 (0.83–1.09)	0.94 (0.81–1.10)
	MidBP	1.14 (1.02–1.28)	1.16 (1.03–1.31)	1.32 (1.06–1.67)
	Glucose	1.05 (0.94–1.81)	1.02 (0.91–1.16)	1.07 (0.72–1.23)
	Cholesterol	0.91 (0.80–1.03)	0.87 (0.76–0.99)	0.81 (0.65–0.98)
	Triglycerides	1.08 (0.95–1.21)	1.10 (0.96–1.27)	0.66 (0.92–1.59)
	MetS	1.06 (0.94–1.21)	1.07 (0.94–1.21)	1.11 (0.91–1.33)

RR, relative risk; MetS, metabolic syndrome; BMI, body mass index; Mid BP, mid blood pressure
[1] Relative risk estimate with attained age as time scale and stratified within the model for cohort, sex and categories of birth year.

Women (*n*=315)			
z-score, crude[1]	z- score, adjusted[2]	z-score. calibrated[3]	Interaction[5] *p*-value
1.07 (0.96–1.20)	1.04 (0.92–1.17)	0.92 (0.79–1.07)	0.45
1.19 (1.07–1.32)	1.22 (1.09–1.36)	1.34 (1.08–1.66)	0.06
1.23 (1.14–1.34)	1.20 (1.10–1.32)	1.98 (1.41–2.76)	0.02
1.10 (0.99–1.23)	1.09 (0.96–1.22)	1.16 (0.96–1.41)	0.08
1.16 (1.04–1.29)	1.00 (0.88–1.22)	0.91 (0.69–1.96)	0.22
1.32 (1.18–1.47)	1.36 (1.22–1.53)	1.58 (1.34–1.87)	0.18

[2]Adjusted for age at baseline, smoking status and for the z-score of analyzed factors i.e. BMI, mid BP, glucose, cholesterol and triglycerides. The MetS adjusted for age at baseline and smoking status
[3] Regression calibration adjusted as for z-score adjusted
[4]Z score for MetS is adj. for age at baseline and smoking status.
[5] P-value for interaction between sex and exposure. Adjusted as in z score adjusted[4].

	Women			
Interaction[4] *p*-value	z-score, crude[1]	z-score, adjusted[2]	z-score, calibrated[3]	Interaction[4] *p*-value
	1.12 (0.95–1.30)	1.01 (0.85–1.20)	1.01 (083–1.23)	
	1.35 (1.17–1.56)	1.35 (1.15–1.57)	1.72 (1.29–2.25)	
	1.21 (1.07–1.35)	1.15 (1.00–1.31)	1.67 (1.00–2.71)	
	1.06 (0.90–1.24)	1.04 (0.88–1.24)	1.06 (0.82–1.39)	
	1.13 (0.96–1.33)	1.04 (0.86–1.24)	1.08 (0.74–1.53)	
	1.34 (1.41–1.57)	1.39 (1.18–1.63)	1.61 (1.27–2.03)	
0.37	1.42 (1.11–1.81)	1.30 (0.99–1.72)	1.34 (0.99–1.83)	0.13
0.91	1.22 (0.91–1.63)	1.06 (0.77–1.46)	1.11 (0.62–1.98)	0.43
0.21	1.31 (1.07–1.60)	1.22 (0.99–1.52)	2.08 (0.96–4.69)	0.03
0.05	1.12 (0.83–1.51)	1.03 (0.75–1.43)	1.05 (0.65–1.72)	0.67
0.84	1.42 (1.06–1.90)	1.18 (0.84–1.65)	1.39 (0.71–2.70)	0.17
0.83	1.59 (1.21–2.10)	1.64 (1.25–2.15)	2.04 (1.38–3.02)	<0.01
0.64	0.93 (0.76–1.14)	0.91 (0.73–1.34)	0.90 (0.70–1.39)	0.15
0.01	1.06 (0.88–1.28)	1.11 (0.91–1.35)	1.21 (0.84–1.72)	0.37
0.45	1.26 (1.10–1.46)	1.29 (1.12–1.49)	2.55 (1.52–4.36)	<0.01
0.22	1.11 (0.93–1.33)	1.18 (0.98–1.43)	1.29 (0.97–1.72)	0.01
0.05	1.00 (0.83–1.21)	0.89 (0.72–1.10)	0.79 (0.52–1.21)	0.70
0.16	1.20 (0.99–1.45)	1.23 (1.01–1.49)	1.35 (1.01–1.78)	<0.01

[2] Adjusted for age at baseline and all exposures BMI, mid BP, glucose, cholesterol and triglycerides. Except MetS which are adjusted for age at baseline
[3] Regression calibrated z-score adjusted as for z-score adjusted.
[4] P-value for interaction between smoking status and exposure. Adjusted as for z score adjusted[3].

Discussion

In this large prospective cohort study of almost 600.000 individuals, with 862 incident cases of pancreatic cancer, a statistically significant association between mid-blood pressure, glucose and the MetS respectively and pancreatic cancer, were found among women, with the strongest association for glucose. In men, there was an indication of a positive association between mid-blood pressure and glucose and risk of pancreatic cancer. Risk estimates obtained after correction for measurement error made the associations somewhat stronger, indicating an underestimation of the true associations.

Why the MetS should be a more important risk factor in women than in men is not clear. The calculation of absolute risks in this paper, indicated a protective effect in women for the low quintiles, but this difference disappeared at high exposure levels. Incidence rates of pancreatic cancer are known to be higher in men than in women, which were confirmed in this paper. Later in life, incidence rates become nearly equivalent [21]. There is at present no support in the literature that women with the MetS or its individual components are more susceptible to developing pancreatic cancer. Estrogens and/or androgens have tumour promoting effects in relation to other cancer forms. Whether or not sex hormones affect the development of pancreatic cancer or if these hormones could modify other risk factors and thereby explain different risk factor profiles in men and women are unclear.

There is only one study on the putative association between the MetS and pancreatic cancer. Russo et al used subjects who simultaneously were prescribed with antihypertensive, lipid lowering and anti-diabetic drugs in a small study of 43 individuals and found a positive association between the MetS and the risk of pancreatic cancer, but only in men [22]}. This was not confirmed by the present study, which indicated an association between mid-blood pressure and glucose levels and risk of pancreatic cancer, whereas the analysis of the MetS z-score did not reveal any significant association. Epidemiological data supports a relationship between obesity and pancreatic cancer [23, 24] and between high glucose levels and pancreatic cancer [25–27], but most studies have reported null association between cholesterol / hypertension and the risk of pancreatic cancer [28, 29]. The results in the present study are in accordance with these findings, except that there was no positive association between BMI and pancreatic cancer in men. In women, a positive association was only seen in the highest quintile vs. the lowest. It is, however, possible, as suggested by Li et al [23], that obesity at a younger age has a more profound effect on risk of pancreatic cancer, than has obesity at an older age.

Smoking is a well-known risk factor for pancreatic cancer and most studies have found a two-fold risk increase [30]. In the present study the risk of pancreatic cancer were analysed in strata of smoking habits, but no consistent pattern were found. It is possible it is due to chance, but in studies on breast and endometrial cancer it has been shown that the risk of cancer are increased in former smokers [31]. To what extent smoking modify the association between metabolic effects and the risk of pancreatic cancer remains to be elucidated.

The main strengths of this study are the large sample size from seven population-based cohorts in Europe and the possibility to perform record linkage with national cancer registries. The validity of these registries has been evaluated previously, and it can be expected that the correctness of the pancreatic cancer diagnosis is almost perfect, although completeness may be somewhat lower [32–34]. However, it is unlikely that misclassification of some pancreatic cancer cases as healthy subjects would have affected the estimates to any great extent. Other major strengths were the repeated health examinations, which allowed us to adjust risk estimates for intra-individual

variation of the analysed exposures and thereby decrease the risk of a misclassification bias related to the measured exposure, a potential regression dilution bias.

All cohorts had data available on BMI and smoking status, which allowed for adjustment for these potential risk factors. A limitation is that there were no data on covariates such as genetic risk factors, alcohol consumption and physical activity. As far as it is known there is no known association between genetic factors associated with pancreatic cancer and metabolic factors. Hence, confounding by such factors ought to have been a minor problem. Alcohol consumption and physical activity have both been related to pancreatic cancer [2, 35]. Alcohol is thought to exert its carcinogenic effect via reactive oxygen production [36] i.e. it acts on the same pathway as the components of the MetS. If this is true, it would have been problematic to include alcohol in the multivariate analysis. The same might be applicable to physical activity. Indeed, Michaud *et al.* [35] have shown that physical activity is inversely related to pancreatic cancer in obese, but not in subjects with a BMI < 25, and it has been shown that physical activity can lower plasma glucose levels [37].

The attendance rate in the various cohorts ranged from 56– 90% ([10], it may therefore be difficult to apply the results in this study to the general population. However, we consider that the internal comparisons and calculations of relative risks are less sensitive to a potential selection bias. Another concern is the different geographical differences between the cohorts and differences in methods of measurement of investigated exposures. To overcome these problems, quintile classification and the z-score were stratified for the individual cohorts. Calculations were furthermore repeated without cohort stratification in the model and did not reveal any material changes in the risk estimates.

Pancreatic cancer is a highly aggressive tumour and most patients who are diagnosed with pancreatic cancer die within a year and the 5-year survival rate is less than 4% [1]. In this study, the majority of cases (83%) had a follow-up after baseline measurement of more than 5 years and exclusion were made for cases diagnosed within one year of health check-up. Poor survival indicative of a rapidly progressive disease, compatible with a short sub-clinical phase, makes the findings in this paper less likely to be due to reverse causality.

Several comparisons were made and the risk of a Type I error has to be considered. The results show a clear pattern when different statistical models are used. This, together with the fact, that significant findings are in line with the à priori hypothesis, supports the view that the results were not simply due to chance. The exception was cholesterol among men which was negatively associated with the risk of pancreatic cancer. This finding will have to be interpreted with caution, considering the exclusion of cases with a follow-up of less than 1 year. Confidence intervals were generally narrow, which indicates that statistical power was good.

The question is how the MetS might promote the development of cancer. One theory is that insulin resistance hold the potential to explain most of the factors associated with the MetS [7]. This is thought to be the main mechanism between obesity and pancreatic cancer, i.e. obesity promotes insulin resistance, which in turn promotes the development of hyperinsulinemia. A hyperinsulinemic state can trigger mitotic activity [27, 38] and in vitro studies have showed that hyperinsulinemia can stimulate cell proliferation in the pancreas [39]. Besides, adipocytes acts, not only as storage sites for triglycerides, they also synthesise and secrete hormones and cytokines, the latter with the propensity for inflammation, which has been suggested to affect the risk of pancreatic cancer [40]. Hyperglycaemia induces elevation of insulin and insulin-like growth factor-I (IGF-1) [41] and glucose may itself have a direct tumour promoting effect. Glucose is

used as an energy substrate in tumour cells, particularly in fast-growing, highly proliferative tumour cells [42]. Excess glucose promotes the formation of reactive oxygen species, which can damage DNA in genes that are important in cell proliferation or cell survival, which in turn can trigger cancer progression [43]. Reactive oxygen stress may also explain the effect of elevated triglycerides and increased oxidative stress in fat has been demonstrated to be an important pathogenic mechanism in the MetS [44]. How cholesterol and hypertension may be linked to cancer development remains unclear, although hypertension has been suggested to increase cancer risk by blocking and subsequently modifying apoptosis and thereby affecting cell turnover [45].

Conclusion

There was a statistically positively significant association between single metabolic factors, as well as for the MetS and pancreatic cancer in women. In men there was a positive association between mid-blood pressure and pancreatic cancer, and an indication of an association between high glucose levels and the risk of pancreatic cancer.

Acknowledgements

Financial support was received by the World Cancer Research Fund (Grant 2007/09),

The Swedish Research Council, The Ernhold Lundström Foundation, The Einar and Inga Nilsson Foundation, The Malmö University Hospital Cancer Research Fund, The Malmö University Hospital Funds and Donations, The Crafoord Foundation and The Mossfelt Foundation.

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