Exocrine pancreas cancer

The European Pancreatic Cancer-Research Cooperative (EPC-RC)

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1.2 Pancreatitis and exogenous risk factors for pancreatic cancer

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Introduction

Pancreatic cancer is a highly lethal disease, with an estimated 29,200 new cases a year in the United States and more than 30,000 in Europe. Its incidence virtually equals its mortality (1,2). The overall five-year survival is about 0.4%, making pancreatic cancer the fourth leading cause of cancer-related death in Western countries (3, 4). One of the reasons for the poor prognosis of pancreatic adenocarcinoma is its tendency to form micrometastases before clinical symptoms arise and before the tumor is detectable by diagnostic imaging techniques. The mechanisms that determine the highly malignant growth and dissemination pattern of pancreatic cancer are poorly understood. Much of the grim prognosis of pancreatic cancer can be attributed to our ignorance of clear risk factors, of premalignant states, and of its tumor biology. The fact that most pancreatic neoplasm are diagnosed at an incurable stage of the disease highlights the need to determine risk factors and to understand their contribution to carcinogenesis.

Environmental risk factors of pancreatic cancer

Smoking is the strongest exogenous risk factor known to be associated with pancreatic cancer. Carcinogens derived from tobacco smoke probably reach the pancreas via the blood stream after being absorbed from the lungs or the upper aero-digestive tract. Nearly all published reports show that exposure to tobacco products increases the risk of pancreatic cancer 2-fold compared to non-smokers. In 1994 Doll et al. reported in the UK the annual male mortality rates for pancreatic cancer in non-smokers, ex-smokers and current smokers with 16, 23 and 35 per 100 000 man years respectively (5). In a Japanese population two cohort studies were able to show a linear dose response curve for tobacco use and pancreatic cancer (6,7). The question what proportion of pancreatic cancer is attributable to smoking habits is of general interest to physicians. The risk of pancreatic cancer in smokers can be estimated by the following formula: attributable risk = P (RR-1)/[(P(RR-1)+1], in which P is the proportion of the population who smoke, and RR the relative risk of pancreatic cancer in smokers compared to non smokers. If P, the prevalence of smoking, is app. 30%-35%, and RR=2, then the estimated attributable risk is 25% (8).

Different dietary regimes have long been suspected to be associated with an increased risk of cancer of the gastrointestinal tract. A large proportion of adults have a daily intake of vitamin pills or dietary supplements some in an attempt to fend off cancer. Nevertheless so far the only substances which were investigated for a potential reduction in pancreatic cancer are the antioxidants alpha-tocopherol and beta-carotene. They have been evaluated in a prospective study in male smokers and did not reduce the frequency of pancreatic cancer over an eight year follow up period (9). Of the various dietary components that have been studied in relation to pancreatic cancer, a high fat content of the diet seems to be the component that has most consistently been found to be associated with pancreatic cancer (10).

Diabetes afflicts approximately 5% of the adult population, but whether this common disorder of the endocrine pancreas is associated with an increased risk of pancreatic cancer is still a matter of debate since diabetes can be one of the early manifestations of the disease. A meta-analysis published in 1995 suggested that diabetics have an about two fold increased risk of pancreatic cancer which could be expressed as the same attributable risk as smoking (11). Subsequent studies disputed this finding.

Alcohol is a major risk factor for pancreatitis which could suggest that it also contributes to pancreatic cancer. Nearly all studies so far have failed to support this notion, including a recent large retrospective cohort study from Sweden (12).

Recent interest has focused on possible genetic links with pancreatic cancers (13). While a number of familial syndromes is associated with pancreatic cancer only a minority of patients with pancreatic cancer have a strong family history of the disease (<4%). Several germline mutations associated with pancreatic cancer have been identified so far (14) and their relevance is reviewed in the chapter by S. Hahn in this volume.

Pancreatic cancer and different varieties of chronic pancreatitis

The association between chronic inflammation and the development of malignancies has been recognized for many years. As early as the year 1863 the German pathologist Rudolf Virchow noted leukoytes in neoplastic tissues and made a connection between inflammation and cancer (15). Nowadays a clear association can be drawn between chronic inflammatory diseases of the gastrointestinal tract like Crohn's disease or ulcerative colitis and an increased cancer risk. For pancreatic cancer this association was only recently confirmed and a consensus conference agreed upon a new classification for pancreatic intraepithelial neoplasia as noninvasive precursor lesions (16). It is therefore not surprising that the one consistent risk factor for pancreatic cancer is chronic pancreatitis. In this chapter we will outline the links between chronic pancreatitis and pancreatic cancer.

Chronic pancreatitis is defined as recurrent bouts of a sterile inflammatory disease characterized by often progressive and irreversible morphological changes, typically causing pain and permanent impairment of pancreatic function. Chronic pancreatitis is histologically closely connected to the transformation of focal necrosis into perilobular and intralobular fibrosis of the parenchyma, pancreatic duct obstruction by pancreatic stones and the development of pseudocysts. In the course of the disease progressive loss of endocrine and exocrine function is common (17, 18). With an incidence of 8.2, a prevalence of 27.4 per 100 000 population and a 0.04% to 5% frequency in unselected autopsy specimens chronic pancreatitis represents a frequent disorder of the gastrointestinal tract. Chronic pancreatitis accounts for a substantial morbidity and health care costs. Annual treatment costs per patient are estimated to approach 17.000 $ and approximately 20.000 Americans are admitted to hospital with the leading diagnosis of chronic pancreatitis. About three times as many are discharged with the diagnosis chronic pancreatitis (19). The 10 year survival rate of patients suffering from alcohol induced chronic pancreatitis is 70%, while 20 year survival rate is only 45%. Mortality is 3.6 fold increased compared to a cohort without chronic pancreatitis (20). Various etiologies are responsible for the development of chronic pancreatitis. In Western countries alcohol consumption is clearly the leading cause (70-90%) of chronic pancreatitis (21). The second most common form of chronic pancreatitis, as of today, is so called idiopathic pancreatitis (25%) (22-23). Patients without identifiable risk factors for chronic pancreatitis are collectively referred to as having idiopathic pancreatitis. This group is constantly decreasing in proportion since Comfort and Steinberg reported in 1952 an inherited form of chronic pancreatitis that follows an autosomal dominant inheritance pattern (24-25). Hereditary pancreatitis represents a genetic disorder closely associated with mutations in the trypsinogen gene and presents with a phenotypic disease penetrance of around 80% for the most common mutations. Shortly after the identification of the first mutations in the trypsinogen gene in association with chronic pancreatitis by Whitcomb et al. another important genetic alteration was reported by Witt et al. (26). This group showed that mutations in the SPINK-1 gene (encoding the pancreatic secretory trypsin inhibitor, PSTI) are associated with idiopathic chronic pancreatitis in children.

Cystic fibrosis is an autosomal-recessive disorder with an estimated incidence of 1:2500 characterized by chronic pancreatic and chronic pulmonary disease. The involvement of the pancreas varies from a complete loss of exocrine and endocrine function to nearly unaffected pancreatic function. Recurrent episodes of pancreatitis can be detected in 1-2% of patients with cystic fibrosis who have normal exocrine function and are rarely seen in patients with exocrine insufficiency (27-28).

Considerable attention, especially in Japan, is nowadays paid to a recently characterized type of steroid responsive chronic pancreatitis, termed autoimmune pancreatitis. This type of chronic pancreatitis mainly presents with a diffuse enlargement of the pancreas, elevated serum lipase levels and, in 70-80% of patients, with obstructive jaundice

(29). Characteristic serum antibodies and a diffuse narrowing of the pancreatic ducts help in establishing the diagnosis.

Several metabolic disorders which are closely linked to hypertriglyceridemia above 1000 mg/dl can be responsible for the development of recurrent bouts of pancreatitis (30). In rare cases chronic calcifying pancreatitis has been reported due to hypercalcemia in long-standing untreated hyperparathyroidism. The latter appears to be exceedingly rare today because serum calcium levels are routinely checked and part of most automated clinical chemistry panels. The pathophysiology of hypercalcemia-induced pancreatitis is still unclear but may be related to an increase in either intracellular calcium concentrations or an excess of calcium in pancreatic juice which could cause precipitation of calcium carbonate in the ducts.

Chronic pancreatitis and the risk of pancreatic cancer

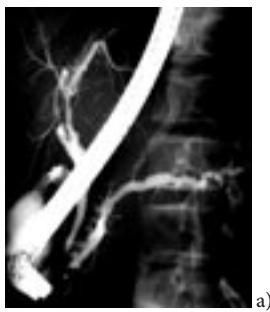
The question of whether or not chronic pancreatitis is a cause of pancreatic cancer arises from the observation that pancreatic cancer itself causes a desmoplastic extracellular matrix reaction that resembles chronic pancreatitis (31). Already in 1913 John B Deaver noted that “It may not be out of place to record my belief that carcinoma of the pancreas is in many instances brought into existence by previous pancreatitis. This is in line with the known fact that chronic irritation predisposes to cancer as is seen in chimney sweep cancer, pipe cancer of the lip, ulcus carcinomatosum of the stomach, cancer of the gallbladder with gallstones and many other forms of the disease elsewhere” (32). To establish an association between chronic alcoholic pancreatitis and an increased risk of pancreatic cancer was especially difficult because many patients survive 20-30 years of chronic pancreatitis and the main portion dies from various complications associated with chronic alcohol abuse such as violent accidents. Therefore early studies on pancreatic cancer were unable to prove chronic pancreatitis as a significant risk factor (33, 34). This was finally achieved in an international cooperative investigation which was conducted by AB Lowenfels and coworkers as a multicenter historical cohort study of 2015 patients with chronic pancreatitis recruited from clinical centers in 6 countries in 1993. This study found a cumulative risk of pancreatic cancer in patients with chronic pancreatitis of 1.8% after 10 years and of 4% after 20 years with a standardized incidence ratio of 14.4. For patients with a minimum of two years follow up the risk of pancreatic cancer was 16.5 fold higher than that of the general population. The risk seemed to be independent of sex, country and the etiology of pancreatitis (35).

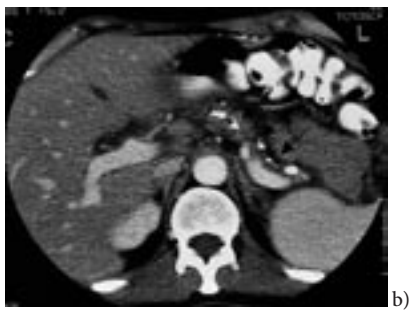
Hereditary pancreatitis as a risk factor for pancreatic cancer

The search for an association between chronic pancreatitis and pancreatic cancer intensified when in 1996 a single point mutation in the third exon of the cationic trypsinogen gene on chromosome 7 (7q35) was found to be associated with hereditary pancreatitis and multiple kindreds were subsequently identified and reported. The G-to-A transition results in an arginine-(R)-(CGC)-to histidine-(H)-(CAC) substitution, numbered R122H, and was predicted to eliminate a fail-safe trypsin hydrolysis site that is necessary to initiate trypsin's self destruction. Since the initial report several other mutations (16 until today) in the trypsinogen gene have been reported, but the R122H mutation is still the most common (36-39). During the last years several attempts have been made to elucidate the role of trypsinogen in the onset of chronic and acute pancreatitis but the question why structural changes in the cationic trypsinogen gene lead to the onset of hereditary pancreatitis has remained a matter of debate. Since pancreatitis has long been regarded as a disease that is caused by proteolytic autodigestion of the organ (40) and because trypsin is known to be a potent activator of other pancreatic zymogens in the gut (41) it has been suggested that the trypsinogen mutations that were found in association with hereditary pancreatitis confer a gain of enzymatic function (24, 42). *In vitro* studies have analyzed the biochemistry of recombinant human trypsinogens, into which pancreatitis-associated mutations were introduced and found that – under defined experimental conditions – either a facilitated trypsinogen autoactivation or an extended trypsin activity can result (43-46). Whether these experimental conditions reflect the highly compartmentalized situation under which protease activation begins intracellularly and *in vivo* (47-48) is presently unknown but the above studies would strongly suggest that either a more effective autoactivation of trypsinogen or an impaired inactivation of trypsin (by degradation or autolysis) would be involved in the onset of hereditary pancreatitis. A number of arguments, however, have been raised against the gain of trypsin function hypothesis of hereditary pancreatitis. Statistically, most hereditary disorders are associated with loss of function mutations that render a specific protein either defective or impair its intracellular processing and targeting (49). Moreover, at least five mutations, A16V (50). D22G (43), K23R (51), N29I (52), R122H (24) that have been found in association with hereditary pancreatitis, are located in different regions of the PRSS1 gene, and would thus be expected to have different structural effects on the trypsinogen molecule. It would therefore be easier to explain their common pathophysiology in terms of a loss of enzymatic function rather than through a gain of enzymatic function. Especially one of these mutations (A16V) also affects the signal peptide cleavage site that is assumed to be involved in the correct processing of trypsinogen (50). Experiments in isolated pancreatic acini and lobules which studied the *in vivo* mechanisms of intracellular zymogen activation have shown that trypsin activity is neither required nor involved in the activation of other digestive proteases and that its most prominent role is in autodegradation (53). This,

in turn, would suggest that intracellular trypsin activity has a role in the defense against other, potentially more harmful, digestive proteases and that structural alterations that impair the function of trypsin would eliminate a protective mechanism rather than generate a triggering event for pancreatitis. Whether these experimental observations obtained on rodent pancreatic acini and lobules have any relevance to human hereditary pancreatitis is presently unknown and cannot be readily assumed without further evidence because human cationic trypsinogen has distinct characteristics in terms of its ability to autoactivate and to autodegrade. A recently reported kindred with hereditary pancreatitis which carries a R122C mutation is very interesting in this context. The single nucleotide exchange in this family is only one position upstream of the one found in the most common variety of hereditary pancreatitis and leads to an amino acid exchange at the same codon (R122C versus R122H). When equal amounts of recombinant protein are used for biochemical studies the enterokinase-induced activation and the autoactivation of Cys-122 trypsinogen are found to be significantly reduced by 60-70% compared to the wild-type enzyme. A possible interpretation of these results would be that Cys-122 trypsinogen misfolds or forms mismatched disulfide bridges under intracellular *in vivo* conditions and therefore confers a dramatic loss of trypsin function that cannot be compensated for by facilitated autoactivation. If this scenario should reflect the *in vivo* conditions within the pancreas it would represent the first direct evidence from a human study for a potential protective role of trypsin activity in pancreatitis. Short of direct access to living human acini from carriers of PRSS1 mutations or a transgenic animal model into which the human PRSS1 mutations have been introduced the question of whether the gain of function hypothesis or the loss of function hypothesis correctly predicts the pathophysiology of hereditary pancreatitis can presently not be resolved. The studies on rodent pancreatic acini and lobules, however, would infer that the role of trypsin in the onset of acute or chronic pancreatitis might be rather different than previously assumed.

Figure 1:


a)


b)

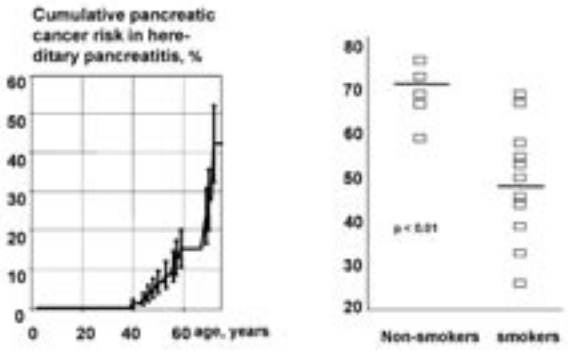
Hereditary pancreatitis is clinically indistinguishable from other forms and varities of pancreatitis.:
a) 14 year old girl with chronic pancreatitis and R122H-mutation
b) 48 year old women with chronic pancreatitis and R122H-mutation

Only very recently the EUROPAC study group presented their work on clinical and genetic characteristics in hereditary pancreatitis. In a multilevel proportional hazard model employing data obtained from the European Registry of Hereditary Pancreatitis this group presented 112 families in 14 countries (418 affected individuals) (54): 58 (52%) families carried the R122H, 24 (21%) the N29I, and 5 (4%) the A16V mutation, 2 had rare mutations, and 21 (19%) had no known PRSS1 mutation. The median (95% confidence interval) time to the start of symptoms for R122H was 10 (8 to 12) years of age, 14 (11 to 18) years for N29I, and 14.5 (10 to 21) years for mutation negative patients (*P* = 0.032). The cumulative risk (95% CI) at 50 years of age for exocrine failure was 37.2% (28.5% - 45.8%), 47.6% (37.1% - 58.1%) for endocrine failure, and 17.5%, (12.2% - 22.7%) for pancreatic resection for pain. Time to resection was significantly reduced for females (P <0.001) and those with the N29I mutation (P = 0.014). Pancreatic cancer was diagnosed in 26 (6%) of all 418 affected patients. Fifteen patients had histological confirmed pancreatic ductal adenocarcinoma. The cumulative risk (95% CI) of pancreatic cancer was 44.0% (8.0% - 80.0%) at 70 years from symptom onset with a standardized incidence ratio of 67% (50% - 82%). Time to cancer did not significantly differ between men and women and the time to the diagnosis of cancer was not significantly influenced by mutation status. This study showed that the risk of pancreatic cancer is negligible up to the age of around 50 years, but thereafter increases markedly in both sexes. A previous study had also shown an estimated lifetime risk of pancreatic cancer of 40% (55). Pancreatic calcification and diabetes were found more frequently in patients who developed pancreatic cancer, compared with age and sex-matched individuals without cancer, suggesting that the risk of pancreatic cancer was directly related to the severity and duration of the inflammatory process. In the 26 patients with pancreatic cancer the study reported a median age of 57 years at diagnosis of cancer in smokers and of 71 years in nonsmokers. In 2001 Lowenfels and coworkers had also shown that in 497 patients with hereditary pancreatitis pancreatic cancer occurred 2 decades earlier in smokers than in non-smokers (56). On the other hand, Hengstler et al. did not find an increased incidence of trypsinogen mutations in patients with sporadic pancreatic adenocarcinoma (57).

Recent genetic studies also revealed an associaton between hereditary or idiopathic pancreatitis and mutations in the serine protease inhibitor Kazal type 1 gene (SPINK1, also known as the pancreatic secretory trypsin inhibitor, PSTI) (26). SPINK-1 mutations are commonly detected in patients who don't present with a family history of pancreatitis and also have no classical risk factors for chronic pancreatitis (58, 59). SPINK-1 is believed to form the first line of defense by inhibiting prematurely activated trypsinogen in the pancreas. The discovery of SPINK-1 mutations therefore provided additional evidence for a role of active trypsin in the development of pancreatitis. Furthermore, tropical pancreatitis, a common form of pancreatitis in Africa and Asia characterized by abdominal pain, intraductal pancreatic calculi and diabetes mellitus in young nonalcoholic subjects, is associated with a high frequency of N34S mutations in the SPINK1

gene (60). In tropical pancreatitis Augustine and Ramesh reported 22 pancreatic cancer cases among 266 patients with tropical pancreatitis over an eight year observation period (8.3%). In this cohort the risk reached its climax after the age of 40 and patients with tropical pancreatitis often displayed features of PanIns (Intraductal Neoplasias) as well as cancer in pancreatic resection specimens (61).

Figure 2:



Left panel: Time from onset of symptoms to pancreatic cancer in hereditary pancreatitis: The figure shows a significant increase in pancreatic adenocarcinoma after the fourth decade of life [54].
Right panel: smoking is an independent risk factor for the development of pancreatic cancer in patients with hereditary pancreatitis [56].

Chari et al. reported that over a 4.5 year period 6 out of 185 patients with tropical pancreatitis died of pancreatic cancer (62). As both studies were conducted before the year 2000 they did not take into account the incidence of SPINK1 mutations. So far only one family was reported with an association between pancreatic cancer and a homozygous N34S mutation as well as symptoms of chronic calcifying pancreatitis (63).

Mutations in the CFTR gene convey is another form of chronic pancreatitis with an underlying genetic cause and early onset of the disease. Cystic fibrosis is an utosomal-recessive disorder with an estimated incidence of 1:2500, characterized by pancreatic exocrine insufficiency and chronic pulmonary disease. The extent of pancreatic involvement varies between a complete loss of exocrine and endocrine function and a nearly unaffected pancreatic function. Recurrent episodes of pancreatitis can be detected in 1-2% of all patients with cystic fibrosis and normal exocrine function and is seen only rarely in patients with exocrine insufficiency. Compared to an unaffected population patients who suffer from idiopathic pancreatitis carry in 17-26 % mutations in the cystic fibrosis conductance regulator (CFTR) gene. Chronic pancreatitis thus represents a third disease entity associated with mutations in the CFTR gene besides cystic fibrosis and infertility due to vas deferens aplasia. It is important to note that pancreatic exocrine insufficiency in patients with cystic fibrosis is a completely different disease entity and not identical to chronic pancreatitis in the presence of CFTR-mutations (27, 28). Several groups have

evaluated the risk of cancer in adults suffering from cystic fibrosis. In 1993 Sheldon et al. reported a cohort of 412 subjects with cystic fibrosis and detected two cases of pancreatic cancer [0.008 expected, p = 0.001, odds ratio 61] (64). The increased incidence of digestive tract cancer, but not cancer in general was confirmed by Neglia et al. among 28,511 cystic fibrosis patients in the United States and Canada (risk ratio 6.5) and Europe (risk ratio 6.4). Only two cases of pancreatic cancer were reported in this group but this exceeded the calculated incidence in the third decade of life highly significantly, resulting in an odds ratio of 31.5 compared to an age matched control cohort (65).

Summary:

The association between long-standing chronic pancreatitis and adenocarcinoma of the pancreas has been clearly established. Pancreatic cancer can develop in all known etiologies of pancreatitis but appears to require 30 to 40 years of inflammation before a statistically significant percentage of patients with chronic pancreatitis develop a malignancy. The only independent risk factor besides a long lasting inflammation that has so far been identified is tobacco use. Therefore all patients with chronic pancreatitis should be advised to refrain from or cease smoking. The second goal to prevent pancreatic cancer is to reduce the extent of pancreatic inflammation. Joan Braganza and her group reported on the toxic effect of oxygen-derived free radicals on the pancreas as a possible pathomechanism for the development of chronic pancreatitis. Oxidative stress caused by agents like nicotine or ethanol can lead to the peroxidation of the lipid bilayer in the cell membrane which consecutively damages the membrane. An excess of oxygen free radicals may overwhelm the protective antioxidant mechanism as shown for some cytochrome –P450 dependent pathways in the liver. This hypothesis initiated a couple of clinical studies which employed antioxidants for the treatment of chronic pancreatitis and which have shown some promising initial results [66-68]. A large European multicenter study (EUROPAC -2) making use of the concept of antioxidant treatment for idiopathic chronic pancreatitis and hereditary pancreatitis will be launched soon. As the risk of pancreatic cancer increases exponentially with the duration of pancreatitis it may be essential to diagnose this lethal disease at a stage before clinical symptoms arise and when surgery may still improve the presently poor prognosis of pancreatic cancer. Which screening strategy might be most effective for the early detection of pancreatic cancer – particularly in the context of chronic pancreatitis – is presently unknown.

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