Pancreatic cancer after remote peptic ulcer surgery


Background: Peptic ulcer surgery may carry an increased risk for pancreatic cancer development. Molecular analysis of K-ras codon 12, frequently mutated in conventional pancreatic cancers, might provide insight into the aetiological mechanisms.

Methods: The relative risk of pancreatic cancer was computed by multivariate and person-year analysis in a cohort of 2633 patients who had undergone gastrectomy. Lung cancer risk was analysed as an indirect means of assessing smoking behaviour. K-ras codon 12 mutational analysis was performed on 15 postgastrectomy pancreatic cancers.

Results: There was an overall increased risk of pancreatic carcinoma of 1.8 (95% confidence interval, 1.3 to 2.6) five to 59 years postoperatively, which gradually increased to 3.6 at 35 years or more after surgery (χ² test for trend, p < 0.05). Multivariate analysis indicated that parameters other than postoperative interval did not influence the risk. Lung cancer risk was significantly increased after surgery, but no time trend was observed. The spectrum and prevalence of K-ras codon 12 mutations were comparable to conventional pancreatic cancer.

Conclusions: Remote partial gastrectomy is associated with an increased risk of pancreatic cancer. Postgastrectomy and non-postgastrectomy pancreatic cancers may share similar aetiological factors, such as smoking. However, the neoplastic process in patients who have undergone gastrectomy appears to be accelerated by factors related to the surgery itself.

Pancreatic cancer is the fifth leading cause of cancer related death. This is mainly the result of its extremely poor prognosis—most cases are detected late in the course of the disease when the neoplasm has spread and curable resection is no longer possible. 1-3 The identification of patients with an increased risk of pancreatic cancer may lead to a higher index of suspicion and to early detection when curative resection is still possible.

Patients with a remote partial gastrectomy for benign ulcer disease may constitute a high risk group for pancreatic cancer, 4-7 although reports are conflicting. 8-11 The mechanism by which partial gastrectomy may contribute to the development of pancreatic cancer is unclear, but an increased production of N-nitroso compounds and nitrosamines in the operated stomach is thought to play a role. 12-14 These carcinogens may not only act locally, but also at sites distant from the stomach, and N-nitroso compounds and nitrosamines have been shown to produce adenocarcinomas of the pancreatic duct in animal models. 15-17 In addition, increased concentrations of cholecystokinin after partial gastrectomy may also mediate pancreatic carcinogenesis. 18-20 Finally, smoking is a major risk factor for both the development of pancreatic adenocarcinoma and for peptic ulcer disease. 21-24 and most patients with peptic ulcers smoke and continue to do so after surgery. 25-26

"The identification of patients with an increased risk of pancreatic cancer may lead to a higher index of suspicion and to early detection when curative resection is still possible"

Activating point mutations in codon 12 of the K-ras oncogene are among the most frequent genetic alterations in pancreatic carcinoma. 27-32 Interestingly, K-ras codon 12 mutations appear to be more common in pancreatic carcinomas from smokers than in pancreatic carcinomas from patients who never smoked. 33 Tobacco specific nitrosamines induce carcinomas of the pancreas in rat models and generate G to A transitions at the second G of a GG pair of the ras oncogene, which is the predominant type of mutation found in pancreatic cancers in humans. 34-36 Thus, the analysis of the prevalence and type of K-ras codon 12 mutation in pancreatic carcinoma occurring after remote peptic ulcer surgery may provide aetiological clues.

We have been following a cohort of 2633 patients who underwent gastrectomy between 1931 and 1960, and in a preliminary analysis we found an almost twofold increased risk for pancreatic cancer in these patients 20 years or more after their surgery. 7 Follow up of this cohort is still complete and 82% of the cohort has now died. Therefore, the present analysis was performed to investigate whether the previously suggested increasing trend for pancreatic cancer risk after peptic ulcer surgery could be further evaluated and definitively established. Specifically, we were interested in whether the longest survivors had a particularly high risk for pancreatic cancer. Furthermore, we performed analysis of codon 12 of the K-ras oncogene in 15 postgastrectomy pancreatic carcinomas to compare these molecular genetic alterations with the mutations encountered in conventional pancreatic cancer in non-operated patients.

Materials and methods

Study population

The characteristics of the study population have been described previously. 4 Briefly, the study population consisted of 2633 patients who underwent gastrectomy for benign conditions in the academic medical center of the University of Amsterdam between 1931 and 1960. There were 2300 men
and 333 women; 207 patients had a Billroth I, and 2343 a Billroth II, and in 83 patients the type of gastrectomy was not specified. The indication for surgery was a duodenal ulcer in 1683 patients, a gastric ulcer in 807 patients, and was not specified in 143 patients. Patient data were collected in 1975 by a review of the records of the departments of pathology and surgery. Surgical specimens of all partial gastrectomies were routinely examined by the pathologist. Therefore, this dual department review of records is thought to have yielded a virtually complete data set.

Patients were traced using the Dutch population register system, in which every citizen has a unique registration card. This allows one to determine the underlying cause of death (in International Classification of Diseases codes) as registered on the official death certificate from the Netherlands Central Bureau of Vital Statistics.33

### Statistical analysis

The computation of person–years at risk for pancreatic cancer started on January 1, 1935 because population mortality rates for pancreatic cancer were only reliably available after that date. The observation time was time from initial surgery to date of death, date of emigration, date of loss to follow up, or to December 31, 1995, the closing date of our study. Person–years at risk according to sex and 5 year age categories from 10 years of age onwards were calculated using a Fortran computer program for cohort analysis.33 At 85 years of age, patients were censored from our study because causes of death above this age were considered too inaccurate for comparison.33 Expected deaths were calculated by multiplying the number of person–years for each 5 year age group and sex by the corresponding age, sex, and calendar time specific death rates. These death rates were obtained from the Netherlands Central Bureau of Vital Statistics, the same source that provided the official cause of death for the deceased in the cohort. Pancreatic cancer deaths during the first five postoperative years were excluded because they might have included pancreatic cancer cases missed at the time of surgery. Standardised mortality ratios of observed over expected pancreatic cancer deaths, and 95% confidence intervals (CI) were calculated for each postoperative interval. One person less than 10 years of age and five individuals who died at the time of surgery were excluded. Multivariate analysis of the risk of pancreatic cancer in the cohort according to postoperative interval, sex, diagnosis, and age at the time of surgery, and type of surgery, was performed assuming a Poisson distribution, as described previously.33

Because smoking is related to peptic ulcer (surgery) and pancreatic carcinoma, it is a potential confounding variable. Patient data of the postgastrectomy cohort were collected by review of the reports from the departments of pathology and surgery and information on smoking behaviour was not reliably available from these sources. Therefore, the effect of smoking was indirectly assessed in the study population by computing the observed over expected mortality for lung cancer, as described above for pancreatic carcinoma.

A $\chi^2$ test for trend was performed to estimate the change in relative risk (RR) for lung cancer and pancreatic cancer during the observation time since initial peptic ulcer surgery.33

### Molecular analysis

Fifteen postgastrectomy pancreatic carcinomas were available for molecular analysis. These carcinomas were obtained from the archives of the Academic Medical Center and other hospitals in The Netherlands; some of the cases were obtained from The Johns Hopkins Hospital in Baltimore, USA. All tumours were primary cancers and the diagnosis was histologically confirmed in all cases. Tumour tissue was carefully microdissected from archival 5 $\mu$m thick haematoxylin and eosin stained sections to provide a sample in which at least 50% of the cells comprised the tissue of interest. DNA was extracted as described previously.33
The protocol for the K-ras codon 12 mutational analysis was described earlier.\textsuperscript{11} DNA is subjected to polymerase chain reaction (PCR) amplification using primers centred around codon 12. One of the primers introduces a restriction site in the PCR products derived from wild-type codon 12 alleles, but not in those derived from mutant codon 12 alleles. Digestion of the PCR products is followed by a second round of PCR amplification, which then yields a PCR product enriched for K-ras codon 12 mutations. The resulting DNA products are denatured and dot blotted on to nylon membranes and subjected to allele specific oligonucleotide (ASO) hybridisation with radioactively labelled probes, specific for each possible K-ras codon 12 mutation, followed by autoradiography.

Cell suspensions with mutant to wild-type ratios of 1 : 100 and 1 : 1000 were used as positive controls in every PCR procedure. The suspensions were made from the human colon cancer cell line SW 480 with a homozygous GGT to GTT mutation at codon 12 of K-ras and the human colon cancer cell line HT 29 with wild-type K-ras. Water was used as a control for contamination, and placental DNA for non-specific hybridisation controls; on each membrane cloned DNA fragments with a known codon 12 sequence complementary to the labelled oligonucleotides are used for the hybridisation of that membrane. All PCR products were hybridised with oligonucleotides with the wild-type sequence to control for amplification of the DNA samples. Enriched and non-enriched PCR products were dot blotted next to each other to check for the digestion and the mutant enrichment. We have previously validated the above mutant enriched PCR with ASO hybridisation through comparison with sequence analysis.\textsuperscript{11}

RESULTS

During the eight additional years of follow up since the previous analysis, another 361 patients died, two more patients emigrated (treated as censored data), and four other formerly emigrated patients returned to the Netherlands (included in our present analysis). At the closing date of the study (1 January 1996) 462 patients were still alive. The official cause of death was obtained from all the 361 patients who had died. Eight additional patients died of pancreatic cancer, making 39 deaths from pancreatic cancer in total. Importantly, follow up was complete for all but eight patients, who were excluded from the analysis.

Table 1 lists the detailed results from the person–year analysis. A total of 35 pancreatic cancer deaths were observed five years or more after peptic ulcer surgery. The overall risk was significantly increased for the complete five to 59 year postoperative interval (RR, 1.8; 95% CI, 1.3 to 2.6). The risk of developing pancreatic cancer increased continuously with increasing time since surgery, reaching significance 35 to 59 years postoperatively. The trend test indicated a significantly increasing trend ($\chi^2$, 1; degrees of freedom (df) = 4.80; $p < 0.05$). Data for both sexes were combined because no interactions by sex were noted and the number of women was too small to make a meaningful statement about them separately.

The multivariate analysis indicated that parameters other than postoperative interval, such as sex, type of surgical procedure, and indication for surgery, did not contribute to the risk of pancreatic cancer. The observed over expected mortality for lung cancer was increased in all postoperative intervals and the overall mortality was also significantly increased (table 2). However, the trend test was not significant ($\chi^2$, 1; df = 0.31; $p > 0.25$). Figure 1 shows the pattern of change of the relative risk of lung cancer and pancreatic cancer during the follow up period.

Molecular analysis

Of the 15 postgastrectomy pancreatic carcinomas analysed, 10 showed a mutation in codon 12 of the K-ras oncogene, four

![Figure 1](http://jcp.bmj.com/)

**Figure 1** Bar chart of the relative risks for pancreatic and lung cancer among patients after gastrectomy for different postoperative intervals. The trend test for pancreatic cancer is significant ($\chi^2$, 1; degrees of freedom (df) = 4.80; $p < 0.05$), whereas the trend test for lung cancer is not ($\chi^2$, 1; df, 0.31; $p > 0.25$).

![Figure 2](http://jcp.bmj.com/)

**Figure 2** Representative autoradiograph of the K-ras codon 12 point mutation analysis. Four nylon membranes, each hybridised with a different radioactively labelled oligonucleotide specific for the sequence of the wild-type codon 12 (left) and the six possible mutations (three of them depicted). On each membrane, the non-enriched PCR products are shown in the left hand lane and the mutant enriched PCR products are shown in the right hand lane. co, hybridisation controls; on each membrane cloned DNA fragments with a known codon 12 sequence complementary to the labelled oligonucleotides are used for the hybridisation of that membrane. H$_2$O, negative water control. pla, placenta DNA. 1 to 5, DNA isolated from postgastrectomy pancreatic cancers with mutations resulting in an amino acid change; sample 2, glycine to valine; samples 1, 3, and 4, glycine to aspartic acid; sample 5, wild-type K-ras codon 12 sequence.
were wild-type, and one could not be amplified. In six of the 10 mutations the normal GGT sequence (glycine) was mutated to GAT (aspartic acid). In three of the 10 mutations, it was mutated to GTT (valine), and in one it was mutated to CGT (arginine) (fig 2).

**DISCUSSION**

The increase in pancreatic cancer risk after gastric surgery for benign conditions is not well established. Several investigators have noted an increased risk of pancreatic cancer after gastrectomy for benign gastrroduodenal disease, whereas others have not. An observation time of at least 20 years since initial peptic ulcer surgery appears to be the discriminating factor between these positive and negative studies.

In our present analysis, the overall (five to 59 years postoperative) pancreatic cancer risk of 1.8 (95% CI, 1.3 to 2.6) confirms the previous preliminary results seen in our cohort. Furthermore, our present analysis provides insight into the pancreatic cancer risk after a long postoperative interval—more than 25 years after the initial surgery. The additional eight pancreatic cancer deaths seen since our previous analysis all occurred 30 years or more after peptic ulcer surgery.

There is a trend of increasing pancreatic cancer risk with increasing time since surgery; in our cohort, the risk gradually increases to a significant 3.6 fold of the expected rate after 35 years or more postoperatively. Multivariate analysis indicated that variables other than postoperative interval had no significant influence on the pancreatic cancer risk.

There are several putative mechanisms through which gastric surgery might enhance pancreatic cancer risk. The hypochlorhydric postoperative stomach provides an environment in which nitrate reducing bacteria can proliferate. This leads to the increased formation of carcinogens, such as nitrosoamines and N-nitroso compounds, which can produce adenocarcinomas of the pancreatic duct in animal models. Interestingly, an increased pancreatic cancer risk was also seen in a cohort of patients with pernicious anaemia, and atrophic gastritis, both of which are accompanied by high gastric pH. Hepatic excretion of carcinogens into the bile and subsequent bile reflux into the pancreatic duct may lead to topical exposure to the carcinogens. Duodenogastric reflux appears to enhance pancreatic carcinogenesis in animal models. An increased production of the polypeptide hormone cholecystokinin (CCK) may have a promoting effect under these circumstances. In the rat azaserine model, CCK stimulates pancreatic carcinogenesis. In humans, the CCK response to oral fat is increased in patients who have undergone gastrectomy compared with normal controls.

Although *Helicobacter pylori* infection as a cause for peptic ulcer disease is firmly established, it is unlikely that this microorganism plays a direct causative role in carcinogenesis of the pancreas after peptic ulcer surgery. Recently, *Helicobacter* DNA was demonstrated in the bile of humans obtained by percutaneous trans-hepatic bile drainage. In another study, comparable serore prevalence rates for *H pylori* were found in both patients with pancreatic cancer and those with gastric cancer, and were significantly higher than the rates seen in the control groups (patients with colorectal cancer and healthy individuals). However, in a large proportion (at least 50%) of patients with peptic ulcer, *H pylori* is eradicated after peptic ulcer surgery as a result of the bile reflux.

“Although *H pylori* infection as a cause for peptic ulcer disease is firmly established, it is unlikely that this microorganism plays a direct causative role in carcinogenesis of the pancreas after peptic ulcer surgery”

Alternatively, factors other than those directly related to the gastric surgery itself could play a role in the increased pancreatic cancer risk. In general, patients with peptic ulcers are smokers and few stop smoking after surgery. Cigarette smoking is the strongest established risk factor for conventional pancreatic cancer. Unfortunately, detailed information on smoking behaviour in our study population is not available. Nevertheless, the observed increased lung cancer risk indicates that smoking in our cohort was more common than in the general Dutch population. However, the pattern of lung cancer risk after gastrectomy is very different from the steadily increasing trend observed for pancreatic cancer risk (fig 1). For lung cancer, the risk is increased after surgery from the beginning and remains constant throughout the postoperative observation period. Thus, although smoking may contribute to the increased pancreatic cancer risk after gastric surgery, the findings of our study suggest that factors related to the surgery itself may accelerate the neoplastic process subsequent to the surgical procedure. Indeed, Mills et al found a significant association between the risk for pancreatic cancer and a history of peptic ulcer surgery among adventists in a study that controlled for smoking. In a previous postmortem study with adjustment for smoking, we also found a significant association between pancreatic cancer risk and remote gastric surgery.

The results of the molecular analysis in our study support the role of smoking as a contributing factor to postgastrectomy pancreatic cancer. The frequency and specific types of K-ras codon 12 mutations in the 15 postgastrectomy pancreatic cancer samples are comparable to the frequency and types of mutations found in non-postgastrectomy pancreatic cancer. The similarities in prevalence and types of mutation in the two groups suggest that similar carcinogens are aetiologically important in both. Further analysis would be required to investigate whether the other genes involved in the development of pancreatic cancer also play a role in pancreatic cancers that develop after remote partial gastrectomy for ulcer disease.

Pancreatic intraepithelial neoplastic lesions were also seen in the tissue surrounding the pancreatic carcinomas in the postgastrectomy cases (fig 3). This suggests that infiltrating pancreatic adenocarcinoma in patients who have undergone gastrectomy occurs via a similar pathway to that postulated for conventional pancreatic cancer. Therefore, an increased index of suspicion after long term gastrectomy is important. In conclusion, an increased risk for pancreatic cancer exists in patients who have undergone gastrectomy, particularly after a long postoperative interval. The underlying aetiological factors may be similar to those that play a role in the development of non-postgastrectomy pancreatic cancer, but the neoplastic process apparently takes place at an accelerated rate.

![Figure 3](http://jcp.bmj.com/) Example of a pancreatic intraepithelial neoplastic lesion, in this case papillary hyperplasia without atypia, seen in the tissue surrounding a pancreatic carcinoma in one of the postgastrectomy cases.
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REFERENCES
Atherosclerosis and a high titre of antinuclear antibodies (ANA) are linked, concludes a recent study. Humoral immunity is implicated in inflammatory processes leading to atherosclerosis in mice, and autoantibodies to atheroma components have been shown in humans. This, however, is the first study of systemic autoimmunity and atherosclerosis in humans.

Grainger and Bethell screened for serum ANA—a marker of systemic autoimmunity—with an indirect immunofluorescent antibody test with HEp 2000 cells used in screening for autoimmune diseases. They compared 40 consecutive patients (aged 53–76) with advanced atherosclerosis (>50% blockage in three coronary arteries) confirmed by coronary angiography and 30 patients (48–74) with no plaques. Neither patients nor immediate (first degree) relatives had an autoimmune disease.

ANA were detected in nearly three quarters of patients with atherosclerosis but less than 20% without (within the range for their age). Overall, the odds of having ANA were significantly higher for coronary artery disease (11.67 (95% confidence interval 3.91 to 17.82; p<0.001)).

A speckled pattern characteristic of nucleolar staining occurred in three of five positive patients without atherosclerosis and 17 of 28 with; other patients showed staining of the centrosome or cytoplasm. Patients with atherosclerosis who had a previous myocardial infarction showed no difference in the proportion with ANA compared with those who had not.

The authors advocate a larger cohort study to permit pairwise matching for age and sex between the groups, but at face value ANA titre may be useful diagnostic marker for coronary artery disease. The tantalising question of the antibody's identity and whether it contributes to plaque development also remains.


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