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 analyses 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 ($\chi^2$ 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.
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.

**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 date. The period of follow up is 1935–95; the age range of the patients is 10–84 years.

expected numbers of lung cancer deaths in an Amsterdam cohort of 2633 patients after gastrectomy (both sexes) in consecutive postoperative intervals compared with the general Dutch population

<table>
<thead>
<tr>
<th>Years since gastrectomy</th>
<th>Observed lung cancer deaths (o)</th>
<th>Expected lung cancer deaths (e)</th>
<th>Ratio o/e</th>
<th>95% CI</th>
<th>p Value (2 sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>2619</td>
<td>8</td>
<td>7.3</td>
<td>1.1</td>
<td>0.5 to 2.2</td>
</tr>
<tr>
<td>5–14</td>
<td>2511</td>
<td>47</td>
<td>29.5</td>
<td>1.6</td>
<td>1.2 to 2.1</td>
</tr>
<tr>
<td>15–24</td>
<td>2087</td>
<td>78</td>
<td>47.4</td>
<td>1.6</td>
<td>1.3 to 2.1</td>
</tr>
<tr>
<td>25–34</td>
<td>1474</td>
<td>80</td>
<td>48.9</td>
<td>1.6</td>
<td>1.3 to 2.1</td>
</tr>
<tr>
<td>35–59</td>
<td>825</td>
<td>49</td>
<td>27.2</td>
<td>1.8</td>
<td>1.3 to 2.4</td>
</tr>
<tr>
<td>5–59</td>
<td>2511</td>
<td>254</td>
<td>152.9</td>
<td>1.7</td>
<td>1.5 to 1.9</td>
</tr>
</tbody>
</table>
The protocol for the K-ras codon 12 mutational analysis was described earlier. 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. All PCR products were hybridised with oligonucleotides with a known codon 12 sequence complementary to the labelled probes, specific for each possible K-ras codon 12 mutation, followed by autoradiography.

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 enriched PCR products are shown in the right hand lane and 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. H2O, 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.

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 (χ², 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 (χ², 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
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 gastroduodenal 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 nitrosamines 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 an increased 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.

Pancreatic intraepithelial neoplastic lesions were seen in the tissue surrounding a pancreatic carcinoma in one of the postgastrectomy cases.

Figure 3 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.

"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 type 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.
Take home messages

- Remote partial gastrectomy is associated with an increased risk of pancreatic cancer, particularly after a long postoperative interval.
- Postgastrectomy and non-postgastrectomy pancreatic cancers may share similar aetiological factors, such as smoking.
- The spectrum and prevalence of Kras codon 12 mutations were comparable to conventional pancreatic cancer.
- The neoplastic process in patients who have undergone gastrectomy appears to be accelerated by factors related to the surgery itself.

ACKNOWLEDGEMENTS
We thank A Kammer from The Johns Hopkins Cancer Registry for assistance with the study, The Central Bureau for Vital Statistics, The Hague, for providing the data on the general Dutch population, and PALGA for tracing the tissue samples. This study was supported by the Netherlands Foundation for Scientific Research (NWO), grant number 950–10–625, and by the Prevention Fund, Grant number 950–10–625, and by the Netherlands Foundation for Scientific Research (NWO), grant number 950–10–625, and by the Prevention Fund, Grant number 950–10–625, and by the Prevention Fund, Grant number 950–10–625, and by the Prevention Fund, Grant number 950–10–625.

REFERENCES

Authors’ affiliations
G.J.A. Offerhaus, M. Tascilar, B.P. van Rees, P.D.J. Sturm, A.C. Tersmette, Department of Pathology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands
G.N.J. Tytgat, Department of Gastroenterology, Academic Medical Center, University of Amsterdam
R.H. Hruban, Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD 21205, USA
S.N. Goodman, Department of Oncology, The Johns Hopkins Medical Institutions
F.M. Giardiello, Department of Gastroenterology, The Johns Hopkins Medical Institutions

www.jclinpath.com
Systemic autoimmunity and atherosclerosis

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.