Circulating Anti-Helicobacter pylori Immunoglobulin A Antibodies and Low Serum Pepsinogen I Level Are Associated with Increased Risk of Gastric Cancer

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Helicobacter pylori infection has been suggested to be associated with an increased risk of gastric cancer, and low levels of serum pepsinogen I (PG I) have been linked to atrophic gastritis, which is a risk factor for gastric cancer. In Finland, 39,268 persons from 25 cohorts participated during 1968–1972 in a health examination survey and were followed for up to 13 years. A nested case-control study was performed on 84 stomach cancer patients identified from the Finnish Cancer Registry and 146 controls matched for age, sex, and municipality. Serum samples drawn at the baseline study were analyzed. An elevated level of serum anti-H. pylori immunoglobulin A (IgA) antibodies (a titer ≥70) and a low serum PG I level (<49 μg/liter) were associated with an increased risk of gastric cancer. The odds ratios were 2.52 (95% confidence interval (CI) 1.14–5.57) for high IgA and 2.68 (95% CI 1.35–5.30) for low PG I. For high immunoglobulin G (IgG) (≥700), the odds ratio was only 1.50 (95% CI 0.70–3.22). When both high IgA and low PG I were present, the odds ratio was 5.96 (95% CI 2.02–17.57). The association of H. pylori infection with cancer became stronger with longer follow-up times, whereas that of low PG I was strongest at shorter follow-up times. Our findings support the hypothesis that H. pylori infection is a prevalent and potentially preventable cause of gastric cancer. They stress the value of IgA antibody determinations and provide new evidence for a pathogenesis leading from prolonged infection through atrophic gastritis to gastric cancer. Am J Epidemiol 1996;144:142–9.
*Helicobacter pylori* infection has also been defined by a positive serum IgG antibody test, which has been shown to be a more sensitive indicator of ongoing infection than morphologic analyses of biopsied samples (18), particularly in the presence of atrophic gastritis (19).

Gastric cancer patients have also been shown to have local mucosal anti-*H. pylori* immunoglobulin A (IgA) antibody responses (20). A diagnostic level of circulating anti-*H. pylori* IgA antibodies has been demonstrated in only about two thirds of infected patients, but the IgA antibody test is sometimes the only positive one of the two serologic tests (21). Both IgG and IgA titers remain stable during infection but decline with successful antimicrobial treatment, and a drop of $\geq 50$ percent within 5–6 months indicates eradication of the bacteria (21).

Lowered serum pepsinogen I levels are regularly found in patients with advanced atrophic gastritis (22), suggesting that it may be a serologic risk indicator for gastric cancer. Considerably reduced levels have been found in gastric cancer patients (23), and in two longitudinal studies the incidence of gastric carcinoma was clearly elevated in men with low pepsinogen I levels (24, 25).

We conducted a prospective, nested case-control study to evaluate the associations of serum anti-*H. pylori* IgA and IgG antibodies and of serum pepsinogen I levels with the incidence of gastric cancer in a large cohort in Finland followed for up to 13 years.

**MATERIALS AND METHODS**

**Study design**

During 1966–1972, the Mobile Clinic Unit of the Social Insurance Institution carried out multiphasic screening examinations in various parts of Finland (26, 27). Total populations or their random samples, a total of 58,440 adults aged 15 years or over, were invited to take part in the study in a number of rural, semiurban, and industrial communities; the participation rate was 82.5 percent. All examinees completed a questionnaire supplying information on previous and current illnesses, smoking habits, and occupations. The subjects were classified as current smokers and nonsmokers. Venous blood samples were drawn and stored at $-20^\circ$C. Blood pressure measurement, determination of height and weight, and serum cholesterol analyses were carried out using standard methods. Hypertension was defined as casual systolic blood pressure $\geq 170$ mmHg and diastolic blood pressure $\geq 100$ mmHg or the use of antihypertensive medication. Frozen serum samples are currently available from persons studied after June 15, 1968. Participants examined before that date and those with previous histories of cancer were excluded. The study population at risk thus comprised 21,172 men and 18,096 women from 25 cohorts.

Almost every cancer case is reported to the Finnish Cancer Registry (28); reporting has been obligatory since 1961. The registry receives information from hospitals, practitioners, pathologic laboratories, and death certificates. Unique personal identification numbers were used to search the registry for cases of cancer occurring in the study population between the date of examination and December 31, 1980. The site of the primary cancer and the date of cancer diagnosis were obtained from the registry. In the cohort, 84 new first primary gastric cancers were identified during the 13-year follow-up. Mean follow-up time was 9.5 years. During the period of our study, the pathologic confirmation rate of gastric cancers has been high and increasing, ranging from 70 percent in 1969 to 80 percent in 1973 and reaching 90 percent by 1981. Tumors of the gastric cardia have been separately coded only since 1970. Of all the 74 gastric cancer cases detected in our cohort since then, only nine were found in the gastric cardia.

A case-control study design nested within the cohort was adopted. Two control subjects were selected for each gastric cancer patient from the final study cohort by individual matching. The control subjects were drawn from the same municipality as the cancer patient, and the ages were matched as closely as possible. In 75 sets, the matching was exact, in seven there was a difference of 1–2 years, and in two, a difference of more than 2 years between cases and controls. Matching for municipality also controlled for the duration of storage of serum samples. The final control group for which serum samples were available for the present study consisted of 146 controls. There were 62 complete sets with two controls per case and 22 sets with only one control.

**Laboratory analyses**

The serum samples were thawed once in 1985 for vitamin and selenium analyses and for the present study again in 1992. Serum samples from each case and its matched controls were analyzed in random order independently of the case-control status, of which the laboratory personnel were also unaware. Any possible effect of systematic variation in the laboratory analyses thus did not affect case-control comparisons within the sets.

Anti-*H. pylori* IgA and IgG antibodies were determined separately by enzyme immunoassay (21). The antigen used was an acid glycine extract from *H. pylori* National Collection of Type Cultures' strain 11637. The absorbance values were converted to re-
ciprocals of the endpoint titers. Separate reference pools were used for IgA and IgG. The lower limits of elevated titers (expressed as reciprocals) were 70 for IgA and 700 for IgG antibodies. The IgA limit coincided with the lowest quartile of the titers in control subjects. With these limits, the sensitivity and specificity of the IgA test were 73.1 percent and 95.1 percent and for the IgG test, 93.7 percent and 93.9 percent. These were determined in a separate series consisting of 439 patients whose biopsy samples were cultured and studied histologically for the presence of helicobacteria in 1992 and early 1993. For IgG antibodies, the lowest quartile limit in this study was 1,300; at this level, the sensitivity of the IgG test was 65.7 percent, and the specificity was 98.1 percent in the separate patient series.

The serum pepsinogen I concentration was determined by the radioimmunoassay method according to Tamm et al. (22) with some modifications. Lactoperoxidase sorbent was used to iodinate pepsinogen I, and the double-antibody solid-phase technique (Decanting Suspension 3; Pharmacia, Uppsala, Sweden) was used to separate the bound and free fractions in radioimmunoassay. Two mCi of Na$_{125}$I (IMS 300; Amersham International, Amersham, United Kingdom), 2 µl of 8.8 mmol/liter of H$_2$O$_2$, and 0.8 mg of lactoperoxidase sorbent were used to iodinate 2 µg of pepsinogen I (Orion Diagnostica, Espoo, Finland) at pH 6.5 in 0.1 mol/liter of acetate buffer. The radioimmunoassay antiserum (Orion Diagnostica) was diluted 1:35,000. Serum samples (diluted 1:10 and 1:2.5) and standards were incubated overnight at 4°C before the second double-antibody solid-phase incubation, which lasted 1 hour at room temperature. The serum dilution curve was parallel with the standard curve in the range of 1–100 µg/liter, using 0.04 mol/liter of phosphate-buffered saline, pH 7.5, containing 1 percent bovine serum albumin. The master curve was adjusted according to the Multicalc transformation scheme (Wallac, Turku, Finland) and used for daily calibrations. In every series of determinations, two control charts were plotted.

Pepsinogen I values of ≤49 µg/liter are regularly found in atrophic gastritis (22) and have also been used to assess the risk of gastric cancer (25). To evaluate the comparability of the results obtained in our laboratory with those obtained in the laboratory used by Parsonnet et al. (25), 317 serum samples were analyzed in both laboratories. There was a close correlation (multiple $R^2 = 0.85$), but our levels were higher. On the basis of the regression equation, our cutoff limit of 49 would correspond to the value 36 in the laboratory of Parsonnet et al., and our cutoff limit of 64 to the value 51.5.

The serum retinol, β-carotene, and α-tocopherol concentrations were determined simultaneously using high-pressure liquid chromatography, and the concentration of serum selenium, by the graphite furnace atomic absorption spectrometric method as described elsewhere (27).

### Statistical methods

The conditional logistic model for matched sets (29) was used to estimate the association between serum IgA, IgG, and pepsinogen I levels and the risk of gastric cancer, including potential confounding and effect-modifying factors in the model when indicated. The relative odds were computed for selected cutoff points of serum IgA, IgG, and pepsinogen I, as well as for quartiles. Statistical significances were tested with the likelihood ratio test based on the model.

### RESULTS

The gastric cancer patients were similar to the control subjects with respect to several baseline characteristics (table 1). Cancer patients were nonsignificantly leaner, more often nonsmokers, hypertensive, and unmarried than the control subjects and, despite

<table>
<thead>
<tr>
<th>TABLE 1. Mean levels of baseline characteristics of gastric cancer patients and their matched control subjects, Finland, 1968–1972</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Cases (n = 84)</td>
</tr>
<tr>
<td>Controls (n = 146)</td>
</tr>
</tbody>
</table>

| Number of cases was 54 and that of controls, 81. |
the matching for municipality, a higher proportion was employed in agricultural occupations.

The threshold values of our laboratories for defining *H. pylori* infection (IgA ≥ 70, IgG ≥ 700) and atrophic gastritis (pepsinogen I < 49 µg/liter) were used to estimate the prevalence of infection and atrophic gastritis and the corresponding odds ratios of gastric cancer (table 2). IgA was elevated in 76 percent of the controls, IgG in 83 percent, and either IgA or IgG in 87 percent, indicating a very high infection rate. Pepsinogen I was low in 36.9 percent of cases and 19.9 percent of controls. The odds ratio of gastric cancer at IgA titers ≥ 70 was 2.52 (95 percent confidence interval (CI) 1.14–5.57), at IgG titers ≥ 700 it was 1.50 (95 percent CI 0.70–3.22), and for pepsinogen I < 49 µg/liter it was 2.68 (95 percent CI 1.35–5.30).

Further analyses were based on dividing all values into quartiles. There was a suggestive increased risk of gastric cancer in the three highest quartiles of anti-*H. pylori* IgA titers, as well as a similar elevated risk in the lowest quartile of pepsinogen I concentration, in comparison with the risk in other quartiles (table 3). In comparison with the lowest quartile, the point estimate of the odds ratio of gastric cancer in persons with higher IgG (> 1,300) was 1.22; for high IgA (> 70), it was 2.29. The corresponding point estimate of the odds ratio associated with a low pepsinogen I level (< 64 µg/liter) was 2.24. These findings were not altered by adjustment for smoking, occupation, serum α-tocopherol, or serum β-carotene, retinol, and selenium (data not shown). Exclusion of the patients with tumors in the gastric cardia slightly strengthened these odds ratios that, for high IgG, were 1.75 (95 percent CI 0.80–3.81); for high IgA, 2.76 (95 percent CI 1.11–6.87); and for low pepsinogen I, 2.37 (95 percent CI 1.19–4.74).

### TABLE 2. Odds ratios of gastric cancer between persons with and without elevated titers of circulating anti-*Helicobacter pylori* immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies and low levels of pepsinogen I (PG I) concentration in the total sample, Finland, 1968–1980

<table>
<thead>
<tr>
<th>Variable</th>
<th>Threshold values</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Odds ratio</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>&lt;70</td>
<td>9</td>
<td>35</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥70</td>
<td>75</td>
<td>111</td>
<td>2.52</td>
<td>1.14–6.57</td>
</tr>
<tr>
<td>IgG</td>
<td>&lt;700</td>
<td>11</td>
<td>25</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥700</td>
<td>73</td>
<td>121</td>
<td>1.50</td>
<td>0.70–3.22</td>
</tr>
<tr>
<td>PG†</td>
<td>≥49</td>
<td>53</td>
<td>117</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;49</td>
<td>31</td>
<td>29</td>
<td>2.68</td>
<td>1.35–5.30</td>
</tr>
</tbody>
</table>

* CI, confidence interval.
† Threshold values expressed as µg/liter.

A study of possible effect modification by several baseline factors revealed statistically significant associations among IgA antibodies, pepsinogen I, and gastric cancer incidence in men but not in women (table 4). The associations were stronger in younger than in older persons and statistically significant in nonsmoking but not in smoking men. However, none of these suggestive effect modifications were statistically significant. The strongest and statistically significant association between gastric cancer risk and high IgA titer was noted at follow-up times beyond 6 years. The increased risk with low pepsinogen I levels was strongest and statistically significant during the first 2 years.

Raised antibody titers were observed in a slightly smaller proportion of cases with low pepsinogen I than in those with a higher pepsinogen I level (raised IgA in 80.6 vs. 94.3 percent; raised IgG in 77.4 vs. 92.5 percent). The study of the simultaneous effect of high IgA titers and low pepsinogen I showed that they were independently associated with cancer risk, and no significant interactions were observed. The odds ratio for a combination of high IgA and low pepsinogen I was 5.96 (95 percent CI 2.02–17.57) in comparison with lower IgA titers and higher levels of pepsinogen I.

### DISCUSSION

We observed a clear-cut association of the risk of gastric cancer with positive IgA tests suggesting the presence of *H. pylori* infection and with low pepsinogen I levels related to atrophic gastritis. Unexpectedly, the positive associations of gastric cancer with IgG were weaker and statistically nonsignificant. In agreement with other prospective studies (15–17), our findings show that a preceding *H. pylori* infection is a risk factor for gastric cancer. Findings from the earlier nested case-control studies were based on the presence of IgG antibodies against *H. pylori* in serum samples collected up to 24 years earlier. In our study, the strongest evidence for the association of infection with gastric cancer was derived from IgA antibodies. The odds ratios between *H. pylori* infection and gastric cancer that we observed were smaller than several of those reported previously.

Low pepsinogen I was associated with an increased risk of gastric cancer. Low pepsinogen I concentrations are associated with atrophic gastritis (22), which is a known risk factor for gastric cancer (7, 8). Two other longitudinal studies have shown that low pepsinogen I levels are associated with an increased risk of gastric cancer (24, 25). However, whereas Parsonnet et al. (25) in their study with a median 15-year follow-up reported that a low pepsinogen I was associated with an increased risk of gastric cancer...
only in the presence of *H. pylori* infection, our findings after a mean follow-up of 9.5 years showed that a low pepsinogen I was associated with increased cancer risk both when antibodies were present and when they were absent. The independent effect of low pepsinogen I can be understood on the basis that antibody production may be reduced and cease when atrophy progresses (see below). These findings in combination with the known association between *H. pylori* infection and superficial gastritis (4, 5) are rather strong evidence for the chain infection: superficial gastritis-chronic atrophic gastritis-gastric cancer.

The stronger association between the risk of gastric carcinoma and infection after a longer follow-up time has been reported previously (16). It may be indicative of a diminishing immune response caused by a de-
The occurrence of various causes of gastric cancer may lead to a high occurrence of atrophic gastritis. Infection is a cause of both atrophic gastritis and gastric cancer. However, not all future cancer patients had low serum pepsinogen I levels for long periods before cancer diagnosis nor did they have a preceding *Helicobacter pylori* infection.

One explanation for the rather low odds ratio estimates may be the very high prevalence of *H. pylori* infection in the population of this age in Finland, which was also reflected in the small difference between infection rates of our cases and controls. However, infection prevalences have also been rather high in some of the other previously studied populations, where reported associations between elevated IgG and gastric carcinoma have been stronger (16, 17). Taking into account the analytic difference in pepsinogen I levels (see Materials and Methods), a comparison of our data with those of Parsonnet et al. (25) suggests that the prevalence of atrophic gastritis (low pepsinogen I) was higher in our data set. If antibody levels decrease with progressive atrophy, the actual effect of the infection may be underestimated in a population with a high prevalence of atrophic gastritis, particularly if follow-up is relatively short. The larger odds ratios in some other studies may also be due to longer follow-up times, younger age structures, higher socio-economic status, and possible selection biases.

The apparent differences in odds ratio estimates between studies may also be due to profound differences between populations, their environments, and living conditions. While both genetic and environmental factors may operate (30), the latter may be more important. Differences between populations are reflected in variations in infection rates and the occurrence of other cancer-promoting and -protective factors operating independently or interacting with the infection. The infection may also predispose the gastric mucosa to the effects of other causes of cancer. A high incidence and prevalence of *H. pylori* infection may lead to a high occurrence of atrophic gastritis. That the occurrence of various causes of gastric cancer may differ between populations is supported by the varying strengths of the associations between different subgroups in both our study (see below) and other studies (17). Indeed, one reason for the difference in seeming importance of infection between studies and populations may be the influence of other causes of gastric cancer in combination with the varying prevalence of infection. Similar considerations may also explain the observed decline in the incidence of gastric cancer (2). The environment in which the majority of our cases and controls lived during the first decades of this century was a rural agricultural Finland with a traditional diet, implying the presence during winter, for example, of a rather abundant intake of nitrosamines in salted and smoked foods, as well as a lack of antioxidants due to poor supplies of vegetables and fruits. The risk of early *H. pylori* infection was high. Together, these factors may explain the very high incidence of gastric cancer in Finland in the 1950s (3).

The statistically significant association of both the infection (IgA antibodies) and atrophic gastritis (low pepsinogen I) with gastric cancer was concentrated in men, nonsmoking men, and younger persons. Some of the suggestive effect modifications were probably due to differences in the occurrence of other risk factors of gastric cancer among these groups.

Many known details of the pathogenetic mechanisms associated with *Helicobacter* infection are obvious and important factors in carcinogenesis. Since most persons acquire the chronic infection in childhood (31), these factors have several decades to exert their effects. The increased rates of mitosis seen in the mucosae during *Helicobacter* infection (32) increase the number of vulnerable periods in the life of the mucosal cells. Infection also leads to a rapid fall in ascorbate concentrations in the mucosae (33) and thus to less effective inhibition of oxidative bursts and release of free radicals. *Helicobacter* strains differ in their production of cytotoxins (34) and the M120,000 cagA protein (20) aggressiveness toward granulocytes (35), and these properties seem to play a role in their capacity to cause ulcer disease. There are similar differences between the cytotoxic (36) and cagA protein-producing (37) strains in their effects toward malignant changes.

Methodologically, our longitudinal study based on a large representative population sample is sound. The cancer follow-up was virtually complete, and there are no obvious sources of bias in the case-control comparisons. The Finnish Cancer Registry's lack of data on the anatomic distribution of gastric cancer prior to 1970 could have been a problem, since previous studies (10, 17) have shown that tumors of the gastric cardia are not related to *H. pylori* infection. However, tumors of the gastric cardia are relatively rare in Finland and, when we excluded them, the associations became slightly stronger. Qualitatively, however, the findings were not substantially altered. Had we excluded gastric cancer cases occurring prior to 1970, we
would have lost part of the early follow-up and reduced the material. Our estimates would have lost some stability. Combining all gastric cancers in the analyses was justified, even though it slightly reduced the strength of associations with IgG and IgA.

Detection of circulating anti-H. pylori antibodies has been shown to be a more sensitive method of diagnosing a Helicobacter infection than bacteriologic and histologic studies on biopsies (18, 19). Most laboratories use only IgG antibody tests, which are positive in >90 percent of culture-positive patients (18, 21). The IgA antibody test that we used is less sensitive (73 percent), but, when previously used in combination with the IgG antibody test, it has been positive in a further 2 percent of culture-positive patients (21). The facts that circulating IgA antibodies were more common than IgG antibodies in gastric cancer patients and that the associated odds ratio was higher than that associated with IgG emphasize the importance of IgA antibodies. The strong mucosal anti-H. pylori IgA response noted in cancer patients (20) thus seems to have a strong counterpart in serum antibodies and is also a powerful indicator of cancer risk.

Our findings are important in both practice and theory. Gastric cancer is known for its poor prognosis and, although declining (2), it is still very common. In some developed populations, H. pylori infection has been calculated to be responsible for up to 60 percent of the cancer cases (15, 17), and therefore prevention would be important. Scattered data from European developed countries (14, 31) suggest that the prevalence of H. pylori infection is declining with time in successive cohorts. This background, in combination with information about the current low Helicobacter infection rates in children in developed countries, implies that, whereas prevention trials relying only on vaccinations of children may be the method of choice in developing countries, trials in developed countries should be concerned with eradication of already existing infections in adults. Since the frequency of H. pylori infections in adults is very high, it would be preferable to select subjects of higher than average risk for therapeutic trials. According to our data, elevated anti-H. pylori IgA titers and low serum pepsinogen I levels are risk factors for gastric cancer and could perhaps be used as aids in such high-risk group selection. Eradication of H. pylori infection has been followed by regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue (MALT) type (38), which is a relatively rare disease. Whether eradication of the infection would prevent the development of the more common malignant process leading to gastric cancer is a question of major biologic importance that only intervention trials can answer.

In conclusion, our findings lend further support to the hypothesis that H. pylori infection is an important cause of gastric cancer, and they provide new evidence supporting the proposal that prolonged infection may exert its effects by causing atrophic gastritis that may proceed to gastric cancer. The observations clearly demonstrate the value of IgA antibody determinations and lend additional credibility to the hypothesis that carcinogenesis results from the combined action of infection and other factors. Indirectly, the findings can be interpreted as suggesting that the process leading to malignant gastric tumors may be preventable by eradication of the infection. If the prospects of prevention of gastric cancer through eradication of infection are to be evaluated, our results can provide a basis for such large-scale intervention trials in high-risk groups.

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