Seroconversion for *Helicobacter pylori*

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**Summary**

The prevalence of *Helicobacter pylori* antibodies increases with age, but it is unknown whether this is due to a constant rate of infection in different age groups, or whether most infection occurs in childhood. Follow-up data on infection rates and the course of infection in an untreated population are scarce. We measured *H pylori* IgG antibody concentrations in patients who were seen at our endoscopy unit between 1979 and 1983.

115 of 164 eligible patients (70%) participated in the study. *H pylori* IgG antibody concentrations were measured in two serum samples taken with a mean interval of 11·5 years. 56 patients tested positive at the first visit. During follow-up, 2 patients became infected (annual infection rate 0.30%, 95% Cl 0.04–1.08%). Evidence of infection disappeared in 6 patients: after gastric surgery in 3 and due to an unknown cause in the remaining 3 patients. A non-significant decrease of infection was shown in different age cohorts during follow-up. Antibody concentrations did not increase with age.

These results strongly support the concept of dominant infection rates in childhood. Elimination of infection may occur in a few patients without eradication therapy.

*Lancet* 1993; 342: 328–31

**Introduction**

*Helicobacter pylori* infection of the gastric mucosa leads to a pronounced local and systemic immune response in nearly all patients. Measurement of IgG serum antibodies by means of enzyme-linked immunosorbent assay (ELISA) is a reliable, inexpensive, and non-invasive method for detection of infection, and for evaluation of the effect of eradication therapy, and for epidemiological studies. Whether the increasing prevalence of *H pylori* antibodies with age, is due to a constant rate of infection in different age groups, or to an age-cohort effect with dominant conversion rates at a younger age, remains to be seen. The latter theory is based upon the assumption of a decreasing rate of infection in younger age cohorts in recent decades due to a rise in living standards. Several authors have reported a correlation between high IgG serum antibody concentrations as reflected by ELISA, and clinical diagnosis. Higher antibody concentrations were found to reflect more active gastritis and a higher prevalence of duodenal ulceration. Follow-up studies reported a correlation between IgG *H pylori* antibody concentrations and the risk of development of gastric carcinoma. Longterm follow-up data on *H pylori* serum antibody concentrations in an untreated population are needed. We measured *H pylori* IgG antibody concentrations in patients at presentation and after 10–13 years.

**Patients and methods**

**Patients**

Between 1979 and 1983, we studied the biochemistry, genetics, and clinical relevance of gastric pepsinogen A and C. After obtaining informed consent, we took gastric biopsy specimens and serum from 230 randomly selected patients referred for upper gastrointestinal endoscopy. Serum samples were stored at −20°C. With the help of general practitioners, hospital patient data, and registry offices for births, deaths, and marriages, follow-up data for 219 subjects (95%) were obtained. 40 had died by 1992; none due to gastric cancer or peptic ulcer disease. Of the remaining 179, 15 had moved abroad or were too ill to ask to participate in the follow-up study. After receiving written and verbal information, 115 (62 men) of the remaining 164 subjects gave their consent to participate. The mean (SD) age at first visit was 50 (14.7) years, with a range of 15–80 years. The subjects were seen in 1992 after a mean follow-up of 11.5 years (range 10–13 years). A history was taken from all participating patients, and information from their general practitioners, specialists, and hospital records was added. Special emphasis was put on determining current and past drug histories and the presence of dyspeptic complaints. The study was approved by the ethical committee of the Free University Hospital.

**Methods**

Stored serum samples from all participants in the study were thawed and 100 μL of each was used. Second serum samples were taken at another visit. Both sera were used for the determination of *H pylori* IgG antibodies with an ELISA method. To standardise the antibody measurement, the mean (SD) value for absorbance of the standard positive reference serum was taken as 1.0 (0.1). The absorbance index (AI) was calculated from the mean of two
readings of optical density (OD) of serum. The results were expressed as:

\[ \text{AI} = \text{Patient's mean OD (n=2)} - \text{mean OD of blank reading (n=2)} \]  
\[ \text{Reference mean OD (n=2)} - \text{mean OD of blank reading (n=2)} \]

All tests were carried out in duplicate. Excellent test reproducibility was found with a mean variance of duplicate test results of less than 5%. An AI of 0.650 or higher was taken as evidence of infection, with 95% sensitivity and 96% specificity, as previously tested in a cohort of individuals referred for upper gastrointestinal endoscopy by correlation with culture of an antral specimen, detection of part of the highly \( H. pylori \)-specific 16 sRNA gene by polymerase chain reaction, \(^{13}\) and histological evaluation of 4 \( \mu \)m Giemsa stained slides of 2 antral and 5 corpus specimens. A decrease in AI of more than 50% to below the cut-off value of 0.650 was taken as evidence of eradication of infection because such a decrease occurred in 97% of patients within 6 months of eradication. In contrast, a rise in AI of more than 100% to above the cut-off value was regarded as evidence of infection during follow-up. These distinctions were made to exclude a false diagnosis of seroconversion during follow-up in those patients with AIs near the cut-off value. Univariate statistical analysis was performed with chi-square and Student's \( t \) tests. For both tests, a \( p \) value below 0.05 was taken as significant.

**Results**

59 of the sera (51%) from the first visit tested negative for IgG \( H. pylori \) antibodies. During 11.5 years of follow-up, 2 of these 59 seronegative patients (3.4%) converted to seropositive; these 2 patients showed a rise in AI of more than 100%. 2 additional patients showed a 57% and 46% rise in AI from below to above the cut-off value, respectively. With our strict criteria for seroconversion, these 2 patients were not thought to have become infected during follow-up. The patient with a 57% rise in AI recently had upper gastrointestinal endoscopy for reasons unrelated to this study; no \( H. pylori \)-like organisms were seen in 2 antral and 5 corpus specimens. Culture of an additional antral specimen was negative.

56 patients had evidence of \( H. pylori \) infection at the first visit. During follow-up, 1 of these patients was treated with bismuth, but he remained \( H. pylori \)-positive. In contrast, 7 of these 56 seropositive patients reverted to seronegative with a decrease in AI of more than 50% from above to below the cut-off value (table). None of these 7 patients was reported to have been treated with bismuth or triple therapy at any time during follow-up, nor had any taken acid-suppressive drugs. 1 patient had a subtotal stomach resection for carcinoma in situ of the gastric corpus in 1989. 2 had a Billroth II resection of the distal stomach for complicated peptic ulcer disease around the time of collection of the first serum sample. The possibility of conversion to seronegativity because of development of mucosal atrophy was excluded in 6 of these 7 seroreverters (excluding the patient with subtotal resection of the stomach) by measurement of serum gastrin and endoscopy.

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>Gastrin (ng/L)</th>
<th>Endoscopic biopsies (atrophy/( H. pylori ))</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>52/M</td>
<td>30</td>
<td>-/-</td>
<td></td>
</tr>
<tr>
<td>57/M</td>
<td>23</td>
<td>-/-</td>
<td>Billroth II resection 1980</td>
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<td>Stomach resection 1989</td>
</tr>
<tr>
<td>68/M</td>
<td>8</td>
<td>Refused</td>
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<tr>
<td>75/M</td>
<td>22</td>
<td>-/-</td>
<td>Billroth II resection 1980</td>
</tr>
<tr>
<td>79/M</td>
<td>30</td>
<td>-/+</td>
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</tr>
<tr>
<td>86/M</td>
<td>38</td>
<td>Not asked</td>
<td></td>
</tr>
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</table>

*Data at end of follow-up period.

Table: Data* of patients who converted from seropositive to seronegative during follow-up

The mean (SD) AI values and their spread in the total patient cohort did not differ for first and follow-up sera: 0.729 (0.51) at first visit and 0.697 (0.50) at follow-up (difference of 4.5%, \( p > 0.1 \), paired \( t \) test). For infected patients, a mean AI of 1.153 (0.260) was found at first visit and 1.042 (0.186) at follow-up (difference of 2.2%, \( p > 0.1 \), paired \( t \) test). As shown in figure 2, 9 of 34 (26%) patients (table); 1 was not asked for endoscopy because of his age of 86 years. In 1 patient the continued presence of \( H. pylori \) infection was shown in endoscopic biopsy specimens, despite a 76% decrease in AI from the high positive to the negative range. Thus, 6 of the 7 seroreverters were shown to have disappearance of infection during follow-up: 3 after gastric surgery; and 3 with no factors that could be held responsible for eradication of the infection.

Correlation of first and follow-up test results for the total patient cohort is given in figure 1. For the subgroup of patients with persistent infection throughout the follow-up period, this correlation was 0.34 (\( p = 0.02 \), \( t \) test) which was much lower than the correlation of 0.70 (\( p < 0.01 \), \( t \) test) found for the total patient cohort. These correlations are consistent with fluctuation of antibody titres during long-term follow-up.

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below 40 years, 30 of 51 (59%) patients between 40 and 60 years of age, and 17 of 30 patients (57%) older than 60 tested positive at the first visit (p > 0.05, chi-square test). Repeated age-cohort studies at the end of the follow-up period showed the following infection rates: 2 of 11 (18%) patients below the age of 40, 22 of 47 (47%) patients between 40 and 60, and 28 of 57 (49%) patients older than 60 years (p > 0.05, chi-square test, figure 2). The correlation between AI and age in infected patients for both visits is given in figure 3. No significant correlation between age and AI was found (r = 0.01 for the first visit in 1980 and r = 0.02 at follow-up in 1992, p > 0.1). Intermittent or continuous longterm treatment with acid-suppressive drugs was given to 28 patients. After exclusion of 6 patients who received treatment for reflux disease, acid-suppressive therapy clearly correlated with H pylori infection in 22 patients who had treatment for dyspeptic complaints: 18 of 56 patients (32%) with H pylori infection received treatment compared with 4 of 59 non-infected patients (p = 0.002, chi-square test).

**Discussion**

*H pylori* is associated with chronic active gastritis. Although a gradual increase of infection prevalence in older age cohorts in developed countries has been noted by various investigators, little is known about the acquisition rate of infection and the mode of acquisition. If most people were infected during youth (below 20 years), a higher infection prevalence at an older age (above 50 years) would be explained by higher infection rates in the past. Follow-up data are scarce and are mostly on patients after attempts to eradicate the infection.4,5 Treatment failures showed stable IgG concentrations during 12 months follow-up. In developed countries, reinfection after successful eradication was seen in 1 of 214 and 2 of 73 patients during a 1 year follow-up period. We found 1 infection in 25 patients with reflux oesophagitis over 4 years of follow-up. In contrast, a 20% reinfection rate was reported in 63 Italian children over an 18 month period.17 These data suggest a higher infection rate during youth, but differences in local epidemiological factors may be responsible. Follow-up data for seroconversion in an untreated population are even more scarce. A Canadian study reported a single seroconversion in 112 seronegative adult patients after a 2 year follow-up. A study in the USA showed an annual seroconversion rate of 0.49% during 7.5 years of follow-up. In this study, we found a seroconversion in 2 of 59 seronegative patients over 11.5 years: an annual 0.30% seroconversion for this adult cohort (95%, CI 0.04–1.08%). This low seroconversion rate supports an age-cohort effect with acquisition of most *H pylori* infections during youth.

*H pylori* infection is widely thought to remain for life, unless eradication treatment is prescribed or gastric mucosal atrophy develops. We took a history from all patients, and further information was obtained from their treating physicians with special emphasis on current and past drug use. Although a retrospective history for an 11-year period could be incomplete, no gastrointestinal investigation, or use of bismuth or triple therapy regimens was reported for any of our 3 patients for whom no explanation for the seroconversion could be found. Negative seroconversion due to development of gastric mucosal atrophy was excluded by endoscopy and measurement of serum gastrin concentrations. We conclude, therefore, that a small proportion of infected patients can eliminate *H pylori* infection. This conclusion is in agreement with a recent report of a clear mismatch between the prevalence of *H pylori* serum antibodies and urea breath test results.49 of 100 healthy volunteers had *H pylori* antibodies, whereas only 24 had a positive 13C-urea breath test. Prevalence of serum antibodies in the absence of detectable *H pylori* bacteria has also been noted by other investigators.21-23 Meyer and co-workers suggest that their data may be evidence of spontaneous elimination of *H pylori* infection. However, this argument is based upon the assumption of persistent positive serum antibody concentrations after eradication of infection. This assumption has since been proven to be invalid by several follow-up studies that showed a strong decrease in antibody concentrations after eradication,44 therefore an extension of sensitivity and specificity testing of the methods of breath testing and serology measurement used by Meyer et al is needed. Nevertheless, our data on negative seroconversion support the hypothesis of possible spontaneous elimination of infection. We were able to eliminate the possibility of past use of bismuth or double/triple therapy regimens. However, incidental eradication of *H pylori* by the widespread use of antibiotics cannot be excluded in our population.

Long-term storage of sera at −20°C and thawing of the samples may have affected our test results. However, the difference of less than 5% between the overall means of test results of first and follow-up visits, suggests no effect of storage and thawing. This accords with the results of Nomura and colleagues who excluded any effect even after storage for 20 years at −20°C.

Veenendaal et al found a weak, but significant positive correlation (r = 0.27) between age and antibody concentrations in 70 patients with *H pylori* infection. They speculate that this finding might reflect progression of chronic superficial gastritis because of a rise in serum pepsinogen A and C. Others, however, disagreed. Our results show a total absence of any correlation between age and antibody concentrations (r = 0.01 and 0.02 for 1980 and 1992, respectively; figure 3). Moreover, no increase in antibody concentration was found after 11.5 years of follow-up. Finally, we show that serum antibody concentrations fluctuate during longterm follow-up. Whether this finding reflects fluctuation in the grade of gastritis activity
and *H pylori* colonisation needs to be investigated. Long-term follow-up studies may shed new light upon the accepted idea of persistent infection with unchanged activity, until atrophic gastritis and intestinal metaplasia develop as end-stage disease. Differences in behaviour of different *H pylori* strains may be important in the definition of gastric cancer risks and may explain discrepant results\(^{6-24}\) of epidemiological studies in different countries.

This study was made possible by financial support of the Netherlands Digestive Diseases Foundation.

References


