Seroconversion and seroreversion in IgG antibodies to Helicobacter pylori: a serology based prospective cohort study

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Abstract

Study objectives—To assess the incidence of seroconversion and seroreversion in IgG antibodies to Helicobacter pylori within a 11 year observation period using these events as markers for acquisition and loss of the infection, respectively.

Design—Population based prospective cohort study

Setting—Northern part of Copenhagen County, Denmark

Participants and methods—A random sample of 2527 Danish adults were examined and blood obtained in 1983 and in 1994. Matching pairs of sera were analysed for the presence of IgG and IgM antibodies to H pylori with an in house enzyme linked immunosorbent assay. Participants who were seronegative at study entry and seropositive at follow up and had a fourfold increase in baseline IgG antibody levels were categorised as seroconverters and regarded as having acquired H pylori infection. Participants who were seropositive at study entry and had at least a fourfold decrease in baseline IgG antibody levels at follow up were assumed to have lost the infection (seroreverters).

Results—The seroprevalence of H pylori infection was 24.7 (95% confidence intervals (95% CI) 23.0, 26.4) % in 1983 and 24.5 (95% CI 22.8, 26.2) % in 1994. A total of 14 participants seroconverted within the observation period (cumulative 11 year incidence proportion: 1.0 (95% CI 0.5, 1.5) %). Having increased IgM antibody levels at study entry significantly increased the likelihood of IgG seroconversion (relative risk 6.4 (95% CI 2.1, 19.6). Seroreversion was seen in 48 participants (cumulative 11 year incidence proportion: 7.7 (95% CI 5.6, 9.8) %).

Conclusions—Changes in H pylori infection status with time are rare in Danish adults. Few adults become infected with H pylori in Denmark. Helicobacter pylori is a human pathogen bacterium that causes chronic gastritis, peptic ulcer disease, and is a probable cause of gastric cancer. Although most cases of H pylori infection are acquired in childhood, cross sectional studies have shown that the prevalence of infection increases steadily with age. It is generally agreed that this age related increase in prevalence is attributable to a cohort phenomenon—that is, that certain birth cohorts have been exposed to H pylori early in life and retain a high infection rate throughout life. First time H pylori infection in adult life is rare and annual incidence rates have been low ranging from 0.006 to 1.1 per cent. Whereas spontaneous eradication of H pylori infection after primary acquisition of the bacteria has been reported in infancy, spontaneous eradication seems to be rare in adults. Once acquired, the bacterium stays in the stomach for life in most cases unless gastric atrophy develops. Incidental eradication of H pylori secondary to antimicrobial treatment of other infections is probably unlikely, but H pylori can be eradicated by a triple or quadruple therapy that combines an antisecretory drug with different antimicrobials. When eradication is successful, serum levels of IgG and IgA antibodies directed against H pylori surface antigens decline significantly within two to six months but do not return to pre-infectious levels.

The aim of this study was to assess the cumulative 11 year incidence proportion of IgG seroconversion and seroreversion as markers for acquisition and loss of H pylori infection, respectively in a random population of Danish adults.

Methods

STUDY POPULATION AND RESPONSE PATTERN

In October 1982, a population study governed by the World Health Organisation for the Monitoring of Trends and Determinants in Cardiovascular Disease (DAN MONICA) was initiated at the Copenhagen County Centre of Preventive Medicine at the Glostrup University Hospital, Denmark. An age and sex stratified sample consisting of 2404 men and 2403 women, (age 30, 40, 50, and 60 years) was drawn from the National Danish Civil Registration System, in which all people who live in Denmark are registered by a unique 10 digit number. After 226 people of foreign extraction had been excluded, 2280 Danish men and 2301 women (study base 1) were invited to a general health examination by a standard letter containing information about the project (fig 1). Also enclosed was a self administered questionnaire concerning medical history, drug consumption within the preceding five years, and health and lifestyle practices. Between November 1982 and February 1984, 3608 people (response rate 78.8 per cent) (m/f=1843/1765) entered the study (fig 1). Data on the non-responder group (n=973) were as previously reported.

By January 1993, 450 members of study base 1 had died and one person had disappeared. The remaining 4130 sample members of
Danish extraction (study base 2) were re-invited to a follow up examination. A total of 2656 people (response rate 64.3 per cent) (m/f=1333/1323) attended the follow up examination that took place between June 1993 and December 1994 (fig 1). Among non-responders at follow up, 460 people were interviewed by telephone, 57 people had died between invitation and scheduled examination, 458 people refused to participate, and 499 people could not be reached.

The project was approved by the Regional Research Ethics Committee of the Copenhagen County.

MATCHING OF SERUM SAMPLES
Serum samples were obtained from 3590 participants at study entry and from 2541 participants at follow up. Sera obtained at study entry were thawed for the first time and analysed in June 1993.30 Sera obtained at follow up were analysed in September 1995 (fig 1). Matching pairs of sera were identified in 2527 cases. This study is based on these eligible pairs of sera.

DETECTION OF IgG AND IgM ANTIBODIES TO *H. PYLORI*
IgG antibodies against a low molecular weight fraction of *H. pylori* antigens and IgM antibodies against heat stable *H. pylori* antigens were measured in duplicate with an in house indirect enzyme linked immunosorbent assays (ELISA) as previously described.30–32 The IgG serology had been validated in a mixed population of 250 Danish adults with dyspeptic symptoms; a population that was comparable to the present study population in terms of sociodemographic

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* The numbers in parentheses are the number of non-responders from whom information was obtained through telephone interviews or postal questionnaires. † Excluded because of foreign extraction.

**Figure 1** Sampling and response pattern in 4581 Danish adults who were invited for a population study in 1982.
Table 1  The IgG seroprevalence % (n+), of H pylori infection in 1983 and 1994 in 2278 Danish adults, by sex and age

<table>
<thead>
<tr>
<th>Age in 1983 (y)</th>
<th>IgG seroprevalence % (n•)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>13.5 (43)</td>
</tr>
<tr>
<td>40</td>
<td>30.5 (97)</td>
</tr>
<tr>
<td>50</td>
<td>27.0 (86)</td>
</tr>
<tr>
<td>60</td>
<td>28.9 (92)</td>
</tr>
<tr>
<td>All†</td>
<td>24.9 (318)</td>
</tr>
</tbody>
</table>

• n is the number of IgG seropositive cases. †IgG seropositivity

Table 2  IgG seroprevalence % (n+), of H pylori infection at study entry (responders and non-responders) and at follow up

<table>
<thead>
<tr>
<th>Anti-H pylori IgG status at follow up</th>
<th>Seronegative</th>
<th>Borderline</th>
<th>Seropositive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seronegative</td>
<td>1315 (52.0)</td>
<td>83 (3.3)</td>
<td>22 (0.9)</td>
<td>1420 (56.2)</td>
</tr>
<tr>
<td>Borderline</td>
<td>102 (4.0)</td>
<td>313 (12.4)</td>
<td>73 (2.9)</td>
<td>488 (19.3)</td>
</tr>
<tr>
<td>Seropositive</td>
<td>14 (0.6)</td>
<td>76 (3.0)</td>
<td>529 (20.9)</td>
<td>619 (24.5)</td>
</tr>
<tr>
<td>Responders*</td>
<td>1431 (56.0)</td>
<td>472 (18.7)</td>
<td>624 (24.7)</td>
<td>2527</td>
</tr>
<tr>
<td>Non-responders*</td>
<td>544 (21.8)</td>
<td>203 (8.0)</td>
<td>303 (12.0)</td>
<td>1050</td>
</tr>
</tbody>
</table>

* Sera were available from 1050 non-responders.

**KEY POINTS**

- Serological signs of acquisition of *H pylori* infection are uncommon in Danish adults. Studies on which factors predispose to the infection should focus on factors exerting their effect in childhood.
- The seroprevalence of *H pylori* infection in Danish adults does not change markedly with time. A single baseline assessment of *H pylori* infection status is probably sufficient for aetiological studies on *H pylori* related diseases.
- Only a minor proportion of people with solitary increased IgM antibodies to *H pylori* develops serological signs of long term *H pylori* infection.

Factors and ethnicity. The sensitivity, specificity, positive predictive value, and negative predictive value was 98.5, 54.0, 76.1, and 96.2 per cent, respectively. Borderline cases were people with indeterminate IgG antibody levels between the cut off points for seronegativity and seropositivity. The assay was previously tested against commercially available assays and yielded similar sensitivity and specificity values when applied to a mixed population.

To rule out changes in the ELISA procedure between the initial and the follow up analysis affected antibody measurements, sera from people who changed antibody status and from people who showed a significant increase in IgG antibody levels within this period were re-tested pair wise on the same ELISA plate.

The 11 year cumulative incidence proportion of IgG seroconversion (11 year seroconversion) according to definition (1) was calculated for participants who remained at risk of acquiring a first time *H pylori* infection at study entry—that is, participants who were seronegative at study entry. When definition (2) was applied the population at risk also included participants with borderline increased IgG antibody levels at study entry. The 11 year cumulative incidence proportion of IgG seroreversion (11 year seroreversion) was calculated in participants who remained at risk of acquiring a first time *H pylori* infection. To assess the validity of a solitary IgM increase as a marker for primary infection, IgG seroconversion rates were compared in this subgroup and in IgG negative people at follow up.

The SPSS statistical package for Windows was used. The 11 year cumulative incidence proportion of IgG seroconversion (11 year seroconversion) according to definition (1) was calculated for participants who remained at risk of acquiring a first time *H pylori* infection at study entry—that is, participants who were seronegative at study entry. When definition (2) was applied the population at risk also included participants with borderline increased IgG antibody levels at study entry. The 11 year cumulative incidence proportion of IgG seroreversion (11 year seroreversion) was calculated in participants who remained at risk of acquiring a first time *H pylori* infection. To assess the validity of a solitary IgM increase as a marker for primary infection, IgG seroconversion rates were compared in this subgroup and in IgG negative people at follow up.

**RESULTS**

**IGG SEROPREVALENCES IN 1983 AND 1994**

Whereas IgG seroprevalences increased significantly with age in women at both attendances IgG seroprevalences did not change significantly after age 40 in men (table 1). The overall seroprevalence of *H pylori* infection remained unchanged within the observation period. When stratified by age, minor and insignificant changes in age specific seroprevalences were seen between 1983 and 1994.
Table 3  The 11 year incidence of seroconversion and seroreversion % (n) in IgG antibodies to H pylori in 2527 Danish adults, by sex and age

<table>
<thead>
<tr>
<th>Age in 1983 (y)</th>
<th>IgG seroconversion % (n)*</th>
<th>IgG seroconversion %, (n)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men % (n)</td>
<td>Women % (n)</td>
</tr>
<tr>
<td>30</td>
<td>0.85 (2)</td>
<td>1.19 (3)</td>
</tr>
<tr>
<td>40</td>
<td>0.52 (1)</td>
<td>0.55 (1)</td>
</tr>
<tr>
<td>50</td>
<td>1.55 (3)</td>
<td>0.57 (1)</td>
</tr>
<tr>
<td>60</td>
<td>2.00 (2)</td>
<td>0.95 (1)</td>
</tr>
</tbody>
</table>

Annual incidence per 1000 persons at risk

<table>
<thead>
<tr>
<th>Men % (n)</th>
<th>N (95% CI)</th>
<th>Women % (n)</th>
<th>N (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.12 (0.35, 1.89)</td>
<td>0.84 (0.17, 1.51)</td>
<td>1.88 (1.02, 2.74)</td>
<td>2.11 (1.20, 3.03)</td>
</tr>
</tbody>
</table>

There was an insignificant tendency towards higher prevalences of H pylori infection in men than in women in 1994.

NON-RESPONDERS AT FOLLOW UP

Cohort members that did not attend the follow up examination (non-responders) were more likely than responders to be old (relative risk 1.7 95% CI 1.5, 1.9) and to report low socioeconomic status (relative risk 1.6 95% CI 1.4, 1.8). The seroprevalence of H pylori infection at study entry was significantly higher in non-responders than in responders (table 2).

IGG SEROCONVERSION

According to definition (1) 14 people seroconverted in IgG antibodies within the observation period (tables 2 and 3). A total of 1431 people were seronegative at study entry and remained at risk of acquiring H pylori infection. The 11 year seroconversion rate for both sexes was 1.0 (95% CI 0.5, 1.5) % (table 3). When definition (2) was used, 38 people seroconverted among 1903 people at risk of acquiring H pylori infection resulting in a 11 year seroconversion rate of 2.0 (95% CI 1.4, 2.6) % (table 3). There was a tendency towards higher seroconversion rates with age in men. No sex differences were seen in seroconversion rates.

IGG SEROCONVERSION IN PEOPLE WITH SOLITARY INCREASED IGM ANTIBODY LEVELS AT STUDY ENTRY

A total of 48 people who had increased IgM antibody levels unaccompanied by an IgG or IgA antibody response at study entry were examined at follow up. Three persons (6.3 per cent) seroconverted in IgG antibodies within the observation period. Relative risk of seroconversion in this subgroup compared with people who were IgG seronegative at study entry was 6.4 (95% CI 2.1, 19.6).

IGG SEROREVERSION (LOSS OF H PYLORI INFECTION)

A total of 48 people were IgG seropositive at study entry and had a fourfold decrease in baseline IgG antibody levels within the observation period, suggesting seroreversion (table 3). The 11 year seroreversion rate was 7.7 (95% CI 5.6, 9.8) %. Among this group, 22 people were seronegative at follow up, whereas the remaining 26 people had antibody levels in the indeterminate region (table 2). There was an insignificant tendency towards higher seroreversion rates among women compared with men (relative risk 1.3 (95% CI 0.8, 2.3)). Young and middle aged people had slightly higher seroreversion rates than older people (table 3).

USE OF ANTIMICROBIALS AND IGG SEROREVERSION

Two people among 48 seroreverters had used antibiotics on one or more occasions within the past five years, whereas the corresponding numbers were 29 among 529 participants who tested IgG seropositive at both attendances.

The relative risk of seroreversion was 0.8 (95% CI 0.2, 2.9) for antimicrobial users compared with those who had not used antimicrobials.

Discussion

In this population based prospective cohort study comprising more than 2500 Danish adults a low incidence of seroconversion in IgG antibodies to H pylori and a high incidence of seroreversion was found. Given that these events reflect acquisition and loss of H pylori infection, respectively, the overall prevalence of the infection may, perhaps also attributable to a cohort phenomenon, decline within the next decades.

The clinical picture of acute H pylori infection has been described in a few anecdotal reports. These infections have manifested mild, but transient dyspeptic complaints, that are likely to go undiagnosed. The low H pylori infection incidence rate found in this study supports the observation that acute H pylori induced gastritis is rarely seen in daily clinical work. The only lower seroconversion rate was found in a Canadian population. The latter study had a short observation period (two to three years) and only one person seroconverted (table 4). H pylori infection rates in Denmark are generally low. For this reason, the risk of being exposed to H pylori is small.

The response rate at follow up was low and non-responders more often emanated from poor socioeconomic levels. As H pylori infections are more frequent in deprived people, it is possible that non-responders were more likely to acquire H pylori infection than responders. This bias could result in an underestimation of the incidence of IgG seroconversion.
Table 4  Annual incidence (%) of first time H pylori infection in comparable studies

<table>
<thead>
<tr>
<th>Author (ref)</th>
<th>Year of publication</th>
<th>Country</th>
<th>H pylori detection method</th>
<th>Number</th>
<th>Ages (y)</th>
<th>Number of seroconverters</th>
<th>Observation period</th>
<th>Number of observation years</th>
<th>Annual incidence (%) and 95% CIs</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parsonnet13</td>
<td>1992</td>
<td>US</td>
<td>IgG ELISA</td>
<td>341</td>
<td>30–50</td>
<td>11</td>
<td>1969 to 1987/88</td>
<td>8.5</td>
<td>0.49 (0.3, 0.9)*</td>
<td>The antigen was a pool of sonicated isolates of H pylori. Sensitivity and specificity exceeded 95%</td>
</tr>
<tr>
<td>Kuipers14</td>
<td>1993</td>
<td>Netherlands</td>
<td>IgG ELISA</td>
<td>115</td>
<td>15–80</td>
<td>2</td>
<td>1979/83 to 1992</td>
<td>11.5</td>
<td>0.30 (0.04, 1.08)</td>
<td>IgG and IgA antibodies were measured with flow cytometric immunofluorescent assay. Only published in abstract form.</td>
</tr>
<tr>
<td>Veldhuyzen van Zanten15†</td>
<td>1992</td>
<td>Canada</td>
<td>IgG and IgA ELISA</td>
<td>175</td>
<td>18–71</td>
<td>1</td>
<td>—</td>
<td>2–3</td>
<td>0.0024</td>
<td>Acid-glycine extracted antigen. Sera from patients with chronic gastritis were tested.</td>
</tr>
<tr>
<td>Cullen16</td>
<td>1990</td>
<td>Australia</td>
<td>IgG ELISA</td>
<td>141</td>
<td>20–65</td>
<td>6</td>
<td>1969/1978/1990</td>
<td>21</td>
<td>0.33 (0.08, 0.59)*</td>
<td>Only published in abstract form.</td>
</tr>
<tr>
<td>Banatvala20</td>
<td>1994</td>
<td>Japan</td>
<td>IgG ELISA</td>
<td>417</td>
<td>—</td>
<td>7</td>
<td>1980 to 1989/90</td>
<td>10</td>
<td>1.1</td>
<td>Participants were endoscoped but not bled at study entry. Acid-glycine extracted antigen. Seroconversion rates increased with age.</td>
</tr>
<tr>
<td>Sipponen18</td>
<td>1996</td>
<td>Finland</td>
<td>IgG and IgA ELISA</td>
<td>181</td>
<td>53 (±2)</td>
<td>12</td>
<td>1974 to 1991</td>
<td>15</td>
<td>0.44 (0.23, 0.80)</td>
<td>Acid-glycine extracted antigen. Seroconversion rates increased with age.</td>
</tr>
<tr>
<td>Rosenstock‡</td>
<td>2000</td>
<td>Denmark</td>
<td>IgG ELISA</td>
<td>2,327</td>
<td>30–72</td>
<td>14</td>
<td>1982/83 to 1994/95</td>
<td>11</td>
<td>0.01 (0.04, 0.14)</td>
<td>Normal population, LMW antigen, specificity 98.5%, sensitivity 54%</td>
</tr>
<tr>
<td>Valle23</td>
<td>1996</td>
<td>Finland</td>
<td>Histology</td>
<td>102</td>
<td>15–55</td>
<td>2</td>
<td>1952 to 1983</td>
<td>32</td>
<td>0.4 (0.04, 1.0)†</td>
<td>Outpatients with dyspepsia. H pylori infection status at study entry was assessed by biopsy.</td>
</tr>
</tbody>
</table>

*Annual incidence per patient year. †The observation period was not reported. ‡Annual incidence proportion in per cent for both sexes.
Incidence of first time Helicobacter pylori infection

frequent in the older age groups. As the likelihood of gastric atrophy increases with the duration of the infection and thereby with age, atrophic gastritis is probably not a valid explanation for the high seroreversion rate.

The applicability of this study would improve, if H pylori infection status was assessed with another diagnostic test. Large scale screening necessitates non-invasive screening methods. Although urea breath tests and western blotting are more accurate than serology, their sensitivity and specificity do not reach 100 per cent. 

The prevalence of solitary increased IgM antibody levels to H pylori in this population was previously reported. Although the IgG seroreversion rate was significantly higher in people who had solitary increased IgM antibodies at study entry as compared with those who were seronegative, most participants with solitary increased IgM levels remained IgG seronegative. An isolated increase in IgM antibody, therefore, may not be a reliable marker for primary H pylori infection in Danish adults. It is also possible that some episodes of first time H pylori infection may have been cleared spontaneously without causing a detectable increase in IgG antibody levels.

Two findings in this study support this theory that the age related increase in the seroprevalence of H pylori infection seen in this and similar studies is attributable to a cohort phenomenon rather than a constant infection rate. Firstly, IgG seroprevalences remained stable between study entry and follow up. Secondly, the seroreversion rate does not account sufficiently for the increase in IgG seroprevalence with age. The prevalences of peptic ulcer disease and gastric cancer are declining in the Western world. More studies are needed to determine whether this is attributable to a decrease in the incidence of H pylori infections or to the gradual extinction of generations who have carried a high prevalence of H pylori infection throughout life.

In conclusion, the rate of IgG seroreversion in H pylori antibodies is low in Danish adults. As loss of the infection, evidenced by IgG seroreversion, was seen more often than seroclearance, it is possible that the prevalence of H pylori infection will decline within the decades to come. This may subsequently bring on a parallel decline in H pylori induced disorders such as peptic ulcer, chronic gastritis and possibly gastric cancer.

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