Music is the medicine of a troubled mind.
Chapter IV

The role of Helicobacter pylori and autoimmunity in serological atrophic corpus gastritis in a Dutch primary care community


Keywords: serological atrophic corpus gastritis, primary care community, pepsinogen, gastrin, Helicobacter pylori infection, parietal cell antibodies

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Abstract

Background & Aims: Atrophic corpus gastritis predisposes to the development of vitamin B12 deficiency and gastric cancer. Little is known about the seroprevalence of atrophic corpus gastritis in primary care communities in Western Europe. The Dutch province Zeeland has a fairly stable resident population with predominantly indigenous people and is therefore an appropriate area for epidemiological investigation. The aim of this study was to investigate the seroprevalence of atrophic corpus gastritis and the involvement of Helicobacter pylori (H.pylori) infection and autoimmunity in a West-European primary care community.

Methods: 997 consecutive subjects (455 males and 542 females; mean age (±SD) 52 (±16) years) in a single Dutch general practice were asked to participate in this study by filling in a questionnaire and donating a fasting blood sample. Gastrin, pepsinogen A and C and antibodies to H.pylori were measured in serum by well-validated immunological methods. Our validated criteria for serological advanced atrophic corpus gastritis, were a serum concentration of PgA < 17μg/l, a PgA/C ratio < 1.6 and a serum level of gastrin > 100ng/l. The resultant atrophic corpus gastritis-group was compared to a nested case-control group of subjects without serological atrophy matched for age and sex, ran-
domly chosen from the total study group of patients. In the atrophic corpus gastritisp

**Results:** 34 subjects (3.4%) fulfilled the serological criteria of advanced atrophic corpus gastritis; 21 (62%) of them compared to 17 of 34 (50%) age- and sex matched nested controls were *H. pylori* positive (n.s.). In the atrophy group 15 subjects had anti-PC (44%) compared to one (3%) in the control group (p<0.005). When comparing to the nested case controls the relative risk of having atrophic corpus gastritis is 1.62 (0.62-4.24) for *H. pylori* infection, and 24 (3-201) for anti-PC. In the atrophy group 5 persons had anti-IF (15%) and 2 in the control group (6%; ns). Four of the 5 patients with atrophy who had anti-IF, also had anti-PC. Seven atrophy persons had both *H. pylori* infection and anti-PC; 5 atrophy subjects had neither anti-*H. pylori* nor anti-PC and anti-IF. Nine of the 25 subjects with atrophic corpus gastritis had lowered vitamin B_{12} levels in serum. Persons with atrophic gastritis did not differ from controls regarding the presence or history of gastric complaints.

**Conclusions:** The seroprevalence of advanced atrophic corpus gastritis in a primary care community in The Netherlands is 3.4%. Both autoimmunity and *H. pylori* infection appear to be relevant for the pathogenesis of atrophic corpus gastritis. When compared to controls the odds ratio of having atrophic corpus gastritis was significantly higher (p<0.025) for parietal cell antibodies (24, 95% CI, 3.00 – 201) than for *H. pylori* antibodies (1.62, 95% CI, 0.62-4.24). In view of the decreasing risk of *H. pylori* infection in the Western world, it is likely that the impact of *H. pylori* on the development of atrophic corpus gastritis will further diminish.

**Introduction**

Atrophic corpus gastritis may lead to vitamin B_{12} deficiency due to reduced intrinsic factor production\(^{11}\). It further predisposes to the development of gastric cancer \(^{14-6}\).

Little is known about the seroprevalence of atrophic corpus gastritis in primary care communities in Western Europe. Early serological identification of individuals with gastric precancerous conditions followed by endoscopy, would be a desirable aim of a screening program in the general population \(^{8,9}\).

The province of Zeeland in The Netherlands is a good region to survey with a stable and predominantly indigenous population. The rural inhabitants of this area are a relatively homogeneous group of Dutch people with large families. Zeeland was up to 50 years ago a fairly isolated part of the country in the geographical delta of the river Schelde. This stable population seems to be an ideal target group for epidemiological screening studies in a primary care community.

Screening tests should ideally be convenient, virtually free of discomfort or risk
and economically attractive. Gastric endoscopy with biopsy is a commonly used test for the diagnosis of chronic gastritis but is invasive and as such has none of these characteristics. Its value is further hampered by sampling problems of biopsies. A reliable serological test would be preferable.

The serological gastric biopsy fulfills the above mentioned screening test criteria and might be a reliable diagnostic instrument to identify gastric mucosal disorders. Taking a serological gastric biopsy means the measurement of the serum concentration of the gastric secretory products pepsinogen A, pepsinogen C and gastrin. Prior to the discovery of Helicobacter pylori, serum pepsinogen levels had been shown to vary with gastric mucosal histology. Samloff demonstrated a progressive fall in serum pepsinogen A (PgA) with increasing degrees of gastric fundus atrophy, reflecting loss of zymogen producing cells. A less marked fall in serum pepsinogen C (PgC) occurs in atrophic corpus gastritis because PgC (unlike PgA) is also produced by pyloric glands of the antrum and in Brunner’s glands in the duodenum. These non-parallel changes result in a progressive lowering of the PgA/C ratio with increasingly severe gastric fundus atrophy. Very low PgA-levels and a low PgA/C ratio are accurate predictors of severe atrophic corpus gastritis and are frequently found in gastric cancer.

In the setting of general practice the diagnostic value of the gastric serum markers has had no attention up to now, probably because of unfamiliarity with the subject among general practitioners. Therefore our present study was undertaken to evaluate the performance of the serological gastric biopsy at the primary care level.

The first aim of this study was to investigate the prevalence of atrophic corpus gastritis by serology in a Dutch primary care community.

The second aim was to determine whether subjects with serological atrophic body gastritis have evidence of H. pylori infection and/or gastric autoimmunity. H. pylori is a major risk factor for chronic gastritis and plays a critical part in the promotion of atrophic gastritis. Gastric autoimmunity plays an important part in the progression of atrophic corpus gastritis after H. pylori infection and also independent of H. pylori infection.

Because H. pylori-associated atrophic corpus gastritis is a risk factor for gastric cancer, it might be clinically important to prevent atrophic gastritis by eradicating H. pylori.

Subjects and Methods

Subjects
In a period of 2 years, a total of 997 consecutive subjects, 455 males and 542 females, mean age 52 years (±SD 16 year), was enrolled from the general practice ’s-Gravenpolder in The Netherlands. The subjects had made an appointment to consult the family doctor for common medical problems and were asked to par-
participate in the study by donating a fasting blood sample and filling in a questionnaire with questions covering upper gastrointestinal symptoms, past gastric diseases, family history of gastric disorders and the use of acid lowering drugs or antibiotics. The minimum age was 18 years while there was no upper age limit. Pregnant patients and patients with gastric resection, renal insufficiency and those using antisecretory agents or antibiotics recently were excluded from the analysis. The study was performed according to the declaration of Helsinki and all patients gave informed consent.

Methods
All serum samples were examined by well-validated radioimmunoassays for pepsinogen A and C and gastrin. H. pylori serology was performed by a validated enzyme immunoassay detecting specific immunoglobulin G against a homogenate of 6 strains of H. pylori. Western blots of this homogenate showed the presence of CagA bands, indicating a cytotoxic variety of H. pylori. The results were expressed as the absorbance index (AI): serum with an AI > 0.32 IgG H. pylori antibody was considered evidence of H. pylori infection.

Our validated criteria for advanced atrophic corpus gastritis, corresponding to pentagastrin refractory achlorhydria or severe hypochlorhydria (PAO < 5mmol/hr), were a serum concentration of PgA < 17 µg/l, a PgA/C ratio < 1.6 and an accompanying serum concentration of gastrin > 100 ng/l. Parietal cell autoantibodies were analysed using a commercially available kit (Autoscreen 1, Scimedx Corporation, Denville, NJ 07834, USA). Serum vitamin B12 levels were measured by radioimmunoassay.

Subjects with serological atrophic corpus gastritis were compared to an age and sex matched nested case-control group without serological evidence of atrophic corpus gastritis randomly chosen from the total study group of participants. In the case of more than one corresponding control subject, one subject was at random chosen.

Statistical analysis
Results were analysed using Chi-square test with Yates correction. The relative risk, its 95 percent confidence interval and comparison of different relative risks were performed using standard statistical methods.

Results
Atrophy findings
34 (3.4%) of the 997 subjects fulfilled the serological criteria of advanced atrophic corpus gastritis, of which 19 were female (56%). The mean age in the atrophy group was 67 years (range 28-91) compared to 52 years (range 18-91) in the total group (Figure 1).
Figure 1. Age distribution of 34 subjects with serological atrophic corpus gastritis (filled bars) compared to the 963 subjects without (open bars).

Figure 2. Helicobacter pylori positivity in relation to age in 997 subjects from a general practice in the Netherlands (filled bars: H. pylori positive; open bars: H. pylori negative).
**H. pylori findings**

In this study of 997 subjects, 272 persons (27%) were *H. pylori* positive; *H. pylori* was positive in 14% of the subjects younger than the mean age of the total group of 52 years, compared to 40% of the subjects older than 52 years. The 50% infection rate starts from the 7th age decade illustrating the cohort effect of *H. pylori* infection in the general population in the Netherlands (Figure 2).

The *H. pylori* infection rate of subjects with serological atrophic corpus gastritis (62%) differed significantly from that of the whole group (27%) (p<0.0001). However, the *H. pylori* infection rate in the 34 subjects with serological atrophic corpus gastritis (62%) did not significantly differ from the 34 subjects in the nested case control group (50%; p = 0.46; table 1), resulting in an odds ratio (95 percent confidence interval) of 1.62 (0.62-4.24). Nevertheless, according to the well-known *H. pylori* cohort effect in Western countries, it is difficult to look for a statistical significance comparing two elderly populations (mean ages 67 and 65 years) where a high anti-*H. pylori* seroprevalence is expected.

**Parietal cell antibody findings**

In the whole cohort of 997 subjects, 3% had a personal or family history of autoimmune disease. In the group with serological atrophic corpus gastritis, this percentage was 6% and in the selected controls nobody had a personal or family history of autoimmune disease.

In the serological atrophic corpus gastritis group, 15 (44%) subjects had parietal cell antibodies, compared to 1 (3%) person in the nested control group (p<0.005, table 1), resulting in an odds ratio of 24 (95% CI, 3-201).

Eight subjects with serological atrophic corpus gastritis and anti-parietal cell antibodies were anti-*H. pylori* negative (table 2). This subset of subjects had a mean age of 63 years (range 28-89), 2 of them did have other autoimmune disorders and 3 of them showed slight macrocytic anaemia.

In the atrophic corpus gastritis group 7 persons had both parietal cell and *H. pylori* antibodies. In contrast, 5 patients had *neither* parietal cell nor *H. pylori* antibodies (table 2).

**Intrinsic factor antibody findings**

In the serological atrophic corpus gastritis group, 5 (15%) persons had intrinsic factor antibodies, compared to 2 (6%) subjects in the nested control group (n.s.).

In the atrophic gastritis group, 4 persons with antibodies to intrinsic factor had also antibodies to parietal cells.

**Possible false-negative *H. pylori* serology**

Four subjects with serological atrophic corpus gastritis had an *H. pylori* absorbance index between 21-30 compared to one person in the control group. These 4 subjects had no antibodies to parietal cells. It cannot be excluded that a relatively high but
The role of Helicobacter pylori

Table 1. Characteristics and findings of 34 subjects with serological atrophic corpus gastritis compared to 34 nested case-control subjects without serological evidence of atrophic corpus gastritis.

<table>
<thead>
<tr>
<th></th>
<th>Serological atrophic corpus gastritis group N=34</th>
<th>Nested case-control group N=34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M / F)</td>
<td>15 / 19</td>
<td>15 / 19</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>67 (28 – 91)</td>
<td>65 (28 – 89)</td>
</tr>
<tr>
<td>History of complaints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>59%</td>
<td>56%</td>
</tr>
<tr>
<td>Seldom</td>
<td>15%</td>
<td>18%</td>
</tr>
<tr>
<td>Sometimes</td>
<td>20%</td>
<td>9%</td>
</tr>
<tr>
<td>Often</td>
<td>6%</td>
<td>11%</td>
</tr>
<tr>
<td>Almost always</td>
<td>0%</td>
<td>6%</td>
</tr>
<tr>
<td>Gastrin (ng/l)</td>
<td>1256 (102 – 4500)</td>
<td>45 (18 – 108)</td>
</tr>
<tr>
<td>Pepsinogen A (.g/l)</td>
<td>6 (1 – 16)</td>
<td>33 (12 – 128)</td>
</tr>
<tr>
<td>Ratio PgA/C</td>
<td>0.5 (0.1 - 1.4)</td>
<td>2.1 (1.4 – 5.0)</td>
</tr>
<tr>
<td>Anti-H. pylori</td>
<td>21 of 34 (62%)</td>
<td>17 of 34 (50%)</td>
</tr>
<tr>
<td>Anti-parietal cells</td>
<td>15 of 34 (44%)</td>
<td>1 of 32* (3%)*</td>
</tr>
<tr>
<td>Anti-intrinsic factor</td>
<td>5 of 34 (15%)</td>
<td>2 of 34 (6%)</td>
</tr>
</tbody>
</table>

* the serum of 2 subjects in the nested case-control group was disturbed by hetero-antibodies
* significant (P < 0.005)

Table 2. Prevalence of anti-H.pylori and anti-parietal antibodies in 34 subjects with serological atrophic corpus gastritis.

<table>
<thead>
<tr>
<th>Anti-parietal</th>
<th>+ ve</th>
<th>- ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ ve</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>- ve</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>13</td>
</tr>
</tbody>
</table>

+ ve = positive
- ve = negative
still normal H. pylori absorbance ratio between 21-30 represents persons with a recently acquired H. pylori infection prior to seroconversion or subjects with an infection in the past, a so-called serological H. pylori scar. Assuming that H. pylori infection in the past has contributed to atrophic gastritis in these 4 subjects, H. pylori may be involved in atrophic corpus gastritis in 25/34 (74%) compared to 18/34 (53%) in the nested case controls (p=0.13), resulting in a relative risk of having serological atrophic corpus gastritis for anti-H. pylori antibodies of 2.5 (0.9-6.8). In table 3 the prevalence of anti-H. pylori and anti-PC antibodies in subjects with serological atrophic body gastritis are presented assuming that the 4 persons with H. pylori absorbance ratio’s between 21-30 are H. pylori positive. Only one person with atrophic body gastritis had neither anti-H. pylori antibodies nor anti-PC antibodies.

**Table 3. Prevalence of anti-H.pylori antibodies adjusted for borderline H. pylori infection and anti-parietal cell antibodies in 34 subjects with serological atrophic corpus gastritis.**

<table>
<thead>
<tr>
<th>Anti-parietal</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>-ve</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>+ve</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>-ve</td>
<td>19</td>
<td>34</td>
</tr>
</tbody>
</table>

+ ve = positive  
- ve = negative

**Vitamin B₁₂ deficiency**

In 9 out of 25 subjects with serological atrophic corpus gastritis serum vitamin B₁₂ was low (< 120 pmol/l).

**History of gastric complaints**

Significant differences in gastric complaints between atrophic gastritis and non-atrophic gastritis participants could not be detected: 59% of atrophic-persons, versus 56% of non-atrophic subjects never had stomach complaints; 15% versus 18% seldom; 20% versus 9% sometimes; 6% versus 11% often and 0% versus 6% had a history of almost always complaints (table 1).

It is noteworthy that, analyzing the whole cohort of 997 subjects, 24% of the individuals had seldom to sometimes gastric complaints and 8% frequently. In table 1 the characteristics of the 34 persons with serological atrophic gastritis compared to their 34 nested case-control subjects are summarized.
Discussion

Our study clearly differs from all other studies on chronic atrophic gastritis because it is family practice based, carried out in a large unselected primary cohort and it uses a nested case-control group without serological atrophy. A limitation of secondary care studies and studies with volunteers (8) on the subject of chronic atrophic gastritis is the use of a highly selected group of patients, often with clinical disease. We enrolled consecutive subjects visiting a general practice for common health questions. The sample is self selected by attendance at the GP’s surgery but the study population can be considered as a reflection of the general population (31). In our consecutive sample of 997 general practice patients, 34 (3.4%) individuals were identified using serological markers, as having atrophic corpus gastritis. It was shown that the prevalence of H. pylori infection (62%) in subjects with serological atrophic corpus gastritis in a primary care community in Western Europe did not appear to be significantly higher compared to a nested control group without serological atrophic corpus gastritis (50%). On the contrary, the prevalence of antibodies to parietal cells, as a manifestation of autoimmunity, was significantly higher (44%) in the serological atrophy group than in the nested control group (3%). The odds ratio of having serological atrophic corpus gastritis was much higher (p <0.025) for parietal cell antibodies (24) than for H. pylori antibodies (1.62). It is therefore attractive to pose that, apart from H. pylori, autoimmunity is involved in the evolution of atrophic corpus gastritis in this part of the Netherlands. About the impact of H. pylori, it is interesting to note that an additional 4 subjects with seroatrophy showed anti-H. pylori IgG-levels just below the serological cut-off point, suggesting previous extinguished H. pylori infection. Inclusion of these 4 subjects augments the overall prevalence of H. pylori to 72%, a percentage which approaches the H. pylori prevalence in atrophic corpus gastritis found by Annibale et al (32). It is also noteworthy that 2 young female subjects with serological atrophic corpus gastritis, aged 28 and 42 years, respectively, had no antibodies to H. pylori but antibodies to parietal cells. At a young age it is very improbable that H. pylori antibodies have already been extinguished.

A study by Bins et al. in 1984 in another part of the Netherlands, investigating the prevalence of pentagastrin refractory achlorhydria in a general population by gastric intubation, revealed a prevalence of 2.4% (33). The difference with a prevalence of 3.4% severe atrophic corpus gastritis in our study might be due to the difference in the age of the study population. Bins et al. studied subjects and their spouses working in a factory resulting in a maximum age of about 65 years. In our study 24 of the 34 subjects with serological atrophic gastritis were over 65 years. Acquired infection with H. pylori in early childhood (34-36) and a host response in developing autoimmune reactions against organ specific tissue like the gastric mucosa are both aetiopathogenic factors in the evolution of atrophic gastritis.

- 57 -
and subsequently via intestinal metaplasia and dysplasia with progression to gastric cancer \(^{(1,37)}\).

These aetiopathogenetic factors may act separately but they may also influence each other leading to increased progression of atrophic changes. This increased progression to gastric atrophy may result in a decrease of the \(H.\ pylori\) infection rate shown in a gastric biopsy study \(^{(38)}\). Although anti-\(H.\ pylori\) antibodies remain positive for many years after \(H.\ pylori\) eradication from the gastric mucosa, it is not possible to exclude with certainty that the \(H.\ pylori\)-negative subjects in our study had been infected by \(H.\ pylori\) long ago \(^{(39)}\).

Our 7 atrophy subjects with both \(H.\ pylori\) and parietal cell antibodies illustrate their possible concomitant and/or sequential part in the progression of atrophic corpus gastritis \(^{(18,38)}\). It has been suggested that antibodies to parietal cells are secondary to the tissue damage induced by the \(H.\ pylori\) infection \(^{(40-42)}\). However, 8 of the subjects with parietal cell antibodies had no evidence of previous \(H.\ pylori\) infection, 2 of this group being quite young, aged 28 and 42 years.

As mentioned above, in 4 of the 5 atrophy subjects with negative serological antibody reaction to both \(H.\ pylori\) and parietal cells, the IgG absorbance index for \(H.\ pylori\) was just below the upper limit of normal, suggesting the possibility that the immune response to \(H.\ pylori\) is extinguishing. Because decades may pass between initiation and detection of gastric atrophy and the atrophic microenvironment promotes spontaneous eradication \(^{(39)}\), substantial misclassification of relevant exposure to \(H.\ pylori\) may occur. This misclassification due to fading \(H.\ pylori\) infection is much more relevant for studies using gastric biopsies than for studies using \(H.\ pylori\) serology. It is noteworthy that we used a type of enzyme immunoassay known to be most successful for documentation of previous \(H.\ pylori\) infection \(^{(26,43)}\).

In a review of the medication history of all the \(H.\ pylori\) negative subjects there was no evidence of use of antibiotics during the 4 years before blood sampling.

This study indicates that most patients with serological atrophic corpus gastritis did not present more frequently with a history of gastric complaints than did control subjects. Thus early detection of atrophic gastritis is not possible on account of complaints \(^{(7)}\). About 35% of patients with serological atrophic corpus gastritis in our study had low vitamin B\(_{12}\) serum levels as a silent clinical condition. Cobalamin supplementation is now possible.

This explorative study has the advantage that the population studied is enrolled in one general practice and that the research is supervised by the primary care physician himself who knows everybody personally and is familiar with the family roots of his patients. Furthermore, this general practitioner happens to be also pharmacist for his own patients, so the use of drugs is accurately documented and controlled.

In conclusion, the seroprevalence of atrophic corpus gastritis, in a Dutch primary care community is 3.4%. This study further shows that in the indigenous West-European population serological atrophic corpus gastritis is due both to \(H.\ pylori\)
The role of *Helicobacter pylori* infection and gastric autoimmunity. In our study at the primary care level serological atrophic corpus gastritis was significantly associated with gastric autoimmunity with an odds ratio of 24.0, while a non-significant odds ratio of 1.62 was found with *H. pylori* infection. In view of the decreasing risk of *H. pylori* infection in the Western world it is likely that the impact of *H. pylori* on the development of atrophic corpus gastritis will further diminish.

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