Is Helicobacter pylori Infection a Necessary Condition for Noncardia Gastric Cancer?

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Although the association between Helicobacter pylori infection and gastric cancer is well established, this association might have been underestimated in epidemiologic studies because of possible clearance of the infection in the course of disease development. The authors addressed this hypothesis in a case-control study from Saarland, Germany (68 cases first diagnosed between 1996 and 1998 and 360 controls), with serologic assessment of H. pylori infection in which various exclusion criteria were used to minimize potential bias from this source. Joint application of three such exclusion criteria (blood sample taken more than 90 days after gastrectomy, advanced (T4) gastric cancer, and CagA positivity in Western blot analysis despite a negative result in anti-H. pylori immunoglobulin G enzyme-linked immunosorbent assay) increased the odds ratio of noncardia gastric cancer from 3.7 (95% confidence interval (CI): 1.7, 7.9) to 18.3 (95% CI: 2.4, 136.7) for any H. pylori infection and from 5.7 (95% CI: 2.6, 12.8) to 28.4 (95% CI: 3.7, 217.1) for CagA-positive H. pylori infections. Furthermore, there was no single H. pylori-negative patient out of 32 patients with noncardia gastric cancer left after additional exclusion of subjects with borderline levels in immunoglobulin G enzyme-linked immunosorbent assay. The H. pylori-gastric cancer relation may be much stronger than previously thought, and H. pylori infection may even be a (close to) necessary condition for development of noncardia gastric cancer.

case-control studies; Helicobacter pylori; stomach neoplasms

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.
bacteria from the gastric mucosa a long time after patho-
physiologic changes possibly leading to gastric cancer have
been initiated.

In contrast to attempts to increase the precision of the esti-
mates of the *H. pylori*-gastric cancer association by meta-
analyses, attempts to increase the validity of the estimates by
overcoming and correcting for the putative underestimation
of the association due to underdetection of *H. pylori* infec-
tion have been scarce. Such underestimation may be
substantial: In one recent case-control study from Sweden
(10), the adjusted odds ratio of serologically defined *H. pylori*
infection for noncardia gastric cancer increased from
2.2 to 21.0, if subjects who were negative by immunoglob-
ulin G (IgG) enzyme-linked immunosorbent assay (ELISA)
but CagA positive by immunoblot analysis were removed
from the reference group of seronegative subjects (a pattern
that might be indicative of past infection with cagA-positive
*H. pylori* strains, as existing clinical data indicate that anti-
CagA antibodies persist longer after loss of the infection
than specific IgG antibodies detected by conventional
ELISA (11)). These intriguing findings suggest that more
detailed epidemiologic analyses aimed at overcoming poten-
tial bias due to underdetection of *H. pylori* infection may
give a more valid estimate of the *H. pylori*-gastric cancer
relation. Following these considerations, we carried out a
detailed reanalysis of a large case-control study on the asso-
ciation of *H. pylori* infection with gastric cancer conducted
in Germany.

**MATERIALS AND METHODS**

**Study design and study population**

This analysis is based on data of a statewide, population-
based study on risk factors, patterns of the diagnostic
process, and prognosis conducted among patients with
various forms of cancer in Saarland, Germany. Details of the
design of this study have been reported elsewhere (12).
Briefly, all patients with a first diagnosis of gastric cancer,
colorectal cancer, or breast cancer between November 1996
and February 1998 who were aged 80 years or less were
eligible for participation contingent on written informed
consent. Patients were recruited during first hospitalization
due to their cancer by 34 of 36 pertinent hospitals partici-
pating in the study in Saarland. Among the eligible subjects
reported to the study center by these hospitals, 2 percent died
before an interview could be conducted, and another 3
percent refused to participate.

In this case-control analysis, patients with a first diagnosis
of histologically verified gastric cancer (cases) are compared
with respect to *H. pylori* infection with patients with a first
diagnosis of histologically verified colorectal cancer, who
served as controls. This approach has also been used in two
previous reports that had specifically addressed the joint
impact of *H. pylori* infection and family history of gastric
cancer (13) and smoking (14) on gastric cancer risk.

**Data collection**

Data collection included personal standardized interviews,
collection of medical information from the hospital charts,
and drawing of blood samples. All interviews were conducted
by a team of four trained interviewers. Most inter-
views could be realized during hospitalization of the
patients, typically a few days after surgery, but in some cases
interviews had to be postponed until after discharge and, in
a few cases, were possible only several weeks to months after
surgery. Following the interviews, nonfasting blood samples
were obtained by venipuncture from 82 percent of the
patients who agreed to this additional component of the
study. In addition, detailed information on clinical features,
including the exact location, stage, and histology of the
tumors, was obtained from hospital charts and pathology
reports.

Serum samples were stored at −80°C and analyzed for the
presence of IgG antibodies to *H. pylori* by an ELISA (GAP
Assay; Bio-Rad Laboratories Diagnostics Group, München,
Germany). According to the manufacturer’s instructions,
levels above 20 units/ml were considered positive, and levels
below 12.5 units/ml were considered negative. Individuals
with borderline levels between 12.5 and 20 units/ml (17
percent of all subjects) were treated as negative in the initial
statistical analyses (which reflects common practice in
serology-based studies of the *H. pylori*-gastric cancer associ-
ation) and excluded in subsequent alternative statistical anal-
yses (see below). The Bio-Rad GAP Assay IgG test kit has
been extensively evaluated, and it performed well compared
with other commercial kits (15). The sera were further
analyzed for the presence of antibodies to the CagA antigen
using a commercial Western blot test (*H. pylori* Western
blot; Autoimmun Diagnostika GmbH (AID), Strasberg,
Germany). All laboratory analyses were carried out in
blinded fashion by trained personnel in a central laboratory
as previously described (13). However, in contrast to our
previous work, in which results of the Western blot analysis
had been available only among patients who were positive in
the IgG ELISA (13, 14), we meanwhile extended the
Western blot analyses to all cases and controls.

**Statistical analysis**

We compared the seroprevalence of *H. pylori* infection
between cases and controls according to the result of the Bio-
Rad GAP Assay test. Among seropositive subjects, a further
distinction was made between CagA-positive and CagA-
negative subjects. The association between the serostatus
of *H. pylori* infection and the risk of gastric cancer was quanti-
fied by odds ratios and their 95 percent confidence intervals
after adjustment for age and gender by multiple logistic
regression. Because of the small number of subjects in some
patient subgroups, logistic regression models using condi-
tional rather than unconditional maximum likelihood estima-
tion were used (16). All analyses were carried out using SAS
statistical software (SAS Institute, Inc., Cary, North Caro-
olina).

To address potential underestimation of the *H. pylori-
 gastric cancer association due to loss of the infection during

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the course of disease, we repeated the analyses after excluding, both separately (one at a time) and sequentially (in a cumulative manner), the following subgroups of study participants for whom *H. pylori* test results might not adequately indicate previous *H. pylori* exposure:

1. Patients with gastric cancer who could only be interviewed and whose blood samples were taken more than 3 months after surgery and patients who had developed gastric cancer after previous gastrectomy. These patients were excluded because seroreversion might be expected in the long run after total or subtotal gastrectomy, even though the prevalence of the infection appears to remain high in the absence of specific treatment (17, 18).

2. Patients with advanced (T4) gastric cancer, which often goes along with severely atrophic epithelium or areas of intestinal metaplasia, conditions under which *H. pylori* may not be able to survive (19).

3. Cases and controls who were seronegative according to the ELISA test for *H. pylori* IgG antibodies but who were found to be CagA positive in the Western blot. Such a pattern might reflect recent clearance of *H. pylori* infection (e.g., due to severe gastric atrophy), because the immune response against the CagA antigen may persist longer than the antibodies detected by IgG ELISA (11).

4. Cases and controls with borderline levels (12.5–20.0 units/ml) in the IgG ELISA, which might again reflect recent clearance of the infection (e.g., due to severe gastric atrophy). This suggestion is supported by recent work (20), which showed that older subjects with borderline IgG levels have a particularly high risk of gastric cancer.

Because previous studies have suggested that *H. pylori* primarily or even exclusively affects the risk of noncardia gastric cancer (5), all analyses were repeated including patients with noncardia gastric cancer in the case group only.

**RESULTS**

Overall, 68 cases and 360 control patients who agreed to give a blood sample and in whom *H. pylori* serostatus could be determined were included. The median ages were 64 years and 66 years among cases and controls, respectively. About 60 percent of both cases and controls were males; 98.4 percent of study participants were of German nationality.

The cases included two patients who had developed cancer at the anastomosis after previous gastrectomy and 11 patients with cancer at the gastroesophageal junction (*n* = 1) or the cardia (*n* = 10). Only 18 percent and 21 percent of the cases were diagnosed at stage T1 and stage T2, respectively, whereas 26 percent of the cases were diagnosed with a stage T4 cancer. All but two gastric cancer patients underwent gastric surgery, and in 76 percent of these patients, interview and venipuncture could be realized before or within 30 days after gastric surgery. There were seven cases (11 percent), however, who could only be interviewed more than 3 months after gastric surgery.

The majority of both cases and controls in the total sample were seropositive for *H. pylori* infection according to the IgG ELISA, but the prevalence was higher among cases (78 percent) than among controls (63 percent) (table 1). In particular, the proportion of subjects with CagA-positive infections was higher among cases (46 percent) than among controls (23 percent), and the differences were even larger when the case group was restricted to patients with noncardia cancer only.

The differences in overall seroprevalence (regardless of CagA status) result in odds ratios of 2.3 (95 percent confidence interval (CI): 1.2, 4.3) and 3.7 (95 percent CI: 1.7, 7.9) for total gastric cancer and for noncardia gastric cancer, respectively (table 2). The corresponding odds ratios for CagA-positive infections are 3.4 (95 percent CI: 1.7, 6.7) and 5.7 (95 percent CI: 2.6, 12.8), respectively, considerably higher than those for CagA-negative infections, which are 1.5 (95 percent CI: 0.8, 3.1) and 2.3 (95 percent CI: 1.0, 5.3), respectively. These odds ratios remained essentially unchanged when additional potential confounding variables, including smoking and level of school education, were controlled for in subsequent analyses.

After exclusion of eight patients with gastric cancer whose serum samples were obtained more than 90 days after gastrectomy or who had developed gastric cancer after previous gastrectomy, five of whom were seronegative, the seroprevalence increased to 83 percent among all gastric cancer patients and to 88 percent among noncardia gastric cancer patients (table 1). As a consequence, the overall odds ratios for *H. pylori* infection increased from 2.3 to 3.3 for total gastric cancer and from 3.7 to 5.3 for noncardia gastric cancer. The odds ratios for CagA-positive infections increased from 3.4 to 5.0 and from 5.7 to 8.5, respectively (table 2). Separate (one at a time) exclusion of 18 (26 percent) patients with advanced (T4) gastric cancer, of two cases and 21 controls who were seronegative according to IgG ELISA but who were CagA positive according to Western blot, or of eight cases and 65 controls with borderline levels of IgG led to a somewhat less pronounced increase in the odds ratios for noncardia cancer and did not materially affect the odds ratios for total gastric cancer.

However, after simultaneous application of the four exclusion criteria, only two seronegative patients were left among 39 remaining gastric cancer patients, corresponding to a seroprevalence of 95 percent. By contrast, there remained 58 of 285 controls who were seronegative, corresponding to a seroprevalence of 80 percent (table 1). As a result, the overall odds ratio for total gastric cancer was strongly increased to 5.0 (95 percent CI: 1.2, 21.4) for all *H. pylori* infections and to 7.2 (95 percent CI: 1.6, 32.3) for CagA-positive infections (table 2). The odds ratio for noncardia gastric cancer increased from 3.7 (95 percent CI: 1.7, 7.9) to 18.3 (95 percent CI: 2.4, 136.7) for all *H. pylori* infections and from 5.7 (95 percent CI: 2.6, 12.8) to 28.4 (95 percent CI: 3.7, 217.1) for CagA-positive *H. pylori* infections, even after application of only the first three of the four exclusion criteria. A closer look at the single *H. pylori*-negative patient with noncardia gastric cancer who was left after application
of these exclusion criteria revealed that this patient, a man aged 60 years, also had quite advanced disease (T3) and a borderline value in the IgG ELISA. Hence, after application of this additional exclusion criterion, there was no *H. pylori*-negative patient left among the remaining 32 cases with noncardia gastric cancer.

**DISCUSSION**

In this in-depth analysis of a case-control study, the proportion of seronegative subjects among cases with gastric cancer strongly decreased if the sample was restricted to patients in whom possible loss of the infection as a consequence of gastric atrophy (and therefore misclassification of previous *H. pylori* exposure) was less likely. As a consequence, much stronger associations of *H. pylori* infection with gastric cancer, and particularly noncardia gastric cancer, emerged if such exclusions were made.

Our results are in agreement with previous findings of a stronger *H. pylori*-gastric cancer association in subgroups of patients in whom loss of the infection as a consequence of the disease process itself is less likely, such as younger patients or patients with early gastric cancer (5, 8). However, in previous analyses such factors were mostly considered one at a time only, and our third criterion, *H. pylori* seronegativity despite CagA positivity, has been applied in only one recent study from Sweden (10). In that study, patients with previous gastrectomy were also excluded, and blood had been taken preoperatively among all participants (an even more stringent criterion than our first exclusion criterion).

In both the Swedish study and our study, the results of a standard analysis without exclusions of subjects with possible disease-induced loss of infection yielded odds ratios that are very similar to those obtained in many other studies and in pertinent meta-analyses, but the odds ratios were much higher if a consequent approach was taken to exclude such subjects. After exclusion of IgG ELISA-negative, CagA-positive subjects from the reference group, the odds ratio for noncardia gastric cancer rose to 21.0 in the Swedish study, a value that is much higher than previously reported estimates and remarkably similar to the estimate of 18.3 obtained in our analysis after application of the first three exclusion criteria (patients with borderline IgG values, our fourth exclusion criterion, were not excluded in the Swedish study). The finding that no single patient among the 32 remaining cases with noncardia gastric cancer was left after application of all four exclusion criteria in our study even raises the question of whether *H. pylori* infection might even be a (close to) necessary cause of noncardia gastric cancer. This suggestion would be supported by a recent cohort study from Japan, in which 36 of 1,246 *H. pylori*-infected subjects, but none of 280 uninfected subjects, developed gastric cancer during a mean follow-up of 7.8 years (21).

However, our study also has limitations. Although it was larger than most single studies reported on the relation of *H. pylori* to gastric cancer to date, the number of patients with gastric cancer was much lower and the confidence intervals...
were much broader than in the meta-analyses that have been
done on this issue (5–9). Therefore, replication of our find-
ings in larger samples is required before firm conclusions can be
drawn. Furthermore, given the limited numbers of patients
retained in the final analyses, the number of covari-
ates that could reasonably be controlled for in multivariable
analyses was small. It appears, however, that confounding
is not a major issue. The odds ratios hardly changed when addi-
tional potential confounding variables were controlled for in
the initial model including all study participants.

We used patients with colorectal cancer rather than
healthy people as controls. There have been suggestions of a
possible weak association between H. pylori infection and the
risk of colorectal cancer, which would imply that the
H. pylori-gastric cancer association might still have been under-
estimated in our study. However, pertinent evidence is
inconclusive, and most recent studies have not supported this
suggestion (22, 23). Although patients who undergo
colorectal surgery frequently receive perioperative antibiotic
treatment, this is unlikely to have influenced the serologic
results, as antibiotic treatment other than the specific combi-
nations used for H. pylori eradication does not seem to have a
relevant impact on the persistence of the infection among
adults (24) and seroreversion would not be expected in the
short run (25). It may also be hypothesized that the serostatus
of colorectal cancer patients might be affected by other
factors such as cachexia or by decreased immune status (an
argument that might also be made for gastric cancer
patients), particularly in the case of advanced disease. We
therefore carried out additional analyses in which not only
cases but also controls whose blood was taken more than 3
months after surgery, as well as controls with advanced (T4)
cancer, were excluded, but these exclusions did not materi-
ally alter any of the results. An advantage of the use of
colorectal cancer as a control group was that cases and
controls were recruited under highly comparable circum-
stances from the same population and during the same
calendar period. It is furthermore reassuring that the point
estimates we obtained in the “usual analyses” (without the
exclusion of subjects with a potential for misclassification of
previous H. pylori exposure) were very well in line with the
summary odds ratios estimated in meta-analyses from
previous studies using population controls. Finally, the
H. pylori prevalence in our control group was very similar to
that reported among comparable age groups from popula-
tion-based studies conducted in Germany (26–28), which
further supports the suggestion that this control group
adequately represents the study base that gave rise to the
cases.

Although the strength of existing meta-analyses lies in the
increasing precision of risk estimates, a major attempt was
made in this analysis, like in the Swedish study (10), to
increase the validity and to reduce the systematic error from
disease-induced changes of infection status to the largest
possible extent, which, at the end, almost led back to indi-
vidual case reviewing. We think that this approach may be a
worthwhile complement to the attempt to increase the preci-
sion of relative risk estimates based on large numbers of
subjects that is made in the meta-analyses. Ideally, of course,
both attempts should be combined, and we would like to

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**TABLE 2.** Odds ratios with 95% confidence intervals, adjusted for age and gender, for the association between Helicobacter pylori infection and gastric cancer derived from the entire sample and after exclusion of defined subgroups of patients in whom interpretation of H. pylori serology may be uncertain, Saarland, Germany, 1996–1998

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gastric cancer, any location</th>
<th>Noncardia gastric cancer only*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall CagA−</td>
<td>CagA+</td>
</tr>
<tr>
<td></td>
<td>OR† 95% CI</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>All patients</td>
<td>2.3 1.2, 4.3</td>
<td>1.5 0.8, 3.1</td>
</tr>
<tr>
<td>Exclusions (one at a time)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;90 days after gastrectomy‡</td>
<td>3.3 1.6, 6.8</td>
<td>2.1 1.0, 4.8</td>
</tr>
<tr>
<td>T4 gastric cancer§</td>
<td>2.2 1.1, 4.4</td>
<td>1.5 0.7, 3.4</td>
</tr>
<tr>
<td>HP−, CagA+¶</td>
<td>2.3 1.2, 4.4</td>
<td>1.5 0.7, 3.2</td>
</tr>
<tr>
<td>Borderline IgG† result</td>
<td>2.5 1.1, 5.9</td>
<td>1.7 0.7, 4.3</td>
</tr>
<tr>
<td>Exclusions (consecutive)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;90 days after gastrectomy</td>
<td>3.3 1.6, 6.8</td>
<td>2.1 1.0, 4.8</td>
</tr>
<tr>
<td>T4 gastric cancer</td>
<td>3.8 1.6, 9.3</td>
<td>2.6 1.0, 7.0</td>
</tr>
<tr>
<td>HP−, CagA+</td>
<td>3.9 1.5, 10.3</td>
<td>2.7 1.0, 7.7</td>
</tr>
<tr>
<td>Borderline IgG result</td>
<td>5.0 1.2, 21.4</td>
<td>3.5 0.8, 15.8</td>
</tr>
</tbody>
</table>

* Excluding one patient with gastric cancer at the gastroesophageal junction and 10 patients with cardiacia.
† OR, odds ratio; CI, confidence interval; IgG, immunoglobulin G.
‡ Exclusion of gastric cancer patients and controls who were IgG negative in enzyme-linked immunosorbent assay and CagA positive in Western blot analysis.
§ Exclusion of gastric cancer patients who were interviewed more than 90 days after surgery or who developed gastric cancer after previous gastrectomy.
¶ Exclusion of gastric cancer patients with T4 cancers.
† Exclusion of gastric cancer patients with T4 cancers.

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encourage combined analyses of previous studies, in which similar approaches to reduce potential misclassification of *H. pylori* infection as the one taken in this study are followed. In particular, it seems that the nested case-control studies, which allow a more direct assessment of the issues addressed in this paper, would be particularly suited for such an investigation. This will require a de novo pooled analysis, however, rather than a meta-analysis of published studies, as the information needed for such an approach is usually not given in pertinent detail in publications.

In summary, our results are in line with previous evidence of a causal relation between *H. pylori* infection and gastric cancer. In agreement with a recent study from Sweden, it suggests, however, that this relation might be much stronger than previously thought (10, 29). Clearly, our findings require corroboration in larger studies or pooled reanalyses of existing studies. If corroborated, they may have far-reaching implications. First, prevention and possibly treatment of *H. pylori* infection might have an even more prominent role in the prevention of gastric cancer than previously thought. Second, the research focus should move from the question of whether and to what extent *H. pylori* infection contributes to noncardia gastric cancer risk to the question of the cofactors responsible for the development of noncardia gastric cancer among the large number of infected people, most of whom do not develop this form of cancer. Such cofactors include characteristics of both the host (13, 14, 29–34) and the infectious agent and may be helpful to focus potential measures of early detection and prevention of gastric cancer (29, 30, 35).

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REFERENCES

28. Seher C, Thierfelder W, Dortsch R. *Helicobacter pylori* prevalence in the German population. (In German). Gesundheitswe-


