Clinical Investigation and Reports

Association of Virulent Helicobacter pylori Strains With Ischemic Heart Disease

Vincenzo Pasceri, MD; Giovanni Cammarota, MD; Giuseppe Patti, MD; Lucio Cuoco, MD; Antonio Gasbarrini, MD; Rita L. Grillo, MD; Giuseppe Fedeli, MD; Giovanni Gasbarrini, MD; Attilio Maseri, MD

Background—Previous studies have reported an association between chronic Helicobacter pylori infection and ischemic heart disease. However, it is not clear whether this association is really due to the virulence of the bacterium or is merely the result of confounding factors (in particular, age and social class).

Methods and Results—We assessed the prevalence of infection by Helicobacter pylori and by strains bearing the cytotoxin-associated gene-A (CagA), a strong virulence factor, in 88 patients with ischemic heart disease (age, 57±8 years; 74 men) and in 88 age- and sex-matched controls (age, 57±8 years; 74 men) with similar social background. Prevalence of Helicobacter infection was significantly higher in patients than in controls (62% versus 40%; P=.004), with an odds ratio of 2.8 (95% CI, 1.3 to 7.4; P<.001) adjusted for age, sex, main cardiovascular risk factors, and social class. Patients with ischemic heart disease also had a higher prevalence of CagA-positive strains (43% versus 17%; P=.0002), with an adjusted odds ratio of 3.8 (95% CI, 1.6 to 9.1; P<.001). Conversely, prevalence of CagA-negative strains was similar in patients and controls (19% versus 23%), with an adjusted odds ratio of 0.8 (95% CI, 0.4 to 1.4).

Conclusions—The association between Helicobacter pylori and ischemic heart disease seems to be due to a higher prevalence of more virulent Helicobacter strains in patients. These results support the hypothesis that Helicobacter pylori may influence atherogenesis through low-grade, persistent inflammatory stimulation. (Circulation. 1998;97:1675-1679.)

Key Words: heart diseases ■ Helicobacter pylori ■ risk factors

Experimental and clinical studies have suggested that inflammatory diseases may have a role in the pathogenesis of ischemic heart disease. Indeed, several epidemiological studies have shown a significant association between ischemic heart disease and various infective diseases, both bacterial and viral, including cytomegalovirus and Chlamydia pneumoniae infections, chronic bronchitis, and dental infections. Mendall et al reported a higher prevalence of Helicobacter pylori infection in patients with ischemic heart disease than in healthy controls. Subsequently, other investigators have assessed the relationship between Helicobacter pylori and ischemic heart disease, reporting a strong positive association, a mild association, and even negative findings. These contradictory results may be explained, at least in part, by the different inclusion criteria of patients and controls used in the various studies and by the strong association of Helicobacter pylori infection with confounding factors, such as age and social class. However, none of the previous studies took into account the genetic polymorphism of Helicobacter pylori. More virulent Helicobacter pylori strains bearing the cytotoxin-associated gene-A (CagA) have a well-recognized pathogenetic role in peptic ulcer disease and gastric cancer and can directly induce enhanced inflammation, whereas CagA-negative strains provoke a significantly lower inflammatory response. Because there are no known determinants of CagA status, we reasoned that the presence of a higher proportion of CagA-positive strains in patients with ischemic heart disease would strongly suggest an association between virulence of Helicobacter pylori and ischemic heart disease. Thus, the aim of our study was to assess the prevalence of infection by more virulent strains of Helicobacter pylori, bearing the CagA antigen, in patients with ischemic heart disease and in an age and sex-matched group of control subjects.

Methods

Patients

We studied 88 consecutive patients (mean age, 57±8 years; 74 men) with severe unstable angina (Braunwald class IIIb, 27 patients), acute myocardial infarction (34 patients), or chronic stable angina for >1 year (27 patients). Patients were at the point of first clinical manifestation of ischemic heart disease. All patients had angiographically confirmed coronary artery disease (>70% diameter stenosis of at least one coronary vessel). The number of diseased vessels was defined as the number of major epicardial vessels with狭窄。
Serological Data
Specific anti–Helicobacter pylori IgGs were measured by use of a commercial ELISA (Enzyghost anti-Hp/IgG) according to the manufacturer’s instructions. Titers were defined as positive or negative according to a cutoff value of 10 U/mL (sensitivity and specificity >95%). Humoral response to CagA protein was assessed by Western blot (Helico Blot 2.0, Genelabs Diagnostics; sensitivity and specificity ≥95%). Fasting total serum cholesterol was also measured. Hypercholesterolemia was defined as total serum cholesterol >240 mg/dL. Fibrinogen levels were measured according to the Clauss method.

Statistics
According to a recent study,20 prevalence of CagA-positive Helicobacter pylori in asymptomatic healthy subjects is ~30%. We hypothesized that patients with ischemic heart disease could have a prevalence of CagA-positive Helicobacter pylori similar to that observed in patients with nonulcer dyspepsia (ie, ~55%).21 Thus, a sample of 88 patients and 88 controls would provide a 98% power to detect this difference (30% versus 55%), with an error 0.01. Continuous variables between groups were compared by t test for normally distributed values (age, body mass index); otherwise, the Mann-Whitney U test was applied. Proportions were compared by Yates-corrected χ² test. Odds ratios and 95% CIs assessing the risk of ischemic heart disease associated with infection by Helicobacter pylori or by CagA-positive Helicobacter were estimated by use of multiple logistic regression, adjusted for age, sex, body mass index, history of smoking and diabetes, presence of hypertension and hypercholesterolemia, and fathers’ social class at birth (manual versus nonmanual work).21 A value of P < .05 (two-tailed) was considered significant. All analyses were performed with GB-STAT V6 software. Results are expressed as mean ± SD.

Results
Study Population
The general features of patients and controls are summarized in Table 1. The two groups were similar in body mass index and social class, but patients had a significantly higher prevalence of classic risk factors for ischemic heart disease (hypercholesterolemia, hypertension, diabetes).

Helicobacter pylori Infection
Anti–Helicobacter pylori IgGs were detected in 55 of 88 patients with ischemic heart disease compared with 35 of 88 controls (62% versus 40%; P = .004; Figure 1). The odds ratio was 2.9 (95% CI, 1.5 to 5.2; P = .001) and 2.8 (95% CI, 1.3 to 7.4; P < .001) after adjustment for age, sex, classic risk factors for ischemic heart disease, and childhood living conditions (Figure 2). Patients who were either seropositive or seronegative for Helicobacter pylori infection were similar in age, sex, number of diseased vessels and coronary stenoses, and prevalence of risk factors for ischemic heart disease; however, seropositive patients tended to come from a lower social class (Table 2). Prevalence of infection by Helicobacter pylori was similar in patients with acute myocardial infarction (22/34, 65%), unstable angina (16/27, 59%), or chronic stable angina (17/27, 63%; P = .91).

Prevalence of CagA-Positive Helicobacter pylori
A total of 38 of 88 patients and 15 of 88 controls were infected by CagA-positive Helicobacter pylori (43% versus 17%; P = .0002; Figure 1). The odds ratio was 4.2 (95% CI, 2.1 to 8.4; P < .0001) and 3.8 (95% CI, 1.6 to 9.1; P < .001) after adjustment for classic risk factors for ischemic heart disease and for childhood living conditions (Figure 2). CagA-positive and -negative patients were similar in age, sex, number of diseased vessels and coronary stenoses, prevalence of risk factors for ischemic heart disease, and father’s social class at birth (Table 3). CagA-negative strains of Helicobacter pylori had a similar prevalence in patients and controls (19% versus 23%; P = .52; Figure 1) and were not associated with an increased risk of ischemic heart disease (odds ratio, 0.8; 95% CI, 0.4 to 1.4; Figure 2). Prevalence of infection by CagA-positive strains was similar in patients with acute conditions (Figure 2).

Table 1. Main Clinical Features of Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=88)</th>
<th>Controls (n=88)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57 ± 8</td>
<td>57 ± 8</td>
<td>1</td>
</tr>
<tr>
<td>Males</td>
<td>74 (84%)</td>
<td>74 (84%)</td>
<td>1</td>
</tr>
<tr>
<td>HP infection</td>
<td>55 (62%)</td>
<td>35 (40%)</td>
<td>.004</td>
</tr>
<tr>
<td>Infection by CagA-positive HP</td>
<td>38 (43%)</td>
<td>15 (17%)</td>
<td>.0002</td>
</tr>
<tr>
<td>Infection by CagA-negative HP</td>
<td>17 (19%)</td>
<td>20 (23%)</td>
<td>.52</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.1 ± 2.8</td>
<td>26.5 ± 2.6</td>
<td>.33</td>
</tr>
<tr>
<td>Fathers with a manual occupation</td>
<td>42 (48%)</td>
<td>47 (53%)</td>
<td>.51</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>36 (41%)</td>
<td>20 (23%)</td>
<td>.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>39 (44%)</td>
<td>0 (0%)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Current smokers</td>
<td>36 (41%)</td>
<td>34 (39%)</td>
<td>.79</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12 (14%)</td>
<td>0 (0%)</td>
<td>.0002</td>
</tr>
<tr>
<td>Peptic ulcer</td>
<td>6 (7%)</td>
<td>2 (2%)</td>
<td>.11</td>
</tr>
</tbody>
</table>

HP indicates Helicobacter pylori; CagA, cytotoxin-associated gene-A.

Figure 1. Prevalence of Helicobacter pylori infection and of cytotoxin-associated gene-A (CagA)–positive or CagA-negative strains in patients and controls.
myocardial infarction (15/34, 44%), unstable angina (11/27, 41%), or chronic stable angina (12/27, 44%; \( P = .95 \)).

### Discussion

The present investigation shows that infection by more virulent CagA-positive strains of *Helicobacter pylori* is significantly associated with ischemic heart disease, whereas CagA-negative strains have a similar prevalence in patients and controls. These findings strongly suggest that the association between *Helicobacter pylori* and ischemic heart disease is related to the virulence of this bacterium.

### Infection by CagA-Positive *Helicobacter pylori* and Ischemic Heart Disease

CagA is a high-molecular-mass (120- to 128-kD) *Helicobacter pylori* antigen, associated with enhanced virulence and cytotoxin production. Recent investigations have shown a clear association between CagA-positive *Helicobacter* strains and severe forms of gastroduodenal diseases, including peptic ulcer and gastric cancer. Although CagA status is only an indirect marker of the expression of *Helicobacter* cytotoxin, which is encoded by a distinct gene, peptic ulcer is more strongly associated with CagA expression than with cytotoxin production. Prevalence of CagA *Helicobacter pylori* has been studied widely in gastroduodenal diseases, but no previous study has assessed the possible role of these more virulent *Helicobacter pylori* strains in ischemic heart disease.

In the present study, we compared the prevalence of CagA-positive *Helicobacter pylori* infection in patients with angiographically confirmed coronary disease and a control group similar for age, sex, and social background. We confirmed the presence of a significant association between *Helicobacter* infection and ischemic heart disease. However, prevalence of CagA-positive strains was higher in patients than in controls and was significantly associated with ischemic heart disease in multivariate analysis. Conversely, CagA-negative strains were clearly not related to ischemic heart disease. These findings may also help to explain the contradictory results of previous studies on the association of *Helicobacter pylori* and ischemic heart disease, because none of these previous studies assessed the relative prevalence of CagA-positive and -negative strains of *Helicobacter*.

### Pathogenetic Mechanisms

The possible mechanisms by which more virulent strains of *Helicobacter pylori* infection could increase the risk of ischemic heart disease are unknown and cannot be deduced from our study. Previous studies have shown a significant association between chronic viral and bacterial infections and vascular pathology, including ischemic heart disease and stroke. Several recent studies have shown that the presence of an inflammatory response has a prognostic value in patients with unstable angina and may predict the long-term risk of cardiovascular events in patients with chronic stable angina and in healthy men. Although various chronic bacterial and viral infections may contribute to this inflammatory response, it is likely that infection by more virulent strains plays a decisive role. Indeed, bacterial cytotoxins are able to induce production of several cytokines (including interleukin-1, interleukin-6, and tumor necrosis factor) that may activate the vascular endothelium to change the hemostatic system by increasing the expression of procoagulant substances (fibrinogen, plasminogen activator inhibitor...
tor-1, tissue factor) and downregulating the fibrinolytic system, and to cause a prolonged endothelial dysfunction.

The results of previous studies on the association between Helicobacter infection and fibrinogen levels have been contradictory, and in the present study, CagA-positive Helicobacter infection was not related to higher levels of fibrinogen. In addition, Helicobacter pylori may induce lipid peroxidation, and oxidized LDL are an important component both of early development and late evolution of atherosclerotic lesions.

We did not find an association between CagA-bearing Helicobacter pylori and severity of coronary atherosclerosis in patients with ischemic heart disease. Indeed, Helicobacter pylori infection is more common in developing countries and CagA strains are more common in the Far East, whereas ischemic heart disease is more prevalent in developed western countries. These findings suggest that CagA-positive Helicobacter pylori can not directly induce coronary atherosclerosis but need the presence of other cofactor(s) to influence the onset and evolution of ischemic heart disease. Moreover, prevalence of Helicobacter pylori and even of CagA-positive strains was similar in patients with acute and chronic coronary syndromes, thus suggesting that Helicobacter pylori is unlikely to have a specific role in different coronary syndromes.

Our case-control study can demonstrate only an association, not a causal relationship between CagA-positive strains and ischemic heart disease. Indeed, infection by Helicobacter pylori could be associated with other risk factors (such as diabetes or obesity), although in the present study the prevalence of diabetes and the body mass index were similar in patients with and without infection by CagA-positive strains. On the other hand, it is also possible that infection may occur during hospitalization after an acute cardiac event (acute myocardial infarction or unstable angina) or after the onset of symptoms of chronic angina, although this is unlikely because infection by Helicobacter pylori usually occurs during early life. However, the most critical issue in case-control studies is the choice of an appropriate control group. Our control group was drawn from blood donors of our hospital and was similar to the patients' group in both geographic and social background. Yet, our control group may not be representative of the entire population, and the association suggested by our data needs to be confirmed by prospective studies.

Conclusions

To the best of our knowledge, this is the first study to have shown an association between ischemic heart disease and chronic infection by virulent strains of a micro-organism, supporting the hypothesis that the pathogenetic link between chronic infections and ischemic heart disease may be the chronic inflammatory response caused by these infections. However, further prospective studies are needed to assess the relationship between early life exposure to Helicobacter pylori (in particular by CagA-positive strains) and subsequent risk of ischemic heart disease. Because Helicobacter pylori infection (even by CagA-positive strains) may be easily eradicated by specific treatments, the accurate definition of this new risk factor may lead to new strategies for the prevention of ischemic heart disease.

References


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