Cytotoxin-Associated Gene-A–Positive *Helicobacter pylori* Strains Are Associated With Atherosclerotic Stroke

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**Background**—It is uncertain whether *Helicobacter pylori* is associated with ischemic syndromes and whether this association is mediated by the induction of atherosclerosis. In this study, we tested the hypothesis that atherosclerotic stroke shows a selective association with virulent *H pylori* strains.

**Methods and Results**—The seroprevalence of infection by *H pylori* and by strains bearing the cytotoxin-associated gene-A (CagA), a strong virulence factor, was assessed by ELISA in 138 patients with large-vessel stroke (group A), in 61 patients with cardioembolic stroke (group B), and in 151 healthy control subjects. The 3 groups had a similar socioeconomic status. Serum levels of C-reactive protein were also measured by ELISA. The prevalence of infection was 71% in group A, 63.9% in group B, and 70.2% in the control group (*P* = NS), whereas the prevalence of CagA-positive strains was higher in group A than in group B (42.8% versus 19.7%, respectively; odds ratio 3.04, 95% CI 1.43 to 6.49; *P* < 0.001) and higher in group A than in the control group (42.8% versus 17.9%, respectively; odds ratio 4.3, 95% CI 2.12 to 8.64; *P* < 0.001), after adjusting for main cardiovascular risk factors and social class. A trend toward a difference in C-reactive protein was observed between CagA-positive (2.00 ± 3.43 [mean ± SD] mg/dL) and CagA-negative (1.31 ± 1.72 [mean ± SD] mg/dL) patients (*P* = 0.072, Mann-Whitney *U* test).

**Conclusions**—The association between *H pylori* and acute cerebrovascular disease seems to be due to a higher prevalence of more virulent *H pylori* strains in patients with atherosclerotic stroke. (*Circulation. 2002;106:580-584.)*

**Key Words:** infection • genes • stroke • proteins

There is increasing evidence, from both clinical and experimental observations, that inflammation plays an important role in the development of atherosclerotic lesions in patients with coronary artery disease and ischemic stroke.1–2 Some studies have suggested that *Helicobacter pylori* may be the etiologic agent of the inflammatory process.3–5 However, the significance of the association remains uncertain because of the conflicting findings reported by various studies.6–9

In patients with coronary artery disease, it has been recently suggested that these contradictory reports may in part be explained by the fact that among different genotypes of *H pylori*,10 only the cytotoxin-associated gene-A (CagA)-positive virulent strains are associated with the disease.11 There are no available data regarding patients with ischemic stroke.

Whereas in patients with coronary artery disease, the disease is caused in almost all cases by atherosclerotic changes of the arteries, in patients with ischemic stroke, the same syndrome may be caused by quite different pathophysiological mechanisms: large-vessel disease is in fact due to extensive atherosclerotic changes, whereas cardioembolic stroke is caused by the abrupt occlusion of a cerebral artery by a clot arising from a distant source.12 Available data suggest that the prevalence of *H pylori* infection may be increased in patients with stroke that is due to large-vessel disease but not in patients with cardioembolic stroke.13–15

In the present study, we show that only CagA-positive strains of *H pylori* are associated with ischemic stroke and that this association is confined to patients with atherosclerotic stroke.

**Methods**

All patients with acute cerebrovascular disease admitted at the Departments of Neurology, Internal Medicine, and Emergency, Tor Vergata University, Rome, Italy, during the year 2000 and part of 2001 were considered eligible for the present study. Every patient included in the study had MRI and CT suggestive of ischemic stroke.

All patients were subjected to an ultrasonographic evaluation of the neck and intracranial arterial vessels. Carotid arteries were assessed and defined by color-flow B-mode Doppler ultrasound.
In some selected cases, in which the results of ultrasound examinations were not completely satisfactory, MRI angiography was performed to define exactly the presence of atherosclerotic lesions.

Patients without clinical and instrumental evidence of atherosclerosis who had atrial fibrillation and/or echocardiographic findings suggestive of possible cardioembolism were classified as having thromboembolic stroke. The remaining patients were diagnosed as having large-artery stroke (ischemia due to cervical or intracranial thromboembolic stroke. The remaining patients were diagnosed as having large-artery stroke (ischemia due to cervical or intracranial thromboembolic stroke). The remaining patients were diagnosed as having large-artery stroke (ischemia due to cervical or intracranial thromboembolic stroke). The remaining patients were diagnosed as having large-artery stroke (ischemia due to cervical or intracranial thromboembolic stroke). The remaining patients were diagnosed as having large-artery stroke (ischemia due to cervical or intracranial thromboembolic stroke).

During hospitalization, global neurological evaluation was always revised by 1 neurologist coordinating the application of study criteria for a proper classification of patients. All instrumental evaluations were performed at the same center (Department of Neuroradiology). Informed consent was obtained from all patients (or, when not possible, by their closest relatives) for the drawing of blood. The control subjects were chosen among the relatives (aged >40 years) of patients admitted to the Department of Internal Medicine for noncardiac noninfectious diseases. The absence of atherosclerosis in control subjects was assessed as follows: by normal 12-lead ECG, by normal echocardiography, by <25% stenosis of the carotid tree (Doppler ultrasonography), and by normal physical examination of the lower limb arteries. All the control subjects with a positive clinical history of cardiac disease were excluded from the study.

In patients, the venous blood was analyzed for H pylori status and CagA status at the time of diagnosis, whereas in control subjects, the same procedure took place at the time of enrollment in the study. At the time of enrollment, demographic data, potential risk factors for H pylori infection (eg, parental social class and/or personal history of peptic ulcer disease), and known risk factors for vascular disease (eg, hypertension, diabetes, smoking, body mass index [BMI], and/or hyperlipidemia) were collected in patients and control subjects.

Patients and control subjects were defined as hypertensive if they had diastolic blood pressure >90 mm Hg and systolic blood pressure >140 mm Hg or if they had been treated for at least 1 year for this disorder. Patients were classified as diabetic if they had fasting levels of glucose >126 mg/dL in 2 distinct instances or if they had been treated for at least 1 year with hypoglycemic drugs. Patients were defined as smokers if they reported a daily habit of ≥10 cigarettes for at least 1 year during the last 10 years, and they were considered hyperlipidemic if they had levels of total cholesterol >220 mg/dL or if they had been treated for at least 1 year with lipid-lowering drugs. BMI (kg/m²) was taken as a measure of obesity. All subjects reporting previous therapy aimed at eradication of H pylori were excluded from the study. Patients and controls lived in the same geographic area (the Italian district Lazio).

The study protocol was approved by the ethics committee of our institution.

Serological Data

All samples were kept at −80°C and were analyzed simultaneously by technicians who were unaware of whether the sample belonged to cases or to controls. Sera were investigated for IgG antibodies to H pylori and to CagA protein by ELISA (Helori and CTX, respectively; Eurospital) according to the manufacturer’s instructions. They were analyzed in duplicate, and results are expressed in units. The reference limits were previously determined in our laboratory by using serum samples from patients infected or not by H pylori. CagA characterization of H pylori isolates was performed by polymerase chain reaction, as previously described. Samples with >7.5 U were considered positive for CagA antibodies, whereas values <5.5 U were considered negative. Samples with values between 5.5 and 7.5 U were considered borderline and were excluded from the study. The interassay variations for IgG to H pylori and IgG to CagA were not >10%.

C-reactive protein (CRP) was assessed by rate nephelometry (Behring NA latex CRP, Behring Institute) and, in samples with <0.25 mg CRP/dL, by enzyme immunoassay (Imx, Abbott Laboratories), calibrated with the World Health Organization’s International Reference Standard for CRP immunoassay; the range of value detected by the assay is 0.005 to 3 mg/dL.

Statistical Analysis

When not otherwise stated, data are presented as mean±SD. A 2-tailed value of P=0.05 was considered statistically significant.

Odds ratios (ORs) and 95% CIs assessing the risk of ischemic stroke associated with infection by H pylori or by CagA-positive strains were estimated by univariate analysis and by multiple logistic regression, adjusted for age, sex, BMI, smoking history, diabetes, presence of hypertension, hypercholesterolemia, personal history of peptic ulcer disease, and fathers’ social class at birth (manual versus nonmanual work). Because CRP is not normally distributed, a nonparametric test (Mann-Whitney U test) was used when this variable was compared between different groups. All analyses were performed with SPSS release 8 software.

Results

Study Population

Three hundred seventy-five patients with stroke and 266 control subjects were considered for the study. One hundred seventy-six patients and 115 control subjects were excluded for the following reasons: diagnosis of stroke subtypes other than large-vessel stroke or cardioembolic stroke (103 patients), association of cardioembolic stroke and clinical or instrumental evidence of atherosclerosis (39 patients), refusal to participate in the study (14 patients and 34 control subjects), previous H pylori eradication (20 patients and 15 control subjects), abnormal ECG (32 control subjects), abnormal echocardiographic findings (13 control subjects), and asymptomatic carotid stenosis (21 control subjects).

Therefore, data are given for 199 patients and 151 control subjects. After diagnostic workup, 138 patients were classified as having large-vessel stroke, and 61 patients were classified as having cardioembolic stroke.

The general features of patients and control subjects are summarized in Table 1. No significant difference regarding risk factors for H pylori infection was detected, but patients with large-vessel stroke had a higher prevalence of classic risk factors for atherosclerosis than the control subjects (male sex, OR 1.63 and CI 1.02 to 2.60; hypercholesterolemia, OR 3.33 and CI 1.67 to 6.65; hypertension, OR 5.33 and CI 3.17 to 8.97; diabetes, OR 5.00 and CI 2.10 to 11.91; and smoking, OR 4.46 and CI 2.61 to 7.60), whereas only a history of hypertension was detected with increased frequency in patients with cardioembolic stroke compared with control subjects (OR 3.26 and CI 1.73 to 6.22).

No significant difference was found between patients with large-vessel stroke and patients with cardioembolic stroke.
Prevalence of \textit{H} pylori Infection and of CagA-Positive Strains in Patients and Control Subjects

Anti-\textit{Helicobacter} IgGs were found in 98 (71\%) of 138 patients with large-vessel stroke, 39 (63.9\%) of 61 patients with cardioembolic stroke, and 106 (70.2\%) of 151 control subjects (\textit{P}=NS).

A total of 59 (42.8\%) of 138 patients with large-vessel stroke, 27 (17.9\%) of 151 control subjects, and 12 (19.7\%) of 61 patients with cardioembolic stroke were infected by CagA-positive \textit{H} pylori (Figure). No significant difference in the prevalence of CagA-positive strains was found between patients with cardioembolic stroke and control subjects, whereas the prevalence of CagA-positive strains was significantly higher in patients with large-vessel stroke than in control subjects (by univariate analysis, OR 3.42 and CI 2 to 5.86 [\textit{P}<0.001]; by multivariate analysis, OR 3.50 and CI 1.81 to 6.75 [\textit{P}<0.001]) and in patients with cardioembolic stroke (by univariate analysis, OR 3.04 and CI 1.49 to 6.23 [\textit{P}<0.001]; by multivariate analysis, OR 3.03 and CI 1.43 to 6.44 [\textit{P}<0.001]) (Table 2).

Levels of CRP in Patients and Control Subjects

The measurement of CRP protein was performed in 124 patients with atherosclerotic stroke, in 55 patients with cardioembolic stroke, and in 134 control subjects.

CRP levels were 1.62±2.92 mg/dL in patients with large-vessel stroke, 1.39±0.79 mg/dL in patients with cardioembolic stroke, and 0.61±1.00 mg/dL in control subjects; the difference between both groups of patients and control subjects was significant (\textit{P}=0.001). Among patients, the CagA-positive patients had higher levels of CRP than did the CagA-negative patients (2.00±3.43 versus 1.31±1.72 mg/dL, respectively), although the difference was of borderline statistical significance (\textit{P}=0.072).

Discussion

Main Findings

In the present study, we found no difference in the prevalence of \textit{H} pylori infection among patients with 2 different stroke subtypes (patients with large-vessel stroke and patients with cardioembolic stroke) and a population of healthy control subjects. However, the prevalence of CagA-positive strains of \textit{H} pylori in patients with large-vessel stroke was more than twice that in patients with cardioembolic stroke and in control subjects, with the latter 2 groups showing similar levels of CagA-positive strains.

CRP, a sensitive marker for the detection of systemic inflammatory response, was similarly increased in both groups of patients compared with control subjects; however, compared with CagA-negative patients, CagA-positive patients showed a trend toward higher levels of CRP.

Clinical and Pathophysiological Implications

This is the first report showing that only 1 genotype of \textit{H} pylori (ie, CagA-positive strain) and only 1 subtype of ischemic stroke (ie, atherosclerotic stroke) are associated with each other.

Our data do not explain whether a causal link exists between the 2 events. However, the fact that compared with CagA-negative patients, CagA-positive patients showed a trend toward higher levels of systemic inflammation gives some support to the hypothesis that virulent \textit{H} pylori strains may induce systemic inflammation, a recognized risk factor for atherosclerosis.\textsuperscript{1,2} Furthermore, it should be considered that the difference in CRP levels between CagA-positive and CagA-negative patients was probably underestimated, inasmuch as CRP evaluation was performed in the first few days after the acute event. The strong aspecific inflammatory response after acute tissue ischemia may well have minimized a preexistent difference due to a chronic low level of inflammation induced by virulent \textit{H} pylori strains.

Perhaps other mechanisms may link infection with CagA-positive strains of \textit{H} pylori to atherosclerotic stroke. For example, it has recently been shown that \textit{H} pylori may be present at the level of the carotid plaques.\textsuperscript{23} Because CagA-positive strains elicit a strong local inflammatory response,\textsuperscript{24} it is possible that their presence may contribute to plaque instability and to the development of ischemic stroke through a local action.
TABLE 2. ORs for Infection by CagA-Positive *H pylori* in Patients and Control Subjects After Adjustment for Possible Confounding Factors

<table>
<thead>
<tr>
<th></th>
<th>Large-Vessel Stroke Patients vs Cardioembolic Stroke Patients</th>
<th>Large-Vessel Stroke Patients vs Control Subjects</th>
<th>Cardioembolic Stroke Patients vs Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>OR 95% CI P</td>
<td>OR 95% CI P</td>
<td>OR 95% CI P</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.73 0.88–3.41 0.11</td>
<td>2.65 1.42–4.92 0.00</td>
<td>1.27 0.66–2.46 0.48</td>
</tr>
<tr>
<td>BMI &gt;28</td>
<td>3.01 0.52–3.27 0.56</td>
<td>0.97 0.46–2.04 0.94</td>
<td>1.01 0.43–2.36 0.98</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.86 0.93–3.76 0.08</td>
<td>5.26 2.75–10.04 0.00</td>
<td>2.00 0.99–4.06 0.05</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.41 0.69–2.86 0.34</td>
<td>5.30 2.82–9.98 0.00</td>
<td>4.21 1.95–9.07 0.00</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.83 0.94–8.55 0.06</td>
<td>5.00 1.70–14.68 0.00</td>
<td>1.63 0.45–5.93 0.46</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>2.21 0.83–5.94 0.11</td>
<td>2.93 1.19–7.22 0.02</td>
<td>1.05 0.37–3.05 0.92</td>
</tr>
<tr>
<td>Father’s manual job</td>
<td>0.85 0.43–1.69 0.64</td>
<td>0.79 0.44–1.42 0.43</td>
<td>0.71 0.37–1.37 0.31</td>
</tr>
<tr>
<td>History of PUD</td>
<td>1.15 0.35–3.81 0.81</td>
<td>0.34 0.12–1.04 0.06</td>
<td>0.88 0.29–2.68 0.82</td>
</tr>
<tr>
<td>CagA positivity</td>
<td>3.03 1.43–6.44 0.00</td>
<td>3.50 1.81–6.75 0.00</td>
<td>1.68 0.73–3.90 0.22</td>
</tr>
</tbody>
</table>

PUD indicates peptic ulcer disease.

From a clinical point of view, our data suggest that infection with CagA-positive strains of *H pylori* represents a risk factor for the development of atherosclerotic stroke. Therefore, a policy of selective eradication of virulent *H pylori* strains might be the most appropriate approach for preventing this disorder in high-risk subjects.

**Our Data in the Context of Available Literature**

Inconclusive results have been obtained from the few studies performed on the relationship between ischemic stroke and *H pylori* infection,13–15,25 although 3 of these studies13–15 placed emphasis on the possible preferential association of *H pylori* with ischemic stroke of atherothrombotic origin. No study is available on the possible relationship between CagA-positive *H pylori* strains and ischemic stroke; therefore, it is possible that these puzzling results may be due to the fact that a variable proportion of *H pylori* strains unable to induce atherosclerosis and a variable proportion of patients without atherosclerotic stroke have been considered in previous studies.

A selective association with CagA-positive strains has been reported in patients with coronary artery disease.11 Although subsequent reports26–31 did not uniformly confirm this finding, even negative studies26,27,30 showed a significant or near-significant difference in the crude prevalence of CagA-positive strains between patients and control subjects, which was attenuated after adjustment for covariates. However, the OR remained >1 in all cases.

**Peculiarity and Limitations of the Study**

The main limit of the present study is represented by the case-control design. Although much attention was paid to avoid any potential bias interfering with the results, it is well known that prospective studies are often not able to confirm the association reported in case-control studies. Thus, further prospective studies are needed to confirm the reported association.

The cross-sectional nature of the present study does not allow us to establish whether CagA-positive strains of *H pylori* play a causal role in determining atherosclerotic stroke.

Furthermore, given the widespread diffusion of *H pylori*, it is theoretically possible that unrecognized factors, independently associated with infection and ischemic stroke, may be responsible for a spurious association. Finally, our control subjects were not representative of the general population, and the inclusion criteria may have led us to select a population at low risk for *H pylori* infection. However, it should be emphasized that in our series, patients with or without ischemic stroke showed almost identical rates of *H pylori* infection.

Peptic ulcer disease and gastric cancer, the only recognized factors selectively associated with infection due to CagA-positive strains,32,33 were similarly distributed in patients and control subjects, in spite of the fact that patients had a higher prevalence of infection with these strains. This finding may be due to the poor relationship between personal history of peptic ulcer disease and endoscopic diagnosis. In any case, given the relatively low prevalence of peptic ulcer disease (~10% in our series), it is improbable that possible misreporting of the disorder by some patients may have influenced our findings.

Although it cannot be theoretically excluded that our control group had an unusually low rate of infection with CagA-positive strains of *H pylori* compared with the general population, the fact that the prevalence of CagA-positive strains in our series (17.9%) was quite similar to that found in healthy subjects of the same geographic area in a previous study11 involving the relationship between *H pylori* and coronary heart disease (18%) makes us confident about the reliability of the prevalence of infection with CagA-positive strains in our control population.

**Conclusions of the Study**

The present study shows for the first time that the possible association between ischemic stroke and *H pylori* infection may be largely due to an increased prevalence of infection with virulent strains in patients with atherosclerotic stroke. The pathophysiological mechanism underlying this association is probably represented by a chronic inflammatory response caused by this infection. Because *H pylori* infection...
caused by any genotype may represent an easily removable risk factor, confirmatory studies assessing the above-reported relationship are clearly needed.

Acknowledgments
We thank Andrea Galanti, MD, and Fabio De Pascalis, MD (Emergency Department, Tor Vergata University, Rome, Italy) for their invaluable help in the enrollment of patients.

References
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