Association Between Cerebral Ischemia and Cytotoxin-Associated Gene-A–Bearing Strains of Helicobacter pylori

Michael R. Preusch; Armin J. Grau, MD, PhD; Florian Buggle, MD; Christoph Lichy, MD; Jan Bartel, MD; Carmen Black, MD; Jochen Rudi, MD, PhD

Background and Purpose—Studies on Helicobacter pylori infection and risk of ischemic stroke yielded variable results. Infection with more virulent H. pylori strains, such as cytotoxin-associated gene-A (CagA)–bearing strains, may be of particular relevance for ischemic diseases. We investigated whether H. pylori and CagA seropositivity are independent risk factors for cerebral ischemia or its etiologic subtypes.

Methods—We determined IgG antibodies against H. pylori and CagA protein (enzyme immunoassays) in 190 patients with acute cerebral ischemia and in 229 age- and sex-matched control subjects selected randomly from the general population.

Results—CagA seropositivity was more common in patients (114/190; 60.0%) than in control subjects (99/229; 43.2%; odds ratio, 1.97; 95% CI, 1.33 to 2.91; P <0.001). This result remained significant after adjustment for age, sex, vascular risk factors and diseases, and childhood and adult social status (odds ratio, 1.84; 95% CI, 1.13 to 3.00; P=0.015). Subgroup analyses yielded similar results in all etiologic stroke subtypes. In contrast, H. pylori seropositivity in general was not associated with increased risk of stroke or its etiologic subtypes.

Conclusions—Our results support the hypothesis of an association between infection with CagA-positive H. pylori strains and acute cerebral ischemia.

Key Words: stroke ■ risk factors ■ infection ■ inflammation

There has been increasing evidence that in addition to established risk factors, markers of inflammation and chronic infectious diseases may be linked to stroke and other ischemic vascular diseases.1 Infection with Helicobacter pylori is among the infectious diseases discussed in this respect. H. pylori is a gram-negative spiral bacterium that can cause gastritis, peptic ulcer, and gastric cancer but often remains asymptomatic. After infection, which occurs mostly in childhood via fecal–oral or oral–oral pathways, it persists in the gastric mucus layer. The infection induces a serum antibody response, which persists during the entire lifetime. Socioeconomic factors influence the age and rate of infection with H. pylori2–3 and low socioeconomic status in childhood appears to be associated with H. pylori seropositivity.4

Seroepidemiologic studies on H. pylori and coronary heart disease or acute myocardial infarction yielded varying results, but the larger studies and those that adjusted for potential confounders were mostly negative or reported moderate effects in multivariate analysis.5 Regarding stroke, a small nested case–control study did not find an increased risk from H. pylori seropositivity.6 In 4 other case–control studies, seropositivity was associated with the risk of atherothrombotic or lacunar stroke but not with other stroke subtypes.7–10 In a prospective study, H. pylori seropositivity did not predict cardiovascular events in women.11 H. pylori strains bearing the cytotoxin-associated gene-A (CagA) are particularly virulent and are associated with increased inflammation. Regarding coronary heart disease, it has been suggested that only those virulent strains are associated with the disease.12–14 Most recently, increased titers of antibodies against CagA strains but not against H. pylori in general were reported in large-vessel stroke but not in cardioembolic stroke.15 Furthermore, CagA seropositivity (but not H. pylori seropositivity in general) was associated with an increased risk of carotid atherosclerosis16 in 1 study but not with increased intima–media thickness in another.17

We performed a case–control study to investigate whether seropositivity for H. pylori in general and for CagA-positive strains in particular are associated with ischemic stroke and transient ischemic attack and their etiological subtypes after...
adjustment for childhood and adult socioeconomic variables and other potential confounders.

Subjects and Methods

We investigated 190 consecutive patients who were hospitalized for acute cerebral ischemia and 229 age- and sex-matched control subjects randomly selected from the general population. Exclusion criteria included inability to give informed consent and subjects aged <18 or >75 years. Subjects had to be native speakers and residents of a defined area around the hospital. Population controls were selected randomly from a 2% random sample of the population registry of the study area that contained name, age, sex, and address. Subjects were first contacted by mail and later by telephone and asked for study participation. The participation rate among eligible control subjects was 69%.

Patients with acute cerebral ischemia had ischemic stroke (acute ischemic lesion on brain imaging or neurological deficits lasting >24 hours; n=127; 68.3%) or transient ischemic attack (neurological deficit of <24 hours without new ischemic lesions; n=58; 31.2%). All patients received cranial computed tomography or an MRI, excluding cerebral hemorrhage, and extracranial and transcranial Doppler sonography, an ECG, and transthoracic or transesophageal echocardiography.

Etiologic stroke subtypes were classified according to criteria of the Trial of Org 10172 in Acute Stroke Treatment (TOAST).18 The history of vascular risk factors and previous vascular diseases was assessed in a personal interview performed by specially trained interviewers using a standardized questionnaire for all patients and control subjects. If a subject was unable to follow the interview, help from a next of kin was accepted. A history of vascular risk factors or other diseases was accepted only if the diagnosis had been made by a physician before cerebral ischemia or the interview, and if standard criteria were fulfilled (hypertension [blood pressure repeatedly >140/90 mm Hg or on antihypertensive treatment] or diabetes mellitus [fasting glucose >125 mg/dL or on antidiabetic treatment]). We took a detailed history of past and present smoking and drinking habits. To assess the subjects’ social status, we asked for the number of years in school and the current profession, or the last profession of those retired or unemployed. Childhood housing conditions and socioeconomic status were assessed by asking subjects their age when fixed hot water supply was available (“Do you remember how old you were when fixed warm water supply was available in your home?”) and by collecting information on the profession of subjects’ parents. According to their profession, subjects were divided into 2 groups: untrained or blue-collar workers and white-collar employees or persons with academic education. The study protocol was approved by our ethics committee. All participating subjects gave informed written consent.

TABLE 1. Demographic Variables and Risk Factors

<table>
<thead>
<tr>
<th>Patients</th>
<th>Population Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>190</td>
<td>229</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.5±11.7</td>
<td>58.9±8.6</td>
</tr>
<tr>
<td>Female gender</td>
<td>65/190 (34.2%)</td>
<td>60/229 (26.2%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>114/187 (61.0%)</td>
<td>77/225 (34.2%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>11/186 (19.9%)</td>
<td>17/224 (7.6%)</td>
</tr>
<tr>
<td>Never</td>
<td>128/188 (68.1%)</td>
<td>114/227 (49.8%)</td>
</tr>
<tr>
<td>Current/ex-smoker (≥6 months)</td>
<td>37/186 (27.5%)</td>
<td>7/227 (3.1%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>26/180 (14.4%)</td>
<td>13/223 (5.8%)</td>
</tr>
<tr>
<td>Previous stroke or transient ischemic attack</td>
<td>19/184 (10.3%)</td>
<td>5/227 (2.2%)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>71/185 (38.4%)</td>
<td>70/224 (31.3%)</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>55/188 (29.3%)</td>
<td>88/228 (38.6%)</td>
</tr>
<tr>
<td>School education ≥10 years</td>
<td>Untrained/blue collar</td>
<td>100/185 (54.1%)</td>
</tr>
<tr>
<td>White collar/academic</td>
<td>85/185 (46.0%)</td>
<td>128/223 (57.4%)</td>
</tr>
<tr>
<td>Fathers’ profession</td>
<td>Untrained/blue collar</td>
<td>139/178 (78.1%)</td>
</tr>
<tr>
<td>White collar/academic</td>
<td>39/178 (21.9%)</td>
<td>76/220 (34.5%)</td>
</tr>
<tr>
<td>Mothers’ profession</td>
<td>Untrained/blue collar</td>
<td>168/183 (91.8%)</td>
</tr>
<tr>
<td>White collar/academic</td>
<td>15/183 (8.2%)</td>
<td>30/226 (13.3%)</td>
</tr>
<tr>
<td>Housing conditions in childhood*</td>
<td>115/154 (44.8%)</td>
<td>79/211 (37.4%)</td>
</tr>
<tr>
<td>H. pylori antibody titers (U)†</td>
<td>11.5 (2.5)</td>
<td>10 (2.4)</td>
</tr>
<tr>
<td>H. pylori seropositivity‡</td>
<td>104/190 (54.7%)</td>
<td>115/229 (50.2%)</td>
</tr>
<tr>
<td>CagA antibody titers (U)†</td>
<td>6.1 (3.5, 12.0)</td>
<td>3.9 (2.3, 8.5)</td>
</tr>
<tr>
<td>CagA seropositivity§</td>
<td>114/190 (60.0%)</td>
<td>99/229 (43.2%)</td>
</tr>
</tbody>
</table>

*Warm water available at ≥20 years of age.
†Median (25th percentile, 75th percentile).
‡≥10 U/mL.
§≥5 U/mL.
Serological Data

Serum samples gained from peripheral venous blood within the first week after cerebral ischemia were stored at −80°C. IgG antibodies against *H. pylori* (Amersham Pharmacia) and against CagA protein (RAVO Diagnostika) were analyzed by enzyme immunoassays. Antibody titers against *H. pylori* (≥10 U/ml) and CagA (≥5 U/ml) were classified as positive according to the instructions of the manufacturer.

Statistical Analysis

We used the χ² test to compare categorical variables and the Mann–Whitney U test for comparison of continuous variables. Odds ratios (ORs) and 95% CIs are given in univariate and multivariate analyses. Logarithmic transformation of serological results was used for analyses of antibody titers as continuous variables. We decided to include the following variables in the multivariate model: age, sex, the generally accepted stroke risk factors (hypertension, smoking, diabetes mellitus, previous cerebral ischemia), and associated diseases (coronary heart disease or peripheral arterial disease) plus all those socioeconomic variables that were significant in univariate analyses. *P* values <0.05 were considered significant. Analyses were performed with the SAS software package (SAS Institute).

### Results

Demographic variables and risk factors are presented in Table 1. Arterial hypertension, smoking, diabetes mellitus, previous cerebral ischemia, coronary heart disease, and peripheral arterial disease were more common among patients than control subjects. Extended school education and a white-collar or academic profession of the subjects themselves and their fathers were encountered less frequently in patients than in control subjects.

*H. pylori* antibody titers were not different between groups, and *H. pylori* seropositivity did not differ between patients (104/190; 54.7%) and control subjects (115/229; 50.2%; OR, 1.20; CI, 0.82 to 1.76; *P*=0.36). However, CagA antibody titers were higher in patients than in control subjects. Similarly, CagA seropositivity was more common in patients (114/190; 60.0%) than in control subjects (99/229; 43.2%; OR, 1.97; 95% CI, 1.33 to 2.91; *P*=0.0006; Table 1). This association remained significant after adjustment for age, sex, vascular risk factors and diseases, and childhood and adult social status (OR, 1.84; 95% CI, 1.13 to 3.00; *P*=0.015; Table 2). In the multivariate model, hypertension, smoking, and previous cerebral ischemia significantly increased, diabetes mellitus tended to increase, and an academic or white-collar profession by subjects’ fathers decreased the odds of cerebral ischemia (Table 2). Analyzing CagA antibody titers as a continuous variable yielded similar results compared with above analyses with dichotomized values in univariate (OR, 1.86; 95% CI, 1.19 to 2.91; *P*=0.0066) and multivariate analysis (model as in Table 2; OR, 1.66; 95% CI, 0.96 to 2.88; *P*=0.072), although statistical significance was not reached after adjustment for covariables.

CagA antibody titers were higher in all etiological stroke subgroups than in control subjects, and in univariate analysis, seropositivity was associated with increased risk of cerebral ischemia for all subgroups. However, after adjustment for other covariables, the risk increase did not reach statistical significance in any of the subgroups (Table 3). *H. pylori*...
seropositivity in general was not associated with the risk of any stroke subgroup (data not shown).

**Discussion**

In our study, increased antibody titers against CagA-bearing strains of *H. pylori* were associated independently with cerebral ischemia, whereas *H. pylori* seropositivity in general was not. The assessment of several potential confounders, including parameters of childhood socioeconomic and living conditions, the random selection of control subjects from the general population, and the relatively high participation rate among control subjects, are strongholds of our study. On the other hand, there are several potential limitations of our study design. As in previous studies, *H. pylori* was not detected by gastroduodenal biopsy, which is considered the gold standard for diagnosis of *H. pylori* infection. In control subjects, the prevalence of CagA seropositivity was only slightly smaller than *H. pylori* seropositivity, and in patients, it was even higher, a result that may mainly be caused by the somewhat arbitrary definitions of cut-off values of different commercially available and approved enzyme immunoassays. Generally accepted cut-off values are lacking, and all classifications may be susceptible to bias. Our analyses on the basis of cut-off values as recommended by the manufacturers are supported by analyses of antibody titers as a continuous variable that showed similar results, although the ORs were slightly smaller, and in multivariate analysis, results did not reach significance. Furthermore, CagA reactivity can be found in ~7% to 10% of *H. pylori*-negative subjects, a phenomenon that may, however, explain our results only to a minor degree. To avoid an ascertainment bias in risk factor evaluation between patients and community controls, we relied on subjects’ reports of diagnoses made previously by physicians not involved in the present study, an approach that may lead to an underestimation of disease prevalence in both groups. However, the prevalence found in our study is in keeping with that of similar studies. Furthermore, the case–control design of our study does not allow clarification as to whether the link between infection and stroke is of a causal nature, and factors that were not assessed in our study may have confounded the association between both entities.

Recent results on *H. pylori* antibody titers and stroke were at variance, although several studies reported an association between atherothrombotic and lacunar stroke and *H. pylori* seropositivity. Conflicting results may be due partly to variable proportions of different *H. pylori* strains in various study populations. Similar to our results, Pietroiusti et al found an association with stroke regarding only CagA seropositivity and not *H. pylori* seropositivity. In contrast to our findings, their study showed increased antibody titers against CagA-bearing strains in atherothrombotic but not cardioembolic stroke. We did not find differences between stroke subgroups regarding the role of CagA seropositivity. However, numbers in subgroups were small, and associations in subgroups did not reach statistical significance in multivariate analysis. Therefore, caution with the interpretation of subgroup analyses is warranted. Coronary atherosclerosis contributes to atrial fibrillation and to other sources of cardioembolism such as left-ventricular akinesia after myocardial infarction. Presuming that CagA-bearing strains contribute to stroke only via promoting atherogenesis, different proportions of coronary atherosclerosis in various study groups may contribute to conflicting results.

*H. pylori* had not been detected in atherosclerotic plaques in several older investigations, however, recent studies showed its presence (e.g., in carotid plaques and its association with upregulated adhesion receptors). *H. pylori* induces cyclooxygenase-1 and cyclooxygenase-2 expression and up-regulates vascular endothelial growth factor expression in several cell types, mechanisms that may contribute to atherogenesis and thrombosis. Cross-reactivity of anti-CagA antibodies with vascular wall antigens is another possible link between *H. pylori* infection and atherosclerosis. *H. pylori* can activate human endothelial cells via NFκB activation, inducing upregulation of adhesion molecules (e.g., intercellular adhesion molecule-1, E-selectin) and proinflammatory chemokines and cytokines, events that stimulate procoagulant mechanisms in the endothelium. *H. pylori* infection may induce antiphospholipid antibody production and decrease levels of vitamin B12 and folic acid while elevating homocystein levels. Some *H. pylori* strains were shown to induce platelet aggregation by binding of von Willebrand factor, mechanisms that increase the risk of thrombosis and embolism. Therefore, *H. pylori* infection may be linked to stroke of atherothrombotic origin but also to other etiologic stroke subtypes.

Recently, evidence has been collected supporting the concept that the number of infectious pathogens to which an individual has been exposed is linked to the progression of atherosclerosis. Each specific infection may contribute only slightly or moderately to atherogenesis and ischemia, whereas the cumulative effects of several chronic infections may contribute to an important degree. *H. pylori* infection may be one of the infections contributing to the global pathogen burden potentially influencing stroke risk.

The link between *H. pylori* infection, particularly with its virulent strains, and ischemic stroke requires further large case–control and mainly prospective studies that also allow study of associations with etiologic stroke subgroups. *H. pylori* infection is a potentially curable condition. Its identification as a stroke risk factor may have important implications for stroke prevention.

**Acknowledgments**

This study was supported by a grant from the Deutsche Forschungsgemeinschaft to A.J.G. (Gr 11023/1–1).

**References**


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*Stroke*. 2004;35:1800-1804; originally published online May 27, 2004;
doi: 10.1161/01.STR.0000131751.35926.48

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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