CagA-positive *Helicobacter pylori* strains may influence the natural history of atherosclerotic stroke

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**Abstract**—Objective: To test the hypothesis that infection with virulent cytotoxin-associated gene-A (CagA)-bearing *Helicobacter pylori* strains influences the atherosclerotic process and the clinical course in atherosclerotic stroke patients. Methods: ELISA was used to assess the seroprevalence of infection by *H. pylori* and CagA-positive strains in 185 patients. Intima-media thickness (IMT) was determined by Doppler ultrasound. Baseline, 1-week, and 1-month NIH Stroke Scale (NIHSS) scores were used to evaluate the short-term clinical course. Results: *H. pylori* infection was found in 79% of patients; 58% of these tested positive for CagA. IMT was higher among CagA-positive patients than among CagA-negative ones (1.13 ± 0.26 mm vs 0.97 ± 0.15 mm; univariate analysis, *p* = 0.0001; multivariate analysis, odds ratio [OR], 2.36; 95% CI, 1.57 to 3.54; *p* = 0.0001) or *H. pylori*-negative ones (1.01 ± 0.17 mm; univariate analysis, *p* = 0.007; multivariate analysis, OR, 1.90; 95% CI, 1.22 to 2.97; *p* = 0.005). CagA-positive patients had poorer initial outcomes based on serial measurements of the NIHSS score (repeated measures analysis of variance, *p* < 0.0001). No significant difference in IMT and NIHSS score was found between *H. pylori*-positive and *H. pylori*-negative patients. Conclusions: Infection with cytotoxin-associated gene-A-positive *Helicobacter pylori* strains in atherosclerotic stroke patients is associated with greater intima-media thickness and poorer short-term outcome compared with cytotoxin-associated gene-A-negative patients.

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Chronic infection and inflammation may play a role in the pathogenesis and outcome of cardiovascular and cerebrovascular disease. In recent years, attention has been focused on the possible causal role of cytotoxin-associated gene-A (CagA)-positive *Helicobacter pylori* strains. Conflicting findings have been reported in cardiovascular disease, whereas recent evidence suggests a role for CagA-positive strains in cerebrovascular disease. In particular, an association between infection with CagA-positive *H. pylori* strains and atherosclerotic stroke has been recently reported. However, the possible clinical and pathophysiologic implications of this association remain unclear. To explore this issue, we evaluated whether intima-media thickness (IMT) of the common carotid arteries, a reliable marker of early atherosclerosis, differs between atherothrombotic stroke patients with or without infection with CagA-positive strains. We also assessed the possible influence of these strains on the short-term clinical course of the disease. Furthermore, because systemic inflammation has been reported to be associated with atherosclerotic damage and a poor clinical course of stroke, we also analyzed the possible association between infection with CagA-positive strains and serologic markers of inflammation in these patients.

**Patients and methods.** Subjects. From May 2000 to May 2002, consecutive patients with first acute ischemic stroke hospitalized at our department of neurology were considered for the study. All patients had CT on admission and MRI within 1 week from the onset of symptoms. Neuroradiologists evaluated images. Patients underwent 12-lead EKG and echocardiography. History of arterial hypertension, diabetes, smoking, and hyperlipidemia was assessed for all subjects. Patients were defined as hypertensive if they 1) 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; 2) were diabetic and had fasting levels of glucose ≥126 mg/dL in two distinct instances or had been treated for at least 1 year with hypoglycemic drugs; 3) smoked, reporting a daily habit of >10 cigarettes per day for at least 1 year during the past 10 years; and 4) were hyperlipidemic and had levels of total cholesterol >220 mg/dL or had been treated for at least 1 year with lipid-lowering drugs.

Body mass index (BMI; kg/m²) was taken as a measure of obesity. Exclusion criteria were history of peptic ulcer disease or gastric cancer, previous therapy aimed at eradication of *H. pylori*, and evidence of connective tissue or hematologic disease. According to the guidelines of our department, carotid endarterectomy or stent was not routinely performed in the acute phase of stroke. Therefore, surgery was considered only for select cases. Based on the possible bias introduced by these isolated patients on the
interpretation of clinical evolution, patients undergoing vascular surgery were excluded from the analysis.

Written informed consent was obtained by patients or, when not possible, by their closest relatives. The Ethics Committee of our Institution approved the study protocol.

Neurologic data. A standardized physical and neurologic examination was carried out on all subjects, and NIH Stroke Scale (NIHSS) was used to evaluate the severity of ischemic event at admission (<24 hours), after 1 week, and at 1 month after the acute event.

According to the criteria of the Trial of Org 10172 in Acute Stroke Treatment (TOAST) investigators, only patients with stroke caused by large artery atherosclerosis were recruited. Serologic data. Venous blood samples were drawn from patients within 48 hours after symptom onset. Samples were kept at −80°C until analysis; the frozen sera were assayed for the presence of immunoglobulin (Ig) G antibodies to H. pylori and the CagA protein and for the level of C-reactive protein (CRP). IgG antibodies against H. pylori were evaluated using ELISA (Pyloriset, ORION Diagnostica, Espoo, Finland). Titers ≥300 units were regarded as positive.

Antibodies to CagA protein were investigated using ELISA (CTX, Eurospital, Trieste, Italy). CagA characterization of H. pylori isolates was performed by PCR. Samples with >7.5 units were considered positive. Interassay variation of the ELISA was <10% for both H. pylori and to CagA, were not >10%. Patients testing negative for H. pylori and positive for CagA were excluded from the study.

CRP was assessed using rate nephelometry (Behring NA latex C100, Behring Institute, Scoppito, l’Aquila, Italy) and in samples with >0.25 mg of CRP/dL using enzyme immunoassay (Imx, Abbott Laboratories, North Chicago, IL), 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. The normal upper limit was considered 3 mg/dL.

Carotid ultrasonography. Extracranial and intracranial vessels were evaluated with a continuous-wave Doppler and color flow B-mode Doppler ultrasound (Esaote Biomedica, Genova, Italy) with a high-resolution 7.5-MHz linear array-imaging probe. A visual scoring was used to define the location and the percentage of diameter stenosis in extracranial arteries.

Steno-occlusive lesions in the internal carotid arteries were assessed and defined according to validated criteria. A plaque was defined as a localized thickening >1.2 mm that did not uniformly involve the entire artery. Measurement of IMT was performed on the common carotid arteries (CCAs), along ~1.5 cm proximal to the flow divider, according to the method described by Leary et al. In brief, a longitudinal image of the distal CCAs was acquired. CCA wall thickness was defined as the mean of the maximum wall thickness for the near and far wall on the left and right side. The vertebrobasilar system was evaluated as described by Bars.

Intracranial vessels were examined using a Multidop X/TCD transcranial Doppler instrument (DWL Elektronische Systeme GmbH, Sipplingen, Germany) and color flow B-mode Doppler ultrasound (Esaote Biomedica). In some selected cases, in which the results of ultrasound examinations of extracranial vessels were not completely satisfactory, MRI angiography was performed to confirm the presence of atherosclerotic lesions.

The same two operators performed all ultrasonographic evaluations. The reproducibility of stenosis and IMT measurements between and within sonographers had previously been checked.

Statistics. Statistical analysis was performed using SPSS software (version 11, Chicago, IL). When not otherwise stated, data are presented as mean ± SD. A two-tailed value of p ≤ 0.05 was considered significant. The χ2 test was used to analyze categorical variables, and Student’s t-test was used to analyze continuous variables.

A non-parametric test (Mann–Whitney U test) was used to compare CRP values between different subgroups.

Multiple logistic regression, considering the effect of cardiovascular risk factors (e.g., age, sex, BMI, smoking, diabetes, hypertension, and hypercholesterolemia), was performed to assess the presence and strength of effect of CRP on the association between H. pylori virulent strains and IMT.

Two-way analysis of variance was used to assess changes of NIHSS score during the follow-up period (within factor) in patients infected and not infected with CagA-positive strains (between factor). A post hoc analysis by Scheffé test was performed if appropriate.

Results. Eight hundred five patients with first acute ischemic stroke were considered for the study. Six hundred nineteen patients were excluded because they had a final diagnosis other than large vessel stroke: cardioembolic stroke (157 patients), lacunar stroke (209 patients), undetermined stroke (220 patients), and stroke caused by other causes (33 patients). One hundred eighty-six patients had atherosclerotic stroke. One patient showing a worsening evolution and with a severe internal carotid stenosis, hemodynamically unstable, was submitted to stenting and therefore excluded from the study.

Thus, 185 patients were included: 55 with intracranial disease, 103 with extracranial disease, and 27 with both localizations. Seventy-nine percent of patients were positive for H. pylori; of these 58% tested positive for CagA. Among H. pylori-positive patients, the percentage of intracranial or extracranial disease was similar in CagA-positive or CagA-negative patients (intracranial, 27% vs 31%; extracranial, 58% vs 52%; both localizations, 15% vs 17%).

Based on our criteria of definition of hypertensive, diabetic, and hyperlipidemic patients, the majority of subjects with these disorders received medical treatment at the time of hospitalization. Among treated patients, differences in the class of administered drugs did not exceed 20% in the three groups of patients when the absolute number of treated patients was at least five in the group with the highest frequency. Regarding other pharmacologic treatment, all included patients had antplatelet therapy with 325 mg aspirin daily for the entire period of the study, with the exception of three patients (two positive and one negative for CagA) who were treated with ticlopidine, 500 mg daily. The clinical characteristics of H. pylori-negative, CagA-positive and H. pylori-positive, and CagA-negative and H. pylori-positive patients are shown in table 1. With univariate analysis, no difference in IMT was observed between uninfected patients and H. pylori-positive and CagA-negative patients (1.01 ± 0.17 mm vs 0.97 ± 0.15; p = 0.269). By contrast, IMT was higher in H. pylori- and CagA-positive patients compared with uninfected ones (1.13 ± 0.26 mm vs 1.01 ± 0.17 mm; p = 0.007); furthermore, for H. pylori-positive patients, those who were positive for CagA showed higher IMT values than those negative for CagA (1.13 ± 0.26 mm vs 0.97 ± 0.15 mm; p = 0.0001), and the difference was still persistent after multivariate analysis considering classic risk factors for atherosclerosis (e.g., age, sex, BMI, active smoking, diabetes, hyperlipidemia, and hypertension): OR, 2.36; 95% CI, 1.57 to 3.54; p = 0.0001 (table 2).

When dividing IMT into quartiles, the prevalence of infection with CagA-positive strains was higher in those patients with the highest quartile of IMT than in those with the lowest one (p for trend across quartiles = 0.0001; table 3).

Concerning CRP values, Mann–Whitney test for group comparison showed higher values in CagA-positive than in CagA-negative patients (median, 1.1; range, 0.1 to 6.0; vs median, 0.3; range, 0.1 to 5.9; p = 0.002) or H. pylori-
negative patients (median, 0.6; range, 0.1 to 2.6; *p* = 0.021).

At baseline, NIHSS score was similar in CagA-positive and CagA-negative patients (10.80 ± 2.8 vs 11.05 ± 2.8; *p* = 0.701). Thirty-two patients were not available for 1-month follow-up visit: 9 patients were lost because they died (6 positive and 3 negative for CagA); 23 (14 positive and 9 negative for CagA) did not return for the visit because they lived or were hospitalized in rehabilitation centers far from our institution. Clinical characteristics of patients lost to follow-up evaluation were similar to their original group of CagA-positive or CagA-negative subjects. Therefore, analysis was restricted to 114 *H. pylori*-positive patients (57% positive and 43% negative for CagA). Among these patients, fever requiring antibiotic therapy developed in 20% (21% positive and 18% negative for CagA).

Changes of NIHSS score after 1 week and at 1 month are shown in the figure. We observed a "group × time effect" (*p* = 0.0003). The post hoc analysis (Scheffé test) showed that NIHSS score was higher in CagA-positive patients than in CagA-negative patients at 1 week (8.35 ± 2.5 vs 7.52 ± 3.1; *p* = 0.009) and at 1 month (7.65 ± 2.2 vs 6.86 ± 1.9; *p* = 0.016).

**Discussion.** A recent report showed a strong correlation between infection with *H. pylori* CagA-positive

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**Table 1** Clinical, ultrasound, and serological variables in *H. pylori* negative and *H. pylori* positive CagA negative or CagA positive strains

<table>
<thead>
<tr>
<th></th>
<th><em>H. pylori</em> – (n = 39)</th>
<th><em>H. pylori</em> +/CagA – (n = 61)</th>
<th><em>H. pylori</em> +/CagA + (n = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66.9 ± 15.8</td>
<td>66.3 ± 14.0</td>
<td>68.8 ± 9.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.3 ± 3.3</td>
<td>26.4 ± 3.6</td>
<td>25.2 ± 4.5</td>
</tr>
<tr>
<td>Male sex</td>
<td>21/39 (53.8%)</td>
<td>44/61 (72.1%)</td>
<td>54/85 (63.5%)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>18/39 (46.1%)</td>
<td>31/61 (50.8%)</td>
<td>29/85 (34.1%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>63/39 (15.4%)</td>
<td>11/61 (18.0%)</td>
<td>17/85 (20%)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>13/39 (33.3%)</td>
<td>20/61 (32.8%)</td>
<td>38/85 (44.7%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25/39 (71.8%)</td>
<td>46/61 (75.4%)</td>
<td>56/85 (65.9%)</td>
</tr>
<tr>
<td>Father’s manual job</td>
<td>24/39 (61.5%)</td>
<td>34/61 (55.7%)</td>
<td>54/85 (63.5%)</td>
</tr>
<tr>
<td>IMT, mm</td>
<td>1.01 ± 0.17$</td>
<td>0.97 ± 0.15*</td>
<td>1.13 ± 0.26*$</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.6 (0.1–2.6)$</td>
<td>0.3 (0.1–5.9)$</td>
<td>1.1 (0.1–6.0)$</td>
</tr>
</tbody>
</table>

For continuous variables, values are indicated as mean ± SD. CRP values are expressed as median and range.

BMI = body mass index; IMT = intima media thickness; CRP = C-reactive protein.

At univariate analysis:

* *p* = 0.0001.
† *p* = 0.002.
‡ *p* = 0.021.
§ *p* = 0.007.

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**Table 2** Odds ratios and 95% confidence intervals for IMT in CagA positive and *H. pylori* negative patients and in CagA positive and *H. pylori* negative patients after adjustment for possible confounding factors

<table>
<thead>
<tr>
<th></th>
<th>95% CI</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>Odds ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.05</td>
<td>0.99</td>
<td>1.09</td>
<td>0.065</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.14</td>
<td>0.54</td>
<td>2.39</td>
<td>0.726</td>
</tr>
<tr>
<td>BMI</td>
<td>0.96</td>
<td>0.85</td>
<td>1.07</td>
<td>0.445</td>
</tr>
<tr>
<td>Current smokers</td>
<td>1.25</td>
<td>0.71</td>
<td>2.2</td>
<td>0.437</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.98</td>
<td>0.27</td>
<td>3.56</td>
<td>0.985</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0.47</td>
<td>0.16</td>
<td>1.44</td>
<td>0.188</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.45</td>
<td>0.83</td>
<td>7.22</td>
<td>0.104</td>
</tr>
<tr>
<td>Father’s manual job</td>
<td>1.05</td>
<td>0.41</td>
<td>2.70</td>
<td>0.918</td>
</tr>
<tr>
<td>IMT</td>
<td>1.90</td>
<td>1.22</td>
<td>2.97</td>
<td>0.005</td>
</tr>
</tbody>
</table>

BMI = body mass index; IMT = intima media thickness.
strains and atherothrombotic stroke; therefore, a relationship between infection with these strains and progressive increase with time of IMT, a marker of early atherosclerosis, has also been reported. In the present study, we explored the possible influence of infection with CagA-positive strains on the degree of IMT and the short-term clinical course of patients with large vessel stroke, in which the atherosclerotic process represents the main pathophysiologic mechanism.

We found that patients with atherothrombotic stroke infected with CagA-positive H. pylori strains, but not those infected with H. pylori CagA-negative strains, had higher levels of IMT and a poorer short-term clinical course than noninfected patients. Furthermore, within H. pylori-positive patients, significantly higher IMT values were found in those positive for CagA than in those negative for CagA. The finding of increased prevalence of infection with these strains in the highest IMT quartiles further strengthened the relationship between infection with CagA-positive strains and IMT.

Moreover, serum levels of CRP, a sensitive marker of systemic inflammation, were higher in CagA-positive patients than in CagA-negative patients. These data suggest that atherothrombotic stroke patients infected with CagA-positive strains represent a subgroup with peculiar pathophysiologic and clinical characteristics and that infection may influence the natural history of the disease in the early and late stages.

Table 3: Relationship between IMT quartiles and infectious status

<table>
<thead>
<tr>
<th>IMT quartiles (range)</th>
<th>CagA positive, %</th>
<th>CagA negative, %</th>
<th>H. pylori positive, %</th>
<th>H. pylori negative, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st (0.61–0.91 mm)</td>
<td>35.1</td>
<td>64.9</td>
<td>80.4</td>
<td>19.6</td>
</tr>
<tr>
<td>2nd (0.91–1.02 mm)</td>
<td>44.1</td>
<td>55.9</td>
<td>73.9</td>
<td>26.1</td>
</tr>
<tr>
<td>3rd (1.02–1.20 mm)</td>
<td>65.8</td>
<td>34.2</td>
<td>82.6</td>
<td>17.4</td>
</tr>
<tr>
<td>4th (1.20–2.42 mm)</td>
<td>86.5</td>
<td>13.5</td>
<td>78.7</td>
<td>21.3</td>
</tr>
</tbody>
</table>

IMT = intima media thickness.

Figure: Evolution with time of NIH Stroke Scale (NIHSS) score in cytotoxin-associated gene-A (CagA)-positive (solid line) and CagA-negative (dotted line) patients. Post hoc analysis indicates that NIHSS was significantly higher in the CagA-positive subgroup compared with the CagA-negative group at evaluation after 1 week (**p = 0.009) and 1 month (**p = 0.016).
events ultimately leading to local inflammation and early arterial damage with increased IMT. According to this hypothesis, the primary link between infection with *H. pylori* CagA-positive strains and atherothrombotic stroke should be represented by antibody response. If this is the case, the eradication therapy may be of minor relevance because antibody response may persist after *H. pylori* clearance.

Despite these limitations, the present data raise the possibility that a short course of antibiotics may be helpful because it can lead to the eradication of *H. pylori* infection in the majority of patients, which may not only lead to decreased levels of acute phase reactants but also could alter the course of the disease. This hypothesis will need to be tested in prospective studies.

Although significant, absolute differences in NIHSS scores and IMT values are rather small, and their clinical significance remains to be clarified. It should be considered that the presence of the organism at the level of the atherosclerotic plaque, suggested by recent studies, might be the cause of ongoing inflammation at the plaque level, which might negatively affect leukocyte and platelet rheology, thus interfering with the compensatory mechanisms after the abrupt interruption of cerebral arterial blood flow.

**References**


804 NEUROLOGY 63 September (1 of 2) 2004