Aims Previous studies suggested an association between infection by cytotoxic CagA-positive *Helicobacter pylori* strains and atherosclerotic stroke. It has been hypothesized that CagA strains could increase the risk for stroke by affecting carotid plaque irregularity. Our aims were: (1) to confirm the association between CagA strains and atherosclerotic stroke, and (2) to assess the association between CagA strains and carotid plaque irregularity.

Methods and results We enrolled 105 consecutive patients affected by atherosclerotic stroke and 130 sex, age, social background-matched controls without relevant vascular diseases. Risk factors for atherosclerotic stroke, *H. pylori* infection and CagA status were evaluated in all subjects. The presence of plaque instability was evaluated by colour Doppler ultrasound. The prevalence of CagA-positive strains was significantly higher in patients than in controls (adjusted OR 2.99, 95% CI 1.52–5.88, \( P = 0.002 \)). The CagA seropositivity was the only factor independently associated with carotid plaque irregularity (adjusted OR 8.42, 95% CI 1.58–44.64, \( P = 0.004 \)).

Conclusion The evidence of significant associations between CagA-positive *H. pylori* strains and the presence of carotid plaque instability support their possible involvement in the pathophysiology of atherosclerotic stroke.

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role in the development of AS. Complicated atherosclerotic lesions are associated with an increased inflammatory response. The strong inflammatory response elicited by CagA-positive strains may induce plaque instability through an immune-mediated release of cytokines and other substances endowed with proinflammatory properties.

Aims of the present study were:

1. To confirm if there is an independent association between the infection by CagA-positive strains of H. pylori and AS.
2. To assess if an association exists between the cytotoxic strains of the bacterium and carotid plaque instability (assessed as presence of plaque surface irregularity at colour Doppler ultrasound).

Methods

We performed a case-control study on 105 subjects with AS and 130 age, social and geographical background-matched controls.

Cases

We studied 105 subjects with AS (56 females and 49 males; mean age 68±8), admitted from March 1999 to May 2000 to the Department of Internal Medicine and Angiology, Gemelli Hospital, Catholic University of the Sacred Heart, Rome, Italy. All patients underwent Computed Tomography or Magnetic Resonance Imaging, basal electrocardiogram (ECG), extracranial (carotid and vertebral) colour Doppler imaging and echoangiography (transthoracic or transoesophageal). Diagnosis of AS was made according to the criteria of the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) investigators.

Controls

The control group consisted of 130 subjects (66 females and 64 males; mean age 66±7 years) without a history or presence of definite or suspected vascular diseases, defined by negative results from basal ECG and ergonomic stress testing (when suggested by the history or basal ECG) and from extracranial colour Doppler imaging.

All subjects were recruited in the outpatient unit of the Department of Internal Medicine. They all came from Rome or surrounding areas. The matching with patients was for sex, age and social background (assessed based on the father’s occupation at birth: manual versus non-manual work).

Diagnoses made in control subjects were as follows: 68 with irritable bowel syndrome, 44 relatives of patients with colorectal cancer undergoing a screening evaluation, 18 miscellaneous other conditions.

History of peptic ulcer was assessed by means of a standardized questionnaire routinely used in our outpatient service in all participating subjects.

Risk factors’ assessment

A standardized interview was performed in both patients and controls to evaluate family history, high blood pressure, diabetes, current smoking habits and alcohol intake. Body mass index (BMI), fasting total serum cholesterol and triglycerides were also evaluated. Subjects were defined as:

1. Hypertensive: if they had diastolic blood pressure >90 mmHg and/or systolic blood pressure >140 mmHg or if they were chronically (>6 months from evaluation) taking anti-hypertensive drugs.
2. Diabetics: if they had fasting levels of glucose >126 mg/dl in two distinct instances or if they were chronically (>6 months from evaluation) taking hypoglycaemic drugs.
3. Current smokers: if they were active smokers or their abstinence from smoking was <30 days at the enrolment visit.
4. Alcohol abuser: if they reported a score >7 on a validated alcohol screening questionnaire (SAAST). The SAAST is a self-report questionnaire used to assess lifetime alcohol dependence. A score of 7 or greater suggests history of alcoholism.
5. Hypercholesterolaemics: if subjects had levels of total serum cholesterol >220 mg/dl or they were chronically (>6 months from evaluation) taking lipid-lowering drugs.
6. Hypertriglyceridaemics: if subjects had levels of triglycerides >180 mg/dl or they were chronically (>6 months from evaluation) taking lipid-lowering drugs.

Moreover, subjects were defined to have a positive familiar history for AS if they had at least one first-degree relative affected by AS.

A signed informed consent was obtained by all participants.

H. pylori and CagA serology

Blood samples were collected in all patients and controls, and stored at −80 °C until analysis. Serologic studies were performed blindly; sera of patients and controls were analysed at the same time. Specific anti-H. pylori IgG antibodies were detected by ELISA (Eurospital, Trieste, Italy) according to the manufacturer’s instructions. Values higher than 10 U/ml (sensitivity and specificity >95%) defined the infectious status. In infected subjects, a serological assay for specific IgG against CagA was also performed (Radim, Pomezia, Italy). Titres were defined as positive or negative according to a cut-off value of 10 U/ml (sensitivity and specificity >95%).

Carotid colour Doppler ultrasound

Bilateral assessment of the carotid arteries was performed by a trained operator unaware of the subjects’ clinical and laboratory findings. The test was carried out with an Acuson 128XP/10 ART; Acuson, USA, with a linear 7.0 MHz probe. Patients were examined in the supine position with the neck rotated 45° in the direction opposite the site being examined. The carotid trunk was identified using both B-mode and pulsed-wave colour Doppler ultrasonography, and the following segments were examined: common carotid artery, carotid bifurcation, and internal carotid arteries. All arteries underwent longitudinal and transverse scanning as well as a flow analysis. Plaque was defined as a protrusion into the vessel lumen of at least 2 mm, as measured from the border between the adventitial and medial layers. The principal characteristics of atherosclerotic carotid plaques (degree, surface characteristics) were evaluated. The degree of stenosis was measured along the longitudinal axis and the patients were divided in two groups (50–74% or >75% of vessel diameter). Concerning surface characteristics, the plaques were considered stable or unstable according to the features of their surface: smooth, irregular (height variations <2 mm) or ulcerated (discrete depression >2 mm in width extending into the media). Due to the small number of ulcerated lesions (n=3), they were combined with the irregular ones for the purpose of analysis.
**Statistical analysis**

Differences in means and proportions were evaluated by Student’s t-test and Chi-square test, respectively. Values of $P<0.05$ were considered to be significant. Conditional logistic regression was used to estimate the odds ratio for matched case-control pairs adjusting for arterial hypertension, diabetes, BMI, familiar history for cerebral ischaemia, alcohol abuse, hypercholesterolaemia, hypertriglyceridaemia, current smoking habit. Subgroup analyses were performed in strata defined by age, sex, and social background.

Logistic regression analysis was also used to assess the association between plaque irregularity and CagA status alone and after adjusting for vascular risk factors. The analysis was performed using SPSS release 6.0 Software.

**Results**

**Study population, prevalence of H. pylori infection and CagA status**

The demographic characteristics of all participants as well as the distribution of the most relevant risk factors for atherosclerotic stroke in both patients and controls are summarized in Table 1.

<table>
<thead>
<tr>
<th>Seropositivity</th>
<th>Controls (n=130)</th>
<th>Patients (n=105)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori</td>
<td>81 (62%)</td>
<td>75 (71%)</td>
<td>1.51 (0.87–2.62)</td>
<td>1.85 (0.94–3.67)</td>
</tr>
<tr>
<td>CagA</td>
<td>30 (23%)</td>
<td>44 (42%)</td>
<td>2.40 (1.37–4.21)</td>
<td>2.99 (1.52–5.88)</td>
</tr>
</tbody>
</table>

A trend toward higher prevalence of H. pylori infection was observed in patients with atherosclerotic stroke with respect to controls, but no statistically significant difference was reached. The anti-H. pylori IgG antibodies were detected in 75 of 105 patients and in 81 of 130 controls (71% vs 62%; $P=0.14$). The crude OR was 1.51 (95% CI, 0.87–2.62) (Table 2). After statistical adjustment for the other vascular risk factors considered (conditional logistic regression), the OR was 1.85 (95% CI, 0.94–3.67; $P=0.08$).

The CagA-positive strains resulted to be significantly more prevalent in patients with atherosclerotic stroke than in controls, both before and after statistical adjustment for the other risk factors considered. In particular, anti-CagA antibodies were detected in 43 of 105 patients and in 30 of 130 normal controls (42% vs 23%; crude OR: 2.4, 95% CI, 1.37–4.21, $P=0.002$) (Table 2). Adjusted OR was 2.99 (95% CI, 1.52–5.88, $P=0.002$).

At the multivariate analysis, only hypertension and diabetes were found to be independently associated with AS (OR 4.03 and 2.92 respectively) besides CagA seropositivity.

**H. pylori infection, CagA status and plaque distribution and irregularity**

Table 2

A significant association was found between CagA positivity and detection of plaque-surface irregularity by colour Doppler imaging. In particular, CagA positivity was found in 80% of irregular plaques with respect to 37.5% of regular plaques (6.67, 95% C.I. 1.78–24.45; $P=0.03$) (Table 3). The association remained significant after
Plaque instability and atherosclerotic stroke

Table 3  Relation between seropositivity for H. pylori and CagA and plaque irregularity (PI) in patients with atherosclerotic stroke before and after adjustment for the classic vascular risk factors

<table>
<thead>
<tr>
<th>Seropositivity</th>
<th>PI+ a (n=22)</th>
<th>PI− b (n=83)</th>
<th>Crude OR</th>
<th>P</th>
<th>Adjusted OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. pylori</td>
<td>18 (80%)</td>
<td>59 (71%)</td>
<td>1.60 (0.42–5.94)</td>
<td>0.51</td>
<td>1.69 (0.35–8.10)</td>
<td>0.51</td>
</tr>
<tr>
<td>CagA</td>
<td>18 (80%)</td>
<td>31 (37%)</td>
<td>6.67 (1.78–24.45)</td>
<td>0.003</td>
<td>8.42 (1.58–44.64)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

a PI+=presence of plaque irregularity.

b PI−=absence of plaque irregularity.

Discussion

This study shows an independent association between the infection by cytotoxic CagA-positive strains of H. pylori and AS. Our study is the first to report also an independent association between infection by CagA-positive strains and the presence of plaque irregularity.

The relationship between the infection with CagA positive strains and AS has been investigated by Pietroiusti and colleagues, showing these strains to be strongly associated to AS independently of the classical vascular risk factors. A recent prospective study by Mayr and colleagues seems to support the hypothesis of such an association, reporting that in subjects infected by CagA-positive H. pylori strains, changes in common carotid artery intima-media thickness during a 5-year follow-up were significantly more marked compared to subjects infected with CagA-negative strains.

The present study identifies a link between CagA-positive H. pylori strains, AS and plaque instability. Irregularities of the atherosclerotic plaque reflect plaque instability, that is closely associated with major ischaemic events.

Several mechanistic models may be invoked to explain the association found. First, it is well known that CagA positive strains are able to induce an enhanced inflammation in the gastric mucosa. Since the inflammation plays a major role in atherosclerosis and complicated lesions are associated with an increased inflammatory response, the strong inflammatory response related to the infection by CagA-positive strains may influence them through an immune-mediated release of cytokines and other substances endowed with proinflammatory properties. Our study was hypothesis-generating, and not aimed at identifying associations between infection markers and inflammation (e.g. C-reactive protein values, a well known sensitive marker for systemic inflammatory response). However, previous cross-sectional studies assessing the role of H. pylori/CagA seropositivity in the atherosclerotic-related diseases did not find any significant association between infection status and C-reactive protein values. We agree with Mayr et al. that the possible synergistic effect of infection, CagA positivity and high C-reactive protein levels on atherosclerotic disease is a problem to be investigated in future studies.

Second, it has been suggested that H. pylori, as well as Chlamydia pneumoniae, could directly provoke inflammation within the atherosclerotic plaques. H. pylori DNA has been found in carotid atherosclerotic lesions and has associated with features of inflammatory cell response. Third, an autoimmune reaction could be postulated. A cross-reactivity between anti-CagA antibodies and vascular wall antigens has been recently demonstrated, suggesting that these antibodies may contribute to the activation of inflammatory cells within atherosclerotic lesions. This activation might eventually lead to plaque destabilization.

We perceive that the present study has some limitations. The independent association found in a case-control study does not allow us to establish a pathogenic link between H. pylori CagA status, plaque instability and atherosclerotic stroke. Prospective data demonstrated that infection with CagA-positive strains significantly increases the risk of carotid atherosclerosis. More studies are needed to confirm prospectively the novel association between CagA and plaque instability found in this study. It may also be argued that the control population we used may not be representative of the general population. However, the prevalence rates of H. pylori infection and CagA positive strains in our control subjects were similar to those observed in control populations from two previous studies conducted in our same geographical area, suggesting that our findings have concurrent validity in the geographical setting of urban central Italy.

In conclusion, the associations found generate the hypothesis for a pathogenic link between cytotoxic CagA-positive H. pylori strains, plaque instability and AS. The relevance of such association should be established by larger prospective observational and interventional studies. Indeed, the pathophysiologic mechanisms underlying this association should also be tested using animal disease models. Confirming and defining CagA-positive H. pylori infection as a novel risk factor for atherosclerotic stroke might open new perspectives for stroke prevention.