cagA status and virulence of *Helicobacter pylori* strains

Results of a French multicentric prospective study

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**SUMMARY**

Previous experimental and epidemiological studies with few patients suggested that the presence of the cagA gene was a virulence factor for *Helicobacter pylori* (H. pylori).

**Aim** — To establish in this large epidemiological cohort study the relationship between the histological virulence of *H. pylori* infection and the cagA status of the bacteria.

**Methods** — This prospective cohort study (6 month follow-up) was conducted on adult patients undergoing endoscopy for upper gastrointestinal symptoms. The cagA status of *H. pylori*-positive patients was established using the polymerase chain reaction (PCR) method on an antral biopsy. A score of histological virulence (inflammation, activity) was recorded on the basis of the Sydney system (on antral, angular and fundic biopsies). Eradication treatment given was not imposed and a clinical follow-up was performed at 3 and 6 months. *H. pylori* eradication was verified by a 13C urea breath test at 3 months.

**Results** — Four hundred and twenty two centers recruited 652 patients (mean age: 51 ± 15 years, 55% female). Upper GI endoscopy was abnormal in 80% of the patients of whom 68% had a gastritis aspect; 38% were infected by *H. pylori*, and among them 51% were cagA-positive. The histological virulence scores associated with the cagA-positive strains were significantly higher than those associated with the cagA-negative strains, globally (P = 0.0035), in the antrum (P = 0.0063), and in the angular (P = 0.046), but not in the fundus (P = 0.05). The cagA status was correlated neither with the symptom severity at inclusion and at 6 months (P > 0.05), nor with the *H. pylori* eradication rate at 3 months (75% in cagA-positive and 70% in cagA-negative strains, P = 0.52).

**Conclusion** — This study on a large cohort of patients confirms the greater histological virulence of *H. pylori* cagA-positive strains. However, this virulence was not associated with more severe symptoms nor with an increase in resistance to *H. pylori* eradication treatment.

Key words: Helicobacter pylori. CagA. Virulence.

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**Material and methods**

**Study population**

In November 1996, a representative sample of 616 gastroenterologists practicing in metropolitan France were selected to conduct a prospective cohort study among outpatients consulting for upper digestive tract disorders. Each participating physician was to include two patients.

Patients included were to be aged at least 18 years, present clinical manifestations warranting upper digestive tract endoscopy in a non-emergency setting and have no contraindications for endoscopy and biopsy (no anticoagulants or antiplatelet drugs during the 4 days before...
endoscopy). The patients were not to have taken H. pylori eradication treatment over the last six months or proton pump inhibitors or antibiotics over the last 1.5 days. Patients were asked to give their written informed consent to the study and agree to participate in follow-up for six months. To avoid selection bias, each physician included the first two patients meeting the inclusion criteria during two consecutive days following study onset.

Method

PATIENT INCLUSION

The following data were recorded for each patient: reason for performing upper digestive tract endoscopy, lesions observed at endoscopy, history of esophageal, gastric, or duodenal disorders, severity of digestive signs as evaluated on a visual analogue scale (VAS), and treatment prescribed at the end of the initial consultation.

Biopsies were taken during the endoscopic procedure from the antrum, the angulus, and the corpus for histopathological examination. A urease test (CLO-test) was performed on two antral biopsies. After 2h incubation, each CLO-test was sent by courier to the bacteriology laboratory of Pellegrin Hospital, Bordeaux, where the biopsies were ground for polymerase chain reaction (PCR) detection of the cagA gene using the cag1-2 probes, then the cag3-4 probes if the first test was negative [10].

PATIENT FOLLOW-UP

In order to verify the efficacy of the eradication treatment chosen by each gastroenterologist, a 1C labeled urea breath test (Respylo-test) was performed three months after inclusion for patients who had a positive CLO-test and who had received eradication treatment. A sample of expired air was collected before and 30 minutes after ingestion of 75 mg labeled urea (citric acid was given to patients prior to the test to inhibit gastric emptying). Analyses were centralized and performed on an ABACA mass spectrometer (Europro Scientific). Delta per 1000 greater than 3.5 was considered positive. Data collected six months after inclusion mainly concerned the intensity of the clinical manifestations and treatments received since inclusion.

HISTOLOGICAL VIRULENCE

Histological virulence was established from the histopathological examination of the gastric biopsies centralized in four laboratories (Bordeaux, Marseille, Nancy, and Paris). The pathologist scored the four Sydney morphological criteria (inflammation, activity, atrophy, intestinal metaplasia) for each of the three biopsy areas (antrum, angulus, corpus) using a scale from 0 to 3 (0 = absent, 1 = minimal, 2 = moderate, 3 = severe) [11]. The histological virulence score was taken as the sum of the inflammation and activity scores, giving a possible score of 0 to 6 for each biopsy zone, and an overall score of 0 to 18. A second score was also calculated with the same method using the sum of the atrophy and intestinal metaplasia scores.

HELIcobacter Pylori infection and cAGa STATUS

H. pylori detection was performed on each patient, using two methods: urease test (CLO-test) and histopathological examination. If these two methods were discordant, the histopathological examination was considered for subsequent analysis.

cagA status was determined on antral biopsies for all H. pylori-positive patients. PCR was performed in a centralized laboratory, was considered positive when one of the two PCR was positive.

CLINICAL MANIFESTATIONS

Dyspepsia was evaluated with a 0-100 VAS. In addition to an overall intensity score estimated by the patient, four subscores were determined for “reflux”, “motor disorder”, “ulcer”, and “intestinal functional disorder” type dyspepsia.

ABBREVIATIONS:

H. pylori : Helicobacter pylori
PCR : polymerase chain reaction
RGO : reflux gastro-esophageen
EVA : échelle visuelle analogue

Statistical analysis

Statistical analyses were performed with SAS (version 6.12, SAS Institute, Cary, North Carolina). The significance threshold was 95% (α = 5%). Univariate analysis included the Pearson chi-square test or Fisher exact test for percentage comparisons, and the Student’s t test or Kruskall-Wallis test for mean comparisons. The Wilcoxon test was also used to compare the bacterial density in the antrum, angulus and corpus. The Student’s t test for paired variables was used to compare the clinical follow-up data.

Variance-covariance was determined to compare mean virulence scores. These multivariate analyses were performed with adjustment for confounding factors (type of endoscopic lesion) and were based on the following linear regression equation: virulence score = f(cagA status + adjustment factors).

Results

The cohort was composed of 652 patients included by 422 independently working gastroenterologists. Mean patient age was 51.3 ± 15 years, 55% were women (sex ratio 0.82), 85% were born in France, and 20% were smokers.

The endoscopy performed at inclusion was abnormal for 523 patients (80.3%): duodenal ulcer (55 patients), gastric ulcer (12 patients), duodenal erosions (37 patients), gastric erosions (52 patients), esophagitis (160 patients), gastritis (354 patients), other lesions (108 patients). For the 354 patients with endoscopic evidence of gastritis, antral localizations predominated (74%).

Chronic gastritis for at least one of the biopsy areas was observed at histology in 394 patients (60.9%): antrum (58.6%), angulus (56.5%), corpus (51.9%).

The H. pylori and cagA status of the study population are summarized in table I. H. pylori status was ascertained for 643 patients. H. pylori-positive and H. pylori-negative patients were comparable for gender (p = 0.916), socio-economic status (p = 0.172), and reason for upper intestinal endoscopy (p = 0.292), but the H. pylori-positive patients were significantly older (53 ± 14.7 versus 50.3 ± 15.9 years, p = 0.037).

Among the 245 H. pylori-positive patients (table II), the cagA status was known for 193 (78.7%). The CLO-test was positive in 215 patients but was a false positive in 22 cases; histological proof of H. pylori infection was found in 52 patients with a negative CLO-test and therefore cagA was not tested in these latter patients. Patients infected with a cagA-positive strain were comparable with those infected with a cagA-negative strain for gender (p = 0.810), age (p = 0.306), socio-economic status (p = 0.750), and reason for endoscopy (p = 0.473). There was no significant difference between patients infected with cagA-positive and cagA-negative strains for endoscopically identified lesions (table II): 83.8% of the 99 cagA-positive patients had at least one endoscopic abnormality compared with 84.0% of the 94 cagA-negative patients (p = 0.969).

The level of histological virulence (inflammation, activity) as assessed by the mean virulence score (table III) was significantly higher in patients with a cagA-positive strain in the antrum and the angulus (p = 0.0063 and 0.0046, respectively). The difference was less marked for the corpus (p = 0.05). Considering all localizations together, cagA-positive strains were significantly more virulent than cagA-negative strains (p = 0.003). The difference was not significant at the antrum level for the presence of atrophy with or without intestinal metaplasia (p = 0.025). After adjustment for the different types of lesions observed at the first endoscopy, the effect of cagA status on histological virulence (inflammation, activity) remained significant (p = 0.003). The mean overall virulence score, adjusted in accordance with the
variance-covariance analysis model, was 11.49 ± 0.90 and 10.01 ± 0.97 for cagA-positive and cagA-negative patients, respectively.

There was a significant difference in the level of histological virulence (inflammation, activity) for bacterial density scored 1 to 3 in the antrum, the angulus, and also the corpus (p < 0.0001) (table IV). This difference was found for both cagA-positive and cagA-negative strains. Conversely, irrespective of the status, symptoms had improved at six months with a significant difference in the mean value on the corresponding VAS was not significant between patients infected with H. pylori and cagA-negative strains, at inclusion (table V) and six months after inclusion. At inclusion, among the patients with reflux symptoms were significantly more severe in patients with cagA-negative strains. Conversely, irrespective of the cagA status, symptoms had improved at six months with a significantly lower score for each type of symptom (p < 0.001). A breath test (Respylo-test®) was performed at three months to examine for eradication of H. pylori in 117 patients who had been given a triple therapy. The cagA status was known for 114 of these patients (57 with peptic ulcer and 57 with non ulcer dyspepsia). The rate of eradication was not significantly different between the cagA negative and positive patients (75.4% and 70.2% eradication, respectively, p = 0.52).

The severity of each type of clinical symptoms as assessed by the mean value on the corresponding VAS was not significantly different between patients infected with cagA-positive and cagA-negative patients, at inclusion (table VI) and six months after inclusion. At inclusion, among the patients with reflux symptoms were significantly more severe in patients with cagA-negative strains. Conversely, irrespective of the cagA status, symptoms had improved at six months with a significantly lower score for each type of symptom (p < 0.001). A breath test (Respylo-test®) was performed at three months to examine for eradication of H. pylori in 117 patients who had been given a triple therapy. The cagA status was known for 114 of these patients (57 with peptic ulcer and 57 with non ulcer dyspepsia). The rate of eradication was not significantly different between the cagA negative and positive patients (75.4% and 70.2% eradication, respectively, p = 0.52).

### Discussion

This prospective open study in a large cohort confirms the greater histological virulence of cagA-positive H. pylori strains compared with cagA-negative strains. The prevalence of H. pylori infection in the study population recruited by gastroenterologists was 38%. This is a low prevalence, but close to that observed in another study conducted in France where the prevalence was 48%, among patients consulting gastroenterologists for upper digestive tract symptoms [12]. The prevalence of cagA-positive strains here was also low (51.3%) probably because of the wide recruitment of unselected patients (no selection for ulcer symptoms). Several studies have suggested that cagA-positive strains are associated with a greater degree of gastric inflammation than cagA-negative strains [13-17]. However, most of these studies have included small numbers of patients. cagA-positive strains, called type I strains, carrying the cag pathogenicity island and considered to be more virulent than the cagA-negative strains, have been observed more frequently among patients with severe gastroduodenal disorders such as gastric or duodenal ulcers, than in patients with uncomplicated chronic gastritis [18]. Furthermore, it has been demonstrated that infection with a cagA-positive strain is a risk factor for the development of atrophic gastritis [19-22], a precursor of gastric carcinoma [9, 23].

The greater virulence of the cagA-positive strains is related to their capacity to trigger a major inflammatory reaction. Patients with gastric ulcer or carcinoma exhibit more pronounced gastric inflammation than H. pylori-infected patients without ulcer. This observation indicates that inflammation plays an important role in the development of local gastroduodenal lesions and that the intensity of the inflammation and its localization could determine the risk of overt lesions. The mechanism of the greater inflammatory response to cagA-positive strains has been partially elucidated. These strains stimulate production of IL-8, a powerful pro-inflammatory mediator, and subsequent recruitment of polymorphonucleotides responsible for the inflammatory infiltrate. Several genes in the cag pathogenicity island cause epithelial cells to release IL-8 [24]. However, strains without the pathogenicity island can also induce an inflammatory response and gastritis, though to a lesser degree. In this case, other virulence factors, intrinsic to all H. pylori strains such as urease, LPS, and perhaps other bacterial antigens, are undoubtedly operating.

In our study, patients infected with cagA-positive strains had a greater degree of gastric inflammation in the antrum and the angulus, but not in the corpus, compared with cagA-negative patients. This could be explained by the distribution of the bacteria in untreated patients, known to be more abundant in the antral than in the corpus region.

Our results are in agreement with earlier reports showing more severe gastric inflammation [13, 14], greater activity, and more pronounced atrophy [14, 16], intestinal metaplasia [14].

### Table I. Patient distribution according to H. pylori and cagA status.

<table>
<thead>
<tr>
<th>H. pylori Status</th>
<th>n (%)</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>245</td>
<td>38.1</td>
</tr>
<tr>
<td>Negative</td>
<td>398</td>
<td>61.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cagA Status</th>
<th>n (%)</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>193</td>
<td>100.0</td>
</tr>
<tr>
<td>Negative</td>
<td>99</td>
<td>51.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cagA status</th>
<th>n (%)</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>94</td>
<td>48.7</td>
</tr>
<tr>
<td>Negative</td>
<td>94</td>
<td>51.3</td>
</tr>
</tbody>
</table>

a Determined for 9 patients. 95% CI: 95% confidence interval.

### Table II. Results of initial upper gastroduodenal endoscopy according to cagA status.

<table>
<thead>
<tr>
<th>Endoscopic findings</th>
<th>cagA status</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n = 99)</td>
<td>Negative (n = 94)</td>
</tr>
<tr>
<td>Normal</td>
<td>16 (16.2)</td>
<td>15 (16.0)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>60 (72.3)</td>
<td>50 (63.3)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>26 (31.3)</td>
<td>18 (22.8)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>5 (6.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Duodenal erosion</td>
<td>9 (10.8)</td>
<td>7 (8.9)</td>
</tr>
<tr>
<td>Gastric erosion</td>
<td>12 (14.5)</td>
<td>6 (7.6)</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>18 (21.7)</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (12.1)</td>
<td>10 (12.6)</td>
</tr>
</tbody>
</table>

* Some patients had several types of lesions, the total is >100%. a Pearson χ². b Fisher exact test.

### Table III. Histological virulence (inflammation, activity) score of each gastric localization according to cagA status.

<table>
<thead>
<tr>
<th></th>
<th>cagA-positive (n = 99)</th>
<th>cagA-negative (n = 94)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Mean score (SD)</td>
<td>Mean score (SD)</td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>98</td>
<td>4.4 (1.1)</td>
<td>94</td>
</tr>
<tr>
<td>Angulus</td>
<td>98</td>
<td>4.2 (1.1)</td>
<td>94</td>
</tr>
<tr>
<td>Corpus</td>
<td>98</td>
<td>3.2 (1.4)</td>
<td>93</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>11.9 (3.0)</td>
<td>93</td>
</tr>
</tbody>
</table>

* Student’s t test. a Theoretical range of the local histological virulence score = 0-6. b Theoretical range of the overall histological virulence score = 0-18.
Finally, the intensity of gastric inflammation (inflammation, activity) score of each gastric localization according to bacterial density score.

| Bacterial density (± SD) | P
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Antrum</td>
<td>2.9 (1.1)</td>
</tr>
<tr>
<td>Angulus</td>
<td>2.5 (1.2)</td>
</tr>
<tr>
<td>Corpus</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Total</td>
<td>6.1 (2.5)</td>
</tr>
</tbody>
</table>

* Kruskall-Wallis test.

| Table V – Severity of initial dyspeptic symptoms according to cagA status.

| cagA-positive (n = 99) | cagA-negative (n = 94) | P
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n (mean (SD))</td>
<td>n (mean (SD))</td>
<td></td>
</tr>
<tr>
<td>Reflux type</td>
<td>76 (27.4 (3.5))</td>
<td>74 (37.9 (3.8))</td>
</tr>
<tr>
<td>Ulcer type</td>
<td>81 (34.6 (3.7))</td>
<td>78 (36.0 (4.0))</td>
</tr>
<tr>
<td>Motor disorder type</td>
<td>79 (19.4 (3.0))</td>
<td>73 (17.6 (2.9))</td>
</tr>
<tr>
<td>Intestinal functional disorder type</td>
<td>79 (19.4 (3.1))</td>
<td>74 (13.7 (2.7))</td>
</tr>
<tr>
<td>Overall symptom intensity</td>
<td>81 (60.0 (4.63))</td>
<td>78 (57.9 (2.7))</td>
</tr>
</tbody>
</table>

Symptom severity evaluated on a visual analogue scale. * Student test. b Kruskall-Wallis test.

REFERENCES


