

Colonisation density and topographic localisation of *Helicobacter pylori* do not depend on the *cagA* status

M Twisk, J G Kusters, A G Balk, E J Kuipers, R J L F Loffeld

Abstract

Aims—To explore the correlation between the *cagA* status of *Helicobacter pylori* and the density and topographic localisation of *H pylori*.

Methods—Gastric antral biopsy specimens were taken from 716 consecutive patients, including 293 *H pylori* positive patients (124 men, 169 women; mean age, 52.6 years; range, 12–87). A serum sample was taken for determination of IgG anti-CagA antibodies (sensitivity of 94.4% and specificity of 92.5%). The density of *H pylori* was assessed semiquantitatively (grades I–IV) in biopsy specimens stained with the modified Giemsa stain. Topographic localisation was classified as follows: score A, *H pylori* closely attached to the mucosa; score B, *H pylori* attached to the mucosa and in the mucus; and score C, *H pylori* solely in the mucus.

Results—CagA antibodies were present in 154 (52.5%) of the patients. There was no significant difference in colonisation density and *cagA* status: grade I, 23 (14%); grade II, 78 (50.6%); grade III, 42 (27.5%); and grade IV, 11 (7.2%) in the *cagA*⁺ strains and 29 (21.2%), 57 (40.8%), 38 (27%), and 15 (11%), respectively, in the *cagA*[−] strains. There was no difference in topographic localisation between *cagA*⁺ and *cagA*[−] *H pylori*. Mean anti-CagA titres were 0.84, 0.84, 0.89, and 0.73 in patients with grades I–IV bacterial density, respectively.

Conclusion—Antibody titres do not correlate with *H pylori* density and there is no difference in density between *cagA*⁺ and *cagA*[−] *H pylori* strains. In addition there is no difference in topographic localisation between *cagA*⁺ and *cagA*[−] *H pylori* strains. (J Clin Pathol 2001;54:771–773)

Keywords: *Helicobacter pylori* topography; *Helicobacter pylori* colonisation; density; antibody titres

Colonisation with *Helicobacter pylori* causes active chronic gastritis and elicits an antibody response that can be used for diagnostic purposes.

An important marker of *H pylori* virulence is the *cag* pathogenicity island.¹ This part of the bacterial genome encodes the CagA protein, the function of which has recently been elucidated.^{2,3} Colonisation with *cagA*⁺ *H pylori* strains is associated with an increased risk for the development of peptic ulcer disease and gastric cancer.⁴

The IgG antibody titre is indicative of the severity of gastritis⁵ and the presence of *cagA*⁺ *H pylori* strains.⁶ Therefore, we reasoned that the colonisation density of *cagA*⁺ *H pylori* strains in the antral mucosa should be significantly higher than that of *cagA*[−] strains.⁷ However, data about the density of *H pylori* in the gastric antrum, its relation to IgG antibodies against CagA, and the topographical localisation of *H pylori* are lacking. For this reason, a cross sectional study was carried out to explore the hypothesis that the presence or absence of the *cag* pathogenicity island in the infecting strain (as assessed by the presence of IgG antibodies against CagA) correlates with bacterial density in the gastric antrum, or the topographic localisation of the strain (that is, predominantly found intimately associated with gastric epithelial cells versus lying more distantly in the mucus layer).

Patients and methods

Consecutive patients referred for upper gastrointestinal endoscopy, because of reflux complaints or dyspepsia, were eligible for inclusion. After informed consent, endoscopy was performed using the Olympus EVIS 100 video endoscope, and antral biopsy specimens were taken for detection of *H pylori* via culture and Gram stain, standard haematoxylin and eosin staining, rapid urease test, and immunoperoxidase staining, as described previously.⁸ In addition, a serum sample was taken and stored frozen at −70°C for the determination of IgG antibodies against *H pylori* and IgG anti-CagA antibodies.

Biopsy specimens were cut and stained according to the modified Giemsa protocol.⁹ Colonisation density of *H pylori* was assessed semiquantitatively at high power (magnification, ×400) in well oriented sections. The density was graded as follows: grade I, sporadic presence of bacteria within the mucus layer only detectable after scrutinised search of the entire biopsy specimen; grade II, clusters of bacteria present; grade III, bacteria covering at least half of the mucosal surface; and grade IV, the entire mucosal surface covered with bacteria. The topographical distribution of *H pylori* was described as follows: score A, *H pylori* closely attached to the mucosa; score B, *H pylori* attached to the mucosa and widely distributed in the gastric mucus; and score C, *H pylori* solely in the mucus.

Specific IgG antibodies against *H pylori* were measured in serum using an in house enzyme linked immunosorbent assay (ELISA) as described previously.¹⁰ An absorbance index

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Accepted for publication
1 May 2001

above 0.32 was considered positive. Sensitivity and specificity of this ELISA for the detection of *H pylori* carriage were 98.5% and 91.7%, respectively.¹⁰

Determination of the *H pylori* *cagA* status was based on the presence of serum IgG antibodies to orv220, a 65 kDa recombinant CagA product purified from *Escherichia coli*.⁹ The presence of these antibodies was assessed by means of ELISA according to previously described methods.¹¹ The ELISA technique has been validated in the USA and yielded a sensitivity of 94.4% and a specificity of 92.5% for the detection of carriage of *cagA*⁺ *H pylori* strains. In addition, we validated the technique for the Dutch population by means of sera from 311 patients assessed to be *H pylori* negative by the combination of negative histology, culture, rapid urease test, and serology for *H pylori*. The mean result +2 SD in this population yielded an optical density cut off value of 0.458. All results above this value were considered positive.

Patients were considered to be *H pylori* positive if one of the histological or microbiological methods yielded a positive result. Only *H pylori* positive patients were included in our present study.

Previous use of acid suppressive treatment (H2 receptor antagonists or proton pump inhibitors) was assessed in all patients, but these patients were not excluded from the study.

Statistical analysis was done with χ^2 test for contingency tables and the *t* test. A *p* value below 0.05 was considered significant.

The study was approved by the medical ethics committee of De Heel Zaans Medisch Centrum.

Results

In total, 716 consecutive patients were studied; of these 345 were *H pylori* positive. From 293 of these patients (124 men, 169 women; mean age, 52.6 years; range 12–87), both serum and histology were available for the determination of IgG anti-CagA antibodies and modified Giemsa staining. There was no difference in age between men and women. CagA specific antibodies were present in 154 (52.5%) of the patients. There was no significant difference in colonisation density and *cagA* status (table 1). In the group of patients showing grade I bacterial density, a significantly higher number of patients received pretreatment with proton pump inhibitors. Table 2 shows the association between bacterial density and use of proton pump inhibitors or H2 blockers. When patients using proton pump inhibitors were excluded from the analysis no changes occurred in the relation between bacterial density and *cagA* status (table 3). Patients with grade I density were excluded from topographical scoring because *H pylori* was present only sporadically. Hence, correct topographic localisation in these cases was judged to be inadequate. There was no significant difference in topographical scores between *cagA*⁺ or *cagA*⁻ *H pylori* strains (table 4). Table 5 shows the relation between bacterial density and topographical score.

Table 1 Bacterial density in the gastric antrum in relation to *cagA* status of *Helicobacter pylori*

Density	Strain characteristics	
	<i>cagA</i> ⁺	<i>cagA</i> ⁻
Grade I	23 (14%)	29 (21.2%)
Grade II	78 (50.6%)	57 (40.8%)
Grade III	42 (27.5%)	38 (27%)
Grade IV	11 (7.2%)	15 (11%)

Not significant.

Table 2 Use of acid suppressive treatment in relation to bacterial density

Density	N	Medication	
		H2 blockers	PPI
Grade I	52	7 (13.5%)	13 (25%)
Grade II	135	46 (34%)	18 (13%)
Grade III	80	26 (32.5%)	5 (6.3%)
Grade IV	26	9 (34.6%)	1 (3.8%)

p <0.001.

PPI, proton pump inhibitors.

Table 3 Bacterial density in relation to *cagA* status after exclusion of patients receiving pretreatment with proton pump inhibitors

Bacterial density	Strain characteristics	
	<i>cagA</i> ⁺	<i>cagA</i> ⁻
Grade I	15 (10.9%)	24 (20.2%)
Grade II	72 (52.5%)	45 (37.8%)
Grade III	39 (28.5%)	36 (30.3%)
Grade IV	11 (8.1%)	14 (11.7%)

Not significant.

Table 4 Topographical localisation of *Helicobacter pylori* in antral biopsy specimens in relation to *cagA* status

Topographical localisation	Strain characteristics	
	<i>cagA</i> ⁺	<i>cagA</i> ⁻
A	38 (29%)	30 (28%)
B	42 (32%)	36 (33%)
C	52 (39%)	43 (39%)

Table 5 Topographical localisation (A, B, and C: see methods) in relation to bacterial density

Density	A + B	C
Grade II	75 (45%)	64 (67%)
Grade III	70 (42%)	28 (29%)
Grade IV	22 (13%)	3 (4%)

p <0.001.

The mean (SD) IgG antibody titres were 0.71 (0.25) in patients with grade I *H pylori* density; 0.72 (0.26) in grade II; 0.74 (0.28) in grade III; and 0.68 (0.22) in grade IV. No significant differences were present. The mean (SD) anti-CagA titre was 0.84 (0.21) in patients with grade I bacterial density; 0.84 (0.21) in grade II; 0.89 (0.21) in grade III; and 0.73 (0.24) in grade IV. These results did not differ significantly. Exclusion of patients receiving treatment with proton pump inhibitors from the analysis had no effect.

Discussion

Helicobacter pylori colonisation causes inflammation of the gastric antrum and the corpus. The degree of inflammation,⁵ the density of *H pylori*, and the characteristics of the colonising strain correlate with each other.

The *cag* pathogenicity island is an important marker of virulence of *H pylori*. *CagA*⁺ strains are associated with increased intensity of gastric inflammation and increased mucosal concentrations of certain cytokines, particularly interleukin 8,¹² and are associated with the development of peptic ulcer and gastric cancer. The IgG antibody response correlates with the severity of gastritis.⁵ From this point of view, it could be argued that *cagA*⁺ *H pylori* strains elicit a higher immune response than *cagA*⁻ strains. In the literature, one report suggested that the density of *H pylori* in the gastric mucosa is higher in *cagA*⁺ than in *cagA*⁻ patients.⁷ The results of our study do not agree with this report. The obvious reason is that the results of quantitative culture, which can be judged as an indirect method, are different from direct visual analysis of biopsy specimens. In our patients no difference in bacterial colonisation density was noted when *cagA*⁺ and *cagA*⁻ *H pylori* strains were compared. Therefore, the presence of a more virulent strain does not necessarily result in gastric inflammation with a higher degree of bacterial colonisation density.

In our study, the *cagA* status of *H pylori* was assessed by the presence of antibodies against CagA, the protein encoded by the *cagA* gene. When these antibodies are present, it is assumed that a *cagA*⁺ *H pylori* strain is present.

It was recently reported that the CagA protein is delivered into gastric epithelial cells by *H pylori*, where it triggers profound changes in the morphology of the host cells.^{2,3} The cytoskeleton is rearranged and a cup shaped pedestal forms beneath the bacterium. This process is thought to require intimate contact (attachment) between *H pylori* and the gastric cells. From these experiments it could be postulated that *cagA*⁺ *H pylori* strains are more closely attached to the epithelial cells; however, our present study showed that there was no difference in topographical localisation between *cagA*⁺ and *cagA*⁻ strains. The fact that topographical localisation is related to bacterial density suggests that topographical localisation is determined by colonisation density or vice versa, and not by the presence of the CagA protein. Finding a relation between density and colonisation is not unexpected because higher density might reflect an increased growth rate, whereas differences in localisation might reflect differences in the availability of nutrients.

It is well known that profound acid suppression with proton pump inhibitors results in a shift in *H pylori* from the antrum to the corpus,

which is reflected by a decrease in bacterial density in the antrum.^{13,14} In the Netherlands, many patients referred for upper gastrointestinal endoscopy have been pretreated with some type of acid suppressive drug. When all patients using proton pump inhibitors were excluded from analysis the results of our study did not change.

Although high IgG antibody titres indicate the presence of a *cagA*⁺ *H pylori* strain, this titre is of no value in determining the colonisation density of *H pylori* in the gastric antrum. In addition, the same is true for antibodies against CagA.

In conclusion, anti-CagA antibody titres do not correlate with antral colonisation density of *H pylori* and there is no difference in bacterial colonisation density between *cagA*⁺ and *cagA*⁻ *H pylori* strains. The topographical distribution of *H pylori* is not determined by *cagA* status.

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J Clin Pathol 2001 54: 771-773

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