Relationship between bacterial load, morbidity and *cag*A gene in patients infected by *Helicobacter pylori*

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Abstract

One hundred and seventy-six biopsies of the gastric corpus and antrum from 97 patients were processed using classical and molecular methods in order to study the relationship between the factor *cag*A of *Helicobacter pylori*, bacterial load and morbidity. Bacterial load in patients with *cag*A was greater than in patients without it, both in the antrum and corpus ($p < 0.01$). There was a statistically significant association between *cag*A and consumption of proton pump inhibitors (adjusted odds ratio 3.11). Haemorrhage of the upper digestive tract was more associated with bacterial load than with the *cag*A gene (adjusted odds ratio 2.34 and 1.12, respectively), but none of these associations yielded statistical significance.

Keywords: *cag*A gene, gastric pathology, *Helicobacter pylori*, quantification, RT PCR

Original Submission: 25 August 2011; **Revised Submission:** 26 March 2012; **Accepted:** 28 March 2012
Editor: F. Megraud
Article published online: 4 April 2012
Clin Microbiol Infect 2012; **18:** E251–E253
10.1111/j.1469-0691.2012.03884.x

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*Cag*A bacterial virulence factor has been considered to be involved in gastric pathology associated with *H. pylori* [1,2]. Our aim was to evaluate the association between the number of microorganisms in the patient's mucosa (bacterial load) and cagA, and to determine whether they are associated with a greater severity of the clinical condition.

One hundred and seventy-six biopsies of the gastric corpus and antrum from 97 consecutive patients in whom there was clinical suspicion of infection by *H. pylori* ($n = 52$ duodenal ulcer, $n = 15$ duodenitis, $n = 24$ gastric ulcer, $n = 6$ gastritis), were processed using Gram stain, culture, rapid urease test, anatomopathological study and DNA extraction.

The quantification of the microorganisms was carried out by amplifying a fragment of the urease gene. This system has an internal control, also designed in our laboratory [3]. In order to correct for the effect of the size of the gastric biopsy the number of human cells in the biopsy sample was quantified by amplifying the human albumin gene. The detection of the *cag*A gene was carried out using a real-time PCR system (RT-PCR) designed and validated in our laboratory [4].

The PCR values obtained were classified into three groups based on the tertiles of each distribution of the number of *H. pylori* microorganisms present according to RT-PCR: scarce (up to 13.0), moderate (13.0–422.5) and abundant (>422.5). The reference group corresponded to the lowest bacterial load category.

In order to estimate associations between the *cag*A gene and associated clinical factors, adjusted ORs were estimated in different multivariate models.

The *cag*A gene was detected in 103 biopsies (58.5%), corresponding to 55 patients (57.3%) (5). *Cag*A-positive patients were on average 7.6 years younger than *cag*A-negative patients ($p = 0.04$) and smokers ($p = 0.02$). No significant differences were found for age, sex or other clinical and lifestyle characteristics analysed.

Bacterial load was much higher in patients with the virulence *cag*A gene: median *cag*A-positive antrum 345.4 (interquartile rank (IR) 1710.5); median *cag*A-negative antrum 58.4 (IR 297.8) $p = 0.002$; median *cag*A-positive corpus 446.6 (IR 5190.6); median *cag*A-negative corpus 26.1 (IR 191.8) $p < 0.001$.

In both the antrum and corpus a strong statistically significant association was found between *cag*A and the number of microorganisms when the bacterial load was divided into three ordinal categories (scarce, moderate and abundant), with a very significant dose–response pattern: crude odds ratio for the highest category (ORH) in the antrum 4.28 (95% CI 1.5–12.7), p trend = 0.009; ORH corpus 12.5 (95% CI 3.0–52.5), p trend < 0.001. These associations became more marked after adjusting for age, sex, upper digestive tract haemorrhage and prior antibiotic treatment (see Table 1).

Table 2 shows the associations between digestive tract morbidity, *cag*A and bacterial load adjusted for each other. *Cag*A is more closely associated with the consumption of proton pump inhibitors than is the bacterial load: adjusted OR *cag*A 3.11 (95% CI 1.0–9.6); ORH bacterial load 2.34. In relation to clinical presentation of upper digestive tract haemorrhage, although none of them yielded statistical significance, association was higher for bacterial load (ORH 2.34, 95% CI 0.6–8.5) than for *cag*A (OR 1.12). However, consumption of NSAIDs (OR 8.99) or male sex (OR 3.26) is more closely associated with upper digestive tract haemorrhage than with *cag*A gene or bacterial load. In the case of a history of complicated ulcer, male sex is the factor most strongly associated (OR 7.20).

Our data show that the *cag*A gene is detected in younger people and it is statistically associated at high bacterial loads with a clear, significant dose–response (p trend) relationship between *H. pylori* load and presence of *cag*A. These data suggest that both parameters combine to produce clinically significant lesions, as compared with colonization by strains of low virulence that multiply very little in the gastric mucosa and so do not cause significant damage [5]. These data are corroborated by the great variability in bacterial load values obtained. In some patients the number of microorganisms in the gastric mucosa was very small and therefore it is unlikely in these cases that the microorganism had an important influence on the patient's pathology [6,7].

Our results suggest that the *cag*A gene is a risk factor independent of bacterial load in patients with a history of consumption of proton pump inhibitors (OR 3.11). This association could be explained by the fact that the virulence gene *cag*A produces greater gastric symptomatology, thus fomenting consumption of proton pump inhibitors. It may also be the case that consumption of these drugs decreases the bacterial load, thus weakening their association with the latter and strengthening their association with *cag*A.

On the other hand, it is striking that clinical presentation in the form of upper digestive tract haemorrhage (our most robust variable as an indicator of the greater severity) seems to be more associated with bacterial load than with the cagA gene. Studies such as that by Molnar *et al.* [8] would support the results of our study, because they found a significantly increased bacterial density in gastric erosions when compared with the healthy part of the gastric mucosa. Nevertheless, neither association with bacterial load nor association with the

TABLE 1. Association between *Cag* A and number of microorganisms in the antrum and corpus, respectively

	N *Cag*A negative	N *Cag*A positive	Crude OR	(95% CI)	Crude p-trend	Adjusted OR[a]	(95% CI)	Adjusted p-trend
Antrum bacterial load > 0								
Scarce (up to 13.0)	18	15	1.00[b]			1.00		
Moderate (13.0–422.5)	16	14	1.05	(0.4–2.8)	.009	1.76	(0.5–5.7)	0.003
Abundant (422.5 through highest)	7	26	4.29	(1.5–12.7)		6.83	(2.0–23.8)	
Corpus bacterial load > 0								
Scarce (up to 46.3)	17	10	1.00			1.00		
Moderate (46.3–781.2)	12	17	2.41	(0.8–7.1)	<0.001	2.91	(0.8–10.6)	<0.001
Abundant (781.2 through highest)	3	22	12.47	(3.0–52.5)		17.26	(3.6–82.0)	

[a]Odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for gender, age (≤vs. >50 years), consumption of NSAIDs in the 7 days prior to the endoscopy (yes/no), and clinical presentation of upper digestive tract haemorrhage (yes/no).
[b]Scarce categories were taken as the reference category.

TABLE 2. Association between digestive tract morbidity and sex, age, consumption of non-steroid anti-inflammatory drugs and *cag*A and bacterial load (number of *H. pylori* microorganisms) adjusted for each other

	UDTH[a]		PPI consumption[a]		History of complicated ulcer	
	OR[b]	(95% CI)	OR	(95% CI)	OR	(95% CI)
*Cag*A positive	1.12	(0.4–3.4)	3.11	(1.0–9.6)	**2.92**	(0.5–18.9)
Moderate antrum *H. pylori* load[c] (13.0–422.5)	1.07	(0.3–3.9)	1.11	(0.3–3.8)	**1.56**	(0.2–10.5)
Abundant antrum *H. pylori* load[c] (422.5 through highest)	2.34	(0.6–8.5)	0.30	(0.1–1.1)	**0.46**	(0.1–3.5)
Sex (male vs. female)	3.26	(1.1–9.7)	1.93	(0.7–5.6)	**7.20**	(0.9–59.5)
Age (≤50 years vs. >50 years)	1.16	(0.4–3.6)	0.97	(0.3–2.8)	**1.65**	(0.3–10.5)
Consumption of non-steroid anti-inflammatory drugs	8.99	(2.2–37.1)	0.80	(0.3–2.3)	**0.27**	(0.0–2.7)

[a]UDTH denotes clinical presentation of upper digestive tract haemorrhage. PPI consumption denotes consumption of protein pump inhibitors.
[b]Odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for sex, age (≤50 years, >50 years), consumption of NSAIDs in the 7 days prior to the endoscopy (yes/no), *Cag*A status and RT-PCR categorized quantification in the antrum as surrogate for bacterial load.
[c]The reference category was antrum bacterial load scarce (up to 13.0 *H. pylori* microorganisms per cell).

cagA gene yielded statistical significance in our study, so this finding deserves further investigations.

In our study, we found that certain clinical factors, such as the consumption of non-steroid anti-inflammatory drugs or male sex, are more strongly associated with upper digestive tract haemorrhage or a history of complicated ulcer than are markers associated with *H. pylori*., This confirms that the process producing alterations in the gastric mucosa is a complex phenomenon in which infectious and non-infectious phenomena interact [9].

In conclusion, the clear dose–response pattern between bacterial load and the *cag*A gene confirm the importance of quantifying the number of microorganisms present in the gastric mucosa [10–12]) by means of molecular techniques when evaluating the clinical importance and severity of infection due to *H. pylori* [13]. This makes it possible to study the effect of the bacterial load on the relationship between digestive tract morbidiy and the *cag*A gene with greater precision, thus contributing to a better understanding of the complex interaction between the microorganism and gastric mucosa [14,15].

Acknowledgements and Funding Section

This work was supported by the collaboration of the Fundación Bienvenida Navarro-Luciano Trípodi (research grants 2009), Generalitat Valenciana Proyectos precompetitivos/ 2008/317 and FIBELX 08/01.

Conflict of interest

The authors have no conflict of interest.

Transparency Declarations

The authors have no conflict of interest.

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