Clinical Usefulness of Virulence Factors of *Helicobacter pylori* as Predictors of the Outcomes of Infection. What is the Evidence?

*Helicobacter pylori* is one of the commonest bacterial pathogens in humans, infecting between 80%–90% of the population in developing countries and between 30%–50% in developed countries (1). Although it was isolated in 1982, it is considered to be part of the natural human flora since time immemorial (2) and probably an historical equilibrium between the bacteria and the host has existed.

*H. pylori* colonizes and proliferates in the mucus layer over the epithelium (3). The ability to survive and grow in gastric acid is apparently linked to its ability to maintain a tolerable pH by activation of internal urease production. In the majority of infected humans, there are no clinical consequences from *H. pylori* infection. Only 15%–20% of them will develop severe gastroduodenal pathology (3) and less than 1% will eventually develop a gastric tumour in their life.

It is known that in many geographical areas no correlation exists between prevalence of infection and the risk of gastroduodenal diseases. Moreover, why *H. pylori* infection produces different outcomes and why a minority of infected develop a clinical disease are remaining questions. *H. pylori* strains appear to be highly diverse (4) and some strains seem to be more virulent than others (5). Therefore, it has been proposed (6) that the type of bacterial strains, in combination with host factors, may condition both the intensity and the pathway of the process. However, the strength of the scientific evidence provided in the literature is variable. The aim of this comprehensive review is to evaluate the most recent published epidemiological evidence on the role of virulence factors of *H. pylori* as markers of severity and specificity of the consequences of *H. pylori* infection. This means its clinical usefulness as predictors of the outcomes of infection.

The markers of bacterial virulence factors so far described, that may influence *H. pylori* putative virulence and/or clinical outcomes, that were analysed are the following (Table I): the cytotoxin CagA, vacuolating cytotoxin (VacA), IceA, BabA2 and Neutrophil activating protein (NAP). We have identified all epidemiological studies published up to December 2002 available in MEDLINE (National Library of Medicine) that investigated the role of these virulence factors of *H. pylori* infection in the risk of gastric cancer or peptic ulcer. All case-control studies or prospective nested case-control studies were considered for the review. Studies based only on serological results of *H. pylori* antibodies not evaluating other virulence factors were excluded. Clinical studies based on a series of cases without controls, as well as some experimental studies using cell lines, were considered only to support or explain findings from etiological studies. We found 20 articles based on 14 case-control studies and 4 nested studies, which are summarized in Tables II and III, respectively. For each study, we evaluated the design and size, the study base, the features of controls, the covariates included and the results for each virulence factor analysed. Comparison of results with *H. pylori* antibodies alone was performed whenever this result was available.

**Pathogenicity island and the cytotoxin associated antigen (CagA)**

The cag pathogenicity island (cagPAI) is a 40 Kilobase segment of DNA that has been acquired, possibly from another organism, and incorporated within the *H. pylori* genome (7). It contains 31 genes, including cagA, cagE, cagH, cagI, and cagL (8). Cytotoxin CagA, is an immuno-

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### Table I. Putative virulence factors of *Helicobacter pylori*

<table>
<thead>
<tr>
<th>Genes</th>
<th>Allelic variants</th>
<th>Proteins</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA</td>
<td>cagA1, cagA2</td>
<td>CagA</td>
<td>CagA is injected by <em>Helicobacter pylori</em> into gastric epithelial cells. There it participates in protein dephosphorylation</td>
</tr>
<tr>
<td>vacA</td>
<td>s1a, s1b, s1c, m1, m2a, m2b</td>
<td>VacA</td>
<td>Increases paracellular permeability and vacuolation of gastric epithelial cells</td>
</tr>
<tr>
<td>babA2</td>
<td></td>
<td>BabA</td>
<td>Mediates adherence of <em>Helicobacter pylori</em> to the epithelial cells</td>
</tr>
<tr>
<td>iceA</td>
<td>iceA1, iceA2</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>napA</td>
<td></td>
<td>NAP</td>
<td>Activates recruitment of neutrophils to gastric mucosa</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Author country</th>
<th>Study base</th>
<th>Number of Observations</th>
<th>Clinical outcome</th>
<th>Covariates</th>
<th>Virulence factor alone</th>
<th>H. pylori alone</th>
<th>Both</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santolaria (28)</td>
<td>Spain Hospital</td>
<td>2001</td>
<td>104</td>
<td>Antibodies CagA/VacA</td>
<td>Peptic ulcer</td>
<td>Age-sex</td>
<td>CagA+ = 5.7 (2.1–15.6)</td>
<td>Not possible to estimate</td>
</tr>
<tr>
<td>Palli et al. (27)</td>
<td>Italy Hospital</td>
<td>2002</td>
<td>346</td>
<td>Antibodies CagA</td>
<td>Gastric ulcer</td>
<td>Age-sex</td>
<td>CagA+ = 0.4 (0.2–0.8)</td>
<td>Esoph/Cardia: CagA+ = 0.7 (0.4–1.1)</td>
</tr>
<tr>
<td>Chow (39)</td>
<td>USA Population</td>
<td>1998</td>
<td>129</td>
<td>Antibodies Cag</td>
<td>Duodenal ulcer</td>
<td>Non-cardia cancer</td>
<td>CagA+ = 1.4 (0.7–2.8)</td>
<td>Non-cardia: CagA+ = 1.3 (0.7–2.3)</td>
</tr>
<tr>
<td>Palli et al. (27)</td>
<td>Italy Hospital</td>
<td>2002</td>
<td>346</td>
<td>Antibodies Cag</td>
<td>Duodenal ulcer</td>
<td>Age-sex</td>
<td>CagA+ = 1.61 (1.06–2.45)</td>
<td>EIA = 0.85 (0.56–1.3)</td>
</tr>
<tr>
<td>Santolaria (28)</td>
<td>Spain Hospital</td>
<td>2001</td>
<td>104</td>
<td>Antibodies CagA/VacA</td>
<td>Gastric ulcer</td>
<td>CagA</td>
<td>CagA+ = 9.5 (3.6–26.8)</td>
<td>No association</td>
</tr>
<tr>
<td>Chow (39)</td>
<td>USA Population</td>
<td>1998</td>
<td>129</td>
<td>Antibodies Cag</td>
<td>Esoph./cardia</td>
<td>CagA</td>
<td>CagA+ = 1.93 (1.01–3.68)</td>
<td>No association</td>
</tr>
<tr>
<td>Queiroz (68)</td>
<td>Brasil Hospital</td>
<td>1998</td>
<td>119</td>
<td>cagA genotyping</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>VagA+ = 1.74 (1.08–2.78)</td>
<td>Immunoblot = 1.46 (0.77–2.77)</td>
</tr>
<tr>
<td>Shimoyama (64)</td>
<td>Japan Hospital</td>
<td>1999</td>
<td>81</td>
<td>Antibodies CagA</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>VagA+ = 0.9 (0.5–1.7)</td>
<td>Not possible to estimate</td>
</tr>
<tr>
<td>Shimoyama (90)</td>
<td>Japan Hospital</td>
<td>1999</td>
<td>81</td>
<td>Antibodies VacA</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>VagA+ = 1.0 (0.41–2.44)</td>
<td>No association</td>
</tr>
<tr>
<td>Yamaoka (73)</td>
<td>USA Population</td>
<td>1999</td>
<td>110</td>
<td>Antibodies CagA/VacA</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>CagA+ = 1.4 (6.7–31.9)</td>
<td></td>
</tr>
<tr>
<td>Enroth (72)</td>
<td>Sweden Hospital</td>
<td>2000</td>
<td>72</td>
<td>cagA/VacA Genotyping</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>CagA+ = 10.4 (4.2–29.7)</td>
<td>CagA–Hp+/Hp+ = 15.0 (6.4–35.2)</td>
</tr>
<tr>
<td>Maca (70)</td>
<td>Japan Hospital</td>
<td>2000</td>
<td>80</td>
<td>Antibodies CagA</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>CagA+ = 5.5 (2.4–12.4)</td>
<td>No association</td>
</tr>
<tr>
<td>Ekström (65)</td>
<td>Sweden Population</td>
<td>2001</td>
<td>298</td>
<td>Antibodies CagA</td>
<td>Gastric cancer (non-cardia)</td>
<td>Age-sex</td>
<td>CagA+ = 2.77 (1.10–6.97)</td>
<td>CagA+ = 2.1 (1.1–3.9)</td>
</tr>
<tr>
<td>Louw (74)</td>
<td>Africa Hospital</td>
<td>2001</td>
<td>48</td>
<td>cagA genotyping</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>CagA+ = No difference</td>
<td>No association</td>
</tr>
<tr>
<td>Brenner (67)</td>
<td>Germany Population</td>
<td>2002</td>
<td>71</td>
<td>Antibodies CagA</td>
<td>Gastric cancer</td>
<td>Age-sex</td>
<td>CagA+ = 5.5 (2.4–12.4)</td>
<td>No association</td>
</tr>
</tbody>
</table>
### Table III. Prospective studies on Helicobacter pylori virulence factors and clinical outcomes

<table>
<thead>
<tr>
<th>Number of Observations</th>
<th>Results: OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>**Author country</td>
<td>Study base</td>
</tr>
<tr>
<td>Nomura (29)</td>
<td>229</td>
</tr>
<tr>
<td>Hawaii 2002</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Limburg (41)</td>
<td>261</td>
</tr>
<tr>
<td>China 2001</td>
<td></td>
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<td></td>
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</table>

CagA protein enhances inflammatory responses and stimulates the synthesis of cytokines and growth factors (16). However, it has also been described that the deletion of cagA has no effect on epithelial secretion of interleukin (IL-8), while deletion of many other genes in the cag PAI does abolish the ability of the bacterium to stimulate IL-8 production (17). It was postulated that the level of the intensity of the inflammatory response was associated with the density of CagA-positive H. pylori strains in the gastric mucosa. One study observed that the density of CagA-positive H. pylori strains in the antral mucosa was higher than that of CagA-negative strains (18), however other studies (19, 20) were not able to confirm this association.

**CagA and peptic ulcer disease.** H. pylori infection is the major cause of peptic ulcer. Once H. pylori has established itself in the stomach, gastritis virtually always develops. Variations in gastritis patterns have been associated with distinct gastric acid response, which apparently determines the risk of peptic ulcer (21). Although it is generally assumed that the risk is higher for those infected by CagA positive bacterial strains (22), the results from studies in a series of cases, comparing prevalence of antibodies against CagA in patients with and without peptic ulcer, are inconsistent. Some studies (23, 24) found that infection with CagA positive strains is more common among people affected by duodenal (DU) and gastric ulcer (GU) than in control patients, while other studies (25, 26) did not observe these differences.

In one case-control study (27) (Table II) the risk of DU and GU was higher in CagA positive subjects compared with seronegative subjects, but the odds ratio (OR) using both

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markers (antibodies against \textit{H. pylori} and anti-CagA antibodies) was similar. In another case-control study (28), serum CagA positive was higher in peptic ulcer patients than in controls, both groups being infected by \textit{H. pylori}. In a nested case-control study (Table III) within a cohort of Japanese-American men (29), the OR in those CagA positive was 1.4 for GU and 2.6 for DU when compared with those CagA negative. It is noteworthy that using \textit{H. pylori} positivity alone as a marker of risk, the OR was higher (4.0 for GU and 2.5 for DU). Finally, it should be also taken into account that most persons infected with \textit{H. pylori} strains that produce CagA and possess \textit{cagA} genotype nonetheless remain asymptomatic. This observation strongly suggests that additional factors are important in peptic ulcer development.

\textit{CagA and cardia and some oesophageal diseases. While duodenal ulcer and gastric cancer of the body and antrum have been declining in most of the world, other diseases of the gastric cardia and distal oesophagus have been increasing, particularly in Western countries (30). These changes have been attributed to the simultaneous fall in the prevalence of \textit{H. pylori} and CagA strains (31).}

This hypothesis is based on the inverse relationship between \textit{H. pylori} infection and CagA positive strains infection, and risk of gastroesophageal reflux disease (GERD), Barrett’s oesophagus and of oesophageal and gastric cardia adenocarcinoma, observed in some clinical and epidemiological studies. Studies that did not evaluate CagA antibodies found that \textit{H. pylori} infection prevalence was lower in patients with oesophageal neoplasm (32), GERD and Barrett’s disease (33, 34), and Barrett’s adenocarcinoma (35) than in control patients. Studies of a series of cases that evaluated the presence of CagA antibodies, observed also a protective effect of colonization by CagA positive strains against Barrett’s oesophagus (36, 37) and GERD (38). Finally, a case-control study (Table II) based on population controls, observed that \textit{H. pylori} infection with CagA positive strain was significantly associated with a reduced risk of oesophageal and gastric cardia adenocarcinoma (39).

However, a combined analysis of 12 nested case-control studies (40) has failed to confirm this protective effect at least for the \textit{H. pylori} infection. Unfortunately information on CagA status was lacking. The overall risk of cardia cancer was 1.0 (95% CI 0.7–1.4) discarding both a protective and an increased risk effect. Moreover, a nested case-control study in China (41) (Table III) observed an unexpected positive association between cardia cancer, \textit{H. pylori} infection and CagA strains, the level of risk being relatively similar for CagA positive antibodies rather than for \textit{H. pylori} antibodies alone. Unfortunately, results of the effect of eradication of \textit{H. pylori} infection on GERD from randomized controlled trials have not solved this issue. While one study (42) concluded that eradication helped to prolong the disease-free interval in patients with GERD, the other (43) did not observe this effect.

The possible mechanism of the putative protective effect of \textit{H. pylori} and/or CagA strains remains incompletely understood. It has been related to the ability of \textit{H. pylori} infection of both reducing intragastric acidity and decreasing the potency of the gastric refluxate in patients with corpus predominant chronic gastritis (44). It remains also to be elucidated whether this protective effect may be related to \textit{H. pylori} infection itself, or only to the colonization by the virulent CagA strains. This possible relationship has important clinical consequences in public health. If some types of \textit{H. pylori} strains are protective against some diseases, the approach of eradication of \textit{H. pylori} infection could, in some cases, become questionable (31).

\textit{CagA and non-cardia gastric cancer. Experimental and epidemiological evidence indicates that gastric cancer (GC) is the result of a long multi-stage and multifactorial carcinogenic process (45) involving the interaction of \textit{H. pylori} infection (6), host susceptibility factors (46) and environmental exposure (47). Most of the relevant inflammatory mediators to the inflammatory response to \textit{H. pylori} infection (IL, tumour necrosis factor (TNF), and human leucocyte antigen (HLA)) display distinct functional variant alleles in the population. TNF (48) and HLA (49, 50) polymorphisms have been associated with the risk of GC. Interleukin-1 gene polymorphism, is associated (51) with increased risk of both hypochlorhydria induced by \textit{H. pylori} infection and gastric cancer. On the contrary, host responses involving other prosecretory IIs could be more related with duodenal ulcer, which needs a high secretory condition (51).

Several epidemiological studies have assessed the role of CagA positive \textit{H. pylori} infection in the risk of gastric cancer. A cross-sectional study in 13 countries (52), found that prevalence of CagA positive \textit{H. pylori} infections varied across countries. However, geographic variation of the incidence rates of gastric cancer was better explained by the variation in the seroprevalence of \textit{H. pylori} infections alone than by differences in the proportion of CagA positive strains. CagA was not associated with the risk of gastric cancer in other cross-sectional studies (53–55). Only one study performed in three geographical areas in Mexico (56) showed an association.

On the other hand, several studies have shown a positive association between infection with CagA positive \textit{H. pylori} strains and the risk of atrophic gastritis and intestinal metaplasia (57–61), recognized precursors of gastric cancer. Infection with CagA positive strains (62), mainly in the corpus, was found to be more associated with glandular atrophy and intestinal metaplasia than infection with CagA negative strains. However, it is clear that CagA infection is not per se a sufficient condition. The majority (93%) of African adults (63) were found to be cagA positive but only in 2% of them, focal atrophy was observed. Furthermore, no differences in mucosal responses between cagA positives and negatives were found in Africa.

Results from case-controls studies (Table II) on either distal gastric cancer or gastric cancer as a whole, are inconsistent. In two studies (64, 65) the risk of GC was higher using
anti-CagA antibodies than using *H. pylori* antibodies alone, although in one of them (64) the OR was relatively low (1.93). Five studies (66–70) reported a relatively high risk for subjects with CagA positive antibodies. However, in these studies, the risk for *H. pylori* alone was not reported or not possible to estimate because all selected controls were infected (68). Two studies (71, 72) did not observe differences in the risk of cancer comparing CagA antibodies with *H. pylori* antibodies and two studies (73, 74) found no association.

In the study from Portugal (66), both the effect of *H. pylori* genotypes and host genetic polymorphisms of IL-1 were investigated. The risk of GC for infection with CagA positive strains was 15 times higher than infection with CagA negative strains, being even higher for the combination of CagA with genetic variants of IL-1B and IL-1RN. However, this complex issue remains partially unsolved. On the one hand, the effect of *H. pylori* alone was not reported. On the other hand, not only several individuals with high-risk genotypes and infected with more virulent strains had only gastritis, but several GC cases had low-risk genotypes and were infected by less virulent strains as well.

Results from prospective studies (Table III) assessing the association between CagA antibodies and non-cardia gastric cancer are also inconsistent. So far, four articles have been published. One nested study (75) found a higher risk in subjects with CagA positive in comparison with CagA negative. However, the OR for CagA positive was lower than the usual risk of *H. pylori* alone. Moreover, levels of anti-CagA antibodies did not correlate with the risk of cancer and it was not possible to estimate the risk of *H. pylori* antibodies alone. A new study in the same cohort (76) found a higher risk for *H. pylori* antibodies than for CagA antibodies. In another nested case-control study in China (41) the OR was also relatively low, being 1.84 for CagA positive strains alone and 1.68 for whole-cell seropositive against *H. pylori*. On the contrary, a nested study in the USA (77) did observe a positive association, the risk of CagA positive being 5.8 while the risk for *H. pylori* alone was 3.6.

**Vacuolating cytotoxin (VacA)**

Vacuolating cytotoxin (VacA) is another putative bacterial virulent factor being responsible for much of the gastric epithelial erosion observed in the infected host. Its vacuolating activity on the epithelial cell membrane increases its paracellular permeability and the activation of inflammatory cells (78), promoting the production and release of nutrients that are necessary for the growth of the bacteria (3). The pathogenic role of the toxin is still controversial. Strains with inactive vacA genes have been isolated from infected patients and VacA-negative mutants can colonize in animal models, indicating that VacA is not essential for colonization of *H. pylori* (79).

The vacuolating cytotoxin gene A (vacA), which encodes VacA, is detected in almost all strains in Japanese people (17) and in 50% of the bacteria isolated in Western populations. While the expression of VacA protein is correlated with the expression of cagA, CagA is not necessary for the expression of VacA (80). The latter is neither part of the PAI nor do cagA mutations affect VacA production (6). For some reason, however, VacA is not usually secreted by *H. pylori* unless the PAI is present (7). Two divergent regions in the gene have been described (81): one in the signal peptide coding region (s1a, s1b, s1c and s2 allelic types) and the other in the mid-region (m1, m2a and m2b allelic types). Some genotypes have been associated with increased pathogenicity: s1a/m1 strains are believed to be the most virulent allelic types associated with peptic ulcer disease in the USA. However, this variant was found in different lesions in Japan (17) not associated with the clinical outcome (82).

The geographical distribution of vacA allelic types of *H. pylori* has been studied (83). Subtype s1c was observed exclusively in isolates from East Asia (81) while in Northern and Eastern European countries up to 89% of strains were subtype s1a. In Spain, Portugal, Central and South America, 89% of strains were s1b subtypes. Subtype m1 was most prevalent in Spain, Portugal, Central and South America, while m2 was more prevalent in East Asia. However, in another study in Japan (84) most strains identified were s1a/ m1 genotype. In a case-control study in Africa (74), where the *H. pylori* infection was not associated with the risk of stomach cancer, the vacA s1 subtype was found in 100% of gastric cancer cases and in 76% of controls.

Studies of virulence factors of *H. pylori* isolates from patients in Japan (85) with different gastroduodenal diseases did not observe differences in VacA antibodies among these diseases. The prevalence of infection (86) with strains type I (expressing CagA and VacA) is very high (79%), irrespective of the gastroduodenal disease of the host, showing that these virulent factors cannot be used as markers of the risk of severity. In other countries, on the contrary, in a series of cases vacA s1, cagA genotypes of *H. pylori* were more likely associated with peptic ulcer (24) and less likely associated with GERD (38). A study with patients infected by *H. pylori* in Colombia and Portugal (62) found that *H. pylori* vacA s1 and vacA m1 genotypes were associated with higher *H. pylori* density, atrophy, inflammatory infiltrates and epithelial damage.

In a cross-sectional study, including strains isolated from Colombia, Japan, Korea and the USA (87) vacA status from patients with peptic ulcer, gastric cancer and atrophic gastritis, was not associated with clinical outcomes. Another similar study (88) found that the vacA s1a/m1 genotype was present in more than 95% of strains, independent of the gastroduodenal disease. In Portugal, (89) it was found that vacA s1 strains were associated with duodenal ulcer, gastric ulcer or gastric carcinoma, while vacA m1 was associated with gastric ulcer or carcinoma but not with duodenal ulcer. In a case-control study in Spain (28), no association between VacA antibodies and peptic ulcer was observed.

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As far as we know, the six case-control studies (Table II) addressing prevalence of VacA and the risk of gastric cancer have failed to detect a consistent association. In two studies (73, 90) measuring VagA antibodies and one based on vacA genotypes (72), no association with gastric cancer risk was observed. In another study (71), an association was observed but the level of risk (OR = 1.7) was lower than the usual level of risk associated with *H. pylori* alone. A study in Africa (74) found a higher proportion of vacA s1 and s2 genotypes in cases than in controls. Finally, the study carried out in Portugal (66) observed a higher risk for infection with vacA s1 and m1 genotypes in comparison with s2 and m2 genotypes. In a study of a series of cases (91) the OR was 3.3, but its relevance is hampered by the fact that 30% of gastric cancer cases were negative for VacA antibodies.

### BabA

The babA2 gene encodes the blood-group (Lewis B) antigen-binding adhesin (BabA2) which binds to the Lewis B-type antigens of human cells. This protein plays a role in the adherence of *H. pylori* to the epithelial cells (92), although this process seems to be independent of the Lewis antigen expression of these cells (93). Additional bacterial proteins and glycolipids promote the adhesion of *H. pylori* to gastric epithelial cells (3). This step induces changes in the host cell, apparently necessary for translocation of the CagA into the gastric epithelial cells.

A series of cases in Germany (94) showed a statistically significant association between the presence of strains sharing the vacAs1, cagA and babA2 genotype, with duodenal ulcer and distal gastric carcinoma. These strains were associated with higher inflammatory response, although this response was associated with *H. pylori* itself and appeared to be independent of BabA and CagA (95). The presence of babA2 seems to be usually associated with the presence of the cagA and vacA s1 strains, but the actual importance of BabA for bacterial colonization and bacterial virulence is unknown. Several adhesins have been identified, although it is highly unlikely that any one particular adhesin could be responsible for a particular clinical outcome of *H. pylori* infection (96).

### IceA

IceA is a gene, whose expression is induced by the contact or adherence between the *H. pylori* and the epithelial cells (97). Two allelic variants have been described: iceA1 and iceA2 (98). The role of iceA as virulent factor is unknown. In cross-sectional studies, the iceA1 gene was associated with peptic ulcer (24) and increased mucosal concentration of IL-8 (98), although it is neither a risk factor for peptic ulcer (99) nor for atrophy, *H. pylori* density, or gastric cancer in Japan (100). In a study in two countries with high risk of GC (62), the iceA1 genotype was associated with gastric epithelial damage in Portuguese patients but not in Colombian patients. A study of a series of cases in South Africa (101) suggested that it may be associated with gastric cancer but a large study in four different countries (87) was unable to associate iceA status with any specific clinical outcome.

### Neutrophil activating protein (NAP)

The neutrophil activating protein (NAP) of *H. pylori* is coded by the gene napA (102). By activating the recruitment of neutrophils to the gastric mucosa, NAP contributes to the inflammatory response. Increasing infiltration of polymorphonuclear cells and macrophages induces occurrence of apoptosis, liberating endogenous oxygen radicals (ROS) and nitrogen oxide species, both bona fide endogenous mutagens (103). Some foods (preserved meat and fish, salted foods) and tobacco smoking are also important sources of ROS and N-nitroso compounds, therefore endogenous and exogenous compounds that could induce DNA oxidative damage and DNA mutations could be critical in the cancer process.

On the other hand, it has been suggested that NAP could have an important role in the immune response, due to its capacity of inducing protection against *H. pylori* after vaccination of mice with NAP (104). In spite of that, as far as we know, no epidemiological study has been published assessing the role of NAP as a marker of risk. Therefore, its clinical relevance remains uncertain (79).

### Conclusions

At the present, there is no doubt about the causal relationship between *H. pylori* infection, gastric and duodenal ulcer, non-cardia gastric cancer and gastric lymphoma. There are, however, several important remaining questions that have scientific and clinical relevance.

Firstly, it is essential to elucidate whether the so-called virulence factors previously described determine the type of the disease. According to the evidence reviewed, none of these markers can be used as predictor of one or more specific clinical outcomes. Moreover, while strain-specific genetic diversity was initially thought to be involved in the organism’s ability to cause distinct diseases, cluster analyses of complete genomic sequences of unrelated *H. pylori* isolates obtained from patients with different gastroduodenal diseases revealed no disease-specific strains (105). Evidence accumulated during the last few years has lead to acceptance today that the nature of the disease depends rather on host and environmental factors than on the type of bacterium (7).

Secondly, it is extremely important to clarify whether the putative virulence factors have predictive value for the presence or future development of severe gastroduodenal disease. This question has relevant clinical consequences because it could be possible to recommend that only those patients infected with more virulent strains should be candidates for any preventive treatment.

An important controversy exists in the scientific community regarding this issue. Some authors have postulated (7,
that CagA seropositivity is associated with increased risk of developing more severe disease. Others, on the contrary, have suggested (107, 108) that, although strains with a functional PAI produce a more severe inflammation which might accelerate the disease process, detection of PAI positive strains has no predictive value for the presence and/or the future development of a clinically significant outcome. Likewise, it was recognized in a recent comprehensive review (79) that, in spite of the enormous progress made in the knowledge of virulence factors, this information has not yet been used in clinical practice.

Although it is not yet possible to exclude the role of some of these virulence markers as predictors of risk, the epidemiological and clinical evidence reviewed in this article does not support this role. The most widely used virulence factors have been CagA and VacA. Relatively few studies have assessed the role of IceA, BabA, while no epidemiological study on NAP was identified. Most of the studies have provided inconsistent and contradictory results. So far, neither CagA nor VacA seroposities are better predictors of increased peptic ulcer risk or stomach cancer risk than antibodies against H. pylori. The fact than more than 90% of strains in East Asia are CagA and VacA positive, suggests that these factors are not sufficient cause for the induction of a severe gastroduodenal disease. Furthermore, no differences were observed when cost and benefits (109) of screening asymptomatic individuals for CagA positive antibodies and for H. pylori positive antibodies alone were compared.

Several methodological issues can account for the observed inconsistencies in the literature. In some studies, limitations in design do not allow for adequate control of confounding factors. Most studies are cross-sectional studies, where prevalence of antibodies against CagA or VacA was compared in a series of cases diagnosed with different diseases, often H. pylori-related. Therefore, the effect of H. pylori alone cannot be evaluated. Moreover, the presence of potential sources of bias may affect the validity of their findings. Eleven of the fourteen case-control studies were hospital based. Since diseases included in the control group were usually associated with H. pylori infection, a selection bias is likely to occur.

Only five articles from three nested studies have been published so far, reporting also inconsistent results. On peptic ulcer, only one cohort study was published (29), in which the risk of CagA was not superior to that of the risk of H. pylori alone. Regarding gastric cancer risk, only one (77) of the four articles found that the level of risk for CagA antibodies was higher than for H. pylori antibodies alone.

An important feature of H. pylori that can explain at least part of the inconsistencies is that a single individual may be simultaneously infected with multiple strains. Moreover, strains within the same individual can change over time, due to endogenous mutations, recombination between strains and chromosomal rearrangements (3, 110). Therefore, several types of different strains can be isolated from the same individual precluding a correct attribution of a phenotype to a given strain.

On the other hand, limitations of serological methods are also of importance. It has been suggested that after eradication or spontaneous disappearance of H. pylori infection, antibodies to cagA persist longer (111) than the IgG antibodies detected with ELISA tests. Therefore, (112) the higher relative risk associated with CagA seropositivity observed in some studies may, in fact, reflect a better ability to classify correctly the true status of the past exposure. Discrepant findings may also result from the analysis of different populations, and the use of distinct methods of assays and/or antigens to detect anti-CagA antibodies (70). In this regard, limitations in test reproducibility have already been reported (113). Serum VacA antibodies showed no relation with vacA genotypes and the detection of CagA antibodies was strongly dependent on the test used (114). It is noteworthy that after comparing four serological tests to determine the CagA and VacA status (115) it was concluded that none of them could be recommended.

Few etiological studies are based on genotyping assessment of strains instead of serological testing. Unfortunately, most studies published so far are cross-sectional studies in which causal inference is not possible. The Portuguese study, using a case-control approach (66), suggested that cagA, vacAsl and vacAonl genotypes of H. pylori may be indicators of GC risk. However, other case-control studies based on genotypes (72, 74) did not observe this effect.

Well-designed and conducted prospective studies are needed to reach a definitive conclusion of the value of putative virulence factors, as predictors of severity of H. pylori infections. These studies should be based on healthy individuals, use an appropriate methodology for detection of past exposure of H. pylori infection, and be able to compare the level of risk between those positive and negative for each factor (including virulence factors and H. pylori antibodies alone). Nonetheless, if host as well as other environmental factors are determinants of the outcome of the H. pylori infection, they should be taken into account simultaneously, to ascertain not only their relative contribution but also the contribution of their putative interactions.

On the other hand, if genetic susceptibility conditions the progression and severity of H. pylori infection, a better understanding of their mechanism of action, particularly of the inflammatory response, is needed. The individual capacity of the host (116) to respond to the bacterium is critical to the colonization level and shows great heterogeneity compatible with the heterogeneity of human response genes. Observations reported in the last decade indicate that those genetic host factors are important in determining the type and severity of the disease (117). It is however clear that a deeper knowledge of the virulence factors together with host genetic factors is necessary before we can really understand the variability of the response to H. pylori infection, ranging from
simple gastritis to peptic ulcer and eventually to gastric cancer.

C. A. González (correspondence)  
Dept. of Epidemiology  
Catalan Institute of Oncology  
Gran Via s/n Km 2.7.  
ES-08097 Hospitalet Barcelona  
Spain

S. Peña  
Dept. of Gastroenterology and Laboratory of Immunogenetics  
VU University Medical Centre Amsterdam  
The Netherlands

G. Capellà  
Translational Research Laboratory  
Catalan Institute of Oncology  
Barcelona  
Spain

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