Importance of *Helicobacter pylori* cagA and vacA status for the efficacy of antibiotic treatment

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Abstract

**Background**—Virulence factors of *Helicobacter pylori* are associated with peptic ulcer disease and may be also associated with the efficacy of treatment.

**Aims**—To determine the relation between the vacA and the cagA status of *H pylori*, clinical disease, and treatment outcome.

**Patients**—121 patients with *H pylori* infection and peptic ulcer disease or functional dyspepsia were treated by quadruple antibiotic therapy in two groups for one and two days, respectively.

**Methods**—DNA was isolated from gastric antral biopsy specimens, taken before and after treatment, and the vacA and cagA status was determined by polymerase chain reaction and reverse hybridisation.

**Results**—Peptic ulcer disease was significantly associated with the vacA s1 type, and cagA positivity, but not with the vacA m type. Treatment efficacy was significantly higher in patients with peptic ulcer disease, or infected with cagA+/vacA s1 strains.

**Conclusions**—The strong association between the cagA and vacA status and peptic ulcer disease was confirmed. Cure rates seem to be higher for patients with cagA+/vacA s1 *H pylori* strains, which is consistent with the higher cure rate observed among ulcer patients compared with functional dyspepsia patients. Therefore, treatment studies may require stratification for presence of ulcers as well as *H pylori* genotypes.

**Keywords**: Helicobacter pylori, virulence; treatment; vacA, cagA

Helicobacter pylori colonises the human stomach; its persistence causes gastritis and peptic ulcers and in some individuals may lead to the development of gastric cancer. Clinical outcome of infection is determined by factors such as the age of acquisition, environment, host factors, and genetic heterogeneity of *H pylori*.1,2 At present, several virulence associated genes have been identified in the genome of *H pylori*: vacA, cagA, and iceA.3**4 vacA is present in all *H pylori* strains and encodes a vacuolating cytotoxin. There are at least four vacA signal sequence types (s1a, s1b, s1c, and s2) and three middle region types (m1, m2a, and m2b).4 Type s1/m1 strains express more cytotoxin activity than type s1/m2 strains, and type s2/m2 strains produce no detectable cytotoxin activity.8**9 Discrimination between the vacA genotypes can be useful for classifying strains that are associated with either functional dyspepsia or peptic ulcer disease. cagA (the cytotoxin associated gene) is not present in all *H pylori* strains, and is considered as a marker for the presence of a pathogenicity island of 35–40 kbp in the bacterial genome. This island contains a number of genes, whose products are associated with increased pathogenicity of *H pylori*—for example, by induction of cytokine production in the gastric mucosa, resulting in a more severe gastritis. This may be the reason why cagA positive strains are more prevalent in patients with peptic ulcers compared with patients with gastritis only.10**13 The presence of cagA is also strongly associated with the presence of the vacA s1 allele.4

*H pylori* infection can be effectively cured by antibiotics. Numerous treatment regimens have been tested over the years. Triple and quadruple regimens containing at least two antibiotics are the most effective treatments.14**15 Despite the success of the current anti-helicobacter therapies, some studies have suggested that the eradication rate among patients with gastritis is lower than among patients with peptic ulcer disease,16 and the causes of this phenomenon have been the subject of speculation. As the cagA and vacA status of *H pylori* is associated with the development of peptic ulcer disease, the aim of the present study was to determine whether the *H pylori* genotype plays a role in the efficacy of quadruple antibiotic therapy.

**Materials and methods**

**PATIENTS AND ANTI-HELICOBACTER TREATMENT**

A total of 121 *H pylori* positive patients clinically diagnosed with duodenal ulcer (n=54), gastric ulcer disease (n=14), or functional dyspepsia (n=53) was studied. All patients were recruited consecutively at one gastroenterology unit and lived in the southeastern part of The Netherlands. The patients took part in two studies to determine the efficacy of particular treatments for *H pylori* infection and were combined for the purposes of this study. Table 1 summarises the patient characteristics.

Forty six patients (33 with peptic ulcer disease and 13 with non-ulcer dyspepsia) were treated in group I with lansoprazole 30 mg twice daily on days 1–4.12 On day 4 they received colloidal bismuth subcitrate 120 mg, tetracycline hydrochloride 250 mg, and metronidazole 250 mg at 0900, 1100, 1300, 1500, 1700, 1900, 2100, and 2300 hours.

**Abbreviations used in this paper**: PCR, polymerase chain reaction.
 Seventy five patients (35 with peptic ulcer disease and 40 with non-ulcer dyspepsia) were treated in group II with lansoprazole 30 mg twice daily on days 1–5. On days 4 and 5 they also received colloidal bismuth subcitrate 120 mg four times daily, tetracycline 500 mg four times daily, and metronidazole 500 mg four times daily.14 Four patients were not available for follow up.

All patients were completely compliant, as determined by administration of medication in the hospital during the one day treatment (group I), pill counts, and patient interviews.

Antral biopsy specimens were placed in 1 ml of thioglycollate broth and transported to the microbiology laboratory within six hours of endoscopy. One biopsy specimen was kept frozen at −70°C and later used for polymerase chain reaction (PCR), and the other was immediately used for culture. Before treatment, all patients were _H pylori_ positive in endoscopic biopsy specimens from antrum and corpus, as determined by the CLO test, histology (Giemsa stain), and culture.

**CULTURE OF _H PYLORI_**

Bacteria were cultured on Belo-Horizonte medium containing brain-heart infusion agar (35 g/l), sheep’s blood (10%), vancomycin (10 mg/l), trimethoprim lactate (5 mg/l), cefsulodine (5 mg/l), and amphotericin (5 mg/l). The plates were incubated in microaerobic conditions at 37°C for seven days. Putative _H pylori_ colonies were confirmed by Gram staining, and tested for catalase, oxidase, and urea hydrolysis.

Metronidazole and clarithromycin susceptibilities were tested by the E test (AB Biodisk, Solna, Sweden) on plates using Belo-Horizonte medium with sheep’s blood (10%). Strains were considered susceptible to metronidazole when the minimal inhibitory concentration was less than 8 µg/ml.

**ANALYSIS OF vacA AND cagA**

For PCR analysis, antral biopsy specimens were used that had been taken immediately before and six weeks after treatment and stored in thioglycollate broth at −70°C. Specimens were homogenised with a sterile micropestle and DNA was isolated by the method of Boom et al.20 The _vacA_ and _cagA_ status was determined by multiplex PCR followed by reverse hybridisation on a line probe assay (LiPA), as described previously.21 22

**STATISTICAL ANALYSIS**

Data were analysed with SPSS (SPSS Inc., Chicago, Illinois, USA) version 8.0. Associations between categorical variables were tested for statistical significance using χ² tests with one degree of freedom; t tests were used for normally distributed data. Logistic regression was performed to analyse the relation between peptic ulcer disease (combining gastric and duodenal ulcers) and the various combinations of _cagA_ and _vacA_ genotypes, and the relation between the success of eradication therapy and the _H pylori_ genotypes. In both analyses the relation with the genotypes was adjusted for age (using categories ≤40, 41–50, 51–60, 61–70, and >70 years) and sex. Additional confounders included in the second analysis were the presence or absence of peptic ulcers, the therapy received (group I and II), and the sensitivity to metronidazole. Covariates (the _H pylori_ genotypes) were included by forward selection on the basis of a likelihood ratio test. A value of p<0.05 was used as a significance level for inclusion, and odds ratios (OR) as well as their 95% confidence intervals (CI) of significant covariates were determined.

**Results**

**EVALUATION OF THE EFFICACY OF TREATMENT BY PCR**

A total of 121 patients was treated as part of a clinical trial with either one or two day quadruple therapy (table 1).17 Group I comprised 46 patients, treated for one day, and all were available for follow up analysis six weeks after therapy by CLO test, culture, and histology, resulting in a cure rate of 57% (26/46). Follow up samples from two patients were not available for PCR analysis. The eradication results were confirmed, except for two cases, where results of the initial tests were inconclusive, and according to the protocol they were initially considered as not cured. In one of these cases, histology and culture were both negative in post-treatment biopsy specimens from antrum and corpus. The CLO test on the corpus specimen was negative but positive on the antrum specimen. PCR on the antrum specimen was also positive and this case was still considered as not cured. In the second case only the corpus specimen was positive in the CLO test. Giemsa staining and culture on both antrum and corpus biopsy specimens, CLO test, culture, and PCR tests on the antral biopsy specimen were all negative. The CLO test result was considered as false positive and therefore this patient was now considered to be cured. Overall, the final cure rate, taking the PCR results into account, was 59% (27/46). Efficacy of treatment was higher in patients with peptic ulcer disease (22/33; 67%) compared with those with functional dyspepsia (5/13; 39%; χ²=3.06; p=0.08). In the second group, a total of 75 patients was treated for two days, but follow up was not available in four patients. The initial cure rate was 72% (51/71) based on negative results of

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<td>Group I (n=46)</td>
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<tr>
<td>PUD (%)</td>
<td>33 (72)</td>
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<td>NUD (%)</td>
<td>13 (28)</td>
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<td>Men (%)</td>
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<td>Age (SD (range))</td>
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<td>Initial cure rate* (%)</td>
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<td>Final cure rate† (%)</td>
<td>27/46 (59)</td>
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<td>Final cure rate NUD (%)</td>
<td>5/13 (39)</td>
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*Based on CLO test, culture, and histopathology results of the post-treatment biopsy specimens.
†Taking PCR on post-treatment biopsy specimens into account.
‡Follow up samples from four cases were not available.
H pylori genotypes and treatment efficacy

The CLO test, histology, and culture on gastric biopsy specimens were analysed. Among the post-treatment biopsy specimens that were scored negative by CLO, culture, and histology, positive PCR results were obtained in five cases, and these were therefore considered as not cured. Thus, the final cure rate, taking PCR results into account, was 65% (46/71) among patients available for evaluation. As the CLO test, histology, and culture on gastric biopsy specimens were used for further statistical analysis. Thus, 108 patients with a single genotype before treatment were excluded from analysis.

To study the relation between the vacA and cagA genotypes and treatment efficacy, post-treatment and post-treatment biopsy specimens were analysed from both patient groups.

Taken together, among the 121 patients, six cases with a single genotype before treatment showed a different vacA/cagA genotype during follow up, of which four were multiple and two single genotypes (table 3). Furthermore, post-treatment biopsy specimens were not available for genotyping in six cases. Among the 109 treated patients available for analysis, therapy was effective in 72 patients, but failed in 37 cases. Table 4 shows the association between ulcer outcome and the presence of ulcers is not significantly related to the vacA s region. Through forward selection, cagA+ was selected as the only significant predictor variable (OR cagA+ versus cagA− = 8.1, CI: 2.9 to 22.6). However, owing to the very close association between cagA+ positivity and presence of the vacA s1 genotype, an approximately equal fit (with similar likelihoods) was obtained when including the vacA s genotype instead of the cagA genotype (OR s1 versus s2 = 9.9, CI: 2.9 to 33.7).

RELEVANCE OF H PYLORI GENOTYPE FOR THE EFFICACY OF ANTIBIOTIC THERAPY

To study the relation between the vacA and cagA genotypes and treatment efficacy, post-treatment and post-treatment biopsy specimens were analysed from both patient groups.

Table 2 shows the cagA status as well as the vacA genotypes determined in pretreatment biopsy specimens. vacA s1/m1, s1/m2, s2/m2, and multiple genotypes were found in 38 (31.4%), 46 (38.0%), 24 (19.8%), and 13 (10.7%) of the 121 cases, respectively. Of the 84 strains containing vacA type s1, 73 (86.9%) were subtype s1a and 11 (13.1%) were s1b.

To investigate the relation between genotypes and peptic ulcer disease, the 13 cases with multiple genotypes were excluded from statistical analysis. Thus, 108 patients with a single vacA/cagA genotype in the pretreatment biopsy specimen were used for further statistical analyses. Univariate analyses (using the χ² test) of the H pylori genotypes yielded the following results. cagA+ was present in 67 (62.0%) of the 108 strains, and its presence was significantly associated with the presence of vacA type s1 (χ²=44.231; p<0.001; group I: p=0.005; group II: p=0.001) and type m1 (χ²=12.114; p=0.001; group I: p=0.236; group II: p=0.001). All s2/m2 cases were cagA+ negative. Peptic ulcer disease was strongly associated with the presence of cagA (χ²=6.641; p=0.002; group I: p=0.017; group II: p=0.012) and vacA s1 (χ²=9.765; p=0.001; group I: p=0.004; group II: p=0.035), but not with the vacA m type (χ²=0.074; p=0.444, group I: p=0.664; group II: p=0.418). These results are in agreement with earlier findings.6 9

Logistic regression analysis relating ulcer disease to H pylori genotypes confirmed that the presence of ulcers is not significantly related to the vacA m region. Through forward selection, cagA+ was selected as the only significant predictor variable (OR cagA+ versus cagA− = 8.1, CI: 2.9 to 22.6). However, owing to the very close association between cagA+ positivity and presence of the vacA s1 genotype, an approximately equal fit (with similar likelihoods) was obtained when including the vacA s genotype instead of the cagA genotype (OR s1 versus s2 = 9.9, CI: 2.9 to 33.7).
Patients with multiple genotypes (n=12), incomplete follow up (n=6), and non-matching pretreatment and post-treatment genotypes (n=6) were excluded from this table.

Multiple vacA genotypes were detected in 12 of 109 patients (11.0%) with a complete follow up and matching pretreatment and post-treatment biopsy specimens (table 5). Therapy failed in six of these patients, and in four, only single genotypes were detected in the post-treatment biopsy specimen; in the remaining two patients, the same combination of vacA genotypes was also detected after treatment. From one patient (table 5, patient 4) with a mixed s1a/s2/m1/m2/cagA+ genotype before therapy, a metronidazole resistant strain was cultured. After treatment, only a s1a/m2/cagA− genotype was identified in the biopsy specimen, and the cultured strain was metronidazole sensitive.

**Discussion**

In order to eradicate *H pylori* from the human stomach, triple and quadruple therapies with bismuth salts, multiple antibiotics, H2 antagonists, and proton pump inhibitors for 4–14 days can reach per protocol cure rates of more than 90%. As compliance is an important factor for treatment failure, shorter therapies have been investigated. Earlier studies have indicated different cure rates between ulcer and non-ulcer patients. Furthermore, the association between peptic ulcer disease and several virulence genes of *H pylori* has been described. The aim of the present study was to address the relation between peptic ulcer disease, the efficacy of *H pylori* eradication, and the status of the virulence associated vacA and cagA genes.

Genotyping of *H pylori* was performed directly on gastric biopsy specimens, which provides information on the actual infection in the stomach. Diagnostic findings of histology, culture, and CLO test were confirmed by PCR in all but six cases, where follow up biopsy specimens were positive by PCR only. This may reflect the extremely high sensitivity of PCR compared with other diagnostic methods. Although PCR detection does not discriminate between live and dead bacteria, the detection of *H pylori* DNA at six weeks after therapy was considered as indicative of failure to eradicate the bacterium.

The great majority of vacA s1 strains were classified as subtype s1a. This finding is consistent with earlier studies on the worldwide distribution of vacA subtypes, which showed that s1a is the predominant subtype in northern and eastern Europe, whereas in...
H pylori genotypes and treatment efficacy

The prevalence of cagA among patients with functional dyspepsia is lower than among patients with peptic ulcers, and the presence of cagA is strongly associated with the vacA s1 genotype.6 8 20 The vacA s1 allele is often present in peptic ulcer patients, whereas genotype s2/m2 is more prevalent in patients without ulcers, and there was no difference between patients with gastric ulcers or duodenal ulcers. These results are in accord with earlier reports.6 25

Genotypes were determined in pretreatment and post-treatment biopsy specimens. Our results indicate a difference in sensitivity to therapy between the H pylori genotypes. Some H pylori strains could be intrinsically more prone to antibiotic treatment than others. The present study indicated that the non toxin-producing s2/m2 strains (which are mostly cagA−) seem to be more resistant to therapy than the toxin producing s1/m1 and s1/m2 strains (which are mostly cagA+). cagA+/s1 strains may proliferate faster than cagA−/s2 strains, and would be therefore more susceptible to antibiotic activity that interferes with the metabolism of a dividing cell. Alternatively, the more virulent genotypes may result in a different physiological environment in the stomach, such as the degree of damage to the gastric epithelium and mucous layer where H pylori is residing. It is conceivable that antibiotics can reach higher concentrations in inflamed mucosa. Thus, cagA−/s2 strains, that produce less severe inflammation, would be less accessible to antibiotics and therefore more difficult to eradicate.

Patients with peptic ulcer disease seem to reach a higher cure rate compared with patients with functional dyspepsia.19, 21, 26 This was first observed in studies using omeprazole dual therapy and has been less apparent with the more effective triple and quadruple therapies, probably owing to the small sample size of most studies.16, 22, 27–31 The present study shows that the presence of peptic ulcer disease, the efficacy of antibiotic therapy, and the cagA and vacA genotypes of the H pylori strains are strongly interrelated.

Compliance was 100% in these patient groups, as patients of group I were treated in the hospital, and patients from group II were closely monitored over the short treatment period. Therefore, lack of compliance among patients is an unlikely explanation for the different treatment outcome.

Current therapy regimens may be particularly suited to eradicate the more virulent (s1/cagA+) H pylori strains. Future studies should determine the genotypes of strains from patients that did not respond to other therapy regimens.

In six cases in which therapy was not successful, genotyping results did not match, and in four cases, the post-treatment biopsy contained multiple genotypes (table 3). Reinfection during the follow up period is extremely unlikely.32 Although only a single genotype was detected before treatment, other genotypes may already have been present before treatment at a low concentration or may have remained undetected owing to sampling variation during endoscopy. Treatment may have shifted the absolute numbers and relative prevalence of the different genotypes. The fact that vacA s2 genotypes were detected in three of the six post-treatment biopsy specimens (table 4) but not in any of the pretreatment biopsy specimens would also be consistent with the finding that s2 strains are more resistant to therapy than s1 strains.

Twelve patients with a complete follow up had multiple genotypes before treatment. Therapy failed in six cases, and in three of these, only a single genotype was found after treatment. Based on the other biopsy tests, therapy seemed to have completely failed in these patients. However, PCR analysis indicated partial eradication. From one of these three patients, containing multiple genotypes in the pretreatment biopsy, a metronidazole resistant strain was cultured. After treatment only a metronidazole sensitive strain was cultured and a single genotype was detected. This indicates that, although antibiotic resistance is an important feature of H pylori, it is not the crucial factor to determine the efficacy of treatment.

The cagA−/s2 strains can be killed with the same antibiotics as the cagA+/s1 strains, but it takes longer for cagA−/s2 strains. It is therefore consistent that the difference in cure rate only becomes apparent in studies which use short treatment regimens. During prolonged treatment, the more resistant cagA−/s2 strains are also adequately killed. Hermida et al showed that after six days of treatment more ulcer patients were cured compared with non-ulcer patients, but this difference disappeared after prolonged (12 day) treatment.30 This finding has important clinical implications. Treatment studies comprising both ulcer and non-ulcer patients may be biased. Most studies to date were performed in ulcer patients and the high cure rates reported may not be equally high in a population of non-ulcer patients. Treatment failures will also lead to an increase in the prevalence of antibiotic resistance. Resistance to metronidazole may be of importance for efficacy of treatment, but was not found to be a dominant factor. Non-ulcer patients carrying cagA−/s2 strains that are also metronidazole resistant, and who stop treatment prematurely might be expected to be the most difficult to cure. Therefore, differences in cure rates between ulcer and non-ulcer patients are clinically most relevant in areas with a high antibiotic resistance rate.

In conclusion, different genotypes of H pylori may result in different clinical outcomes and respond differently to antibiotic therapy. These findings suggest that treatment studies should be stratified with respect to the presence of peptic ulcers, as well as different H pylori vacA or cagA genotypes.