

## 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.

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*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*.<sup>1 2</sup> At present, several virulence associated genes have been identified in the genome of *H pylori*: *vacA*, *cagA*, and *iceA*.<sup>3-6</sup> *vacA* is present in all *H pylori* strains and encodes a vacuolating cytotoxin.<sup>7</sup> There are at least four *vacA* signal sequence types (s1a, s1b, s1c, and s2) and three middle region types (m1, m2a, and m2b).<sup>8</sup> Type s1/m1 strains express more cytotoxin activity than type s1/m2 strains, and type s2/m2 strains produce no detectable cytotoxin activity.<sup>8 9</sup> 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.<sup>10-13</sup> The presence of *cagA* is also strongly associated with the presence of the *vacA* s1 allele.<sup>8</sup>

*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.<sup>14 15</sup> 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,<sup>16</sup> 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 south-eastern 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.<sup>17</sup> 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.

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Table 1 Characteristics of patients in groups I and II

|                         | Group I (n=46) | Group II (n=75) | Significance (group I v group II) |
|-------------------------|----------------|-----------------|-----------------------------------|
| PUD (%)                 | 33 (72)        | 35 (47)         | $\chi^2=7.281$ ; $p<0.01$         |
| NUD (%)                 | 13 (28)        | 40 (53)         |                                   |
| Men (%)                 | 31 (67)        | 49 (65)         | $\chi^2=0.054$ ; $p=0.816$        |
| Age (SD (range))        | 15.7 (23–85)   | 13.6 (23–82)    | $p=0.830$                         |
| Initial cure rate* (%)  | 26/46 (57)     | 51/71‡ (72)     | $\chi^2=2.908$ ; $p=0.08$         |
| Final cure rate† (%)    | 27/46 (59)     | 46/71 (65)      | $\chi^2=0.442$ ; $p=0.506$        |
| Final cure rate PUD (%) | 22/33 (67)§    | 26/34 (76)§     | $\chi^2=0.792$ ; $p=0.373$        |
| Final cure rate NUD (%) | 5/13 (39)      | 20/37 (54)      | $\chi^2=0.936$ ; $p=0.330$        |

\*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.

§Difference between final cure rate of patients with peptic ulcer disease (PUD) compared with patients with non-ulcer dyspepsia (NUD) was not significant in group I ( $\chi^2=3.06$ ;  $p=0.08$ ), but was in group II ( $\chi^2=3.903$ ;  $p=0.04$ ).

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.<sup>18</sup> 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  $-70^{\circ}\text{C}$  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), cefsulidine (5 mg/l), and amphotericin (5 mg/l). The plates were incubated in microaerobic conditions at  $37^{\circ}\text{C}$  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  $\mu\text{g/ml}$ .

#### 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  $-70^{\circ}\text{C}$ . Specimens were homogenised with a sterile micropestle and DNA was isolated by the method of Boom et al.<sup>19</sup> The *vacA* and *cagA* status was determined by multiplex PCR followed by

reverse hybridisation on a line probe assay (LiPA), as described previously.<sup>20 21</sup>

#### 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  $\chi^2$  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  $\leq 40$ , 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).<sup>17</sup> 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. Based on 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 antral 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%;  $\chi^2=3.06$ ;  $p=0.08$ ).

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

Table 2 Relation between *Helicobacter pylori* *cagA* and *vacA* genotypes in pretreatment biopsy specimens and clinical diagnosis

| <i>vacA</i> genotype | Duodenal ulcer (n=54) |               | Gastric ulcer (n=14) |               | Functional dyspepsia (n=53) |               | Total      |
|----------------------|-----------------------|---------------|----------------------|---------------|-----------------------------|---------------|------------|
|                      | <i>cagA</i> +         | <i>cagA</i> - | <i>cagA</i> +        | <i>cagA</i> - | <i>cagA</i> +               | <i>cagA</i> - |            |
| <b>Group I</b>       |                       |               |                      |               |                             |               |            |
| s1/m1                | 7                     | 1             | 2                    | -             | 1                           | 2             | 13 (28.3%) |
| s1/m2                | 9                     | 4             | 2                    | 2             | 2                           | 1             | 20 (43.3%) |
| s2/m2                | -                     | 1             | -                    | 1             | -                           | 5             | 7 (15.2%)  |
| Multiple             | 4                     | -             | -                    | -             | 1                           | 1             | 6 (13.0%)  |
| <b>Group II</b>      |                       |               |                      |               |                             |               |            |
| s1/m1                | 9                     | -             | 3                    | 1             | 11                          | 1             | 25 (33.3%) |
| s1/m2                | 11                    | 1             | 2                    | -             | 8                           | 4             | 26 (34.6%) |
| s2/m2                | -                     | 3             | -                    | 1             | -                           | 13            | 17 (22.7%) |
| Multiple             | 4                     | -             | -                    | -             | -                           | 1             | 7 (9.3%)   |
| <b>Total</b>         | <b>44</b>             | <b>10</b>     | <b>9</b>             | <b>5</b>      | <b>25</b>                   | <b>28</b>     | <b>121</b> |

the CLO test, histology, and culture on gastric biopsy specimens taken six weeks after therapy. Post-treatment biopsy specimens from these 71 cases were also available for PCR analysis. 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 in group I, efficacy of treatment in this group was also higher among patients with peptic ulcer disease (26/34; 76%) compared with those with functional dyspepsia (20/37; 54%;  $\chi^2=6.85$ ;  $p<0.01$ ).

#### ANALYSIS OF THE PRETREATMENT BIOPSY SPECIMENS

To assess the relation between clinical symptoms and *H pylori* genotypes, pretreatment biopsy specimens were analysed. 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  $\chi^2$  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 ( $\chi^2=44.231$ ;  $p<0.001$ ; group I:  $p=0.005$ ; group II:  $p<0.001$ ) and type m1 ( $\chi^2=12.114$ ;  $p=0.001$ ; 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* ( $\chi^2=6.641$ ;  $p=0.002$ ; group I:  $p=0.017$ ; group II:  $p=0.012$ ) and *vacA* s1 ( $\chi^2=9.765$ ;  $p=0.001$ ; group I:  $p=0.004$ ; group II:  $p=0.035$ ), but not with the *vacA* m type ( $\chi^2=0.074$ ;  $p=0.444$ , group I:  $p=0.664$ ; group II:  $p=0.418$ ). These results are in agreement with earlier findings.<sup>6,9</sup>

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).

#### RELEVANCE OF *H PYLORI* GENOTYPE FOR THE EFFICACY OF ANTIBIOTIC THERAPY

To study the relation between the *vacA* and *cagA* genotypes and treatment efficacy, pretreatment 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 treatment outcome and the *cagA* and *vacA* genotypes. Again, the cases containing multiple genotypes (12 of the 109 cases) were excluded. Univariate analyses (using the  $\chi^2$  test) showed the following relations. Therapy was significantly more effective in ulcer patients compared with non-ulcer patients ( $\chi^2=6.356$ ;  $p=0.012$ ; group I:  $p=0.108$ ; group II:  $p=0.024$ ). *cagA*+ strains seemed to be more easily eradicated than *cagA*- strains ( $\chi^2=10.638$ ;  $p=0.001$ ; group I:  $p=0.036$ ; group II:  $p=0.015$ ). Similarly, therapy was more effective for *vacA* s1 strains than for s2 strains ( $\chi^2=4.845$ ;  $p=0.028$ ; group I:  $p=0.025$ ; group II:  $p=0.247$ ), but there was no significant

Table 3 *Helicobacter pylori vacA* and *cagA* status of cases with non-matching pretreatment and post-treatment genotypes

| Patient | Genotype in pretreatment biopsy | Genotype in post-treatment biopsy |
|---------|---------------------------------|-----------------------------------|
| 1       | s1a/m1/ <i>cagA</i> +           | s2/m2/ <i>cagA</i> -              |
| 2       | s1a/m2/ <i>cagA</i> +           | s1a/m1/m2/ <i>cagA</i> +          |
| 3       | s1a/m1/ <i>cagA</i> +           | s1a/s2/m1/m2/ <i>cagA</i> +       |
| 4       | s1a/m2/ <i>cagA</i> +           | s1a/s2/m2/ <i>cagA</i> +          |
| 5       | s1a/m1/ <i>cagA</i> +           | s1a/m2/ <i>cagA</i> +             |
| 6       | s1a/m2/ <i>cagA</i> -           | s1a/m1/m2/ <i>cagA</i> +          |

Table 4 Relation between presence or absence of peptic ulcer disease, *cagA* and *vacA* genotypes in pretreatment biopsy specimens, and efficacy of one to two days of intensified quadruple eradication treatment

| Clinical status      | No of cases | Successful eradication | <i>cagA</i> status | Successful eradication | <i>vacA</i> status | Successful eradication |
|----------------------|-------------|------------------------|--------------------|------------------------|--------------------|------------------------|
| Peptic ulcer disease | 54          | 79.6% (43/54)          | pos: 39            | 87.2% (34/39)          | s1: 39             | 86.1% (34/39)          |
|                      |             |                        | neg: 15            | 60% (9/15)             | s2: 0              | –                      |
| Non-ulcer dyspepsia  | 43          | 55.8% (24/43)          | pos: 20            | 70% (14/20)            | s1: 9              | 44.4% (4/9)            |
|                      |             |                        | neg: 23            | 43.5% (10/23)          | s2: 6              | 83.3% (5/6)            |
|                      |             |                        |                    |                        | s1: 20             | 70% (14/20)            |
|                      |             |                        | s2: 0              | –                      |                    |                        |
| s1: 7                | 57.1% (4/7) |                        |                    |                        |                    |                        |
|                      |             |                        |                    | s2: 16                 | 37.5% (6/16)       |                        |

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.

relation with the *vacA* m region type ( $\chi^2=1.046$ ;  $p=0.306$ ; group I:  $p=0.690$ ; group II:  $p=0.371$ ).

Among the 109 strains cultured from pretreatment biopsy specimens, 75 strains (68.8%) were sensitive to metronidazole, and 14 (12.8%) were resistant. The cure rate was lower in patients infected with metronidazole resistant strains compared with patients infected by metronidazole sensitive cases, but this difference did not reach statistical significance ( $\chi^2=3.018$ ;  $p=0.08$ ). All strains were sensitive to clarithromycin. The antibiotic susceptibility of 20 (18.3%) strains could not be determined.

Logistic regression analysis was used to assess the relation between outcome of therapy with the *H pylori* genotypes. If adjusted for the potential confounders age, sex, presence of ulcer disease, and therapy received, the only significant covariate was the *vacA* s genotype (OR s1 versus s2 = 3.6, CI: 1.02 to 12.3). In contrast to the relation between peptic ulcer disease and genotypes, none of the other genotypes was selected when the *vacA* s genotype was excluded from the analysis. It could, however, be argued that ulcer disease should not be included as a confounder as presence of peptic ulcer disease itself is highly dependent on the *H pylori* genotypes, as shown in the first part of the study. When excluding ulcer disease as a confounder, the association with the *vacA* s genotype (again the only significant covariate) was much more pronounced (OR s1 versus s2 = 4.6, CI: 1.4 to 15.0). Furthermore, when *vacA* s genotype was excluded from the analysis, *cagA* was selected as a significant covariate (OR *cagA*+ versus *cagA*- = 3.0, CI: 1.17 to 7.9), which is consistent with the strong association between *cagA*+ and the *vacA* s1 genotypes.

Table 5 Pretreatment and post-treatment *vacA* and *cagA* status of cases containing multiple genotypes in the pretreatment biopsy specimen

| Patient | Genotype in pretreatment biopsy | Treatment outcome | Genotype in post-treatment biopsy |
|---------|---------------------------------|-------------------|-----------------------------------|
| 1       | s1a/m1/m2/ <i>cagA</i> +        | Cured             | –                                 |
| 2       | s1a/s2/m1/m2/ <i>cagA</i> +     | Cured             | –                                 |
| 3       | s1a/s2/m1/m2/ <i>cagA</i> +     | Not cured         | s1a/s2/m1/m2/ <i>cagA</i> +       |
| 4       | s1a/s2/m1/m2/ <i>cagA</i> +     | Not cured         | s1a/m2/ <i>cagA</i> -             |
| 5       | s1b/s2/m1/m2/ <i>cagA</i> +     | Not cured         | s1b/m1/ <i>cagA</i> +             |
| 6       | s1/m1/m2/ <i>cagA</i> -         | Not cured         | s2/m2/ <i>cagA</i> -              |
| 7       | s1a/s1b/m2/ <i>cagA</i> +       | Cured             | –                                 |
| 8       | s1a/m1/m2/ <i>cagA</i> +        | Cured             | –                                 |
| 9       | s1a/s2/m2/ <i>cagA</i> -        | Not cured         | s1a/s2/m2/ <i>cagA</i> -          |
| 10      | s2/m1/m2/ <i>cagA</i> +         | Cured             | –                                 |
| 11      | s1a/s2/m1/m2/ <i>cagA</i> +     | Cured             | –                                 |
| 12      | s1a/s2/m1/m2/ <i>cagA</i> +     | Not cured         | s2/m2/ <i>cagA</i> -              |

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,  $H_2$  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.<sup>16–22</sup> Furthermore, the association between peptic ulcer disease and several virulence genes of *H pylori* has been described.<sup>4–6</sup> 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.<sup>23</sup> 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

southern Europe s1b is highly prevalent.<sup>24</sup> 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.<sup>6, 8, 20</sup> 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.<sup>6, 25</sup>

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.<sup>16, 22, 26</sup> 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.<sup>16, 22, 27-31</sup> 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 patients 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.<sup>32</sup> Although only a single genotype was detected before treatment, other genotypes may already have been present before treat-

ment 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.<sup>30</sup> 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.

1 Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. *Clin Microbiol Rev* 1997;10:720-41.

2 Taylor DN, Blaser MJ. The epidemiology of Helicobacter pylori infection. *Epidemiol Rev* 1991;13:42-59.

- 3 Blaser MJ. Intrastrain differences in *Helicobacter pylori*: a key question in mucosal damage? *Ann Med* 1995;27:559-63.
- 4 Atherton JC. The clinical relevance of strain types of *Helicobacter pylori*. *Gut* 1997;40:701-3.
- 5 Mobley HL. Defining *Helicobacter pylori* as a pathogen: strain heterogeneity and virulence. *Am J Med* 1997;100:2S-11S.
- 6 van Doorn LJ, Figueiredo C, Sanna R, et al. Clinical relevance of the *cagA*, *vacA*, and *iceA* status of *Helicobacter pylori*. *Gastroenterology* 1998;115:58-66.
- 7 Leunk RD. Production of a cytotoxin by *Helicobacter pylori*. *Rev Infect Dis* 1995;13(suppl 8):S686.
- 8 Atherton JC, Cao P, Peek RMJ, et al. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995;270:17771-7.
- 9 Atherton JC, Peek RMJ, Tham KT, et al. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* 1997;112:92-9.
- 10 Kuipers EJ, Perez-Perez GI, Meuwissen SG, et al. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. *J Natl Cancer Inst* 1995;87:1777-80.
- 11 Censini S, Lange C, Xiang Z, et al. *cagA*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996;93:14648-53.
- 12 Covacci A, Censini S, Bugnoli M, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993;90:5791-5.
- 13 Beales IL, Crabtree JE, Scunes D, et al. Antibodies to *CagA* protein are associated with gastric atrophy in *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 1996;8:645-9.
- 14 de Boer WA, Tytgat GNJ. The best therapy of *Helicobacter pylori* infection. *Scand J Gastroenterol* 1995;30:401-7.
- 15 Graham DY, de Boer WA, Tytgat GN. Choosing the best anti-*Helicobacter pylori* therapy: effect of antimicrobial resistance. *Am J Gastroenterol* 1996;91:1072-6.
- 16 van der Hulst RW, Weel JF, Verheul SB, et al. Treatment of *Helicobacter pylori* infection with low or high dose omeprazole combined with amoxicillin and the effect of early retreatment: a prospective randomised double blind study. *Aliment Pharmacol Ther* 1996;10:165-71.
- 17 de Boer WA, van Etten RJXM, Schade RW, et al. One-day intensified lansoprazole-quadruple therapy for cure of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 1997;11:109-12.
- 18 de Boer WA, van Etten RJXM, Coremans A, et al. Two-day "weekend" lansoprazole-quadruple therapy for *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 1998;12:77-81.
- 19 Boom R, Sol CJ, Salimans MM, et al. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990;28:495-503.
- 20 van Doorn LJ, Figueiredo C, Rossau R, et al. Typing of the *Helicobacter pylori vacA* gene and detection of the *cagA* gene by PCR and reverse hybridization. *J Clin Microbiol* 1998;36:1271-6.
- 21 van Doorn LJ, Figueiredo C, Sanna R, et al. Expanding allelic diversity of *Helicobacter pylori vacA*. *J Clin Microbiol* 1998;36:2597-603.
- 22 Labenz J, Leverkus F, Börsch G. Omeprazole plus amoxicillin for cure of *Helicobacter pylori* infection. Factors influencing the treatment success. *Scand J Gastroenterol* 1994;29:1070-5.
- 23 van Doorn LJ, Henskens YMC, Nouhan N, et al. The efficacy of laboratory diagnosis of *Helicobacter pylori* is related to bacterial density and *vacA*, *cagA*, and *iceA* status. *J Clin Microbiol* (in press).
- 24 van Doorn LJ, Figueiredo C, Mégraud F, et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. *Gastroenterology* 1999;116:823-30.
- 25 Weel JF, van der Hulst RW, Gerrits Y, et al. The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and *Helicobacter pylori*-related diseases. *J Infect Dis* 1996;173:1171-5.
- 26 Catalano F, Privitera U, Catanzaro R, et al. Duodenal ulcer and non-ulcer dyspepsia: first results of *Helicobacter pylori* susceptibility to eradicating therapy [abstract]. *Gastroenterology* 1996;110:A78.
- 27 Schmid CH, Ross SD, Whiting GW, et al. Omeprazole plus antibiotics in the eradication of *H pylori* infection: a met-regression [abstract]. *Gut* 1996;39(suppl 2):A37.
- 28 Luna P, Del Castillo G, Farias R, et al. Efficacy and tolerability of a 7-day triple scheme with pantoprazole, amoxicillin, and clarithromycin for eradication of *H pylori* [abstract]. *Gastroenterology* 1997;112:A202.
- 29 Pieramico O, Zanetti MV, Innerhofer M. Role of gastroduodenal disease and age on the outcome of omeprazole-based dual and triple therapy for *H pylori* eradication [abstract]. *Gastroenterology* 1997;112:A91.
- 30 Hermida C, Fernandez-Munoz J, Perez-Poveda JJ, et al. Triple therapy omeprazole + amoxicillin + clarithromycin for *H pylori* infection, 6 versus 12 days. Results and cost analysis [abstract]. *Gut* 1997;112:A94.
- 31 Tucci A, Poli L, Paparo GF, et al. Weekend therapy for the treatment of *Helicobacter pylori* infection. *Am J Gastroenterol* 1998;93:737-42.
- 32 Lai JYC, de Boer WA, Driessen WMM, et al. Follow-up after cure of *Helicobacter pylori* infection with 4 days of quadruple therapy. *Aliment Pharmacol Ther* 1996;10:645-50.



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

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