The treatment of helicobacter pylori infection and its sequelae with emphasis on nitroimidazole resistance
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Chapter 9

Reliability of biopsy-based diagnostic tests for *Helicobacter pylori* after treatment aimed at its eradication.

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Abstract.

**Objective:** Recurrence of *H. pylori* after apparently successful treatment mostly represents resurgence of the infection, rather than a new one. Therefore, the reliability of biopsy-based tests after treatment was investigated.

**Methods:** ≥4 weeks after treatment, antral biopsy samples were taken for culture, histology, urease test and PCR and a corpus specimen for culture. Treatment failure was defined as ≥2 tests positive. If one test was positive a $^{13}$C-urea breath test was performed and considered conclusive.

**Results:** 197 patients were evaluated. Endoscopy was performed 53 (27-92) days after treatment. 22 patients with missing test results and 19 patients on acid-suppressive drugs were excluded. In 140 of 156 patients (89.7%) *H. pylori* was eradicated. Sensitivity and specificity of culture of antrum was respectively: 100% and 100%, culture of corpus: 100% and 100%, rapid urease test: 87% and 99%, haematoxylin/eosin stain: 94% and 95%, Giemsa stain: 81 and 99%, and PCR: were 88% and 100%.

**Conclusion:** Although all biopsy-based tests are reliable after treatment, culture is the biopsy-based test of first choice as it is the most accurate and gives additional information on antibiotic resistance.
Introduction

Eradiation of *Helicobacter pylori* (*H. pylori*) is the treatment of choice in all infected patients with duodenal or gastric ulcer disease as it prevents ulcer recurrence and cures the patient (1,2). The currently accepted definition of successful treatment is failure to detect the micro-organism by any method at least four weeks after the end of anti-*H. pylori* therapy, as it is assumed that recrudescence of the infection will occur within four weeks after completion of treatment if the micro-organism is not completely eradicated (3).

Diagnosis of *H. pylori* infection can be based on detection of the micro-organism in gastric biopsy samples by culture, histology, polymerase chain reaction (PCR), or rapid urease test. Non-invasive tests include serology, urea breath tests using either $^{13}$C or $^{14}$C, and tests for *H. pylori* antigens in stool specimens. Several studies have examined the accuracy of these tests in the untreated patient (4-6). Data on the reliability of these tests in the assessment of eradication, however, are sparse. In this study, performed in conjunction with two different treatment trials (7,8), we evaluated six different biopsy based tests (culture, PCR, haematoxylin/eosin stain (HE), Giemsa stain, and a rapid urease test of the antrum and culture of the corpus) in the assessment of eradication at least four weeks after the completion of treatment.

Methods

All patients participating in the study were treated for a culture proven *H. pylori* infection with one of three different treatment regimens. Regimen A consisted of colloidal bismuth subcitrate 120 mg (Denol®, Yamanouchi Pharma B.V., Leiderdorp, The Netherlands), metronidazole 250 mg, and tetracycline 250 mg all four times daily for two weeks. Regimen B consisted of omeprazole 40 mg (Losec®, Astra Pharmaceutica B.V., Rijswijk, the Netherlands) and amoxicillin 1000 mg (Flemoxinsolutab®, Yamanouchi Pharma B.V., Leiderdorp, the Netherlands) both twice daily for two weeks. Regimen C consisted of a one-week course of omeprazole 40 mg, amoxicillin 1000 mg, and tinidazole 500 mg (Fasigyn®, Pfizer B.V., Rotterdam, the Netherlands), all twice daily.

To assess the treatment result, the patients were endoscoped at least four weeks after the completion of treatment. Endoscopes and biopsy equipment were thoroughly cleaned with a detergent and disinfected with 2% glutaraldehyde in an automatic washing machine between all procedures. Biopsy specimens were taken within three cm of the
pylorus, one for culture, one for PCR, one for a rapid urease test, and two for histologic examination. One more was taken from the gastric body for culture. Culture was performed as described previously (9). In short, biopsy specimens were placed in 1 ml of thioglycolate broth and transported to the laboratory immediately after endoscopy. They were rubbed on two selective agar plates, one plate with Belo-horizonte medium, containing brain heart infusion agar (35 g/ml), sheep blood (10%), vancomycin (6 mg/l), nalidixic acid (20 mg/l), amphotericin B (2 mg/l), and 2,3,5-triphenyltetrazolium chloride (40 mg/l) and one plate with Campylobacter selective medium containing Columbia agar (40 g/l), sheepblood (5%), vancomycin (10 mg/l), trimethoprim (5 mg/l) and polymyxine B (2500 U/l) (CSM-medium). The plates were incubated at 36°C under micro-aerophilic conditions (5% oxygen, 85% nitrogen and 10% carbon dioxide) using a system of automatic jar evacuation-replacement (Anoxomat®, Mart BV Microbiology Automation, Lichtenvoorde, the Netherlands) and were examined after two and three days. Colonies were confirmed to be \textit{H. pylori} by Gram staining, catalase, oxidase, and urease activity. PCR, using primers as described by Clayton et al. (10) was performed as previously described [11]. A home made test containing one ml of a 10% urea solution and phenol red as a pH indicator was used as rapid urease test (5,12). It was considered positive if it turned red within one hour (5). Histological examinations using the haematoxylin/eosin (HE) and the Giemsa stain on paraffin embedded slides were performed by one pathologist (HBO). The two stains were examined independently at different occasions and the pathologist was blinded to the results of his previous examination using the other stain. The patient was regarded to be still \textit{H. pylori}-infected if at least two tests were positive. If only one of the tests was positive, while all other tests were negative, a $^{13}$C-urea breath test was performed as described previously (13) and considered conclusive. In determining this "gold standard" the two histological stains were regarded as one test and the test was considered positive if either stain showed \textit{H. pylori}. In the assessment of the individual test performance, however, both stains were evaluated separately. Apart from the endoscopist who read the rapid urease test, all investigators were blinded to the other test results and the endoscopic diagnosis. Sensitivity, specificity, positive and negative predictive values, and accuracy were calculated in the usual way.
Results
One hundred ninety-seven patients (115 males, 58%) with a mean age of 54 years (range 21-90) participated in the treatment trials (7,8). One hundred twenty-two patients had peptic ulcer disease (84 duodenal ulcer and 38 gastric ulcer), 62 patients had functional dyspepsia, 12 patients were treated because of the need for chronic acid suppression with proton pump inhibitors (PPI) (14) and one because of a strong family history of gastric carcinoma.

Forty-seven patients were treated with regimen A, 50 with regimen B and 100 with regimen C. The second endoscopy was performed 53 days (range 27-92) after the end of treatment. One patient underwent endoscopy one day too early. As it was assumed, however, that one day would not have a significant impact on the test results, this patient was not excluded. Twenty-two patients had to be excluded because one or more test data were missing. At the time of the second endoscopy 6 patients used H₂-receptor antagonists (H₂RA) and 13 used PPI. As both H₂RA (15) and PPI (16-19) may influence the accuracy of the test results these 19 patients were also excluded from the final analysis.

Figure 1. Number of positive tests for H. pylori (culture, PCR, rapid urease test and histology (HE or giemsa stain) of the antrum en culture of the corpus) in 156 patients after treatment.
Table 1. Reliability of biopsy-based tests for H. pylori after treatment.

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of Cases</th>
<th>True Negative</th>
<th>False Positive</th>
<th>False Negative</th>
<th>True Positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive Predictive Value (%)</th>
<th>Negative Predictive Value (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>156</td>
<td>140</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (98-100)</td>
</tr>
<tr>
<td>Giemsa</td>
<td>156</td>
<td>140</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (98-100)</td>
</tr>
<tr>
<td>HE</td>
<td>156</td>
<td>140</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (98-100)</td>
</tr>
<tr>
<td>Culture corpus</td>
<td>156</td>
<td>140</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (98-100)</td>
</tr>
<tr>
<td>Culture atium</td>
<td>156</td>
<td>140</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (98-100)</td>
</tr>
<tr>
<td>PCR</td>
<td>156</td>
<td>140</td>
<td>0</td>
<td>2</td>
<td>16</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (79-100)</td>
<td>100 (97-100)</td>
<td>100 (98-100)</td>
</tr>
</tbody>
</table>

Note: HE represents the hematoxylin/eosin stain. Negative, false positive, false negative and positive are given as number of cases. Sensitivity, specificity, positive and negative predictive values and accuracy are presented as percentage (95% confidence interval).
By our criteria, *H. pylori* was eradicated in 140 of the remaining 156 patients (89.7%). The number of positive tests in each patient and their distribution are presented in figure 1. At odds with all other tests, the urease test was positive in one case, the HE stain in 6 cases, the Giemsa stain in one case, and both the HE and the Giemsa stain in another case. In all these patients $^{13}$C-urea breath tests were negative.

The HE stain and the Giemsa stain were difficult to interpret in 16 and 5 cases, respectively. In these cases the test was finally considered negative, but the pathologist was unable to exclude a few remaining rods with certainty. In most of these slides there was still some inflammation present, accompanied by amorph material on the mucosal surface. According to our gold standard, all of these patients were classified as *H. pylori* negative.

Sensitivity, specificity, positive and negative predictive values, and accuracy of all tests are shown in table 1.

**Discussion**

Several studies have reported high recurrence rates of *H. pylori* infection after apparently successful eradication (20). In most of these studies, however, recurrence occurred in the first year after the completion of treatment, suggesting that these recurrences in fact represented resurgence of a suppressed infection rather than a new one (21-24). Therefore, the reliability of the tests used to establish eradication of the infection in these studies is to be questioned. Several studies examined the reliability of a negative test after treatment, but only four of them were published as full paper (25-39). In most studies one single test was selected as the gold standard, resulting in an a priori accuracy of that test of 100%. This makes interpretation of these studies difficult. A few studies tried to avoid that bias and classified the *H. pylori* status of each patient on an individual basis (27,29,38). Comparable to three other studies (32,34,39), we avoided to select a single test as the gold standard, by stipulating that a positive test should be confirmed by another positive test.

A possible drawback of this study is that true eradication of the infection in patients classified as 'H. pylori negative' was not confirmed by long term follow-up. In a similar treatment study performed in our hospital, however, the same endoscopic follow-up protocol was used and all but one of one of ninety successfully treated patients were still *H. pylori* negative after a median follow-up of 6 years (chapter 11). This suggests that
also in the present study the micro-organism was indeed successfully eradicated in patients classified as 'H. pylori negative'.

In our study all biopsy based tests proved to be accurate in the assessment of eradication, but, in comparison with the other tests histology seemed to have a somewhat lower accuracy. We acknowledge that this is likely to be observer dependent, but as histological examination by the same pathologist was highly accurate (sensitivity 96%, specificity 99%) before treatment (5), it is probably also related to the posttreatment situation. Apparently, as was also demonstrated by the cases in which the test was finally considered negative, although the pathologist was not completely certain, it is sometimes difficult to differentiate between amorph material and a few remaining bacteria, especially if some inflammation is still present. It is conceivable that the performance of histology can be improved by using special stains (like the Warthin-Starry silver stain or an immunostain) as is suggested by the results of other investigators (25,37), but these stains are more costly and technically more demanding. Moreover, in one study the accuracy of the Warthin-Starry silver stain after treatment was even lower than that of the rapid urease test (32).

In this posttreatment situation with probably low bacterial density and hence low urease activity the cheap rapid urease test had an unexpected high sensitivity. If the patient leaves the endoscopy ward with a negative test there is a 99% chance of true eradication.

In our study both culture of antral and corpus specimens were 100% accurate. This shows that culture enables reliable H. pylori detection even after treatment when bacterial load is probably low. In contrast with the results of Lamouliatte et al.(28) but confirming our previous experience (11) PCR did not detect any more treatment failures than culture. Our data confirm the results of others (40,41) and show that the biopsy site (antrum or corpus) is less important than an adequate culture technique.

In summary, we have shown that nearly all biopsy based tests are reliable in the assessment of eradication. Histology, however, especially when using the HE stain, may induce diagnostic uncertainty and a positive test should be confirmed by at least one other positive test. In our view, culture is the biopsy-based test of first choice. It has intrinsically 100% specificity and when performed properly is highly sensitive. Moreover, it gives additional information on antibiotic resistance, a frequently occurring problem after treatment failure (42,43).
References.


