Diagnostic tests for *Helicobacter pylori*: Comparison and influence of non-steroidal anti-inflammatory drugs

A S Taha, P Boothman, I Nakshabendi, J Reid, C Morran, C G Gemmell, F D Lee, R D Sturrock, R I Russell

**Abstract**

**Aims:** To evaluate the efficacy of culture, histology, CLO-test, Helico-G and Pyloriset tests in diagnosing *Helicobacter pylori* in the presence or absence of non-steroidal anti-inflammatory drugs (NSAIDs).

**Methods:** Of 134 patients studied, 75 had taken NSAIDs. At endoscopy, biopsy specimens were taken for culture, histology, and CLO-test. Blood was also taken for enzyme linked immunosorbent assay (ELISA) (Helico-G) and latex agglutination (Pyloriset) tests.

**Results:** The sensitivity, specificity, and predictive values of histology and CLO-test, compared with culture, ranged from 90% to 97%, regardless of NSAID intake. In the 59 patients not taking NSAIDs Helico-G had a sensitivity of 75% (p < 0.05) and a specificity of 61%; Pyloriset's sensitivity and specificity were, respectively, 63% (p < 0.05) and 67%. In the 75 patients taking NSAIDs the sensitivity of Helico-G was 81% and its specificity 45% (p < 0.05); Pyloriset had a sensitivity of 61% (p < 0.05) and a specificity of 50% (p < 0.05).

**Conclusion:** These findings suggest that *H pylori* is more reliably diagnosed by culture, histology, and CLO-test than by the serological tests used in this study, especially in patients treated with NSAIDs.

The increasing realisation of the importance of *Helicobacter pylori* in the development, treatment, and recurrence of peptic ulceration has made the identification of these organisms an essential part of the investigation and management of peptic ulcer disease. Several tests have been proposed, including culture, histology, urease activity, and serological tests. The value of such methods in general and the serological tests in particular has not been fully established. Their application to patients treated with non-steroidal anti-inflammatory drugs (NSAIDs), who are at a particular risk of developing peptic damage, has not been adequately investigated.

**Methods**

Patients over the age of 18 years were recruited from the gastroenterology and rheumatology clinics. NSAIDs had to have been taken for a minimum of four weeks before endoscopy. Patients were excluded if they had a history of gastric surgery or if they had received antibiotics or drugs intended to heal ulcers within two weeks of endoscopy.

Informed consent was obtained and endoscopy carried out, using 3–6 mg midazolam intravenously for sedation, within three hours of taking 10 ml of venous blood for serology tests. Biopsy specimens were taken from healthy looking mucosa in the gastric antrum, at least 2 cm away from the edge of the ulcer, in patients with ulcers, for culture (one specimen), histology (two specimens), and CLO-test (one specimen). In patients with ulcers biopsy specimens were also taken from the ulcer edge and base to exclude malignancy which, if found, precluded patients from the final analysis.

*H pylori* was detected by culture and histology, as previously described. Gastritis was classified according to the Whitehead system, modified to cover chemical and lymphocytic gastritis.

Urease activity was tested for by inserting one antral biopsy specimen, immediately after it had been obtained, into the gel pellet of the CLO-test slide (Delta-West Ltd, Bentley, Western Australia). The slides were reviewed for the positive red colouration at three and 24 hours.

Serum IgG *H pylori* antibodies were measured using a quantitative commercial IgG ELISA (Helico-G) according to the manufacturer's instructions (Porton Cambridge, Maidenhead, England). In summary, a standard curve was prepared and 100 μl serum aliquotes were dispensed into microwell strips precoated with *H pylori* cell membrane-derived antigen. The microwells were incubated at 37°C for one hour, washed in buffer, and 100 μl antibody conjugate added, to be incubated again for 30 minutes and washed as above. Substrate chromogen (100 μl of tetramethylbenzidine) was added, shaken for 10 minutes, and the reaction was then stopped using 50 μl of 2M sulphuric acid. Each microwell was then read at an absorbance of 450 nm in an ELISA reader (Labsystems, Uxbridge, England). An antibody titre of 10 units or more was considered indicative of infection with *H pylori*.

To test for IgG *H pylori* antibodies by latex agglutination, Pyloriset (Orion Diagnostica, Finland) was used. The test uses latex particles coated with acid extracted antigen of *H pylori*. A drop (40 μl) of patient's serum, already...
Table 1  Patients’ demographic details

<table>
<thead>
<tr>
<th></th>
<th>Patients receiving NSAIDs</th>
<th>Patients not receiving NSAIDs</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td>75</td>
<td>59</td>
<td>134</td>
</tr>
<tr>
<td>Males</td>
<td>22</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>Females</td>
<td>53</td>
<td>41</td>
<td>94</td>
</tr>
<tr>
<td>Age (years) median (interquartile ranges)</td>
<td>56 (44-66)</td>
<td>47 (36-65)</td>
<td>53 (39-65)</td>
</tr>
<tr>
<td>Smokers</td>
<td>27</td>
<td>21</td>
<td>48</td>
</tr>
<tr>
<td>Drinkers</td>
<td>34</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Abdominal symptoms</td>
<td>36</td>
<td>39</td>
<td>75</td>
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Table 2  Types of NSAIDs and second line drugs used

<table>
<thead>
<tr>
<th>NSAIDs</th>
<th>Number</th>
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<tbody>
<tr>
<td>Indomethacin</td>
<td>17</td>
</tr>
<tr>
<td>Naproxen</td>
<td>9</td>
</tr>
<tr>
<td>Fenbufen</td>
<td>8</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>7</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>6</td>
</tr>
<tr>
<td>Ibuprofen</td>
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</tr>
<tr>
<td>Piroxicam</td>
<td>5</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>5</td>
</tr>
<tr>
<td>Nabumetone</td>
<td>5</td>
</tr>
<tr>
<td>Others</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Second line drugs</th>
<th>Number</th>
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<tbody>
<tr>
<td>Sulphasalazine</td>
<td>15</td>
</tr>
<tr>
<td>Sodium aurothiomalate</td>
<td>15</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>7</td>
</tr>
<tr>
<td>Penicillinum</td>
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Table 3  H pylori positivity in 59 patients not treated with NSAIDs (No %)

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>Patients positive for H pylori</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
</tr>
<tr>
<td>Symptomatic patients (n = 39)</td>
<td>32 (82)</td>
</tr>
<tr>
<td>Endoscopic findings:</td>
<td></td>
</tr>
<tr>
<td>Normal (n = 53)</td>
<td>36 (68)</td>
</tr>
<tr>
<td>Ulcers (n = 6)</td>
<td>5</td>
</tr>
<tr>
<td>Histological findings:</td>
<td></td>
</tr>
<tr>
<td>Normal (n = 14)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Chronic superficial gastritis (n = 40)</td>
<td>33 (83)</td>
</tr>
<tr>
<td>Chronic atrophic gastritis (n = 3)</td>
<td>3</td>
</tr>
</tbody>
</table>

Results

A total of 134 patients entered the final analysis: 75 had taken NSAIDs (table 1). Patients in both groups were comparable in their ages, smoking and drinking habits, and the prevalence of abdominal complaints. The detail of NSAIDs and second line drugs used by patients with rheumatoid arthritis (NSAID group) are presented in table 2. The NSAIDs taken by most patients in this group are known for their potent anti-inflammatory activity and relatively strong ulcerogenic potential. The median duration of their intake was two years.

Patients not treated with NSAIDs, who are positive for H pylori according to various tests, are shown in table 3. They are subgrouped according to the presence or absence of symptoms, and endoscopic and histological findings. Similar numbers of H pylori positive patients were identified by culture, histology, and CLO-test in various subgroups. Although such numbers were greater but not significantly different from those identified by the serological tests, the latter included more false positive cases which in turn resulted in lower specificity (table 4).

H pylori positive patients in the NSAID group, classified according to their symptoms and endoscopic findings, are shown in table 5. The serological tests, Helico-G in particular, diagnosed greater numbers of H pylori positive patients receiving NSAIDs, but the importance of this finding is limited by the low specificity of such tests (see below). It is also worth noting that patients treated with NSAIDs had a total of 30 cases of ulcers or erosions (30/75, 40%), and only 18 of these (18/30, 60%) were associated with abdominal complaints.

The histological abnormalities in patients receiving NSAIDs and the corresponding numbers of H pylori positive cases are shown in table 6. The major abnormality was that of chronic superficial gastritis, followed by chemical gastritis. H pylori was identified in more than 60% of cases of chronic superficial gastritis by all tests used, with the greatest proportion (83%) diagnosed by Helico-G test. Six of 11 (54%) cases of chemical gastritis were associated with peptic damage, compared with 18 of 36 (50%) with H pylori, and six of 29 (21%); p < 0·05 in patients without chemical gastritis or H pylori.

The sensitivity, specificity, and predictive values of various tests, compared with culture, are summarised in table 4. Histology and CLO-test had the highest sensitivity, specificity, and predictive values, regardless of NSAID intake. Helico-G and Pyloriset tests had the lowest specificity and negative predictive values in patients treated with NSAIDs. Compared with Pyloriset, Helico-G had higher sensitivity and positive values but lower specificity and negative predictive values, in the presence or absence of NSAIDs.

Using culture, histology, and CLO-test, the prevalence of H pylori in the NSAID group was 43–48% compared with 64–69% in patients not treated with NSAIDs (p < 0·01); no significant differences were detected using Helico-G or Pyloriset tests. H pylori IgG titres measured by Helico-G in patients taking NSAIDs were also comparable with those in patients not receiving such drugs. Of 15 patients treated with gold and NSAIDs, five were positive for H pylori by culture, and this did not alter the interpretation of the above mentioned findings. The presence or absence of rheumatoid factor did not seem to influence the sensitivity or specificity of the serological tests used in this study.

Table 3  H pylori positivity in 59 patients not treated with NSAIDs (No %)

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</tr>
</tbody>
</table>
Table 4  Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of tests used, with culture taken as a standard

<table>
<thead>
<tr>
<th>Histology</th>
<th>CLO-Test</th>
<th>Helico-G</th>
<th>Pyloriset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients not taking NSAIDs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>95%</td>
<td>93%</td>
<td>75%*</td>
</tr>
<tr>
<td>Specificity</td>
<td>94%</td>
<td>94%</td>
<td>61%</td>
</tr>
<tr>
<td>PPV</td>
<td>95%</td>
<td>93%</td>
<td>76%</td>
</tr>
<tr>
<td>NPV</td>
<td>95%</td>
<td>95%</td>
<td>61%</td>
</tr>
<tr>
<td>Patients taking NSAIDs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97%</td>
<td>90%</td>
<td>81%</td>
</tr>
<tr>
<td>Specificity</td>
<td>90%</td>
<td>97%</td>
<td>45%*</td>
</tr>
<tr>
<td>PPV</td>
<td>97%</td>
<td>90%</td>
<td>83%</td>
</tr>
<tr>
<td>NPV</td>
<td>90%</td>
<td>97%</td>
<td>44%</td>
</tr>
</tbody>
</table>

Significant differences after correction for multiple comparison: *: p < 0.05, McNemar's test, compared with histology.

Discussion

This study shows that culture, histology, and CLO-test have comparable sensitivity, positive and negative predictive values in diagnosing *H. pylori* infection. Such characteristics do not seem to be influenced by treatment with NSAIDs. On the other hand, Helico-G and Pyloriset were less discriminatory, and the intake of NSAIDs was associated with lower specificity and negative predictive values with both of these serological tests.

Detecting *H. pylori* in gastric biopsy specimens may be influenced by sampling errors. We believe that we have minimised this by studying multiple histological sections made through each specimen and by assessing additional samples by culture and CLO-test as well as histology. The minimal differences in the sensitivity and specificity between these three methods can be explained by the presence of very few organisms in some biopsy specimens, and this would be detected as a light growth by culture. On the other hand, the higher number of false positive results diagnosed by the serology tests probably reflects previous infection with *H. pylori*. The sensitivity of Helico-G found in our study is comparable with that of another report. However, we were unable to confirm the recently reported sensitivity and specificity levels of Pyloriset.

To our knowledge, all *H. pylori* diagnostic tests have been assessed in patients not treated with NSAIDs. There is evidence to indicate that the natural history of peptic ulceration can be altered by such agents. Firstly, although more than 30% of patients taking NSAIDs might develop peptic damage, many such lesions can be completely asymptomatic, as shown by this study and others. Secondly, NSAIDs seem to reduce the prevalence of *H. pylori* infection, as suggested by data produced at our units and elsewhere. Such features of NSAID induced peptic damage might limit the value of finding a positive IgG related serological test in predicting ulcer disease. This needs to be taken into consideration especially when serological testing is not supplemented with endoscopic, culture, or histological assessments.

The reason for the low prevalence of *H. pylori* in the presence of NSAIDs remains a matter of speculation. It could be due to direct toxic action against the organisms, or it could be indirectly related to the effect of NSAIDs on the gastric mucosa. Aspirin and indomethacin increased basal and maximally stimulated gastric acid secretion. Aspirin and indomethacin also inhibited mucus secretion. This rise in gastric acid and the interference with the mucus layer by NSAIDs might in turn make it difficult for *H. pylori* organisms to survive under such unfavourable conditions.

The median duration of NSAID intake in this study was two years. Assuming that some patients became negative for *H. pylori* during this time, the behaviour of their serum antibody titres could not be predicted as the precise time of their conversion was not known. Recent studies have suggested that such titres might fall or remain unchanged.

In this study chemical gastritis was only found in patients taking NSAIDs. *H. pylori* IgG antibodies were detected in five patients with chemical gastritis (5/11, 45%) while the presence of *H. pylori* organisms was confirmed in only one of these cases. This discrepancy between the serological and biopsy tests might again support the suggestion that the positive serology reflects previous infection with *H. pylori*.

Table 5  *H. pylori* positivity in patients taking NSAIDs with symptoms or endoscopic abnormalities (no/%)  

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>Patients positive for <em>H. pylori</em></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>Culture</td>
<td>Histology</td>
<td>CLO-Test</td>
<td>Helico-G</td>
</tr>
<tr>
<td>Symptomatic patients (n = 36)</td>
<td>17 (47)</td>
<td>19 (53)</td>
<td>16 (44)</td>
<td>24 (67)</td>
</tr>
<tr>
<td>Endoscopic findings:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n = 45)</td>
<td>19 (42)</td>
<td>23 (51)</td>
<td>19 (42)</td>
<td>29 (64)</td>
</tr>
<tr>
<td>Erosions (n = 8)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>3 (38)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>Gastric ulcers (n = 14)</td>
<td>9 (64)</td>
<td>9 (64)</td>
<td>7 (50)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Duodenal ulcers (n = 6)</td>
<td>6 (75)</td>
<td>6 (75)</td>
<td>6 (63)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>Symptomatic endoscopic lesions (n = 18)</td>
<td>9 (50)</td>
<td>10 (56)</td>
<td>8 (44)</td>
<td>12 (67)</td>
</tr>
</tbody>
</table>

Table 6  *H. pylori* positivity in patients taking NSAIDs with histological abnormalities (no/%)  

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>Patients positive for <em>H. pylori</em></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>Culture</td>
<td>Histology</td>
<td>CLO-Test</td>
<td>Helico-G</td>
</tr>
<tr>
<td>Chronic superficial gastritis (n = 46)</td>
<td>30 (65)</td>
<td>34 (74)</td>
<td>29 (63)</td>
<td>38 (83)</td>
</tr>
<tr>
<td>Lymphocytic gastritis (n = 4)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Chemical gastritis (n = 11)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Normal histology (n = 14)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
pylori which could have been eradicated, or at least altered, by NSAID intake.\(^\text{17}\)

Peptic ulcers or erosions associated with NSAID intake were found in 54% of patients with chemical gastritis and 50% of those with \(H\) pylori, compared with only 21% of other patients in the NSAID group. This agrees with our recent data suggesting that the prevalence of NSAID induced peptic damage seems to be increased by the presence of chemical gastritis or \(H\) pylori.\(^\text{17}\)

In conclusion, culture, histology, and CLO-test have comparable sensitivity and specificity which do not seem to be influenced by NSAID intake. However, IgG antibody serology tests (Helico-G and Pyloriset) seem to be less useful than the above tests involving the use of gastric biopsy specimens. Such differences are further emphasised in patients treated with NSAIDs. The use of improved serological tests should still be considered because of their non-invasive nature, low cost, time and labour saving potential.

We thank Porton Cambridge, Maidenhead, England and Orion Diagnostics, Finland, for donating some of the Helico-G and Pyloriset kits. We also thank Miss Stephanie McLaughlin for her help in the statistical analyses, Miss Jacqueline Kennedy for her secretarial assistance, and Merck Sharp and Dome for their financial support.


18 McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages. \textit{Psychometrika} 1947;12:157-76.


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