Aim. To assess five main histological features of gastritis in gastric mucosa colonized with Helicobacter pylori before and after the treatment.

Methods. Histologic assessment of H. pylori-associated gastritis was performed according to the Sydney classification before and after the treatment in 97 patients. Two additional parameters – the presence of lymphocytic aggregates and coccoid forms of bacteria – were also analyzed. Helical and coccoid forms of H. pylori were detected by immunohistochemistry in biopsies after the treatment.

Results. Whereas acute epithelial damage was quickly repaired, some of the local responses to bacteria, e.g., lymphoid aggregates and intestinal metaplasia, persisted after treatment. Higher H. pylori and coccid density was found before and after treatment in patients with intestinal metaplasia (p=0.020). Correlation between H. pylori and mucosal atrophy was found only after treatment (p=0.009). Immunohistochemical staining was more sensitive in detecting of H. pylori than Giemsa staining (p=0.007) in cases where, using only Giemsa staining, it was not possible to distinguish coccoid forms of H. pylori from other cocci.

Conclusion. After treatment, H. pylori-associated gastritis showed reduction of acute and chronic inflammation, but lymphoid aggregates and intestinal metaplasia persisted. Immunohistochemistry of different forms of H. pylori may be a valuable technique in monitoring the success of the treatment.

Key words: biopsy; campylobacter pylori; gastritis; gastritis, atrophic; helicobacter infections; helicobacter pylori; metaplasia, intestinal; stomach diseases

There is a large body of evidence that H. pylori takes part in the pathogenesis of chronic gastritis and peptic ulcer disease (1-7). Incorporation of H. pylori colonization in classifications of chronic gastritis with topography and morphology led to the Sydney classification (3,8). The most important characteristic of this system is grading of five main histological features of gastritis, which allows accurate assessment of changes that occur in the gastric mucosa (8).

Several tests, both invasive and non-invasive, are available for the diagnosis of H. pylori infection. The invasive methods include the demonstration of H. pylori in the biopsy sample of gastric mucosa during endoscopy. H. pylori can also be demonstrated by urease test, in vitro cultures, and molecular methods (9-12). In clinical trials and clinical practice, accurate detection of H. pylori before and after eradication therapy has proved to be of critical importance for the evaluation of the treatment outcome (6). Anti-H. pylori antibody used on paraffin-embeded sections provides a more specific way to distinguish coccoid forms of H. pylori from other cocci (13,14).

The aim of this study was to assess five main histological features of gastritis in gastric mucosa colonized with H. pylori before and after the treatment. Whereas the recognition and density of H. pylori in gastric biopsies before treatment is not difficult to assess by Giemsa staining, small number of microorganisms after the treatment cannot be visualized by this staining method. Therefore, the biopsies obtained after the treatment were immunostained with specific H. pylori antibody in addition to the standard Giemsa staining.

Patients and Methods

The study included 157 consecutive patients with duodenal ulcer, non-ulcer dyspepsia, and gastric ulcer admitted to the out-patient clinic in the Zagreb University Hospital, Zagreb, Croatia. Only patients with histological evidence of gastritis and positive H. pylori by Giemsa staining, and with
The standard avidin-biotin immunoperoxidase method. The differences were considered significant if p<0.05. We used the Wilcoxon test, chi-square test, and Spearman's rank correlation.

**Results**

After the specific therapy, statistically significant differences were observed for the density of *H. pylori* colonization, chronic inflammation, and activity (for all p=0.001); and the presence of cocci (p=0.030). The degree of atrophy, presence of intestinal metaplasia and lymphocytic aggregate formation failed to show significant change between the pre- and post-treatment biopsy specimens (Table 1).

In post-treatment biopsies, 36 cases were positive by Giemsa staining, whereas only 18 cases were immunohistochemically positive. The majority of biopsies with a low density of bacteria (grade 1) were immunohistochemically negative (Table 2).

**Discussion**

This study showed that the restoration of gastric mucosal integrity after eradication of *H. pylori* is much more complex than a simple disappearance of microorganisms and neutrophils. Whereas acute epithelial damage is quickly repaired, some of the local responses to the bacteria, such as lymphoid aggregates and epithelial intestinal metaplasia, persisted for 3-4 months after treatment in our patients. According to some authors, these changes may persist long after the eradication therapy for *H. pylori*-associated chronic gastritis (15-18).

Although it has been suggested that *H. pylori* is involved in the malignant transformation of intestinal metaplasia, bacteria are not often detected in the gastric mucosa of patients with intestinal

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**Table 1. Assessment of pre- and post-treatment gastric mucosa biopsies in 97 patients with *H. pylori*-associated gastritis**

<table>
<thead>
<tr>
<th>Pathological parameter</th>
<th>No. of patients with Sydney gastritis score <em>a</em></th>
<th>p <em>b</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Acute inflammation</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td><em>H. pylori</em> density</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>Atrophy</td>
<td>57</td>
<td>53</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>Lymphatic aggregates</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Presence of cocci</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*a*Before and after eradication therapy.  
bFor probability significant difference of findings before and after the treatment.

**Table 2. Post-treatment detection of *H. pylori* by Giemsa staining versus immunohistochemistry**

<table>
<thead>
<tr>
<th>Density grade</th>
<th>Giemsa detection</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>negative</td>
</tr>
<tr>
<td>0</td>
<td>61 (62.9)</td>
<td>57</td>
</tr>
<tr>
<td>1</td>
<td>26 (26.8)</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>7 (7.2)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3 (3.1)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>97 (100.0)</td>
<td>79</td>
</tr>
</tbody>
</table>
metaplasia (19-21). Altered milieu in the areas of intestinal metaplasia, including an increase in secretory IgA and reduced acidity in comparison to the normal gastric mucosa, may explain the decreased colonization of H. pylori observed in the metaplastic mucosa (22). In our study we noticed a statistically significant positive correlation between H. pylori density and intestinal metaplasia in control biopsies obtained 3-4 months after the eradication therapy.

The relationship of H. pylori colonization and gastric mucosa atrophy was examined with regard to the reports that bacteria may be absent in severely atrophic mucosa (19,23). Our results showed that H. pylori density did not correlate with the atrophy of gastric mucosa in primary biopsy, but was associated with gastric atrophy in control biopsies (p=0.009), indicating that H. pylori may be actively involved in the development of mucosal atrophy.

With regard to the fact that the confirmation of H. pylori eradication and detection of reinfection are important for appropriate patient management, pathologists have sought more reliable methods for the detection of H. pylori in biopsy specimens, including immunohistochemistry, polymerase chain reaction and, more recently, in situ hybridization (24-30). One of the advantages of immunohistochemistry is the ability to detect low numbers of organisms, often difficult to detect using traditional staining methods, and to identify the coccoid forms of H. pylori, which otherwise cannot be reliably identified by conventional methods. Our results showed that some H. pylori detected by Giemsa staining were not confirmed using immunohistochemistry, particularly in low bacterial density (grade 1). It seems that the majority of these bacteria belonged to another species of microorganisms.

Ashton-Key et al (31) have proposed that an agreement could be reached by using immunohistochemistry in gastric biopsy specimens with chronic gastritis characteristics but which are H. pylori-negative by conventional staining methods or have coccoid forms of bacteria. This should also be done for post-treatment specimens from gastric lymphoma patients to ensure therapy efficiency. Application of immunohistochemistry in H. pylori detection seems to be a valuable technique in the evaluation of gastric biopsies in patient monitoring after the treatment for H. pylori.

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### References


Table 3. Correlation of the H. pylori and cocci density with atrophy and intestinal metaplasia before and after antibiotic treatment of H. pylori-associated gastritis

<table>
<thead>
<tr>
<th>Correlated variables</th>
<th>Correlation coefficients* and p values for findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-treatment</td>
</tr>
<tr>
<td>H. pylori density vs. intestinal metaplasia</td>
<td>0.23</td>
</tr>
<tr>
<td>H. pylori density vs. atrophy</td>
<td>0.05</td>
</tr>
<tr>
<td>Cocci density vs. intestinal metaplasia</td>
<td>0.41</td>
</tr>
<tr>
<td>Cocci density vs. atrophy</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*aSpearman’s rank correlation (\( \rho \).)


