Specific detection of *Helicobacter pylori* and non-*Helicobacter pylori* flora in small- and large-cell primary gastric B-cell non-Hodgkin’s lymphoma

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Summary

**Background:** Primary gastric non-Hodgkin’s lymphomas possibly develop in response to local infection by *Helicobacter pylori* (*H. pylori*). We investigated the presence of *H. pylori* and non-*H. pylori* flora histologically in small- and large-cell primary gastric lymphomas using a specific staining method.

**Materials and methods:** Specimens of 52 cases of primary gastric lymphoma (17 small cell, 35 large cell) were stained with modified Giemsa (MG) and immunohistochemically using a polyclonal antibody against *H. pylori* (IHC).

**Results:** Thirty-two cases (61.5%) (small cell 76% versus large cell 53%, P > 0.05) showed immunoreactivity for *H. pylori* in the mucosa surrounding the tumor. Remarkably, there was localization of *H. pylori* in the neck of the gastric glands in 3 cases. Non-*H. pylori* flora was seen in 35 cases (76.3%) (small cell 53% versus large cell 74%, P > 0.05). In 20 cases, this non-*H. pylori* flora was mixed with *H. pylori*. Five cases showed no bacterial flora at all.

**Conclusions:** (1) Using immunohistochemistry, the prevalence of gastric lymphoma cases with *H. pylori* (61.5%) approximates that of *H. pylori* in the normal population. (2) No statistical difference was found between the occurrence of *H. pylori* and non-*H. pylori* bacterial flora in small- versus large-cell lymphoma. (3) Our results suggest that *H. pylori* may not be the only etiologic factor in primary gastric lymphoma.

**Key words:** bacterial flora, *Helicobacter pylori*, immunohistochemistry, MALT, primary gastric lymphoma

Introduction

Studies investigating the pathogenesis of gastric carcinomas have found an important role in the etiology of this condition for *Helicobacter pylori* (*H. pylori*) [1]. This bacterium may also be involved in the etiology of primary gastric lymphoma, which is the commonest extranodal non-Hodgkin’s lymphoma. Although it accounts for only a minority of gastric neoplasms, its incidence is increasing [2]. *H. pylori* is considered to cause a follicular gastritis in the stomach and to be responsible for the development of lymphoid tissue in the stomach, where normally no organized lymphoid tissue is present. It is hypothesized that malignant lymphoma could evolve from this acquired mucosa-associated lymphoid tissue (MALT). The observation that eradication therapy for *H. pylori* in some cases can lead to regression of primary low-grade gastric lymphomas may support this hypothesis [3–5].

Studies investigating the presence of *H. pylori* in gastric mucosa surrounding MALT lymphoma using non-specific staining methods have found the bacterium in 41% to 92% of cases. These differences prompted us to study *H. pylori* with a more sensitive and specific immunohistochemical staining method [6, 7]. Despite the fact that most gastric lymphomas are large-cell lymphomas, most studies on the occurrence of *H. pylori* have focused on small-cell gastric MALT lymphoma. It may, however, also be interesting to look for the presence of *H. pylori* in large-cell lymphoma. Finally, by using this specific staining technique for the detection of *H. pylori* in addition to a nonspecific staining method (modified Giemsa), we were also able to evaluate the presence of other bacteria present in primary gastric lymphomas and therefore could evaluate a possible role for non-*H. pylori* flora in the pathogenesis of gastric lymphoma.

Materials and methods

Gastric lymphoma cases were selected from the files of the pathology departments of ten hospitals in the provinces of Limburg and Brabant, the Netherlands. Clinical data were retrieved to establish that the lymphoma was primary gastric, i.e., the stomach was the only or most massively affected organ, and no former nodal disease was present. All formalin-fixed, paraffin-embedded tissue blocks from either biopsies or gastrectomies were collected. Only cases in which not only tumor tissue but also normal gastric mucosa was present were included in this study, for two reasons: first, to assure a more detailed classification of MALT (i.e., more chance to detect a low-grade MALT component in high-grade lymphoma), and second, because of the possibility that the occurrence of *H. pylori* could be confined to ‘normal’ mucosa. Hematoxylin-eosin stained sections were reviewed by two pathologists (J. W. Arends, F. J. Bot), and diagnosis of MALT lymphoma was based on the criteria described by Isaacson et al. [8–10], i.e., presence of reactive B-cell fol-
lies, surrounded by the tumor cell infiltrate consisting of centrocyte-like cells, selectively invading epithelial structures to form characteristic lymphoepithelial lesions. When the tumor contained fields of large blastic cells, cases were regarded as large-cell (high-grade) lymphoma. Unless these large-cell tumors contained a low-grade component displaying the typical features of MALT, no diagnosis of large-cell MALT lymphoma could be made and they were diagnosed as large B-cell lymphoma. Using these criteria, 52 cases could be selected (31 men, 21 women, mean age at diagnosis: 62 years). At least two tissue blocks were present for each case, with a maximum of 14 blocks. Based on the classification, 17 patients had small-cell lymphoma, in all of which MALT features could be detected; 26 had a large-cell lymphoma of MALT with a small-cell MALT component and nine patients had a large B-cell lymphoma of the stomach without a small-cell MALT component.

Diagnosis of H. pylori was made by assessment of modified Giemsa (MG) and immunohistochemistry (IHC) stained serial sections of 4 μm. MG staining was performed as described by Gray [11]. For the immunohistochemical staining, a purified H. pylori antiserum was used (DAKO E474, ITK Diagnostics BV, Uitgoorn, the Netherlands) in a 1:500 dilution. Staining was performed as described by Loffeld et al. [6] without pepsin treatment and with biotin-conjugated swine-anti-rabbit antibodies (DAKO E431) as a second layer, followed by StreptABC-HRP (DAKO K377). All slides were examined at a magnification of 400 times, by two observers. In the MG stain, H. pylori was diagnosed by its characteristic S-shaped appearance and the typical localization in or on the gastric mucus layer. H. pylori positivity was defined as the presence of immunoreactivity. By joint evaluation of both IHC and MG stains, non-H. pylori flora was assumed when bacterial flora observed in MG were negative in IHC staining. These bacteria were not further characterized in this study. The presence of H. pylori was scored on a semi-quantitative scale: grade 0: no bacteria seen; grade 1: sporadic bacteria observed; grade 2: bacteria present in almost all high power fields; grade 3: bacteria abundantly found in all high power fields, also lying in clusters. The chi-square test (and in case of small samples, Fisher's exact test) was applied for statistical analysis. A P-value of less than 0.05 was considered to be significant.

Results

Detection of H. pylori by IHC revealed a positive reaction in 32 cases (61.5%). Figure 1 shows an example of both an MG and an IHC stain in the same lymphoma, both showing H. pylori flora. In all cases, H. pylori was detected in the mucosa surrounding the tumor. It was never detected inside the lymphoma tissue. The presence of H. pylori varied from grade 1 to grade 3. In 12 cases grade 1 was the highest grade, in 6 cases grade 2, and in 10 cases we noticed grade 3. Using the MG stain, H. pylori was suspected in 4 additional cases, which were negative on IHC. The MG-stained sections were also examined for the presence of other bacteria, apart from H. pylori. We noticed this phenomenon in 3 cases (2 small-cell MALT cases, 1 large-cell lymphoma of MALT origin).

Discussion

Gastric MALT lymphomas account for the majority of extranodal MALT lymphomas, which is paradoxical, since the normal gastric mucosa is devoid of any organized lymphoid tissue. Therefore, the hypothesis was formulated that there should be a stimulus for the development of MALT in the stomach. Since the description of H. pylori by Warren [12], research on H. pylori has extended exponentially [13, 14], and its role in the pathogenesis of gastritis, peptic ulcer, and gastric cancer [1, 15, 16] has been investigated. In the case of gastric lymphoma, the development of lymphoid tissue is important in its pathogenesis, and studies have shown that stomachs
Table 1. *Helicobacter pylori* and non-*Helicobacter pylori* flora in low-grade MALT lymphomas, high-grade lymphomas with a low-grade MALT component, and high-grade gastric lymphomas without a low-grade MALT component.

<table>
<thead>
<tr>
<th>Staining results</th>
<th><em>H. pylori</em></th>
<th>Non-<em>H. pylori</em> flora only</th>
<th>Both <em>H. pylori</em> as well as non-<em>H. pylori</em> flora (mixed)</th>
<th>No flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small-cell MALT (n = 17)</td>
<td>13 (76.5%)</td>
<td>3 (17.6%)</td>
<td>6 (35.3%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>Large cell (n = 35)</td>
<td>19 (54.3%)</td>
<td>12 (34.3%)</td>
<td>14 (40.0%)</td>
<td>4 (11.4%)</td>
</tr>
<tr>
<td>With a small-cell MALT component (n = 26)</td>
<td>16 (61.5%)</td>
<td>5 (26.9%)</td>
<td>12 (46.2%)</td>
<td>3 (11.5%)</td>
</tr>
<tr>
<td>Without proven MALT component (n = 9)</td>
<td>3 (33.3%)</td>
<td>5 (55.6%)</td>
<td>2 (22.2%)</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td>Total (n = 52)</td>
<td>32 (61.5%)</td>
<td>15 (28.8%)</td>
<td>20 (38.5%)</td>
<td>5 (9.6%)</td>
</tr>
</tbody>
</table>

*a* χ²-statistics were used to determine differences in the distribution of *H. pylori*, non-*H. pylori* flora only, mixed flora and no flora, between small-cell MALT vs. large-cell cases, small-cell MALT vs. large cell with a small-cell MALT component, small-cell MALT vs. large cell without certain MALT component, and large-cell MALT vs. large cell without a certain MALT component.

* Significant difference in distribution of *H. pylori* (P = 0.042, Fisher's exact test).

infected by *H. pylori* show acquired lymphoid follicles and aggregates [17, 18]. This lymphoid tissue may be a precursor of gastric lymphoma. Several groups have investigated the occurrence of *H. pylori* in gastric lymphomas, either by histological detection or by serology. The reported frequency of *H. pylori* in primary gastric lymphoma varies considerably, from 41% in a study by Calvert et al. [19], 50% in a study by Karat et al. [20], 59% in one by Miettinen et al. [21], 80% in one by Muller et al. [22], to a maximum of 92% in a study by Wotherpoon et al. [23] when detected histologically. In spite of a common approach, using a routine hematoxylin-eosin staining, and only staining cases which were negative and/or in which additional material was present with the MG stain, considerably varying percentages of *H. pylori* were reported.

Using IHC for the detection of *H. pylori* in all cases allowing a more specific identification was an important feature of our study. We could detect *H. pylori* by immunohistochemistry in 32 out of 52 primary gastric lymphoma cases (61.5%). Including the cases which were (false)-positive in MG and negative in IHC, this percentage was 69.2%. Besides the choice of a more or less specific and/or sensitive staining method, additional explanations like atrophic changes in the body mucosa [21, 24], differences in fixation of the specimens [25], availability of gastrectomy specimens [26], presence of non-tumorous mucosa, previous eradication therapy of *H. pylori* (which plays no important role in the present series, because all patients were included before 1992), extent of intestinal metaplasia [27], and experience of the observers to identify *H. pylori* can explain variations in detected frequencies. The percentage of *H. pylori* found in this study is close to the mean percentage found in the asymptomatic European population of this age (62.4%) [28]. Although no serological studies of *H. pylori* in the asymptomatic population of the same region are available, Loffeld et al. found a percentage of about 70% in dyspeptic patients [29]. In earlier studies, which reported higher percentages of *H. pylori*, an association with primary gastric lymphoma was suggested because of these higher percentages. Our findings of more commonly observed percentages make it plausible that *H. pylori* is not the sole causative factor in gastric lymphoma but that additional etiologic factors are necessary for the development of MALT and gastric lymphoma.

Comparing the findings in small-cell primary gastric MALT lymphomas and the total of large-cell gastric lymphomas (i.e., with or without a small-cell MALT component), no significant difference in the occurrence of *H. pylori* was found. Assuming that *H. pylori* provides an antigenic drive for the proliferation of gastric lymphoma [30, 31], this suggests that large-cell lymphomas could still be dependent on this drive. The finding of lower frequencies of *H. pylori* in large-cell lymphomas without a small-cell MALT component compared to both small MALT (significant) as well as large-cell lymphomas with a small-cell MALT component (not significant) might suggest that *H. pylori* plays a minor role in lymphomas without a MALT origin. Moreover, these cases showed the most intense non-*H. pylori* flora, which might suggest a more important role of non-*H. pylori* flora in these lymphomas.

**Figure 2.** Immunohistochemical staining of a small-cell primary gastric lymphoma of mucosa-associated lymphoid tissue, in which *Helicobacter pylori* is seen deep in the gastric pits, at the level of the neck of the gastric glands (arrows). In this case, *Helicobacter pylori* was not seen in or on the mucus layer. Original magnification 312×.
However, it should be borne in mind that in these cases little mucosal tissue was present, which interfered both with the detection of \textit{H. pylori} as well as with the diagnosis of MALT. Furthermore, the high intensities of non-\textit{H. pylori} flora in this study may be the result of local changes induced by the tumor, like an increased pH, causing a more intestinal-like bacterial flora.

The observation of \textit{H. pylori} in three cases in the neck of the gastric glands without their presence in the superficial mucosa is interesting. To our knowledge, such a localization of \textit{H. pylori} has never been described before. We hypothesize that this unusual localization could be a result of a nonactive infection with \textit{H. pylori}, retracted in the deep layers of the mucosa but not completely removed by the immune system. This equilibrium and its disturbances may play a role in the development of organized lymphoid tissue in the stomach.

In conclusion, our results support the occurrence of \textit{H. pylori} in primary gastric B-cell non-Hodgkin's lymphoma is not different from its occurrence in the normal (European) population. Furthermore, we found no significant difference between small-cell MALT lymphoma and large-cell lymphoma with a proven MALT component, and we found a high frequency of other bacteria. Although there is evidence for a role of \textit{H. pylori} in the etiology of primary gastric lymphoma, the abundance of non-\textit{H. pylori} flora suggests that \textit{H. pylori} might not be the only etiologic factor in the development of gastritis, MALT, and primary gastric lymphoma. For this reason, and because the presence of \textit{H. pylori} in some cases is difficult to establish using nonspecific staining methods, its histological presence is best examined by IHC, for example, when considering eradication therapy for gastric lymphoma. Furthermore, one should realize that small-spectrum antibiotic therapy directed to \textit{H. pylori} might not eradicate other bacteria that could be a persisting drive for the development of MALT or gastric lymphoma.

References