Diagnosis and Posttreatment Follow-Up of Helicobacter Pylori-Positive Gastric Lymphoma of Mucosa-Associated Lymphoid Tissue: Histology, Polymerase Chain Reaction, or Both?

By A. Savio, G. Franzin, A.C. Wotherspoon, G. Zamboni, R. Negrini, F. Buffoli, T.C. Diss, L. Pan, and P.G. Isaacson

LOW-GRADe GASTRIC lymphoma of mucosa-associated lymphoid tissue (MALT) has been shown to be associated with colonization of the gastric mucosa by Helicobacter pylori (H pylori) in more than 90% of cases.1,2 In gastric biopsies with a dense mucosal lymphoid infiltrate, the distinction between a reactive lymphoid population and a low-grade MALT lymphoma can be difficult3-5 and is greatly helped by the demonstration of B-cell monoclonality. Monoclonality can be inferred by the immunocytochemical demonstration of Ig heavy chain restriction, which is possible using conventional formalin-fixed paraffin-embedded biopsy specimens. However, the interpretation of the staining in small, often crushed, biopsies is frequently difficult. The development of the polymerase chain reaction (PCR) for the identification of clonal B-lymphocyte populations by the analysis of Ig heavy chain gene and its application to paraffin-embedded biopsy specimens provides a specific and sensitive tool for the identification of neoplastic B-cell populations and therefore be of use in the distinction between florid H pylori-associated gastritis and low-grade MALT lymphoma.

The assessment of monoclonality is fundamental in the management of patients with lymphoma both in the diagnosis and in the evaluation of response to treatment. Recent studies have shown rapid histologic regression of gastric MALT lymphoma after the eradication of H pylori that has been accompanied by PCR evidence of loss of the monoclonal B-cell population.6,7 In this study, we have formally evaluated the role of histology and PCR in the diagnosis of routinely fixed, paraffin-embedded gastric biopsies with a dense lymphoid infiltrate and in the follow-up of cases of low-grade MALT lymphoma treated by H pylori eradication.

MATERIALS AND METHODS

Diagnostic procedures. All gastric biopsies showing a dense mucosal lymphoid infiltrate were retrieved from the surgical files of the Department of Histopathology, Ospedale S. Orsola, Brescia, Northern Italy for the period between 1991 and 1993. Sixty-nine cases were obtained (36 female and 33 male patients: age range, 16 to 84 years) and sections from routinely processed paraffin-embedded tissue were stained with hematoxylin and eosin (H&E) and a modified giemsa stain to detect H pylori. These were reviewed by four histopathologists (A.S., G.F., A.C.W., and P.G.I.). The H pylori status was noted and the lymphoid infiltrate was assessed for features of low-grade MALT lymphoma using established criteria.8 To assess alterations in the histologic appearances in subsequent biopsies, the confidence of a diagnosis of lymphoma was objectively scored using molecular regression have monoclonal-amplified products 17 and 24 months after negative histology. In 3 cases, the histology of the biopsies was considered indeterminate or discordant. In 1 of these cases, the histologic features were obscured by crush artefact. In a second case, there was molecular evidence of monoclonality in the absence of histologic features suggestive of lymphoma; this persisted after H pylori eradication. An additional single case originally diagnosed as reactive developed a PCR detectable clonal population 29 months after original evaluation in the absence of histologic features of lymphoma but in the presence of persistent H pylori infection. These findings suggest that the histologic assessment of gastric biopsies remains the method of choice for the diagnosis of lymphoma in gastric endoscopic biopsies with a dense mucosal lymphoid infiltrate. PCR provides a useful technique to support the diagnosis if clonal amplification products are found. The significance of PCR detected clonality in the absence of histologic evidence of lymphoma is uncertain but may represent a stage of tumor progression/regression when the clonal population is insufficient to be detected by conventional histology. This is supported by the evidence that PCR-detectable monoclonality can persist after treatment and the dissappearance of histologically detectable lymphoma.

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a previously published system\(^6\) (Table 1) in which scores up to 3 were considered reactive and grades 4 to 5 neoplastic.

In each case, PCR was performed on DNA extracted from unstained sections from the same paraffin blocks using primers to the joining region and the framework (Fr) 3 part of the variable region bromide, and viewed under electrophoresis on 10% polyacrylamide gels, stained with ethidium were considered reactive and grades 4 to 5. The products were subjected to formed in which the Fr2 primer was substituted with a primer to stained sections from the same paraffin blocks using primers to the heavy chain gene using a seminested technique. The products were subjected to

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Histologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Scattered plasma cells in lamina propria. No lymphoid follicles</td>
</tr>
<tr>
<td>1</td>
<td>Chronic active gastritis</td>
<td>Prominent lymphoid follicles with surrounding mantle zone and plasma cells. No LELs.</td>
</tr>
<tr>
<td>2</td>
<td>Chronic active gastritis with florid lymphoid follicle formation</td>
<td>Lymphoid follicles surrounded by small lymphocytes that infiltrate diffusely in lamina propria and occasionally into epithelium</td>
</tr>
<tr>
<td>3</td>
<td>Suspicious lymphoid infiltrate in lamina propria, probably reactive</td>
<td>Lymphoid follicles surrounded by CCL cells that infiltrate diffusely in lamina propria and into epithelium in small groups</td>
</tr>
<tr>
<td>4</td>
<td>Suspicious lymphoid infiltrate in lamina propria, probably lymphoma</td>
<td>Presence of dense diffuse infiltrate of CCL cells in lamina propria with prominent LELs</td>
</tr>
</tbody>
</table>

Data from Wotherspoon et al.\(^6\) Abbreviations: CCL, centrocyte-like; LEL, lymphoepithelial lesion.

In each case, PCR was performed on DNA extracted from unstained sections from the same paraffin blocks using primers to the joining region and the framework (Fr) 3 part of the variable region of the Ig heavy chain gene using a seminested technique.\(^6\) In biopsies with a histologic score of 3 to 5 without PCR evidence of clonality using this primer combination, a separate amplification was performed in which the Fr3 primer was substituted with a primer to the Fr2 part of the variable region.\(^6,11\) The products were subjected to electrophoresis on 10% polyacrylamide gels, stained with ethidium bromide, and viewed under UV light. For each case, the reactions were run in duplicate and only those cases with a dominant band of identically sized fragments were considered to contain a monoclonal B-cell population. Reagent preparation, amplification, and product analysis were performed separately to avoid contamination. Molecular weight markers and positive (previously characterized B-cell malignant lymphoma centroblastic-centrocytic) and negative (no DNA) controls were included for each reaction.

**Treatment and staging.** All patients received anti-H pylori therapy consisting of a combination of amoxycillin, tinidazole, colloidal bismuth carbonate, and/or omeprazole. When eradication was not achieved, alternative antibiotic regimes were devised based on the results of an antibiotic.

Patients in whom the biopsy showed histologic features suggestive or diagnostic of low-grade MALT lymphoma or in whom a monoclonal B-cell population was identified underwent staging procedures that included a bone marrow biopsy, chest x-ray, abdominal ultrasonography, oropharyngeal examination, biochemical profile including lactate dehydrogenase (LDH) and \(\beta2\)-microglobulin assay, and a full blood count with differential white blood cell count.

**Follow-up.** Each patient underwent endoscopy 2 to 4 months after completion of the anti-H pylori therapy and biopsies were taken. In 27 cases, cultures were performed to confirm eradication or to obtain an antibiotic.

Patients with histologic or molecular evidence of low-grade MALT lymphoma underwent repeat endoscopy at approximately 6-month intervals after H pylori eradication. On each occasion, the procedure was undertaken by the same endoscopist (F.B.) who performed several biopsies from the same gastric region previously involved by lymphoma. Biopsy specimens were assessed histologically by the same four pathologists and PCR was performed using the primer combinations that had previously produced a monoclonal pattern. Amplification products from all specimens from an individual case were run side by side on a single gel to confirm the persistence or disappearance of the clonal population. Cases in which the initial biopsies were assessed as showing a reactive lymphoid infiltrate were followed-up by endoscopy and biopsy only after a specific request by the patient.

**RESULTS**

Sixty-nine patients were identified in whom gastric biopsies showed a dense mucosal lymphoid infiltrate. All patients had initially presented with nonspecific upper gastrointestinal symptoms. H pylori organisms were identified in the initial biopsy specimens in all cases by examination of the H&E- and/or giemsa-stained sections.

Histologic evaluation showed a reactive lymphoid infiltrate (score 2 to 3) in 55 cases and features considered to be suggestive or diagnostic of low-grade MALT lymphoma (score 4 to 5) in 13 cases. One case showed severe crush artefact that obscured the histologic features. PCR gave monoclonal products in a total of 11 cases. Nine of 13 (69%) cases with a histologic score of 4 to 5 showed molecular evidence of monoclonality. PCR of the case showing crush artefact showed a monoclonal pattern. One of the 55 cases (2%) with no histologic evidence of lymphoma (score 2) showed a clonal amplification product. In 1 case originally considered to be reactive (score 2), follow-up biopsies showed an increase in the severity of the lymphoid infiltrate to score 3, but PCR of this biopsy showed evidence of a clonal population. On the basis of the combined histologic and molecular results, the cases were divided into three groups: 53 cases of reactive lymphoid infiltrate, 13 cases of low-grade MALT lymphoma, and 3 cases in which the features were either indeterminate (due to crush) or showed discordance between histology and molecular results.

**Reactive lymphoid infiltrate.** Review of the endoscopic appearances of 53 patients (27 female and 26 male patients; age range, 17 to 86 years) diagnosed as reactive lymphoid infiltrates showed gastritis in 27 cases, erosions in 9, and ulceration in 18. In 27 cases, the follow-up period was less than 8 months, but in the remainder the mean follow-up period was 23 months. Eradication of H pylori was achieved in 40 patients (76%), but in 1 case bacterial relapse/reinfection was noted. In those cases in which eradication was successful, a rapid decrease in the lymphoid infiltrate was observed, with complete reversion to normal a mean of 18 months after therapy.

**Low-grade MALT lymphomas.** The clinical, histologic, and molecular data are summarized in Table 2. In 7 patients, the predominant symptom was epigastric pain that was associated with hematemesis in a single case. Two cases had a previous history of duodenal ulcer. Gastroscopy showed active or healed ulceration in 6 cases (3 single and 3 multiple), erosions in 2, gastritis in 4, and a normal appearance in 1 case. Ulcers and erosions were located in the antrum and angulus in 5 cases and in the distal body in 1. The endoscopic appearances could not discriminate between cases of reactive
lymphoid infiltrate and low-grade MALT lymphoma. In all cases, staging procedures showed that the lesion was confined to the stomach (stage IE).

Retrospective examination of previous biopsies in 4 patients showed histologic evidence of lymphoma 9 to 52 months before the commencement of anti-H pylori therapy. In these cases, the histologic appearances of lymphoma remained unaltered until antibiotic therapy was instituted.

Eradication of H pylori was achieved in 12 cases (92%), of which 4 required more than one antibiotic combination. In 1 case, eradication was not achieved despite 5 different antibiotic combinations. Histologic regression (score 2 to 3) was seen in 11 of 12 (92%) patients and was evident in the first posttreatment biopsy performed 2 to 4 months after eradication in 8 cases. The biopsy appearances in regressed cases were characterized by an “empty” lamina propria composed of loose connective tissue with scattered plasma cells but devoid of organized collections of lymphoid cells, with loss of gastric glands that had previously been destroyed by the neoplastic infiltration (Fig 1A and B). In 3 cases, histologic regression preceded complete eradication of H pylori organisms. All 11 cases remained disease and infection free 25 to 43 months after eradication.

Molecular regression after eradication of H pylori and determined by the disappearance of the clonal amplification band seen at diagnosis was observed in 6 of the 9 (67%) cases originally monoclonal by PCR. In 1 case, synchronous histologic and molecular regression was seen, but, in the remaining 3 cases, molecular regression lagged behind histologic remission by 10, 12, 16, and 28 months, respectively. Two cases have remaining molecular evidence of a clonal population 17 and 24 months after histologic regression.

In the single case (case no. 13) in which H pylori eradication has not been achieved, the histologic appearances have fluctuated after different antibiotic therapies (score 5-2-3-4-3), with no significant histologically detectable difference in the colonization of the gastric epithelium by the organism. In this case, PCR has always failed to show a clonal population.


discussion

The clinical presentation of the cases of low-grade MALT lymphoma in this study reinforces the nonspecific nature of the digestive symptoms in patients subsequently found to have lymphoma. In addition, the endoscopic findings suggest that an appearance of gastritis or a normal gastric mucosa can harbor low-grade MALT lymphoma. Biopsy of all patients undergoing gastroscopy for upper gastrointestinal symptoms is advised to exclude a possible underlying malignancy.

Table 2. Outcome After Anti-H Pylori Therapy of 13 Cases of Low-Grade MALT Lymphoma: Clinico-Pathologic and Molecular Data

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex</th>
<th>Onset Symptoms</th>
<th>Gastric Endoscopy</th>
<th>Hist Score</th>
<th>PCR</th>
<th>Mo of Follow-Up</th>
<th>Mo Since Erad</th>
<th>Final Hist</th>
<th>Final PCR</th>
<th>Mo Erad/Hist Regr</th>
<th>Mo Erad/Mol Regr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68/F</td>
<td>Dyspepsia</td>
<td>Multiple ulcers</td>
<td>5</td>
<td>M</td>
<td>50</td>
<td>19</td>
<td>P</td>
<td>3</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>68/F</td>
<td>Epig. pain</td>
<td>Gastritis</td>
<td>5</td>
<td>M</td>
<td>30</td>
<td>28</td>
<td>1</td>
<td>P</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>34/F</td>
<td>Epig. pain</td>
<td>Gastritis</td>
<td>5</td>
<td>M</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>P</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>36/M</td>
<td>Epig. pain</td>
<td>Erosions</td>
<td>4</td>
<td>M</td>
<td>45</td>
<td>36</td>
<td>1</td>
<td>P</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>41/M</td>
<td>Dyspepsia</td>
<td>Erosions</td>
<td>5</td>
<td>M</td>
<td>27</td>
<td>14</td>
<td>2</td>
<td>P</td>
<td>13</td>
<td>8</td>
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<tr>
<td>6</td>
<td>65/F</td>
<td>Erathemph.</td>
<td>Single ulcer</td>
<td>5</td>
<td>M</td>
<td>30</td>
<td>21</td>
<td>3</td>
<td>M</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>70/M</td>
<td>Dyspepsia</td>
<td>Multiple scars</td>
<td>4</td>
<td>M</td>
<td>23</td>
<td>18</td>
<td>1</td>
<td>P</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>67/F</td>
<td>Dyspepsia</td>
<td>Gastritis</td>
<td>5</td>
<td>M</td>
<td>55</td>
<td>16</td>
<td>5</td>
<td>M</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>76/F</td>
<td>Epig. pain</td>
<td>Single scar</td>
<td>4</td>
<td>M</td>
<td>79</td>
<td>27</td>
<td>2</td>
<td>3</td>
<td>M</td>
<td>52</td>
</tr>
<tr>
<td>10</td>
<td>59/M</td>
<td>Dyspepsia</td>
<td>Gastritis</td>
<td>5</td>
<td>P</td>
<td>28</td>
<td>28</td>
<td>1</td>
<td>P</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>54/M</td>
<td>Dyspepsia</td>
<td>Single ulcer</td>
<td>4</td>
<td>P</td>
<td>29</td>
<td>25</td>
<td>2</td>
<td>P</td>
<td>4</td>
<td>-3</td>
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<tr>
<td>12</td>
<td>45/M</td>
<td>Dyspepsia</td>
<td>Gastritis</td>
<td>5</td>
<td>P</td>
<td>24</td>
<td>24</td>
<td>2</td>
<td>P</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>36/M</td>
<td>Dyspepsia</td>
<td>Normal</td>
<td>5</td>
<td>P</td>
<td>28</td>
<td>Not Erad</td>
<td>3</td>
<td>P</td>
<td>Not Erad</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Hist, histologic score; Erad, eradication of H pylori; Diagn, diagnosis; Mol, molecular; M, monoclonal; P, policlonal.

* Remission preceding a complete eradication of H pylori.

Indeterminate/discordant cases. The clinical, histologic, and molecular data are summarized in Table 3. In 1 case, the original biopsies showed severe crush artefact. Although crushing of cells and nuclear smearing has been suggested to be a feature suggestive of a neoplastic infiltrate, the histologic features of the preserved areas in this biopsy were not thought to be sufficient for a diagnosis of lymphoma. Endoscopy showed a single healed scar but no obvious tumor mass. PCR analysis of this biopsy showed monoclonality. A second biopsy 2 months after the completion of anti-H pylori therapy showed no histologic evidence of lymphoma, and PCR of this biopsy showed disappearance of the original clone. This case remains disease and infection free 25 months after eradication.

One case showed histologic features of a reactive lymphoid infiltrate (score 2) but a PCR-detectable monoclonal population. Endoscopy of this case showed gastritis but no ulcer or mass. Follow-up biopsies showed continued absence of histologic features of lymphoma and molecular regression 23 months after eradication.

A third case initially showed histologic features of chronic follicular gastritis (score 2), with no evidence of a clonal population by PCR. A repeat biopsy 29 months later showed a more intense lymphoid infiltrate (score 3) but no overt histologic evidence of lymphoma; H pylori infection persisted. PCR of this material produced a clonal pattern.
Fig 1. (A and B) Empty lamina propria. Characteristic histologic aspect of gastric low-grade MALT lymphoma regression after the eradication of H pylori: loose connective tissue with scattered plasmacells with loss of gastric glands. (H&E; original magnification x 100 and x 200, respectively.)

nancy. The finding of early, stage IE disease in all the patients in this study contrasts with a recent report of frequent extragastric extension of low-grade MALT lymphoma. Both this and the minimal gastroscopic abnormalities in these cases may be explained by a high endoscopy rate in this institution.

The histologic distinction between a florid reactive lymphoid infiltrate and low-grade lymphoma of MALT type can be difficult. Several investigators have identified features that are helpful in the diagnosis of gastric MALT lymphoma, including the presence of lymphoepithelial lesions, Dutcher bodies, and marked to moderate cytologic atypia. Extension of centrocyte-like cells into the lamina propria, mild cytologic atypia, and crushing/smearing of lymphocytes are features considered to suggest a lymphoma diagnosis. Many biopsy specimens do not show all these features, which results in histologic suspicion without diagnostic certainty. Immunocytochemical staining of the small routinely fixed and paraffin-embedded gastric biopsies can be problematic, and assessment of stains for Ig light chain restriction in this material can be difficult to interpret. PCR from routinely processed paraffin-embedded material provides a powerful tool for the identification of neoplastic lymphoid populations by the examination of the Ig heavy chain and T-cell receptor genes. Application of PCR to small biopsies could potentially be used to distinguish between benign and neoplastic infiltrates. Our finding of PCR-detectable clone in 69% of cases with histologic features suggestive or diagnostic of low-grade MALT lymphoma is in accordance with previously published studies comparing Southern blot and PCR-detectable monoclonality and the detection of clonal populations in frozen and paraffin-embedded material. The high false-negative rate (30%) means that histologic examination of gastric endoscopic biopsies remains the method of choice for the diagnosis of low-grade MALT lymphoma. When a
positive result is obtained, PCR provides a useful confirmation of the histologic impression of lymphoma, but can only be interpreted in the context of the histology.

Regression of gastric low-grade MALT lymphoma after the eradication of H pylori has previously been reported. To date, no study has assessed the role of PCR in the diagnosis and follow-up of these patients or documented the timing of histologic and molecular regression of lymphomas responding to antibiotic therapy. In the 4 cases in this study that showed histologic features of lymphoma on retrospective examination of previous biopsies, no histologic change was seen 9 to 52 months before anti-H pylori therapy. This absence of spontaneous remission is further evidence that the lymphoma regression is specifically related to the eradication/suppression of H pylori.

The significance of persistent PCR-detectable monoclonality after histologic regression in some cases is undetermined. The in vitro demonstration of the MALT lymphoma cells’ T-cell–dependent proliferation when exposed to H pylori strains specific for an individual tumor suggests that eradication of the organism removes the T-cell drive to an immunologically competent malignant clonogenic cell. It has also been suggested that MALT lymphoma cells can terminally differentiate into nonproliferative but clonal plasma cells. The lag phase between histologic and molecular regression might therefore be explained by the time interval between the removal of proliferative drive and the terminal differentiation and senescence of the differentiating but nonproliferating lymphoma cells.

In this study, PCR showed monoclonality in the original biopsies in 2 cases lacking histologic features of lymphoma. In 1 case, crush artefact obscured the histology and might have raised a suspicion of lymphoma. A repeat biopsy was suggested, which was performed 2 months after completion of anti-H pylori therapy; at that time, no evidence of lymphoma was found using histology or PCR. Whether or not this represents an inappropriate PCR result will never be known.

A definite false-positive molecular result was found in 1 case. The endoscopic features were reported as showing gastritis, and the histology of the biopsy specimens showed follicular gastritis with a mild lymphoplasmacytic infiltrate in the lamina propria but no evidence of lymphoma. Repeat biopsies after eradication of H pylori showed persistence of an identically sized product, which suggests the presence of a genuine clonal population. The significance of this finding remains undermined. However, this might represent the presence of a small lymphoma population within the mucosa that may have become evident in the future.

In 1 case initially diagnosed as reactive, a PCR-detectable clonal population evolved in the absence of histologic evidence of lymphoma but with the persistence of H pylori infection. In this case, the histologic features have changed with an increased lymphoid infiltrate in the lamina propria of lymphocytes, some of which show Dutcher bodies, a feature suggestive of but not diagnostic of lymphoma (score 2 increased to 3). This might be another false-positive result or might represent the emergence of a clonal population from within an H pylori-associated gastritis. Further biopsies may elucidate the nature of the PCR-detectable monoclonality in this case.

The presence of a PCR-detectable population in the absence of histologic features of lymphoma might therefore present a stage in tumor regression in those cases with H pylori eradication or tumor progression in new cases at a time when the clonal population is sufficiently large to be detected by PCR (approximately 5% of the infiltrate) but not by conventional histology.

In cases in which regression was achieved in this series, no relapse was detected after 14 to 36 months (mean, 24 months) follow-up. The indolent nature of low-grade MALT lymphoma, the possibility of reinfection and recrudescence of lymphoma, and the possibility of transformation and T-cell–independent proliferation of any residual quiescent neoplastic cells suggests that continuous regular follow-up is required. Large trials with prolonged follow-up are required to assess the long-term survival and efficacy of antibiotic therapy for low-grade gastric MALT lymphoma.

REFERENCES

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A Savio, G Franzin, AC Wotherspoon, G Zamboni, R Negrini, F Buffoli, TC Diss, L Pan and PG Isaacson