Treatment of low-grade gastric MALT lymphoma using *Helicobacter pylori* eradication

Lečenje MALT limfoma želuca niskog stepena maligniteta eradikacijom *Helicobacter pylori* infekcije

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**Abstract**

**Background/Aim.** Lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) of the stomach usually occurs as a consequence of *Helicobacter pylori* (*H. pylori*) infection. The aim of this study was to investigate the long-term effect of treatment of low-grade gastric MALT lymphoma with the *H. pylori* eradication method. **Methods.** In the period 2002–2012 in 20 patients with dyspepsia, mean age 55.1 years, the endoscopic and histologic diagnosis of gastric MALT lymphoma in the early stages were made. Histological preparations of endoscopic biopsy specimens were stained with hematoxylin-eosin (HE), histochemical and immunohistochemical methods. **Results.** Endoscopic findings of gastritis were documented in 25% of the patients, and 75% of the patients had hypertrophic folds, severe mucosal hyperemia, fragility, nodularity, exulcerations, and rigidity. Histopathologically, pathognomonic diagnostic criterion were infiltration and destruction of glandular epithelium with neoplastic lymphoid cells, the so-called lymphoepithelial lesions. In all 20 patients *H. pylori* was verified by rapid urease test and Giemsa stain. After the triple eradication therapy complete remission of MALT lymphoma was achieved in 85% of the patients, with no recurrence of lymphoma and *H. pylori* infection in the average follow-up period of 48 months. In 3 (15%) of the patients, there was no remission of MALT lymphoma 12 months after the eradication therapy. Of these 3 patients 2 had progression of MALT lymphoma to diffuse large-cell lymphoma. **Conclusion.** Durable complete remission of low-grade gastric MALT lymphoma is achieved in a high percentage after eradication of *H. pylori* infection, thus preventing the formation of diffuse large-cell lymphoma and gastric adenocarcinoma.

**Key words:** lymphoma, b-cell, marginal zone; stomach neoplasms; helicobacter pylori; drug therapy; remission induction; prognosis; histology.

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**Apstrakt**

**Introduction**

Primary lymphomas of stomach are rare tumors, which make less than 5% of primary stomach neoplasms. However, they are the most common extranodal lymphomas, representing 4–20% of all extranodal lymphomas.\(^1\)\(^2\). Lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is an extranodal lymphoma of the marginal zone, which contains morphologically heterogenic small B-cells with marginal zone (cells similar to centrocytes), cells that are similar to monocytes, small lymphocytes and singular immunoblasts, as well as cells similar to centroblasts\(^3\)\(^4\). Infiltration of epithelium with neoplastic cells and forming of lymphoepithelial lesion is typical in epithelial tissues. Lymphoepithelial lesion appears as an indicator of MALT lymphoma at biopsy samples, which is the result of atypical lymphocytes invasion and reactive lymphoid follicles of tissue\(^5\).

Most of MALT lymphoma are low-grade malignancy, small number are initially manifested as mid-grade malignancy non Hodgkin’s lymphoma (NHL), which can develop as the evolution of low-grade malignancy lymphoma. Under normal circumstances, there is no organized lymphoid tissue in musoca of stomach, except poorly present lymphocytes. Besides that, most of MALT lymphomas develop in the stomach. This paradox can be explained by the fact that MALT and its product MALT lymphomas develop in the stomach, because in 70% of cases MALT lymphoma develops as the result of *H. pylori* infection. Several cytogenetic alterations are identified, most commonly trisomy 3 or t (11;18). Mutations are usually identified in NHL and are mostly not present in MALT lymphoma, although there are reports on the presence of BCL2 and TP53. However, specific genetic abnormalities which would be responsible for pathogenesis of MALT lymphoma are still not identified\(^6\)\(^7\).

In 1991, Wooterspoon et al.\(^8\) announced for the first time that the patients with gastritic MALT lymphoma were ordinarily infected with *H. pylori*. After histomorphological examinations, recent epidemiological, molecular biological and experimental examinations showed the key role of *H. pylori* in the development and progression of gastric MALT lymphoma. These examinations led to the revolutionary shift in treatment of these patients. The tumor was cured with antibiotic therapy for the first time in the history of medical oncology\(^9\).

The aim of this prospective non-randomized study was to investigate the long-term effect of low-grade gastric MALT lymphoma treatment applying *H. pylori* eradication.

**Methods**

In a period 2002–2012 in 20 patients (11 or 55% male and 9 or 45% female), average age 55.1 + 10.76 (32–73) years MALT lymphoma of the stomach was diagnosed in early stage, with localization in mucosa and/or submucosa.

Upper gastrointestinal endoscopy was done using video gastrosopes Olympus GIF-Q165 to all of the patients with symptoms of dyspepsia. At least 10 biopsies were taken from mucosa of the stomach for the histological examination, 3 of which from the antrum (the front, the back and incisura angularis), 3 from the corpus (the front wall of the corpus, the back wall of the corpus and fundus) and at least 4 biopsies from suspicious lesion. Two additional biopsies were taken (one from the antrum, 20 mm from the pylorus towards big curve, and from the fundus) for *H. pylori* quick urease test.

Endoscopic biotic material was fixated in 10% formaldehyde solution for 24 h. Then, laboratory testing of tissue in autotechnicon, with molding in paraffin and cutting of paraffin molds that were 3 μm thick was performed. Deparaffinized, enlightened through xylesen and rehydrated histological preparations were then colored using the following methods: classical hematoxylin-eozin (HE) method, histochemical methods like acian blue-periodic acid Schiff (AB-PAS, pH 2.5), van Gieson and modified Giemsa, immunohistochemical method Avidine-Biotine-Complex (ABC) by using antibodies on pancytoceratine, as a common marker for all tumors of epithelial origin, antibodies on CD20, as a marker for B-cell origin of lymphoma, antibodies on CD3, as the marker of T-cell origin and Ki-67, as the marker for mitotic activity of tumor cells.

Routine screening was done in all the patients in order to evaluate the eventual propagations of lymphoma. Routine screening included biohumoral analysis, ultrasound examination of upper abdomen, and radiografic examination of lungs and heart. Computed tomography (CT) and endoscopic ultrasound were also done in several patients.

**Results**

In 12 of the 20 (60%) patients diagnosed with MALT lymphoma of the stomach, the symptoms of dyspepsia were present, in the form of epigastric pain, nausea and intumescence of the abdomen, while 8 (40%) patients had alarming symptoms (poor apetite, weight loss, hematemesis and/or melena).

Endoscopic finding of gastritis, often present with nodular aspect, had 5 (25%) out of 25 patients, and the other 15 (75%) patients had hypertrophy of crease, a strong hyperemia of mucosa, fragility, nodularity, ulcerations and rigidity (Figure 1).

![Fig. 1 – Endoscopic appearance of mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach antrum: edema, hyperemia, mucosal fragility and nodularity.](image)

Most of the patients had the described changes that were located in the lower part of the corpus and the antrum of the stomach in 7 of 15 (46.7%) patients, only in the antrum in 4 (26.7%) patients, or only in the corpus of the stomach in 4 (26.7%) patients.

From pathohistological perspective, in the early phase of MALT lymphoma, lymphoid follicles look like normal lymphoid follicles, which are hyperplastic with reactive germinative centers and with multiplied small B-lymphocytes of marginal zone, often densely distributed in lamina propria of mucosa. Because of the similarity with centrocytes, monocytes and plasmocytes they are classified in centrocytoid, monocytoid and plasmacitoid types (Figure 2).

Atypical lymphoid cells type, centroblast and immunoblast, were rare and mixed with the dominant cells of marginal zone. Malignant cells did infiltration, destruction, and replacement of glandular parenhyma. Therefore, pathognomonic diagnostic criteria were infiltration and destruction of glandular epithelium by the nearby neoplastic lymphoid cells, apropos “lymphoepithelial lesion”, verified by immunohistochemical epithelial marker pancytokeratin (Figure 3). Antibodies on CD20, as the mutual marker for B-cell origin of lymphoma were positive (Figure 4). Antibodies on CD3 were negative, which excluded T-cell origin of MALT lymphoma. Mitotic activity verified by marker Ki-67 was negligible in MALT lymphoma, while in case of its progression to lymphoma of higher grade of malignity a significant increase of this marker was detected.

In all the 20 (100%) patients _H. pylori_ was verified by bioptic urease test and the histologically modified Giemsa method. The triple eradication therapy was administered for 10 days, with protonic pump inhibitor in double dose, with clarithromycin 2 × 500 mg and with amoxicilin 2 × 1000 mg or metronidazole 3 × 500 mg. Two months after eradication therapy, control examinations of upper endoscopy and histological examination of endoscopic biopsies were done. Then, endoscopic-histological control examinations were done once in every 6 months. In case of histological verification of full remission of MALT lymphoma in the two consecutive results done once in every 6 months, the examination was done once a year. The average period of monitoring all of the 20 patients was 48 ± 19.8 months (from 2 to 98 months).

Eradication of _H. pylori_ infection was considered succes-
eradication therapy, in 8 out of 17 (47%) 6 months after, and in 4 out of 17 (23.5) 12 months after the therapy (Table 1).

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<thead>
<tr>
<th>Period after eradication therapy (months)</th>
<th>Number of patients</th>
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<th>%</th>
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<tr>
<td>2</td>
<td>5</td>
<td>29,4</td>
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<tr>
<td>6</td>
<td>8</td>
<td>47</td>
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<td>12</td>
<td>4</td>
<td>23,5</td>
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<td>Total</td>
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**MALT – mucosa associated lymphoid tissue**

In the average period of follow-up (48 ± 19.8 months) there were no relapses of MALT lymphoma, non relapses of *H. pylori* infection in the patients with complete remission that was accomplished. In 3 (15%) of the patients with localisation of MALT lymphoma in mucosa and submucosa there was not remission during the 12 month follow-up. Chemotherapy was administered due to worsening of symptoms (poor appetite, weight loss), also due to persistence of endoscopic and histological signs of MALT lymphoma. In 2 of these 3 patients there was the progression of MALT lymphoma in diffuse large-cell lymphomas, after 2 and 5 years of the MALT lymphoma diagnose. Both patients died during the repeated chemotherapy.

In the patients with full remission of MALT lymphoma accomplished, there was a regression of endoscopic changes. Histologically, in the patients with complete remission of lymphoma *lamina propria* looked “empty”, with the loss of glands. Rare lymphocites and monocites were spilled in the fur, and there were some focal accumulations of small lymphocites. There were no lymphoepithelial lesions. Epithelium was normal with the adequate secretion of mucine. Sometimes the empty spaces were filled with the typical foveolar hyperplasia (Figure 5).

![Figure 5 - Effect of *H. pylori* eradication therapy: a rare lymphocytic infiltrate with typical foveolar hyperplasia of the covering epithelium (HE, x 250).](image)

**Discussion**

MALT lymphoma is slightly more common in females, the average age of 65 years, and with the highest prevalence in seventh and eight life decade, although it can occur in children, adolescents, and younger people. In our patients, MALT lymphoma of the stomach was similarly present in both males and females (males 55% and females 45%), and with the average age of 55 years.

In most of cases with MALT lymphoma it is diagnosed during upper gastrointestinal endoscopy, which is done due to usual dyspeptic symptoms. Alarming symptoms, as vomiting, weight loss, hematemesis and melena are rarely present. In our study alarming symptoms were often present in 40% of patients.

Histological diagnosis of MALT lymphoma is often unexpected for an endoscopist because of the fact that only endoscopic signs of gastritis are found in more than 50% of cases, while in 41% of the cases there are singular or multiple active or rehabilitated ulcerations, while in 5% of cases there is erosion. Irregular and serpentine hypertrophic folds, which are described as a typical endoscopic finding are present in 3% of patients and 1% of tumor mass. All together, endoscopic finding which would be characteristic for lymphomas is present in only the third of the number of patients. In our study, a fewer patients (25%) with MALT lymphoma had only endoscopic signs of gastritis, which could be explained by the fact that within the previous years we did not routinely take biopsies of all patients with gastritis. A small number of biopitic samples makes the initial diagnosis of lymphoma more difficult. Since gastric MALT lymphoma can be multifocal, and diffuse large-cell lymphoma can exist at the same time, it is recommended to take more biopsy samples. There is no standardized protocol of taking biopsies, but multiple biopsies of every endoscopic lesion are recommended, as well as biopsies of macroscopically unchanged mucosa of all the main gastric regions (antrum, angulus, corpus and fundus), from the front and the back wall, and from the small and large curve. The identical protocol of taking biopsies should be applied on control endoscopic examinations, for the sake of adequate histologic comparation, which guided us in our study.

Since there is no specific marker for immunohistochemical typisation of MALT lymphoma, histological HE method is “the gold standard” of lymphoma diagnosis. The pathohistological diagnosis is often difficult in the early phase of MALT lymphoma. The most common diagnostical dilemma is the differentiation between chronic atrophic gastritis and MALT lymphoma. That is why it is recommended to take multiple biopsies and to discover lymphoepithelial lesions with lymphocites that have polymorphic and atypical nucleus and increased mitotic activity. The main diagnostic criterion that favors the diagnosis of MALT lymphoma is quantitative. The lymphoid infiltrate has to be thick and occupies most of the *lamina propria*, leading to the destruction and replacement of glandular structure, which is characteristic lymphoepithelial lesion. Among those present lymphoid follicles interfollicular space should be completely filled with lymphocytes. The main cellular components of MALT lymphoma are cells similar to centrocytes, monocitoid cells, small circular lymphocytes, plasma cells, and scattered, individual, large centroblasts or immunoblasts similar cells. In gastritis dominate plasma cells and inflammatory activity manifested by granulocyte-leukocyte infiltration.
Histological parameters are usually sufficient for the diagnosis of gastric MALT lymphomas and rare lymphomas of the stomach, other similar histological structure, and follicular and "mantle cell" type. Nevertheless, the analysis of the panel of immunohistochemical markers types CD20, CD79a, CD3, CD5, CD10 and Cyclic D1 confirm the diagnosis of gastric MALT lymphoma. The neoplastic cells of MALT lymphoma showed expression of B-cell markers CD20 and CD79a. Dense CD20+ B cell infiltrate that invades and replaces the glandular structure is a common finding in MALT lymphoma. Markers CD5, CD10, BC6, and cyclin D1 were always negative in MALT lymphoma and allow exclusion of other small B cell lymphomas. There are also a number of "scattered" CD3+ non-neoplastic T-cells. Staining of pancytokeratin provides better visibility lymphoepithelial lesions of MALT lymphoma. In our patients histological parameters were crucial for the diagnosis of gastric MALT lymphoma, also. For diagnostic confirmation of MALT lymphoma, we used immunohistochemical markers, such as antibodies to pancytokeratin, a common marker for the tumors of epithelial origin and antibody to CD20, a marker for B-cell lymphoma origin, who were positive and antibodies to CD3, which was negative, excluding the T-cell origin of lymphoma.

The exact prevalence of H. pylori infection in MALT lymphoma is unknown and varies depending on the study from 50% to almost 100%. This variability could be explained by the number of tests used for detection of H. pylori and their kind. If you use only one test more likely are false negative results. As for the types of tests, the prevalence of H. pylori infection is higher when using serological and urea based test compared to biopsy. This is explained by extensive mucosal lesions in MALT lymphomas that may lead to the reduction in H. pylori colonization, to undetectable levels. Also, the prevalence of H. pylori infection depends on the depth of invasion of lymphoma, so the lymphoma limited to the mucosa and submucosa prevalence of infection is higher than in lymphomas with a deeper propagation. These results support the hypothesis that H. pylori is present in the early stage of MALT lymphoma, but later, with the progression of lymphoma may result in the loss of H. pylori. In all of our patients with MALT lymphoma H. pylori infection was proven. Such a high percentage of H. pylori infection in our patients could be explained by the fact of a large number of biopsy samples, and the two tests used for the detection of H. pylori (biopsy urease test and Giemsa stain) and that MALT lymphomas were at an early stage, limited to the mucosa and/or submucosa.

According to Maastricht IV Consensus, eradication of H. pylori is the first line therapy of MALT lymphoma of low-grade malignancy. In 60–100% cases of MALT lymphoma remission is achieved with eradication of H. pylori infection. Detection of genetic alterations, such as translocations t (11, 18) (q21; q22) lead to poor therapeutic response to antibiotics and recommended for consideration other therapeutic modalities for MALT lymphoma, such as radio- or chemotherapy. Molecular biology techniques, such as polymerase chain reaction (PCR) methods contribute to the diagnosis of B cell clonality detection of MALT lymphoma, although a negative PCR result does not rule out MALT lymphoma.

Similar to data in the literature, in 85% of our patients there was a complete remission of MALT lymphoma after eradication of H. pylori infection. Complete remission of lymphoma involves the absence of endoscopic and histological signs of lymphoma in two consecutive follow-ups. Histologically, neoplastic lymphoid infiltrate and lymphoepithelial lesions are completely withdrawn. Residual scattered lymphocytes and plasma cells are present in the lamina propria, and regressive changes such as stromal fibrosis and empty lamina propria devoided of glands.

In our study, complete remission of MALT lymphomas was usually achieved 6 months after the eradication therapy (47%), after 2 months (29.4%) and after 12 months (23.5%). In a prospective study, Hong et al. achieved complete remission in 78% of patients after 6 months, while in 93% it was achieved after 12 months of eradication therapy. In a systematic review by Zullo et al. complete remission of lymphoma occurs in approximately 5 months, but in a small number of patients after a much longer period (3–4 years). Therefore, remission of lymphoma is a continuous process that can be fast (2–3 months), but in many patients longer (4–12 months or over 24 months).

According to data in the literature, the majority of patients with MALT lymphoma after successful eradication of H. pylori take years to maintain stable remission. In some studies with long-term monitoring the average of 5% or 10% of relapses of lymphoma were shown. A higher percentage of relapse is shown in MALT lymphomas with previously undiagnosed foci of high grade malignancy. Overall, relapse of MALT lymphoma is mainly related to the recolonisation of H. pylori. In the absence of these reasons, the transient relapse of MALT lymphoma is described, which is self-limiting with repeated spontaneous remission. In our study, there was no recurrence of MALT lymphoma during the average follow-up of 48 months, which may be explained by the absence of reinfection, as well as the lack of focus of lymphoma with a high degree malignancy. Currently, it indefinite endoscopic-histologic follow-up of patients per year who are in stable remission is recommended, in order to detect possible reinfection and relapse of lymphoma. In patients with histological remission achieved, the presence of persistent monoclonal B-cell population, detected by PCR, is the risk factor for relapse of lymphoma. Therefore, these patients are recommended for more intensive monitoring, and in the case of at least three negative findings by PCR analysis monitoring may be in longer intervals.

Remission was not achieved in 25% of our patients, possible because there was a translocation t (11, 18), which we could not, for technical reasons, found out. In cases with no complete remission of early stage MALT lymphoma after eradication of H. pylori infection achieved strategy of “watch-and-wait” for a period of 24 months prior to the consideration of alternative treatments are generally considered adequate. The interval could be shorter than 24 months in case of perigastric lymph node development and widespread disease. However, a large number of protocols recommend that after 12 months of successful eradication therapy it can be considered that there is no response.

to antibiotic therapy, so another form of treatment should be considered. In our study, the patients with no remission of MALT lymphoma after H. pylori eradication after 12 months were sent to chemotherapy due to the worsening of clinical symptoms and persistence of endoscopic and histological signs of MALT lymphoma. Besides the possibility of the absence of MALT lymphoma remission, recurrence of lymphoma and MALT lymphoma transformation to diffuse large cell lymphoma, there is a 6 times higher risk of developing adenocarcinoma of the stomach, compared to the general population.

The shortcomings of our study are a relatively small series of patients, and no molecular techniques such as PCR used for the detection of B-cell clonality and genetic abnormalities.

### References


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