Long-Term Outcomes of Gastric Mucosa-Associated Lymphoid Tissue Lymphomas after *Helicobacter pylori* Eradication Therapy

SHIHO TERAI, KATSUNORI IIJIMA, KATSUAKI KATO, NAOHIRO DAIRAKU, TATSUHIKO SUZUKI, MASAYOSHI YOSHIDA, TOMOYUKI KOIKE, YASUSHI KITAGAWA, AKIRA IMATANI, HITOSHI SEKINE, SHUICHI OHARA and TOORU SHIMOSEGAWA

Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan

Mucosa-associated lymphoid tissue (MALT) lymphomas are localized primarily in the gastrointestinal tract and are characterized by an indolent nature and favorable outcome with specific therapy. Gastric MALT lymphomas are closely linked to *Helicobacter pylori* (*H. pylori*) infection, for which eradication therapy is recognized as an effective primary treatment for the disease. However, there is little information about long-term outcomes after the therapy. In the present study, we elucidated the long-term outcomes of 74 patients (*70 H. pylori*-positive and 4 negative cases) followed up by endoscopy at least 12 months after exclusive eradication therapy alone. The median follow-up period was 46 months. When the remission status was estimated at the time point of 12 months post-eradication, the numbers of patients with complete remission (CR), histologically residual disease with macroscopic normalization (hRD), partial remission with more than 50% tumor reduction (PR) or no response (NR) were 56, 12, 2 and 4, respectively. During follow-ups of over 12 months post-eradication, 11 of the 12 hRD cases were belatedly induced to CR but one CR case histologically relapsed into hRD. One of the 2 PR cases eventually turned into hRD 20 months later. Therefore, 66 CR, 3 hRD, 1 PR, and 4 NR cases (including 3 *H. pylori*-negative) were identified at the last follow-up of the present study. All 74 patients were followed up without any second-line therapies, but none exhibited disease progression. Thus, the long-term outcome of localized gastric MALT lymphoma after *H. pylori* eradication therapy was favorable. A watch and wait strategy may be a reasonable approach for hRD since the majority might be in the process of turning into delayed CR.


© 2008 Tohoku University Medical Press

*Helicobacter pylori* (*H. pylori*) colonizes the gastric epithelium and the chronic gastric inflammation thereby induced is linked to various disease outcomes. First, *H. pylori* is a well-established cause of peptic ulcer diseases and eradication therapy remarkably prevents their recurrence. Additionally, *H. pylori* infection has been recognized as one of the most important

*H. pylori* infection is also associated with gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The concept of MALT lymphoma was established by Isaacson and Wright (1983). Since the first report of the regression of gastric MALT lymphoma by *H. pylori* eradication therapy (Wotherspoon et al. 1993), many reports have provided evidence confirming its effectiveness (Bayerdorfer et al. 1995; Roggero et al. 1995; Neubauer et al. 1997; Nakamura et al. 2001; Fischbach et al. 2004). Thus, *H. pylori* eradication therapy today has become recognized as a first-line treatment for gastric MALT lymphoma. However, there are few data on the long-term outcome of gastric MALT lymphoma after eradication therapy in a large prospective series.

There is also a controversy concerning the appropriate timing for defining a failure of eradication therapy. Regarding the time course from successful *H. pylori* eradication to the subsequent regression of gastric MALT lymphoma, some previous studies reported that the majority of the patients achieved complete remission within the first 3 months following treatment (Lee et al. 2004; Nakamura et al. 2005; Wundisch et al. 2005). Thus, once *H. pylori* infection is successfully eradicated, the gastric MALT lymphoma may disappear in response to the therapy in a relatively short term. Therefore, the determination of a failed response to *H. pylori* eradication therapy has so far been made at 12 months after the therapy, and the second-line therapies such as radiation or chemotherapy have been applied to patients who did not achieve complete remission at that time (Ruskone-Fourmestraux et al. 2001; Lee et al. 2004; de Mascarel et al. 2005). For example, Wundisch et al. (2005) reported a delayed remission of gastric MALT lymphoma of between 12 and 28 months after the bacteria eradication in 12 of 96 patients who eventually achieved complete remission. Hence, a watch and wait strategy has been considered suitable for such patients without administering a second-line therapy (Fischbach et al. 2002, 2004).

In this study, we present the long-term outcomes of gastric MALT lymphoma after *H. pylori* eradication therapy in a large prospective series of patients, with special attention to how the states of remission evaluated at 12 months after the eradication changed subsequently during additional long-term follow-ups. The present study might provide useful information for managing patients with residual histologic lymphoma in practical clinical settings.

**Patients and Methods**

**Patients**

From April 1995 to August 2006, 85 consecutive patients were newly diagnosed with stage IE or IIE-1 gastric MALT lymphoma according to Musshoff’s modification (Musshoff 1997) of the Ann Arbor staging system, in which the lymphoma cells were localized in the stomach or infiltrated into contiguous lymph nodes without distant metastasis, and were cared for at Tohoku University Hospital. Of these 85 patients, 7 cases followed-up for less than 12 months and 4 cases treated with radiation as second-line therapy were excluded from the analysis (Four patients received radiotherapy [30Gy] at 4, 5, 11, or 21 months after *H. pylori* eradication therapy, respectively, because they were judged as having no response to the first-line eradication therapy.). Consequently, 74 patients followed-up for more than 12 months after *H. pylori* eradication therapy alone were enrolled in the present analysis. The diagnosis of gastric MALT lymphoma was based on the World Health Organization classification and the scoring system of Wotherspoon et al. (1993) by histological examinations with a plurality of endoscopic biopsy samples obtained at every endoscopic examination. Tissue specimens were immunohistochemically stained for CD3 and CD20/CD79a to confirm the monoclonal expansion of B cells. Patients with involvements in large cell components revealing diffuse, solid or sheet-like proliferations, were excluded from the category of gastric MALT lymphoma.

The initial stages of lymphomas were determined with physical examination, blood cell count and serum chemistry analysis, computed tomography (CT) scan, endoscopic ultrasonography, gallium scintigraphy, bone marrow examination, and colonoscopy. The status of *H. pylori* infection was diagnosed by a rapid urease test, histologic examination, serum anti *H. pylori* antibody, and $^{13}$C urea breath test. *H. pylori* infection was defined
as positive when one or more of the diagnostic methods applied were positive, and defined as negative if all the results were negative. The chimeric transcript of the API2-MALT1 gene, which is known to be unnaturally generated as a consequence of the chromosomal translocation (11;18) (q21;q21), was investigated in 26 patients by a specific reverse transcription polymerase chain reaction (RT-PCR) (Liu et al. 2002). The investigation with RT-PCR was conducted prospectively in consecutive patients with gastric MALT lymphoma irrespective of the H. pylori status from 2002 to 2004, and thereafter mainly in H. pylori-negative patients. The study was conducted in accordance with the Helsinki Declaration as revised in Tokyo, and informed consent was obtained from each subject.

Treatment and follow-up

All patients enrolled in the present study received H. pylori eradication therapy with a combination of a proton pump inhibitor and antibiotics (1,500 mg/day amoxicillin plus 800 mg/day clarithromycin or 1,000 mg/day metronidazole) for 7 days as the first eradication therapy, even if infection was undetected. If the first eradication therapy failed, a second treatment with alternate antibiotics was administered. In turn, successful eradication was achieved in all H. pylori-positive patients.

Based on a combination of macroscopic and histopathological findings, the effectiveness of H. pylori eradication therapy on gastric MALT lymphoma was classified into five groups according to Wundisch et al. (2005): complete remission (CR); histologic residual disease (hRD); partial remission (PR); no response (NR); and progressive disease (PD). The state of CR was defined as both macroscopic and histopathologic disappearance of lymphoma in two consecutive investigations. hRD was defined as the state in which residues of lymphoma cells revealing a monotonous infiltrate of centrocyte-like cells and/or lymphoepithelial lesions (Wotherspoon’s score of 4-5) were detected in post-treatment biopsy specimens despite normalization of the macroscopic findings. The findings of hRD are equal to those of minimal residual disease (MRD) described by Fischbach et al. (2002, 2004) or responding residual disease (rRD) by Copie-Bergman et al. (2003). In assessing as CR or hRD, a discolored flat mucosal area similar to focal mucosal atrophy was considered to be a normalization of macroscopic findings (Urakami et al. 2000; Ishihara et al. 2002). On the other hand, PR was defined as a tumor reduction of > 50% macroscopically. NR was defined as no changes in macroscopic findings and PD was defined as an increase in the tumor size and an advance in clinical stage after the eradication therapy.

Follow-up endoscopic examinations with multiple biopsies from the lesions were performed at 1 month after the eradication therapy, then every 1-3 months until reaching CR, and thereafter every 6 months after CR. When the patient was judged as NR or PD, a second-line treatment (radiation therapy or chemotherapy) was considered within 6 months after eradication. In the cases of hRD, the patients were carefully followed up without second-line treatment with the patients’ consent as long as the macroscopic findings exhibited no exacerbations. Kaplan-Meier method was used to estimate the duration of CR.

RESULTS

Clinical features of the patients

The clinical features of the 74 patients enrolled in the present study (mean age 63 years old; range 35-80 years old) are shown in Table 1. H. pylori infection was detected in 70 of 74 (95%) patients. As shown by endoscopic observations, gastric MALT lymphomas were predominantly located in the gastric body (42/74, 57%) and were mostly classified into the superficial type (57/74, 77%). The invasion depth of gastric MALT lymphoma in the patients enrolled in the present study was confirmed to be limited to the mucosa and/or submucosal layer of the gastric wall by endoscopic ultrasonography. In addition, from the results of the whole body CT scan, gallium scintigraphy, and bone marrow examination, all patients were classified into stage IE 69/74, 93%) or stage IIE-1 (5/74, 7%). Of 26 patients with the RT-PCR assessment, the API2-MALT1 chimeric transcript was detected only in 3 cases. This chimeric gene was prevalently identified in H. pylori-negative patients compared with H. pylori-positive patients. (3/4 = 75% vs 0/22 = 0%) (Table 1).

Remission induction

The median follow-up period after H. pylori eradication therapy in overall patients was 46 months (range 12-108 months). Of 74 patients treated with H. pylori eradication therapy, 66 cases (89%) achieved CR without any additional treatments by the time of the follow-up examina-
Long-Term Outcome of MALT Lymphoma

The median time span to reach CR after successful *H. pylori* eradication was 6 months. When the efficacy of the eradication therapy was evaluated from the viewpoint of *H. pylori* infection status, 65 of 70 *H. pylori*-positive patients (93%) and 1 of 4 *H. pylori*-negative patients (25%) achieved CR. Gastric MALT lymphomas located in the antrum showed a higher remission rate than those in other locations (CR rates: fundus, 73%; body, 90%; antrum, 100%), and the superficial type also exhibited a high remission rate in comparison to the others (CR rates: superficial type, 96%; other type, 65%). Concerning (a) the invasion depth and (b) the clinical stage, CR rates were as follows: (a) 56/64 (88%) patients with limited submucosal invasion and 2/2 (100%) with the deeper invasion, and (b) 61/69 (88%) of stage IE patients and 5/5 (100%) of stage IIE-1. The rates of CR were also affected by the presence of the API2-MALT1 chimeric gene, such that 21 of 23 patients (91%) without API2-MALT1 chimera achieved CR, but 3 API2-MALT1-positive patients failed in CR induction.

Changes in remission status during long-term follow-up

Changes in the clinical outcomes of the patients enrolled in the study from the time point at 12 months post-eradication to the last follow-up are summarized in Fig. 1. Six patients were lost for further endoscopic follow-up after confirmation of CR after a median period of 39 months (range 17-60 months).

When the remission status of all patients was assessed at the time point of 12 months after successful *H. pylori* eradication, 2 patients were defined as PR, 4 patients were defined as NR, and the remaining 68 patients showed normalization of the macroscopic findings. Of these 68 patients, 56 cases were judged as CR since they also showed histological disappearance of lymphoma cells while the remaining 12 patients were judged as hRD due to histological residues of lymphoma cells. None was evaluated as PD at that time.

During follow-ups of 56 CR cases for a median of 47 months, 3 patients dropped out from the CR status due to histological relapses at 12, 27, and 39 months after reaching CR (Fig. 2). Consequently, the median of sustained CR in these patients was 37 months (range 6-102). In 2 of 3 patients with histological relapse, the lesions spontaneously disappeared by the next examination without any treatment. In the other relapsed patient, the hRD status persisted through subsequent examinations from the relapse point at 39 months to the last observation at 45 months. None of these 3 patients showed any evidences of re-infection of *H. pylori*.

Of 12 patients diagnosed as hRD at the time point of 12 months post-eradication, 11 cases belatedly turned into CR after a median follow-up period of 22 months (range 14-40), and the remaining one sustained hRD until 27 months after eradication. Nevertheless, all these cases showed neither exacerbations of macroscopic

| TABLE 1. Clinical characteristics of enrolled 74 patients with gastric MALT lymphoma. |
|---------------------------------|-----------------|
| Sex                            |                 |
| Male                           | 24              |
| Female                         | 50              |
| **H. pylori status**           |                 |
| Positive                       | 70              |
| Negative                       | 4               |
| Predominant location of lesion |                 |
| Fundus                         | 15              |
| Body                           | 42              |
| Antrum                         | 17              |
| Endoscopic appearance          |                 |
| Superficial type               | 57              |
| Other types                    | 17              |
| Depth invasion by EUS          |                 |
| Mucosa and/or submucosa        | 64              |
| Muscularis and/or beyond       | 2               |
| Not detected                   | 8               |
| Clinical stage                 |                 |
| IE                             | 69              |
| IIE-1                          | 5               |
| API2-MALT1                     |                 |
| Positive                       | 3               |
| Negative                       | 23              |
| Unknown                        | 48              |
findings nor advance in the clinical stage during the observation period.

Of 2 patients judged as PR at 12 months, the one was induced to hRD at 20 months but the other persisted in PR until 15 months post-eradic-
cation. Four patients diagnosed as NR at 12 months have been carefully followed up without any additional treatment under the patients’ con-
sent. None of them have shown any signs of disease progression until the last observation at a median of 73 months (range 49-84). Three of the 4 NR patients were \textit{H. pylori}-negative and API2-
MALT1-positive cases.

Overall, by the last follow-up examination at a median 46 months post-treatment, 8 patients (3 hRD, 1 PD, and 4 NR cases) did not achieve CR despite successful \textit{H. pylori} eradication. No transformation into diffuse large B-cell lymphoma was recognized in any patients enrolled in the present study. At present, no patient has died of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Changes in remission status of gastric MALT lymphoma during long-term follow-up after \textit{H. pylori} eradication therapy. Excluding 11 patients from 85 gastric MALT lymphoma patients diagnosed at Tohoku University Hospital from April 1995 to August 2006 due to short-term follow-ups or having received eradication therapy, the remission status of 74 patients was evaluated at 12 months after successful \textit{H. pylori} eradication and at the last follow-up at a median of 47 months. All patients were followed-up by endoscopic examination after eradication therapy alone, hence additional therapies were not applied for all patients between the two time-points. Numbers in parentheses represent numbers of \textit{H. pylori}-negative patients.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{The survival curve for the duration of complete remission in 56 patients with gastric MALT lymphoma who achieved complete remission after \textit{H. pylori} eradication. Local relapses were observed in 3 patients. Circles represent occurrences of disease relapse. Kaplan-Meier method was used to estimate duration of CR.}
\end{figure}
lymphoma and 1 died of other causes. During the follow-ups, no concomitant gastric carcinoma was found in any patients.

**DISCUSSION**

This large scale, prospective, long-term follow-up study confirmed again the favorable outcome of *H. pylori*-positive gastric MALT lymphoma treated by eradication of the infection. In particular, although histologically residual lymphoma despite normalization of the endoscopic findings was persistent during a certain period of time after the successful eradication in some of the patients, they also showed favorable long-term outcomes.

A recent review reported that the rate of CR of *H. pylori*-positive gastric MALT lymphoma after the eradication therapy varied from approximately 60% to 90% (Stolte et al. 2002). In the present study, the overall CR rate of *H. pylori*-positive gastric MALT lymphoma was as high as 93% at the end of the present study. This value was rather high compared with those of previous reports. One possible reason for this high CR rate may be related to the low prevalence of the API2-MALT1 chimeric transcript in the intended patient group, insofar as all of the 22 *H. pylori*-positive patients investigated by RT-PCR were negative for the API2-MALT1 chimera gene although only a limited number of the patients were investigated. The presence of the chromosomal translocation (11;18) (q21;q21) is known to be a marker for limited responsiveness to *H. pylori* eradication therapy in gastric MALT lymphoma (Liu et al. 2002).

Concerning the influence of the *H. pylori* infection status on the effectiveness of the eradication therapy, only 1 of 4 *H. pylori*-negative gastric MALT lymphoma achieved CR in the present analysis. In addition to these cases, there were 3 other *H. pylori*-negative and API2-MALT1-positive patients who were excluded from the present analysis because they received second-line radiotherapy after a determination of NR. Thus, overall, only 1 of 7 patients with *H. pylori*-negative gastric MALT lymphoma achieved CR in the present study. The patient’s stomach had not shown gastric atrophy endoscopically and histologically before eradication, which is known to occasionally prevent the detection of *H. pylori* infection leading to false *H. pylori*-negatives. Such resistance to the bacterial eradication therapy in *H. pylori*-negative gastric MALT lymphoma is consistent with previous reports (Akamatsu et al. 2006; Nakamura et al. 2006). Conversely, Raderer et al. (2006) have recently demonstrated that *H. pylori* eradication therapy was effective in 4 of 6 (67%) patients with *H. pylori*-negative gastric MALT lymphoma although, in their report, the API2-MALT1 chimeric transcript was detected in only 1 of 6 patients. In contrast, other reports showed a high prevalence of the translocation in patients with *H. pylori*-negative gastric MALT lymphoma (50-100%). (Nakamura et al. 2003; Ye et al. 2003; Nakamura et al. 2006), which is consistent with our current study. Differences in the prevalence of the translocation in the patient groups of these studies may explain the diverse outcomes in *H. pylori*-negative gastric MALT lymphoma. The actual mechanism by which antibiotic therapy is able to exert curative effects on *H. pylori*-negative gastric MALT lymphoma remains unknown, but other bacteria such as *Helicobacter heilmannii* might be associated with the development of gastric MALT lymphoma as was described in a previous report (Morgner et al. 2000). In any case, because *H. pylori* eradication therapy is safe and effective for gastric MALT lymphoma potentially including some sort of *H. pylori*-negative cases as well, antibiotic therapy can be considered worthwhile.

In this study, *H. pylori* eradication therapy was applied to patients with stage IE or stage IIE-1 gastric MALT lymphoma, and the CR rates were not different between the two groups. The result was consistent with that of one previous paper (Nakamura et al. 2001), but appeared to differ from those of other reports (Ruskone-Fourmestraux et al. 2001; Levy et al. 2002) which described that perigastric lymph node involvement might be a negative predictive factor for the responsiveness of gastric MALT lymphoma to *H. pylori* eradication therapy. A possible reason for these discordant results may be the difficulty in
distinguishing between tumor invasion and inflammatory lymph node swelling. Further studies are required to resolve this issue.

Although many previous studies reported the efficacy of *H. pylori* eradication for gastric MALT lymphoma, thus far, only a few studies addressed the issue of long-term persistence (Fischbach et al. 2004; Nakamura et al. 2005; Wundisch et al. 2005). In a large study from Germany, 120 patients with *H. pylori*-positive gastric MALT lymphoma were followed-up after eradication for a median of 75 months (Wundisch et al. 2005). Another study of a large series from Japan was intended to assess 96 gastric MALT lymphoma with or without areas of diffuse large B cell lymphoma for a median of 38 months (Nakamura et al. 2005). Both studies revealed a favorable long-term outcome of gastric MALT lymphoma after *H. pylori* eradication. The present study including 74 patients with gastric MALT lymphoma followed-up for a median of 46 months also confirmed their favorable outcome after *H. pylori* eradication. The application of second-line therapy for such patients is still controversial, i.e., second-line therapy was previously considered to be applicable for patients who failed in the induction of CR by *H. pylori* eradication at 12 months after the successful eradication (Ruskone-Fourmestraux et al. 2001; Lee et al. 2004; de Mascarel et al. 2005). However, recent studies conversely proposed a watch and wait strategy for patients with a minimal residue of gastric MALT lymphoma based on the favorable natural course (Fischbach et al. 2002). In the present study, we also applied a watch and wait strategy for patients with hRD and carefully followed them up without any second-line treatments as long as their macroscopic findings continued to indicate remission. Consequently, we also found a favorable natural course in such patients after *H. pylori* eradication, namely that 11 of 12 hRD cases at 12 months finally achieved CR after a median follow-up of 22 months, while the remaining 1 patient persisted in hRD after 27 months post-eradication. This seems to be consistent with a recent long-term follow-up study describing that patients with hRD showed no progression but, ultimately, achieved delayed CR (Wundisch et al. 2005). Overall, 3 patients were judged as hRD at the last observation, including one persistent hRD case, one histological relapse case after achieving CR, and one changed from PR at 20 months. It seems difficult to estimate whether hRD will be persistent or finally induced to delayed CR, but our results support the validity of the watch and wait strategy for such patients as long as they can be carefully followed-up.

When the effectiveness of the treatment on gastric MALT lymphoma was evaluated at 12 months after successful *H. pylori* eradication, a substantial portion (16%: 12/74) of the patients in the current study showed histologically residual lymphoma cells despite normalization of the macroscopic findings. The application of second-line therapy for such patients is still controversial, i.e., second-line therapy was previously considered to be applicable for patients who failed in the induction of CR by *H. pylori* eradication at 12 months after the successful eradication (Ruskone-Fourmestraux et al. 2001; Lee et al. 2004; de Mascarel et al. 2005). However, recent studies conversely proposed a watch and wait strategy for patients with a minimal residue of gastric MALT lymphoma after eradication represents potentially missed remnant foci of initial lymphoma by sampling error or true relapse (Hong et al. 2006). In this study, the histological relapse spontaneously disappeared without any treatment in two cases. The other 1 patient with persistent histologic relapse was carefully followed-up without any second-line treatments. We, for the present, consider that histological relapse of gastric MALT lymphoma after eradication can be managed with a watch and wait strategy, as long as the macroscopic findings continue to be favorable.

In this study, 4 patients judged as NR were followed up without any second-line therapies and none of them, so far, have shown disease progression during their long-term follow-ups of as long as a median 73 months (range 49-84).
may reflect the indolent nature of the disease. However, many studies reported that a transformation to a high grade sometimes occurred during follow-up after eradication (Morgner et al. 2000; Fischbach et al. 2004; de Mascarel et al. 2005; Wundisch et al. 2005). Accordingly, such patients in NR are still considered to require intensive follow-ups. In order to determine the indication for second-line therapy in NR patients, some biological markers for the prediction of subsequent transformation would be useful in the clinical practice. It has been reported that the presence of the API2-MALT1 chimeric transcript, which is a useful marker for poor responsiveness to eradication therapy, could also represent the nature of the gastric MALT lymphoma (Starostik et al. 2002; Akamatsu et al. 2006). Consistent with these reports, 3 of 4 NR patients (all 3 are H. pylori-negative) in the present study were positive for the API2-MALT1 chimera gene.

In conclusion, this large scale, long-term follow-up study revealed a favorable outcome of gastric MALT lymphoma after the eradication of H. pylori. Histologically residual lymphoma infiltration despite the normalization of endoscopic findings was observed in some patients. A careful follow-up without second-line therapy is a reasonable approach to care for such patients since the majority of them should eventually achieve CR.

References


