

Clinicopathologic Comparison between the API2-MALT1 Chimeric Transcript-positive and -negative Gastric Low-grade B-Cell Lymphoma of Mucosa-associated Lymphoid Tissue Type

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Little is known about the clinicopathological differences between API2-MALT1 chimeric transcript-positive and -negative gastric low-grade B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) type. The aim of this study was to clarify those differences in gastric MALT lymphoma. Twenty-three patients with gastric MALT lymphoma were enrolled in a unicenter study. *Helicobacter pylori* (*H. pylori*) infection status and clinical stages were investigated. Antibacterial treatment was performed for every patient. Responsiveness of MALT lymphoma to this treatment was assessed by means of regular follow-up endoscopy combined with biopsy. All cases were examined for API2-MALT1 chimeric transcript by means of RT-PCR and sequencing analyses. *H. pylori* infection status was assessed as positive in 20 patients and negative in three. With regard to responsiveness to antibacterial treatment, complete remission was observed in two patients, partial remission in 12 and no change in nine. API2-MALT1 chimeric transcript was detected in seven patients, all of whom showed no change in response to antibacterial treatment. API2-MALT1 positivity was found to be significantly correlated with responsiveness to antibacterial treatment ($P=0.0001$), absence of *H. pylori* infection ($P=0.0198$), and gross cobblestone mucosa observed endoscopically ($P=0.0198$). For the other factors (age, sex, dominant site of lesion, high-grade component, infiltrated layer of gastric wall, nodal involvement or clinical stages), there were no differences between API2-MALT1 chimeric transcript-positive and -negative cases. Gastric API2-MALT1 chimeric transcript-positive MALT lymphoma generally features unresponsiveness to antibacterial treatment, and is thought to be unrelated to *H. pylori* infection in its pathogenesis. Our findings indicate the presence of different clinical subtypes in gastric MALT lymphomas.

Key words: MALT lymphoma — API2-MALT1 — t(11;18)(q21;q21) translocation

Gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type is regarded as a distinct lymphomatous entity.^{1,2} It is strongly related to *Helicobacter pylori* (*H. pylori*) infection in terms of its histopathologic, epidemiological, and clinical characteristics.^{3,4} *H. pylori* infection has been documented in up to 90% of patients with low-grade MALT lymphoma.^{3,4} This infection provides the antigenic stimulus, mediated by mucosal T cells, that is needed for sustaining the growth of gastric MALT tumors.⁵ Furthermore, these findings have recently led to the proposition of eradication of *H. pylori* as a novel treatment option for gastric MALT lymphoma patients.⁶ Several independent studies have shown that this treatment may induce complete remission in over 70% of localized cases,^{7,8} and antibiotic treatment is now recommended as the first clinical therapeutic modality for *H. pylori*-positive gastric MALT lymphoma.

t(11;18)(q21;q21) translocation is the most frequent chromosome aberration, occurring in 30% to 40% of

MALT lymphomas.^{9,10} This translocation was first described by Levine *et al.*,¹¹ and subsequently identified in some cases of MALT lymphomas.¹² Recently, we¹³ and Dierlamm *et al.*¹⁴ demonstrated that the API2 gene at 11q21, which is involved in the anti-apoptotic signal transduction pathway, is fused in-frame with a novel gene, *MALT1/MLT*, at 18q21 in t(11;18)(q21;q21) of MALT lymphomas, resulting in API2-MALT1/MLT chimeric products. Furthermore, we reported that gastric MALT lymphomas without regression in response to *H. pylori* eradication expressed the API2-MALT1 chimeric transcript mediated by t(11;18)(q21;q21) translocation and that this gene aberration was thus a good predictive marker for responsiveness to anti-*H. pylori* treatment.^{15–17} A few studies have supported this result,^{18,19} but a detailed clinicopathologic comparison between API2-MALT1 chimeric transcript-positive and -negative gastric MALT lymphoma in terms of clinical factors has not been reported to our knowledge. These factors include age, sex, dominant site of lesion, endoscopic appearance, the presence or absence of high-grade components, infiltrated layer of the gastric wall,

nodal involvement, clinical stage, status of *H. pylori* infection, and responsiveness to antibacterial treatment.^{18, 19)}

The aim of this study was to clarify the clinicopathologic differences between API2-MALT1 fusion transcript-positive and -negative gastric MALT lymphomas. Special attention has been paid to interrelationships among *H. pylori* infection status, responsiveness to antibacterial treatment, and the detection or non-detection of API2-MALT1 chimeric transcript. All the patients in this study were diagnosed, treated and followed up at our center.

MATERIALS AND METHODS

Case selection Between October 1994 and August 2001, 23 patients with low-grade gastric MALT lymphoma were enrolled in a unicenter study. Partial inclusion of high-grade components was demonstrated by biopsy or in resected specimens of five cases. In all cases, the endoscopic biopsy specimens were pathologically diagnosed by two pathologists at our hospital. Of the five to eight biopsy specimens randomly obtained from any abnormal area of the stomach, two or three were immediately frozen and stored for molecular analysis. Another two to four specimens were obtained from endoscopically normal areas of the stomach for the evaluation of the location and spread of MALT lymphoma. *H. pylori* infection was examined in all cases by culturing endoscopic biopsy specimens obtained from the greater curvature of the gastric antrum and the upper gastric body. Serum anti-*H. pylori* immunoglobulin G (IgG) antibody titers were measured by means of an enzyme-linked immunosorbent assay (ELISA) HM-CAP (Epi, Inc., New York, NY).

Histological diagnosis and criteria MALT lymphoma was diagnosed according to established histologic criteria,⁶⁾ comprising (1) a dense, diffuse infiltrate of marginal-zone centrocyte-like B cells with round to slightly irregular nuclear contours, often with abundant pale cytoplasm; (2) the presence of lymphoepithelial lesions, characterized by infiltration and disruption of gastric glands or crypts by groups of neoplastic lymphoid cells, and (3) minor criteria in support of, but not essential for diagnosis, including the presence of residual secondary follicle centers with or without intact mantles and of follicular colonization, defined as replacement of follicle centers by neoplastic lymphoid cells. Immunophenotypic expression of pan-B-cell antigens, such as CD20 and CD79a, and lack of expression of CD5 and CD10 supported the diagnosis. The presence of high-grade components was also evaluated. As defined by de Jong and colleagues,²⁰⁾ a high-grade component was considered to be present when compact clusters or sheets of large atypical lymphoid cells (centroblast-like or lymphoblast-like cells) were observed in at least 1% or more of the neoplastic lymphoid population.

Clinical staging of the disease The degree of the disease

was classified according to the Lugano staging system, a modification of the Ann-Arbor classification.²¹⁾ Staging involved a clinical physical examination, ultrasonography of the abdomen, gastric endoscopic ultrasonography (EUS), computed tomography (CT scan) of the abdomen and thorax, gallium scan, ileocolonoscopy together with upper gastrointestinal tract endoscopy, Walderyer's ring endoscopy, and bone marrow biopsy. Gastric EUS was performed before treatment to assess the depth of infiltration according to the criteria of Palazzo and colleagues.²²⁾ Lymph nodes detected by EUS around the duodenum and stomach were considered as infiltrated by lymphoma if they were spherical, hypoechoic, and 1 cm or more in diameter.

Study design After clinical staging, a two-week antibacterial treatment course (clarithromycin, 200 mg twice daily, amoxicillin, 500 mg thrice daily, and lansoprazole, 30 mg once daily) was started for every patient with clinical stage I or III. Patients were followed up at 6 to 8 weeks after the antibacterial treatment with gastric endoscopy using biopsy specimens from the same site as those used for the pretreatment examination and CT scan of the abdomen. At this time, culturing of biopsy specimens and ¹³C-urea breath test were also performed to evaluate *H. pylori* eradication. If the ¹³C-urea breath test showed a $\delta^{13}\text{C}$ level below 2.5‰, *H. pylori* infection was assessed as negative.

The treatment for *H. pylori* was considered successful for patients who showed negative results for both cultured specimens and the breath test. Subsequently patients were followed up with the same procedures every 3 months for the first year, every 4 months for the second year, every 6 months for the third year, and annually for the fourth year or later. Complete remission (CR) of the lymphoma was defined as a histological score for MALT lymphoma of 2 or less, as described by Wotherspoon *et al.*,⁶⁾ on post-treatment biopsy specimens at any time during the follow-up period. The lesion was considered to be in partial remission (PR) when the regression was endoscopically evident, but the post-treatment biopsy samples showed a histological score of 3 or more. Patients exhibiting no endoscopic or histological response six weeks after the completion of eradication were considered to represent no change (NC). CR or PR was defined as characteristic of responders and NC as characteristic of non-responders. The latter were subjected to alternative treatment strategies. For localized stages (stage I and III), radiation therapy or surgery was recommended, and chemotherapy for advanced stages (stage II2, IIE, and IV). Informed consent was obtained from all patients with regard to the aims and protocol of the study. Although chemotherapy was recommended to the patients whose pretreatment stage was evaluated as advanced (stage II2, IIE, and IV), antibacterial treatment was performed when they refused chemotherapy and expressed a preference for antibacterial treatment.

RT-PCR and nucleotide sequencing studies for API2-MALT1 chimeric transcripts The tissues obtained by endoscopic mucosal resection or biopsy of gastric MALT lymphoma before eradication treatment were used for this study. Five micrograms of total RNA prepared with the guanidium-isothiocyanate method was converted to cDNA by reverse transcriptase and PCR was conducted as described previously.²³⁾ The primer pair of API2/S1203–1222 (5'-GTTCTACTACTGTGCAATG-3') and MALT1/AS1030–1049 (5'-CAAAGGCTGGTCAGT-TGTTT-3') was used. The PCR products were electrophoresed on a 1.2% gel and stained with ethidium bromide. The amplified fragments were sequenced with the dideoxy chain termination method using an ABI PRISM Dye Termination Cycle Sequencing Ready Reaction kit (Perkin Elmer, Foster City, CA) to determine whether they were API2-MALT1 chimeric products.

Statistical analysis The Wilcoxon test was used to compare the means for continuous variables between groups with the results expressed as mean standard deviations (SD). Associations between categorical variables were evaluated with the χ^2 test and Fisher's exact test. Variables were evaluated with the Mann-Whitney *U* test. Two-sided *P* values of 0.05 or less were considered to indicate statistical significance. Since the number of subjects was limited, only univariate analysis was conducted.

RESULTS

Clinicopathologic features Table I shows the clinicopathological findings of the 23 enrolled cases of gastric MALT lymphoma. Twelve of the 23 patients were male and 11 female. Their median age was 54 years (mean 57, range 37–87 years). The median duration of follow-up was 14 months (mean 18, range 2–77 months). Twenty of the 23 patients showed evidence of *H. pylori* infection in serology and/or culture findings. Antibacterial treatment was given to all patients. *H. pylori* infection of all 20 cases except one (case no. 15) was assessed as negative after antibacterial treatment on the basis of results obtained with ¹³C-urea breath test, histology, and culture. Case no.15 showed negative histology and culture results for *H. pylori* infection after antibacterial treatment, but demonstrated marginal positivity with ¹³C-urea breath test (3.2‰). The anti-*H. pylori* IgG antibody titers in this case were 5.7 EV before treatment and 5.0 EV 12 months after treatment (cut-off value, 1.7 EV). These results may indicate that a small amount of *H. pylori* had survived somewhere in his stomach.

Endoscopic examination indicated that the tumor had infiltrated predominantly to the gastric body in 18 cases, to the antrum in two, to both the angulus and antrum in one, and to the whole stomach in two. At the time of diagnosis, the gross appearance of the lymphoma varied, with

rough mucosa observed in one case, atrophic mucosa in two cases, ulcer or ulcers in five, erosions in two, early gastric cancer-like lymphoma in three, discolored mucosa in three, edematous mucosa in one, cobblestone in three and submucosal tumor-like lymphoma in three.

Histopathological examination of the biopsy specimens revealed a low-grade MALT lymphoma in 18 cases and a low-grade tumor with high-grade components in five. Infiltrated layers were identified by EUS as the mucosa in 12 cases, the submucosa in seven and the serosa in one. Lymph node (LN) involvement was also evaluated by EUS as negative in 17 cases and positive in three (cases 15, 16, and 18). Two (cases 15 and 18) of these three cases also showed bone marrow involvement. Eighteen cases were diagnosed as stage I according to the Lugano staging system, one case as stage III1 and two cases as stage IV.

RT-PCR and nucleotide sequencing studies for API2-MALT1 chimeric mRNA API2-MALT1 chimeric transcript was detected in seven (nos.17 to 23) of the 23 cases analyzed with RT-PCR (Table I). Fig. 1 shows that the amplified fragments varied in size from case to case. The nucleotide sequence analysis indicated that all of the seven amplified fragments were fusion products of API2-MALT1. RT-PCR analysis was conducted in a blind fashion, and no clinical information was given to the person in charge of RT-PCR analysis.

Therapeutic response and outcome With regard to responsiveness to antibacterial treatment, CR, PR, and NC of the lymphoma were observed in two, 12, and nine cases, respectively. Among the nine patients (nos. 15 to 23) who failed to achieve CR or PR, two (cases 16 and 19) underwent total gastrectomy and one (case 22) received radiation therapy (total dose 40 Gy). The other seven patients did not want additional treatment in spite of our advice, and have been followed up for a median period of 12 months (mean 21, range 2–77 months). All of these seven patients have survived with disease, but the clinical course has not shown progress.

Clinicopathologic comparison between API2-MALT1-positive and -negative MALT lymphomas Table II summarizes clinicopathological differences between API2-MALT1 chimeric transcript-positive and -negative MALT lymphomas. It should be noted that three factors (responsiveness to antibacterial treatment, *H. pylori* infection, and cobblestone mucosa in gross appearance) were statistically significant. Age, sex, dominant site of lesion, high-grade components, infiltrated layer of gastric wall, nodal involvement, and clinical stage showed no differences between these two groups.

Positivity of the API2-MALT1 chimeric transcript was mostly related to responsiveness to antibacterial treatment (*P*=0.0001), since all of the seven API2-MALT1-positive lymphomas were included in the non-responders. The remaining two (cases 15 and 16) of the nine non-respond-

Table I. Clinicopathological Findings of the 23 Enrolled Cases of Gastric MALT Lymphoma

| No. | Age | Sex | <i>H.pylori</i> | Histological grade of pretreatment biopsy | Histological grade of last biopsy | Dominant gastric site of lesion | Endoscopic appearance of gastric MALT lymphoma | Histology | Infiltrated layer assessed by EUS | Nodal involvement assessed by EUS | Metastatic lesion | Clinical stage | Response to antibacterial treatment | RT-PCR (API2-MALT1) | Additional treatment after antibacterial treatment | Follow-up periods (months) | Outcome |
|-----|-----|-----|-----------------|---|-----------------------------------|---------------------------------|--|-----------|-----------------------------------|-----------------------------------|---------------------|----------------|-------------------------------------|---------------------|--|----------------------------|-----------------------|
| 1 | 53 | M | + | 5 | 3 | Body | Resembling early gastric cancer type IIc | LH | sm | - | - | I | PR | - | - | 31 | alive without disease |
| 2 | 87 | M | + | 4 | 3 | Body | Ulcers and erosions | L | sm | - | - | I | PR | - | - | 30 | alive without disease |
| 3 | 54 | F | + | 5 | 3 | Body | Discolored area | L | m | - | - | I | PR | - | - | 29 | alive without disease |
| 4 | 43 | M | + | 5 | 3 | Antrum | Multiple erosions | L | m | - | - | I | PR | - | - | 29 | alive without disease |
| 5 | 53 | M | + | 5 | 2 | Body | Resembling early gastric cancer type IIc | LH | m | - | - | I | CR | - | - | 23 | alive without disease |
| 6 | 66 | F | + | 5 | 3 | Whole stomach | Discolored area with ulcers and erosions | L | sm | - | - | I | PR | - | - | 17 | alive without disease |
| 7 | 69 | F | + | 5 | 3 | Body | Discolored area with ulcers and erosions | LH | sm | - | - | I | PR | - | - | 16 | alive without disease |
| 8 | 39 | F | + | 5 | 3 | Body | Resembling early gastric cancer type IIc | LH | m | - | - | I | PR | - | - | 16 | alive without disease |
| 9 | 37 | F | + | 4 | 2 | Body | Discolored area | L | NA | NA | - | I | CR | - | - | 13 | alive without disease |
| 10 | 56 | M | + | 5 | 3 | Antrum | Erosions | L | m | - | - | I | PR | - | - | 10 | alive without disease |
| 11 | 52 | M | + | 5 | 3 | Body | Multiple erosions | L | m | - | - | I | PR | - | - | 8 | alive without disease |
| 12 | 53 | F | + | 4 | 3 | Body | Discolored area | L | m | - | - | I | PR | - | - | 7 | alive without disease |
| 13 | 54 | F | + | 5 | 3 | Body-Angulus | Ulcers and erosions | L | m | - | - | I | PR | - | - | 6 | alive without disease |
| 14 | 59 | M | + | 4 | 3 | Body | Erosions | L | m | - | - | I | PR | - | - | 3 | alive without disease |
| 15 | 62 | M | + | 5 | 4 | Body | Atrophic mucosa | L | m | + | Bone marrow | IV | NC | - | - | 17 | alive with disease |
| 16 | 72 | F | + | 5 | 5 | Body | Submucosal tumor-like | LH | se | + | Regional lymph-node | III | NC | - | Total gastrectomy | 3 | alive without disease |
| 17 | 69 | M | + | 5 | 5 | Body | Cobblestone appearance with multiple ulcers | L | NA | NA | - | I | NC | +(ex7-ex5) | - | 77 | alive with disease |
| 18 | 63 | F | + | 5 | 5 | Whole stomach | Edematous mucosa with erosions | L | sm | + | Bone marrow | IV | NC | +(ex7-ex8) | - | 46 | alive with disease |
| 19 | 39 | M | + | 5 | 5 | Body | Submucosal tumor-like | L | sm | - | - | I | NC | +(ex7-ex5) | Total gastrectomy | 12 | alive without disease |
| 20 | 70 | F | + | 5 | 5 | Body | Cobblestone appearance with multiple ulcers | L | NA | NA | - | I | NC | +(ex7-ex5) | - | 11 | alive with disease |
| 21 | 49 | M | - | 5 | 5 | Body | Irregular and rough mucosa | L | m | - | - | I | NC | +(ex7-ex3) | - | 14 | alive with disease |
| 22 | 50 | F | - | 5 | 5 | Body | Cobblestone appearance with erosions | L | m | - | - | I | NC | +(ex7-ex5) | Irradiation therapy | 11 | alive without disease |
| 23 | 70 | M | - | 5 | 5 | Body | Submucosal tumor-like | L | sm | - | - | I | NC | +(ex7-ex5) | - | 2 | alive with disease |

M, male; F, female; L, low-grade MALT lymphoma; LH, low-grade MALT lymphoma with high-grade component; m, mucosa; sm, submucosa; se, serosa; NA, not available; CR, complete regression; PR, partial regression; NC, no change; RT-PCR, reverse transcriptase polymerase chain reaction (see Ref. 30 about exon numbers of *API2* and *MALT1* genes involved in fusion transcript). Histological grade was assessed according to Wotherspoon's classification. Clinical stage was assessed according to the modified Ann-Arbor classification adopted at the Lugano workshop.

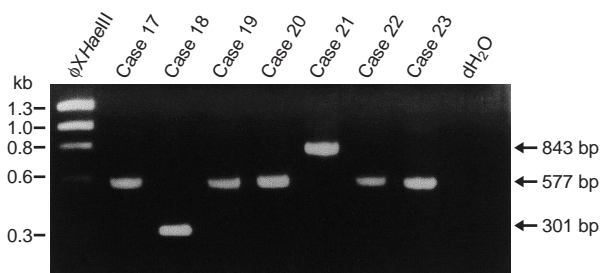


Fig. 1. RT-PCR analysis for API2-MALT1 fusion transcript. Sizes of the amplified fragments are shown on the right (see Table I for exon structures). The sizes of ϕ X/*Hae*III markers are shown on the left.

ers were found to be negative for API2-MALT1 chimeric transcript. Positivity was further significantly related to the absence of *H. pylori* infection and to endoscopic cobblestone appearance of mucosa (both $P=0.0198$).

Fig. 2 shows a representative endoscopic picture and histological finding of case 22, which had a positive API2-MALT1 chimeric transcript and no regression in response to antibacterial treatment.

DISCUSSION

Most gastric MALT lymphomas are thought to occur in MALT acquired in response to *H. pylori* and develop by stepwise accumulation of genetic abnormalities. This theory is called the *H. pylori* theory.²⁴ Recent studies including ours have shed light on the genetic mechanism of the pathogenesis and disease progression of MALT lymphomas. The *API2* gene, an inhibitor of apoptosis, and the novel *MALT1/MLT* gene have been found to be altered by t(11;18)(q21;q21), which represents the most frequent structural chromosomal abnormality in extranodal low-grade MALT lymphoma.^{13, 14}

An increasing number of studies have shown that recurrent t(11;18)(q21;q21) translocation is the sole aberration present in some marginal zone B-cell lymphomas of MALT type, but is not found in large cell MALT lymphomas.^{10, 14, 25} Therefore, this translocation may define a subgroup of lymphomas with a relatively high genetic stability and low capacity to evolve towards large-cell, more aggressive variants. In contrast, t(11;18)-negative low-grade B-cell lymphomas of MALT type are characterized by frequent gains on chromosome 3 and DNA amplifications on 2p13–p15.²⁵ These t(11;18)-negative MALT lymphomas can be assumed to progress from low grade to high grade in accordance with the *H. pylori* theory.²⁴

The study presented here compared the clinicopathological features of API2-MALT1 chimeric transcript-positive MALT lymphoma with those of its negative counterpart.

Table II. Clinicopathological Differences between API2-MALT1 Chimeric Transcript-positive and -negative MALT Lymphomas

| | API2-MALT1-positive | API2-MALT1-negative | Univariate analysis |
|---|---------------------|---------------------|---------------------|
| No. of cases | 7 | 16 | |
| Age (y) (mean) (range) | 58.6 (39–77) | 56.8 (37–87) | NS** |
| Sex | | | |
| M | 4 | 8 | NS* |
| F | 3 | 8 | |
| <i>H. pylori</i> | | | |
| Positive | 4 | 16 | 0.0198* |
| Negative | 3 | 0 | |
| Responsiveness to antibacterial treatment | | | |
| Responder | 0 | 14 | 0.0001* |
| Non-responder | 7 | 2 | |
| Dominant site of lesion | | | |
| Proximal | 6 | 12 | NS* |
| Distal | 0 | 3 | |
| Whole stomach | 1 | 1 | |
| Endoscopic appearance, main features | | | |
| Rough mucosa | 1 | 0 | NS* |
| Atrophic mucosa | 0 | 2 | NS* |
| Ulcer | 0 | 5 | NS* |
| Erosions | 0 | 2 | NS* |
| Early gastric cancer-like | 0 | 3 | NS* |
| Discolored mucosa | 0 | 3 | NS* |
| Edematous mucosa | 1 | 0 | NS* |
| Cobblestone mucosa | 3 | 0 | 0.0198* |
| Submucosal tumor-like | 2 | 1 | NS* |
| High-grade components | | | |
| Positive | 0 | 5 | NS* |
| Negative | 7 | 11 | |
| EUS assessment of infiltrated layer | | | |
| Mucosa | 2 | 10 | NS* |
| Submucosa | 3 | 4 | |
| Submucosa propria | 0 | 0 | |
| Serosa | 0 | 1 | |
| Nodal involvement | | | |
| Positive | 1 | 2 | NS* |
| Negative | 4 | 13 | |
| Clinical stage | | | |
| I | 6 | 14 | NS** |
| II1 | 0 | 1 | |
| II2 | 0 | 0 | |
| IV | 1 | 1 | |

* Fisher's exact test, ** Mann-Whitney *U* test.

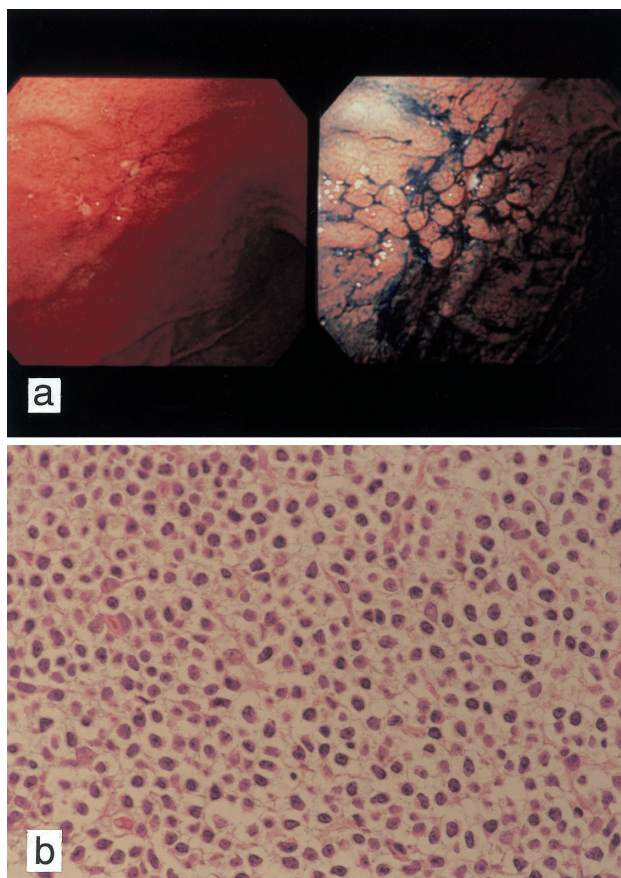


Fig. 2. Endoscopic pictures and histology of gastric MALT lymphoma (case 22). a: Endoscopy revealed a cobblestone appearance, which was detected clearly by spreading indigocarmine (left, conventional endoscopy; right, indigocarmine-spread endoscopy). b: Histology reveals low-grade B-cell lymphoma of MALT type. (Hematoxylin-eosin staining, original magnification $\times 150$).

API2-MALT1 positivity was found to be closely related to unresponsiveness to antibacterial treatment ($P=0.0001$), absence of *H. pylori* infection ($P=0.0198$) and endoscopic cobblestone appearance of mucosa ($P=0.0198$). Other factors, including clinical stages and the presence or absence of high-grade components, showed no differences. Liu *et al.*¹⁹⁾ reported that seven out of nine API2-MALT1 fusion-positive patients were at stage IIE or higher, and suggested that this genetic alteration is associated with more advanced stages of the disease.²⁶⁾ In contrast with their finding, our present study demonstrated that only one of seven API2-MALT1 chimeric transcript-positive cases was at clinical stage IV and that all of the others were at stage I. The stage IV patient has survived without symptoms for 46 months, as have all the stage I cases. Moreover, among

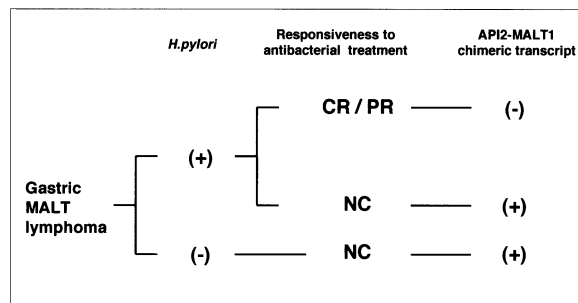


Fig. 3. Relationships among status of *H. pylori* infection, responsiveness to antibacterial treatment and API2-MALT1 chimeric transcript in gastric MALT lymphoma.

these API2-MALT1-positive cases, no progression of clinical stages, high-grade transformation, or chromosomal aberrations have been detected, thus indicating their high biological stability.²⁵⁾ Their clinical behavior can therefore be regarded as highly indolent. This implies that more radical treatment of this type of MALT lymphoma does not need to be initiated immediately after antibacterial treatment, although longer clinical follow-up is needed to reach a definitive conclusion.

There were two non-responders (cases 15 and 16) that showed negativity of the API2-MALT1 chimeric transcript. We suggested that in case 15, *H. pylori* eradication was incomplete and that its infection persisted even after antibacterial treatment, because the $\delta^{13}\text{C}$ value of the urea breath test (3.0‰) only slightly exceeded the cut-off value (2.5‰) and *H. pylori* antibody titers did not decrease. In case 16, it appears that the follow-up period (only 3 months) was too short to assess the responsiveness to antibacterial treatment. However, it cannot be ruled out that another, as yet undetermined gene alteration may be responsible for the unresponsiveness to antibacterial treatment.

H. pylori-negative gastric MALT lymphoma did not respond to antibiotic treatment.²⁷⁾ Its histogenesis was inconsistent with the *H. pylori* theory because MALT was not acquired in response to *H. pylori*. To our knowledge, no reports on API2-MALT1 chimeric transcripts of *H. pylori*-negative MALT lymphoma have been published except for this and one previous study.¹⁶⁾ Liu *et al.*¹⁹⁾ investigated API2-MALT1 chimeric transcripts only in *H. pylori*-positive MALT lymphoma, and Baens *et al.*²⁸⁾ stated that no correlation was found between API2-MALT fusion and *H. pylori* status. In a series studied by Kalla *et al.*, status of *H. pylori* infection was assessed as negative in some cases which had already received antibiotic treatment.²⁹⁾ Our study found all the *H. pylori*-negative MALT lymphomas to be positive for API2-MALT1 chimeric tran-

script, suggesting that this genetic alteration may arise independently of *H. pylori* infection.

Among several endoscopically observed gross appearances of gastric MALT lymphoma, only cobblestone mucosa significantly correlated with API2-MALT1 positivity. We previously reported that polypoid-type MALT lymphoma with submucosal tumor-like appearance was unresponsive to antibacterial treatment.¹⁵⁾ In the current study, too, three cases with such gross appearance, consisting of two API2-MALT1-positive and one -negative lymphomas, did not respond to *H. pylori* eradication. Both of these appearances, cobblestone mucosa and submucosal tumor-like, may thus be clinically relevant for predicting unresponsiveness to antibacterial treatment.

Fig. 3 shows the relationships among status of *H. pylori* infection, responsiveness to antibacterial treatment, and API2-MALT1 chimeric transcript in gastric MALT lymphoma. The data presented here suggest that low-grade gastric MALT lymphoma can be practically classified into two types in terms of API2-MALT1 chimeric transcripts.

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