【 CASE REPORT 】

The Diagnosis of Gastric Mucosa-associated Lymphoid Tissue Lymphoma by Flow Cytometry and Fluorescence *in situ* Hybridization of Biopsy Specimens

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Abstract:

Mucosa-associated lymphoid tissue (MALT) lymphoma and reactive inflammatory lymphoid changes are frequently difficult to distinguish based on a routine histological differential diagnosis. We were unable to diagnose gastric MALT lymphoma histologically using specimens obtained by endoscopy, although a flow cytometry (FCM) analysis demonstrated clonality of neoplastic cells by separating cells by CD45 gating. Furthermore, a fluorescence *in situ* hybridization (FISH) analysis showed trisomy 18. We therefore diagnosed gastric MALT lymphoma with trisomy 18. We recommend that FCM and FISH analyses of biopsy specimens be considered for diagnosing gastric MALT lymphoma if this diagnosis is suspected based on endoscopic findings.

Key words: mucosa-associated lymphoid tissue lymphoma, trisomy 18, flow cytometry, fluorescence *in situ* hybridization, reactive inflammatory lymphoid changes

Introduction

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is a neoplasm that originates from marginal zone B cells and that primarily or secondarily involves the gastrointestinal tract, lungs, salivary glands, thyroid, ocular adnexa, liver, and skin (1, 2). Among these sites, the stomach is the most frequently involved in cases of MALT lymphoma. Eighty-eight percent of patients with MALT lymphoma are infected with *Helicobacter pylori* (3), and several genetic alterations are reportedly associated with the pathogenesis of MALT lymphoma (4). When diagnosing MALT lymphoma using biopsy specimens, basic histopathological images stained with Hematoxylin and Eosin (H&E) staining are the most important. However, it is often difficult to histologically diagnose MALT lymphoma, as neoplastic cells are scarce in biopsy specimens (5-7).

In the present case, we were unable to diagnose gastric MALT lymphoma histologically; however, we were able to make a conclusive diagnosis of MALT lymphoma by flow cytometry (FCM) and fluorescence *in situ* hybridization (FISH) analyses of biopsy specimens obtained by endoscopy.

Case Report

A 79-year-old man visited Fukuyama National Hospital because of sudden abdominal pain exacerbation. The patient's history included cerebral infarction and atrial fibrillation; however, he had no history of gastrointestinal or hematological diseases. He had no notable family history. His physical examination showed overall abdominal pain, with the strongest pain in the epigastrium, and positive peritoneal irritation signs. There was no evidence of hepatosplenomegaly or peripheral lymphadenopathy.

His laboratory data were as follows: WBC, 10,100/μL;
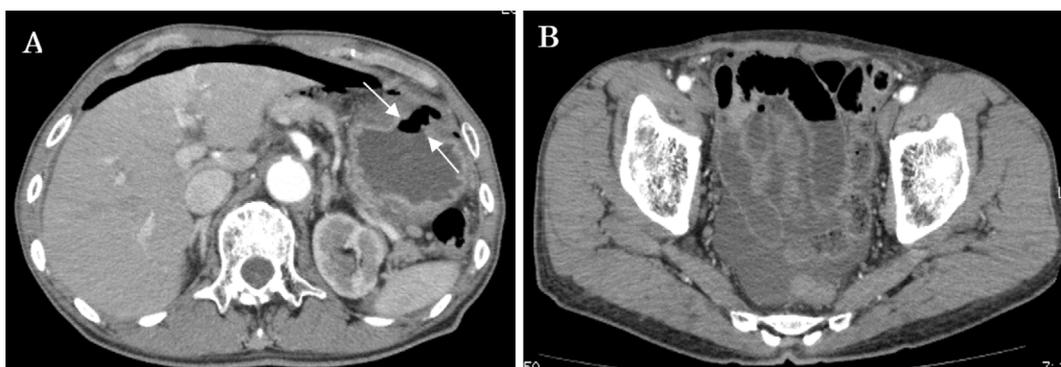
Figure 1. Contrast-enhanced computed tomography. A: Showing the perforated region in the anterior wall of the upper gastric body (arrows) and large amounts of free air. B: Showing small amounts of ascites.
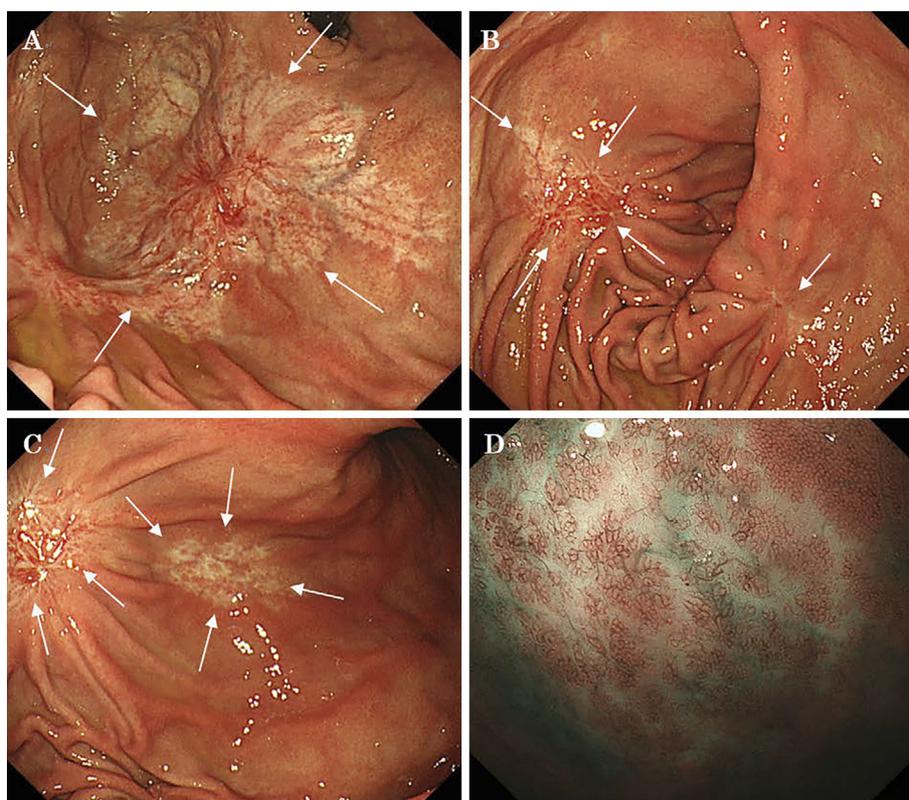
Figure 2. A gastrointestinal endoscopic examination. A: A discolored, extensive, flat lesion with fold convergence at the anterior wall of the upper gastric body was considered to be perforated because of a remarkable scar (arrows). B, C: A large number of discolored depressed lesions throughout the stomach were strongly suspected of being gastric MALT lymphoma (arrows). D: A magnified observation demonstrated a lack of gastric pits and the presence of abnormal vessels. MALT: mucosa-associated lymphoid tissue

Hb, 6.7 g/dL; C-reactive protein (CRP), 2.14 mg/dL; soluble interleukin-2 receptor (sIL-2R), 801 U/mL; serum *H. pylori*-IgG was negative, and a urea breath test (UBT) did not confirm *H. pylori* infection. Contrast-enhanced computed tomography (CE-CT) revealed a perforated region in the anterior wall of the upper gastric body, large amounts of free air, and small amounts of ascites (Fig. 1). In addition, CE-CT of the neck, chest, abdomen, and pelvis revealed no lymph node enlargement or organ involvement besides the stomach. The patient was diagnosed with acute generalized peritonitis with gastric perforation, and emergency surgery was performed. Operative findings revealed a perforated region, measuring 7 mm in diameter in the anterior wall of the upper gastric body that included a surrounding indurated area. A histopathological examination of biopsy specimens of the perforated lesion, obtained during surgery, showed no malignant (neoplastic) cells; therefore, surgical greater omentum filling was performed.

Two months after the surgery, a gastrointestinal endoscopic examination was performed, revealing a discolored,
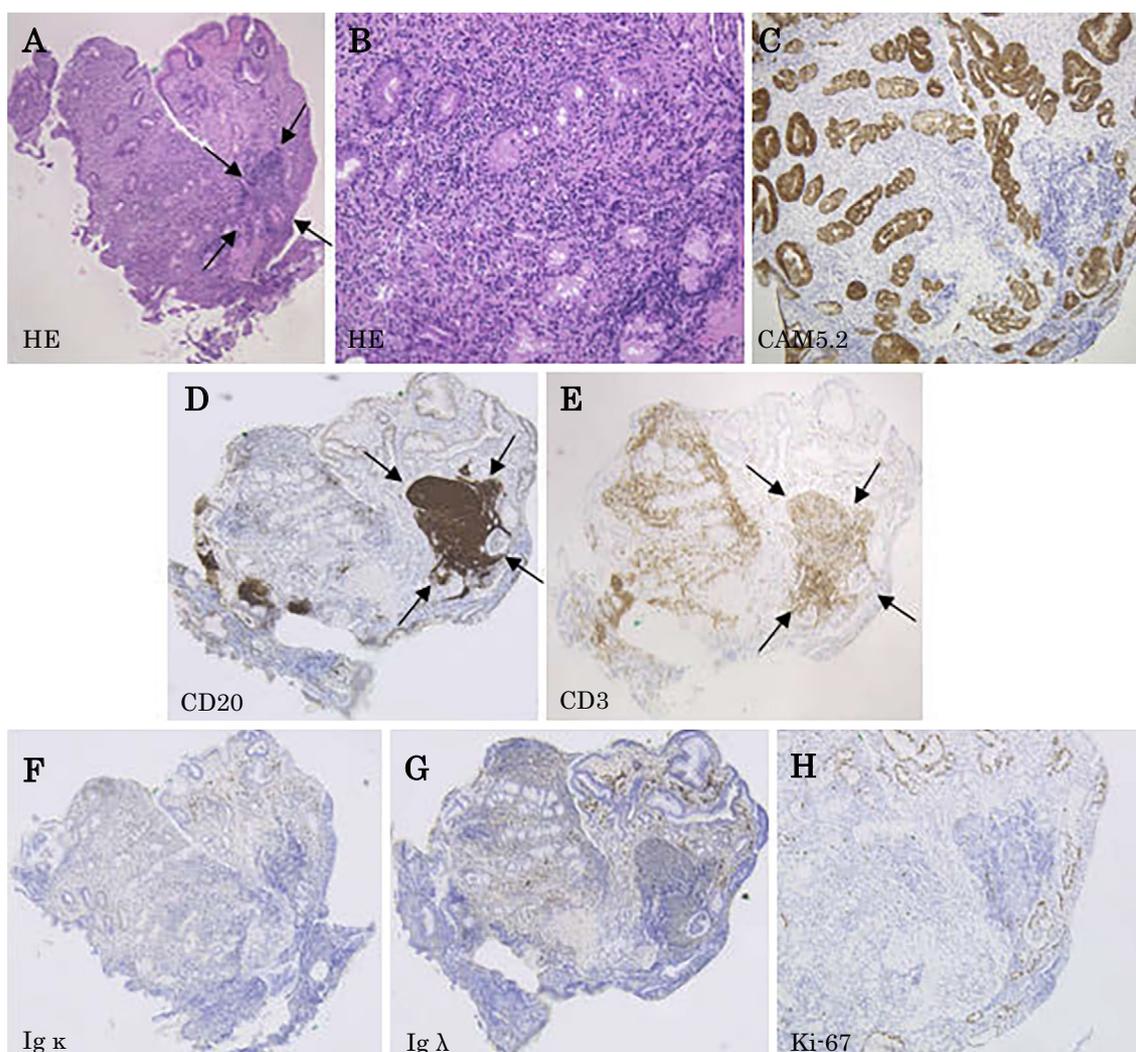
Figure 3. Pathological images in the biopsy specimens. A: Dense lymphoid infiltration in the lamina propria of the mucosa was observed (arrows) (Hematoxylin and Eosin staining, ×4). B, C: Lymphoepithelial lesions (LELs) were not observed, even by CAM5.2 staining (B, ×20; C, ×10). D, E: Immunohistochemical staining showed that more lymphocytes were positive for CD20 than for CD3 (arrows) (×4). F, G: An *in situ* hybridization analysis showed no immunoglobulin light chain restriction (×4). H: The Ki-67 labeling index was low (×10).

extensive, flat lesion with fold convergence at the anterior wall of the upper gastric body (Fig. 2A). The lesion was considered to be perforated because of a remarkable scar. Furthermore, endoscopic findings revealed a large number of discolored depressed lesions throughout the stomach (Fig. 2B, C). A magnifying observation demonstrated a lack of gastric pits and the presence of abnormal vessels (Fig. 2D). Based on the endoscopic findings, we strongly suspected gastric MALT lymphoma. We performed eight biopsies of different discolored depressed lesions; three specimens were used for histopathological study, three for the FCM analysis, and two for the FISH analysis.

In the biopsy specimens of these lesions, dense lymphoid infiltration in the lamina propria of the mucosa was observed by an H&E stain analysis (Fig. 3A). CAM5.2 staining was not able to detect lymphoepithelial lesions (LELs) (Fig. 3B, C). Immunohistochemical staining showed that

more lymphocytes were positive for CD20 than for CD3 (Fig. 3D, E) and negative for CD5 and CD10, indicative of B-cell characteristics. However, an *in situ* hybridization analysis showed no immunoglobulin light chain restriction (Fig. 3F, G), and the Ki-67 labeling index was low (Fig. 3H). We were unable to diagnose gastric MALT lymphoma based on these findings alone.

We next simultaneously analyzed the FCM and FISH findings. FCM was performed on three biopsy specimens obtained by endoscopy (Fig. 4). Lymphocytes were identified by CD45 gating, and a group of cells with a slight decrease in CD45 expression, which were considered neoplastic cells, was identified. An FCM analysis revealed that most of the cells were positive for CD20 and expressed immunoglobulin light chain lambda, indicative of immunoglobulin light chain restriction. In addition, a FISH analysis for t(11; 18)(q21;q21) translocation revealed no fusion genes of
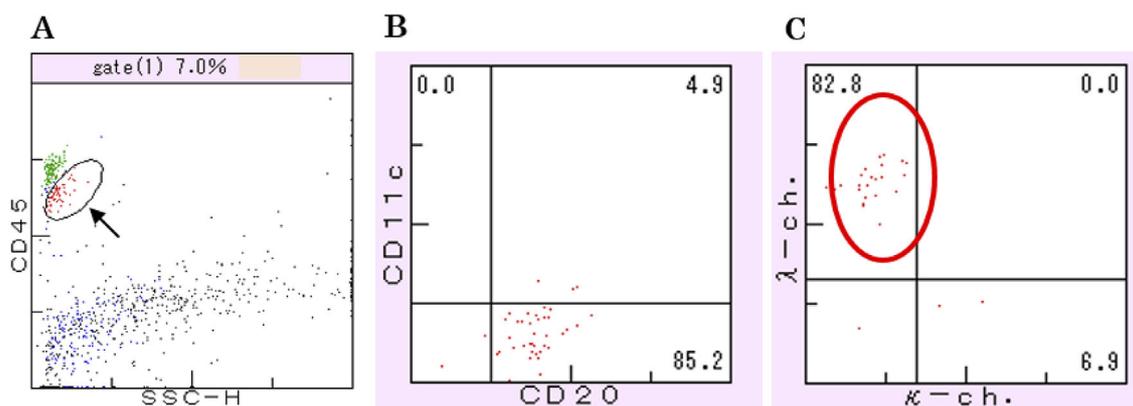
Figure 4. Flow cytometry (FCM) showing immunoglobulin light chain restriction. A: CD45 is a marker of lymphocytes; however, neoplastic cells have slightly decreased CD45 expression. After gating all cells in the three biopsy specimens by CD45 and side scatter, we were able to identify a group of cells with slightly decreased CD45 expression that also had a rough internal structure (arrow). B, C: An FCM analysis revealed that most of the cells were positive for CD20 and expressed immunoglobulin light chain lambda, indicative of immunoglobulin light chain restriction.
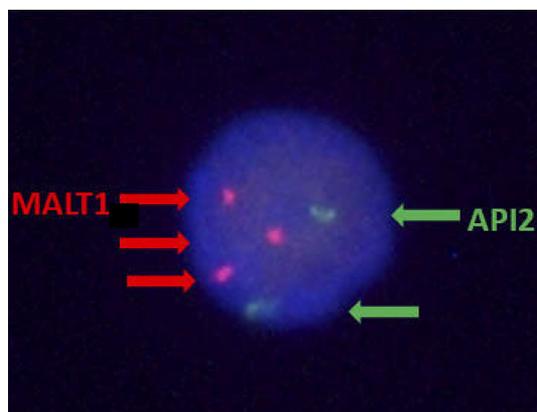
Figure 5. Fluorescence *in situ* hybridization (FISH) images of the neoplastic cells. In the FISH analysis for t(11;18) (q21;q21) translocation, the *API2* gene is visualized as a green signal, *MALT1* as a red signal, and fusion genes of *API2-MALT1* as yellow signals. The results revealed no fusion genes, although extra (three) copies of *MALT1* were identified in 40.0% of the investigated cells (arrows). A FISH analysis showed trisomy 18 in 40.0% of lymphoma cells, suggesting clonality of the neoplastic cells.

API2-MALT1, although extra (three) copies of *MALT1* were identified in 40.0% of the investigated cells (Fig. 5). Chromosome banding of the bone marrow aspirate showed a normal karyotype of 46, XY, indicating no congenital chromosomal abnormalities. Positron emission tomography revealed no tracer uptake, and colonoscopy revealed no colorectal involvement. Based on these findings, we diagnosed the patient with primary gastric MALT lymphoma with trisomy 18. Radiation was administered as curative therapy.

Discussion

MALT lymphoma of the stomach comprises 40-50% of gastric (malignant) lymphoma, and accounts for only 1-5% of gastric malignancies (8).

The gold standard for diagnosing MALT lymphoma is the histological detection of the infiltration of centrocyte-like cells, lymphoepithelial lesions (infiltration of lymphoma cells around the epithelium) and the proliferation of lymphocytes in the lamina propria of the mucosa (1, 9). Immunoglobulin light chain restriction adds further diagnostic value and is important in the differential diagnosis with benign lymphoid infiltrates (10).

However, histologically diagnosing MALT lymphoma is often difficult. Only 50-75% of the cases are diagnosed by both endoscopy and histology using biopsy specimens (5-7). Several reasons underlie the difficult diagnosis of MALT lymphoma. First, endoscopically, gastric MALT lymphoma often shows morphological features similar to gastritis (11). Therefore, the histological findings of MALT lymphoma are often indistinguishable from those of gastritis consisting of reactive inflammatory lymphoid changes. Second, the number of neoplastic cells is often low in biopsy specimens (12). Therefore, large amounts of normal lymphocytes in the background obscure the lymphoma cells (neoplastic cells). Third, histologically, some gastric MALT lymphomas have few characteristic morphologic features. Finally, obtaining biopsies of the tumor cells is difficult because neoplastic cells exist in the lamina propria of the mucosae or submucosa (9). Suekane et al. (13) recommended that endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) be performed in patients considered to have primary early gastric lymphoma and for whom a definite diagnosis cannot be made by biopsy specimens alone.

In addition, Ono et al. (14) reported that magnifying endoscopic findings of nonstructural areas and abnormal vessels may be useful for a target biopsy to aid in diagnosing gastric MALT lymphoma. The sensitivities of these findings for the diagnosis were 76.9% and 85.7%, respectively, and

the specificities were 87.5% and 85.7%, respectively (14).

In our case, the endoscopic findings revealed a large number of discolored depressed lesions throughout the stomach. A magnifying observation demonstrated a lack of gastric pits and the presence of abnormal vessels. Based on these endoscopic findings, we strongly suspected gastric MALT lymphoma. However, the histological findings of a target biopsy of nonstructural areas with abnormal vessels, suspected to be MALT lymphoma, revealed dense lymphoid infiltration in the lamina propria of the mucosa with no LELs [Wotherspoon score, grade 3 (12)]. Immunohistochemical staining showed that more lymphocytes were positive for CD20 than for CD3; however an *in situ* hybridization (ISH) analysis showed no immunoglobulin light chain restriction. Given these findings, we were unable to confirm that the patient had gastric MALT lymphoma because of so few infiltrating lymphoid cells and no immunoglobulin light chain restriction. Therefore, the infiltrating lymphoid cells were initially misdiagnosed as reactive inflammatory lymphoid changes rather than MALT lymphoma by a pathologist.

Accordingly, we simultaneously performed FCM of three biopsy specimens obtained by endoscopy. CD45 is a marker of lymphocytes (15); however, neoplastic cells have slightly decreased CD45 expression (15). After gating all cells in the three biopsy specimens by CD45, we were able to identify a group of cells with slightly decreased CD45 expression. In addition, an FCM analysis of that group of cells demonstrated immunoglobulin light chain restriction. We were therefore able to precisely detect only neoplastic cells (lymphoma cells). Thus, by CD45 gating, the FCM analysis demonstrated the clonality of the neoplastic cells.

FCM is generally used in the diagnosis of B-cell lymphomas in hematopathology. The determination of clonality was based on combining the histogram pattern and percentage of cell positivity for the tested antigens. An abnormal kappa/lambda ratio was defined as outside the range of 1.0 to 3.0 for bone marrow aspirates and 1.0 to 2.0 for all other specimens. In our FCM, a kappa/lambda ratio outside these ranges, combined with other antigen expression patterns, generally prompts a closer investigation for a neoplastic clonal population (16).

Furthermore, a FISH analysis showed trisomy 18 in 40.0% of the investigated cells, suggesting clonality of the neoplastic cells. Given the above results, the patient was diagnosed with primary gastric MALT lymphoma with trisomy 18.

Aneuploidy - most commonly trisomy 18, trisomy 3, or both - is frequently observed in t(11;18)(q21;q21)/*API2-MALT1*-negative MALT lymphoma (17-20). The presence of extra copies of *MALT1* is significantly associated with the progression or relapse of lymphoma (21, 22), and it is an independent prognostic factor for a worse event-free survival, as determined by a Cox multivariate analysis (21). Previous studies have suggested that numerical gains, such as trisomy 3 or 18, are associated with the high-grade transformation of MALT lymphoma (18, 23). In addition, Taji et al. reported

that, among 13 patients with gastric MALT lymphoma, 2 had trisomy 18 (24). Both of these patients with trisomy 18 achieved a complete response to eradication therapy for *H. pylori*. Isikawa et al. reported the case of a patient with gastric MALT lymphoma with trisomy 18 (22). Eradication therapy resulted in partial regression, and radiotherapy was thus initiated. Although complete remission was achieved with the radiotherapy, local recurrence in the stomach was detected 16 months after the completion of treatment. These findings suggest that gastric MALT lymphoma with trisomy 18 is relatively sensitive to eradication therapy. However, careful follow-up is required in these patients in order to timely detect any relapse.

Extra copies of *MALT1* (3 and occasionally 4 copies), suggestive of partial or complete trisomy (or occasionally tetrasomy) 18, were detected in 18 (25%) of 71 cases (21). Therefore, extra copies of *MALT1* are likely of diagnostic value. However, because trisomy 18 also develops in other non-Hodgkin lymphomas, such as follicular lymphoma, diffuse large B-cell lymphoma and peripheral T-cell lymphoma (25-27), the patient could not be diagnosed with gastric MALT lymphoma based solely on the presence of trisomy 18.

Gastric disorders involving lymphocytes are divided into two major groups. Malignant lymphoma, including MALT lymphoma, represents the monoclonal neoplastic proliferation of lymphocytes, while reactive inflammatory lymphoid changes are characterized by the hyperplastic and polyclonal aggregation of lymphocytes (10). Although MALT lymphoma and reactive inflammatory lymphoid changes are distinct clinicopathological entities, they are frequently difficult to distinguish in a routine histological differential diagnosis.

For example, the differential diagnoses of MALT lymphoma includes reactive inflammatory processes, lymphoepithelial sialadenitis, Hashimoto thyroiditis, and other small B-cell lymphomas (follicular lymphoma, mantle cell lymphoma, and small lymphocytic lymphoma) (1). Distinction from reactive inflammatory processes is mainly based on the presence of destructive infiltrates of extrafollicular B-cells, typically with the morphology of marginal zone cells. In borderline cases, immunophenotyping or a molecular genetic analysis to assess B-cell clonality is necessary to help establish or exclude a diagnosis of MALT lymphoma, although molecular studies may also demonstrate clonal B-cells in some non-neoplastic MALT proliferations or persistent clonal populations in gastric MALT lymphomas even after histological complete remission. The distinction of MALT lymphoma from other small B-cell lymphomas is based on a combination of characteristic morphologic and immunophenotypic features (1). In addition, FCM and FISH analyses of biopsy specimens are useful for making an exact diagnosis of MALT lymphoma and distinguishing between MALT lymphoma and reactive inflammatory lymphoid changes.

We must not forget that histological findings are the gold standard for the diagnosis of MALT lymphoma. However, we often cannot histologically diagnose malignant lym-

phoma, particularly MALT lymphoma. We therefore recommend that FCM and FISH analyses of biopsy specimens obtained by endoscopy always be considered for diagnosing gastric MALT lymphoma if this diagnosis is suspected on the basis of endoscopic findings.

Multiparameter analyses, including FCM and FISH, were useful for making an accurate differential diagnosis between MALT lymphoma and reactive inflammatory lymphoid changes and may help prevent progression or relapse of MALT lymphoma.

The authors state that they have no Conflict of Interest (COI).

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