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Antisecretory medication is associated with decreased *Helicobacter pylori* detection in gastric marginal zone lymphoma☆,☆☆,★,★★

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ABSTRACT

Helicobacter pylori status influences the prognosis and management of gastric extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), so accurate determination of *H pylori* status is of clinical importance. The low rate of histologic *H pylori* positivity among gastric MALT lymphoma cases at our institution prompted investigation for possible causes. A case series of 24 patients as having gastric MALT lymphoma (with no diffuse large B-cell component) in a tertiary care setting between 1997 and 2010 was identified, and clinical records were reviewed. Immunohistochemical staining for *H pylori* and BCL10 was performed. This study received institutional review board approval (protocol number M13-033). Thirty-nine percent of cases (9/23) were *H pylori* positive by histology, and 4 additional patients had positive serologic results; overall, 57% of cases (13/23) were positive for *H pylori*. Treatment with antisecretory medications was associated with a lower likelihood of histologic positivity (13% among treated patients vs 75% among untreated; $P = .04$). Nuclear localization of BCL10 was seen in 2 cases and was not associated with *H pylori* status. Antisecretory medications decrease the likelihood of histologic detection of *H pylori* in gastric MALT lymphoma cases. Incorporation of results of serologic or other testing is needed to ensure correct classification with respect to *H pylori* status.

1. Introduction

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) arises most commonly in the stomach [1]. The paradoxical prevalence of MALT lymphoma in an organ normally lacking organized lymphoid tissue has been attributed to the association of gastric MALT lymphoma with infection by *Helicobacter pylori*, which can cause chronic gastritis with lymphoid infiltrates [2]. Persistent antigenic stimulation and an accumulation of genetic mutations can lead to the development of a neoplastic B-cell clone [1]. Most published studies have documented *H pylori* infection in a significant majority of patients with gastric MALT lymphoma [3].

Accurate assessment of *H pylori* infection status is important at the time of gastric MALT lymphoma diagnosis, as antibiotic therapy is a mainstay of first-line treatment of stage I_E and II_E *H pylori*–positive MALT lymphoma, and *H pylori* eradication induces protracted remission, if not cure, in most cases [4]. *H pylori* infection may be diagnosed by histologic examination of gastric biopsy specimens or by noninvasive approaches, including rapid urease test, urea breath test, serology, and monoclonal stool immunoassays. Studies report variation in the sensitivity of these methods, although histologic examination and the rapid urease test are consistently found to be among the most sensitive and specific [5–8].

The present study was prompted by the empirical observation that many of the gastric MALT lymphoma cases at our institution were described as *H pylori*–negative. Although such a disparity between actual local results and expected findings based on the published literature could simply represent a chance finding due to small sample size, other potential contributing factors included variation in disease characteristics in different populations, gastric biopsies not representing the patients' true *H pylori* status, or falsely negative cases due to insufficient sensitivity of methods used for *H pylori* testing. The objective of this study was to identify factors underlying the low observed prevalence of *H pylori* infection among patients diagnosed as having gastric MALT lymphoma at our institution.

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2. Materials and methods

Cases of gastric MALT lymphoma were identified using the laboratory information system of the Department of Pathology and Laboratory Medicine at the University of Vermont Medical Center. The University of Vermont Medical Center is an acute care hospital affiliated with the University of Vermont and serves as both a community hospital for the Burlington, Vermont, metropolitan area and a tertiary referral center for patients throughout Vermont and northern New York. Approximately 1 million people reside in the hospital's catchment area. Excluding cases with a diffuse large B-cell component, 24 cases of gastric MALT lymphoma were diagnosed between 1997 and 2010. The archived slides were examined, the histologic diagnosis of MALT lymphoma was confirmed, and formalin-fixed, paraffin-embedded tissue blocks were selected from each case for further study.

The patients' electronic health records were reviewed. Demographic data, results of prior testing for *H pylori*, details of gastric biopsy procurement (number, location, and prior antisecretory medication usage), and lymphoma staging data were recorded; data were in many instances incomplete, as many years had elapsed since some of the diagnoses were originally made. *Antisecretory medications* were defined as proton pump inhibitors (PPIs) and H2 receptor antagonists. Cancer stage was determined using the Lugano staging system [9]. The *proximal stomach* was defined as the cardia, fundus, high body, and mid body, whereas the *distal stomach* was defined as the antrum and lower body [10].

A patient was considered to be *H pylori* positive if evidence of infection was demonstrable by one or more methods (histology including immunohistochemical [IHC] staining for *H pylori*, and/or serologic testing) [11]. For IHC staining for *H pylori* and BCL10, slide mounted 5-μm tissue sections cut from formalin-fixed, paraffin-embedded tissue were dewaxed by three 5-minute washes in xylene, followed by rehydration through graded ethanol washes (100%, 95%, 70% and 50%; 2× 3 minutes in each). After rinses in Milli-Q ultra-pure water (EMD Millipore, Billerica, Massachusetts), heat-induced epitope retrieval was performed by immersing the slides in Target Retrieval solution pH 6.0 (Dako North America Inc, Carpinteria, California) and heating at 100°C for 15 minutes in a Decloaking Chamber Pro pressure cooker (Biocare Medical, Concord, California). Slides were then allowed to cool in the pressure cooker unit for another 20 minutes. After three 5-minute rinses in TBST (25 mmol/L Tris, 0.15 mol/L NaCl, 0.05% Tween 20), slides were immersed in 3% H_2O_2/TBST for 15 minutes as to inactivate any endogenous peroxidase in the tissues. After three 5-minute washes in TBST, slides were immersed in protein block, serum-free (Dako), for 15 minutes to block nonspecific protein binding sites in the tissues. Primary antibody was then applied (Table 1).

As a negative control test, IHC was also performed substituting primary antibody with a mouse monoclonal (mAb) IgG1 antibody to *Aspergillus niger* glucose oxidase (Dako). After TBST washes, secondary detection was performed by incubating the slides for 30 minutes at room temperature (RT) with EnVision+ Dual Link polymer horseradish peroxidase reagent (Dako). After a further series of TBST washes, slides were incubated for ~6 minutes with DAB+ chromogen substrate (Dako) and then rinsed with tap water. Tissues were then counterstained with hematoxylin for ~7 minutes, rinsed with TBST and water, and then dehydrated through 50%, 70%, 95%, and 100% ethanol. Finally, slides were coverslipped over Cytoseal mountant (ThermoFisher Scientific, Waltham, Massachusetts) for viewing by bright-field microscopy.

All slides for each biomarker were stained in the same batch to ensure consistency of staining intensity. Positive and negative control staining yielded appropriate results. Because of limited archived tissue, not all studies could be performed on all cases. Sections stained for BCL10 slides were reviewed by 2 pathologists (K.B.S. and M.R.L.), as were the *H pylori* IHC slides (K.B.S. and R.W.); review was conducted in a blinded manner with respect to results of previous or concurrent staining, and consensus regarding staining interpretation was reached for each case. BCL10 nuclear localization was considered to be positive when BCL10 was detected in more than 10% of the nuclei of tumor cells [12–15]. Descriptive statistics were gathered, and findings in observed in *H pylori*–positive and *H pylori*–negative subgroups were compared using the Fisher exact probability test and Student *t* test as appropriate.

This study was approved by the institutional review board of the University of Vermont (protocol number M13-033).

3. Results

3.1. Clinical features

Among the 24 patients, 9 (38%) were female. Age at diagnosis ranged from 36 to 90 years (median, 73.5 years; mean, 68.8 years; Table 2). Four patients (17%) had undergone one or more gastric biopsies prior to the biopsy diagnostic for lymphoma. Nine patients (38%) had a documented history of treatment with an antisecretory medication: 6 had been treated using PPIs (lansoprazole, 1; omeprazole, 2; omeprazole and esomeprazole, 1; pantoprazole, 1; unspecified PPI; 1); one had been treated with famotidine; one had been treated with both famotidine and pantoprazole; and one had received an unspecified antisecretory therapy. Lymphoma of mucosa-associated lymphoid tissue was found in the proximal stomach in 10 patients, the distal stomach in 3 patients, and both proximal and distal stomach in 4 patients; specific localization data were unavailable for the remaining 7 patients. On average, 6.7 biopsies (range, 2-18) were taken in each patient. Of the patients who had full staging workups, a majority (63%) presented with stage I_E disease, whereas 26% and 11% of patients presented at stages II_E and IV, respectively (Fig. 1). All but 4 cases had one or more stains performed in addition to hematoxylin and eosin (H&E) at the time of initial diagnosis; of those 4, 3 had *H pylori*–like organisms visible in H&E-stained sections (Fig. 2). Special stains used included Steiner (positive in 6 of 13 cases), Warthin-Starry (negative in 2 cases), Alcian blue (negative in 1 case), and IHC staining for *H pylori* (negative in 4 cases). Serologic testing was performed in 9 cases and was positive in 7.

3.2. H pylori testing

Results of IHC staining for *H pylori* were in agreement with the prior histologic diagnosis in all but 3 cases (Table 2). In 2 of these, the prior positive diagnoses had been made using either Steiner or Warthin-Starry staining, but IHC staining yielded no evidence of *H pylori*. In the other instance, prior IHC staining had been determined to be negative for *H pylori*, but staining for purposes of this study revealed rare organisms. Overall, 39% of biopsies (9/23) were positive for *H pylori* by IHC staining (Fig. 2). As 4 of the 15 cases negative by IHC had serologic evidence of infection, a total of 54% of patients (13/24) were found to be *H pylori* positive. No significant differences between *H pylori*–

Table 1
Immunohistochemistry antibodies

Antigen	Antibody clone	Source	Dilution/Time	Positive control tissue
BCL-10	Mouse monoclonal antibody, clone: SPM520	Pierce Biotechnology, Rockford, IL	1:10, 30 min, RT	Tonsil
H pylori	Rabbit pAb	Pierce Biotechnology	1:50, 30 min, RT	H&E–verified *H pylori*–infected tissues