Accuracy of biopsies for *Helicobacter pylori* in the presence of intestinal metaplasia of the stomach

STOMACH

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ABSTRACT

Background/Aims: Gastric cancer is the second leading cause of cancer-related death worldwide. The majority of gastric cancers is "intestinal-type" adenocarcinoma, caused in part by *H. pylori* infection. Chronic gastritis leading to atrophy and intestinal metaplasia (IM) can result in cancer. Studies have demonstrated reversibility of mucosal atrophy following *H. pylori* eradication. Concern has been raised regarding the sensitivity of gastric biopsy for *H. pylori* detection in the context of IM.

Materials and Methods: All cases of IM on gastric biopsy from a single gastroenterologist's outpatient practice were retrospectively reviewed from February 1, 2006 until May 31, 2012.

Results: In total, 105 IM cases were found, of which 37 (35.2%, 95% CI: 26.3-45.2) were *H. pylori*-positive on biopsy. Charts of the remaining 68 patients were reviewed for availability of other tests, namely urea breath test (UBT) and serology. Of 43 *H. pylori*-negative patients who underwent a UBT, 10 were positive for the infection (23.3%, 95% CI: 12.3-39.0). Amongst patients with coexisting autoimmune gastritis (AIG), 4 out of 9 (44.4%, 95% CI: 15.3-77.3) also had evidence of *H. pylori* infection by UBT, despite negative histology.

Conclusion: For cases of gastric IM with negative histology for *H. pylori*, UBT should be considered, even in cases of AIG, as this may alter the management and clinical course for patients.

Keywords: Gastric intestinal metaplasia, *H. pylori*, urea breath test, test accuracy

INTRODUCTION

Gastric cancer is the fourth leading cause of cancer worldwide and second leading cause of cancer-related death (1). The majority of gastric cancers is well-differentiated or "intestinal-type" adenocarcinoma, believed to be caused in part by *H. pylori* infection (1).

H. pylori was recognized as a class I carcinogen by the WHO in 1994, based mainly on epidemiological data. It is believed to cause intestinal-type gastric adenocarcinoma by inducing chronic active gastritis, which over decades evolves to multifocal atrophic gastritis, intestinal metaplasia (IM), dysplasia, and eventually cancer (2). Several studies have demonstrated the reversibility of mucosal atrophy following *H. pylori* eradication (3,4).

Gastric IM is considered to be a precancerous condition, and while there is controversy regarding the reversibility

of IM after eradication of *H. pylori* infection, several trials suggest a benefit (5-10). There has also been demonstration of stability of IM severity scores up to 10 years post-eradication of *H. pylori*, suggesting at the very least that treating the organism may prevent progression of this pre-cancerous condition over time (11-13).

While gastric biopsy is considered by most to be the "gold standard" for diagnosing *H. pylori* infection, with sensitivity and specificity exceeding 95% (14), the organism is known to *selectively* colonize gastric mucosa or gastric metaplastic tissue. With IM, colonization is impaired, possibly due to local production of antimicrobial factors (15) but also hypochlorhydria, hindering the stability of *H. pylori*. Because of this, we hypothesize that biopsy alone for the detection of *H. pylori* infection may be less accurate in the context of IM, particularly if the biopsies fail to sample non-metaplastic tissue. Hence,

urea breath test (UBT) may perform better, as it samples the urease activity of the entire stomach-more importantly, a larger surface area of non-metaplastic tissue.

MATERIALS AND METHODS

This retrospective study was conducted at the Jewish General Hospital, a McGill University-affiliated institution (Montreal, Canada). Approval from the hospital's ethics committee was obtained. Out-patient clinical charts from the practice of a single gastroenterologist (PG) were reviewed, from February 1, 2006 until May 31, 2012, looking for patients with IM. Facturation.net, an online billing service used by the principal investigator (PG), was searched to generate a list of patients bearing diagnostic code 5350, indicative of "gastritis," for the study period. This code was chosen for the search, since it is used consistently as a surrogate billing code for gastric IM by PG, due to lack of a separate dedicated code for this specific diagnosis by the billing service. It was also thought to be the most likely diagnosis to encompass most IM cases.

Charts of patients with a histological diagnosis of IM of the stomach were examined, and data were extracted for patient's age, sex, date of gastroscopy, presence or absence of *H. pylori* on biopsy specimen, and diagnosis of autoimmune gastritis (AIG). For the subgroup of patients without evidence of *H. pylori* on biopsy, results of urea breath test (^{13}C or ^{14}C-UBT) and *H. pylori* serology were also recorded, if these tests were performed.

All patients had a minimum of two antral and two gastric body biopsies. Giemsa staining was performed for pathologic assessment of *H. pylori* infection. The slides were read by any one of the four hospital pathologists specializing in gastrointestinal (GI) pathology.

Inclusion criteria were age ≥18 and presence of IM on at least one gastric biopsy report. Exclusion criteria were diagnosis of gastric adenocarcinoma, mucosa-associated lymphoid tumor (MALT), or other gastric malignancy. The study was approved by the hospital's ethics committee.

Statistical analysis

Basic descriptive statistics were calculated for the study variables. Estimates for sensitivity, specificity, and positive predictive value (PPV) and negative predictive value (NPV) of the gastric biopsy for the detection of *H. pylori* in the presence of IM were calculated using sample proportion estimates of conditional probabilities (16). Approximate 95% confidence intervals were constructed using the score interval and the Yates continuity correction.

RESULTS

A total of 105 patients with histologically proven IM of the stomach were identified (Figure 1). Indications for gastroscopy included dyspepsia, reflux symptoms, abdominal pain, bloating, weight loss, and iron deficiency anemia. Of the 105 patients, 57 (54.3%) were female. Mean age at endoscopy was 62.7 (range: 24-85). Thirty-seven patients (35.2%, 95% CI: 26.3-45.2) had evidence of *H. pylori* infection by histologic assessment.

Of the remaining 68 patients with histologic absence of *H. pylori* infection, 38 (55.9%) were female. Sixty-three patients were also sent for a UBT, with results available for 43 of these patients (68.3% of the cohort of patients who were negative for *H. pylori* as per histology). Ten of the 43 patients with absence of *H. pylori* on biopsy had a positive UBT result.

Only 14 of the 68 histologically negative patients had serological testing for *H. pylori,* of which 4 (28.6%) were positive. One was in a patient with positive UBT, and 3 had negative UBT results. Three (21.4%) patients with positive UBT had negative serology. The diagnostic flowchart is shown in Figure 2.
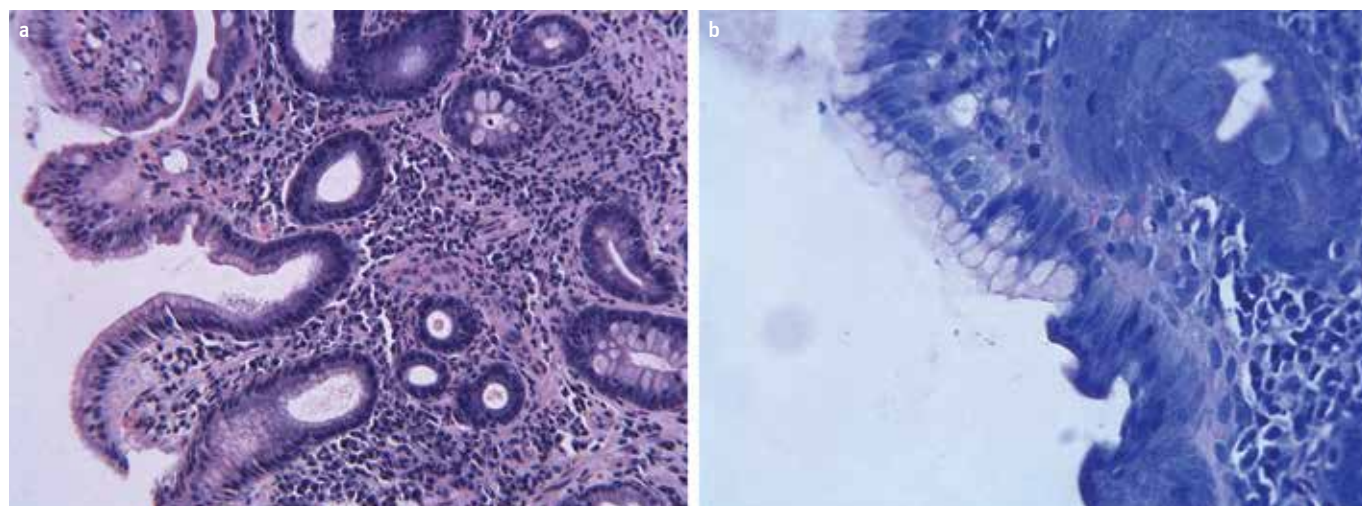

Figure 1. a, b. Gastric antrum of a study patient. Chronic active gastritis with polymorphonuclear leukocytes and intestinal metaplasia in the glands (hematoxylin and eosin stain, magnification x200) **(a)**. Intestinal metaplasia with goblet cells and absence of *H. pylori* organisms (Giemsa stain, magnification x400) **(b)**.
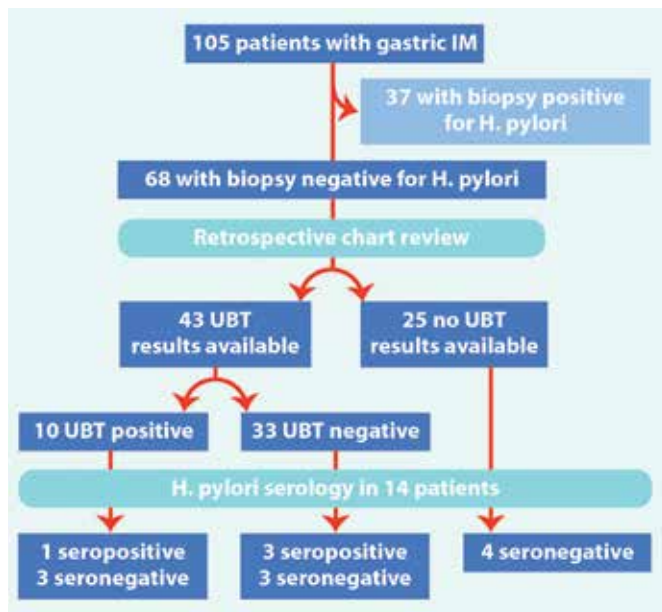
Figure 2. Flow diagram of patients included in the study. IM: intestinal metaplasia; UBT: urea breath test.

Of the 105 patients with IM of the stomach, 11 (10.5%, 95% CI: 5.6-18.4) had simultaneous histologic features diagnostic of AIG. Two of these patients also had *H. pylori* on histology, while 4 out of 9 (44.4%, 95% CI: 15.3-77.3) without *histological* evidence of *H. pylori* had a positive UBT. Thus a total of 6/11 (54.5%, 95% CI: 24.6-81.9) with AIG also had evidence of *H. pylori* infection.

The accuracy of gastric biopsies relative to UBT was assessed assuming a null false-positive rate for both biopsy and UBT or, equivalently, perfect specificity and positive predictive value (PPV). This yielded an estimated sensitivity of gastric biopsy for the diagnosis of *H. pylori* in IM patients of 78.7% (37/47, 95% CI: 63.9-88.8) and an estimated negative predictive value (NPV) of 76.7% (33/43, 95% CI: 61.0-87.7). The latter indicates that nearly one-quarter of all negative biopsy results in patients with IM of the stomach are false negatives. For the subset of patients with AIG and IM, the sensitivity of gastric biopsy for the diagnosis of *H. pylori* was 33.3% (2/6, 95% CI: 6.0-75.9), with an NPV of 55.6% (5/9, 95% CI: 22.7-84.7), meaning that almost half of all negative biopsy results were false negatives.

Because results were unavailable for 5 patients who were never sent for UBT and for 20 biopsy-negative patients for whom UBT was ordered (either because the patients never completed the exam or because the results were lost or outstanding at the time of analysis), a sensitivity analysis was performed to evaluate a range of plausible values for the NPV of gastric biopsy. Assuming the best-case scenario whereby all 25 such cases were assumed to be UBT-negative, the estimated false-negative rate for gastric biopsy falls to 14.7% (10/68, 95% CI: 7.7-25.8), implying an estimated NPV of 85.3% (95% CI: 74.2-92.3). Sensitivity would remain the same, as would specificity and PPV, each assumed to be perfect.

DISCUSSION

While gastric biopsy is still recognized by many as the "gold standard" test for the diagnosis of *H. pylori* infection (14), this is based on studies representing mostly patients without mucosal atrophy or IM of the stomach. The concern is whether gastric biopsy has lower accuracy in the context of IM because of *H. pylori*'s impaired ability to grow on metaplastic tissue.

Our study is not the first to question the accuracy of gastric biopsy for *H. pylori* diagnosis in the context of IM but reinforces the discrepancy in the results of biopsy as compared to UBT in this patient population. Some trials have compared biopsy with non-invasive tests in patients with *atrophic gastritis* of the stomach (17,18). In a study by Kokkola et al. (17), histology and ^{13}C-UBT were positive in 30% and 28%, respectively, showing comparable sensitivity but significantly lower than the 82% positivity rate of serology. Karnes et al. (18) compared gastric biopsy with serology, demonstrating good correlation between the two testing modalities in the general population but a discrepancy in the subgroup with atrophic gastritis, where 33% had positive histology as opposed to 86% with positive serology. One argument is that serology could remain positive for months post-eradication; so, at least part of the noted discrepancy could reflect decreased specificity on behalf of serological testing, as opposed to the solely impaired sensitivity of biopsy to pick up *H. pylori* infection. In the present study, too few patients had serology to draw meaningful conclusions; however, 6 out of 12 serology results were discrepant with UBT results, suggesting that the former test is much less reliable in the patient population studied. We do not at this time recommend serology as a means of evaluating for *H. pylori* in cases of gastric IM.

Interestingly, a Korean study comparing culture, CLO test (rapid urease test), histology, and serology demonstrated sensitivities, specificities, and predictive values >90% for all diagnostic modalities in patients *without* atrophic gastritis or IM (19). Meanwhile, the sensitivity of CLO test and specificity of serology were markedly reduced in patients with atrophic gastritis and even more so in cases of IM, prompting the authors to recommend a combination of at least two testing modalities in patients with IM (19). While histology (Giemsa staining) was deemed more reliable than CLO test or serology in this context, the accuracy of histology was also impaired with progression of atrophic gastritis and/or IM, with sensitivity and specificity dropping below 90% for moderate-severe cases (19).

Another study evaluated the accuracy of CLO test, Giemsa stain, and culture in detecting *H. pylori* infection in patients with atrophic gastritis with or without IM (20). All invasive tests performed worse in patients with atrophic gastritis and even poorer with IM. While CLO test seemed to be more affected than histology, Giemsa staining saw its detection rate for *H. pylori* drop from 91-100% in the absence of IM to 65.1% in the presence of IM with regard to antral biopsies (20).

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In our study, a positive UBT was considered indicative of active infection, even in the absence of *H. pylori* on biopsy, due to the very high specificity of UBT. Indeed, in a recent review comparing invasive and non-invasive diagnostic tests for *H. pylori*, UBT demonstrated the highest sensitivity, specificity (99%), and positive predictive value (99%) of all modalities (21).

While the accuracy of a gastric biopsy for *H. pylori* in the presence of IM was still reasonable, with 78.7% sensitivity and 76.7% NPV, there is still a non-negligible risk of a false-negative result. Because eradication of the offending agent may reduce the risk of advancing carcinogenesis, failure to detect and treat *H. pylori* infection in as many as 20% to 25% of patients is clinically relevant.

Our study also demonstrated an important miss rate for *H. pylori* infection based on histology alone for patients with AIG, a group already at higher risk of gastric cancer than the general population. Firstly, over half (54.5%) of patients with AIG also had evidence of bacterial infection by biopsy and/or UBT, suggesting the possibility that IM may have also been related to *H. pylori* rather than being exclusively attributed to hypochlorhydria from autoimmune loss of parietal cells. Moreover, it may support emerging evidence of a causative link between *H. pylori* and autoimmune gastritis, as evidenced by reports of a significant association between *H. pylori* infection and presence of APCA and antibodies to an intrinsic factor (AIFA) (22). In our study, 44% of AIG patients with negative histology had evidence of infection on UBT. Because of the impaired sensitivity of histology in this patient population and the risk of progression of IM to more advanced neoplasia over time, we suggests that patients with gastric IM in combination with AIG also have dual testing to more reliably exclude *H. pylori* infection.

Limitations of our study include its retrospective nature, small patient cohort (limited to one physician's practice), and the fact that only Giemsa staining was performed for the histologic diagnosis of *H. pylori*, which may have negatively affected the performance of biopsy. Had the study been prospective, further histological testing could have been performed in cases with discrepant UBT results, such as silver staining or immunohistochemical testing for *H. pylori*. Another limitation is the fact that the slides were evaluated by a single GI pathologist, as opposed to two or even three blinded pathologists. While these may be perceived as weaknesses of the study, they actually make it more clinically relevant, since, outside of scientific trials or tertiary care centers, it is common for Giemsa stain alone to be used for diagnosis of *H. pylori* and for the slides to be reviewed by a single pathologist, except in cases of dysplasia or suspected carcinoma where a second opinion is sought. Lastly, another limitation of this study is not having documented use of proton pump inhibitors, which may also affect the performance of histology for *H. pylori* detection.

Despite its limitations, our data raise questions about the reliability of histology used as the sole test for detection of *H. pylori*

when gastric IM is present, particularly in the way histology is performed in routine clinical practice. Given the evidence of stability or even regression of IM after eradication of *H. pylori*, it may be worthwhile considering dual testing (i.e., biopsy in combination with UBT or stool antigen testing where available) in this patient population. Prospective studies are needed to determine the cost-effectiveness of multiple tests for *H. pylori* in patients with IM and the subsequent impact of *H. pylori* eradication on the development intestinal-type gastric adenocarcinoma.

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