

# Gastric biopsies: The gap between evidence-based medicine and daily practice in the management of gastric *Helicobacter pylori* infection

Hala El-Zimaity MD, Stefano Serra MD, Eva Szentgyorgyi MD, Rajkumar Vajpeyi MD, Amir Samani MD PhD

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**BACKGROUND:** Many consider histology to be the gold standard for *Helicobacter pylori* detection. Because the number and distribution of *H pylori* organisms vary, particularly in patients taking proton pump inhibitors (PPIs), the American Gastroenterological Association recommends discontinuing PPIs two weeks before endoscopy, and taking biopsies from both the body and antrum.

**OBJECTIVE:** To assess the influence of clinical practice on the histopathological detection of *H pylori* infection.

**METHODS:** Electronic patient records were evaluated for the sites of gastric sampling and PPI use at endoscopy. One hundred fifty cases with biopsies taken from both antrum and body were randomly selected for pathological re-review with special stains. The gastric regions sampled, *H pylori* distribution and influence of clinical factors on pathological interpretation were assessed.

**RESULTS:** Between 2005 and 2010, 10,268 biopsies were taken to detect *H pylori*. Only one region was sampled in 60% of patients (antrum 47%, body 13%). Re-review of biopsies taken from both antrum and body indicated that the correct regions were sampled in only 85 (57%) patients. Of these, 54 were *H pylori* positive and 96 were *H pylori* negative. *H pylori* was present in the antrum in only 15% of the patients and body only in 21%. Of 96 *H pylori*-negative patients, two were reinterpreted as positive. Forty-seven per cent of patients were taking PPIs at endoscopy, contributing to both false-negative and false-positive diagnoses.

**CONCLUSION:** Despite national and international guidelines for managing *H pylori* infection, the American Gastroenterological Association guidelines are infrequently adhered to, with PPIs frequently contributing to false diagnosis; sampling one region only increases the likelihood of missing active infection by at least 15%.

**Key Words:** Endoscopic accuracy; Diagnostic accuracy; *Helicobacter pylori*; Histopathological diagnosis; Management guidelines

*Helicobacter pylori* infection remains clinically relevant, particularly in centres that serve the general population with a high mean age and a high number of immigrants (1-3). The infection plays an active role in many diseases, including peptic ulcer disease, gastric mucosa-associated lymphoid tissue (MALT) lymphoma and adenocarcinoma, dyspepsia, iron-deficiency anemia, idiopathic thrombocytopenic purpura and, in some patients, coronary artery disease (4-18). Active infection should be eradicated in patients with uninvestigated dyspepsia, active peptic ulcer disease, a high risk of gastric cancer and gastric MALT lymphoma (18,19). Importantly, to reduce the risk of gastric atrophy, active *H pylori* infection should be investigated and eradicated before administering proton pump inhibitors (PPIs) to patients with gastroesophageal reflux disease (20).

**Les biopsies gastriques : l'écart entre la médecine fondée sur des données probantes et la pratique quotidienne de prise en charge de l'infection gastrique à *Helicobacter pylori***

**HISTORIQUE :** Bien des gens considèrent l'histologie comme la référence pour déceler l'infection à *Helicobacter pylori*. Puisque le nombre et la répartition des organismes à *H pylori* varient, notamment chez les patients prenant des inhibiteurs de la pompe à protons (IPP), l'American Gastroenterological Association recommande d'arrêter de prendre des IPP deux semaines avant l'endoscopie et de prélever des biopsies à la fois dans le corps et l'antré.

**OBJECTIF :** Évaluer l'influence de la pratique clinique sur la détection histopathologique de l'infection à *H pylori*.

**MÉTHODOLOGIE :** Les chercheurs ont évalué les dossiers électroniques des patients pour déterminer les foyers de prélèvement gastrique et l'utilisation des IPP à l'endoscopie. Ils ont sélectionné au hasard 150 cas qui avaient subi des biopsies prélevées dans l'antré et dans le corps afin de procéder à un nouvel examen pathologique à l'aide de colorations spéciales. Ils ont évalué les foyers gastriques des prélèvements, la répartition du *H pylori* et l'influence des facteurs cliniques sur l'interprétation pathologique.

**RÉSULTATS :** Entre 2005 et 2010, 10 268 biopsies ont été prélevées pour déceler le *H pylori*. Le prélèvement a été effectué dans un seul foyer chez 60 % des patients (antré 47 %, corps 13 %). Le nouvel examen des biopsies prélevées dans l'antré et dans le corps a indiqué que les prélèvements provenaient des bons foyers chez seulement 85 (57 %) des patients. De ce nombre, 54 étaient positifs au *H pylori* et 96 y étaient négatifs. Le *H pylori* était présent dans l'antré de seulement 15 % des patients et dans le corps de seulement 21 % des patients. Chez les 96 patients négatifs au *H pylori*, deux ont été réinterprétés comme positifs. Quarante-sept pour cent des patients prenaient des IPP à l'endoscopie, contribuant à la fois à des diagnostics faux négatifs et faux positifs.

**CONCLUSION :** Malgré les lignes directrices nationales et internationales sur la prise en charge de l'infection à *H pylori*, les lignes directrices de l'American Gastroenterological Association sont peu respectées, et les IPP contribuent souvent à un faux diagnostic. Le prélèvement dans un seul foyer accroît d'au moins 15 % la possibilité de rater l'infection active.

Although several tests are available, many consider histopathological diagnosis of *H pylori* infection to be the 'gold standard' (21,22). Obtaining corpus biopsies in addition to antral biopsies has been shown to increase diagnostic accuracy (22,23); however, because PPIs decrease *H pylori* density, distribution and shape (24,25), their use renders the bacteria more difficult to detect. Therefore, the diagnostic accuracy of histopathology is dependent on which regions are sampled, pathological interpretation (26) and whether the patient is taking PPIs.

The American Gastroenterological Association (AGA) and American College of Gastroenterology (ACG) recommend discontinuing PPIs two weeks before endoscopy, and taking biopsies from both the body and antrum (9,18). The present study evaluated both

Department of Pathology, University Health Network, Toronto, Ontario

Correspondence: Dr Hala El-Zimaity, Toronto General Hospital, 200 Elizabeth Street, Toronto, Ontario M5G 2C4. Telephone 416-340-4551,

fax 416-340-5175, e-mail hala.el-zimaity@uhn.ca

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**TABLE 1**  
**Gastric regions sampled in biopsies in which endoscopy indicated antrum and corpus biopsies were obtained\***

Gastric region	n (%)
Antrum and body	85 (56.67)
Antrum and cardia	8 (5.33)
Body and cardia	15 (10)
Antrum only	16 (10.67)
Body only	26 (17.33)
Total	150 (100)

\*Only one patient had biopsies taken as recommended by the updated Sydney System (2 antrum, 2 corpus, 1 incisura)

daily endoscopy practice and its influence on the histopathological diagnosis of *H pylori* infection. We aimed to establish whether gastroenterologists regularly sample the gastric antrum and body; the frequency of PPI use at endoscopy; and how these endoscopic practices influenced pathological interpretation. In patients who were *H pylori* positive, we also evaluated the effect of biopsy site on diagnosis. The present study addressed the influence of everyday practice on diagnostic efforts.

## METHODS

### Selection of biopsy specimens

The pathology files at Toronto General Hospital (Toronto, Ontario) were reviewed for biopsy specimens submitted specifically for *H pylori* diagnosis between 2005 and 2010. The anatomical sites from where the biopsies were taken were noted to establish whether AGA guidelines had been followed for sampling the body and antrum.

Biopsies from 150 patients, in which both the antrum and corpus had been sampled at endoscopy, were randomly chosen for further clinical and pathological analysis. In this group of 150 patients, it was established whether both regions were correctly sampled (by analysis of the histology of the gastric mucosa – transitional mucosa was grouped with antral mucosa), and the density of *H pylori* within each region. The electronic patient record system was used to evaluate whether the patients were taking PPIs at the time of endoscopy.

To investigate the sampling pattern when the endoscopy report indicated that only one region was sampled, the histology pattern in 200 consecutive specimens that satisfied that criterion was reviewed.

### Histological and immunohistochemical staining

Hematoxylin and eosin sections were retrieved from the archives and two 4 µm sections were cut from each block. These were stained with dual silver/periodic acid-Schiff (27) and anti-*H pylori* rabbit polyclonal antibody (760-2645, Ventana Medical Systems Inc, USA; according to manufacturer's instructions), respectively. Each set of three slides was separately randomized and coded for use within the study.

### Histopathological examination of the slides

The slides were examined independently by four expert gastrointestinal pathologists and one trainee histopathologist. Observers were blinded to the clinical information of the patients, and had no knowledge of their own or their colleagues' interpretation of the other slides.

A visual analogue scale graded from 0 (absent) to 5 (high) was used to score *H pylori*, acute inflammation and chronic inflammation on each slide (28). A score of 1 indicated that only one or two bacteria were observed in the entire biopsy specimen, while a score of 5 indicated that the surface was covered with bacteria and bacterial aggregates. A biopsy specimen was considered to be positive when all observers agreed that bacteria with the characteristic morphological features of *H pylori* were present in at least one of the special stain (dual silver/periodic acid-Schiff- or immunohistochemistry-stained) slides. Slides exhibiting discrepancy among observers were resolved by joint re-examination to reach consensus interpretation.

### Data analysis

All scores were entered into a database and analyzed using Stata/SE version 11.2 (StataCorp, USA). The Fisher's exact test or, when appropriate, the  $\chi^2$  test (both two-tailed), were used for comparison of proportions;  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Evaluation of overall sampling pattern

From 2005 to 2010, gastric biopsies from 10,268 patients were specifically examined for *H pylori* infection in the pathology department of Toronto General Hospital. Specimens received from 6143 patients (60%) had biopsies taken from one region only, 4827 (47%) patients had biopsies taken from the antrum only and 1316 (13%) patients had biopsies taken from the body only. Thus, biopsies from both the antrum and corpus were taken only in 40% of the patients at endoscopy.

### Evaluation of sampling accuracy

**Biopsies in which the endoscopy report indicated that two regions were sampled:** In the 150 biopsy sets randomly chosen from patients in whom the endoscopy report indicated that biopsies had been taken from both the antrum and body, 701 individual biopsies (265 [38%] antrum, 393 [56%] body and 43 [6%] cardia) were reviewed. Each patient had a minimum of two biopsies available for examination (mean 6, median 4, range two to 12 biopsies).

Although the endoscopy report indicated that antral and body biopsies were obtained from each of these patients, histopathological review confirmed that biopsies originated from both regions in only 85 (57%) patients. In the remaining biopsies, the gastric region sampled was body only in 26 patients (17%), antrum only in 16 patients (11%), antrum and cardia in eight patients (5%), and body and cardia in 15 patients (10%) (Table 1).

**Biopsies in which the endoscopy report indicated that only one region was sampled:** In the 200 consecutive specimens the endoscopy report indicated antral biopsies were obtained from 178 patients (89%) and oxyntic biopsies were obtained from 22 patients (11%).

When the endoscopy report indicated antral biopsies were obtained, histology review confirmed that the biopsies were from the antrum in only 119 (66.85%). In the remaining specimens, the gastric region sampled was antrum and body in 47 (26.40%), antrum and duodenum in one (0.56%), body in 10 (5.62%) and transitional mucosa in one (0.56%).

When the endoscopy report indicated oxyntic biopsies were obtained, histology review confirmed oxyntic mucosa in 18 (81.82%). In the remaining specimens, three (13.63%) had antral biopsies, and one (4.56%) had antral and oxyntic mucosa.

Therefore, an extrapolation suggested that that 47% of patients in the entire cohort had biopsies from one region of the stomach only (30% [one-half of specimens in which endoscopy indicated only one region was sampled] and 17% of patients in which the endoscopy report indicated both regions were sampled but incorrect sampling was determined pathologically). The largest source of error appeared to be that endoscopists did not take biopsies far enough distally to ensure that the gastric antrum was actually sampled in the biopsies.

### Evaluation of histopathological sensitivity

From the histological re-assessment, 56 (37%) patients were positive for *H pylori* and 94 (63%) were negative. At routine signout, six of 56 (10.7%) patients had incorrectly been interpreted as *H pylori* negative. The biopsies from patients with a false-negative diagnosis either did not have any active inflammation or only had mild active inflammation. The average number of biopsies taken in patients with a false negative was three (range one to seven). In comparison, the mean number of biopsies taken in patients with a true-positive diagnosis was five (range two to 11).

Of 94 patients negative for *H pylori* on reassessment, two (2%) had been interpreted as positive for the infection at routine signout. Biopsies from these patients had mild active inflammation only. Active

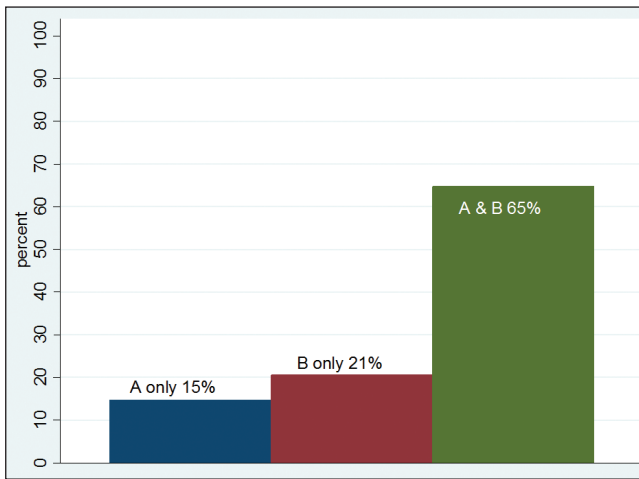


Figure 1) Bacteria distribution in biopsy sets that had both antrum (A) and body (B). A & B Antrum and body

TABLE 2  
Biopsy patterns in patients with a true-positive diagnosis compared with a false-negative diagnosis

Site	True positive	False negative
Antrum and body	33 (66)	1 (16.67)
Antrum and cardia	4 (8)	1 (16.67)
Body and cardia	3 (6)	0 (0)
Antrum only	7 (14)	2 (33.33)
Body only	3 (6)	2 (33.33)
Total	50 (100)	6 (100)

Data presented as n (%). False-negative results were from biopsy sets with few bacteria per biopsy, or from biopsy sets where the bacteria were present in one region. The antrum and body were correctly sampled in 33 of 50 (66%) patients correctly diagnosed with the infection. In contrast, the antrum and body were correctly sampled in only one of six (17%) patients who received a false-negative diagnosis (P=0.0194)

inflammation was focal and, in one case, was limited to an area with intestinal metaplasia. The sensitivity, specificity, negative predictive value and positive predictive value of histopathological analysis for *H pylori* in the present study were 89.29%, 97.87%, 93.88% and 96.15%, respectively.

The influence of sampling pattern (regions examined) on diagnostic accuracy

The antrum and body had been correctly sampled in 34 of 56 (61%) patients positive for *H pylori* infection. Bacteria were identified in the antrum only in five (15%) patients, and in the body only in seven (21%) patients, as shown in Figure 1. This confirms that obtaining biopsies from both the antrum and the body increases the probability of correctly diagnosing active infection.

In patients who received a true-positive diagnosis, the antrum and body were correctly sampled in 33 of 50 (66%) cases. In contrast, the antrum and body were correctly sampled in only one of six (17%) patients who received a false-negative diagnosis (P=0.0194), as shown in Table 2 and Figure 2.

Overall, sampling bias was apparent in many of the biopsy sets and was a major contributor to a false-negative diagnosis. *H pylori* density was generally low in biopsies with a false-negative reading (mean score of 1 [scale 0 to 5]). In these biopsy sets, only a few bacteria were present per biopsy, or the bacteria were distributed only focally within each fragment or were present in only a few of the available biopsy fragments (sampling bias for the infection).

PPI use and diagnostic accuracy

Seventy-one patients (47%) were taking PPIs at the time of endoscopy, as shown in Table 3. Of the patients with a false-negative diagnosis, four of six (67%) were taking PPIs at the time of biopsy. In

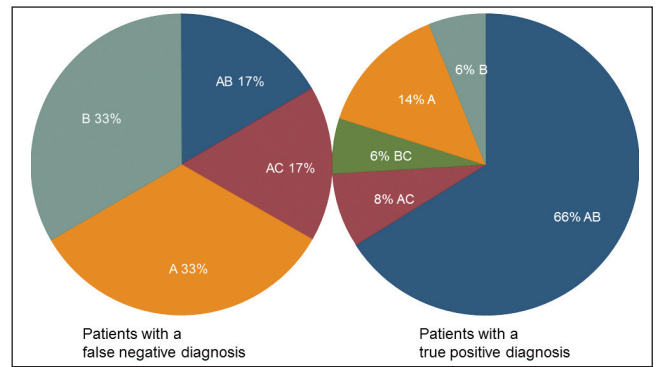


Figure 2) The influence of regions sampled on diagnostic accuracy. Biopsy material from both the antrum (A) and body (B) were more likely to be sampled in patients with a true-positive diagnosis (P=0.0194). AB Antrum and body; AC Antrum and cardia; BC Body and cardia

TABLE 3  
The frequency of proton pump inhibitor (PPI) use among patients

PPI use	Category				Total
	False negative	False positive	True negative	True positive	
No, n	2	1	48	28	79
Yes, n (%)	4 (67)	1 (50)	44 (48)	22 (44)	71 (47)
Total, n	6	2	92	50	150

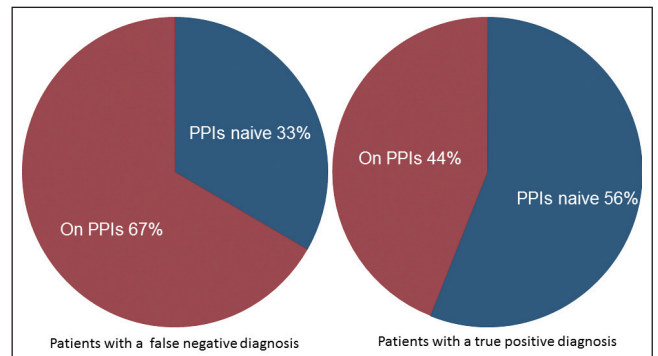


Figure 3) Frequency of proton pump inhibitor (PPI) use among patients

contrast, of the 50 patients correctly diagnosed positive for the infection (ie, true positives), 22 (44%) were taking PPIs at the time of endoscopy, as shown in Figure 3 (P=0.29). Parietal cell hypertrophy was observed in only one patient. Similarly, 44 of 92 (48%) patients with a true-negative diagnosis were taking PPIs, and histopathological examination confirmed PPI use in 17 (39%).

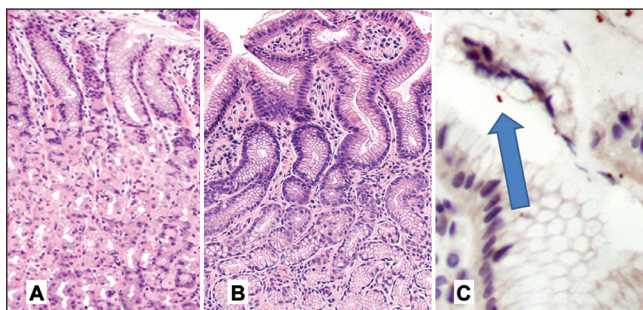
Furthermore, many of these patients exhibited chronic active inflammation. This suggests that PPI use is an important contributor to the inability to detect *H pylori* in patients with an otherwise typical *H pylori* pattern of chronic active inflammation.

DISCUSSION

Despite national and international guidelines (18,21) for the clinical management of *H pylori* infection, *H pylori* bacteria can be – and frequently are – missed on endoscopy. Factors that may cause a false-negative result include sampling the wrong area, taking an inadequate number of biopsies and/or continued use of PPIs at the time of endoscopy. All of the above factors are encountered in daily practice, including in the present study. As a result, each year, the incorrect diagnosis is made and many patients are inappropriately managed.

An important question is the probability of an incorrect diagnosis in patients undergoing endoscopy with subsequent histological





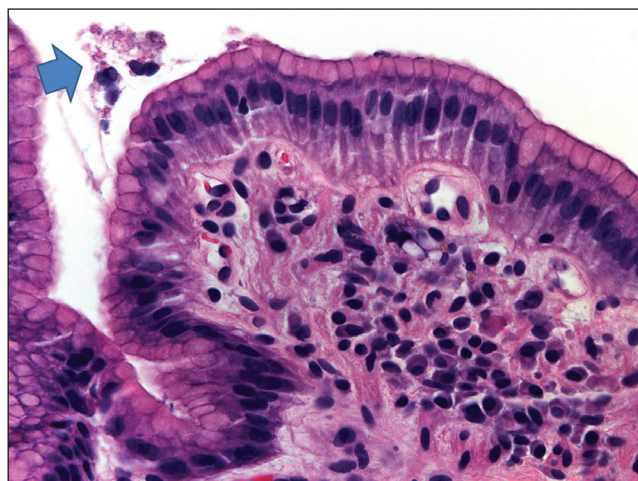
**Figure 4)** With proton pump inhibitor use, *Helicobacter pylori*-associated inflammation is absent or significantly reduced (A), oxyntic mucosa (hematoxylin and eosin, original magnification  $\times 5$ ). B Antral mucosa (hematoxylin and eosin, original magnification  $\times 10$ ). In addition, fewer bacteria (arrow) are present (C); *H pylori* monoclonal antibody (original magnification  $\times 10$ )

assessment of the biopsy. In the present study, 5% (one in 20) were misclassified during routine practice. In the false-negative group, the microorganisms were either rarely present, with only one or two bacteria per slide, or the distribution of microorganisms was in only a few biopsies per set, which may have been restricted to one region (antrum or body). In the absence of significant active inflammation, chronic inflammation was interpreted to be negative for *H pylori*, irrespective of severity. These results are consistent with previous studies that show *H pylori* may be present in biopsy specimens that are histologically close to normal, especially in the gastric body (23,29,30). *H pylori* is more likely to be missed if the classical histological picture is not present on the hematoxylin and eosin sections (31). This is particularly exaggerated with PPI use, for which bacterial density is low and typically more proximal (21,22) (Figure 4).

The present study confirms that obtaining biopsies from both the antrum and the body increases the probability of diagnosing active infection (32). These data are consistent with previous data in which a combination of a biopsy from angulus incisura and one from the greater curvature of the corpus is needed to correctly identify all treatment failures (23). To prevent missing a true-positive result when intestinal metaplasia is present at the incisura, it is optimal that corpus biopsies, as well as biopsies from the antrum closer to the pylorus than the incisura, are taken in addition to a biopsy from the incisura angularis (33).

With the decline in the incidence of *H pylori* infection in the Western world, the need to exclude other diseases, such as Barrett's esophagus, has taken precedence in routine clinical and pathological practice. The sampling behaviour among gastroenterologists in the present study may reflect this change, or perhaps a lack of awareness of the need to take both corpus and antrum biopsies to accurately exclude active infection. As such, the problem of false-negative diagnoses in the present study (10.7%) is likely to be an underestimate, given that the present study only re-examined biopsy sets that included both antrum and corpus from each patient. We excluded 60% of biopsy sets taken exclusively for *H pylori* diagnosis because the endoscopist had sampled only one region (antrum only in 47% and body only in 13%). Of the remaining patients, only 57% sampled both the antrum and body (with antrum alone sampled in 11% and body alone in 26%), as shown in Table 2. Thus, when both gastric regions were sampled, a large source of error was that biopsies were not taken far enough distally to ensure that the antrum was adequately sampled. In patients positive for the infection, inadequate sampling of both body and antrum can result in a miss rate of 15% when only antral biopsies are taken (organisms migrate proximally under a variety of circumstances, including in PPI use [21,22]), and a miss rate of 21% if only oxyntic biopsies are reviewed.

In the present study, biopsies from two patients (2%) were incorrectly interpreted to be positive for the infection (ie, false positive). Active inflammation in these biopsies was mild and focal. The



**Figure 5)** Apices of mucous cells may be cut in a plane such that they may resemble a curved organism. Hematoxylin and eosin stain, original magnification  $\times 40$

apices of mucous cells may be cut in a plane such that they may resemble a curved organism (Figure 5). Also, chains of cocci may be apparent and these may occasionally be confused with *H pylori*. It is possible that in some instances cocci represent degenerate forms of the organisms (34), although they can also be of both oral and duodenal origin. Both patients subsequently received triple therapy for *H pylori* infection with no side effects; so they were not seriously affected by the false-positive diagnosis. Nonetheless, it is important to point out the risks of unnecessary antibiotic use with respect to idiosyncratic or allergic reactions that are potentially fatal (35,36).

While it is possible to blame the operator (in this case, the pathologist), one also must question the context in which the mistake was made. False-negative and false-positive diagnoses are particularly observed in patients using PPIs and in patients who recently received antibiotics (31), but also if biopsies are only taken from one part of the stomach instead of both antrum and oxyntic mucosa (36).

The AGA and ACG guidelines emphasize the need to test symptomatic patients for *H pylori* before prescribing PPIs (9,21). PPIs were being used by 47% of patients at the time of endoscopy, with a higher frequency among patients who received a false-negative diagnosis (67% among patients with false-negative diagnosis versus 48% among patients with a true-negative diagnosis [ $P=0.37$ ]). *H pylori* flourishes in a pH range of 3.5 to 5 (37) and, therefore, PPI use decreases the number of bacteria present, as well as facilitating proximal migration of the bacteria (38). The lower bacterial count can increase the probability of a false-negative diagnosis given the small number of bacteria present. Therefore, PPI use may be a major contributor to the inability to detect *H pylori* histologically in patients who have an active infection, and gastrointestinal signs and symptoms known to be related to *H pylori* such as ulcer-like dyspepsia. Furthermore, many patients on PPIs experience chronic gastric inflammation (39). Because pathologists rely on inflammation as an indicator of active infection, this can increase the probability of a false-positive diagnosis. This suggests that PPI use is a major contributor to both false-negative and false-positive diagnoses.

In the Canadian population, individuals belonging to high-risk groups for *H pylori* infection total more than four million, based on birth origin and/or area of residence ([www.cdhf.ca/digestive-disorders/statistics.shtml#hpylori](http://www.cdhf.ca/digestive-disorders/statistics.shtml#hpylori)). *H pylori* infection is a potentially modifiable risk factor in many chronic diseases (gastric and nongastric) (40-42) and it is likely that *H pylori* infection accounts for a substantially under-recognized global burden of disease. In addition to identifying the true positive population, diagnostic accuracy is important to exclude false positives because inappropriate *H pylori* eradication treatment can precipitate pseudomembranous colitis, particularly in

elderly patients (43,44). In addition, patients with *H pylori*-negative duodenal ulcers appear to experience a significantly worse outcome when treated empirically (45).

The number and location of the gastric biopsies is important for the accurate identification of *H pylori* because biopsies series taken from the antrum or body would result in only a significant false-negative diagnosis. If endoscopists truly wish to maximize their yield of *H pylori*, they must note that the highest yield is from biopsies taken from the antrum. To obtain antral mucosa, biopsies must be taken distally enough to obtain histological antral mucosa, which needs to be quite close to the pylorus. This still results in a false-negative rate of 13%, emphasizing the need for biopsies from oxyntic mucosa as well as antral mucosa. Furthermore, if the antral mucosa is not sampled, either deliberately or accidentally, the false-negative rate for *Helicobacter*, if present, rises to 30%. Routine sampling for *Helicobacter* should, therefore, always include biopsies from both the antral and oxyntic mucosa.

Given the design of the present study, one question that could not be answered was the true false-negative rate. In the present study, it was apparent that the morphological appearance of *H pylori* infection (chronic active gastritis that could be maximal in either the antral or oxyntic mucosa), was accompanied by parietal cell hypertrophy,

indicative of hypergastrinemia, but practically of PPI use. It would, therefore, be sensible for the endoscopist to indicate whether the patient is on, or has recently been on PPIs or antibiotics, or had recently undergone *Helicobacter* eradication therapy, all of which can reduce the number of organisms to low or undetectable levels. Pathologists should be aware that the presence of an inflammatory pattern compatible with *H pylori*, and PPI-related changes in parietal cells, may be indicative of a false-negative biopsy. In addition, pathologists should also consider lymphocytic gastritis in which the bacterial load is low, especially in the oxyntic mucosa; lymphocytic gastritis is a significantly less common cause of gastritis that responds to *Helicobacter* eradication therapy (46). Finally, atrophic gastritis, especially with extensive intestinal metaplasia, may also result in a false-negative set of biopsies (33).

The joint AGA and ACG guidelines are provided to direct best possible care. Despite widespread dissemination and teaching of the guidelines, they are not adhered to in everyday practice. Practicing endoscopists need to be aware that they need to take biopsies from both (distal) antrum and oxyntic mucosa (ideally greater and lesser curve of both), and both clinicians and pathologists need to be aware that PPI use appears to be an important cause of a false-negative *H pylori* diagnosis, especially when the pattern of inflammation present suggests that organisms should be present.

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