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

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

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

<table>
<thead>
<tr>
<th>Gastric region</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum and body</td>
<td>85 (56.67)</td>
</tr>
<tr>
<td>Antrum and cardia</td>
<td>8 (5.33)</td>
</tr>
<tr>
<td>Body and cardia</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Antrum only</td>
<td>16 (10.67)</td>
</tr>
<tr>
<td>Body only</td>
<td>26 (17.33)</td>
</tr>
<tr>
<td>Total</td>
<td>150 (100)</td>
</tr>
</tbody>
</table>

*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 which 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 χ² 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
Why do we still miss *Helicobacter pylori*?

Can J Gastroenterol Vol 27 No 10 October 2013 e27

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).

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 the inability to detect *H pylori* in patients with an otherwise typical *H pylori* pattern of 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
with proton pump inhibitor use, Helicobacter pylori-associated inflammation is absent or significantly reduced (A), oxyntic mucosa (hematoxylin and eosin, original magnification ×5). B Antral mucosa (hematoxylin and eosin, original magnification ×10). In addition, fewer bacteria (arrow) are present (C); H pylori monoclonal antibody (original magnification ×10).

The apices of mucous cells may be cut in a plane such that they may resemble a curved organism. Hematoxylin and eosin stain, original magnification x40.

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

The apices of mucous cells may be cut in a plane such that they may resemble a curved organism. Hematoxylin and eosin stain, original magnification x40.

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 the 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 behavior 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 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 the 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 behavior 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
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 histologic 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 there is 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 stomach should be biopsied) and gastric cancer.

REFERENCES


