Utilization Rate of *Helicobacter pylori* Immunohistochemistry Is Not Associated With the Diagnostic Rate of *Helicobacter pylori* Infection

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Background: Utilization rates of immunohistochemistry (IHC) for the diagnosis of *Helicobacter pylori* infection may vary by laboratory and/or pathologists. IHC for *H. pylori* is not performed routinely in our practice. Instead, it is used in selected cases at the pathologists' discretion (and according to their specific criteria). The purpose of this study was to determine if IHC utilization rates correlated with rates of detecting *H. pylori* infection.

Materials and Methods: We searched our records and investigated all gastric biopsies for 1 calendar year. *H. pylori* diagnostic rate and IHC utilization rate was calculated for each pathologist.

Results: Overall, the rate of diagnosis was 12.1% and the IHC utilization rate was 45.2%. Individual pathologists had *H. pylori* diagnostic rates ranging from 3.6% to 34.1% (median: 11.1%) and IHC utilization ranging from 17.1% to 95.2% (median: 42.2%). The rate of detection of *H. pylori* infection among pathologists showed no significant correlation with rates of IHC utilization (Pearson coefficient = 0.121).

Conclusions: Increasing use of IHC is not independently associated with the diagnostic rate of infection. Ultimately, if we assume that the case mix was similar for each pathologist, it suggests that more liberal criteria to order IHC does not result in more infections diagnosed.

Key Words: *Helicobacter pylori*, *H. pylori*, gastritis, immunohistochemistry, IHC, gastrointestinal pathology, diagnostic rate, IHC utilization

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Helicobacter pylori is one of the most common bacterial infections worldwide, estimated to have infected ~50% of the world[1] and identified in 12% of gastric biopsies in the United States.[2] First identified as a pathogenic organism for peptic ulcers by Marshall and Warren[3] in 1982, *H. pylori* is a gram-negative rod that is either spiral or comma-shaped and measures 2.4 to 4.0 μm.[1,4] Since its discovery and identification, numerous studies have been published, firmly establishing the association of *H. pylori* with chronic gastritis, peptic ulcers, and gastric malignancy.[3,5,6] Because of these associations, there is great pressure on both clinicians and pathologists to accurately identify and treat patients with *H. pylori* infection.[7]

Current testing approaches for *H. pylori* include routine histology with or without special stains, rapid urease testing, molecular testing with polymerase chain reaction (PCR), serological antibody testing, urea breath tests, and fecal antigen test.[8,9] Although there is no defined consensus with regard to the "gold standard" testing protocol for the diagnosis of *H. pylori* infection, endoscopic gastric biopsies with histologic evaluation is generally accepted as a good method of diagnosing *H. pylori* and related pathologies. The major advantage of endoscopic biopsy-based histologic evaluation is that it provides near perfect sensitivity and specificity for the detection of *H. pylori* organisms.[7,8] An additional benefit is the possibility of diagnosing other inflammatory and neoplastic conditions (either distinct from, or as a consequence of *H. pylori* infection). Careful microscopic evaluation of routine hematoxylin and eosin (H&E)-stained preparation is usually sufficient to identify *H. pylori* organisms.[10,11] However, a variety of ancillary special stains (eg, Giemsa, Warthin-Starry, acridine orange, toluidine blue, Alcian blue, Diff-Quik) and immunohistochemical (IHC) antibodies against *H. pylori* are available to further aid the identification of *H. pylori* bacteria in tissue sections.[8–12] These ancillary tests provide benefit in some situations, but their availability raises questions. First, should all gastric biopsy cases be subjected to ancillary testing, and if so which test, and if not, which cases should be tested?

The convenience, quicker turn-around time, and putative ability to capture more positive cases, along with (potential) financial incentives in a fee-for-service environment have led many practices to adopt protocol "up-front" ancillary testing. The major downside to up-front staining is that it may be unnecessarily increasing the overall

costs associated with a pathologist's interpretation of a gastric biopsy.

Batts et al[8] provided guidelines for the use of *H. pylori* ancillary testing based on expert consensus among members of the Rodger C. Haggitt Gastrointestinal Pathology Society. The major point the authors emphasize is the fact that the vast majority of *H. pylori* infections can be diagnosed on H&E stain with high sensitivity and specificity of 91% to 100%.[9,13,14] The group recommended judicious use of IHC based on histologic findings and strongly recommends against up-front testing for *H. pylori* organisms. Chronic active gastritis (lymphoplasmacytic and neutrophilic inflammation) in which *H. pylori* organisms are not detected by routine stain is a definite indication for ancillary testing. The authors further suggest testing in chronic inactive gastritis with at least moderate gastritis.[8] Unfortunately, the distinction between mild chronic gastritis, without the need for further testing, and moderate chronic gastritis which should be tested, is incompletely defined. In contrast, the Houston update to the Sydney classification of gastritis seemed to suggest testing all gastric biopsies, to ensure a careful examination and due to perceived efficiency benefits. At other points in the manuscript, the authors made the recommendation somewhat more targeted by saying staining should be performed in "inflamed" biopsies in which *H. pylori* could not be detected by H&E.[15] This group discussed the limitations in diagnosing inactive chronic gastritis, including an imperfect definition of normalcy, geographic variation in gastric immune cell concentrations, and variably applied pathologic criteria.[15] Given these tremendous obstacles to a precisely defined abnormal state, it is not surprising that a practical point of distinction between "mild chronic inactive gastritis" and "moderate chronic inactive gastritis" was not offered.

At our institution, the use of ancillary testing for *H. pylori* is at the discretion of the attending pathologist. As a practical matter, special histochemical stains are rarely, if ever, used by our pathologists for this purpose, whereas the immunohistochemical stain (anti-*H. pylori* antibody; BioCare Medical, LLC, Concord, CA) is the default ancillary stain when one is considered necessary. No reflexive testing is performed and no strictly enforced rules are in place with regard to when an IHC stain should be ordered. Individual pathologists have no financial incentives to use or not use IHC. Thus, we hypothesized that there would be variability in *H. pylori* IHC usage rates among pathologists. We further hypothesized that since most pathologists would see a similar case mix, the difference in utilization rates would most likely be the result of varying criteria for testing. We assumed that different pathologists would have different thresholds for when an immunohistochemical test was required. We believe this to be a reasonable assumption due to the lack of broadly accepted diagnostic criteria and testing algorithms, as discussed above. On the basis of this assumption, we determined if those pathologists who used IHC more frequently had a higher *H. pylori* diagnostic rate than those using IHC less frequently. We also investigated if pathologists with subspecialty practice in gastrointestinal (GI) pathology used IHC at a different rate than general surgical pathologists.

MATERIALS AND METHODS

Following approval by the Columbia University Institutional Review Board [IRB-AAAO2353 (Y1M00)], all gastric biopsy pathology reports from the New York Presbyterian/Columbia University Medical Center during the calendar year 2013 were retrieved and retrospectively reviewed. The initial database query selected for any cases that had a part-type text "stomach," a term that is always included in specimen part-type assignment of stomach biopsies. Cases accessioned with multiple parts were included, as long at least 1 part of the case was from the stomach. Gastroesophageal junction and gastric cardia specimens were excluded as biopsies at these sites are commonly performed for evaluation of reflux-related pathologies and *H. pylori* is less frequently diagnosed in these sites.

Resulting data consisted of the case number, case pathologist, part-type information, final diagnosis, and comments/notes section texts queried from the Cerner CoPath Plus database. This was then tabulated onto a spreadsheet for data review. For each case, we reviewed the final diagnosis text, comments, and addenda (if applicable) in order to tally the following: (1) the number of gastric biopsies in the case, (2) the number of *H. pylori* positive biopsies, (3) the number of IHC stains ordered, and (4) the number of positive IHC stains.

These annotated data were imported as a comma-separated values file into RStudio with R version 3.2.0 (64-bit) to facilitate data manipulation and statistical analyses. We calculated the diagnostic rate of *H. pylori* positive cases and the number of IHC stains ordered on a per case basis. For our subgroup analysis, the IHC usage rate and diagnostic rates of *H. pylori* of GI versus general surgical pathologists were compared. At our institution, GI biopsies are divided between a subspecialized GI pathology service and a general surgical pathology service according to a load-balancing system that depends dynamically on several factors including daily case volume and optimization of resident education. We defined a GI pathologist (n = 8) as a faculty member who has completed a GI pathology fellowship and who rotates on the subspecialized service.

Statistical Methods

Using R software, we calculated the Pearson product-moment correlation coefficient to determine if diagnostic rate and IHC utilization rates among pathologists significantly correlated. The Welch *t* test was used to measure differences between the mean diagnostic rates and the mean IHC utilization rates within the GI versus general surgical pathologists subgroups.

RESULTS

Distribution of Cases and Pathologists

A total of 3751 cases matching our selection criteria were identified dispersed among 19 pathologists (Table 1). The median total case load was 127 and ranged from 41 to 518 per attending pathologist. GI pathologists (n = 8) had a median of 311 cases and a range of 42 to 518 cases and 11 general surgical pathologists had a median of 120 cases and a range of

TABLE 1. Summary Characteristics, Diagnostic Rates, and Immunohistochemical Utilization Rates by Individual Pathologists

Characteristics of Pathologists (n = 19)	
Cases per pathologist	
Range	41-518
Median	127
Diagnostic rate of *Helicobacter pylori*, by pathologist	
Range	3.6%-34.1%
Median	11.10%
IHC utilization rate, by pathologist	
Range	17.1%-95.2%
Median	42.20%

IHC indicates immunohistochemistry.

41 to 228. The median *H. pylori* diagnosis rate was 11.1% and the median IHC utilization was 42.2%, respectively, with wide ranges of diagnostic rates (3.6% to 34.1%) and IHC utilization rates (17.1% to 95.2%, Fig. 1). One individual was an outlier with a high *H. pylori* diagnosis rate of 34.1% with an IHC utilization rate of 72.7%, but we attributed this to a very low case load (44 cases) for this individual.

Overview of the Diagnostic Data

The overall *H. pylori* diagnostic rate was 12.1% with 455 of 3751 cases positive for *H. pylori* infection (Table 2). *H. pylori* IHC was performed in 1695 of 3751 cases, a 45.2% utilization rate. Individual pathologists' utilization rates ranged broadly from 17.1% to 95.2% (median: 42.2%), supporting our assumption that the pathologists used different thresholds for ordering IHC. We found that the IHC stain was positive for *H. pylori* in 140 of 1695 (8.3%) cases in which it was used.

Our subgroup analyses divided the data among GI pathologists (n = 8) versus general surgical pathologists (n = 11) (Tables 3, 4).

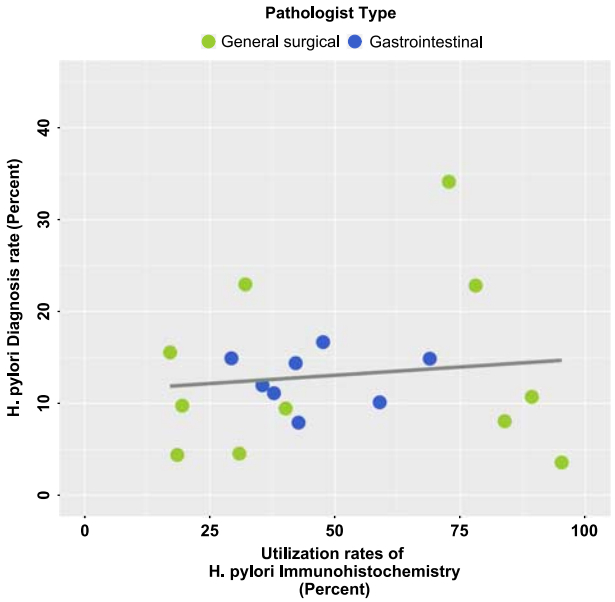

FIGURE 1. Utilization rates of *H. pylori* IHC and diagnoses of individual pathologists. Each data point represents the mean IHC utilization and diagnostic rates for an individual pathologist. No significant correlation between the utilization rate and the diagnostic rate was seen (Pearson product-moment correlation coefficient = 0.121, $P = 0.623$). IHC indicates immunohistochemistry.

TABLE 2. Distribution of Cases

	Total Cases	No. Cases	Distribution of Total Cases (%)
Total cases	3751		
Positive for *H. pylori*		455	12.1
Negative for *H. pylori*		3296	87.9
IHC utilized cases	1695	45.2%	
Positive for *H. pylori*		140 (8.3%)*	3.7
Negative for *H. pylori*		1555 (92.7%)*	41.5
Non-IHC (H&E) cases	2056	54.8%	
Positive for *H. pylori*		315 (15.3%)*	8.4
Negative for *H. pylori*		1741 (84.7%)*	46.4
H. pylori positive cases	455	12.1%	
Diagnosis made on H&E		315 (69.7%)*	8.4
Diagnosis aided by IHC		140 (30.7%)*	3.7
By training	3751		
GI (n = 8)		2436	64.9
General (n = 11)		1315	35.1

*Denotes percentage within the subgroup.
GI indicates gastrointestinal pathologist; H&E, hematoxylin and eosin; *H. pylori*, *Helicobacter pylori*; IHC, immunohistochemical stain.

IHC Usage Versus Diagnostic Rate

A scatterplot of IHC utilization rate versus diagnostic rates of each pathologist is presented (Fig. 1). Statistical analysis showed no significant correlation between IHC utilization rate and diagnostic rate (Pearson product-moment correlation coefficient = 0.121, $P = 0.623$).

TABLE 3. Cases Signed Out by General Pathologists

	Total Cases	No. Cases
Total cases	1315	
Positive for *H. pylori*		172 (13.1%)*
Negative for *H. pylori*		1143 (86.9%)*
IHC utilized cases	641	
Positive for *H. pylori*		47 (7.3%)*
Negative for *H. pylori*		594 (92.7%)*
Non-IHC (H&E) cases	674	
Positive for *H. pylori*		125 (18.5%)*
Negative for *H. pylori*		549 (81.5%)*
H. pylori positive cases	172	
Diagnosis made on H&E		125 (72.7%)*
Diagnosis aided by IHC		47 (27.3%)*

*Denotes percentage within the subgroup.
H&E indicates hematoxylin and eosin; *H. pylori*, *Helicobacter pylori*; IHC, immunohistochemical stain.

TABLE 4. Cases Signed Out by GI Pathologists

	Total Cases	No. Cases
Total cases	2436	
Positive for *H. pylori*		283 (11.6%)*
Negative for *H. pylori*		2153 (88.4%)*
IHC utilized cases	1054	
Positive for *H. pylori*		93 (8.8%)*
Negative for *H. pylori*		961 (91.2%)*
Non-IHC (H&E) cases	1382	
Positive for *H. pylori*		190 (13.7%)*
Negative for *H. pylori*		1192 (86.3%)*
H. pylori positive cases	283	
Diagnosis made on H&E		190 (67.1%)*
Diagnosis aided by IHC		93 (32.9%)*

*Denotes percentage within the subgroup.

GI indicates gastrointestinal pathologist; H&E, hematoxylin and eosin; *H. pylori*, *Helicobacter pylori*; IHC, immunohistochemical stain.

General Surgical Versus GI Pathologists

The subgroup analyses revealed that the diagnostic rate (12.7% vs. 13.3%, Welch *t* test, $P = 0.87$) and the IHC utilization rate (45.4% vs. 52.5%, Welch *t* test, $P = 0.51$) did not differ between pathologists with subspecialty practice in GI pathology versus those with general surgical pathology practice (Fig. 2).

DISCUSSION

We observed an institutional *H. pylori* diagnostic rate of 12.1%, a number within the reported diagnostic range (5% to 31.7%) of *H. pylori* in gastric biopsies in North America.[1,8,9] Overall, we demonstrated that the increasing frequency of IHC usage did not correlate with a higher rate of *H. pylori* diagnosis. In addition, we observed that the subspecialty GI pathology practice did not correlate with IHC utilization rates or differences in the diagnostic rates.

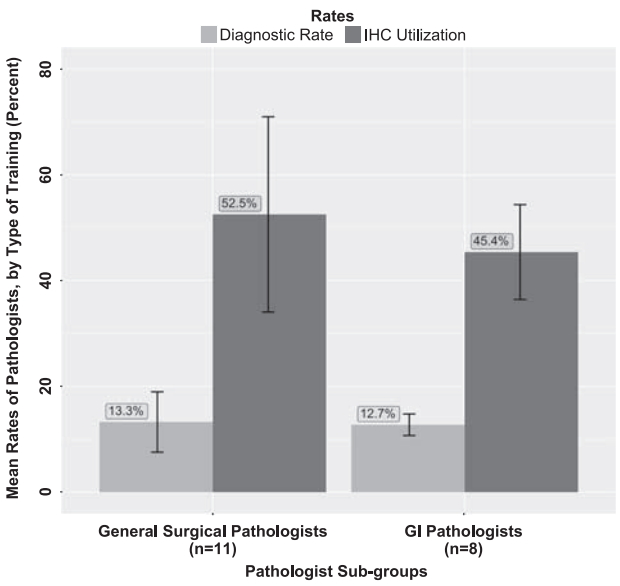

FIGURE 2. Comparison of IHC utilization rates and diagnostic rates of *H. pylori* between general surgical (n = 11) and gastrointestinal pathologists (n = 8). The subgroup analyses show that both the diagnostic rates (Welch *t* test, $P = 0.87$) and the IHC utilization rates (Welch *t* test, $P = 0.51$) are not significantly different between the 2 subgroups. The error bars indicate SE ranges. GI indicates gastrointestinal; IHC, immunohistochemistry.

Given our rigorous quality assurance program (which includes random rereview of cases), we assumed that neither false positives nor false negatives occurred with meaningful frequency. Given that IHC is ordered at the discretion of the attending pathologist at our institution and given that each pathologist sees a similar case mix, we suggest that the variability in utilization rates correlates to different thresholds at which IHC is ordered. The lack of correlation between IHC utilization rate and diagnostic rate indicates that there is no benefit to expanded testing criteria. In fact, such expanded criteria may be detrimental, in that they increase health care expenditures.

This study is not without limitations, such as its single-center setting. This study did not aim to compare sensitivity and specificity of H&E with IHC, and certainly there is a distinction to be made between sensitivity and diagnostic rate. This study is concerned solely with diagnostic rates. Given that our overall diagnostic rate of 12.1% is very similar to a study of nearly 80,000 gastric biopsies in the United States (12.0%) we feel confident that our diagnostic rate at least closely approximates true sensitivity.[2]

As we did not investigate the specific histologic characteristics which were associated with positive IHC, we cannot provide suggestions with regard to when IHC should and should not be ordered. However, we agree with others who have stated that IHC is not necessary in normal or nearly normal gastric biopsies or in biopsies in which the *H. pylori* bacteria can be appreciated by routine H&E stain. Furthermore, a recent guideline published by American Gastroenterological Association (AGA) affirms that the routine use of ancillary special staining would provide limited value in diagnosing *H. pylori* infections while increasing overall costs, in patients with dyspepsia.[16] In contrast, most experts would agree that it is a requirement in cases of chronic active gastritis in which organisms cannot be identified by routine stain.[4,8,11,12,16]

CONCLUSIONS

It is common in daily practice to encounter gastric biopsies with some degree of chronic inactive gastritis. More research is needed to determine the optimal level of inflammation that should be present in such cases to warrant IHC. Put another way, we could improve efficiency of care if there were evidence-based guidelines that allowed reliable distinction between "mild" chronic inactive gastritis in which IHC is not indicated and "moderate" chronic inactive gastritis in which it may be. Absent those data, practicing pathologists must set their own threshold for ordering IHC. This study answers the

question "if *H. pylori* IHC is ordered on an as needed basis (which is in keeping with current expert consensus), does ordering it more liberally result in significantly higher rates of detection?" The answer to that question is an unequivocal "no."

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