

How Reliable are General Histopathologists in Detection of *Helicobacter pylori* on Routine Histology?

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Abstract

Background: Many studies agree that routine histology is less sensitive and less specific than special stains and immunohistochemistry (IHC) with false-positive and false-negative rates. **Objectives:** The aim was to evaluate the accuracy of four general histopathologists in documenting *Helicobacter pylori* (Hp) bacteria in gastric biopsies (stained with H and E) by using IHC and modified Giemsa stain (MG), also to assess the specificity of MG and whether it can replace IHC. **Materials and Methods:** Twenty seven reported gastric biopsies were collected from each of four pathologists (9 reported as negative for Hp, 9 reported as scant Hp, and 9 as abundant Hp). The pathologists selected were known to have different thresholds for Hp. The biopsies were stained with MG and IHC and they were read by two pathologists. **Results:** The four pathologists showed different thresholds for labeling luminal gastric pits' structures as Hp. Overall, there were 5/36 false-negative cases (13.9%) and 26/72 false-positive cases (36%), most of them in the category reported as scant Hp detected. The results of MG were concordant with that of IHC in 107/108 cases. **Conclusion:** The overall accuracy rate of Hp detection on routine histology was 71%; false positivity was much more common than false negativity, especially when only scant Hp reported to be present. MG is a reliable stain and if done properly, it can replace the more costly IHC. When Hp identification is crucial from the clinical management of view, special stains and/or IHC should be requested.

Keywords: Gastritis, *Helicobacter pylori*, immunohistochemistry, modified giemsa stain, routine histology

INTRODUCTION

Since the discovery of *Helicobacter pylori* (Hp) bacteria in 1984 by Marshal and Warren,^[1] its role in the pathogenesis of chronic gastritis, peptic ulcer disease, MALTOMA, and gastric adenocarcinoma was highlighted. Many diagnostic techniques have been developed which include invasive and noninvasive tests. The usefulness, advantages, disadvantages, false negativity, and false positivity of those tests are heavily investigated.^[2-4] Despite the availability of those tests for the detection of Hp bacteria, none of them is universally accepted as the gold standard. In one study published in 2014, nested polymerase chain reaction was regarded to be the gold standard for Hp detection.^[5] Logically, the availability of many reliable noninvasive tests should have made invasive tests (gastroscopy and biopsy) obsolete, taking into account that histology is time consuming, labor intensive, and expensive. Nonetheless, upper gastric endoscopy is widely performed; it allows visualization of the mucosa and taking biopsies for histological examination.

The microscopic examination allows for the assessment of important features like the severity and the type gastritis, the

presence or absence of intestinal metaplasia, gastric atrophy, and any malignant transformation.^[6,7]

In addition, to those histological features, detection of Hp bacteria is one of the important elements of the gastric pathology report as Hp's role is well known in gastric pathologies and is recognized as the most common cause of gastritis and gastric lymphoma.^[8]

Hp bacteria can be visualized by routine histology, it appears as a Gram-negative, spiral-shaped bacterium whose main reservoir is the human stomach and sometimes the duodenum.^[9,10]

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However, these small bacteria could be easily confused with luminal debris within the gastric epithelial surface, and this is a potential source of inter-observer variability among histopathologists.^[11]

To enhance the detection of Hp organisms, many laboratories perform special stains, such as Giemsa stain (GS), modified GS (MG), Genta stain, Warthin–Starry stain (WS), and many others.

Over the years, researchers have investigated the sensitivity of routine histology in detection of the Hp and many researchers compared the sensitivity and specificity of different special stains and immunohistochemistry (IHC) in the detection of Hp bacteria.^[12]

Numerous studies concluded that IHC is the best method for Hp detection on histological sections.^[13] In addition, IHC is able to distinguish coccoid forms of the bacteria from other luminal non-Hp organisms, it also helps in the differentiation of luminal debris and cell borders from proper bacteria and from the observers' point of view IHC slide requires a shorter screening time.^[14]

The aim of the study was to assess the accuracy of general histopathologists in detecting Hp organisms on routine histology using IHC and MG and assessment of the sensitivity and specificity of MG in Hp detection and whether it can replace IHC?

MATERIALS AND METHODS

Study design and samples

Hundred and eight tissue blocks of gastric biopsies (formalin-fixed, paraffin-embedded) were collected from the pathology archive of Rizgary Teaching Hospital and two private laboratories (Erbil city Kurdistan region-Iraq). The cases were reported by four different pathologists with perceived different thresholds of identifying HP in gastric biopsy specimens. For each pathologist, 27 gastric biopsy tissue blocks were collected which were the most recent (from February 2020 going backward), 9 reported as negative for Hp, 9 reported as scant/low-density Hp, and 9 biopsies reported as abundant/numerous Hp identified. The only selection criteria were the Hp status in the pathology report, other details of the pathology reports were ignored like the severity or gastritis, intestinal metaplasia, or other features.

MG stains and Hp IHC were prepared and the slides were read by two of the four pathologists assessed in the study, one of them was perceived to have a low threshold for Hp detection and one was perceived to have high threshold for it.

For the IHC, the paraffin blocks were recut and 4-μm thick sections were prepared. The antibody used was ready-to-use polyclonal rabbit antibody from Dako/Agilent and was prepared using the EnVision FLEX detection system as per the manufacturer's protocol which included standard steps of IHC technique.

For the MG (MGG) staining, after cutting, dewaxing, and steps of rehydration, May Grunwald's was applied for 3 min, followed by washing with a weak stream of tap water, then GS was applied for 17 min, followed by washing again with tap water. Then, the slides were incubated for 10 min at 37° followed by mounting and coverslipping.

The data were analyzed using standard statistical analysis programs.

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. The study was approved by the Ethics Committee at Hawler Medical University.

RESULTS

As illustrated in the table [Table 1], cases reported as negative for Hp, the four pathologists achieved an accuracy of 86.3 (31/36 were correct). The accuracies among the individual four pathologists were 100%, 88.9%, 66.7%, and 88.9%, respectively. Pathologist number 1 (low threshold for Hp detection) achieved 100% accuracy in this category. Pathologist number 4 (high threshold for Hp detection) has one false negative, although he stressed in the report that the morphology of gastritis was highly suggestive of Hp gastritis despite the lack of Hp on routine histology.

In the category where Hp was reported as numerous, the overall accuracy was 83.3 (30/36 correct). The accuracy of the four pathologists were 66.7%, 77%, 88.9%, and 100%, respectively, pathologist number 4 (high threshold for Hp detection) achieved 100% accuracy in this category.

In the third category where Hp bacteria were reported as (scant/low density), the overall accuracy was 44.4% and individually was 22.2%, 44.4%, 55.6, and 55.6%. In this category, pathologist 1 has significant false-positive diagnoses close to 80%, but even pathologist 4 who is perceived to have a high threshold for Hp detection has about 45% false-positive cases. In this study, MG was consistent with IHC in 107/108 cases.

DISCUSSION

Despite the presence of many noninvasive tests for the detection of Hp bacteria, histological detection remains an important tool and can guide the clinician in making proper decisions in individual cases.

There are many histological techniques (special stains and IHC) to assist the pathologist in histological detection of Hp.

Many studies have evaluated the specificity and the sensitivity of those techniques and many of them have shown different detection rates between routine H and E and special stains.^[15]

Hp organisms appear as small curved structures in the gastric pits, many other structures could mimic Hp and pathologists

have different thresholds in interpreting these luminal findings as Hp [Figure 1].

Figure 1 illustrates debris that is easily recognized as junk (a and b) and debris that is easily confused with Hp bacteria (c and d). Figures 2 shows debris and proper Hp bacteria on MG (a and b) and debris and proper Hp bacteria on IHC (c and d) [Figures 2].

In this study, the four pathologists involved are all general pathologists and none of them is a specialist gastrointestinal (GI) pathologist, they are numbered 1, 2, 3, and 4. Number one was perceived by the gastroenterologists as having a low threshold for Hp detection (numerous positive cases), while pathologist 4 was perceived to have a high threshold with numerous negative cases.

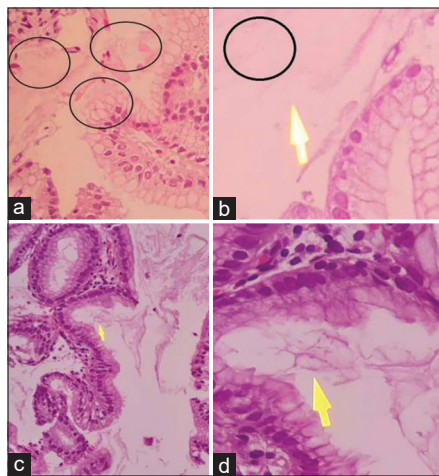


Figure 1: (a and b) (H and E $\times 400$), Examples of luminal debris and cell borders that is easily recognized and unlikely to be reported as *Helicobacter pylori*. (c and d) (H and E $\times 400$), Luminal debris and dirt which are easily confused with *Helicobacter pylori*

The current results confirm that pathologists have different thresholds in interpreting small dark luminal structures in the gastric surface epithelium as true Hp bacteria. It also shows the limitation of routine histology in proper Hp detection when the bacteria are scant.

This variability of pathologists in Hp detection was documented in other studies. A Scandinavian study published in 2013 found that the agreement between four pathologists in the detection of Hp on H and E was moderate. In that study, 82 cases were reviewed and the pathologists made a positive diagnosis in 56% to 84% of the specimens (significant heterogeneity, $P < 0.01$).^[16]

Another study published in 1999 has focused on the pathologist's experience in the bacterial detection. It compared

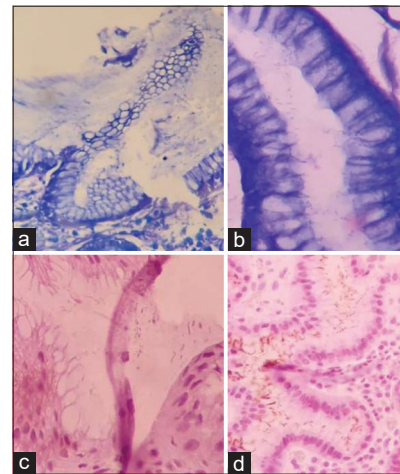


Figure 2: (a-Modified Giemsa stain $\times 400$) nonbacterial debris, (b-modified Giemsa stain $\times 400$) numerous *Helicobacter pylori*. (c-immunohistochemistry stain $\times 400$) nonbacterial debris, (d-immunohistochemistry stain $\times 400$) abundant true *Helicobacter pylori* bacteria

Table 1: The results of *Helicobacter pylori* immunohistochemistry on previously reported gastric biopsies by four pathologists

Hp detection by H and E and IHC	Path 1	Path 2	Path 3	Path 4	Total
High-density Hp by H and E	9	9	9	9	36
False positive by IHC	2	3	1	0	6
True positive by IHC	7	6	8	9	30
Accuracy rate (%)	77.8	66.7	88.9	100.0	83.3
Low-density Hp by H and E	9	9	9	9	36
False positive by IHC	7	5	4	4	20
True positive by IHC	2	4	5	5	16
Accuracy rate (%)	22.2	44.4	55.6	55.6	44.4
Negative for Hp by H and E	9	9	9	9	36
False negative by IHC	0	1	3	1	5
True negative by IHC	9	8	6	8	31
Accuracy rate (%)	100.0	88.9	66.7	88.9	86.1
Total	27	27	27	27	108
Overall accuracy rate (%)	67	67	41	81	71

Path 1: Pathologist number with a low threshold for Hp detection (low threshold for identifying luminal debris as Hp), Path 2 and path 3: Pathologists 2 and 3 with a medium threshold for Hp detection, Path 4: Pathologist 4 with a high threshold for Hp detection. Hp: *Helicobacter pylori*, IHC: Immunohistochemistry, H and E: Hematoxylin and eosin

the accuracy of Hp detection by expert and nonexpert pathologists. While the level of experience was not strictly defined, the expert pathologist showed high sensitivity (98.8%) and specificity (100%), as compared to the nonexpert pathologist (sensitivity 92.6%; specificity 90.3%), emphasizing an inter-observer variability influenced by the level of experience.^[17] The effect of the expertise of the pathologist is also emphasized in other studies.^[18-20]

However, in our study, all pathologists involved have comparable levels of experience, but they had different training backgrounds.

On the other hand, the agreement in Hp detection was very high (reaching 98%) in another study that included four pathologists (all of them were American board certified). In that study, the pathologists' very high concordance rate was based on the examination of slides stained with the triple stain (Sliver, H and E and Alcan blue at pH of 2.5) and not on routine histology; furthermore, the biopsies in that study were all taken with a jumbo forceps yielding larger than average tissue pieces, and the biopsies were processed by a staff specialized in handling GI samples.^[21] Hence, the high rate of concordance achieved in that study cannot be compared to our results.

The false-positive and false-negative cases, which one is more common?

Our study highlighted a significant rate of false-positive Hp detection that reached 36% and most of them in the category of (low-density Hp) group, while the rate of false negatives was about 14%.

This rate of false-positive detection in our study – while still high – is comparable to the findings of another Scandinavian study in which the issue of overdiagnosis (false positivity) was documented.

In that study, four observers assessed the presence of Hp in gastric biopsies taken from 40 patients (20 negative and 20 positive cases confirmed by culture). Histological slides were stained with MG, WS, and IHC. Of the 20 negative cases that were seen by four observers (yielding 80 scores), there were 37, 14, and 8 false-positive scores by all observers for MG, WS, and IHC, respectively.

The authors suggested that non-Hp flora may have accounted for the overdiagnosis of Hp in gastric biopsies. Kappa values for the variance between the four observers were 0.28 for MG, 0.57 for WS, and 0.83 for IHC.^[22]

In the above study, the disagreement was based on special stains and IHC and if H and E was used alone, the disagreement could have been wider.

False positivity was also a problem in a Turkish study in which 78 antral biopsies were studied, 66 of them were Hp positive by GS and H and E, but only 56 were positive by IHC.^[23]

On the other hand, a study conducted in the UK which included 20 cases reviewed by 20 pathologists creating 400 reports has

found that the false-positive diagnoses were much less common than false-negatives, but like our study, the results from cases where Hp was numerous were more consistent, with a detection rate of 89% (as opposed to 90% in our study).^[24]

This difference between our findings and the above British study is likely to be due to the nature of training and the threshold every pathologist set for himself in calling luminal structures as Hp organisms, another probable factor is the quality of the histological preparations which can affect the interpretation of luminal debris. The quality of H and E staining can be variable from one laboratory to another and each laboratory should assess its H and E stain quality in the detection of Hp.^[25]

Are special stains needed? Is modified Giemsa stain reliable?

Many special stains are available for histological identifications of Hp, carbofuchsin was one of the first used following Hp discovery.^[26] Currently, the commonly used special stains for Hp detection are GS, MG, WS, and Genta stains. Less common stains like the modified Diff-Quik stain have also been used.^[27] The sensitivities and specificities of these stains are variable in the literature.

One study reported the sensitivity of Hp detection for MG, WS, and IHC to be $90.0 \pm 10.0\%$, $70.0 \pm 14.1\%$, and $83.8 \pm 11.1\%$, respectively, and the specificities were $53.8 \pm 19.3\%$, $82.5 \pm 9.6\%$, and $90.0 \pm 0.0\%$, respectively.^[22]

Another study assessed the usefulness of Genta stain against IHC in which 100 positive cases and 100 negative cases by Genta stain were assessed by IHC, the results showed very good sensitivity and specificity of Genta stain with only three false negative and two false positive recorded.^[28]

Another study investigated the sensitivities of H and E, GS, and Genta stain, it found them to be comparable (H and E, 92%; GS, 88%; and Genta, 91%), but the H and E specificity (89%) was significantly lower than that of the special stains (98%). Unsurprisingly, the sensitivity for all these three stains was significantly lower at low Hp density than at high density (H and E, 70% vs. 98%; GS, 64% vs. 96%; Genta, 66% vs. 97%).^[11]

In another study, the sensitivity of WS was 94.4%,^[3] while triple stain (Steiner, H and E, and Alcian blue at pH 2.5) was used by other researchers and was found to be as sensitive as the WS for the detection of Hp and significantly more sensitive than H and E alone.^[29]

Gram's stain was used in another study and the sensitivity and specificity for Hp detection were 92% and 100%, respectively.^[30]

MG proved to be more reliable than McMullen and silver stain, but from the observer point of view, the silver stain slide was easier to read because the silver coating makes the bacteria look thick larger.^[12]

However, the sensitivity of MG stain was not that promising in another study which included 207 cases, 124 of them were positive by IHC (64.6%). Of those 124 cases, only 91 were positive by MG (73.3%) and only 48 cases were positive by H and E.^[13] The sensitivity of MG in the above study was significantly lower than many other studies including ours.

In the above quick review, it is clear that the sensitivity and specificity of those special stains are not consistent. The likely explanation is the quality and to a lesser extent the observer's experience.

In the author's practical experience, the quality of staining techniques is variable in different laboratories for individual stains; in some laboratories, GS is no superior to well-prepared H and E slide, while in other laboratories, the stain is very helpful and the same is true for WS, so the quality of the staining techniques is probably the main reason for the differences of sensitivities and specificities of those special stains, and in the author's experience, if WS is prepared well, it is much easier to read than other special stains.

In the current study, MG was as good as IHC with over 99% (107/108) concordance, although detection of Hp bacteria on the IHC was much easier and less time consuming than the MG.

Is immunohistochemistry needed?

Unlike the variability in the sensitivity and specificity of different special stains for Hp detection, IHC is more consistent and the reliability and superiority of IHC over special stains have been confirmed in numerous studies and review articles.^[12,13,31]

Some authors emphasized the ability of IHC to detect the coccoid forms of the bacteria. The coccoid form could be easily confused with other non-Hp bacteria and in this situation, IHC is far more reliable than special stains.^[22,23,32,33] The coccoid form of the bacteria is thought to be a form of bacterial adaptation to an adversarial environment.^[34]

Other studies highlighted the need of IHC in patients treated for Hp eradications, they found that IHC was more accurate than classical methods in identifying any residual Hp after eradication therapy and that IHC should, therefore, be used when the aim is achieving eradication.^[35] IHC was also more accurate when the rapid urease test (RUT) is negative (in cases of mild gastritis).^[36]

Despite all these published works supporting the use of IHC, an American study recommended that IHC for Hp is not necessary because the sensitivity and specificity of H and E stain between four observers were 100% in third observer and 95% by the fourth observer.^[33] The finding of the latter study is not in line with the majority of the published articles that addressed this issue. Besides, the introduction of so many histological special stains in itself is strong evidence proving the limitation of the routine histology. The sensitivity and specificity of detection of HP by routine histology are reported to be between 69%–93% and 78%–90%, respectively.^[14]

In the current study, a significant number of false positive cases were identified and confirmed by IHC.

CONCLUSION

We conclude that general histopathologists have different thresholds for detection of Hp on routine histology depending on their experiences and training background and are more likely to make a false-positive Hp detection. The overall accuracy rate of H and E for Hp detection was 71%.

When the histological identification of the bacteria is essential, gastroenterologists should request further confirmatory histological techniques other than routine histology.

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Conflicts of interest

There are no conflicts of interest.

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