Appropriate Use of Special Stains for Identifying *Helicobacter Pylori*: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society

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Abstract

Helicobacter pylori is a major cause of gastroduodenal disease, gastric cancer, and lymphoma, and thus, there is great interest in its detection and eradication. Several methods of *H. pylori* detection are available, including a variety of histochemical and immunohistochemical stains that may be applied to histologic sections. Although these stains were developed to enhance *H. pylori* detection among infected patients, changing practice models, financial considerations, and a perceived need for rapid turnaround of biopsy cases have led to their widespread use beyond that which was initially intended. Indeed, a recent survey of the Rodger C. Haggitt Gastrointestinal Pathology Society membership revealed that nearly 50% of pathologists perform at least one ancillary stain for *H. pylori* "up front" on all gastric biopsies prior to review of initial sections. Although ancillary stains for *H. pylori* are widely utilized, their added value to routine evaluation of hematoxylin and eosin (H&E) stained sections has never been demonstrated. They are largely unnecessary since *H. pylori* are readily demonstrated in H&E stained sections of biopsies obtained from most infected patients. Failure to identify bacteria by H&E evaluation generally reflects their absence in biopsy material; pathologists rarely, if ever, detect *H. pylori* in "normal" biopsies. The purposes of this review are to critically evaluate the literature regarding the utility of ancillary stains for *H. pylori* detection and to propose practice guidelines for their use. It is our hope that these recommendations will provide helpful information to surgical pathologists, gastroenterologists, and other interested parties, such as third-party payors.

Introduction

The link between *Helicobacter pylori*, previously known as "*Campylobacter pylori*", infection and gastritis and peptic ulcer disease was established in the seminal publication by Marshall and Warren in 1984 (1). This discovery sufficiently impacted science such that the authors were awarded the Nobel Prize in Physiology or Medicine in 2005. It is now inarguably clear that *H. pylori* is the dominant cause of gastritis worldwide. Major disease associations with *H. pylori* include duodenal and gastric ulcers, chronic gastritis, atrophic gastritis, iron deficiency, MALT-type lymphomas of the stomach, and gastric adenocarcinoma (2). Indeed, *H. pylori* is considered a Class 1 carcinogen by the World Health Organization (3).

Eradication of *H. pylori* is commonly recommended when it is identified (4). Strong recommendations for eradication are made if active peptic disease, untreated confirmed history of peptic ulcer disease, low grade gastric MALT-type lymphoma or locally excised early gastric cancer are present. However, the role of *H. pylori* eradication in non-ulcer dyspepsia, unexplained iron deficiency, populations at risk for gastric cancer, co-existent non-steroidal anti-inflammatory drug (NSAID) use, and gastroesophageal reflux disease (GERD) is controversial (5).

Helicobacter pylori infection always induces some degree of chronic inflammation of the gastric mucosa, although the severity of inflammation may vary depending on the duration of infection as well as the presence, or absence, of bacterial virulence factors

including CagA (6). Most infections are associated with moderate to severe chronic gastritis characterized by a band-like superficial infiltrate rich in mononuclear cells and plasma cells, often in combination with neutrophilic inflammation (*i.e.* chronic active gastritis) of gastric pits and surface epithelium. Persistent inflammation leads to intestinal metaplasia and atrophy of the gastric mucosa. Successful *H. pylori* eradication causes a fairly rapid disappearance of neutrophils and gradually diminishing inflammation, but intestinal metaplasia tends to persist (7).

Pathologists rarely, if ever, detect *H. pylori* in "normal" biopsies, yet many laboratories perform ancillary stains for the bacteria in a wide variety of situations. Indeed, a recent survey of the Rodger C. Haggitt Gastrointestinal Pathology Society membership revealed that nearly 50% of pathologists with specific interest in gastrointestinal pathology perform at least one ancillary stain for *H. pylori* "up front" on all gastric biopsies prior to review of initial sections. These practices contribute to escalating health care costs, although their added value to routine evaluation of hematoxylin and eosin (H&E) stained sections has never been demonstrated. The purposes of this review are to critically evaluate the literature regarding the utility of ancillary stains for *H. pylori* detection and to propose practice guidelines for their use. It is our hope that these recommendations will provide helpful information to surgical pathologists, gastroenterologists, and other interested parties, such as third-party payors.

Techniques for *Helicobacter Pylori* Detection

Helicobacter pylori detection methods can be divided into non-invasive and invasive techniques based on whether or not tissue is obtained by biopsy. While the focus of this review is the appropriate use of histochemical and immunohistochemical stains once tissue has been obtained, a brief discussion of the various means of detecting *H. pylori* is *apropros*, as the results of such testing could influence stain utilization.

Non-Invasive Tests

Anti-Helicobacter antibodies

Anti-helicobacter antibodies form in almost all patients with *H. pylori* infection and they are detectable by readily available serologic tests that show a very high sensitivity (90-97%) for *H. pylori* infection (8). Specificity for *H. pylori* infection is also reasonably high. However, antibodies persist for a considerable period of time after successful eradication of the organism and, thus, serologic positivity does not necessarily imply ongoing infection. The likelihood of finding *H. pylori* by invasive means in a patient with known negative serologic studies is very low. Only 2% of *H. pylori* infected patients are seronegative for both IgG and IgA (9). On the other hand, the likelihood of finding active *H. pylori* infection in patients with positive serologic studies probably depends on epidemiologic factors. Positive *H. pylori* serologies are highly associated with current infection in parts of the world where *H. pylori* is endemic and treatment is either largely unavailable or not clinically indicated. The likelihood of a serology-positive individual having *H. pylori* infection is much lower and may approach 50% in

non-endemic areas where readily available antibiotic therapy is available, such as the United States.

Helicobacter antigen stool assay

Stool antigen testing utilizes polyclonal or monoclonal antibodies directed against bacterial antigens in an enzymatic immunoassay. These assays detect *H. pylori* in nearly 95% of patients with active infection (10). Treatment with proton pump inhibitors does not decrease their sensitivity (11).

Urease breath testing

The assay is based on the principle that urease activity is present in the stomachs of *H. pylori* infected individuals. Patients ingest urea labeled with a carbon isotope (^{13}C or ^{14}C), which is cleaved by urease to produce labeled CO2 that is detected on the exhaled breath. The urease breath test detects active infection with high sensitivity (>95%) and specificity (>95%), although treatment with proton pump inhibitors does decrease its sensitivity (11, 12). Both stool assays and urease breath tests are preferred methods of detecting ongoing *H. pylori* infection by non-invasive means (8).

Invasive Tests

Rapid urease tests

A number of commercially available rapid urease, or *Campylobacter*-like organism (CLO), tests are available. These assays rely on the urease activity of *H. pylori* to change the color indicator of a substrate to which tissue biopsy fragments are directly applied.

The tests are inexpensive once the biopsy has been obtained and have high specificity and sensitivity, although the sensitivity is lower when *H. pylori* organisms are present in small numbers (8). These assays do not allow assessment for morphologic disease patterns of the mucosa (*e.g.* atrophic gastritis, malignancy) when used independent of histologic examination and are probably redundant when performed in combination with histologic biopsy interpretation.

Culture for organisms

Cultures for *H. pylori* can be performed directly from biopsy samples and have essentially 100% specificity for *H. pylori* infection. Several issues preclude their widespread use. First, they have lower sensitivity than other assays. Second, cultures are fastidious in nature and require immediate attention since *H. pylori* organisms are fragile outside their native environment (8). Third, cultures are similar to the CLO test in that they do not allow morphologic assessment of the mucosa.

Molecular testing for Helicobacter pylori

Helicobacter pylori can be detected by *in situ* hybdridization or polymerase chain reaction (PCR). Although their performance characteristics are high, both tests detect organisms, regardless of whether they are alive or dead and are susceptible to contamination (8). These assays are also expensive and are not generally used for routine evaluation of patients. Molecular testing does not allow for morphologic assessment of mucosal biopsy samples.

Histologic examination

Histologic evaluation of H&E stained sections of the gastric mucosa detects *H. pylori* infection with sensitivities approaching 100% in some studies. Ancillary histochemical and immunohistochemical stains enhance detection even further in specific situations. The former utilize dyes that directly stain the organisms, whereas the latter involves indirect staining of organisms by means of an antibody to *H. pylori* tagged to a dye. A host of histochemical stains have been used to identify *H. pylori* and include Wright-Giemsa, Toluidine blue, "Genta", Warthin-Starry, Alcian blue, and many others. Immunohistochemical stains remain the gold standard for *H. pylori* detection and have near 100% sensitivity and specificity. However, immunohistochemical stains are more costly than histochemical stains and their availability in remote and third world areas is limited. For these reasons, there is still interest in the application of various histochemical stains to gastric mucosal biopsies for *H. pylori* detection.

Assessing the optimal choice of special stains requires some historical context. *Helicobacter pylori* organisms tend to be present in relatively large numbers in the mucus lining of the foveolar gastric surface and show a predilection for the antrum (Figure 1). This distribution of organisms was characteristic of most patients in the pre-proton pump inhibitor era and is still seen in some areas of the world. Comparisons amongst the various histochemical stains and between histochemical and immunohistochemical stains show very little difference in sensitivity and specificity when organisms are abundant (13). However, the widespread availability of proton pump inhibitors has clearly altered the features of *H. pylori* infection. Organisms are present in smaller numbers, are more

likely to be present in the deeper pits of the proximal stomach, and display coccoid and intracellular forms in patients who receive acid suppression (Figure 2) (14). Recent data suggest that most histochemical stains have sensitivities in the 60-75% range compared to immunohistochemistry, which shows a sensitivity of 100% (9, 15, 16). Thus, many authors now advocate use of immunostains rather than histochemical stains if ancillary stains are indicated (Table 1) (9, 15, 16, Sepulveda, 2008 #62, 17). Pathologists may detect organisms in immunohistochemical stained sections that would likely not be identified by histochemical staining.

The primary differential diagnoses of *H. pylori* organisms include oral flora contaminants and *H. heilmannii* infection. The former are easily distinguished from *H. pylori*. Contaminants may consist of mixed cocci and bacilli, the latter of which are thicker and larger than *H. pylori* organisms. Oral contaminants also tend to be located in luminal mucin and show no specific relationship to the foveolar epithelium, whereas *H. pylori* organisms are always seen in close proximity to gastric epithelium. *Helicobacter heilmannii* are nearly twice as long as *H. pylori* and are much thicker. They have a pronounced corkscrew appearance distinct from the curvilinear shape of *H. pylori* (Figure 3). Notably, histochemical stains do not distinguish *H. pylori* from other bacteria, including *H. heilmannii*. Commercially available antibodies directed against *H. pylori* organisms also cross-react with *H. heilmannii* and, thus, ancillary stains may be unhelpful in this regard.

Detection of Helicobacter: Financial and Ethical Issues

The purpose of this section is to provide data-based opinions regarding the appropriate and clinically indicated use of special stains when detecting *H. pylori* in tissue specimens. Although we will discuss the "cost effectiveness" of ancillary stains, there is virtually no literature providing a *bona fide* cost analysis of the role of random gastric biopsies, let alone special stain use, in detection of *H. pylori*. A true cost effectiveness discussion would include data regarding financial costs of special stains in the context of morbidity prevented and mortality delayed. However, no current data demonstrate that detecting organisms in patients with low bacterial load prevents *H. pylori*-related medical care expenses in the future, nor are there available data regarding special stains costs per year of life saved. Nonetheless, we can provide informed opinion regarding the scenarios in which special stains for *H. pylori* are appropriately applied. For the sake of clarity, we will present information in a question and answer format that outlines a number of clinical scenarios in which special stains for *H. pylori* might be ordered. Although we recommend immunohistochemical stains for *H. pylori* and will limit comments below to use of immunostains, we recognize that some pathologists may opt to use histochemical stains for reasons dictated by the natures of their practices.

1. How often should I expect to find H. pylori in gastric biopsies?

The frequency of *H. pylori* gastritis depends on whether or not patients are from endemic areas. Infection risk is linked to lower socioeconomic status and, thus, *H. pylori* gastritis is more common in equatorial countries, urban areas, and regions with suboptimal

sanitation where infection rates approach 90% (18). In contrast, approximately 30–40% of the U.S. population was infected with *H. pylori* in 2000 and a disproportionate number these patients were elderly individuals who acquired *H. pylori* as children (19). It is anticipated that the incidence of *H. pylori* infection in North America will decline as this group ages and *H. pylori* is systematically eradicated upon detection. Recent data suggest that the prevalence of *H. pylori* infection in North America varies from state to state. It is higher among Medicaid patients compared to those with other types of insurance (20). As of 2010, reported rates of infection range from a low of 3.9% in Kansas to a high of 31.7% in Puerto Rico, as well as relatively high prevalence in Louisiana (24%) and North Carolina (16%) (20-22).

2. Is there anything the pathologist can recommend to the endoscopist regarding sampling that will maximize the likelihood of finding H. pylori if it is present?

There is a relationship between the number of gastric biopsy samples obtained and the rate of *H. pylori* identification. Mucosal atrophy is associated with a decreased likelihood of finding *H. pylori* in the antrum, so multiple samples of antrum and body should be obtained when extensive intestinal metaplasia is present (23). In the pre-proton pump inhibitor era, one sample of the antrum had 80-90% sensitivity for detection of *H. pylori* and two samples (either both antral or antral and body) increased the sensitivity to 95-96% (24). However proton pump inhibitors may cause a shift of bacteria from the antrum to the more proximal stomach (25). Proton pump inhibitor use also decreases the number of organisms in both antrum and body. Thus the Sydney System recommendation of obtaining two samples from the antrum, two from the gastric body,

and one from the incisura angularis (26) seems particularly appropriate today. These are best placed in one cassette, thereby allowing one special stain to evaluate both antrum and body, while also affording an optimal chance to characterize the background mucosa on a single slide.

3. What does the gastric mucosa look like when H. pylori organisms are present?

Greater than 90% of gastric biopsies that contain *H. pylori* show chronic active gastritis characterized by at least moderate lymphoplasmacytic infiltrates and neutrophils (Figure 4) (21). Lymphoid aggregates, particularly those with germinal centers, are a helpful clue to the presence of *H. pylori*. Infection of fundic mucosa produces a dense band of mononuclear cell rich inflammation under the surface epithelium (Figure 4). Chronic gastritis without neutrophils (*i.e.* chronic inactive gastritis) is less commonly associated with ongoing *H. pylori* infection, although it may be seen after successful treatment of *H. pylori* gastritis or in patients who receive antibiotic therapy with partial efficacy against the organism. Therapy quickly diminishes the neutrophilic component of the inflammation, but a chronic inactive gastritis pattern of inflammation may persist for some time (7). Development of intestinal metaplasia is also associated with a decreased likelihood of *H. pylori* detection, particularly when metaplasia is extensive (21).

4. How effective is an H&E stain for detecting H. pylori among infected patients?

The H&E stain is universally regarded as a very effective means of detecting *H. pylori*. Sensitivities range from 70-94% and may be improved by prolonged exposure to hematoxylin as well as fastidiousness and patience on the part of the examining

pathologist (15, 21, 27, 28). Smith *et al* found that evaluation of H&E sections detected *H. pylori* with a sensitivity of 91% compared to immunohistochemistry, but noted that one must evaluate a mean of 5.75 high-power fields (range 1-25) in H&E stained sections compared to a maximum of 3 high-power fields required to detect *H. pylori* by immunohistochemistry when organisms are present (21). The specificity of H&E for *H. pylori* is very high and, thus, there is no incremental value to performing special stains when organisms are apparent in H&E stained sections. Confirmatory immunohistochemical stains may be indicated when diagnostic features are equivocal, such as cases in which organisms are rare or their morphologic appearance is altered.

5. Is a clinical request of "rule out H. pylori" an indication for ancillary stains?

There is a poor correlation between the endoscopic appearance of the stomach and the presence of *H. pylori* or severity of inflammation. Severe endoscopic "gastritis" may yield histologically normal biopsies, whereas endoscopically normal stomachs may harbor a brisk *H. pylori* gastritis (29, 30). Data suggest no relationship between a clinician request to rule out *H. pylori* and histologic detection of organisms (15). A request to evaluate for the presence of *H. pylori* in the setting of previously treated infection is probably reasonable, but unlikely to provide useful information because treatment failures occur in a minority of instances. Patients with both successfully and unsuccessfully treated gastritis have persistent chronic inflammation for weeks to months following cessation of therapy, so this finding alone has no predictive value when assessing subsequent biopsies.

6. Is it appropriate to perform, report, and bill for special stains if H. pylori organisms are detected in routine (H&E) stains? *Helicobacter pylori* organisms are, in most cases, readily seen with routine (H&E) stains when they are present (13, 15, 21, 22, 28). The specificity of H&E stained sections for *H. pylori* is very high and the organism can be easily distinguished from others in the differential diagnosis, such as *H. heilmannii*, albeit this organism is generally treated in a similar fashion so distinguishing the two is of little practical utility. Confirmatory immunohistochemistry is only justified when organisms are not clearly recognized or have unusual morphologic features that prevent a definitive diagnosis. There is no incremental value to performing special stains if *H. pylori* organisms are clearly visible with H&E stained sections. We cannot envision a scenario in which special stains used in this context could be viewed as cost effective.

7. Is it appropriate to perform, report, and bill for special stains if there is a chronic active gastritis but H. pylori organisms are NOT visible by routine (H&E) stain?

The "chronic active" gastritis pattern of inflammation is present in approximately 30% of gastric biopsies, and, when present, is associated with *H. pylori* infection in nearly 75% of cases (Figure 4) (22). The positive predictive value of moderate lymphoplasmacytic and neutrophilic inflammation for *H. pylori* infection is >90%. However, bacteria may be scarce in patients who receive proton pump inhibitors and are acid-suppressed. One may overlook the infection when very few organisms are present, involvement of the surface is patchy, bacteria are present within gastric epithelial cells, and/or when bacteria appear as coccoid forms rather than curvilinear rods (Figure 2) (21). Other causes of *H. pylori*-negative chronic active gastritis cases include idiopathic inflammatory bowel

disease, Epstein Barr virus infection, and less common etiologies, such as poorly characterized immune-mediated disorders and infections (25, 31). Of these, inflammatory bowel disease is the most practically relevant: 26% of pediatric patients with Crohn disease and 13% of ulcerative colitis patients have *H. pylori*-negative chronic active gastritis compared with just 2% of controls (32). Based on these observations, we conclude that the presence of chronic active gastritis is a strong indication for performing ancillary stains when H&E stained sections fail to demonstrate *H. pylori*, although they are unlikely to demonstrate *H. pylori* organisms when serologic studies are negative.

8. Is it appropriate to perform, report, and bill for special stains if there is a chronic inactive gastritis and H. pylori organisms are not visible by routine (H&E) stain?

Although *H. pylori* infection occasionally causes a chronic inactive gastritis with relatively mild mononuclear cell-rich inflammation and an absence of neutrophils, the vast majority of chronic inactive gastritis cases lack *H. pylori* organisms by any detection method. Unfortunately, data regarding the utility of ancillary stains to detect *H. pylori* in patients with chronic inactive gastritis are limited because most studies have not evaluated this patient group as a separate category and minimal criteria for distinguishing normal mucosa from chronic inactive gastritis are not well defined (26). Wang *et al* found that immunohistochemistry detected *H. pylori* organisms in only 5.3% of chronic inactive gastritis cases, compared to 73.5% of chronic active gastritis cases (22). Hartmann noted only 1 of 30 (3%) *H. pylori*-positive gastritis samples displayed only "mild" gastritis, whereas all remaining cases showed more substantial inflammation (15). However, clinicians are interested in detecting even mild infections to treat, and

potentially decrease, future morbidity and, thus, may pressure pathologists to "rule out" *H. pylori* despite its low prevalence in chronic inactive gastritis. Indeed, the chronic inactive gastritis cohort is the largest group of patients in which the use of ancillary stains for *H. pylori* detection should be considered.

Ancillary stains are unnecessary if *H. pylori* organisms are visible in H&E stained tissue sections containing chronic inactive gastritis, but their use in cases that lack *H. pylori* organisms by H&E evaluation is strongly recommended in several situations. Immunohistochemical stains directed against *H. pylori* are suggested whenever chronic inactive gastritis cases display well-formed lymphoid follicles with germinal centers, since this finding is highly predictive of underlying *H. pylori* infection (21). Gastric biopsies from patients with co-existent gastric or duodenal ulcers not clearly associated with chemical injury or reactive gastropathy should also be evaluated with additional stains. Failure to detect *H. pylori* among these individuals may result in substantial patient harm. Finally, biopsies from patients with co-existent gastric lymphoma, particularly MALT-type lymphoma, or adenocarcinoma should be evaluated with immunohistochemistry for *H. pylori*. Eliminating the organism in these situations may modify the disease course or management to some degree.

Use of immunohistochemistry to identify *H. pylori* organisms in patients with chronic inactive gastritis is also reasonable in other circumstances, although this practice is probably not cost effective and promises a low yield of *H. pylori* detection. Biopsies that show moderate or severe mononuclear cell-rich inflammatory infiltrates in the superficial

mucosa are more likely to be associated with *H. pylori* than those that show only minimal, or mild, chronic inflammation and, thus, use of ancillary stains may be considered when biopsies contain substantial chronic inflammation. Samples obtained from patients at high risk for *H. pylori* infection from an epidemiologic standpoint may be evaluated with immunohistochemistry when they show a mild degree of inflammation. Patients who are known to have negative *H. pylori* serologic studies are very unlikely to have *H. pylori* in gastric biopsies that are detectable by any method and, thus, ancillary stains are not recommended in this setting.

9. Is it appropriate to perform, report, and bill for ancillary stains if the stomach is histologically normal?

While some expert pathologists claim to have rarely seen *H. pylori* in otherwise normal stomach biopsies, the literature suggests that this finding is extremely uncommon and essentially non-existent (22). There are no data regarding the long-term morbidity and mortality related to *H. pylori* among these very rare patients. Thus, it is difficult to justify the costs of evaluating normal mucosal biopsies with ancillary stains for *H. pylori* at the current time.

10. Is it appropriate to perform, report, and bill for ancillary stains if biopsies display a classic chemical (reactive) gastropathy?

Chemical (reactive) gastropathy is characterized by the presence of foveolar hyperplasia, reactive epithelial cell changes with mucin depletion and mild nuclear enlargement, and variable fibrosis of the lamina propria with prominent vascular channels (Figure 5).

Chronic inflammation is minimal and, although neutrophils may be detected, they are only identified in the context of an erosion or ulcer. The likelihood of detecting *H. pylori* by any method is essentially nil when a pure chemical gastropathy pattern is present (22). However, the presence of chemical gastropathy does not preclude co-existent *H. pylori* gastritis. Thus, it is reasonable to perform *H. pylori* immunohistochemistry when patterns of chemical gastropathy and chronic active gastritis co-exist and organisms are not detected in H&E stained sections.

11. For what types of unusual gastritis is it appropriate to perform, report, and bill for special stains if H. pylori organisms are not detected in H&E stained sections?

Helicobacter pylori has been implicated as a cause, or mimic, of lymphocytic gastritis, granulomatous gastritis, and eosinophilic gastritis, although the role of this organism in the development of these disorders is unclear. At least 50% of the world population is infected with *H. pylori* and, thus, any reported association between the organism and these diseases may simply be coincidental.

Lymphocytic gastritis, as defined by >25 intraepithelial lymphocytes (IELs) per 100 epithelial cells, may be seen in association with gluten sensitivity or as a cause of either "varioliform" gastritis or a hypertrophic gastropathy resembling Menetrier's disease (33). Although fewer than 5% of *H. pylori*-associated gastritis cases contain adequate numbers of IELs to mimic lymphocytic gastritis, *H. pylori* gastritis is far more common than the latter, thus, accounts for 29% of patients who carry a diagnosis of "lymphocytic gastritis" (34). For this reason, Carmack *et al* suggest that patients with chronic gastritis,

intraepithelial lymphocytosis and *H. pylori* should be treated for *H. pylori* and undergo repeated endoscopic evaluation with biopsy in 6 months. Resolution of lymphocytosis would support a diagnosis of *H. pylori* gastritis with increased IELs rather than lymphocytic gastritis (35).

Granulomatous gastritis can be secondary to systemic conditions (*e.g.* sarcoidosis, mycobacterial or fungal infections) or localized to the stomach, in which case it is classified as "isolated granulomatous gastritis". Rare cases of isolated granulomatous gastritis have been linked to *H. pylori* (36). We suggest that detection of *H. pylori* in a case of unexplained granulomatous gastritis should prompt *H. pylori* eradication therapy followed by resampling in 6 months to ensure resolution.

"Histologic eosinophilic gastritis" is a term suggested in the United States to describe the finding of more than 30 eosinophils per high power field in at least five examined fields (normal range <9/HPF) (37). The link between *H. pylori* and eosinophilic gastritis is tenuous at best. Very rare reports describe a possible association between *H. pylori* and eosinophilic gastritis and a large U.S. study of 60 patients with "histologic eosinophilic gastritis" failed to identify any patients with *H. pylori* infection by immunohistochemistry (38, 39). A large Chinese study also found that only 15% of patients with eosinophilic gastroenteritis were infected with *H. pylori* compared to 58% of controls (40). These data do not lend much evidence supporting a relationship between *H. pylori* and eosinophilic gastritis. However, the rare nature of "histologic eosinophilic gastritis" probably permits use of *H. pylori* immunohistochemistry in these cases.

12. Is it appropriate to perform "up front" ancillary stains for H. pylori in every gastric biopsy?

There is probably no down side to performing "up front" special staining on all gastric biopsies for the sake of expediency and/or convenience of the pathologist or laboratory, provided the patient is not billed for the stains that were not indicated based on H&E findings. In this case, the laboratory must determine whether additional time on the part of the histotechnologist, slides, and reagents justify the expense. Histochemical stains are generally less sensitive than immunohistochemical stains (60-75% compared to virtually 100%). Therefore, "up front" histochemical stains do not necessarily prevent a third line of staining (immunohistochemistry) in the H&E-negative, histochemistry-negative gastric biopsy that shows features suggestive of *H. pylori* infection, such as chronic active gastritis. If "up front" histochemical stains are performed and additional immunohistochemical stains are necessary, charges should be issued for only one technique.

One cannot ethically support billing for "up front" ancillary stains to detect *H. pylori* in all cases. Special stains add no value when *H. pylori* organisms are seen by H&E stain. Most gastric biopsies show normal histology, chemical gastropathy, or a very mild degree of chronic inflammation in H&E stained sections and have a low frequency of *H. pylori* detection. We do not believe that a persuasive argument for the cost effectiveness of "up front" ancillary stains has been made.

13. Is it appropriate to perform, report, and bill for special stains in an inflamed biopsy of the cardia?

Inflammation of the gastric cardia may reflect *H. pylori* infection, gastrosophageal reflux disease (GERD), or even a mild degree of acid injury in asymptomatic patients. *Helicobacter pylori* gastritis can affect the entire stomach ("pangastritis") and extend into the cardia to produce chronic active gastritis in that region, but *H. pylori*-related inflammation localized to the gastric cardia with sparing of the body and antrum has not been described (41). Inflammation limited to the cardia most commonly reflects GERD, which tends to produce a less dense chronic inflammation with fewer plasma cells, lymphoid aggregates, and neutrophils than *H. pylori* pangastritis involving the cardia (42).

It is difficult to justify performing special stains for *H. pylori* on inflamed cardia biopsies when reasonable sampling of the distal stomach shows uninflamed mucosa without *H. pylori*. On the other hand, ancillary stains may be indicated when chronic active inflammation is detected in the cardia, but biopsies of the distal stomach have not been obtained. This is particularly true if the patient is at risk for *H. pylori* infection and has either a positive or unknown *H. pylori* serology result. Ancillary stains for *H. pylori* have essentially no utility in cases of mild, inactive "carditis".

14. Is it appropriate to perform ancillary stains for H. pylori in esophageal samples?

Some practices perform ancillary stains for *H. pylori* on every esophageal biopsy with columnar mucosa. We can find no sound medical reason to justify this practice.

15. Is it appropriate to perform ancillary stains for H. pylori in duodenal samples?

Helicobacter pylori do not normally inhabit intestinal mucosa. Patients with peptic duodenitis who develop gastric foveolar metaplasia may harbor *H. pylori* organisms in metaplastic epithelium (43). However, involvement of metaplastic epithelium in the duodenum does not occur in the absence of *H. pylori* infection of the stomach, so "up front" ancillary stains of duodenal biopsies are not indicated. Ancillary stains of duodenal samples would only be reasonable in a very narrow set of circumstances, namely when gastric metaplasia is present in a patient with no available gastric biopsies and a positive or unknown *H. pylori* serology status.

Summary and Conclusions

Approximately 5-20% of gastric biopsy cases in North America harbor *H. pylori*. Infection rates show regional variation and are higher in lower socioeconomic areas. Optimal sampling to characterize gastritis and detect *H. pylori* includes biopsies of the antrum (preferably two sites), body (preferably two sites) and incisura angularis. These biopsies should be placed in a single formalin bottle to maximize efficiency and reduce costs. Most cases of *H. pylori* infection can be diagnosed based on H&E evaluation alone because biopsies from infected patients are essentially never normal; they are nearly always associated with increased chronic inflammation and frequently show neutrophils. We recommend use of immunohistochemistry when special staining for *H. pylori* is indicated because it generally shows superior sensitivity compared to histochemical stains. If immunostains are not available or affordable, use of histochemical stains may be considered but likely add little value over a well-performed and carefully reviewed H&E stained slide in the current age. Ancillary stains for *H. pylori* should not be performed or billed when organisms are detected in H&E stained sections of any gastric biopsy, nor are they indicated when assessing esophageal or duodenal biopsies. They have no utility when applied to normal gastric biopsies or those that show chemical (reactive) gastropathy alone. *Helicobacter pylori*-negative H&E stains may be supplemented with immunohistochemistry when chronic inflammation is present in gastric biopsies, including the cardia, although the yield of these stains is generally low and depends on the severity of inflammation present. Ancillary stains should not be performed simply because pathologists are requested to "rule out" *H. pylori*

unless the patient has been previously treated for infection. In our opinion, routine "up front" special stains are of dubious cost effectiveness and uniformly billing the patient/payor in all cases is indefensible. If an individual business chooses to perform such staining, but only reports and bills those that are justified based on the H&E findings, we cannot strongly object. However, billing the patient/payor for both an initial histochemical stain as well as a subsequent immunohistochemical stain is not appropriate in the modern era.

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Figure Legend

Figure 1. Innumerable curvilinear *H. pylori* organisms are present within the mucus layer adherent to foveolar epithelium (A). Organisms show strong immunopositivity with the *H. pylori* antibody (B). Although use of immunostains in this situation may be useful for teaching purposes, they are of no added value to H&E stained sections in the management of patients.

Figure 2. Patients who have been incompletely treated for *H. pylori* infection or received proton pump inhibitor therapy have far fewer bacteria in biopsies and typically require immunohistochemical stains for their detection. Rare bacteria are present in the mucus layer (A) and pit lumina (B). Infrequent coccoid forms are present in patients receiving proton pump inhibitor therapy (C), many of which are intracellular (D).

Figure 3. *Helicobacter heilmanii* are longer than *H. pylori* and have a pronounced corkscrew appearance. They are also positive with the commercially available *H. pylori* immunostain and, thus, immunohistochemistry is of no value in distinguishing between these species.

Figure 4. Chronic active *H. pylori*-associated gastritis diffusely involves the antral mucosa. Sheets of plasma cells and lymphocytes are present between the gastric pits and show relative sparing of the deeper mucosa (A). *Helicobacter pylori* infection of the proximal stomach produces a

superficial chronic gastritis with a band of mononuclear cell-rich inflammation subjacent to the foveolar epithelium (B).

Figure 5. Chemical (reactive) gastropathy shows mucin depletion in regenerative-appearing foveolar epithelial cells, but substantial chronic inflammation of the lamina propria is lacking. Such cases are unlikely to show *H. pylori* organisms by any detection method.

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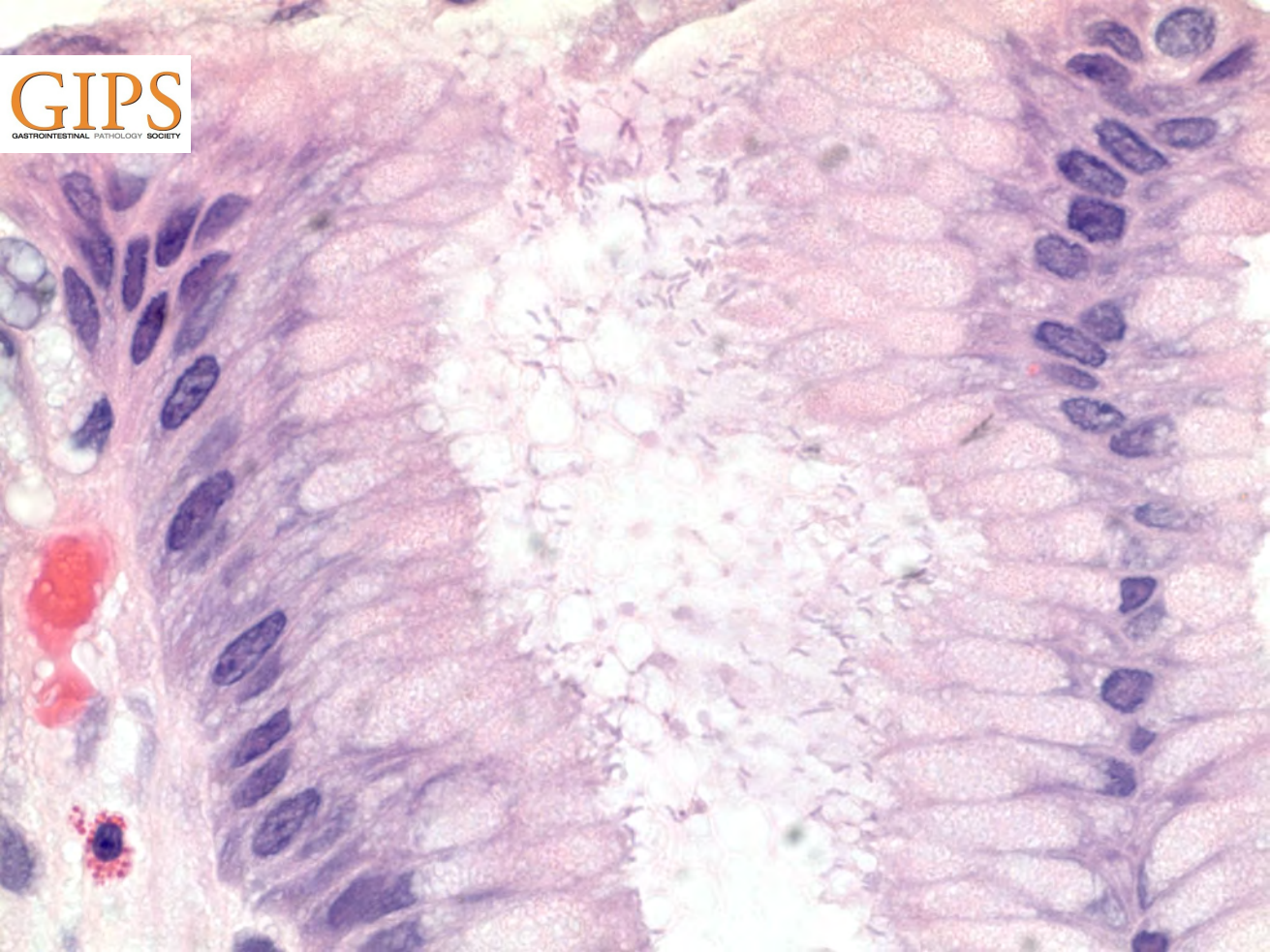
Table 1. Utility of Ancillary Stains in Detecting *H. Pylori* when Evaluating Gastric Biopsies.

Morphologic Findings	Likelihood of *H. Pylori* Detection in H&E Stained Sections	Justification for Special Stains
Normal gastric mucosa	Extremely low	Not indicated
Chemical (reactive) gastropathy	Extremely low	Not indicated if chemical injury is only abnormality
		Appropriate if associated with superimposed chronic gastritis
Chronic active gastritis	High (at least 75%)	Not indicated if H&E demonstrates organisms
		Not indicated if serologic studies are known to be negative
		Appropriate if H&E is negative for *H. pylori*
Chronic inactive gastritis	Low (approximately 5%)	Not indicated if serologic studies are known to be negative, but probably justified in most other cases
		Appropriate if gastroduodenal ulcers are present
		Appropriate if gastric MALT-type lymphoma or adenocarcinoma is present
		Appropriate if duodenal lymphocytosis is present
		Appropriate in patients with prior *H. pylori* treatment
		Appropriate in high-risk demographic areas
Isolated chronic active carditis	Low	Appropriate
Isolated chronic inactive carditis	Extremely low	Not indicated, unless gastric biopsies are unavailable and serologic studies are positive
Barrett's esophagus	Essentially none	Not indicated
Lymphocytic gastritis	Moderate (approximately 30%)	Appropriate
Granulomatous gastritis	Low-moderate	Appropriate
Eosinophilic gastritis	Extremely low	Appropriate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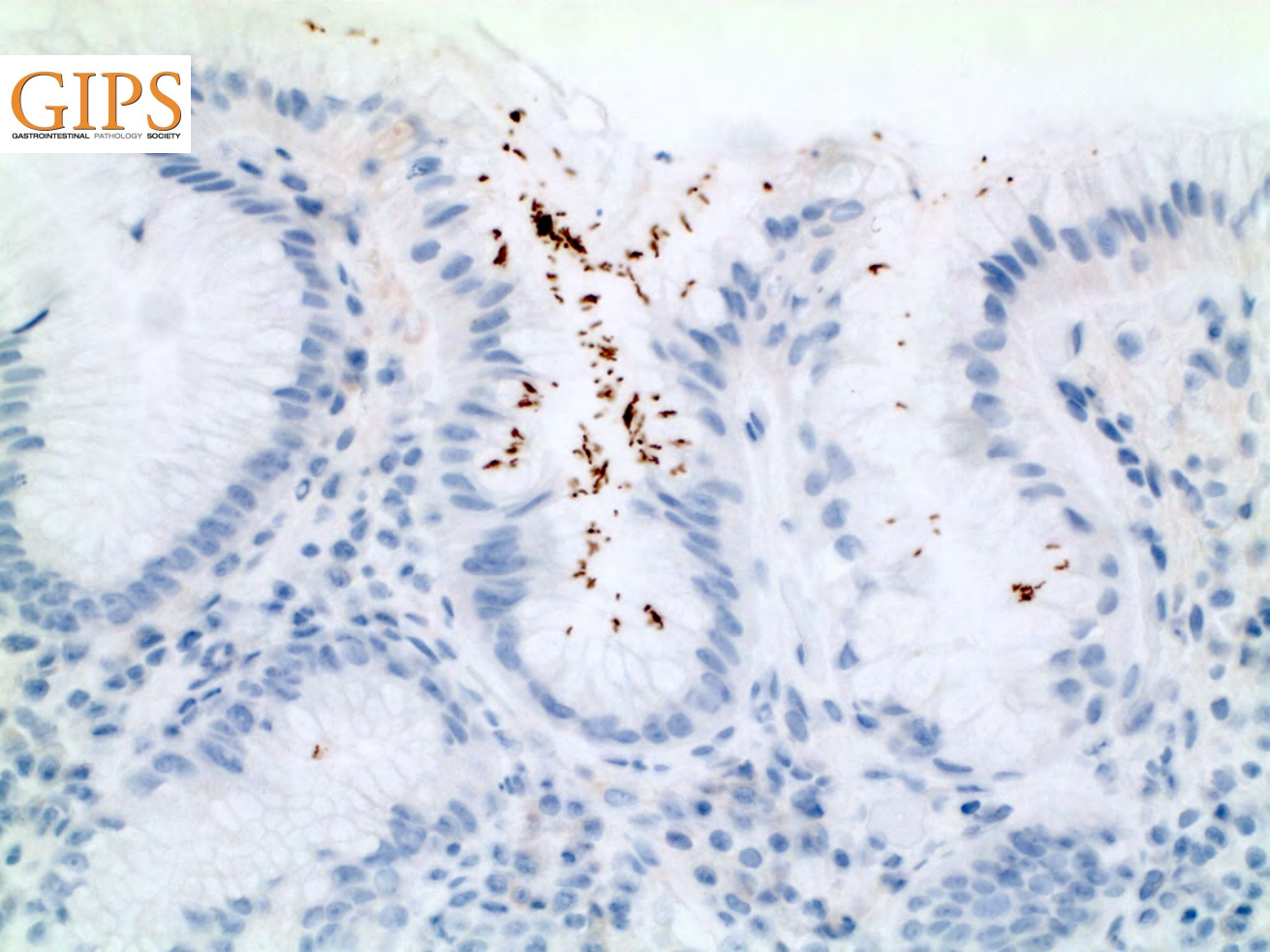
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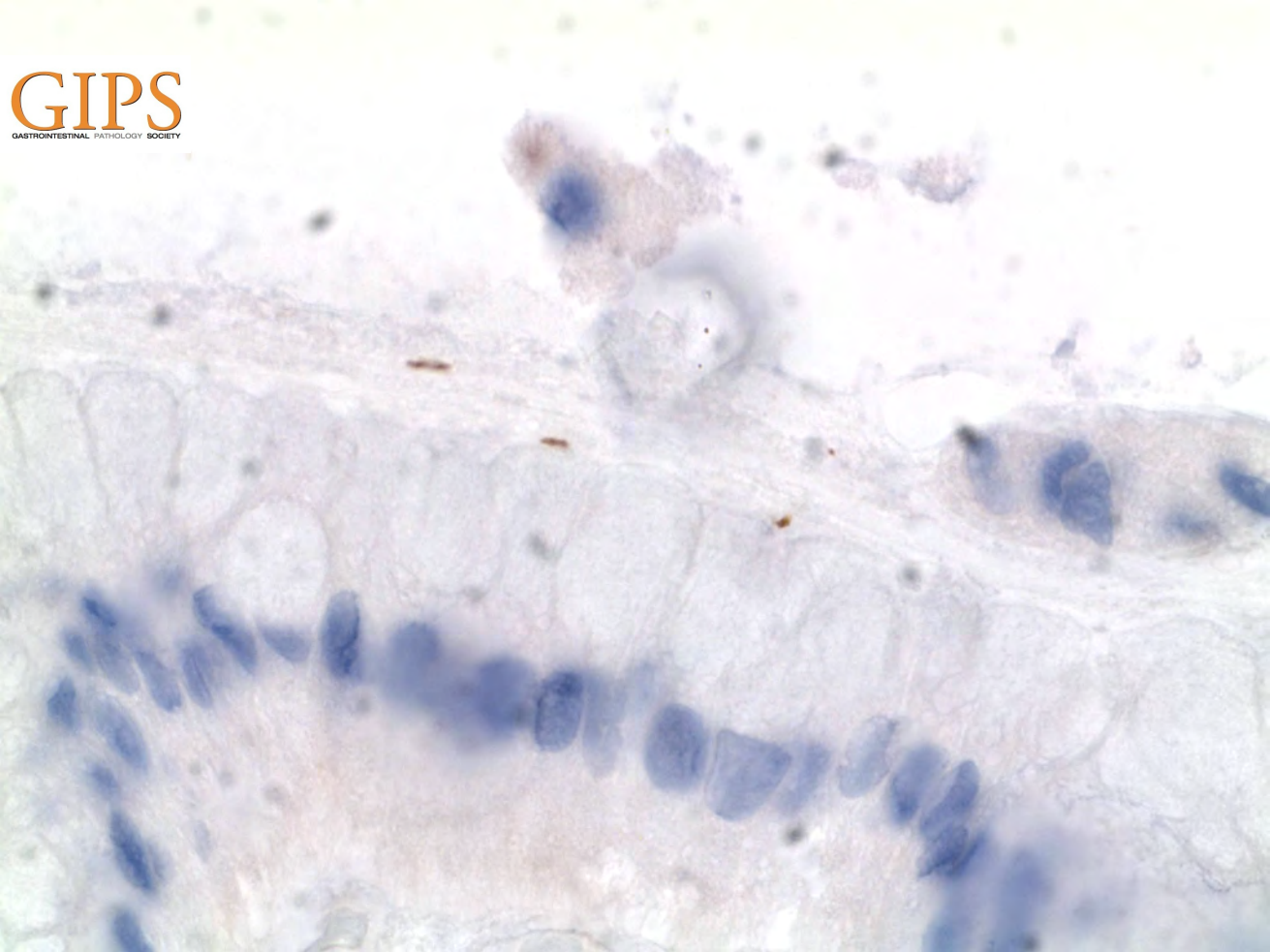

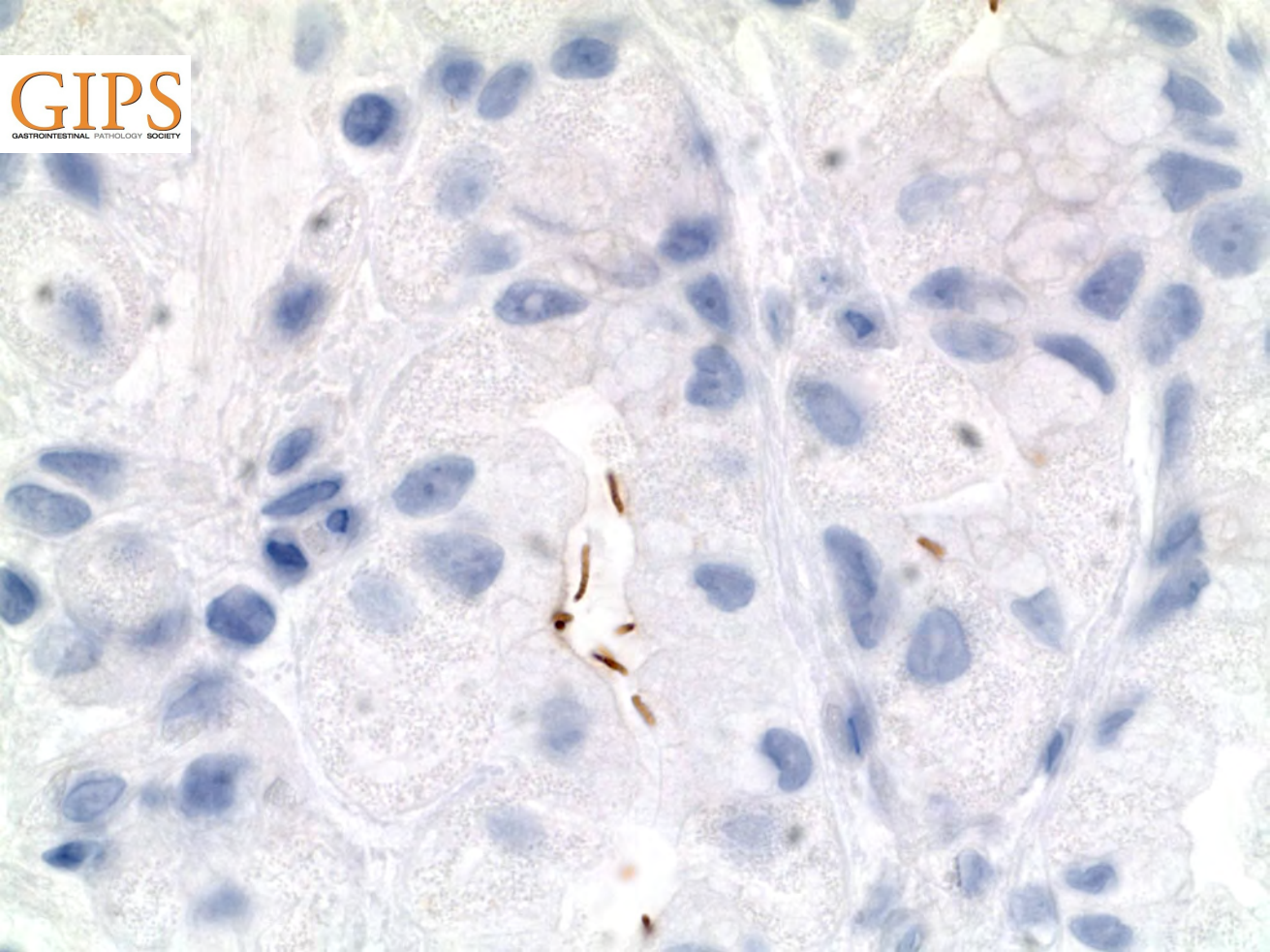
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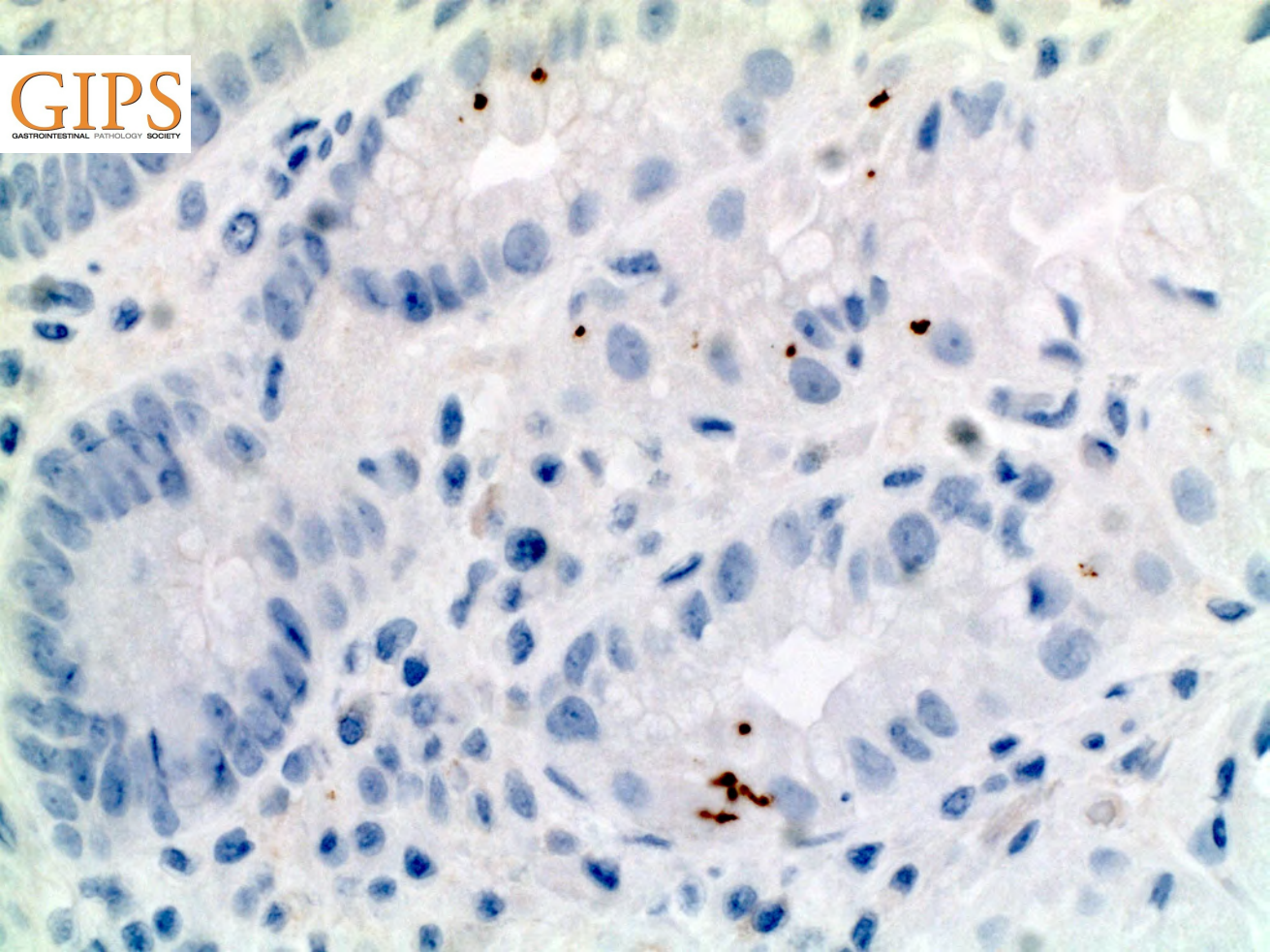
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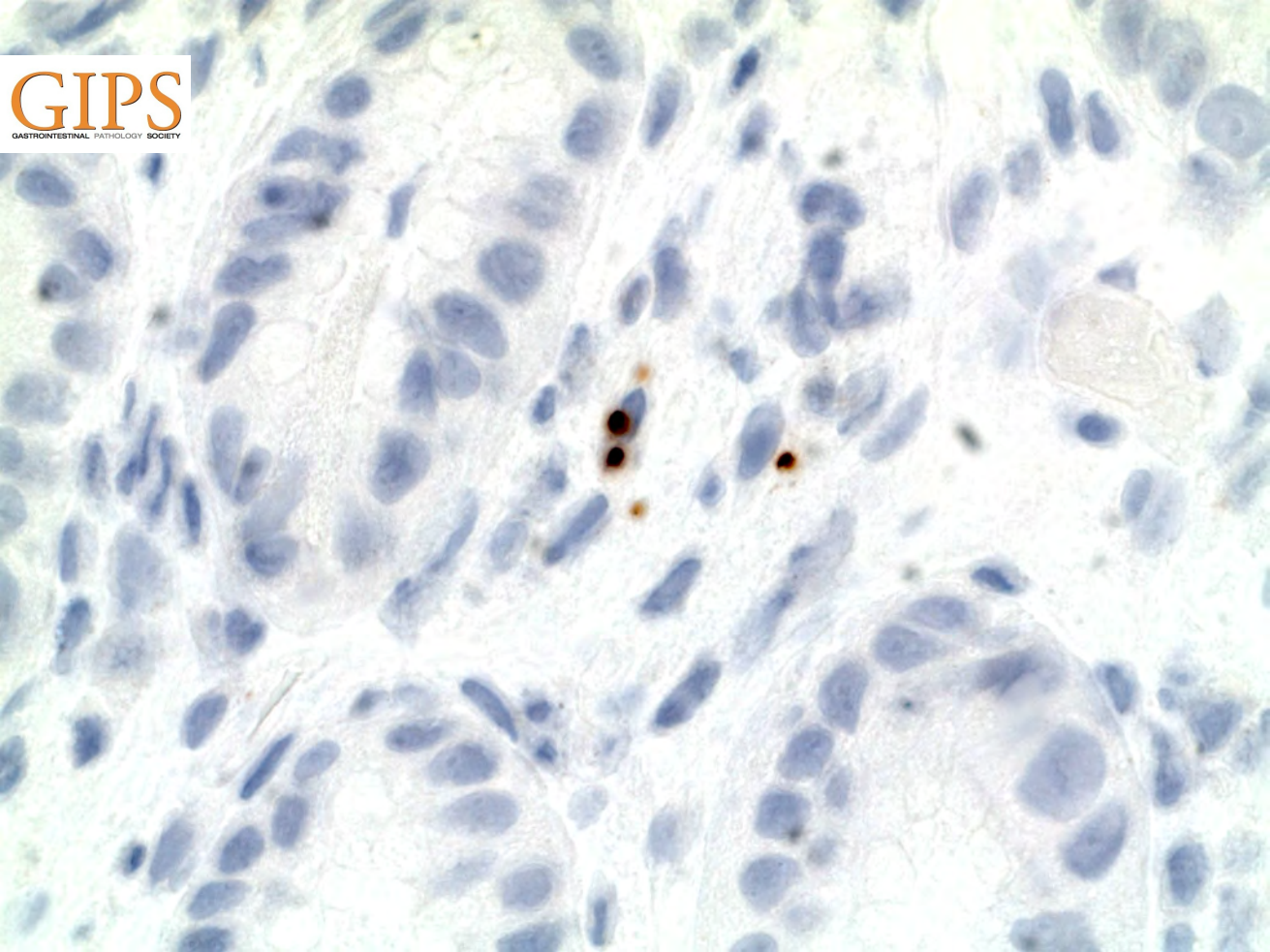
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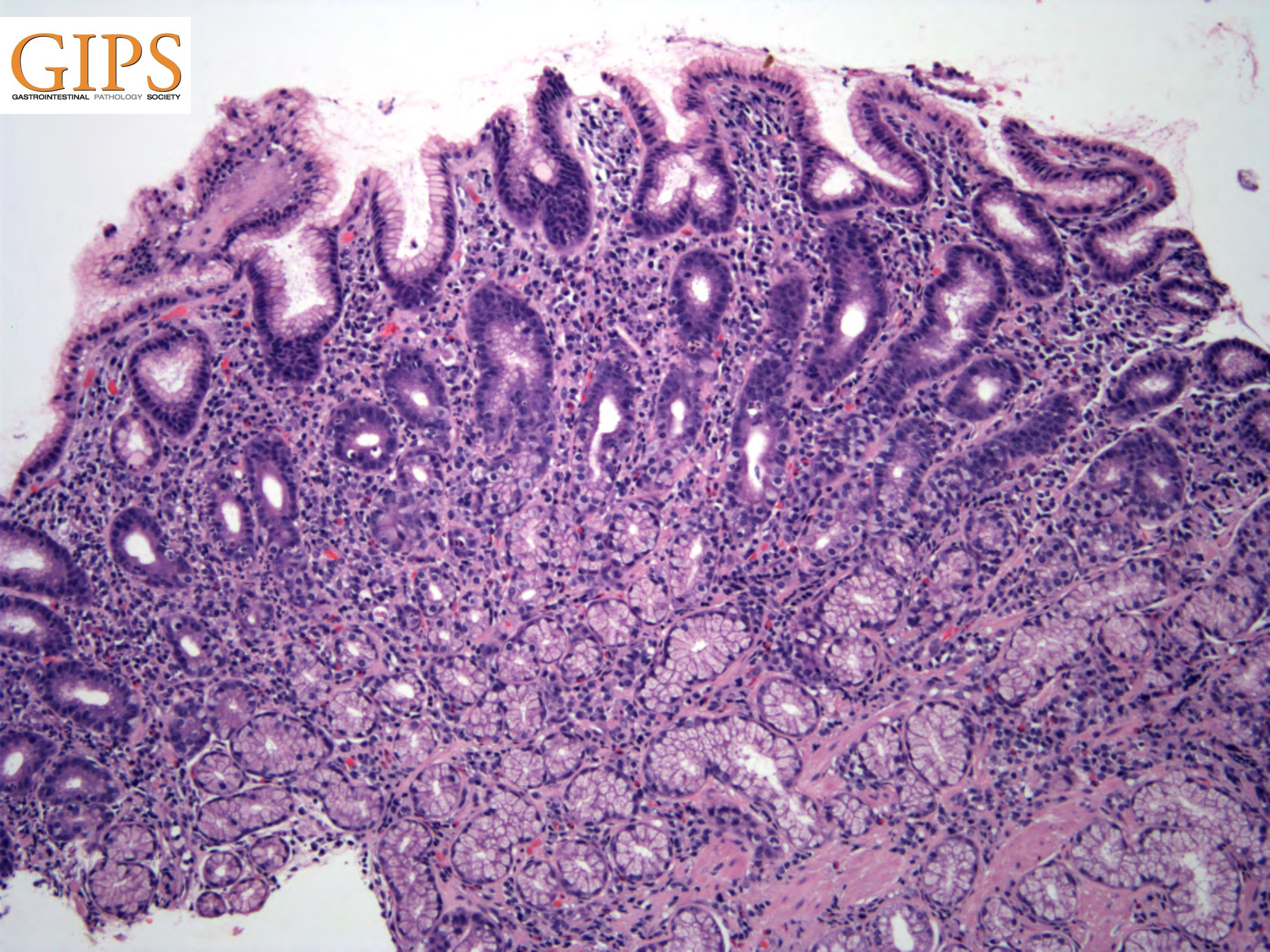

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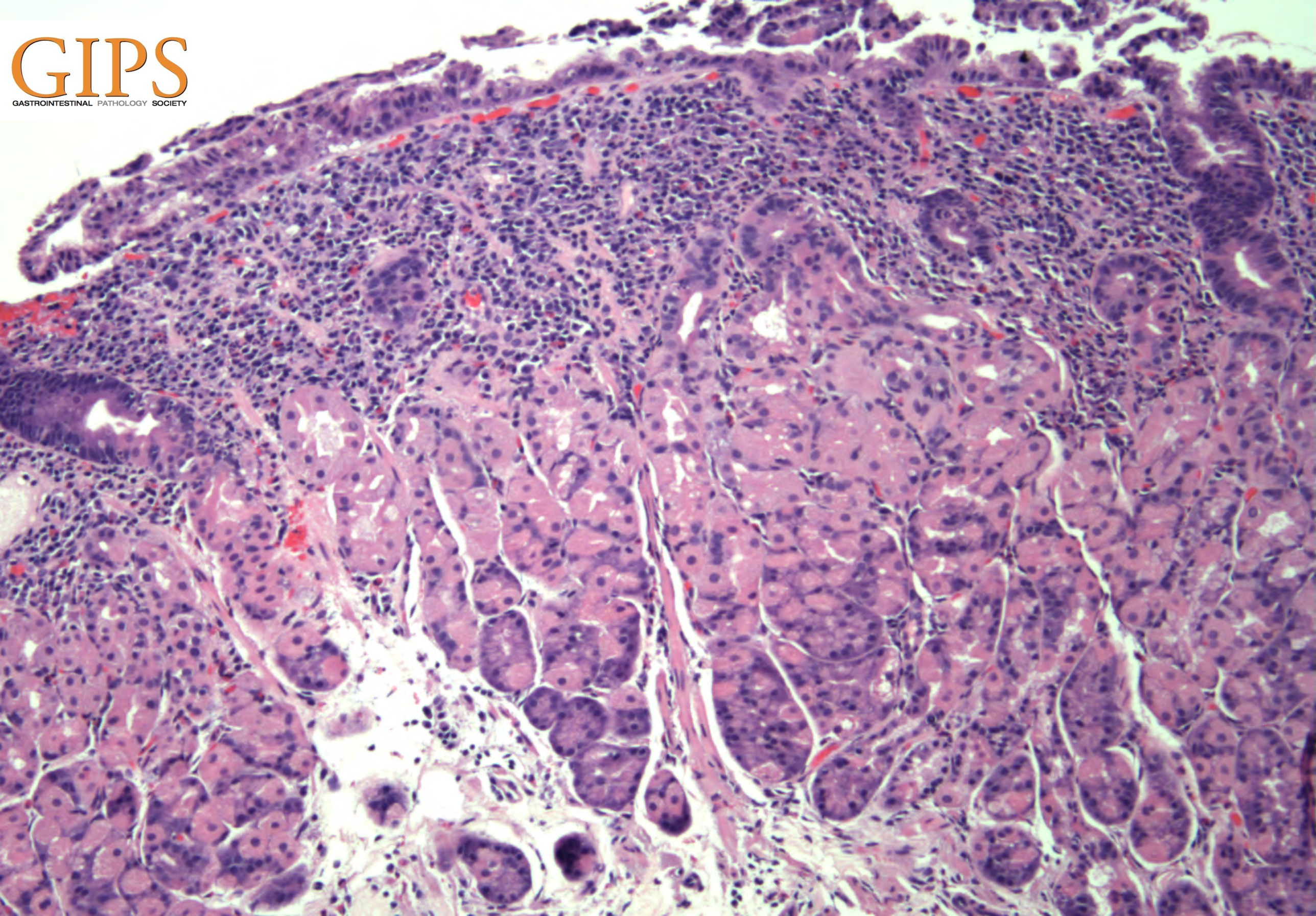

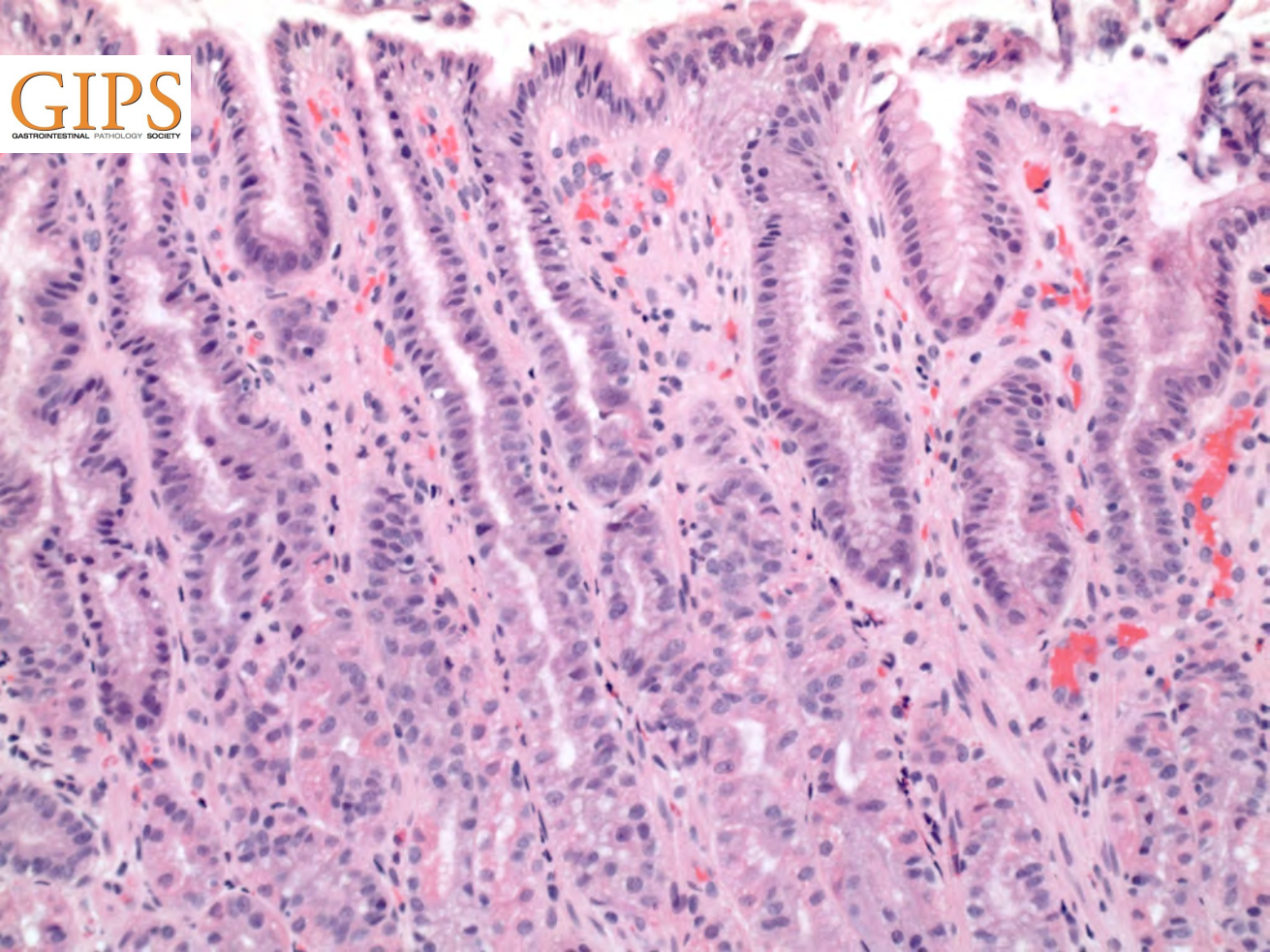
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