USE OF ROUTINE SPECIAL STAINS FOR GASTRIC ANDESOPHAGEAL BIOPSIES

Abstract:

Problem: *Special stains such as the Giemsa, the Alcian blue (AB) and the Periodic Acid Schiff (PAS) are widely used for gastric and/or esophageal biopsies for the diagnosis of Helicobacter pylori (H. pylori) and intestinal metaplasia (IM). The purpose of our study was to determine if these stains are actually needful*

Methods: *We retrospectively studied 209 gastric and esophageal biopsies. We evaluated the H. pylori status on hematoxylin and eosin (H&E) stained slides and the presence of IM and finally we examined the special stains.*

Results: *23% of cases were H. pylori-positive. H&E stain had a high degree of accuracy (92,1%). The Giemsa stain was positive in 23,7% and negative in 71,8%. The Giemsa was useful in 16,7% biopsies. The AB revealed goblet cells in 4,8% cases. AB sensibility was 90,9%. From the 208 slides stained with PAS, 4,3% were classed IM-positive. PAS sensibility was 90%. The two special stains (AB and PAS) have made no diagnostic gain.*

Conclusions: *Routine special stains for every single gastric and/or esophageal biopsy are not required and H&E assessment combined with selective ordering of these stains will identify all cases of H. pylori gastritis and IM.*

Key Words: *Helicobacter pylori, intestinal metaplasia, gastritis, special stain.*

INTRODUCTION:

A careful reading of the Hematoxylin and eosin (H&E) stained biopsies is usually sufficient for the diagnosis of Helicobacter pylori (*H. pylori*) and intestinal metaplasia (IM). However, special stains are performed systematically and not selectively by several laboratories, including our own, to bring out these lesions. Special stains, as Giemsa, are widely used to improve the detection of *H. pylori* [1, 2]. The mucin often has a slightly basophilic tinge and stains intensely blue with the Alcian blue (AB) as well as the Periodic acid schiff (PAS) but also the subgroup of N acetylated sialomucins. However, the realization of special stains increases significantly the time and the expenses required for diagnosis [3, 4]. The purpose of this study was to evaluate the contribution of these stains in the reading biopsies and determine whether they are essential for every gastric and / or esophageal biopsy.

Methods:

This retrospective study included consecutive gastric and esophageal biopsies addressed by the adult gastro enterology department, over a six-month period. During this period we received 209 biopsies from 139 patients, complaining of digestive symptoms, who incured an upper gastrointestinal endoscopy. In our laboratory's normal procedure, all biopsies had two levels of H&E stained serial sections. The length of time of H&E staining was about 5 minutes. For each biopsy are also prepared three special routine stains: a Giemsa stained slide for the detection of *H. pylori* and AB and PAS for the identification of IM. We first examined the H&E stained slides and determined the level of sampling (esophageal, antral or fundic). Then, we evaluated the *H. pylori* status and the presence of IM. Finally, we examined the special stains and noted the results.

H. pylori: The *H. pylori* visualized initially on H&E stained slides, is objectified based on its characteristic morphological criteria (small, basophilic and curved bacillus which is comma-shaped) and its distribution (plated on epithelial cells especially in crypts). The bacteria's presence was classed as: positive, negative or inconclusive. In a second step, we analyzed the corresponding Giemsa stained slide to better visualize the *H. pylori* which stained intensely blue and evaluated the concordance with the H&E stained biopsy using the same scale. The *H. pylori* infection is considered present if the bacterium is detected by either stains H&E or Giemsa. No other resource to detect *H. pylori* was used (no Immunohistochemical stains or breath test).

IM: IM was first identified on H&E stained slide based on morphological criteria: columnar cells with basal nuclei and cytoplasmic mucin shaped like a goblet or barrel. The biopsy was classed as: positive (if one or several positive goblet cells have been identified) or negative (no goblet cell has been seen). Then, we examined the corresponding slides stained with PAS which stains the mucin red and the AB which stains the mucin intensely blue. The same method as that used in classing H&E stained slides was chosen to class PAS and AB stained biopsies. No other resource to detect IM was used (no Immunohistochemical stains).

Results:

We examined 209 biopsies from 139 consecutive patients. Most of the biopsies were antral (111/53,1%) and fundic (93/44,5%). The remaining biopsies were from esophagus (5/2,4%).

H. pylori: On the total of 209 biopsies, *H. pylori* was identified in 48 H&E stained slides, its prevalence was 23%. It was absent in 156 (74,6%) H&E biopsies. Five cases (2,4%) were inconclusive for *H. pylori*. All esophageal biopsies were *H. pylori*-negative on both stains. The results obtained using H&E and Giemsa stain are presented in table 1. H&E had an accuracy of 63,2% and a specificity of 92,1%. The results of the two stains were concordant in 173 biopsies (82.8% of all biopsies) with kappa = 0.58: *H. pylori* was identified by both tests in 36 biopsies (17,2%), both tests were *H. pylori*-negative in 136 biopsies (65,1%) and the presence of *H. pylori* remained inconclusive in one biopsy (Table 2). The two tests were not concordant in 36 biopsies (17,2%). Thereby, Giemsa stain made a diagnostic gain in 35 biopsies (16,7% of total biopsies). It helped to visualize *H. pylori* in 19 biopsies from the 156 *H. pylori*-negative on H&E stain (12,18% of the negative H&E slides / 9,1% of all biopsies). Twelve slides (25% from those regarded as *H. pylori*-positive on H&E stain and 5,7% from all biopsies) were identified *H. pylori*negative on Giemsa. It asserted the diagnosis in 4 biopsies from the 5 inconclusive ones (80% of the inconclusive H&E slides / 1,9 % of all biopsies), so two biopsies were *H. pylori*positive (40%) and the two others were *H. pylori*-negative (40%). Finally, one Giemsa stained slide regarded as inconclusive for *H. pylori* was classed as *H. pylori*-negative on H&E stain. For these discordant cases, there were no inflammation cells and no neutrophils so it was considered as *H. pylori*-negative.

IM: IM was present in 11 H&E sections (5,3% in all biopsies sites) and absent in 198 cases (94,7%). The distribution of site biopsies with IM on H&E, AB and PAS are presented in Table 3. The AB has therefore made no diagnostic gain since all negative biopsies on H&E stain were also negative on AB stain. Comparing the identification of IM on PAS and H&E stains, concordance was in 99.5% (207 biopsies, one biopsy was unstained with PAS because of depletion of materiel biopsy): 100% of the negative biopsies (198 biopsies) and 90% of H&E-positive biopsies. The two stains were not concordant in only one section (0,5%) because the two slides were cut on different levels into the paraffin block. We conclude that PAS has made no diagnostic gain.

Discussion:

HP: *H. pylori* is a bacterium which can almost be identified with H&E stain only [5,6]. Its visibility on slides is enhanced with special stains such as Giemsa, Genta, Toluidine Blue and Warthin-Stary stains or with immunohistochemical antibodies [1, 2, 5,7]. Such special techniques are a waste of time and money [3, 4]. *H. pylori* can be quickly identified on the H&E-stained slide in most cases when the acute inflammation is dense and have numerous organisms at the luminal surfaces. Wight and Kelly found that this was the most common pattern; a special stain wasn't needed for these cases [8].

Laine et al stated that when more than only sparse numbers of *H. pylori* organisms are present, the H&E has a high degree of accuracy (98%) similar to Giemsa (96%) and Genta (97%) stains [9]. Our study found a low degree of accuracy (63,2%) probably because there was not a sufficient contrast between bacteria and surrounding tissue [10]. The identification of the organisms can apparently be enhanced by doubling the H&E staining period from 5 to 10 minutes [11]. Several histologic features are indicators to the likely presence of *H. pylori*. Faigel et al showed that the best positive predictor is the presence of neutrophils (86% positive predictive value) especially if combined with chronic inflammation (92% positive predictive value) [12]. Lymphoid follicles with germinal centers also indicate current or past *H. pylori* infection. Not all cases of moderately inflamed tissue will be infected as autoimmune gastritis, Crohn's disease and lymphocytic gastritis. Currently, many pathologists use special stain if *H. pylori* can't be convincingly seen in H&E biopsies. We found that *H. pylori* was identified in 48 H&E slides (23%). On Giemsa stained sections, *H. pylori* was found in 57 cases (27,3%). Giemsa made a diagnostic gain in 35 biopsies (16,7% of all biopsies). Our results assert that the H&E stain is usually sufficient for the diagnosis of *H. pylori* and highlight that a special stain is greatest for those cases in which organisms are not seen. Several studies [6, 10, 13, 14] stated that the sensibility of Giemsa was 80%. A recent study suggests that Giemsa is not needed since it is not a specific stain [15]. Other methods can be used to determine infection such as the toluidine blue stain which is easily made and requires four minutes to be done but Wright's study showed that this stain was not reliable for the detection of *H. pylori*. Its sensitivity was 73% and its specificity was 90%. Immunohistochemical stains using ☐-*H. pylori* antibody (DAKO®) and breath tests can also be used but they are unnecessary [8, 9].

IM: Regarding AB, it is commonly used to identify IM. The advantages to performing this routine stain remain controversial. We think that the argument for not using a routine special stain for every single biopsy is even stronger when considering the AB stain and its role in the detection of IM. The goblet cells that define IM contain mucin that has a slight basophilic tinge on H&E. This distinguishes them from the "pseudogoblet" cells, in which the distended cytoplasm lacks that tinctorial quality, and the shape of the cell cytoplasm is less distinctly defined than goblet cells [8]. The AB stains the goblet cells intensely blue but it may also stain other columnar cells a pale blue. These 'columnar blues', in the absence of goblet cells, may lead the unwary pathologist into diagnosing a non-existent IM [16]. This may possibly consign the patient to repeated surveillance endoscopies for a non-existent Barrett's esophagus. In our study, the AB has made no diagnostic gain since all negative biopsies on H&E stain were also negative on AB stain. Our study provides evidence for our own and others recommmandations that routine AB of potential Barrett's mucosa is not necessary. Regarding PAS, it objectifies the neutral mucins but also the subgroup of Nacetylated sialomucins since carbohydrates stain magenta. In our laboratory this special stain is performed routinely to see IM. Our study stated that PAS has made no diagnostic gain. In the literature no study has evaluated the contribution of the PAS in visualizing IM. The use of routine stains in esophageal biopsies will facilitate detection of Barrett's mucosa and so identifying those patients that may then be considered for future endoscopic surveillance for dysplasia. Gastric IM is a pathologic finding that should be commented upon when noticed, but special techniques for enhancing its detection are not necessary and represent a waste of time and money. We agree with the AGA Chicago Workshop's observation that, in select cases of esophageal biopsies, specially those in which goblet cells are present, the AB-PAS stain can help confirm the diagnosis and avoid over-diagnosis of IM [16]. However, PAS-AB sections may be interpreted with difficulty because of its a heterogeneity [17]. Several authors have noted that this special stain is not specific since the glandular epithelium of the cardia and distal esophagus is stained, which can potentially lead to false positives [17]. Some studies have suggested that goblet cells are not always visible [18]. The attractive advantage to the immunohistochemical stain is that it distinguishes the goblet cells from the pseudo-goblet cells using the anti-mucin antibody such as MUC1 antibody [19]. Silva concluded that immunohistochemical stain could be used rather than histochemistry to classify of IM [20]. However, other studies have reported that the immunohistochemical stain is not greater than the H&E [21].

Conclusion:

Routine special stains for detection of *H. pylori* and IM are not required for most gastric and/or esophageal biopsies. The best positive predictor is the presence of neutrophils, combined with chronic inflammation. If *H. pylori* eradication treatment has failed, then the presence of neutrophils is a sensitive marker of that failure and if organisms are not seen on H&E, then special stain or immunohistochemical stain should be performed.

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Tables:

TABLE 1: Proportion of Site Biopsies with Helicobacter pylori on Hematoxylin & eosin and Giemsa stains.

	Antrum		Fundus	
	H&E	Giemsa	H&E	Giemsa
H. pylori positive/total biopsies	30 (27%)	34 (30, 6%)	18 (19, 35%)	23 (24, 7%)
H. pylori negative/total biopsies	77 (69, 4%)	77 (69, 4%)	74 (79, 55%)	68 (73, 2%)
H. pylori Inconclusive/total biopsies	4 (3, 6%)	0 (0%)	1 (1, 1%)	2 (2, 1%)

H&E: Hematoxylin and eosin, H. pylori: Helicobacter pylori

Table 2: Correlation between Hematoxylin and eosin and Giemsa Stains on Helicobacter pylori Status.

	H&E / total biopsies	Giemsa HP+ / H&E	Giemsa HP- / H&E	Giemsa Inconclusive / H&E
H&E HP positive	48 (23%)	36 (75%)	12 (25%)	0 (0%)
H&E HP negative	156 (74, 6%)	19 (12, 18%)	136 (87, 18%)	1(0, 64%)
H&E HP Inconclusive	5 (2, 4%)	2 (40%)	2 (40%)	1 (20%)

H&E: Hematoxylin and eosin, HP: Helicobacter pylori, +: positive, -: negative.

Table 3: Proportion of site biopsies with intestinal metaplasia on Hematoxylin and eosin (H&E), Alcian Blue (AB) and Periodic acid Schiff (PAS) stains.

	Antrum (IM/total biopsies)	Fundus (IM/total biopsies)	Esophagus (IM total biopsies)
H&E	9 /111 (8, 1%)	1/93 (1, 1%)	1/5 (20%)
AB	8/111 (7, 2 %)	1/93 (1, 1%)	1/5 (20%)
PAS	7/110 (6, 4%)	1/93 (1, 1%)	1/5 (20%)

AB: Alcian Blue, H&E: Hematoxylin and eosin, IM: intestinal metaplasia, PAS: Periodic acid Schiff.