Histological detection of *Helicobacter pylori*

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A number of different techniques can be used to establish the diagnostic of an infection with Helicobacter pylori. Histologic analysis belongs to direct, invasive (which needs endoscopy) methods.

Biopsy sampling for gastric mucosa gives us information about HP status and about the type, grade and extent of inflammation, atrophy and intestinal metaplasia in the stomach.

Sydney System and Houston updating

The Sydney System (1,2,3) is an early attempt to provide guidelines for biopsy sampling and for classification and grading of gastritis. It has further been improved by the Houston updating (1994) (4). They provide information about the topography, the morphology and the etiology of the lesions. The key of the system is that the mucosa and both the gastric and body are routinely biopsied and in particular that five histological parameters (chronic inflammation, activity, atrophy, intestinal metaplasia, and HP status) are routinely read from the biopsies and recorded in the histopathology report. The recommendations are actually the folllowing :

1. Five biopsy specimens are taken, two from the antrum within 2 to 3 cm of the pylorus, one from the distal lesser curvature, and the other from the distal greater curvature, two from the corpus about 8 cm from the cardia (one from the lesser and the other from the greater curvature) and one from the incisura angularis. Any lesion visible at endoscopy has to be biopsied.
2. Samples from antrum, corpus and incisura angularis, (and any other lesion) should be separately identifiable.
3. Transmission of information to the pathologist about the patient's endoscopic findings, clinical history and biopsy sites is essential for successful clinicopathologic correlation in gastritis.
4. A special stain for HP should be carried out before declaring an inflammed biopsy specimen negative.

The modified Giemsa seems to be the cheaper and a very effective stain but the choice of stain (Whartin-Starry, new Genta, Cresyl violet, acridine orange) is a matter of local preference. The new Genta stain (5) combines three commonly available stains (Steiner silver stain, hematoxylin-eosin, and alcian blue at pH 2.5) into a single procedure and should permit optimal detection of HP in tissue sections while simultaneously it should allow the histolopathologic evaluation of all characteristics of the gastric mucosa, including identification of intestinal metaplasia. Cohen and Laine (6) evaluated the utility of different stains and found that the sensitivities of hematoxylin-eosin (HE), new Genta and Giemsa stains were similar to each other at both low HP density and high density ; specificity was excellent (98-100%) for the Genta and Giemsa stains at both low and high density and the HE stain at high density ; the specificity was decreased (90%) for HE at low density of HP. Moreover, 10% of the Genta stains were technically unsatisfactory. The immunohistochemistry is not needed in routine but it is said to be helpful in identification of coccoid forms (7), or of few HP with atypical forms, especially after treatment. In our opinion, examination at $\times$ 100 objective, under oil immersion could be recommended for detection of HP, chiefly when the density of HP is low.

The five parameters (chronic inflammation, activity, atrophy, intestinal metaplasia and HP status) are graded as following : 0 = absence, + = mild, ++ = moderate, +++ = severe (in Sydney System) or marked (in Houston system).

There is no standard for chronic inflammation and geographical variations are detected but it can be considered that more than 2 to 5 mononuclear leucocytes in the lamina propria or 2 to 3 mononuclear leucocytes between 2 glands, observed with $\times$ 40 objective are not more normal. It is the same when more than 5 intraepithelial lymphocytes are observed for 100 epithelial cells. After treatment, the chronic inflammation of the lamina propria can persist during more than one year. When there is no chronic inflammatory infiltrate in a gastric mucosa, HP will not be found : the negative predictive value of chronic inflammation for detection of HP is 100% (6).

Presence of activity is a marker of presence of HP ; the density of polymorphonuclear and HP are correlated (4).

Lymphoïd follicles indicate that HP is present or was present and is now eradicated. The positive predictive value of the presence of HP in gastric biopsies with lymphoid follicles is 96% (8). They are more often present in biopsies from children (9,10) and they give

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an almost caracteristic nodular aspect in the antral mucosa (9). It was been demonstrated that lymphoïd follicles can be found in all gastric biopsies from patients infected with HP, when sufficient specimens are taken and serial sections are made (11). Mucosal atrophy is caracterised by a loss of glands but actually, there is no consensus over an univoqual image of mucosal atrophy. Dense inflammatory infiltrate of the lamina propria discards sometimes the glands ; giving a false impression of mucosal atrophy, which disappear after treatment (12). When fibrosis appear in the lamina propria, we can be sure that atrophy exists.

The last criteria to be considered is intestinal metaplasia. There are three different types of intestinal metaplasia. The former, the type I, or complete type or "small intestinal" type, demonstrates all cells types present in the small intestine. It contains sialomucins and it represents minimal associated risk for cancer. The type II is intermediate between I and II and contains goblet cells and gastric cells containing neutral and sialomucins. The type III, or incomplete type or "colic" type, demonstrates only goblet cells, with sulfomucins, and any sialomucins. It is associated with a high risk of cancer (2.7 to 5.8 more than type I and II) (4). Special stains (Alcian bleu and PAS at pH 2.5, ...) are recommended to identify these different types of intestinal metaplasia but are limited to research and not used in routine. Interobserver variations in the assessment and grading of gastritis-related parameters is quite satisfactory on average (interobserver agreement reach 83% to 94%) indicating that the diagnostic and grading criteria suggested by the Sydney-Houston system can be applied consistently by histopathologists, and used in routine clinical pratice. Good interobserver concordance is the case particularly in the assessment of inflammation, both acute and chronic, and in the assessment of HP load, but interobserver agreement is worst in the assessment of atrophy (lost of glands) particularly in antral biopsies (13,14). A new visual analog scale was introduced in Houston for the graded variables and a set of guidelines for its application have been designed.

The histological approach reveal also findings that indicate special forms of gastritis, such as eosinophilic, lymphocytic, reactive or granulomatous gastritis, or Helicobacter Heilmannii gastritis (1,2,3,4,15).

The results gathered in the Sydney-Houston system are to be compared with the clinical entities. Several authors like Sipponen and Stolte (15) and Rubin (12) describe different types of HP+ gastritis corresponding to clinical phenotypes. When gastric mucosa is normal, there is almost no risk to develop gastric ulcer or cancer (ulcer developped only in 1/133 patients with normal gastric mucosa during ten years followup) (15). Non-ulcer pangastritis is usually found in asymptomatic HP infected individuals without ulcers. Duodenal ulcer is associated with HP+ gastritis clearly more marqued in the antrum than in the fundus. Gastric ulcer and cancer are commonly associated with HP+, diffuse multifocal atrophic gastritis in the antrum and the fundus. The lesions are more extensive in cancer. Diffuse corpus-predominant atrophic gastritis is associated with autoimmune gastritis.

Sensitivity and specificity

The sensitivity of histological tests, like the specificity depends on the expertise of the pathologist and the density of HP. There are many studies (17,18,19,20,21) which provide information about the sensibility and the specificity before treatment : these are high, varying between 88 and 99% for the former and 90 and 100% for the last with modified Giemsa. It is lower with Haematoxylin-eosin, but this stain is needed for the observation of the mucosa. There are few studies over evaluation of post-treatment biopsies. Rollan *et al.* (22) studied 59 patients with HP+ duodenal ulcer, treated during two weeks with amoxicillin-metronidazole (n = 36) and omeprazole amoxicilline-tinidazole (n = 23). Four weeks after the treatment, they were tested for HP infection. The infection status was established by a concordance of tests results. The diagnostic accuracy of histological detection of HP with Whartin-Starry stain was 93.2% (positive predictive value = 78.6% ; negative predictive value = 97.8%). The best results were obtained with urea-breath test and rapid urease test on biopsy. In our experience (23), based on 153 consecutive HP+ patients 4 to 6 weeks after treatment with a proton pump inhibitor + Amoxicillin and clarithromycin), the sensitivity of histological detection of HP is 93.5% if we consider antral (± body) biopsies and 83.9% if we consider body biopsy alone. The specificity is 98.7% for antral (± body) biopsies and 100% for body biopsies.

Cytology

Cytology is another method for detecting HP but it doesn't provide information about the gastric mucosa (24). It is rapid. Its sensitivity varies between 56-100%, depending of the stain, its specificity between 58-93%. Stain with Giemsa seems to be better than Gram (17).

Advantages and disavantages of the histological and cytological methods (25)

- Histology provides information about HP status, gastric mucosal lesions and specific forms of gastritis ; it provides further opportunities for assessing the risk and likelihood of various gastritis disorders (16) ; cytology doesn't.
- Histology, and cytology are available in the majority of centers.
- This histologic technique needs minimum 24 hours but another 24 hours are needed for sending the report. The cytologic examination is possible in one hour in the endoscopic room when a microscope is available.

— These approaches permit retrospective analysis.
— The histologic sampling gives semi-quantitative grading of the five parameters tested.
— Typing the strains and definition of their sensibility to antibiotics is not possible.
— The reproductibility is good for grading the inflammation and the HP status but not so good for grading atrophy and intestinal metaplasia. In the future, studies are to be done to define the effciency of the visual analog scale introduced in the Houston-System on the agreement between different observers.
— Cost of these tests are 1866,– bf for biopsy and 1069,– bf for cytology.

Conclusions

Histological approach is reliable for grading HP status and gastritis and for defining other special forms of gastritis. It can provide information about atrophy and intestinal metaplasia, which are pronostic factors. Sensibility and specificity are high but depend of the expertise of the pathologist. They also depend on the density of HP, and thus they are better when HP detection is considered as a diagnostic method rather than as a control of eradication. Special stains improve the sensibility and the specificity : the modified Giemsa seems to be a very efficient and cheap stain. It must be employed when an inflammatory gastric mucosa is observed without HP visible on Haematoxylin-eosin. Microscopic examination of slides with × 100 objective improves also the results, chiefly when the density of HP is low. Immunoperoxydasic stain should be reserved, in selected cases, to identification of few HP with atypical forms, or for coccoïd forms.

The choice of histology (and cytology) as tests for the detection of HP is to be discussed in the different clinical situations : diagnostic aim or control of eradication, clinical phenotypes (non ulcerous dyspeptic patients, peptic ulcer, duodenal ulcer, gastric neoplasia), age of the patients, ...

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