Campylobacter pylori detection in gastric mucosa: Association with gastritis

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ABSTRACT: In order to evaluate the association between *Campylobacter pylori* and gastritis, two biopsies were taken from the duodenal bulb, antrum, body and fundus (and from lesions if there were any) in 100 consecutive patients referred to this gastroscopic clinic. For each site, one biopsy was for histology and *C. pylori* detection by Warthin-Starry staining, and the second biopsy was for culture. In addition, for each patient a gastric brushing was Gram stained. Twenty-one patients were excluded from the study. Among the remaining, 33 patients had positive biopsy culture for *C. pylori* and, of these, 30 (91%) had gastritis (including 23 with active chronic gastritis). The culture sensitivity increased with the number of biopsies. Forty-two patients had a positive brushing specimen, of which 30 (71%) had gastritis. Gram stain on a brushing specimen had a sensitivity of 84.8% in comparison with the biopsy culture. Of the 23 patients with positive Warthin-Starry stain, 19 (83%) had an histology of gastritis. There was a strong correlation between the presence of *C. pylori* in the stomach mucosa and the gastritis. The incidence of *C. pylori* associated gastritis is similar in Quebec to other parts of the world. The biopsy culture is a simple and specific test, and at least two biopsies are necessary for a good sensitivity. Gram stain on a brushing specimen is an adequate test for rapid detection of *C. pylori* in the stomach. **Can J Gastroenterol** 1988;2(3):94-98.

Key Words: *Campylobacter*, *Campylobacter-like organism*, *Campylobacter pylori*, Gastritis

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REPORTS OF SPIRAL BACTERIA IN THE human stomach have occurred sporadically over the last century (1,2). However, it was only in 1982 that a micro-aerophilic campylobacter-like organism was isolated by Marshall and Warren (3) from gastric antral biopsy specimens. This initial publication sparked off world wide enthusiastic research into this bacterium now named *Campylobacter pylori* (4,5). Since this time, a number of studies have shown an association between the presence of *C. pylori* in gastric mucosa and histologically confirmed gastritis. However, there is still some discussion whether the organism has a causative role or is simply a secondary invader.

With this study, it was confirmed that *C. pylori* was correlated with gastritis and then different methods for *C. pylori* detection in the human stomach were evaluated. The literature was also reviewed, emphasizing principal arguments in favour of the causative relationship of the bacterium with gastritis. Finally, some basic notions for a *C. pylori* detection pro-

tol in patients undergoing endoscopy are suggested.

PATIENTS AND METHODS

Endoscopy: One hundred consecutive patients were included who were referred for gastroscopy on clinical grounds from January to March 1987. Informed consent was obtained from all patients for endoscopy and biopsies. Biopsies were obtained from the duodenal bulb, antrum, body, fundus and from lesions if there were any. Two biopsies were taken in each area, one for culture and the other for histology, giving eight to 10 tissue specimens from each patient. Also, one brushing specimen from the antrum was spread on a glass slide and air dried. An endoscopist recorded the clinical history, medication and drug use, alcohol consumption, clinical diagnosis and endoscopic findings.

Microbiology: Samples for culture were immediately immersed in 5 mL of thioglycolate broth, transported to the laboratory within 2 h of collection and macerated in 2 mL of the same broth. One drop was then plated on blood agar (trypticase soy agar with sheep blood 5%) and chocolate agar (GC agar, bio-X 1% and hemoglobin 1%) and incubated microaerophilically in anaerobic jars for seven days at 37°C. The gaspak (Campy-Pak, BBL) was replaced every 24 h. *C. pylori*, when isolated, was identified using direct examination with Gram stain, oxydase, catalase, oxydation-fermentation test with purple bromocresol, nitrate reduction, hippurate hydrolysis, urease (Christensen's urea medium), indole, and susceptibility to cephalothin and nalidixic acid. The brushing specimens were fixed and stained by Gram method.

Histology: The biopsies for histological examination were fixed in formalin and routinely processed. Sections were stained with hematoxylin and eosin and assessed for gastritis using the same criteria as Marshall (3), that is normal stomach, chronic gastritis or active chronic gastritis. Chronic gastritis indicates inflammation with no increase in polymorphonuclear leukocytes but with increased or normal number of lymphoid cells and edema, congestion or cell damage. Active chronic gastritis is indicated if polymorphonuclear leukocytes are

increased in number, if a few infiltrate one gland neck or pit, or if they are scattered throughout the superficial epithelium. In addition, other sections from all biopsies were stained by the Warthin-Starry method (6) and examined for presence or absence of small curved bacilli on the surface of the epithelium.

Data analysis: The results were recorded in each department (gastroenterology, microbiology and pathology) independently. For statistical analysis of the findings, the Fisher's exact test or the χ^2 test was used depending on sample size. For the study of the correlation between the presence of *C. pylori* and gastritis, only biopsies from the antrum, body and fundus were considered. A patient was considered positive when there was at least one positive site for *C. pylori* by culture and/or histology.

RESULTS

From the 100 patients, 21 were excluded (two for having taken antibiotics in the week preceding the endoscopy, three for upper digestive neoplasia, one for incomplete clinical data, one for uninterpretable culture and 14 for peptic ulcers). The number of peptic ulcers was too small to study adequately any association with *C. pylori*.

C. pylori was isolated from 75 biopsies of 33 patients. The bacteria were curved or S-shaped Gram-negative rods. In electronic microscopy, they had three to five sheathed flagella arising from one end of the cell. The biochemical feature was typical of *C. pylori*: microaerophilic growth in two to four days at 37°C; positive reaction for oxydase and catalase; inert reaction in oxydation-fermentation test; negative reaction for hippurate hydrolysis, indole and nitrate reduction; very strong positive reaction for urease (5 to 10 mins); susceptibility to cephalothin and resistance to nalidixic acid.

Among the 79 patients remaining in the study, 40 had normal histology and 39 had gastritis (chronic or active chronic). These two groups were closely matched for sex distribution, age, medication, excessive alcohol consumption and upper digestive tract surgery.

The correlation between gastritis and the presence of *C. pylori* defined by biopsy culture was impressive (Table 1). Among the 33 patients with at least one

TABLE 1

Correlation between *Campylobacter pylori* culture and histological examination of gastric mucosa in 79 patients

Histology	Culture	
	Positive*	Negative
Gastritis†		
Active chronic†	23	4
Chronic	7	5
Normal†	3	37

* *C. pylori* was isolated from at least one biopsy.
† $P < 0.0001$ (χ^2 test)

positive biopsy culture, 91% had gastritis (of which 77% were active chronic) and only 9% had normal histology. Table 2 shows the number of positive patients by culture according to different combinations of biopsy sites. The number of positive patients was similar whatever the biopsy site but increased with the number of sites.

With the Gram stain on the brushing specimen, a good correlation between gastritis and presence of *C. pylori* was obtained (Table 3). In comparison with the culture, the Gram sensitivity was 84.8% since 28 of the 33 positive patients by culture were also Gram-positive.

Sections stained with the Warthin-Starry method were not all easy to interpret. When there was a doubt, often caused by artefacts, the specimen was considered negative. However, a correlation between gastritis and *C. pylori* was established by this method (Table 3). Only 23 cases were positive but 83% had gastritis and only 17% had a normal histology.

Finally, the endoscopic findings were compared with the histological diagnosis. There were 20 cases with endoscopic diagnosis of gastritis. One-half of these patients had gastritis by histology

TABLE 2

Number of positive patients by culture according to different combinations of biopsy sites

Biopsy sites	Patients with positive culture(s)*
Antrum	22
Body	22
Fundus	23
Antrum and body	27
Antrum and fundus	28
Body and fundus	28
Antrum, body and fundus	33

* *C. pylori* was isolated from at least one biopsy

TABLE 3

Correlation between Gram and Warthin-Starry results for *C pylori* and histological examination of gastric mucosa in 77 patients

Histology	Gram		Warthin-Starry	
	Positive	Negative	Positive†	Negative
Gastritis*	30	9	19	19
Normal	12	26	4	35

Gram and Warthin-Starry were lacking in two patients; † Bacteria detected in at least one biopsy; * Active chronic or chronic gastritis; $P < 0.0005$ (χ^2 test) gastritis versus normal

and the remaining were normal. In the group with endoscopic diagnosis of normal gastric mucosa, 30 had normal biopsies and 29 had gastritis confirmed by histology.

DISCUSSION

Findings about the identification of the *C pylori* were similar to those of other authors (1,7). The very strong urease activity is the striking feature, indeed *C pylori* is the sole *Campylobacter* species with a positive urease test (8) and this test could suffice in itself to presumptive identification.

A high correlation was found between the presence of *C pylori* and gastritis in the biopsies. Thirty of 33 (91%) of the patients who tested positive for *C pylori* also had gastritis. It was confirmed that the incidence of *C pylori* associated gastritis in Quebec was similar to that found in other parts of the world. Marshall (7) in Australia discovered 100% (52 of 52) of the patients who tested positive to *C pylori* also had gastritis while Jones (9) in the United States had a 96% (26 of 27) correlation and Taylor (10) in Alberta, Canada obtained a 95% correlation. Other authors had similar data (11-14).

Despite its strong association with gastritis, the pathogenic role of *C pylori* is still controversial. Is it simply a colonizer of a mucosa altered by gastritis or the primary disease etiology? Several observations support the latter hypothesis. First, *C pylori* in the stomach seems to provoke a local (12,13,15,16) and systemic immune response (9,11,17-20). Second, *C pylori* is present in primary gastritis and not in secondary gastritis, ie, with known predisposing cause (14,21-23). Third, *C pylori* appears particularly adapted to gastric epithelial cells in which it is associated with well described specific lesions (7,15,16,24-26). Further, few treatment studies have been done but the available data up to now are very

much in favour of a pathogenic role of *C pylori*. Bismuth compounds or amoxycillin clear the organism and the associated gastritis (27-29). Finally, one of the most convincing arguments for the pathogenic action of *C pylori* comes from the demonstration of third and fourth Koch's postulates. Two volunteers with normal stomach developed symptomatic gastritis after ingestion of *C pylori* suspension (15,16). All these observations support a cytopathogenic activity by *C pylori* and militate against the hypothesis that the organism is associated with gastritis merely by colonizing the mucosa after inflammation has occurred.

C pylori associated gastritis is diagnosed by histological and/or microbiological examination of gastric biopsy specimen. The culture of biopsy specimen is the most specific test. The typical identification profile of the bacterium provides very little risk of false-positive culture. The technique is simple and may be performed in a routine laboratory. Numerous causes of false-negative result are possible, such as recent antibiotic therapy, but can be avoided without difficulty (7). As demonstrated in Table 2, the culture sensitivity increases with the number of biopsies. However, although the number of positive patients is similar whatever the observed site, it cannot be concluded that the choice of biopsy site does not influence the sensitivity of the culture. As the biopsy forceps were not sterilized between each area and as the order of biopsies was always the same, a cross-contamination of culture from preceding samples was theoretically possible, particularly from the antrum, which is positive in most positive patients. Hazell *et al* (30), in a study of *C pylori* distribution within the stomach, (in which they cleaned and rinsed biopsy forceps between each site) suggested taking biopsies from both the antrum and body because the bacterium may be limited

to one of these areas. Unfortunately, their method was not perfect since the endoscope lumen was not sterilized between each biopsy.

The principal disadvantage with the culture is the delay of several days before obtaining results. A Gram-stained brushing specimen is useful because results may be available within 1 h, ie, when the patient is still in the gastroscopy clinic. Present results demonstrated a good sensitivity of 84.8% in comparison with culture but a decreased specificity since 10 patients with negative culture and normal histology were positive on Gram-stain. Unfortunately, it is impossible to establish if these positive Gram stains are true positive since there was no culture from the brushing specimens. Also, present results cannot be compared with others since most studies used Gram-staining on biopsy specimens instead of brushing specimens and none evaluated the test specifically.

Other rapid detection tests have been previously investigated. Urease tests performed directly on biopsy specimens obtained different results depending on technique. Its sensitivity varied from 50% to 100% (14,25,31-35) but there is a commercially available urease test (CLO Test) with high specificity and sensitivity (36,37). Furthermore, a recent study demonstrated a correlation between the urease reaction time and the grades of inflammation and number of bacteria seen in biopsies (32). The ^{13}C -urea breath test also exploits the strong urease activity of *C pylori* and its initial results are encouraging (38). Detection of the bacterium by phase contrast microscopy (39) or immunofluorescence (40) is possible but, like the ^{13}C -urea breath test, is not available in many hospitals.

As with most previous studies, the Warthin-Starry stain was used for histological detection of *C pylori*. Data with this stain were less interesting than with culture and Gram stain. The test was time consuming and difficult to interpret because there were a lot of artefacts. Some pathologists suggest, as an alternative, the Giemsa, hematoxylin and eosin or fluorescent acridine orange tests (10,41,42). In a recent study, the bacterium was detected with the hematoxylin and eosin stain in 95% of positive culture biopsies (29).

CONCLUSION

Despite several major reasons to consider *C. pylori* as a pathogen, some continue to refute this idea because the bacterium is found in apparently healthy subjects. However, as with other bacteria, such as group A streptococcus in the pharynx, a carrier state is possible. Although further clinical trials on *C. pylori* infection treatment are required to confirm its pathogenicity, an investigation of different gastroduodenal diseases, particularly gastritis, should include an attempt to detect *C. pylori* within the stomach in addition to the usual histological colouration. At least two biopsies are necessary, one from the antrum. A rapid detection of the bacterium by Gram stained brushing specimen is very simple but not specific enough. The rapid urease test deserves much consideration, however, it should be evaluated in the clinical setting before being used routinely. Any kind of rapid test should be confirmed by culture. Special staining of biopsies to find the bacterium is not indispensable if rapid test and culture are done.

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La détection du *Campylobacter pyloridis* dans la muqueuse gastrique: son association avec la gastrite

RESUME: Afin d'étudier l'association existant entre le *Campylobacter pyloridis* et la gastrite, deux biopsies ont été prélevées au niveau du bulbe duodénal, de l'antrum, du corps, et du fond (et les lésions, le cas échéant) chez 100 patients consécutifs référés à la clinique gastroscopique. Pour chaque site, une biopsie était destinée à l'histologie et à la détection du *C. pyloridis*, et la seconde à la culture. De plus, pour chaque patient, un brossage gastrique subissait une coloration de Gram. Vingt-et-un patients ont été exclus de cette étude. Parmi les autres, 33 patients avaient une biopsie positive pour le *C. pyloridis* et parmi ceux-ci, 30 (91%) souffraient d'une gastrite (dont 23, d'une gastrite chronique en évolution). On relève une augmentation de la sensibilité de la culture avec le nombre de biopsies. Quarante-deux patients ont eu une pièce de brossage positive, et parmi eux, 30 (71%) étaient atteints de gastrite. La coloration de Gram appliquée sur les pièces de brossage avait une sensibilité de 84.8% comparée aux cultures de biopsies. Des 23 patients avec une coloration de Warthin-Starry positive, 19 (83%) avaient une histologie de gastrite. Il existe une forte corrélation entre la présence du *C. pyloridis* dans la muqueuse stomacale et la gastrite. L'incidence du *C. pyloridis* associé à la gastrite est similaire au Québec, Canada et dans les autres régions du monde. La culture des biopsies est un test simple et spécifique, qui requiert deux biopsies au moins pour une bonne sensibilité. La coloration de Gram sur une pièce de brossage constitue un test adéquat pour détecter rapidement la présence du *C. pyloridis* dans l'estomac.

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