

## Sampling Efficiency in the Diagnosis of *Helicobacter pylori* Infection and Chronic Active Gastritis

PETER NEDENSKOV-SØRENSEN,<sup>1\*</sup> STEINAR AASE,<sup>2</sup> ARVID BJØRNEKLETT,<sup>3</sup> OLAV FAUSA,<sup>3</sup>  
AND GEIR BUKHOLM<sup>1</sup>

*Institute Group of Laboratory Medicine, The National Hospital, University of Oslo,<sup>1</sup> and Department of Pathology<sup>2</sup> and Medical Department A,<sup>3</sup> Rikshospitalet, The National Hospital, Oslo, Norway*

Received 23 August 1990/Accepted 31 December 1990

**The methods and sampling procedures used in the diagnosis of *Helicobacter pylori* infection and chronic active gastritis were evaluated. Five biopsy specimens for bacteriological cultivation and three specimens for histological examination were obtained endoscopically from a defined area of the gastric antral mucosae of 83 patients. An increase in the number of biopsy specimens for cultivation from one to five revealed only one more *H. pylori*-infected patient. *H. pylori* was isolated from 31 of 83 patients. Three technically adequate samples for histological examination were obtained from each of 74 patients. Of these 74 patients, chronic active gastritis was diagnosed by demonstration of typical histological changes in all three specimens from each of 20 patients, in two of three specimens from each of 3 patients, and in one of three specimens from 1 patient. The results indicate that one biopsy specimen is sufficient for the isolation of *H. pylori*, whereas several specimens may be necessary for the histological diagnosis. Chronic active gastritis was found in four patients not infected with *H. pylori*; on the other hand, *H. pylori* was isolated from nine patients who showed no signs of chronic active gastritis in any of three samples.**

The diagnosis of *Helicobacter pylori*-associated chronic active gastritis (CAG) is based on (i) demonstration of infection with *H. pylori* and (ii) demonstration of CAG by histomorphological examination. In several studies a significant correlation between infection with *H. pylori* and CAG has been found (3-8). However, the discrepancies in different studies might be explained by insufficient diagnostic procedures or by an uneven distribution of *H. pylori* on the gastric mucosae (3-8). Consequently, it has been advocated that several samples should be taken to ensure a correct diagnosis (3, 6).

The aim of the present study was to determine whether the diagnostic efficiency would improve if multiple biopsy specimens were taken and whether the same correlations between *H. pylori* infection and CAG would be revealed after such an increase.

### MATERIALS AND METHODS

**Patients.** Eighty-three patients referred for upper gastrointestinal endoscopy were studied consecutively and prospectively. Patients who had undergone gastric operations and patients who were treated with antimicrobial agents during the last month prior to endoscopy were excluded from the study, as were patients with ongoing bleeding and patients who used nonsteroidal antiinflammatory drugs. The study included 48 men (mean age, 49.7 years; range, 16 to 81 years) and 35 women (mean age, 50.2 years; range, 16 to 81 years). The patients were referred for gastroscopy for various reasons, of which the major were dyspepsia (24%), abdominal pain (10%), or portal hypertension with esophageal varices (14%). The remaining patients had other diseases.

**Endoscopy and biopsy.** For endoscopy and biopsy, Olympus instruments were used (GIF K 10 or Q 10 endoscopes and biopsy forceps type FB 24K). Biopsy specimens, five for

bacteriological cultivation and three for histological investigations, were taken from a defined area of the antral mucosa. This area was a sector of 30°, with its apex located in the pylorus and having the greater curvature as its axis of symmetry. The specimens were randomly taken from an area within 5 cm of the pylorus. The specimens for cultivation were taken consecutively and were numbered 1 to 5. The forceps were not disinfected between each of the five biopsies.

**Bacteriological examination.** (i) **Preparation of specimens.** Each specimen for bacteriological examination was placed in a well in a solution of 25% glucose-0.45% NaCl (six-well tissue culture plate, Falcon). The samples were kept at 4°C and processed in the laboratory within 4 h. Each specimen was divided into two parts; the major part of the material was ground in a porcelain mortar in 10 to 12 drops of saline. Two or three drops of the resultant suspension were inoculated onto each of the culture plates used for cultivation. The smaller part of the biopsy specimen was used for direct microscopic examination (see below).

(ii) **Cultivation of bacteria.** Brain heart infusion agar (0418; Difco) enriched with 7% horse blood and the same medium containing amphotericin B (2 µg/ml), trimethoprim (5 µg/ml), and vancomycin (3 µg/ml) were used for cultivation. In addition, medium containing human blood (Oxoid CM 233 with 0.07% glucose and 5% human blood) was used for primary isolation. One or two of the plates were inoculated with each of the biopsy specimens, resulting in three to six plates used for each biopsy specimen. The plates were incubated under microaerobic conditions (Gas Generating Kit; Anaerobic System Oxoid) (in anaerobic jars without a catalyst) at 35°C for up to 6 days. The plates were examined for bacterial growth, and whenever possible, typical colonies from the nonselective medium were selected for further identification. To estimate roughly the amount of growth of *H. pylori*, the following scheme was used: sparse growth, less than 10 colonies per plate; moderate growth, 10 to 50

\*Corresponding author.

TABLE 1. Isolation of *H. pylori* by cultivation from five gastric antral biopsy specimens<sup>a</sup>

No. of patients	<i>H. pylori</i> isolated from biopsy specimen no. <sup>b</sup> :				
	1	2	3	4	5
30	+	+	+	+	+
1	-	+	-	-	+
52	-	-	-	-	-

<sup>a</sup> Five antral biopsy specimens from each of 83 patients were examined.

<sup>b</sup> The biopsy specimens were taken consecutively and were numbered from 1 to 5.

colonies per plate; heavy growth, more than 50 colonies per plate.

(iii) **Identification of bacteria.** Bacteria were examined for Gram staining properties and micromorphology, oxidase reaction (tetramethyl-*p*-phenylenediamine), and urease and catalase activities.

(iv) **Microscopic examination of bacteriological smears.** A small part of each biopsy specimen (approximately 20 to 25%) was squeezed onto a glass slide. The smears were allowed to dry and were then fixed in methanol for 2 min and stained with acridine orange in an acetate buffer (pH 4.0) for another 2 min. Two randomly selected slides of specimens from each patient were examined in a Nikon UV incident light microscope for at least 10 min (magnification,  $\times 1,250$ ).

**[<sup>14</sup>C]urea breath test.** In the fasting state, and usually on the day of endoscopy, the patients were given a dose of 185 kBq of [<sup>14</sup>C]urea dissolved in 100 ml of orange juice. Expired CO<sub>2</sub> (1 mmol) was sampled in hyamine-hydrochloride solution before and 10 min after ingestion of the dose. The samples were analyzed in a scintillation counter, and the amount of expired <sup>14</sup>CO<sub>2</sub> was calculated as a percentage of ingested [<sup>14</sup>C]urea. A positive test result was defined as the expiration of more than 0.5% of the ingested [<sup>14</sup>C]urea activity (2).

**Histopathological examination.** Biopsy specimens for light microscopy studies were fixed in 4% formaldehyde, dehydrated in ethanol, and embedded in paraffin. Sections were stained with hematoxylin, azophloxin, and saffron. At the histological examination, a diagnosis of chronic gastritis was made when the lamina propria contained an increased number of inflammatory cells consisting predominantly of lymphocytes. CAG was diagnosed (1, 9, 10) when polymorphonuclear granulocytes infiltrated the epithelial structures, in addition to the signs of chronic gastritis. A section was defined as technically adequate if it contained the whole thickness of the mucosa, including the surface epithelium, down to the muscularis mucosae. All of the examinations were performed blinded.

## RESULTS

**Bacteriological examination.** (i) **Cultivation.** *H. pylori* was isolated from 31 of 83 patients (Table 1). An increase in the number of biopsy specimens from one to five revealed only one more patient who harbored this bacterium. *H. pylori* was isolated from the second and fifth biopsy specimens from this patient; it was isolated from all five specimens from the other patients. *H. pylori* was the only bacterium that was isolated from 15 of the patients; from 11 of these 15 patients, all of the five biopsy samples yielded heavy growth of *H. pylori*, whereas some specimens from the remaining four patients showed only sparse or moderate growth. Mixed

bacterial flora including *H. pylori* was obtained from 16 patients. From 28 patients bacteria different from *H. pylori* were isolated, and from 24 patients no bacteria were isolated at all. The one patient from whom *H. pylori* was isolated from two biopsy specimens only was reexamined twice at 3-month intervals, and *H. pylori* was cultured from four biopsy specimens at the second examination and from all five specimens at the third examination. From this patient very few colonies of *H. pylori* grew on the culture plates together with a few other bacteria. In cases in which bacteria other than *H. pylori* were isolated, the growth medium showed a mixed flora, with a dominance of gram-positive bacteria that presumably originated from the oropharynx. We did not find a case in which a single type of bacterium different from *H. pylori* dominated. In all cases in which *H. pylori* was found, it was able to grow on the different media used. We found, however, that a few isolates grew with substantially larger colonies on the medium supplemented with human blood, but this difference may have been due to batch variation. In some of the cases in which a heavy mixture of different bacteria grew, the selective medium was the only one from which *H. pylori* could be isolated.

(ii) **[<sup>14</sup>C]urea breath test.** As an additional test for the presence of metabolically active *H. pylori* on the gastric mucosae, 79 of the 83 patients were tested by the [<sup>14</sup>C]urea breath test. *H. pylori* was isolated from 29 of these 79 patients. Of the 29 *H. pylori*-infected patients, 28 also showed a positive [<sup>14</sup>C]urea breath test (median, 2.93%; range, 0.73 to 6.30% of the ingested [<sup>14</sup>C]urea). None of the 50 patients from whom *H. pylori* was not isolated showed any reaction by the breath test (median, 0.05%; range, 0.00 to 0.18% of the ingested [<sup>14</sup>C]urea dose). For the patient from whom two of the five biopsy samples revealed sparse *H. pylori* growth, the breath test result was negative.

(iii) **Direct microscopy of smears.** Furthermore, to obtain an indication of the presence of metabolically inactive bacteria or bacteria that did not grow under the described conditions, smears of the samples were stained with acridine orange and examined microscopically. Compared with cultivation, microscopy had a specificity of 100% and a sensitivity of 87%. In 34% of the smears, bacteria different from *H. pylori* were found when culture was negative for *H. pylori* and other bacteria. Very often, large diplococci were seen. Typical *H. pylori* was not found microscopically when cultivation was negative.

**Histomorphological examinations.** Three technically adequate biopsy specimens were obtained from 74 of the 83 patients. CAG was diagnosed in 24 of these 74 patients. In 20 patients, all three biopsy specimens showed CAG, whereas in three patients, CAG was diagnosed in two specimens, and in one patient, CAG was diagnosed in only one of the three specimens. In 10 patients, chronic gastritis without granulocytes in the epithelium was demonstrated, and was thus classified as chronic nonactive gastritis (Table 2).

**Correlation between bacteriology and histomorphology.** *H. pylori* was isolated in pure culture from 6 of the 24 patients with CAG and together with other bacteria from another 14 patients. Bacteria different from *H. pylori* were isolated from four patients with CAG. Of the 10 patients with chronic nonactive gastritis, *H. pylori* was isolated in pure culture from four patients and together with other bacteria from two patients. Thirty-one patients were given a diagnosis of atrophy, fibrosis, or both, and *H. pylori* was isolated in pure culture from 1 patient and other bacteria were isolated from 19 of these patients. No bacteria were isolated from 11 patients. In nine subjects, we found histologically normal

TABLE 2. Comparison of results of cultivation and histological diagnosis of gastric antral biopsy specimens

Cultivation result	No. of patients with the following histological diagnosis <sup>a</sup> :				Total
	CAG	CG <sup>b</sup>	Atrophy, fibrosis, or both	Normal	
<i>H. pylori</i> in pure culture	6	4	1	2	13
<i>H. pylori</i> in mixed flora	14	2	0	0	16
Only bacteria different from <i>H. pylori</i>	4	0	19	3	26
No bacteria	0	4	11	4	19
Total	24	10	31	9	74

<sup>a</sup> Three biopsy specimens were obtained from 83 patients, but all three samples from only 74 of these patients were technically adequate for histological examination.

<sup>b</sup> CG, Chronic nonactive gastritis.

gastric mucosae, and from two of these patients, *H. pylori* was isolated in pure culture, while from three patients only other bacteria were isolated. No bacteria were isolated from the remaining four patients with normal histology (Table 2).

**Endoscopy.** We attempted to classify macroscopic findings at endoscopy according to a scheme based on (i) normal mucosa, (ii) number of petechiae, (iii) number of erosions, and (iv) persisting folds in the prepyloreal region. We found, however, no significant correlation between these macroscopic findings and the presence of *H. pylori* (data not shown).

## DISCUSSION

The isolation of *H. pylori* and the diagnosis of CAG require reliable procedures and methods. The correlation between colonization with *H. pylori* and the histologically defined condition CAG has been demonstrated in several studies. Results of the present study indicate that one biopsy specimen, in most cases, is sufficient for the isolation of *H. pylori*, whereas several samples may be needed for histological examination. An increase in the number of biopsy specimens for cultivation from one to two added only one more patient to the *H. pylori*-infected group. An additional increase to three, four, and five biopsy specimens revealed no more infected patients. Thus, the probability of increasing the isolation rate of *H. pylori* by taking more biopsy specimens is low. That cultivation of *H. pylori* was effective was supported by the [<sup>14</sup>C]urea breath test. There were no patients with a positive reaction by the breath test and a negative result by cultivation of *H. pylori*. Thus, we assumed that the *H. pylori* isolation rate in this study was close to optimal given the procedures for transportation and cultivation of the biopsy specimens used in this study.

The benefit of increasing the number of specimens for histology was obvious in the diagnosis of CAG. The sampling procedure and the processing of specimens for histological examination appear to be prone to errors. Some of the specimens contained too little epithelium for an adequate histological examination, and other samples disintegrated during biopsy, transportation, or processing. Also, some samples were incorrectly oriented during cutting, leaving too little epithelium for a satisfactory examination. In addition, a few specimens were lost during the embedding process. Even in a technically perfect sample, the diagnosis of CAG may be based upon a few granulocytes infiltrating the

epithelium, and consequently, granulocytes may not be included in the section examined. Therefore, only data for the 74 patients from whom three technically adequate specimens were available for histological examination are included in the rest of this discussion. One biopsy specimen from each of 20 patients was adequate for the diagnosis of CAG. An increase to two specimens revealed three more cases of CAG, and taking of three specimens added one more patient. Therefore, we conclude that even if three technically perfect biopsy specimens are available for histology, the diagnosis may be missed in a few cases.

The correlation of infection with *H. pylori* and the presence of CAG depends on the sensitivity of both of the two diagnostic procedures. If only one biopsy specimen had been taken for histological examination, 18 patients colonized with *H. pylori* would have been diagnosed as having CAG, whereas after three biopsy specimens the corresponding number was 20. In addition, we found that nine patients without signs of CAG were colonized with *H. pylori*, whereas four patients with CAG were not infected with *H. pylori* (Table 2). These results confirm the correlation between infection with *H. pylori* and the presence of CAG, but they also indicate that CAG and infection with *H. pylori* may occur independently. Although our methods for isolating *H. pylori* seemed to be sensitive, we cannot exclude the possibility that forms of *H. pylori* exist that are not able to grow under the conditions described here. Also, other bacteria may be associated with CAG, but this study was not designed to answer this question. In the present study, cultivation of the gastric biopsy specimens was performed under microaerobic conditions. This may have excluded growth of strictly aerobic or anaerobic bacteria. In acridine orange-stained smears, bacteria different from *H. pylori* were often found, even when culture was negative. In particular, large unidentified cocci were often found, but rods were also found. These structures might represent bacterial forms that are unable to grow under the same conditions as those required for *H. pylori* growth. The possible role, if any, of these bacteria in the pathogenesis of chronic gastritis needs to be further elucidated.

In the present series, 31 patients were found histologically to have some degree of atrophy of the mucosal glandular structures, very often in combination with mucosal fibrosis. This combination is often seen in elderly patients. *H. pylori* was infrequently isolated from this group (1 of 31 patients), and from 11 of these patients, no bacteria were isolated. This may indicate that an atrophic or fibrotic mucosa is not suited for the attachment of *H. pylori*.

In conclusion, when the diagnosis of *H. pylori* infection was made by cultivation, only a marginal increase in sensitivity was achieved by increasing the number of biopsy specimens from one to two. In contrast, even when three samples were available for histological examination, the diagnosis of CAG could have been overlooked in a few patients.

## REFERENCES

1. Aase, S., K. Elgjo, O. Fausa, A. Bjørneklett, P. Nedenskov-Sørensen, and G. Bukholm. 1988. Bacteria of the gastric antrum and their relation to chronic gastritis. *APMIS* 96:273-279.
2. Bjørneklett, A., O. Fausa, P. Nedenskov-Sørensen, G. Bukholm, and S. Aase. 1989. The <sup>14</sup>C-urea breath test in the diagnosis of *Campylobacter pylori* infection, p. 65-68. In F. Megraud and H. Lamouliatte (ed.), *Gastrointestinal pathology and Campylobacter pylori*. Elsevier Science Publishers B.V., Amsterdam.
3. Goodwin, C. S., J. A. Armstrong, and B. J. Marshall. 1986. *Campylobacter pyloridis*, gastritis and peptic ulceration. *J. Clin.*

- Pathol. 39:353-365.
4. Goodwin, C. S., E. D. Blicow, J. R. Warren, T. E. Waters, C. R. Sanderson, and L. Easton. 1985. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J. Clin. Pathol.* 38:1127-1131.
  5. Jones, D. M., A. M. Lessells, and J. Eldridge. 1984. *Campylobacter* like organisms on the gastric mucosa: culture, histological, and serological studies. *J. Clin. Pathol.* 37:1002-1006.
  6. Morris, A., M. R. Ali, P. Brown, M. Lane, and K. Patton. 1989. *Campylobacter pylori* infection in biopsy specimens of gastric antrum: laboratory diagnosis and estimation of sampling error. *J. Clin. Pathol.* 42:727-732.
  7. Nedenskov-Sørensen, P., A. Bjørneklett, O. Fausa, G. Bukholm, S. Aase, and E. Jantzen. 1988. *Campylobacter pylori* infection and its relation to chronic gastritis. An endoscopic, bacteriologic and histomorphologic study. *Scand. J. Gastroenterol.* 23:867-874.
  8. Taylor, D. E., J. A. Hargreaves, L.-K. Ng, R. W. Sherbaniuk, and L. D. Jewell. 1987. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. *Am. J. Clin. Pathol.* 87:49-54.
  9. Whitehead, R. 1979. Mucosal biopsy of the gastrointestinal tract, p. 26-29. The W. B. Saunders Co., Philadelphia.
  10. Whitehead, R., S. C. Truelove, and M. W. L. Gear. 1972. The histological diagnosis of chronic gastritis in fiberoptic gastro-scope biopsy specimens. *J. Clin. Pathol.* 25:1-11.