Helicobacter pylori infection in patients with Barrett's oesophagus: a prospective immunohistochemical study

O Ricaurte, J-F Fléjou, C Vissuzaine, D Goldfain, A Rotenberg, G Cadiot, F Potet

Abstract

The prevalence of *Helicobacter pylori* infection in patients with Barrett's oesophagus was studied prospectively. A sensitive immunohistochemical staining of *H pylori* was performed in oesophageal and gastric biopsies of 73 patients from a surveillance group with this condition. *H pylori* was detected in 11 cases of Barrett's mucosa (15%) and in 26 gastric mucosa specimens (35.6%). All cases positive in Barrett's mucosa were also positive in the stomach. In Barrett's oesophagus, *H pylori* was never found on specialised epithelium. The percentage of Barrett's mucosa showing inflammatory changes was similar in specimens with and without *H pylori*, both for chronic (81% v 79%) and acute (9% v 10%) infiltrates. These results indicate that *H pylori* infection does not play an aetiological role in Barrett's oesophagus and that colonisation of the metaplastic mucosa by this bacteria is related with the presence of gastric type mucosa in the oesophagus and of *H pylori* infection in the stomach.

(J Clin Pathol 1996;49:176-177)

Keywords: Barrett's oesophagus, *Helicobacter pylori*, immunohistochemistry.

Barrett's oesophagus is a pathological condition characterised by the replacement of squamous lining epithelium of the distal oesophagus by columnar epithelium of gastric or intestinal type. This condition used to be a complication of gastroesophageal reflux. Histologically it has been categorised by Paull *et al* into cardiac or junctional type, fundic type, and intestinal or specialised type¹; in this latter type there is an increased risk of developing oesophageal adenocarcinoma. Since the original description of *Helicobacter pylori* in the stomach, its presence has been reported in variable proportions in retrospective studies of patients with Barrett's oesophagus, using various diagnostic methods.²⁻⁸ From these it is clear that controversy persists about the possible role of *H pylori* in the natural history of Barrett's oesophagus. The aim of this study was to determinate prospectively the frequency of gastric and oesophageal *H pylori* infection in a group of patients with Barrett's oesophagus included in a surveillance programme by means of a sensitive immunohistochemical method.

Methods

Seventy three patients with a histopathological diagnosis of Barrett's oesophagus who had been included in a surveillance programme since 1992-1994 were selected prospectively. Barrett's oesophagus was defined by the presence of a columnar mucosa in the lower oesophagus, of any type (cardiac, fundic, specialised) when extending to more than 3 cm above the gastro-oesophageal junction, or with at least one area of specialised mucosa for short segments of Barrett's mucosa.⁹ The patients were 57 males and 16 females, age range 28-81 years. Four quadrant oesophageal endoscopic biopsy specimens at intervals of 2 cm and a fundic biopsy specimen were taken in each case. Deparaffinised sections were stained with the anti-*H pylori* rabbit primary polyclonal antibody (Dako NB471) diluted 1 in 10. This antibody reacts with heat stable somatic antigens of the whole bacteria.¹⁰ In the following steps, an avidin-biotin-peroxidase complex procedure enhanced by a microwave pretreatment was used. *H pylori* chronic antral gastritis was used as a positive control, while negative controls were incubated with TRIS buffered saline (TBS, pH 7.6). The immunoreaction for *H pylori* was considered positive when morphologically typical bacteria were seen, located at the surface of the epithelium or in the canaliculi of parietal cells. The presence or absence of chronic inflammation and activity were evaluated.

Results

Forty four patients (60%) had specialised Barrett's mucosa on their oesophageal biopsy specimens, associated with gastric-type mucosa in 33 cases, of either cardiac or fundic type. In the remaining 29 cases, only gastric-type mucosa was present in Barrett's oesophagus. *H pylori* was detected in the oesophageal biopsy specimens in 11 cases (15%) (10 male and one female patient, age range 28-78 years) and in the gastric biopsy specimens of 26 patients (35.6%). All patients with *H pylori* in Barrett's oesophagus also had the bacteria in gastric biopsy specimens. *H pylori* was found in the oesophagus only in junctional (10 cases) and fundic (one case) types of metaplastic mucosa and never in the specialised type mucosa. The percentage of specimens of Barrett's mucosa showing inflammatory changes was similar in

Bichat-Claude
Bernard Hospital,
Paris, France:
Department of
Pathology
O Ricaurte
C Vissuzaine
F Potet

Department of
Gastroenterology
G Cadiot

Department of
Pathology, Beaujon
Hospital, Clichy,
France
J-F Fléjou

Department of
Gastroenterology,
Dreux Hospital,
Dreux, France
D Goldfain
A Rotenberg

Correspondence to:
Dr J-F Fléjou, Service
d'Anatomie et de Cytologie
Pathologiques, Hôpital
Beaujon, F-92118 Clichy
Cedex, France.

Accepted for publication
5 September 1995

Reported series of H pylori infection in patients with Barrett's oesophagus

Author (ref)	No of patients	H pylori positive		Diagnostic method
		Stomach	Barrett's	
Talley ²	23		12 (52%)	Warthin-Starry
Paull ¹	26	10 (38.4%)	4 (15.4%)	Giemsa
Houck ⁴	34		0	Giemsa
Fallingborg ⁵	46 ^a		12 (26%)	Culture
	133 ^b		6 (4.5%)	Culture
Walker ⁶	7		2 (29%)	Culture
Francoual ⁷	11	4 (44.4%)	0	Culture
GOSPE ⁸	100		19 (19%)	Warthin-Starry, Giemsa
Present series	73	26 (35.6%)	11 (15%)	Immunohistochemistry

^a Biopsy specimens after gastroscopy.

^b Biopsy specimens before gastroscopy.

specimens with and without *H pylori*, both for chronic (81% v 79%) and acute (9% v 10%) infiltrates.

Discussion

H pylori infection plays an important role in the aetiopathogenesis of chronic gastritis, duodenal and gastric ulcers, and gastric carcinoma. However, its potential role in Barrett's oesophagus has not been completely defined. The detection rate of the bacteria using different techniques has been reported at between 0% and 52% in cases of Barrett's oesophagus,²⁻⁸ while its presence in the stomach has been reported at between 38% and 44% in the same patients (table).³⁷ In the present study the frequency of *H pylori* was low in oesophageal biopsy specimens (15%) as well as in gastric biopsy specimens (35.6%). Specialised metaplastic epithelium was not colonised by the bacteria

and the presence of *H pylori* in the oesophagus was always associated with gastric infection. This finding has been reported previously in retrospective studies^{7,8,13} indicating that *H pylori* colonisation of Barrett's mucosa is probably a consequence of gastric infection. We did not find any differences regarding the inflammatory changes in oesophageal biopsy specimens of patients either with or without *H pylori*. We conclude from our results that *H pylori* does not play an important role in Barrett's oesophagus; its presence in the metaplastic oesophageal mucosa needs the presence of a gastric-type mucosa in the oesophagus, together with a gastric infection.

- 1 Paull A, Trier JS, Dalton MD, Camp RC, Loeb P, Goyal RK. The histologic spectrum of Barrett esophagus. *N Engl J Med* 1976;295:476-80.
- 2 Talley NJ, Cameron AJ, Shorter RG, Zinsmeister AR, Phillips SF. Campylobacter pylori and Barrett's oesophagus. *Mayo Clin Proc* 1988;63:1176-80.
- 3 Paull G, Yardley JH. Gastric and oesophageal Campylobacter pylori in patients with Barrett's esophagus. *Gastroenterology* 1988;95:216-8.
- 4 Houck JA, Lucas JG. Absence of Campylobacter-like organisms in Barrett's esophagus. *Arch Pathol Lab Med* 1989; 113:470-2.
- 5 Fallingborg J, Agnholt J, Moller-Petersen J, Christensen LA, Lomborg S, Sondergaard G, et al. Campylobacter pylori in esophagus. *Dig Dis Sci* 1989;34:1802-3.
- 6 Walker SJ, Birch PJ, Stewart M, Stoddard CJ, Hart CA, Day DW. Patterns of colonisation of Campylobacter pylori in the oesophagus, stomach and duodenum. *Gut* 1989; 30:1334-8.
- 7 Francoual S, Lamy Ph, Le Quintrec Y, Lubinski J, Petit JC. Helicobacter pylori: has it a part in the lesion of the gastroesophageal reflux? *J Infect Dis* 1990;162:1414.
- 8 Gruppo Operativo Per Lo Studio Delle Precancerosi Esofagee (GOSPE). Helicobacter pylori in Barrett's oesophagus. *Histopathology* 1991;18:568-70.
- 9 Dent J, Bremner CG, Collen MJ, Haggitt RC, Spechler SJ. Barrett's oesophagus. *J Gastroenterol Hepatol* 1991;6:1-22.
- 10 Andersen LP, Holck S, Poulsen CO. Campylobacter pylori detected by indirect immunohistochemical technique. *APMIS* 1988;96:559-64.

J Clin Pathol 1996;49:177-179

Use of buffered formaldehyde in the enzymatic digestion of inflamed mucosa

J Meenan, D O Obradov, H van Dullemen, G N J Tytgat, S J H van Deventer

Abstract

Mucosal inflammation is associated with altered expression of cell membrane molecules. Disaggregation of tissue for flow cytometry may introduce artefactual changes. In an attempt to prevent the induction of artefacts, cells were fixed prior to isolation. The addition of 0.1% buffered formaldehyde to the collagenase/dispase digestion of mucosal biopsy specimens from patients with inflammatory bowel disease enhances detection of CD3, CD11b, CD16, CD63, and CD14. No significant effect was noted for CD19, CD67 or CD45. The expression of CD3, CD11b and CD45 correlated with the degree of endoscopic inflammation. Dilute buffered

formaldehyde may be a useful adjunct to the enzymatic isolation of cells from mucosal specimens, by protecting surface antigens from digestion or alterations in expression.

(*J Clin Pathol* 1996;49:177-179)

Keywords: paraformaldehyde, flow cytometry, inflammation.

Flow cytometry is more flexible than standard immunohistochemistry, both in terms of cell numbers analysed and the combination of antigens examined. However, prior to analysis, tissue samples must be disaggregated by mechanical, ion chelation or enzymatic methods.^{1,2}

Department of Gastroenterology and Hepatology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Correspondence to: Dr J Meenan, Department of Gastroenterology and Hepatology, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

Accepted for publication 20 July 1995