Effect of acid-suppressive therapy on *Helicobacter pylori* production of interleukin-8 in the gastric mucosa

Masahiro Yoshinaga MD¹, Akira Ohtani MD¹, Satoru Tsuruta MD¹, Tetsuya Kato MD¹, Eishi Higashi MD¹, Yukio Yamada MD¹, Naohiko Harada MD², Hajime Nawata MD²

M Yoshinaga, A Ohtani, S Tsuruta, et al. Effect of acid-suppressive therapy on *Helicobacter pylori* production of interleukin-8 in the gastric mucosa. *Can J Gastroenterol* 2000;14(4):277-282.

BACKGROUND: Recent studies have shown that acid-suppressive therapy increases the severity of *Helicobacter pylori*-associated gastritis in the corpus.

PURPOSE: To evaluate interleukin (IL)-8 production in the gastric corpus mucosa before and during acid-suppressive therapy in *H pylori*-infected patients.

PATIENTS AND METHODS: Ten patients with reflux esophagitis (five *H pylori*-positive and five *H pylori*-negative) were treated with omeprazole 20 mg. Serum gastrin concentrations, *H pylori* colonization density and mucosal IL-8 levels in the corpus were investigated at entry and two weeks after starting therapy. IL-8 levels were measured by ELISA. The organism density was determined, and scored according to area occupied by the bacterial colonies. The presence of *H pylori* was assessed by rapid urease testing and histological finding of gastric biopsy specimens.

RESULTS: In *H pylori*-positive patients, concentrations of IL-8 during therapy significantly exceeded those before therapy

(36.2±6.8 versus 18.3±3.8 pg/mg protein; P<0.05) without altering *H pylori* density. In *H pylori*-negative patients, IL-8 levels were similar before and during therapy (6.1±2.7 versus 6.3±3.0 pg/mg protein). Concentrations of gastrin during therapy were significantly higher than those before therapy in all patients.

CONCLUSIONS: The results suggest that acid suppression increases mucosal IL-8 levels in *H pylori*-infected patients with reflux esophagitis.

Key Words: *Helicobacter pylori*; Interleukin-8; Proton pump inhibitor; Reflux esophagitis

Effet du traitement antiacide sur la production d'interleukine-8 dans la muqueuse gastrique dans les cas d'infection à *Helicobacter pylori*

CONTEXTE : Des études récentes montrent que le traitement antiacide accroît la gravité de la gastrite associée à *Helicobacter pylori* dans la muqueuse du corps de l'estomac.

voir page suivante

¹Department of Internal Medicine, Chikuhou Hospital; ²Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Correspondence and reprints: Dr Masahiro Yoshinaga, Department of Internal Medicine, Fukuoka Teishin Hospital, 2-6-11 Yakuin, Fukuoka 810-8798, Japan. Telephone +81-92-741-3315, fax +81-92-781-2563, e-mail m-yoshi@winter.try-net.or.jp

Received for publication November 17, 1998. Accepted April 28, 1999

BUT : Évaluer la production d'interleukine-8 (IL-8) dans la muqueuse du corps gastrique avant et durant le traitement antiacide chez les patients infectés à *H. pylori*.

MÉTHODE : Dix patients souffrant d'oesophagite peptique (cinq à *H. pylori* positif et cinq à *H. pylori* négatif) ont été traités à l'aide d'oméprazole, 20 mg. On a mesuré la concentration de gastrine dans le sang, la densité de colonisation par *H. pylori* et le taux d'IL-8 dans la muqueuse du corps au début du traitement et deux semaines plus tard. Le taux d'IL-8 a été déterminé à l'aide du test ELISA; la densité du micro-organisme a été notée selon la région occupée par les colonies de bactéries et la présence d'*H. pylori* a été évaluée par une analyse rapide de l'uréase et des observations histologi-

ques des échantillons de tissu gastrique prélevés à la biopsie.

RÉSULTATS : Chez les patients à *H. pylori* positif, le taux d'IL-8 durant le traitement dépassait largement le taux mesuré avant le traitement ($36,2 \text{ pg/mg} \pm 6,8$ c. $18,3 \text{ pg/mg} \pm 3,8$; $P < 0,05$), sans modification de la densité d'*H. pylori*. Chez les patients à *H. pylori* négatif, ce taux est resté stable avant et durant le traitement ($6,1 \text{ pg/mg} \pm 2,7$ c. $6,3 \text{ pg/mg} \pm 3,0$). La concentration de gastrine s'est montrée passablement plus élevée durant le traitement qu'avant chez tous les patients.

CONCLUSION : Les résultats donnent à penser que la suppression d'acide augmente la concentration d'IL-8 dans la muqueuse gastrique chez les patients infectés à *H. pylori* souffrant d'oesophagite peptique.

Helicobacter pylori infection elicits an infiltration of gastric mucosa with neutrophils, eosinophils, lymphocytes and plasma cells (1,2). This constellation of inflammatory responses constitutes chronic active gastritis and may remain clinically silent or progress to create the background for the development of diseases such as peptic ulcer, gastric adenocarcinoma or primary gastric B cell lymphoma (1,2). Interleukin (IL) -8 has been reported to be a factor causing neutrophil chemotaxis and activation (3). Previous studies have indicated that gene expression and the production of IL-8 are enhanced in *H. pylori*-infected patients with chronic gastritis, duodenal ulcer or nonulcer dyspepsia (NUD) (4-8). Moreover, this IL-8 concentration is correlated with the degree of neutrophil infiltration and histological severity in *H. pylori*-associated gastritis (7,9).

Recent studies have revealed that profound suppression of gastric acid is associated with increased severity of corpus gastritis caused by *H. pylori* (10) and have suggested that patients with reflux esophagitis and *H. pylori* infection treated with omeprazole are at increased risk of atrophic gastritis (11). However, these studies were evaluated in terms of the gastric histological features. The effect of acid suppression on *H. pylori*-associated mucosal production of IL-8 in the corpus is not clear.

We, therefore, obtained the corpus mucosa from *H. pylori*-infected patients with acid-related disorders before and during acid-suppressive therapy, and evaluated the effect of acid-suppressive therapy on the concentrations of *H. pylori*-produced IL-8 in the corpus mucosa.

PATIENTS AND METHODS

Patients: Five *H. pylori*-positive patients were chosen for this study with the following criteria: complaints of heartburn for longer than one month; diagnosed with reflux esophagitis by baseline endoscopy; *H. pylori* assessed at diagnosis by rapid urease testing and histological finding of gastric biopsy specimens; and taking no form of acid-suppressive therapy, antibiotics, bismuth-containing medication or nonsteroidal anti-inflammatory drugs in the month before the entry. Patients ranged in age from 31 to 68 years (mean age 49.4 years). Three patients were male (60%), and all patients had positive findings in both a rapid urease testing and histological examination (Giemsa staining) for *H. pylori* assessment.

Five *H. pylori*-negative controls were selected according to the same criteria, but with negative findings in both rapid urease tests and histology. Controls ranged in age from 59 to 67 years (mean age 64.2 years); four were male (80%) and all had negative findings in both a rapid urease testing and histological examination for *H. pylori*. All 10 patients gave informed consent.

Study design: At entry, fasting blood samples were obtained from all 10 patients to determine serum gastrin concentrations. They underwent baseline upper gastrointestinal endoscopy to obtain biopsy specimens. Following these procedures, they were treated with omeprazole 20 mg/day. Two weeks after starting acid-suppressive therapy, the patients underwent the same examinations that were performed at entry. In *H. pylori*-positive patients, biopsy specimens were used for the cultural and mucosal assessment of IL-8 levels. In *H. pylori*-negative patients, these specimens were used for IL-8 evaluation. The mucosal IL-8 contents and concentrations of serum gastrin during therapy were compared with those before therapy in *H. pylori*-negative patients, as were the mucosal IL-8 contents, serum gastrin concentrations and the *H. pylori* colonization density in *H. pylori*-positive patients. This protocol was approved by the ethical committee of Chikuhou Hospital, Fukuoka, Japan.

Endoscopic procedures: All endoscopies were performed under local anesthesia using a videoscope. Biopsy samples were obtained from the greater curvature of the corpus 50 cm from the incisors. Three samples were obtained from those who were *H. pylori*-negative, and five from those who were *H. pylori*-positive. Three samples were used for mucosal IL-8 assessments in all patients; the rest of the samples were used for *H. pylori* culture in *H. pylori*-positive patients.

***H. pylori* culture:** Two biopsy specimens were spread out with an applicator and placed in Columbia blood agar medium (pH 7.4; Oxoid, Basingstoke, United Kingdom) containing 5% horse blood, vancomycin (10 µg/mL), amphotericin B (5 µg/mL), cefsulodin (5 µg/mL) and trimethoprim (5 µg/mL), and incubated at 37°C under microaerophilic conditions (5% oxygen, 10% carbon dioxide and 85% nitrogen) for up to seven days. The organisms were identified as *H. pylori* by Gram staining, colony morphology, and positive oxidase, catalase and urease reactions. The *H. pylori* colonization density was scored and graded from 0 to 3. The *H. pylori*

TABLE 1
Comparison of mucosal interleukin (IL) -8 contents and serum gastrin levels in *Helicobacter pylori*-positive patients and in *H pylori*-negative patients

	<i>H pylori</i> -positive	<i>H pylori</i> -negative
IL-8 (pg/mg protein)	18.3±3.8*	6.1±2.7
Gastrin (pg/mL)	100.8±12.2*	39.5±1.5

* $P < 0.05$ *H pylori*-positive versus -negative

density score was defined as zero if the *H pylori* colonization was negative. Scores of 1 to 3 were defined as follows: a score of 1 was given if the *H pylori* colonies comprised less than half of the whole culture; a score of 2 was given if the colonies occupied an area equal to or greater than half and less than three-quarters of the whole culture; and a score of 3 was given if the *H pylori* colonies occupied an area equal to or greater than three-quarters of the whole culture. In the preliminary evaluation, the number of *H pylori* (the colony forming unit/mL) was 10^2 in those given a score of 1, 10^4 in those given a score of 2 and 10^6 in those given a score of 3.

Measurement of mucosal IL-8: Three biopsy samples were immediately stored at -20°C and homogenized in 3 mL phosphate-buffered saline (PBS) (pH 7.4) for 1 min using an Ultra Turrax homogenizer (Janke & Kunkel Co, Staufen, Germany). Supernatants obtained by centrifugation (1500 g for 30 mins) were frozen at -70°C in polypropylene tubes until assayed. Supernatants of homogenates were measured for IL-8 levels by ELISA according to the methods established by Ida et al (12). This assay uses the quantitative immunometric 'sandwich' enzyme immunoassay technique. Microtitre plates (Maxisorp 96-well, Nunc, Denmark) were coated with 0.5 $\mu\text{g/mL}$ rabbit antihuman IL-8 polyclonal antibody (Toray Fuji Bionics, Tokyo, Japan) in 100 μL PBS at 4°C overnight and blocked with 0.5% bovine serum albumin (BSA) in PBS at 25°C for 2 h. The plates were washed with 400 μL washing solution (twofold diluted PBS containing 0.025% Tween 20), followed by the addition of 100 μL of reaction buffer (0.1 M Tris-HCl, pH 8.0) containing 0.25% BSA, 0.05% Tween 20 and 0.5% each of normal mouse and rabbit serum). Duplicate 50 μL aliquots of samples or standard IL-8 were added and incubated at 25°C for 1 h. The plate was shaken on a microplate mixer throughout the incubation time. After washing, 0.5 $\mu\text{g/mL}$ mouse anti-IL-8 monoclonal antibody (Toray Fuji Bionics, Tokyo, Japan) labelled with horseradish peroxidase (Boehringer Mannheim, Mannheim, Germany) in 100 μL of PBS containing 0.25% BSA, 0.05% Tween 20 and 0.3 M sodium chloride was sequentially added and incubated at 25°C for 30 mins. After three washing steps, 100 μL of the substrate solution (0.1 M sodium acetate-citrate containing 0.006% hydrogen peroxide and 0.2 mg/mL 3,3',5,5'-tetramethylbenzidine, pH 5.5) was added and incubated at 25°C for 30 mins. Finally, the colorimetric reaction was stopped by adding 10 μL of 1 N sulphuric acid, and the absorbance at 450 nm (reference at 595 nm) was measured. The IL-8 concentration of each sample was derived by comparing the optical density of the sam-

Figure 1 Comparative concentrations of mucosal interleukin (IL)-8 before acid-suppressive therapy (0 WK) with those during therapy (2 WKS) in *Helicobacter pylori*-positive patients. Concentrations of IL-8 during therapy significantly exceeded those before therapy ($P < 0.05$)

ple with the standard curve. This assay accurately measures natural, recombinant, endothelial cell-derived and monocyte-derived human IL-8, with no measurable cross-reactivity to other cytokines. The detection limit of this assay was 12.5 pg/mL, and the inter-/intra-assay variability was less than 6%.

Statistical analysis: Values are given as means $\pm$ SE. The two-tailed Mann-Whitney U test was used for unpaired comparisons between groups. For paired comparisons before and during acid-suppressive therapy, two-tailed Wilcoxon signed rank tests were used. A value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Table 1 shows the comparative levels of serum gastrin and mucosal IL-8 in the corpus between two groups of patients. The mucosal levels of IL-8 in *H pylori*-positive patients significantly exceeded those in *H pylori*-negative patients (18.3 $\pm$ 3.8 versus 6.1 $\pm$ 2.7 pg/mg protein; $P < 0.05$). The levels of serum gastrin in *H pylori*-positive patients also significantly exceeded those in *H pylori*-negative patients (100.8 $\pm$ 12.2 versus 39.5 $\pm$ 1.5 pg/mL; $P < 0.05$).

Figures 1 and 2 illustrate the comparative concentrations of mucosal IL-8 before acid-suppressive therapy and during therapy in two groups of patients. In *H pylori*-positive patients (Figure 1), concentrations of IL-8 during therapy significantly exceeded those before therapy (36.2 $\pm$ 6.8 versus 18.3 $\pm$ 3.8 pg/mg protein; $P < 0.05$). In *H pylori*-negative patients (Figure 2), levels of IL-8 were similar before and during therapy (6.1 $\pm$ 2.7 versus 6.3 $\pm$ 3.0 pg/mg protein).

Figure 2) Comparative concentrations of mucosal interleukin (IL)-8 before acid-suppressive therapy (0 WK) and those during therapy (2 WKS) in *Helicobacter pylori*-negative patients. Levels of IL-8 were similar before and during therapy

Figure 3) Comparative frequency of *Helicobacter pylori* colonization in the corpus before acid-suppressive therapy (0 WK) and during therapy (2 WKS). The median *H pylori* density scores before and during therapy were similar

The frequency of *H pylori* colonization in the corpus during acid-suppressive therapy was compared with that before therapy in Figure 3. The median *H pylori* density score was 1.5 (range 1.0 to 2.0) before acid-suppressive therapy. Dur-

TABLE 2
Comparison of serum gastrin levels at entry and two weeks after starting acid-suppressive therapy in *Helicobacter pylori*-negative and -positive patients

Patients	Gastrin (pg/mL)	
	Entry	After two weeks
<i>H pylori</i> -positive	100.8±12.2	470.0±67.3*
<i>H pylori</i> -negative	39.5±1.5	124.8±20.4*

* $P < 0.05$ entry versus after two weeks

ing therapy, the *H pylori* score was 1.0. This degree of remission of *H pylori* density was not statistically significant.

Table 2 compares serum gastrin levels at entry with those two weeks after starting acid-suppressive therapy in *H pylori*-negative and -positive patients.

Concentrations of gastrin during acid-suppressive therapy significantly exceeded those before therapy in two groups of patients (470.0±67.3 versus 100.8±12.2 pg/mL in *H pylori*-positive patients [$P < 0.05$]; 124.8±20.4 versus 39.5±1.5 pg/mL in *H pylori*-negative patients [$P < 0.05$]).

DISCUSSION

In the present study, levels of gastric mucosal IL-8 and serum gastrin in *H pylori*-positive patients exceeded those in *H pylori*-negative patients. This result agrees with that found in previous studies (4,7,9,13) and shows the accuracy of measurement of the mucosal IL-8 contents in the present study.

The main finding of the present study is that acid-suppressive therapy increases mucosal IL-8 levels in the corpus of *H pylori*-infected patients with reflux esophagitis. To our knowledge, no studies have directly shown the increased production of gastric mucosal IL-8 in the corpus during acid-suppressive therapy in *H pylori*-infected patients. We have not evaluated whether neutrophil numbers were increased according to the increased mucosal IL-8 content in this study. However, previous studies have already shown that acid-suppressive therapy, consisting of proton pump inhibitor administration, worsens the histological features of corpus or fundus lesions in *H pylori*-infected patients with reflux esophagitis, peptic ulcer or gastric erosions (10,14-16). The present study indicates that the previous reported neutrophil chemotaxis is accelerated by increasing the mucosal IL-8 contents.

The *H pylori* density in the corpus during acid-suppressive therapy or under conditions of acid hyposecretion remains to be evaluated. Previous studies indicate that the frequency of *H pylori* in the corpus during acid-suppressive therapy or in acid hyposecretion exceeds that before therapy or that occurring with normal acid secretion (15,17,18). Conversely, recent studies have revealed that the density of *H pylori* in the corpus is similar between these two conditions (10,19,20). The present study showed that the density of organism during acid-suppressive therapy was similar to that at entry. However, more studies are needed because small numbers of subjects were examined in the present study. The present study proposes that local acid secretion may inhibit

directly or through other factors (eg, suppressing the formation of ammonia) the production of the mucosal IL-8. Solcia et al (14) suggested that the transformation from ammonium chloride to ammonia by acid suppression may be attributed to the inflammation in the gastric mucosa caused by *H pylori*. However, further examinations are needed to elucidate whether ammonia enhances the mucosal IL-8 contents in the *H pylori*-infected stomach.

Uemura et al (19) indicated that, in *H pylori*-infected duodenal ulcer patients, acid-suppressive therapy enhanced neutrophil infiltration, despite no significant change in the density of *H pylori* and the IL-8 contents in the corpus. On the contrary, their study showed that in the antrum, neutrophil chemotaxis was reduced after administration of famotidine, despite no significant change in the density of *H pylori* and the IL-8 contents (19). This discrepancy was not clarified in their study, and further examinations may need to be done.

H pylori-infected patients with peptic ulcer should undergo eradication of infection and not simply be prescribed a proton pump inhibitor (21-24). On the contrary, it is controversial whether *H pylori*-positive nonulcer patients require eradication of infection. Lee and O'Morain (25) summarized the national guidelines on *H pylori* eradication in eight European countries; most panels do not consider *H pylori* eradication in gastroesophageal reflux in their guidelines, but one panel considers it to be a relative indication for eradication (25). A few panels consider NUD to be an indication for eradication after investigation and in patients with severe or recurrent symptoms (26), whereas others do not consider it an indication. In the guidelines of the Canadian *Helicobacter pylori* consensus conference, gastroesophageal reflux is not recommended for eradication (27).

NUD and gastritis are considered to be indications for eradication in patients with severe, long lasting or recurrent symptoms (27). Recent studies show that long term acid suppression in *H pylori*-positive patients with reflux esophagitis causes progression of gastric mucosal inflammation in the corpus and fundus (11,16). According to the Canadian guidelines, if a patient with gastroesophageal reflux on long term proton pump inhibitor therapy is already known to be *H pylori*-positive, treatment may be offered on a case-by-case basis (27). The present study implies that IL-8 contents in the corpus are increased with omeprazole administration in *H pylori*-infected patients with reflux esophagitis. Thus *H pylori*-infected patients with nonulcer disease may need attention when they are treated with proton pump inhibitors or H_2 blockers.

Although intragastric pH was not examined directly, the serum gastrin levels were investigated at entry and during acid-suppression therapy in the present study. The present study showed that the serum gastrin levels during therapy significantly exceeded those at entry in both *H pylori*-positive and -negative patients. This finding suggests that this therapy can effectively suppress gastric acid secretion.

CONCLUSIONS

The present study indicates that mucosal IL-8 contents are increased by treatment with omeprazole 20 mg in the corpus of *H pylori*-infected patients with reflux esophagitis. This finding suggests that acid suppression may enhance the mucosal IL-8 of nonulcer patients with *H pylori* infection.

ACKNOWLEDGMENT: The authors thank Ms Yuriko Fukuda (SRL Inc, Tokyo, Japan) for technical assistance.

REFERENCES

- Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273-5.
- Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis: the updated Sydney System. *Am J Surg Pathol* 1996;20:1161-81.
- Matsushima K, Baldwin ET, Mukaida N. Interleukin-8 and MCAF. Novel leukocyte recruitment and activating cytokines. *Chem Immunol* 1992;51:236-65.
- Moss SF, Legon S, Davies J, Calam J. Cytokine gene expression in *Helicobacter pylori* associated antral gastritis. *Gut* 1994;35:1567-70.
- Crabtree JE, Lindley IJ. Mucosal interleukin-8 and *Helicobacter pylori*-associated gastroduodenal disease. *Eur J Gastroenterol Hepatol* 1994;6(Suppl 1):S33-8.
- Crabtree JE. Immune and inflammatory responses to *Helicobacter pylori* infection. *Scand J Gastroenterol Suppl* 1996;215:3-10.
- Gionchetti P, Vaira D, Campieri M. Enhanced mucosal interleukin-6 and -8 in *Helicobacter pylori*-positive dyspeptic patients. *Am J Gastroenterol* 1994;89:883-7.
- Crowe SE, Alvarez L, Dytoc M, et al. Expression of interleukin 8 and CD54 by human gastric epithelium after *Helicobacter pylori* infection in vitro. *Gastroenterology* 1995;108:65-74.
- Ando T, Kusugami K, Ohsuga M, et al. Interleukin-8 activity correlates with histological severity in *Helicobacter pylori*-associated antral gastritis. *Am J Gastroenterol* 1996;91:1150-6.
- Kuipers EJ, Uytterlinde AM, Pena AS, et al. Increase of *Helicobacter pylori*-associated corpus gastritis during acid suppressive therapy: implications for long-term safety. *Am J Gastroenterol* 1995;90:1401-6.
- Kuipers EJ, Lundell L, Klinkenberg-Knol EC, et al. Atrophic gastritis and *Helicobacter pylori* infection in patients with reflux esophagitis treated with omeprazole or fundoplication. *N Engl J Med* 1996;334:1018-22.
- Ida N, Sakurai S, Hosoi K, Kunitomo T. A highly sensitive enzyme-linked immunosorbent assay for the measurement of interleukin-8 in biological fluids. *J Immunol Methods* 1992;156:27-38.
- Konturek PC, Konturek SJ, Bobrzynski A, et al. *Helicobacter pylori* and impaired gastric secretory functions associated with duodenal ulcer and atrophic gastritis. *J Physiol Pharmacol* 1997;48:365-73.
- Solcia E, Villani L, Fiocca R, et al. Effects of eradication of *Helicobacter pylori* on gastritis in duodenal ulcer patients. *Scand J Gastroenterol Suppl* 1994;201:28-34.
- Logan RP, Walker MM, Misiewicz JJ, Gummett PA, Karim QN, Baron JH. Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole. *Gut* 1995;36:12-6.
- Eissele R, Brunner G, Simon B, Solcia E, Arnold R. Gastric mucosa during treatment with lansoprazole: *Helicobacter pylori* is a risk factor for argyrophil cell hyperplasia. *Gastroenterology* 1997;112:707-12.
- Stolte M, Eidt S, Ohsman A. Difference in expression of *Helicobacter pylori* on gastritis in the antrum and body of the stomach. *Z Gastroenterol* 1992;28:229-33.
- Ruiz B, Correa P, Fontham ET, Ramakrishnan T. Antral atrophy, *Helicobacter pylori* colonization, and gastric pH. *Am J Clin Pathol* 1996;105:96-101.
- Uemura N, Oomoto Y, Mukai T, et al. Gastric corpus IL-8 concentration and neutrophil infiltration in duodenal ulcer patients. *Aliment Pharmacol Ther* 1997;11:793-800.
- El-Omar EM, Oien K, El-Nujumi A, et al. *Helicobacter pylori* infection and chronic gastric acid hyposecretion. *Gastroenterology* 1997;113:15-24.
- The Maastricht Consensus Report. European *Helicobacter pylori*

- Study Group. Current European concepts in the management of *Helicobacter pylori* infection. Gut 1997;41:8-13.
 22. Sol AH. Consensus conference. Medical treatment of peptic ulcer disease. Practice guidelines. Practice Parameters Committee of the American College of Gastroenterology. JAMA 1996;275:622-9.
 23. Thomas S, Rosmalen CF, van der Laan JR. Summary of the updated guideline 'Gastric Complaints' of the Dutch College of General Practitioners. Ned Tijdschr Geneesk 1997;141:989-92.
 24. NIH Consensus development panel on *Helicobacter pylori* in peptic ulcer disease. *Helicobacter pylori* in peptic ulcer disease. JAMA 1994;272:65-9.
 25. Lee J, O'Morain C. Who should be treated for *Helicobacter pylori* infection? A review of consensus conferences and guidelines. Gastroenterology 1997;113:S99-106.
 26. Buckley M, Culhane A, Drumm B, et al. Guidelines for the management of *Helicobacter pylori*-related upper gastrointestinal diseases. Irish J Med Sci 1996;165(Suppl 5):1-11.
 27. Hunt R, Thomson ABR, Consensus conference participants. Canadian *Helicobacter pylori* Consensus Conference. Can J Gastroenterol 1998;12:31-41.
-
-