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Randomized study comparing omeprazole with ranitidine as anti-secretory agents combined in quadruple second-line Helicobacter pylori *eradication regimens*

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SUMMARY

Background: Few data are available on the efficacy of second-line *H. pylori* eradication regimens.

Aim: To compare the efficacy of either omeprazole or ranitidine in a second-line quadruple regimen in patients with duodenal ulcer or erosive duodenitis.

Patients and methods: A total of 37 patients with erosive duodenitis and 119 with duodenal ulcer who have failed eradication of *H. pylori* with double or triple regimens, without metronidazole, were randomly assigned to receive tripotassium dicitrato bismuthate 600 mg t.d.s. + metronidazole 500 mg t.d.s. + tetracycline hydrochloride 500 mg t.d.s. combined with either omeprazole 20 mg b.d. (group O, 78 patients) or ranitidine 300 mg b.d. (group R, 78 patients) for 14 days. *H. pylori* eradication was verified by histology, rapid urease test and ^{13}C-urea breath test.

Statistics: t-test, χ^2-test.

Results: A total of 143 patients had a post-treatment endoscopy. Eradication rates were: intention-to-treat: group O 77% (67–87), group R 76% (66–85), $P = 0.85$; per protocol analysis: group O 86% (77–95), group R 82 (71–93), $P = 0.58$. Side-effects were frequent but mild.

Conclusions: Omeprazole 20 mg b.d. and ranitidine 300 mg b.d. were equally effective as antisecretory agents combined in a second-line quadruple eradication regimen.

INTRODUCTION

The pathogenic role of *Helicobacter pylori* in chronic active gastritis and its association with duodenal ulcer disease in 95–99% of patients is well established.[1] Therefore the 1994 National Institute of Health (NIH) consensus development conference recommended the eradication of *H. pylori* in all patients with documented peptic ulcer disease.[2] The ideal eradication regimen remains elusive: previously used double regimens (omeprazole plus amoxycillin) had a success rate ranging from 30 to 80%.[3]

The aim of this study was to compare the efficacy of either omeprazole or ranitidine combined in a quadruple regimen including tripotassium dicitrato bismuth, metronidazole and tetracycline hydrocloride as a second-line treatment in patients with erosive duodenitis or duodenal ulcer in whom a non-metronidazole-based anti-*H. pylori* therapy had previously failed. Currently used eradication regimens show efficacy ranging from 80 to 95%.[4] Quadruple therapy comprising a proton pump inhibitor in combination with bismuth triple therapy appears to produce the highest eradication rates

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(96%; range 92–97%), when used as first line treatment.[4] Cimetidine or ranitidine comprised in quadruple regimens seem to be equally effective (95%).[5] On the other hand quadruple therapy comprising famotidine in combination with bismuth triple therapy succeeds in 89% of *H. pylori* positive patients.[6] The reasons for quadruple therapy failure are unclear, but compliance and metronidazole-resistance are thought to be important.[7] Persistent *H. pylori* infection is presumably more difficult to cure: Few and surprisingly small-scale studies dealing with second-line eradication therapy have been published.[8–14] Maastricht consensus recommendations for triple eradication therapy failures supported the use of quadruple therapy as second-line treatment choice.[15]

PATIENTS AND METHODS

Consecutive patients ranging in age from 18 to 80 years, with erosive duodenitis or duodenal ulcer who had failed to eradicate *H. pylori* with double or triple regimens, but without metronidazole, were enrolled in the study for the time period between March 1 1995 and February 28 1998, after verbal and written informed consent had been obtained.

Erosive duodenitis was defined as the presence of multiple superficial erosions (more than five) in the duodenal bulb, lined with fibrin, and all of them with a diameter smaller than 5 mm, on an oedematous, erythematous and friable mucosa. Lesions larger than 5 mm were considered to be duodenal ulcers.

A single antral specimen was taken for rapid urease test (CLO-test, Ballard Medical products, Osborne Park, Western Australia). Additionally, four biopsies were taken at the baseline endoscopy (at least 4 weeks after finishing the first line treatment) for histological examination (two antral and two corporal). Both histopathological results and rapid urease test were positive at baseline endoscopy.

Patients were excluded if they had known allergy to one of the drugs to be used, if they had current complications of ulcer disease (perforation, active bleeding or pyloric stenosis) and if they needed maintenance treatment with omeprazole. Additional exclusion criteria were: liver or kidney diseases; severe cardiac or pulmonary diseases; alcoholism; drug abuse or any other conditions associated with poor patient compliance; suspected or confirmed malignancy; pregnancy; and breast feeding. Patients on non-steroidal anti-inflammatory drugs or acetylsalicylic acid, on a daily basis, were excluded.

Directly after confirmation of first line treatment failure, patients were randomly allocated to quadruple therapy with tripotassium dicitrato bismuthate 600 mg t.d.s. (De Nol, Gerolymatos, Greece-tablets of 300 mg corresponding to 120 mg Bi_2O_3), tetracycline hydrochloride 500 mg t.d.s. (Hostacycline Hoechst, Germany), metronidazole 500 mg t.d.s. (Flagyl Rhone Poulenc Rover, France) and either omeprazole 20 mg b.d. (Losec Astra, Sweden), group O, or ranitidine 300 mg b.d. (Zantac Glaxo, Greece), group R, for 14 days. The antisecretory agent was continued in half a dose for another 28 days. Tripotassium dicitrato bismuthate was taken before meals, antibiotics with meals and antisecretory agents 15 min before breakfast and dinner for days 1–14 and before dinner for days 15–42 (half a dose). Randomization was non-blinded. Rapid urease test reading and histological evaluation were both performed by personnel unaware of the prescribed regimen.

Patients received both a verbal and a written explanation about the rationale of antibiotic treatment and the importance of complying with the regimen. They were advised to take skipped doses as an extra dose in the evening of the same day. The use of alcohol was discouraged. Patients were pre-warned about side-effects and asked to communicate with the gastroenterology unit before abandoning treatment.

H. pylori assessment was also performed 4–6 weeks after the end of treatment by histopathological examination and rapid urease test.

For histopathological examination, haematoxylin/eosin and modified Giemsa stained biopsy samples of the antrum ($n = 2$) and the corpus ($n = 2$) were examined. One antral biopsy was embedded in a rapid urea test (CLO test) for urease detection and read after 2 and 24 h. Two hours positive reading was regarded as positive, while 24 h negative reading was regarded as negative. Twenty-four hour positive results were regarded as equivocal and considered as negative only if histology was negative.

H. pylori infection was considered to be present if both histopathological results and rapid urease test were positive and defined as being eradicated if both histopathology and rapid urease test were negative. For conflicting results, as well as for patients that refused endoscopy, ^{13}C-urea breath test (*Helicobacter* test INFAI, INFAI-Institut fur Biomedizinische Analytic & NMR-Imaging GmbH, Bochum, Germany) was performed, following the instructions of product manufacturer, 3–6 months after the end of treatment. Patients with

a positive ^{13}C-urea breath test were considered to be treatment failures (a commercial product was used). Sample analysis was performed using isotope ratio mass spectrometry. *Helicobacter* infection was considered to be present when the 30-min value of ^{13}C exceeded the baseline-value by 0.4%.

Thus patients without contradictory results by both rapid urease test and histology had an evaluation 4–6 weeks after the end of the treatment, while those with conflicting results were followed-up for up to 6 months after treatment completion.

The treatment outcome was assessed by intention-to-treat and per protocol analysis. All patients who received the treatment, regardless of their eligibility for the study, were included in the safety analysis. The intention-to-treat population included all patients who had taken at least one dose of study medication. It was assumed that *H. pylori* had not been eradicated if the patient did not return for post-treatment endoscopy and ^{13}C-urea breath test. The per protocol population included only those eligible patients who had had endoscopy after treatment. The χ^2-test was used when comparing group differences and eradication rates between groups. The student's *t*-test was used to determine differences between mean values. A $P < 0.05$ was considered to be statistically significant. Univariate analysis were performed to assess risk factors for *H. pylori* eradication failures.

The medical ethics committee of the institution approved the study design.

RESULTS

A total of 156 patients were included in the study and randomly assigned to the triple therapy plus omeprazole (group O, $n = 78$) and triple therapy plus ranitidine (group R, $n = 78$) groups, respectively. Eighty had received a double regimen (omeprazole 20 mg b.d. and amoxiciline 500 mg q.d.s. for 15 days, OA) and 76 a triple regimen (omeprazole 20 mg b.d., amoxiciline 1 g b.d., clarithromycin 500 mg b.d., OAC). Baseline characteristics of the patients are shown in Table 1. No differences were identified between group R and group O, including the sub-group of patients who refused endoscopy but accepted the urea breath test.

Follow-up was available for 71 patients who received the triple therapy plus omeprazole regimen (group O, 91% follow-up) and 72 who received the triple therapy plus ranitidine regimen (group R, 92% follow-up). In 57 patients out of 71 from group O and 50 out of 72 from group R, a post-treatment endoscopy was performed. Another 36 patients (14 from group O and 22 from group R) refused post-treatment endoscopy but accepted the ^{13}C-urea breath test. Twenty-nine patients (11 from group O, 16 from group R) did so because they felt well and did not wish to proceed with one more endoscopy, while seven (three from group O, four from group R) refused endoscopy because they felt disappointed by the persistence of symptoms.

Table 1. Demographic and clinical data of patients included in the study

	All patients			Endoscopy refused		
Demographic/clinical characteristics	Triple therapy + omeprazole ($n = 78$)	Triple therapy + ranitidine ($n = 78$)	*P*	Triple therapy + omeprazole ($n = 14$)*	Triple therapy + ranitidine ($n = 22$)*	*P*
Mean age-years (s.d.)	47 (44–50)	49 (46–52)	0.35	46 (38–54)	49 (42–54)	0.38
Male/female	44/34	43/35	0.87	6/8	12/10	0.49
Smokers/non-smokers	34/44	35/43	0.87	7/7	11/11	1.00
Al Con/no Al Con*	7/71	7/63	0.95	1/13	4/18	0.35
Previous treatments OA/OAC	40/38	40/38	1.00	7/7	10/12	0.79
Initial diagnosis						
Erosive duodenitis	19	18	0.85	4	3	0.27
Duodenal ulcer	59	60	0.85	10	19	
Additional diagnoses						
Oesophagitis	8	6	0.58	1	2	0.84
Gastric ulcer	11	13	0.66	1	6	0.14
Erosive gastritis	7	8	0.79	2	0	0.07

Alc Con/no Alc Con: every day alcohol consumers/alcohol non consumers. Triple therapy + omeprazole/Triple therapy + ranitidine*: The results for patients who accepted a urea breath test but refused endoscopy are presented.

	Cured over all percentage	Triple therapy plus omeprazole	Triple therapy plus ranitidine	*P*
All patients				
Intention-to-treat (n = 156)	77 (70–83)	77 (67–87)	76 (66–85)	0.85
Per protocol analysis (n = 107)	84 (77–91)	86 (76–95)	82 (71–93)	0.58
Patients received OA as first line treatment				
Intention-to-treat (n = 80)	77 (68–87)	78 (64–91)	78 (64–91)	1.00
Per protocol analysis (n = 57)	83 (72–93)	86 (73–100)	82 (67–97)	0.95
Patients received OAC as first line treatment				
Intention-to-treat (n = 76)	75 (65–85)	76 (62–91)	74 (59–88)	0.79
Per protocol analysis (n = 53)	83 (73–94)	85.7 (72–100)	80 (63–97)	0.58

Table 2. Eradication of *H. pylori* on basis of intention-to-treat. per protocol analysis and initial treatment

Initial endoscopic diagnosis	Triple therapy + omeprazole (95% CI)	Triple therapy + ranitidine (95% CI)	*P*
Duodenal ulcer			
Intention-to-treat (n = 119)	80 (69–90)	70 (58–82)	0.22
Per protocol analysis (n = 83)	86 (76–97)	74 (59–88)	0.14
Erosive duodenitis			
Intention-to-treat (n = 35)	68 (45–91)	94 (80–100)	0.06
Per protocol analysis (n = 25)	83 (59–100)	100	0.12

Table 3. Eradication of *H. pylori* on basis of intention-to-treat. per protocol analysis and initial endoscopic diagnosis

From those patients who had an endoscopy performed. 37 out of 57 from group O and 30 out of 50 from group R had both negative rapid urease test and negative histology; three from group O and three from group R had both positive rapid urease test and positive histology. In 17 patients from group O and 17 from group R the results of rapid urease test and histology were conflicting and the ^{13}C-urea breath test was additionally performed. The urea breath test was positive in five out of the above mentioned 17 patients from group O. Six out of 17 patients from group R had a positive urea breath test ($P = 0.71$). Thus 49 out of 57 patients with post-treatment endoscopy in group R eradicated *H. pylori* as did 41 out of 50 in group O.

Of those who refused endoscopy, three out of 14 patients from group O and four out of 22 patients from group R had a positive ^{13}C-urea breath test.

The results on the basis of the intention-to-treat. per protocol analysis and initial treatment are shown in Table 2. The results on the basis of the intention-to-treat. per protocol analysis and initial endoscopic diagnosis (erosive duodenitis. duodenal ulcer) are shown in Table 3. The intention-to-treat analysis showed a trend in favour of ranitidine for *H. pylori* eradication in the presence of erosive duodenitis on the initial endoscopy, but there was no statistical significance ($P = 0.06$). A higher number of losses during follow-up of duodenitis was observed in group O (three out of 19) compared to group R (one out of 16). There were no statistical differences in *H. pylori* eradication between treatment groups. Intention-to-treat eradication rates for patients who received a first line triple regimen were 76% (62–91) for group O and 74% (59–88) for group R. For patients who received a first line double regimen, intention-to-treat eradication rates were 78% (64–91) for both study groups. Factors such as sex, alcohol consumption, smoking or initial endoscopic diagnosis did not seem to affect treatment success. The age group between

Table 4. Total intention-to-treat eradication rates according to age, sex, smoking and drinking habits. Univariate analysis

Parameter	Eradication rate	P
Age		
20–39 years (n = 55)	82 (71–92)	< 0.01*
40–59 years (n = 58)	64 (51–77)	
60–80 years (n = 40)	88 (77–98)	
Gender		
Males (n = 87)	74 (64–83)	0.37
Females (n = 69)	80 (70–89)	
Smoking habits		
Smokers (n = 66)	77 (67–87)	0.89
Non-smokers (n = 87)	76 (67–85)	
Alcohol consumption		
Non alcohol consumers (n = 142)	78 (71–84)	0.27
Alcohol consumers (n = 14)	64 (36–93)	
Initial endoscopic diagnosis		
Duodenal ulcer (n = 119)	75 (67–83)	0.26
Erosive duodenitis (n = 37)	83 (71–97)	

P for χ^2 analysis. *chi-square between the three age groups (d.f. = 2).

40 and 59 presented a reduced eradication rate (Table 4).

Side-effects

All patients were asked, on the 14th day of treatment, whether they had experienced any side-effects. The number and percentage of patients reporting side-effects, graded according to symptom severity are shown in Table 5. Generally, side-effects were mild and did not interfere with compliance. Only 27 out of 71 patients (38%) in group O and 25 out of 72 (35%) in group R were completely free of side-effects. The assessment of mild symptoms was performed during the day 14 communication, while patients with severe symptoms tended to communicate earlier, to stop treatment prematurely. Eleven patients stopped treatment prematurely: five out of 71(7%) of group O (one because of oral candidiasis, two because of allergic reactions and two because of diarrhoea—one of them developed mild pseudomembranous colitis) and six out of 72 (8.3%) of group R (two because of intense nausea, one because of oral candidiasis, one because of allergic rash and two because of bismuth toxicity—one case of mild apathy and ataxia and one with confusion and multiple premature ventricular complexes).

All serious side-effects (with the exception of allergic reactions—three patients) appeared during the second week of treatment. Five out of the other eight patients who received at least 1 week of eradication treatment were successfully eradicated. Pseudomembranous colitis successfully responded to metronidazole treatment over 14 days. Both patients who presented with bismuth toxicity had increased blood creatinine levels when symptoms appeared (1.5 and 2 mg/dL, respectively); both suffered from non-insulin dependent diabetes mellitus.

DISCUSSION

In this prospective randomized, non-blinded study we evaluated two quadruple regimens, comprising ranitidine or omeprazole plus bismuth triple therapy, as second-line treatment. Both regimens seemed to be equally effective (intention-to-treat 76.9% for group O and 74.3% for group R) in patients with duodenal ulcer. In patients with erosive duodenitis regimen there was a trend in favour of ranitidine comprising regimen concerning *H. pylori* eradication (P = 0.06). Both efficacy was much lower than the one described when those regimens had been used as first line treatment.[4, 5] Side-effects were equally and frequently presented (62%

Table 5. Number and percentage of patients reporting side-effects

	Triple therapy + omeprazole			Triple therapy + ranitidine			
Side-effect	None	Mild	Severe	None	Mild	Severe	P
Nausea	51 (74%)	19 (24%)	1 (1%)	45 (63%)	25 (35%)	2 (3%)	0.24
Vomiting	70 (99%)	1 (1%)	0	70 (97%)	1 (1%)	1 (1%)	0.57
Allergic rash	68 (96%)	2 (3%)	1 (1%)	71 (99%)	1 (1%)	0	0.30
Metallic taste	44 (62%)	25 (35%)	2 (3%)	51 (71%)	20 (28%)	1 (1%)	0.31
Diarrhoea	60 (85%)	8 (11%)	3 (4%)	65 (90%)	6 (8%)	1 (1%)	0.30
Dizziness	58 (82%)	12 (17%)	1 (1%)	51 (71%)	19 (26%)	2 (3%)	0.13

The P-values refer to the comparison between patients with and without the side-effect, ignoring the severity.

for group O and 65% for group R) but there were rather mild and rarely interfered with compliance.

The trial was not conducted in a double-blind manner because patients had to pay for their own medications. Nevertheless *H. pylori* eradication was evaluated by personnel unaware of the treatment regimen.

When used as first line regimen, metronidazole-containing bismuth triple therapy showed a reduction in efficacy from 92% in patients with sensitive *H. pylori* strains to 44% in patients infected with resistant strains.[16] It is suggested that the addition of an acid suppressor to the bismuth triple therapy regimen (quadruple) could overcome the reduced efficacy of the triple regimen resulting from metronidazole resistance. Nevertheless, a 10–16% reduction in regimen efficacy is expected for resistant strains.[5, 17] Although we had not tested *H. pylori* resistance in our population, the expected resistance was between 37.8 and 54.1%, taking into consideration published data for the prevalence of metronidazole resistance in Greece (46%).[18]

De Boer's *et al.* study using ranitidine comprising quadruple regimen as first line treatment, as well as our study using the same regimen as second-line treatment has shown no difference in quadruple treatment efficacy when omeprazole is replaced by ranitidine.[5]

Although some consider erosive duodenitis to be a separate entity, most consider it to be a variety of duodenal ulcer.[19, 20] In the intention-to-treat analysis, losses during follow-up, are considered as failures. Thus the higher number of losses in follow-up of group O (three in group O and one in group R), could be the reason why the ranitidine regimen showed a trend of higher efficacy in patients with erosive duodenitis. Nevertheless, data concerning second-line eradication treatments for erosive duodenitis are missing and the statistical trend appearing in our study needs further research.

Factors such as age, sex, smoking and drinking habits as well as pre-treatment with omeprazole do not seem to affect treatment success of triple therapy.[21] Neither do they seem to affect the final result in our study. Women are more likely to harbour metronidazole resistant strains (73% vs. 38% in Hong Kong, 54% vs. 18% in UK), nevertheless sex did not affected final result.[22] This could be due to the low difference in metronidazole resistance between sexes in Greece (50% vs. 44%).[18]

The previous use of amoxycillin in a first line regimen led us to choose tetracycline, to which resistance is usually low.[23] The replacement of amoxycillin by tetracycline increases efficacy of classical triple therapy.[24] Tetracycline seems to have *in vitro* synergism with metronidazole, especially for resistant strains.[25]

Post-therapeutically, the rapid urease test is thought to present 86% sensitivity when histology is thought to be the gold standard.[26] Nevertheless, when we have taken into consideration long-term follow-up and various diagnostic tests in our unit, histology did not seem to represent the gold standard to validate conflicting results between the rapid urease test and histology.[27] Thus for this group of patients, the ^{13}C-urea breath test was chosen as an additional test: this is considered as reliable as histology in everyday practice and seemed to be ideal for overcoming low histology sensitivity in our laboratory.[28]

Side-effects seem to be dose-dependent (mainly for metronidazole and bismuth), and time-dependent (when side-effects questionnaire is completed).[29, 30] The addition of omeprazole seems to slightly reduce the side-effect profile of classical triple therapy which is usually around 50%, ranging between 7% and 72% depending on the side-effect scoring system.[31, 32] In this study, side-effects were frequent: 62% for triple therapy comprising omeprazole and 65% for that comprising ranitidine. Nevertheless side-effects which led to treatment cessation were rather infrequent: 7% for group O and 9.2% for group R. In our study it is noteworthy that three out of 148 of our patients developed rather serious side-effects: pseudomembranous colitis and bismuth toxicity. Bismuth neurotoxicity has been attributed to increased plasma concentrations (over 100 μg).[33] Quadruple therapy with omeprazole can increase the bismuth blood level.[34] Renal function impairment could contribute to the increase in bismuth concentrations: Both of the patients in our study who presented with bismuth toxicity suffered from diabetes.[35] Sub-clinical renal impairment could not be excluded in either of them. Pseudomembranous colitis has been rarely described as a side-effect of various eradication regimens; occasionally it can be extremely severe.[36–38]

Based on the results of this study and the above mentioned data the approach proposed by the Maastricht consensus seems to be reasonable.[15] That is to: select one triple regimen as the first line treatment, omitting either metronidazole or clarithromycin in order to avoid resistance to both drugs (which would make it difficult to find an effective second-line treatment), then select a quadruple regimen as a second-line treatment: both omeprazole and ranitidine could be used in these regimens without any difference to the final result.

REFERENCES

1 Marshall BJ. *Helicobacter pylori*. Am J Gastroenterol 1994; 89(Suppl.): 116–28.
2 NIH. Concencus Conference. *Helicobacter pylori* in peptic ulcer disease. J Am Med Assoc 1994; 272: 65–9.
3 Collins R, Beatle S, Xia HX, *et al*. High-dose omeprazole and amoxycillin in the treatment of *Helicobacter pylori*-associated duodenal ulcer. Aliment Pharmacol Ther 1993; 7: 313–5.
4 Van der Hulst RWM, Keller JJ, Rauws EAJ, Tytgat GNJ. Treatment of *Helicobacter pylori* infection: review of the world literature. Helicobacter 1996; 1: 6–19.
5 de Boer WA, Driessen WMM, Potters HVPJ, Tytgat GNJ. Randomized study comparing 1 and 2 week of quadruple therapy for eradicating *H. pylori*. Am J Gastroenterol 1994; 89: 1993–7.
6 Borody TJ, Andrews P, Fracchia G, Brandl S, Shortis NP, Bae H. Omeprazole enhances efficacy of triple therapy in eradicating *Helicobacter pylori*. Gut 1995; 37: 477–81.
7 Rauws EAJ. Reasons for failure of *Helicobacter pylori* treatment. Eur J Gastroenterol Hepatol 1993; 5(Suppl. 2): S92–5.
8 Borody TJ, Andrews P, Shortis NP. Features of patients who fail all attempts at *Helicobacter pylori* eradication. Am J Gastroenterol 1994; 89: 1365.
9 Borody TJ, Shortis NP, Chongnan J, Reyes E, O'Shea JE. Eradication failure after *H. pylori* treatment—further therapies. Gastroenterology 1996; 110: A67(Abstract).
10 Reilly TG, Ayres RCS, Poxon V, Walt RP. *Helicobacter pylori* eradication in a clinical setting: success rates and the effect on the quality of life in peptic ulcer. Aliment Pharmacol Ther 1995; 9: 483–90.
11 Seppala K, Sipponen P, Nuutinen H, Farkkila M, Anisimova S, Kosumen TU. Intent to treat metronidazole resistant *H. pylori* infection to 100% of cure therapy with quadruple therapy and its modification. Gastroenterology 1995; 108: A216-(Abstract).
12 Moshkowitz M, Konikoff FM, Peled Y, *et al*. One week therapy with omeprazole, clarithromycin and tinidazole for *Helicobacter pylori*: differing efficacy in previously treated and untreated patients. Aliment Pharmacol Ther 1996; 10: 1015–9.
13 Gisbert JP, Boixeda D, Moreno L, *et al*. Which therapy should we use when a previous *Helicobacter pylori* eradication therapy fails? Gut 1997; 41: A26(Abstract).
14 Coelho LGV, Vieira WLS, Passos MCF, *et al*. Quadraple therapy for eradication failure or *H. pylori* reinfection in ulcer patients. Gut 1997; 41: A94(Abstract).
15 The European Helicobacter Pylori Study Group. Current European concepts in management of *Helicobacter pylori* infection. The Maastricht Concencus Report. Gut 1997; 41: 8–13.
16 Noach LA, Langenberg WL, Bertola MA, Dankert J, Tytgat GN. Impact of metronidazole resistance on the eradication of *Helicobacter pylori*. Scand J Infect Dis 1994; 26: 321–7.
17 Van der Hulst RWM, Van de Ende A, Homan A, Roorda P, Dankert J, Tytgat GNJ. Influence of metronidazole resistance on efficacy of quadruple therapy for *Helicobacter pylori* eradication. Gut 1998; 42: 166–9.
18 Mentis A, Balatsos B, Kastanas K, Tzouvelis L. *In vitro* susceptibility of *Helicobacter pylori* in metronidazole. Hell J Gastroenterol 1992; 5: 305–7.
19 Gibinsky K, Rybecka J, Norwak A, Czarnecka K. Seasonal occurrence of abdominal pain and endoscopic findings in patients with gastric and duodenal ulcer disease. Scand J Gastroenterol 1982; 17: 481–5.
20 Gisbert JP, Boixeda D, de Argila CM, Bermejo F, Redondo C, de Rafael L. Erosive duodenitis: prevalence of *Helicobacter pylori* infection and response to eradication therapy with omeprazole plus two antibiotics. Eur J Gastroenterol Hepatol 1997; 9: 957–62.
21 de Boer WA, Driessen WMM, Jansz AR, Tytgat GNJ. Effect of acid suppression on efficacy of treatment for *Helicobacter pylori* infection. Lancet 1995; 345: 817–20.
22 Banarvala N, Davies GR, Abdi Y, *et al*. High prevalence of *Helicobacter pylori* metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastrointestinal disease. Gut 1994; 35: 1562–6.
23 Walsh JH, Peterson WL. The treatment of *Helicobacter pylori* infection in the management of peptic ulcer disease. N Engl J Med 1995; 333: 984–91.
24 Chiba N, Rao BV, Rademaker JW, Hunt RH. Metaanalysis of the efficacy of antibiotic therapy in eradicating *Helicobacter pylori*. Am J Gastroenterol 1993; 87: 1716–27.
25 Xia H, Daw MA, Sant S, Beattie S, Keane CT, O'Morain CA. Clinical efficacy of triple therapy in *H. pylori* associated duodenal ulcer. Eur J Gastroenterol Hepatol 1993; 5: 141–4.
26 Laine L, Suchower L, Johnson E, Ronca P, Neil G. Accuracy of CLO test after *Helicobacter pylori* therapy. Gastrointest Endosc 1998; 47: 250–3.
27 Michopoulos S, Balta A, Mentis A, *et al*. Invasive methods and clinical follow up to estimate *H. pylori* eradication success. Gastroenterology 1998; 114: A227(Abstract).
28 Epple HJ, Kirstein FW, Bojarski C, *et al*. ^{13}C-urea breath test in *Helicobacter pylori* diagnosis and eradication. Scand J Gastroenterol 1997; 32: 308–14.
29 Borody TJ, Brandl S, Andrews P, Ferch N, Jankiewicz E, Hyland L. Use of high efficacy, lower dose triple therapy to reduce side effects of eradicating *Helicobacter pylori*. Am J Gastroenterol 1994; 89: 33–8.
30 Thijs JC, Zwet AA, Oey HB. Efficacy and side effects of a triple drug regimen for the eradication of *Helicobacter pylori*. Scand J Gastroenterol 1993; 28: 934–8.
31 de Boer WA, Driessen WMM, Jansz AR, Tytgat GNJ. Quadruple therapy compared with dual therapy for eradication of *Helicobacter pylori* in ulcer patients. Eur J Gastroenterol Hepatol 1995; 7: 1189–94.
32 de Boer WA, Driessen WMM, Potters HVPJ, Tytgat GNJ. Randomized study comparing 1 and 2 week of quadruple therapy for eradicating *H. pylori*. Am J Gastroenterol 1994; 89: 1993–7.
33 Gorbach SL. Bismuth therapy in gastrointestinal diseases. Gastroenterology 1990; 99: 863–75.

34 Philips RH, Whitehead MW, Delves HT, Thompson RPH, Powell JJ. Is «quadruple therapy» safe? Gut 1997; 41: A89(Abstract).
35 Weller MPI. Neuropsychiatric symptoms following bismuth intoxication. Postgrad Med J 1988; 64: 308–10.
36 Hudson N, Brydon WG, Eastwood MA, Ferguson A, Palmer KR. Successful *Helicobacter pylori* eradication incorporating a one-week antibiotic regimen. Aliment Pharmacol Ther 1995; 9: 47–50.
37 Award RW, Ward KL, Hawker PC. Fulminant pseudomembranous colitis following triple therapy for *Helicobacter pylori*. J R Coll Surg Edinb 1994; 39: 319.
38 Houben MHMG, van de Beek D, van't Hoff BWM, *et al.* PPI-triple therapy failure: re-treatment with PPI-triple therapy or quadruple therapy? Gastroenterology 1998; 114: A153 (G0627).

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Open-Label Study of a Regimen Consisting of 1 Week of Lansoprazole, Clarithromycin, and Amoxicillin Followed by 3 Weeks of Lansoprazole in Healing Peptic Ulcer and Eradicating *Helicobacter pylori*

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ABSTRACT

Objective: **The aim of the study was to assess the efficacy of a regimen consisting of 1 week of low-dose triple therapy with lansoprazole, amoxicillin, and clarithromycin followed by lansoprazole alone for an additional 3 weeks in the eradication of *Helicobacter pylori* and the healing of peptic ulcer.**

Methods: **Patients aged ≥16 years with active peptic ulcer diagnosed by endoscopy and *H pylori* infection were eligible for this prospective, open-label, 3-center study. A triple-drug regimen was used that consisted of lansoprazole 30 mg once daily, amoxicillin 1 g BID, and clarithromycin 250 mg BID for 7 days. Ulcer healing and *H pylori* eradication were assessed endoscopically 8 to 9 weeks after the start of treatment. *H pylori* was determined to be eradicated if both histologic examination and rapid urease testing (4 biopsy samples, antrum [2] and body [2]) were negative.**

Results: **Fifty-five patients who tested positive for *H pylori*, 49 with duodenal ulcer (DU) and 6 with gastric ulcer (GU), aged 16 to 78 years, were enrolled in the study. Ten patients were lost to follow-up and 1 withdrew from the study because of side effects; 44 patients were included in the per-protocol analyses. *H pylori* was eradicated in 34 patients, 62% (95% CI, 0.477–0.746) and 77% (95% CI, 0.662–0.885) in the intent-to-treat and per-protocol analyses, respectively. Ulcers were healed in a total of 38 patients (34 DU, 4 GU), 69% in the intent-to-treat population (95% CI, 0.552–0.809) and 86% in the per-protocol population (95% CI, 0.727–0.948). In patients with DU, the rate of healing was 69% in the intent-to-treat analysis (95% CI, 0.546–0.817) and 89% in the per-protocol analysis (95% CI, 0.752–0.971).**

Conclusions: **One week of therapy with lansoprazole 30 mg once daily, amoxicillin 1 g BID, and clarithromycin 250 mg BID, followed**

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by 3 weeks of treatment with lansoprazole 30 mg once daily, effectively healed peptic ulcer but was only moderately effective in eradicating *H pylori*.

***Key words:* amoxicillin, clarithromycin, *Helicobacter pylori*, lansoprazole, peptic ulcer, triple therapy. (*Curr Ther Res Clin Exp.* 2000;61:406–413)**

INTRODUCTION

Triple-drug regimens consisting of lansoprazole 30 mg once daily or 30 mg BID with various doses of clarithromycin and metronidazole, amoxicillin and clarithromycin, amoxicillin and metronidazole, or azithromycin and metronidazole for 1 or 2 weeks have been reported to be efficacious in eradicating *Helicobacter pylori* and healing peptic ulcers,[1–10] with success rates >90% in some studies.[3,5,9]

In 2 studies, lansoprazole 30 mg once daily was combined with clarithromycin 200 mg BID and metronidazole 250 mg BID,[4] and clarithromycin 250 mg BID and metronidazole 400 mg BID for 7 days. *H pylori* was eradicated in 88%[4] and 86%[1] of patients, whereas, in another study,[11] the combination of lansoprazole 30 mg once daily with clarithromycin 250 mg BID and amoxicillin 1 g BID for 7 days eradicated *H pylori* in 82% of patients.

Sieg et al[9] recently reported that 1 week of treatment with lansoprazole 15 mg BID in combination with amoxicillin 1 g BID and clarithromycin 500 mg BID eradicated *H pylori* in 87% of patients, similar to the 94% eradication obtained with lansoprazole 30 mg BID. All peptic ulcers were healed in both treatment groups at the follow-up examination. Thus, lansoprazole 30 mg once daily combined with 2 antibiotics appears to eradicate *H pylori* in a high percentage of patients, although usually <90%. Nevertheless, such low-dose regimens should be examined, since they do not generally seem to differ significantly in efficacy from the more expensive regimens using double doses of proton-pump inhibitors.

The present study was undertaken to assess the efficacy of a regimen consisting of 7 days of low-dose triple-drug therapy with lansoprazole, clarithromycin, and amoxicillin, followed by lansoprazole alone for an additional 3 weeks, in eradicating *H pylori* and healing peptic ulcer.

PATIENTS AND METHODS

This prospective, open-label, 3-center study enrolled patients aged 16 to 78 years with active peptic ulcer (duodenal ulcer [DU] or gastric ulcer [GU]) who tested positive for *H pylori* infection. *H pylori* colonization was established by histologic examination (modified Giemsa stain) and/or rapid urease testing (CLOtest, Delta West Pty Ltd., Bentley, Western Australia)

of 2 biopsy samples taken from the antral mucosa of the stomach. Patients were considered positive for *H pylori* and eligible for the study if the result of either test was positive.

Other exclusion and inclusion criteria were similar to those described elsewhere.[12] Outpatients and inpatients of either sex who had been hospitalized for <1 week and underwent endoscopy for epigastric pain, dyspepsia, and/or upper gastrointestinal bleeding were eligible for the study.

The following individuals were excluded: patients with a bleeding ulcer, previous gastric surgery, or associated pyloric channel or esophageal ulceration; patients who had taken corticosteroids, nonsteroidal anti-inflammatory drugs, any antiulcer or antibiotic treatment during the previous 4 weeks, or concomitant therapy with any investigative drug; patients with severe disease (eg, renal, hepatic, hematologic, cardiac, endocrine, or neurologic disease); or patients who abused alcohol or drugs. Women who were pregnant, lactating, or using inadequate contraception were also excluded.

A triple-drug regimen consisting of lansoprazole 30 mg once daily, amoxicillin 1 g BID, and clarithromycin 250 mg BID was given for 7 days, followed by lansoprazole 30 mg once daily for an additional 3 weeks. Patients were instructed to take the drugs before meals. Patients were seen for clinical evaluation by the end of the third week. Patients' compliance was monitored with a detailed questionnaire covering the number of drugs taken each day and the time they were taken.

A second endoscopic examination was scheduled 4 to 5 weeks after the end of the treatment (ie, 8 to 9 weeks after the start of treatment or whenever symptoms recurred) to confirm ulcer healing and *H pylori* eradication. Four biopsy samples were obtained (antrum 2, body 2) and were examined histologically and by rapid urease testing for the presence of *H pylori*. *H pylori* was considered eradicated if both histologic and rapid urease test findings were negative. Histologic examination was carried out by experienced pathologists who were blinded to study visit and treatment. The ulcers were considered healed if the former ulcer site was totally epithelialized.

The study was performed in accordance with the Declaration of Helsinki. Oral informed consent was given by all patients.

A computer program (CIA, microcomputer program 1.1, *BMJ* and Martin Gardner 1991) was used to calculate the CIs.

RESULTS

Fifty-five patients who were positive for *H pylori*, 49 with DU and 6 with GU, were included in the study. Their baseline characteristics are shown in Table I. Ten patients (18%) were lost to follow-up, and 1 female pa-

Table I. Baseline characteristics (N = 55).

Age (y)	
Mean ± SD	48.47 ± 12.29
Range	16–78
Sex, no. (%)	
Male	35 (64)
Female	20 (36)
Smoker, no. (%)*	31 (56)
Bleeding history, no. (%)	11 (19)
Ulcer diameter (mm)	
Mean ± SD	7.2 ± 2.18
Range	5–16

* More than 10 cigarettes/d.

tient (2%) withdrew because of side effects (rash and diarrhea). Thus, 44 patients completed the study and were included in the final analysis; all 44 patients underwent endoscopy according to the protocol.

Results are shown in Table II. *H pylori* was eradicated in 34 patients, 62% of the intent-to-treat population (95% CI, 0.477–0.746) and 77% of the per-protocol population (95% CI, 0.662–0.885). Ulcers were healed in 38 patients (34 DU, 4 GU), 69% of the intent-to-treat population (95% CI, 0.552–0.809) and 86% of the per-protocol population (95% CI, 0.727–0.948). In patients with DU, the incidence of healing was 69% (95% CI, 0.546–0.817), and 89% (95% CI, 0.752–0.971) in the intent-to-treat and per-protocol analyses, respectively. Ulcers did not heal in 6 patients, 2 with GU (both *H pylori* positive) and 4 with DU (1 *H pylori* positive).

Side effects (eg, loose stools, headache, taste changes, constipation, nausea, and tiredness) were reported in 10 (23%) of the 44 patients (4 women between 39 and 60 years of age with DU; 6 men between 21 and 59 years of age with DU). Side effects were generally mild, and none of the patients stopped therapy as a result. Grade II symptomatic esophagitis

Table II. *Helicobacter pylori* eradication and ulcer healing.

	Intent-to-Treat Analysis (N = 55)	Per-Protocol Analysis (n = 44)
Eradication		
No. (%)	34 (62)	34 (77)
95% CI	0.477–0.746	0.662–0.885
Healing		
All patients		
No. (%)	38 (69)	38 (86)
95% CI	0.552–0.809	0.727–0.948
Patients with DU		
No. (%)	34/49 (69)	34/38 (89)
95% CI	0.546–0.817	0.752–0.971

DU = duodenal ulcer.

was observed on the second endoscopy in 1 patient; she completed the study successfully (ie, *H pylori* was eradicated and the DU was healed).

All assessable patients took ≥85% of each prescribed medication.

DISCUSSION

Triple-drug regimens consisting of a proton-pump inhibitor (omeprazole, lansoprazole, or pantoprazole) plus clarithromycin and a nitroimidazole drug (metronidazole, tinidazole, or ornidazole) for 7 days have shown eradication rates >90% for *H pylori*.[13–26] In particular, a large international, multicenter study of a 7-day regimen combining omeprazole and 2 antibiotics (amoxicillin + clarithromycin, metronidazole + clarithromycin, or amoxicillin + metronidazole) demonstrated high eradication rates, with some combinations exceeding 90%.[27]

Lansoprazole in combination with 2 antibiotics was shown to have a high degree of efficacy in eradicating *H pylori*, comparable to that of omeprazole, in a meta-analysis of randomized, controlled clinical trials published in English from 1993 to 1996.[25] The authors concluded that lansoprazole appeared to be an option for eradication of *H pylori*.

In this study, we demonstrated that 7-day triple therapy with lansoprazole 30 mg once daily, amoxicillin 1 g BID, and clarithromycin 250 mg BID eradicated *H pylori* in a substantial (77%) percentage of patients, although not as high as would be expected. Indeed, this triple-drug regimen has been studied in another population,[11] with a per-protocol eradication rate of 82%. The results were even better when clarithromycin was given in a dosage of 500 mg BID (93% eradication) instead of 250 mg BID[5] and when lansoprazole was given in a dosage of 15 mg BID (87% eradication).[9] The results of the present study are comparable with those of Lim et al[28] after 7 days of treatment with lansoprazole 30 mg BID, amoxicillin 1 g BID, and clarithromycin 250 mg BID. Low eradication rates are generally attributed to poor patient compliance and resistance of *H pylori* to antibiotics. Patient compliance was satisfactory in the present study. Metronidazole-resistant *H pylori* strains have been reported in 46% to 54% of Greek patients with gastritis and/or peptic ulcer, but clarithromycin-resistant *H pylori* strains are rare and amoxicillin-resistant strains are even rarer.[29–31]

When we used a regimen consisting of lansoprazole 30 mg BID, clarithromycin 500 mg BID, and amoxicillin 1 g BID, followed by lansoprazole 30 mg once daily for 3 additional weeks (A. Archimandritis, unpublished data, 2000), the eradication rate for *H pylori* was better, exceeding 90%, but the rate of ulcer healing (90%) was unchanged. These results, which are in agreement with the international literature, show that in triple-

drug therapies for *H pylori*, lansoprazole should be given in a dosage of 30 mg BID to obtain the best eradication rate.

CONCLUSIONS

One week of therapy with lansoprazole 30 mg once daily, amoxicillin 1 g BID, and clarithromycin 250 mg BID, followed by 3 weeks of treatment with lansoprazole 30 mg once daily, effectively heals peptic ulcer but is moderately effective in eradicating *H pylori*.

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References:

1. Harris AW, Pryce DI, Gabe SM, et al. Lansoprazole, clarithromycin and metronidazole for seven days in *Helicobacter pylori* infection. *Aliment Pharmacol Ther.* 1996;10:1005–1008.
2. Misiewicz JJ, Harris AW, Bardhan KD, et al. One week triple therapy for *Helicobacter pylori*: A multicentre comparative study. Lansoprazole Helicobacter Study Group. *Gut.* 1997;41:735–739.
3. Caselli M, Trevisani L, Tursi A, et al. Short-term low dose triple therapy with azithromycin, metronidazole and lansoprazole appears highly effective for the eradication of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol.* 1997;9:45–48.
4. Kihira K, Satoh K, Saifuku K, et al. Comparison of ranitidine and lansoprazole in short-term low-dose triple therapy for *Helicobacter pylori* infection. *Aliment Pharmacol Ther.* 1997;11:511–514.
5. Louw JA, Van Rensburg CJ, Moola S, et al. *Helicobacter pylori* eradication and ulcer healing with daily lansoprazole, plus 1 or 2 weeks co-therapy with amoxicillin and clarithromycin. *Aliment Pharmacol Ther.* 1998;12:881–885.
6. Harris AW, Misiewicz JJ, Bardhan KD, et al. Incidence of duodenal ulcer healing after 1 week of proton pump inhibitor triple therapy for eradication of *Helicobacter pylori*. The Lansoprazole Helicobacter Study Group. *Aliment Pharmacol Ther.* 1998;12:741–745.
7. Nishikawa K, Sugiyama T, Ishizuka J, et al. Eradication of *Helicobacter pylori* using 30 mg or 60 mg lansoprazole combined with amoxicillin and metronidazole: One and two weeks of a new triple therapy. *J Gastroenterol.* 1999;34(Suppl 11):72–75.
8. Nakagawa M, Ooishi M, Yoda Y, et al. A comparative study: One week treatment versus two weeks treatment with lansoprazole, amoxicillin and clarithromycin for the eradication of *Helicobacter pylori* in patients with gastric and duodenal ulcer. *Nippon Rinsho.* 1999;57:144–147. In Japanese.

9. Sieg A, Sellinger M, Schlauch D, et al. Short-term triple therapy with lansoprazole 30 mg or 60 mg, amoxicillin and clarithromycin to eradicate *Helicobacter pylori*. *Aliment Pharmacol Ther.* 1999;13:865–868.

10. Wong BCY, Xiao SD, Hu FL, et al. Comparison of lansoprazole-based triple and dual therapy for treatment of *Helicobacter pylori*–related duodenal ulcer: An Asian multicentre double-blind randomized placebo controlled study. *Aliment Pharmacol Ther.* 2000; 14:217–224.

11. Cammarota G, Tursi A, Papa A, et al. *Helicobacter pylori* eradication using one-week low-dose lansoprazole plus amoxicillin and either clarithromycin or azithromycin. *Aliment Pharmacol Ther.* 1996;10:997–1000.

12. Archimandritis A, Charitopoulos N, Diamantis T, et al. Comparison of sucralfate and ranitidine twice daily in duodenal ulcer treatment: A multicenter randomized double-blind study. *J Clin Gastroenterol.* 1991;13:380–383.

13. Bazzoli F, Zagari RM, Fossi S, et al. Short term low-dose triple therapy for the eradication of *Helicobacter pylori*. *Eur J Gastroenterol Hepatol.* 1994;6:773–777.

14. Moayyedi P, Axon ATR. Efficacy of a new one week triple regimen in eradicating *Helicobacter pylori*. *Gut.* 1994;35(Suppl 2):S62. Abstract.

15. Bell GD, Powel KU, Burridge SM, et al. Rapid eradication of *Helicobacter pylori* infection. *Aliment Pharmacol Ther.* 1995;9:41–46.

16. Goddard A, Logan R. One-week low-dose triple therapy: New standards for *Helicobacter pylori* treatment. *Eur J Gastroenterol Hepatol.* 1995;7:1–3.

17. Yousfi MM, El-Zimaity HMT, Al-Assi MT, et al. Metronidazole, omeprazole and clarithromycin: An effective combination therapy for *Helicobacter pylori* infection. *Aliment Pharmacol Ther.* 1995;9:209–212.

18. Labenz J, Adamek RJ, Idstrom JP, et al. Duodenal ulcer healing and *Helicobacter pylori* eradication by one-week low-dose triple therapy with omeprazole, clarithromycin and metronidazole. *Gut.* 1995;37(Suppl 2):A12. Abstract.

19. Labenz J, Stolte M, Ruhl GH, et al. One-week, low-dose triple therapy for the eradication of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol.* 1995;7:9–11.

20. Moayyedi P, Sahay P, Tompkins DS, Axon ATR. Efficacy and optimum dose of omeprazole in a new 1-week triple therapy regimen to eradicate *Helicobacter pylori*. *Eur J Gastroenterol Hepatol.* 1995;7:835–840.

21. Misiewicz JJ, Harris AW, Bardhan KD, et al. One week low-dose triple therapy for eradication of *H. pylori*: A large multicentre, randomised trial. *Gastroenterology.* 1996;110(Suppl):A198. Abstract.

22. Goddard AF, Logan RPH, Lawes S, et al. Metronidazole or tinidazole in combination with omeprazole and clarithromycin for the eradication of *H. pylori*: A randomised and double-blind comparison. *Gastroenterology.* 1996;110(Suppl):A117. Abstract.

23. O'Connor HJ, Loane J, Cunnane K. One week triple therapy for *Helicobacter pylori* incorporating high-dose clarithromycin. A prospective study. *Gastroenterology.* 1996; 110(Suppl):A214. Abstract.

24. Adamek RJ, Szymanski C, Pfaffenbach B, et al. Modified short-term triple therapy with pantoprazole, clarithromycin, and metronidazole for cure of *Helicobacter pylori* infection. *Dtsch Med Wochenschr.* 1995;120:358–360.

25. Bazzoli F, Pozzato P, Zagari M, et al. Efficacy of lansoprazole in eradicating *Helicobacter pylori*: A meta-analysis. *Helicobacter*. 1998;3:195–201.

26. Tzivras M, Archimandritis A, Balatsos V, et al. One-week therapy with omeprazole, clarithromycin and metronidazole or ornidazole, followed by 3 weeks' treatment with omeprazole, eradicates *Helicobacter pylori* equally and heals duodenal ulcer. *Eur J Gastroenterol Hepatol*. 1997;9:1185–1189.

27. Lind T, van Zanten SV, Unge P, et al. Eradication of *Helicobacter pylori* using one-week triple therapies combining omeprazole with two antimicrobials: The MACH 1 Study. *Helicobacter*. 1996;1:138–144.

28. Lim AG, Walker C, Chambers S, Gould SR. *Helicobacter pylori* eradication using a 7-day regimen of low-dose clarithromycin, lansoprazole and amoxicillin. *Aliment Pharmacol Ther*. 1997;11:537–540.

29. Mentis A, Balatsos V, Kastanas K, Tzouvelekis L. In vitro susceptibility of *Helicobacter pylori* to metronidazole. *Hell J Gastroenterol*. 1992;5:305–307.

30. Wurzer H, Rodrigo L, Stamler D, et al. Short-course therapy with amoxicillin-clarithromycin triple for 10 days (ACT-10) eradicates *H. pylori* and heals duodenal ulcer. *Alim Pharmacol Ther*. 1997;11:943–952.

31. Mentis A, Kallergi K, Georgopoulos S, et al. Third European Multicentre Study on *Helicobacter pylori* Resistance: Results of the Greek participation. In: Ladas S, ed. *Abstract Book for the Fourth Hellenic Meeting for* Helicobacter pylori. Athens: Technogramma; 1999:202. Abstract.

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HELICOBACTER PYLORI

Rapid urease test is less sensitive than histology in diagnosing *Helicobacter pylori* infection in patients with non-variceal upper gastrointestinal bleeding

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Abstract

Background and Aims: The validity of the rapid urease (CLO) test to diagnose *Helicobacter pylori* infection in patients with bleeding ulcers has been questioned. The aim of this paper is to evaluate the validity of the CLO test in comparison with histology in diagnosing *H. pylori* infection in patients with acute upper gastrointestinal bleeding (UGB), irrespective of non-steroidal anti-inflammatory drug (NSAID) use.

Methods: Upper gastrointestinal endoscopy was performed within 24 h of admission for all patients with UGB admitted to the Department of Pathophysiology, Medical School, Athens, for a period of 12 months. Patients with variceal bleeding, previous gastric operation, recent treatment with proton pump inhibitors (< 2 months) and those with a history of *H. pylori* eradication therapy were excluded from the study. At least four biopsies (two from the antrum and two from the body) were obtained for the CLO test and histology (modified Giemsa).

Results: Seventy-two consecutive patients (aged 18–90 years, 51 men, 21 women) were included. Forty-six patients (64%) used NSAID. Thirty-two patients (44%) were found to be positive for *H. pylori* infection by the CLO test, while 44 patients (61%) were found to be positive on histology ($P < 0.045$, 95% CI, 0.004–0.331). The sensitivity and specificity of the CLO test were 68 and 93% respectively; positive and negative predictive values were 94 and 65%, respectively. The age of the patient and visible blood in the stomach did not influence results of either the CLO or histology.

Conclusions: The CLO test, performed within 24 h of hospital admission in patients with UGB, irrespective of NSAID use, is unreliable for the detection of *H. pylori* infection. The age of the patient and the presence of blood in the stomach do not seem to influence these results.

Key words: biopsy-based tests, *Helicobacter pylori*, non-steroidal anti-inflammatory drugs, rapid urease test, upper gastrointestinal bleeding.

INTRODUCTION

Diagnosis of *Helicobacter pylori* infection in clinical practice is usually made by two biopsy based methods; the rapid urease test and/or histology. The rapid urease test, in particular the CLO test, is considered to be a quick and reliable test for the initial diagnosis of *Helicobacter pylori* infection, with high sensitivity and specificity.[1,2] Histological examination of the gastric mucosa after suitable staining provides further diagnostic accuracy, but is quite expensive and time consuming. Therefore, if the CLO test is negative,[3] additional biopsies are usually obtained, processed and evaluated to confirm the *H. pylori* status of the patient.

It has been shown that *H. pylori* eradication prevents recurrence of bleeding[4,5] and it has been suggested that

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if the eradication therapy is started in hospital immediately after the control of bleeding, better patient compliance might be ensured.[6] Compliance is considered a major factor in successful eradication of *H. pylori*.[7] Therefore, in patients with bleeding peptic ulcers, it is particularly useful to know their *H. pylori* status so that eradication therapy can be initiated as soon as possible. The CLO test is usually performed as the first endoscopic test to diagnose *H. pylori* infection because of its simplicity, rapidity and reliability.[3]

However, we observed (A. Archimandritis, unpubl. data, 1996) that in patients with acute ulcer bleeding, the prevalence of *H. pylori* infection as diagnosed by using the CLO test was unexpectedly low and roughly similar to that reported by serology in healthy adults.[8] In addition, the low prevalence of *H. pylori* infection in bleeding Greek patients was found by others,[9] who also used the CLO test. Others reported significant false-negative rates in patients with duodenal ulcer bleeding when the rapid urease test was used for the detection of *H. pylori*.[10,11]

Thus, the present prospective study was undertaken to evaluate the validity of the CLO test in comparison with histology examination, to diagnose *H. pylori* infection in patients with non-variceal upper gastrointestinal bleeding (UGB) as they are usually seen in routine clinical practice (i.e. irrespective of the ingestion of non-steroidal anti-inflammatory drugs (NSAID)).

METHODS

In this prospective study, upper gastrointestinal endoscopies were performed on all patients with upper gastrointestinal UGB within 24 h of admission to the Department of Pathophysiology, Medical School, Athens, over a period of 12 months. Patients with variceal bleeding, patients who had undergone any type of gastric operation in the past, recent (less than 2 months') treatment with proton pump inhibitors and previous use of any *H. pylori* eradication therapy were excluded. Before endoscopy, the stomach was washed out with normal saline through a nasogastric tube. At least four biopsies (two from antral and two from the body mucosa) were obtained for the CLO test (Delta West Pty Ltd, Bentley, WA, Australia) and histological (modified Giemsa stain) diagnosis of *H. pylori* infection. One biopsy sample each from the antral and body mucosa were used for each test. The antral and body mucosal specimens were studied together in the same pellet for the CLO tests. Histological diagnosis was made by an experienced pathologist (PD) who was unaware of the results of the CLO test.

Blood in the stomach was defined as the presence of visible blood in the stomach (fresh blood or 'coffee ground' material).

Statistical analysis

The χ^2 and Fisher's exact tests were used, as appropriate, for the statistical analysis. A P value < 0.05 was considered to be significant.

RESULTS

Seventy-two consecutive patients (mean age 56.4 years, range 18–90 years; 51 men, 21 women) were included in the study. Aspirin and non-aspirin NSAID use before bleeding was reported by 46 patients (64%), 24 patients (33%) were smokers and 12 patients (17%) were heavy drinkers (consumption of more than approximately 60 g alcohol per day). Duodenal ulcers only (DU) were found in 42 patients (58%); a gastric ulcer (GU) and/or gastric erosions were found in 18 (25%), while 12 patients (17%) had a mixed pattern of lesions, consisting of gastric and duodenal pathology. Visible blood in the stomach (fresh blood or 'coffee ground' material) was found in 16 patients (22%).

RESULTS

Table 1 shows the number of patients who were found to have *H. pylori* infection by the CLO and histology tests. From the results of the CLO test, 32/72 patients (44%) were found to be *H. pylori* positive, while 44/72 (61%) were found to be positive by histology. The difference in detection between the two tests was significant (χ^2 $P=0.045$, 95% confidence interval (CI) 0.004–0.331).

Thirty patients were positive and 26 were negative by both methods; two patients were *H. pylori* positive by CLO (CLO (+)) and negative by histology (histology (–)), while 14 were *H. pylori* negative by CLO (CLO (–)) and positive by histology (histology (+)). The sensitivity and specificity of the CLO test were 68 and 93%, respectively; the positive and negative predictive values of the CLO test were 94 and 65%, respectively.

Influence of blood in the stomach

The results of the CLO test and histology did not differ significantly ($P=0.471$) in patients who had blood in their stomach (Tables 1,2). Thus, eight of 16 patients (50%) were CLO (+) and 11/16 (69%) were histology (+). One patient was CLO (+), histology (–) and four were CLO (–), histology (+). Thus, in this group of patients, the sensitivity of the CLO test was estimated as 64% (Table 2).

Table 1 Number of patients found to be *Helicobacter pylori* positive by each method

	All patients ($n=72$)	Blood in the stomach ($n=16$) (22%)	NSAID users ($n=46$) (64%)
CLO (+)	32 (44)	8 (50)	19 (41)
Histology (+)	44 (61)	11 (69)	24 (52)
P	0.045	0.471	0.403

Figures in parentheses are percentages.

Table 2 Comparison of the rapid urease test (CLO) and histology with regard to the presence of visible blood in the stomach

	Blood ($n=16$)	P	No blood ($n=56$)	P
CLO (+)	8 (50)		24 (43)	
CLO (–)	8 (50)	0.471	32 (57)	0.089
Sensitivity of test	64%		70%	
Histology (+)	11 (69)		33 (59)	
Histology ( )	5 (31)		23 (41)	
Histology (+), CLO ( )	4 (25)		10 (18)	
Histology (–), CLO (+)	1 (6)		1 (2)	

Figures in parentheses are percentages. There was no significant difference in *Helicobacter pylori* positivity by the CLO test between those with or without visible blood.

Table 3 Comparison of the rapid urease test (CLO) and histology for *Helicobacter pylori* with regard to the use of non-steroidal anti-inflammatory drugs (NSAID)

	NSAID use ($n=46$)	P	No NSAID use ($n=26$)	P
CLO (+)	19 (41)		13 (50)	
CLO (–)	27 (59)	0.403	13 (50)	0.044
Sensitivity	71%		65%	
Histology (+)	24 (52)		20 (77)	0.047
Histology (–)	22 (48)		6 (23)	
Histology (+), CLO (–)	7 (15)		7 (27)	
Histology (–), CLO (+)	2 (4)		0	

Figures in parentheses are percentages.

In patients without blood in the stomach, 24/56 (43%) were CLO (+) and 33/56 (59%) were histology (+); the difference was not significant ($P=0.089$). One patient was CLO (+), histology (–) and 10 were CLO (–), histology (+). In this group of patients the sensitivity of the CLO test was estimated as 70%, which is not different from that in patients with blood in the stomach ($P=0.72$).

Influence of non-steroidal anti-inflammatory drug ingestion

Of the patients who reported ingestion of NSAID, 19/46 (41%) were CLO (+) and 24/46 (52%) were histology (+), but the difference was not significant ($P=0.403$, Table 1). Two patients were CLO (+), histology (–) and seven were CLO (–), histology (+). In this group of patients, the sensitivity of CLO test was estimated as 71%.

The results of histology differed between NSAID users and those who did not use NSAID ($P=0.047$, 95% CI 0.013–0.468; Table 3).

In the 26 patients who did not ingest NSAID, the following results were found: 13 patients (50%) were CLO (+) and 20 (77%) were histology (+). The difference in positive results between CLO and histology was significant (χ^2 test, $P=0.044$, 95% CI 0.008–0.573). No patient was CLO (+), histology (–) while seven were CLO (–), histology (+). In this group of patients, the sensitivity of the CLO test was estimated as 65%, which was not different from those that did not use NSAID ($P=0.75$).

Influence of age

The age of the patient does not seem to influence the results of the CLO test ($P=0.616$), even when comparing patients older or younger than 60 years ($P=0.477$, 95% CI –0.125–0.337 for CLO). It also should be pointed out that age of patient does not seem to influence the histology results either (Table 4).

In those older than 60 years ($n=38$), 23 were CLO (+) and 22 were histology (+); no patient was CLO (+), histology (–), while seven were CLO (–), histology (+). In patients younger than 60 years ($n=34$), 17 were CLO (+) and 22 were histology (+). Two patients were CLO (+), histology (–), while seven were CLO (–), histology (+). Thus, the sensitivity of the CLO test was estimated as 68% in both age groups.

DISCUSSION

It is generally accepted that, for routine clinical practice, the rapid urease test is the most useful tool for the diagnosis of *H. pylori* infection, because of its high sensitivity and specificity.[3,12] However, the diagnostic accuracy of RUT in cases of bleeding ulcers has been questioned. Lai *et al.* reported significant false-negative rates, not related to the severity of bleeding, in patients with bleeding DU and no history of NSAID ingestion;[10] Hawkey *et al.* reported that 23% of bleeding patients who were positive by serology had a negative CLO test that was not explained by recent drug use.[13]

Colin *et al.* questioned the accuracy of rapid urease tests, histology and culture as compared with serology in patients with bleeding ulcers irrespective of NSAID use.[14] They concluded that the decreased sensitivity of these biopsy based tests was associated with two factors: delay between the bleeding episode and biopsies with regard to rapid urease tests and culture, and the age of the patients with regard to rapid urease tests and histology. Sensitivity was not influenced by NSAID intake.

Some influence of age on *H. pylori* diagnosis by biopsy based methods sounds reasonable, given that the

Table 4 Results of the rapid urease test (CLO) and histology in various age groups

	Age group (years) (*n*)					
	18–29	30–39	40–49	50–59	60†–69	70+
CLO (+)*	4	4	1	8	9	6
CLO (–)	4	4	4	5	12	11
Histology (+)‡	5	6	1	10	14	8
Histology (–)	3	2	4	3	7	9
Histology (+), CLO (–)	2	2	0	3	5	2
Histology (–), CLO (+)	0	1	0	1	0	0

*There was no significant difference in the CLO test between the various age groups ($P = 0.616$). †There was no significant difference in the CLO test between those younger or older than 60 (Fisher's exact test; $P = 0.477$, 95% CI −0.125–0.337), CLO sensitivity = 68%. ‡There was no significant difference in the histology results between the various age groups, $P = 0.201$. There was no significant difference in histology between those younger or older than 60 (Fisher's exact test; $P = 0.631$, 95% CI: −0.164–0.307); CLO sensitivity = 68%.

older the patient, the greater the possibility of gastric atrophy and intestinal metaplasia, which reduces the sensitivity of the biopsy based methods.[15] However, we did not find any influence of age in the present study.

As far as serology is concerned, we have previously reported our results on the first 55 patients of this study, 29 (53%) of whom had taken NSAID.[16] *Helicobacter pylori* serology was performed by using a commercially available kit (*Helicobacter pylori* IgG, DIESSE Diagnostica Senese, Siena, Italy). The sensitivity and specificity of serology were 88 and 93%, respectively, by using histology samples prepared with a modified Giemsa stain as the gold standard. *Helicobacter pylori* positivity was found in 89% by serology (IgG+), 40% by the CLO test and in 56% by histology. It should also be mentioned that four serological tests to diagnose *H. pylori* infection in non-bleeding patients who were taking NSAID were shown to be of low sensitivity and, in particular, of low specificity in comparison with biopsy based tests and culture.[17]

For these reasons (i.e. the relatively low sensitivity and specificity of serology tests), we have not included our results of serology in the present study.

Lee *et al.* recently studied the influence of blood in the stomach on the diagnosis of *H. pylori* infection in 68 patients with bleeding peptic ulcer. Patients with recent (< 1 month) NSAID ingestion were excluded.[18] They found that all the biopsy based diagnostic methods had decreased sensitivity while the urea breath test and serology were positive in 95–92% and 100–98% in patients with or without blood in the stomach, respectively. They concluded that the presence of blood in the stomach was associated with decreased sensitivity of the biopsy based tests; in particular, the CLO test. Leung *et al.* recently suggested that the presence of blood adversely affects the performance of the rapid urease tests through the buffering effect of serum albumin on the pH indicator, rather than through a direct inhibition on the urease activity.[19]

However, our results did not support any influence of visible blood in the stomach on the sensitivity of the CLO test. It should be noted, however, that the stomach wash out prior to endoscopy may underestimate the prevalence of blood or 'coffee ground' material in the stomach and explain why we did not find any influence of visible blood on the sensitivity of the CLO test.

It has been reported that the sensitivity and specificity of the CLO test and histology samples after suitable staining are roughly equivalent.[20] In addition, we have previously shown that in a mixed population of non-bleeding patients with dyspepsia (duodenal ulcer, gastric ulcer, functional dyspepsia), there was no difference in the efficiency of the CLO test and histology (Giemsa stain) in diagnosing *H. pylori* infection.[21] However, the results of the present study clearly showed that in bleeding patients the CLO test had low sensitivity compared with histology in diagnosing *H. pylori* infection. This finding is in agreement with all the presented series, mostly in abstract form, until now. According to our results, the CLO test had low sensitivity in bleeding patients irrespective of NSAID use. However, histology samples had significantly more positive results in non-NSAID users (77%) than in NSAID users (52%). Indeed, it appears to be logical that patients with gastroduodenal lesions due to NSAID use would have a lower prevalence of *H. pylori* infection. Alternatively, this finding could be due to some influence of NSAID on *H. pylori*.

We can not offer any plausible explanation for the low sensitivity of CLO at present. It could be due to some changes in the microenvironment of *H. pylori* induced by the bleeding process *per se*, which may affect the ability of *H. pylori* to produce urease. Nevertheless, the fact remains that in bleeding patients seen in the clinical setting, irrespective of NSAID ingestion, the CLO test misrepresents the prevalence of *H. pylori* infection.

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REFERENCES

1 El-Zimaity HMT, Al-Assi MT, Genta RM, Graham DY. Confirmation of successful therapy of *Helicobacter pylori* infection: Number and site of biopsies or a rapid urease test. *Am. J. Gastroenterol.* 1995; **90**: 1962–4.

2 Marshall BJ, Warren JR, Francis GJ, Langton SR, Goodwin CS, Blincow ED. Rapid urease test in the management of *Campylobacter pylori* associated gastritis. *Am. J. Gastroenterol.* 1987; **82**: 200–10.

3 Cohen H, Laine L. Endoscopic methods for the diagnosis of *Helicobacter pylori*. *Aliment. Pharmacol. Ther.* 1997; **11** (Suppl. 1): 3–9.

4 Rokkas T, Karameris A, Mavrogeorgis A, Rallis E, Giannikos N. Eradication of *Helicobacter pylori* reduces the possibility of rebleeding in peptic ulcer disease. *Gastrointest. Endosc.* 1995; **41**: 1–4.

5 Jaspersen D, Koerner T, Schorr W, Brennenstuhl M, Roschka C, Hammar CH. *Helicobacter pylori* eradication reduces the rate of rebleeding in ulcer hemorrhage. *Gastrointest. Endosc.* 1995; **41**: 5–7.

6 Kung NNS, Sung JJY, Yuen NWF. Anti-*Helicobacter pylori* treatment in bleeding ulcers: Randomized controlled trial comparing 2-day versus 7-day bismuth quadruple therapy. *Am. J. Gastroenterol.* 1997; **92**: 438–41.

7 Graham DY, Lew GM, Malaty HM *et al.* Factors influencing the eradication of *Helicobacter pylori* with triple therapy. *Gastroenterology* 1992; **102**: 493–6.

8 Archimandritis A, Bitsikas J, Tjivras M *et al.* *Helicobacter pylori* infection in Greece in healthy people and in patients with peptic ulcer and with dyspepsia without ulcer. *J. Clin. Gastroenterol.* 1993; **16**: 257–8.

9 Tagaris A, Kamberoglou D, Tzitzi D, Doulgeroglou B, Tzias B, Lagoudakis M. *Helicobacter pylori* (HP), non-steroidal anti-inflammatory drugs (NSAIDs) and other risk factors in upper gastrointestinal hemorrhage. *Gastroenterology* 1995; **108**: A230.

10 Lai KC, Hui WM, Lam SK. Bleeding ulcers have high false negative rates for antral *Helicobacter pylori* when tested with urease test. *Gastroenterology* 1996; **110**: A167.

11 Buckley M, Lee J, O'Morain C. The problem ulcer; bleeding, perforation, *Helicobacter pylori*-negativity and intractability. In: Hunt RH, Tytgat GNJ, eds. Helicobacter pylori: *Basic Mechanisms to Clinical Cure 1996*, Dordrecht: Kluver Academic Publishers. 1996; 295–303.

12 Hunt RH, Malfertheiner P, Yeomans ND, Hawkey CJ, Howden CW. Critical issues in the pathophysiology and management of peptic ulcer disease. *Eur. J. Gastroenterol. Hepatol.* 1995; 7: 685–99.

13 Hawkey GM, Everitt S, Pearson GM, Stack WA, Hawkey CJ. Non-steroidal anti-inflammatory drugs and *Helicobacter pylori* as independent risk factors for peptic ulcer bleeding. *Gastroenterology* 1997; **112**: A144.

14 Colin R, Bigard MA, Notteghem B *et al.* Poor sensitivity of direct tests for detection of *Helicobacter pylori* on antral biopsies in bleeding ulcers. *Gastroenterology* 1997; **112**: A93.

15 Atherton JC. Non-endoscopic tests in the diagnosis of *Helicobacter pylori* infection. *Aliment. Pharmacol. Ther.* 1997; **11** (Suppl. 1): 11–20.

16 Archimandritis A, Tzivras M, Souyioultzis S *et al.* High rates of false negative rapid urease test (CLO) in patients with upper gastrointestinal bleeding (UGB). *Gut* 1997; **41** (Suppl. 1): A76 (Abstract).

17 Taha AS, Reid J, Boothmann P *et al.* Serological diagnosis of *Helicobacter pylori* evaluation of four tests in the presence or absence of non-steroidal anti-inflammatory drugs. *Gut* 1993; **34**: 461–5.

18 Lee CL, Tu TC, Yang RN *et al.* Does blood in the stomach influence the diagnosis of *H. pylori* infection in patients with bleeding peptic ulcer? *Gut* 1997; **41** (Suppl. 1): A76 (Abstract).

19 Leung WK, Sung JJ, Siu KL, Chan FK, Ling TK, Cheng AF. False-negative biopsy urease test in bleeding ulcers caused by the buffering effects of blood. *Am. J. Gastroenterol.* 1998; **93**: 1914–18.

20 Marshall BJ. *Helicobacter pylori*. *Am. J. Gastroenterol.* 1994; **89** (Suppl): S116–28.

21 Archimandritis A, Bitsikas J, Anastasakou E *et al.* *Helicobacter pylori* infection in healthy Greeks and patients with peptic ulcer and non-ulcer dyspepsia. *Hell. J. Gastroenterol.* 1992; **5**: 288–93.

Prospective evaluation of a whole-blood antibody test (FlexPack HP) for in-office diagnosis of *Helicobacter pylori* infection in untreated patients

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Objectives **Rapid, reliable in-office tests are needed for applying the adopted screen-and-treat strategy in *Helicobacter pylori*-positive young dyspeptic patients.**

Design **We have evaluated the performance characteristics of a whole-blood antibody (WBA) test for the detection of *H. pylori* infection under in-office conditions.**

Methods **In a prospective double-blind study, 183 untreated patients referred to a tertiary centre for endoscopy because of dyspepsia were studied. Patients were defined as *H. pylori*-positive if two out of three tests (histology, rapid urease test, Gram staining of biopsy smears) were positive, and *H. pylori*-negative if all three tests were negative. An in-office test detecting IgG antibodies to *H. pylori* (FlexPack HP, Abbott Diagnostics) was used with capillary blood and compared with an ELISA detecting IgG (quantitative) and IgA (qualitative) *H. pylori* serum antibodies.**

Results **Of the 183 patients, 139 were defined as *H. pylori*-positive. The in-office test had 79% sensitivity, 95% specificity, 98% positive and 59% negative predictive value. The respective values for IgG serum antibodies were 94, 70, 91 and 79% and those for IgA antibodies were 86, 82, 94 and 64%. About 50% of the false-negative in-office tests had a serum IgG antibody titre > 100 units. Co-evaluation of our data with published reports suggested that both the median sensitivity and negative predictive value of the kit are significantly inferior when performed with whole-blood (five studies) compared with serum (nine studies) (82 versus 92% and 82 versus 93% respectively, $P < 0.035$).**

Conclusions **Improvement of the performance characteristics of FlexPack HP in-office test is needed. However, the test may be a useful tool for identifying *H. pylori*-positive patients in younger age groups who could be managed without upper gastrointestinal endoscopy. *Eur J Gastroenterol Hepatol* 12:727–731 © 2000 Lippincott Williams & Wilkins**

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Introduction

Helicobacter pylori is the aetiological factor in chronic *H. pylori* gastritis, and is implicated in the pathogenesis of peptic ulcer disease [1,2]. Eradication of *H. pylori* infection results in resolution of gastritis and cure of peptic ulcer [3]. Detection of *H. pylori* infection may be performed by invasive methods such as endoscopy with biopsy culture, histology or rapid urease tests, or non-invasive techniques, such as the ^{13}C-urea breath test and serology [4].

According to the Maastricht (Europe) and the National Institute of Health (USA) consensus recommendations [5,6], *H. pylori*-infected dyspeptic patients younger than 45 years, without alarming symptoms, such as weight loss, dysphagia and anaemia, may be managed without upper gastrointestinal endoscopy. This strategy is cost-effective, but it depends on the accuracy of the diagnostic method used to detect *H. pylori* infection. Several commercially available enzyme-linked immunosorbent assay (ELISA) kits that detect anti-IgG and/or IgA antibodies quantitatively or qualitatively have been used over the last decade [7–9]. These tests are simple, quick and of low cost, but are performed in a laboratory. Recently, whole-blood *H. pylori* antibody (WBA) tests have been developed, which give the result within a few minutes [7,8]. They are performed by the physician in the office and are called 'in-office tests' or 'near the patient tests'. However, the European *Helicobacter pylori* Study Group has suggested

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that 'Rapid (office) serological test kits at present need further validation' [10].

FlexPack HP (Abbott Diagnostics, Illinois, USA) is a rapid WBA test [11,12]. It is a one-step test but its sensitivity and specificity have not been adequately evaluated under in-office conditions [13,14]. We have therefore prospectively evaluated the performance characteristics of FlexPack HP and compared it with that of an ELISA detecting IgG (quantitative) and IgA (qualitative) *H. pylori* serum antibodies.

Methods

This prospective double-blind study included patients referred to a tertiary centre for endoscopy because of dyspepsia. None of the patients had had *H. pylori* eradication therapy in the past and none had received antibiotics in the two months before the study. Patients with gastric surgery and those on acid suppression therapy were excluded. The protocol of the study was approved by the Ethical Committee on Human Studies, Department of Internal Medicine, University of Athens, in March 1997.

Study design

Before endoscopy, 10 ml of venous blood was obtained, centrifuged at 3000 *g* for 15 min and the serum stored at −20 °C until analysis for *H. pylori* anti-IgG and IgA antibodies (Milenia *H. pylori*, IgG, IgA, DPC, Biermann GmbH, Bad Nauheim, Germany). Capillary blood was then obtained by finger pricking into a capillary tube and whole-blood IgG antibodies to *H. pylori* were determined by FlexPack HP by independent personnel according to the manufacturer's instructions. A positive test was recorded if two pink lines (i.e. test and control) were visible, and negative if only the control line was observed in the viewing window.

During endoscopy, five gastric mucosa biopsies were obtained using Olympus FB-24Q biopsy forceps. One biopsy from the lesser curvature was used to perform a rapid urease test (CLO test, Delta West PTCL, Bentley, Western Australia). The result of the CLO test was read at 2 h by a single trained observer who was blinded to the endoscopic findings and the results of the in-office test.

Two biopsies from the antrum were separately crushed between two microscope slides, so that they produced four biopsy smears. All four smears were Gram-stained and evaluated by a bacteriologist for the presence of spiral Gram-negative bacteria. The bacteriologist was unaware of the results of the other tests. The remaining two biopsies, one from the lesser curvature of the antrum and the second from the gastric body, were immersed in formalin and sent to the pathology department. Modified Giemsa staining was used to detect *H. pylori* in biopsy specimens by a pathologist who was blinded to the results of the other *H. pylori*-detecting tests.

Serum IgG and IgA antibodies were measured by a bacteriologist who was unaware of the results of the other tests. *H. pylori* serum IgG antibody titres were determined by an enzyme immunoassay (Milenia *H. pylori* IgG, DPC Biermann GmbH, Germany) [15] according to the manufacturer's instructions. ELISA units were calculated from a standard curve. A cut-off value of ≥ 44 units/ml defined a positive and ≤ 36 units/ml a negative test. *H. pylori* serum IgA antibodies were detected qualitatively using an enzyme immunoassay (Milenia *H. pylori* IgA, DPC Biermann GmbH, Germany). Evaluation of the *H. pylori* IgA assay was carried out by direct comparison of the optical density (OD) of each patient serum sample with the OD of the controls. A sample was considered positive if its respective OD was 10% higher and negative if the OD was 10% lower compared with the OD of the reference serum.

A patient was considered *H. pylori*-positive if at least two out of the three tests, i.e. CLO test, histology and biopsy smears, were positive and *H. pylori*-negative if all three tests were negative. Patients for whom only one test was positive were excluded from further analysis.

Statistical analysis

The sensitivity, specificity, predictive values and diagnostic accuracy were determined for the whole-blood in-office test and the serum ELISA (IgG, IgA) tests, by using the combined results of the invasive tests (CLO test, histology, biopsy smears) as the gold standard. The positive predictive value (PPV) was calculated as the proportion of true-positive tests among all positive tests. The negative predictive value (NPV) was calculated as the proportion of true-negative tests among all negative tests. Diagnostic accuracy was calculated as the percentage of the patients who were correctly scored (true-positive plus true-negative) among all patients tested. Results were stored using dBASE software (Microsoft Access 97, Microsoft Corp., Seattle, Washington State, USA) and analysed by the statistical package Statgraphics Plus 2.1 (Manugistics Inc., Statistical Graphics Corp., Rockville, MD, USA). Qualitative data were assessed by the χ^2 test with Yates' correction, as appropriate. Numerical data were analysed by the non-parametric Mann–Whitney (Wilcoxon) two-sided *U* test [16]. A P value < 0.05 was regarded as significant.

Results

One hundred and eighty-nine consecutive patients were studied. Four patients were excluded because

only one out of the three tests used for detection of *H. pylori* infection (CLO test, histology, biopsy smears) was positive. Two additional patients were also excluded because of borderline (36–44 U/ml) IgG ELISA titre, leaving 183 remaining patients (121 men and 62 women, median age 48 years, range 17–76) who fulfilled the inclusion criteria used in this study. Ninety-nine patients had duodenal ulcer, 16 gastric ulcer and 68 non-ulcer dyspepsia. Seventy-nine patients (55 men, 24 women) were younger than 45 years (median 35, range 17–45), including 37 patients with duodenal ulcer and 42 with non-ulcer dyspepsia. Overall 139 (77%) patients were defined as *H. pylori*-positive and 44 as *H. pylori*-negative, according to the combined results of the CLO test, histology and microscopy (gold standard).

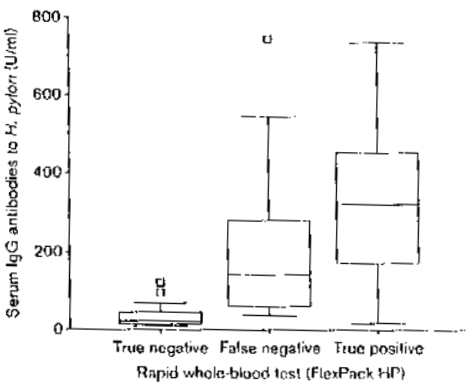
Of the 139 *H. pylori*-positive patients, the in-office test identified 110, IgG ELISA 131 and IgA ELISA 119 patients. Of the 44 gold standard *H. pylori*-negative patients, the in-office test identified 42, IgG ELISA 31 and IgA ELISA 36 patients. Two *H. pylori*-negative patients were falsely identified as positive by the in-office test, 13 by IgG ELISA and eight by IgA ELISA. Twenty-nine *H. pylori*-positive patients were falsely identified as negative by the in-office test, eight by IgG ELISA and 20 by IgA ELISA. The sensitivity, specificity, PPV, NPV and overall diagnostic accuracy of the in-office test (FlexPack HP) and those of the serum IgA and IgG antibodies (ELISA) to *H. pylori* are shown in Table 1. There were no statistically significant differences in the performance characteristics of the WBA in-office test, serum IgG or IgA antibodies to *H. pylori* between the total group studied and the subgroup of patients who were younger than 45 years. However, the in-office test had a significantly lower sensitivity ($\chi^2 = 12.47$, $P < 0.001$) but higher specificity ($\chi^2 = 8.04$, $P < 0.004$) compared to ELISA IgG antibodies to *H. pylori*. All tests had an overall diagnostic accuracy < 90%.

The WBA in-office test had a high PPV (98%), but a low NPV (59%). To investigate whether this low NPV was related to a low IgG antibody titre to *H. pylori*, we compared serum IgG titre among true-positive, true-negative and false-negative WBA in-office tests. Fig. 1 shows that about 50% of the false-negative in-office tests had serum IgG antibody titre to *H. pylori* > 100 ELISA units.

Performance characteristics of FlexPack in serum and blood

A Medline search identified 11 studies evaluating performance characteristics of the FlexPack HP (FlexSure HP) kit. Eight of these studies used patients' serum [9,11–13,17–20] and five, including the present one, used whole blood [13,14,21,22]. Table 2 shows the calculated median values for sensitivity, specificity,

Fig. 1



Box-and-whisker plots of serum *H. pylori* IgG antibody titre of patients who were true-positives, true-negatives and false-negatives by the rapid whole-blood antibody (WBA) in-office test. The box includes 50% of the results (i.e. those falling between the 25th and 75th percentiles = the interquartile distance). The median value is represented as a horizontal line inside the box. Outliers, i.e. points more than 1.5 times the interquartile range from the end of the box, are shown as open squares. It is evident that in about 50% of the false-negative in-office tests, serum IgG antibody titres (ELISA) were greater than 100 units.

Table 1 **Comparison of the whole-blood antibody in-office test (FlexPack HP) and enzyme-linked immunosorbent assay (Milenia IgG, IgA) for serodiagnosis of *Helicobacter pylori* infection**

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	ODA (%)
All patients					
FlexPack HP	79 (73–85)*	95 (92–98)**	98 (96–100)	59 (52–66)	83 (78–88)
Milenia IgA	86 (81–91)	82 (76–88)	94 (91–97)	64 (57–71)	85 (80–90)
Milenia IgG	94 (91–97)*	70 (63–77)**	91 (87–95)	79 (73–85)	89 (85–94)
Patients younger than 45 years					
FlexPack HP	79 (70–88)	97 (93–100)	97 (93–100)	75 (65–85)	86 (78–94)
Milenia IgA	75 (65–85)	77 (68–86)	84 (76–92)	67 (57–77)	76 (67–86)
Milenia IgG	96 (92–100)	74 (64–84)	85 (77–93)	92 (86–98)	87 (80–95)

Median values are presented with 95% confidence intervals. A combination of CLO test, histology and biopsy smears was used as the gold standard to define *H. pylori*-positive or -negative patients. PPV, positive predictive value; NPV, negative predictive value; ODA, overall diagnostic accuracy; * $P < 0.001$, ** $P < 0.004$.

Table 2 **Performance characteristics of FlexPack HP (FlexSure HP) kit**

	Total number of patients	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Serum	1983	92 (74–96)	93 (74–98)	89 (75–97)	93 (77–95)
Whole blood	962	82 (76–84)	79 (52–95)	80 (69–98)	82 (59–84)
Statistical difference[1]		$U = 5$, $P = 0.023$	$U = 10$, $P = 0.11$	$U = 9$, $P = 0.27$	$U = 3$, $P = 0.033$

Values (median and range) have been calculated from eight studies [9,11–13,17–20] using patients' serum and five studies [13,14,21,22], including the present one, using whole blood in-office.
PPV, positive predictive value; NPV, negative predictive value.
[1]Mann–Whitney (Wilcoxon) two-sided U test.

PPV and NPV of the kit when it was used with serum or whole blood. Comparison of the median performance characteristics of the test between serum and blood showed that the sensitivity and NPV of the kit were significantly higher in serum than in whole-blood samples ($P = 0.023$ and $P = 0.033$, respectively).

Discussion

H. pylori has had a major impact on clinical practice. Guidelines for management of *H. pylori* infection have been published in Europe (the Maastricht Consensus Report) and in the USA (National Institutes of Health Consensus Statement) [5,6]. These guidelines adopt the screen-and-treat strategy for young dyspeptic patients with *H. pylori* infection. Avoiding endoscopy in those who are *H. pylori*-positive, but without alarming symptoms, is a cost-effective approach, as it may reduce endoscopy workload without missing significant disease. These young (< 45 years) *H. pylori*-positive patients may be treated by primary care physicians with highly effective *H. pylori* eradication therapies and avoid hospital and specialist referrals.

Currently available non-invasive methods to screen dyspeptic patients for *H. pylori* infection are the ^{13}C-urea breath test, detection of *H. pylori* antibodies in the laboratory by ELISA, *H. pylori* antigen detection in stool specimens, and office-based (in-office) rapid whole-blood *H. pylori* antibody tests. The latter incorporate high-molecular weight cell-associated proteins (HM-CAP), which are highly specific for *H. pylori* IgG antibodies [12]. They do not require laboratory facilities, may be used in primary care settings by the primary care physician, and the result is read within 5 min [11,12].

In the present study, we used the Milenia ELISA kit to detect IgG antibodies to *H. pylori* and compare it with the in-office test. Medline search identified only one paper (in Spanish) evaluating the performance characteristics of the Milenia ELISA test [23]. In that study, the sensitivity, specificity, PPV and NPV were lower compared with our data. However, the problem of poor test performance, and low specificity in particular, may arise when a test that was developed and validated in one population is used in a different population with different strains of *H. pylori*. In our hands, the kit had overall performance characteristics within the range reported for other commercially available kits [7]. It is also important to note that the specificity of the IgG ELISA kits is usually lower than the respective sensitivity, because patients *H. pylori*-positive by serology may be negative by assessment of biopsies, due to a prior infection.

The most widely available tested in-office kit is FlexSure HP (Smith Kline) which has been replaced by FlexPack HP (Abbott). Eight published reports have evaluated the performance characteristics of FlexSure or FlexPack rapid in-office tests, using patients' serum [9,11–13,17–20] instead of whole blood. They report on nine groups including a total of 1983 patients (Table 2). In most of these publications, the test was performed in a laboratory [9,11,12,17], probably by experienced personnel.

Only four publications have tested this kit with whole-blood under real in-office conditions [13,14,21,22]. In the study by Sadowski *et al.* [13], five North American centres participated, with a total of 393 patients. *H. pylori*-positive patients were defined by histology or a rapid urease test (gold standard). The kit (FlexSure HP) had a sensitivity of 84%, specificity of 74%, PPV of 74%, NPV of 84% and overall accuracy of 79%. The authors commented that the in-office test has significantly lower sensitivity than the HM-CAP ELISA. Leung *et al.* investigated 161 Chinese patients [14]. Histology, rapid urease test and ^{13}C-urea breath test were used as gold standard. The sensitivity of FlexPack HP was 82%, specificity 84%, PPV 86% and NPV 79%. In this study, the authors conclude that the low sensitivity of the kit makes it far from ideal for in-office patient testing. Shirin *et al.* [21] studied 94 consecutive symptomatic patients in Israel. Histology and the rapid urease test were used as the gold standard. The kit (FlexPack) had a sensitivity of 84%, but relatively low specificity (52%) when compared with the gold standard, limiting its use in the population studied. Finally, Chey *et al.* [22] studied 131 patients using histology as the gold standard. FlexPack had a rather low sensitivity of 76% and specificity of 79%.

Our data on FlexPack performance characteristics are comparable to those of published reports [13,14,21,22]. Comparison of the median performance characteristics between serum and whole blood indicates that when whole blood is used the sensitivity and NPV of the kit are significantly inferior to the results obtained with serum samples (Table 2). When whole-blood is used, the kit has an unacceptably low NPV (median 82%, range 59–84%). As the data in Fig. 1 show, the results of the kit were falsely negative in many patients with an IgG serum antibody titre much greater than 100 ELISA units.

The reason for the unacceptably low NPV of the kit is unclear, and our study was not designed to answer this question. However, we speculate that the discrepancy between serum and whole-blood results may be because the latter are performed by clinicians and not by experienced laboratory personnel. Despite the fact that Sadowski *et al.* did not find any significant difference between FlexSure HP serum and whole-blood results [13], clinicians may misinterpret cases with poor readability manifested as a faintly positive result. The low sensitivity of FlexPack HP when it is used with whole blood is clearly related to the low NPV of the kit. However, this may not be due to inadequate kit performance, but may be related to challenges associated with the collection of capillary blood from fingerstick, such as proper preparation of the puncture site and speed of sample collection to avoid coagulation [13].

Despite the fact that the overall performance characteristics of the FlexPack HP test are not ideal, as we have shown, the PPV of the test is high. If the major purpose of in-office testing is to eliminate the need for endoscopy in young *H. pylori*-positive dyspeptic patients, then patients identified as *H. pylori*-positive by the test could reliably be assumed to be truly positive. They could thus be treated for the infection without endoscopy. Only a very small proportion of negative patients will falsely test positive and so not have the endoscopy that would otherwise be indicated. The larger numbers of infected patients who falsely test negative would simply undergo endoscopy as normal and would not be compromised. Missing positive patients would only come to harm if they then missed out on appropriate therapy.

We conclude that more accurate office-based tests are needed. However, in-office tests can be used to serve the screen-and-treat strategy for *H. pylori* infection, which has been adopted for the management of dyspepsia [5,6], as they have a high PPV, i.e. they reliably detect *H. pylori*-infected patients.

References

1 Dixon MF. *Helicobacter pylori* and peptic ulceration: histopathological aspects. *J Gastroenterol Hepatol* 1991; 6:125–130.
2 Marshall BJ. *Helicobacter pylori*. *Am J Gastroenterol* 1994; 89(suppl 8):S116–S128.
3 Hopkins RJ, Girardi LS, Turney EA. Relationship between *Helicobacter pylori* eradication and reduced duodenal and gastric ulcer recurrence: a review. *Gastroenterology* 1996; 110:1244–1252.
4 Van Zwet AA, Megraud F. The year in *Helicobacter pylori* 1998. Diagnosis. *Curr Opin Gastroenterology* 1998; 14(suppl 1):S27–S33.
5 European *Helicobacter pylori* Study Group. Current European concepts in the management of *Helicobacter pylori* infection. The Maastricht consensus report. *Gut* 1997; 41:8–13.
6 American Gastroenterological Association. American Gastroenterological Association medical position statement: evaluation of dyspepsia. *Gastroenterology* 1998; 114:579–581.
7 Vaira D, Holton J, Menegatti M, Landi F, Ricci C, Ali' A, *et al.* Blood tests in the management of *Helicobacter pylori* infection. *Gut* 1998; 43(suppl 1):S39–S46.
8 Megraud F. Diagnosis and candidates for treatment of *Helicobacter pylori* infection. *Gastroenterology* 1997; 113(suppl 6):S93–S98.
9 Meijer BC, This JC, Kleibeuker JH, van Zwet AA, Berrelkamp RJP. Evaluation of eight enzyme immunoassays for detection of immunoglobulin G against *Helicobacter pylori*. *J Clin Microbiol* 1997; 35:292–294.
10 Malfertheiner P, Megraud F, O'Morain C, Bell D, Bianchi Porro G, Dellenre M, *et al.* Current European concepts in the management of *Helicobacter pylori* infection – the Maastricht consensus report. *Eur J Gastroenterol Hepatol* 1997; 9:1–2.
11 Sharma TK, Young EL, Miller S, Cutler AF. Evaluation of a rapid, new method for detecting serum IgG antibodies to *Helicobacter pylori*. *Clin Chem* 1997; 43:832–836.
12 Schrier WH, Schoengold RJ, Baker JT, Norell JL, Jaseph CL, Okin Y, *et al.* Development of FlexSure HP – an immunochromatographic method to detect antibodies against *Helicobacter pylori*. *Clin Chem* 1998; 44:293–298.
13 Sadowski D, Cohen H, Laine L, Greenberg P, Goldstein J, Mihalov M, *et al.* Evaluation of the FlexSure HP whole blood antibody test for diagnosis of *Helicobacter pylori* infection. *Am J Gastroenterol* 1998; 93:2119–2123.
14 Leung WK, Chan FK, Falk MS, Suen R, Sung JJ. Comparison of two rapid whole-blood tests for *Helicobacter pylori* infection in Chinese patients. *J Clin Microbiol* 1998; 36:3441–3442.
15 Gerstenecker B, Bode G, Nilius W, Ghadessi M, El Shassi S. Two immunoassays for detecting *H. pylori* IgG [Abstract]. *Clin Microbiol Infect* 1999; 5 (suppl 3):263.
16 Armitage P, Berry G. Distribution free methods. In: *Statistical Methods in Medical Research*, 2nd edn. London: Blackwell; 1987. pp. 406–422.
17 Graham DY, Evans DJ, Peacock J, Baker JT, Schrier WH. Comparison of rapid serological tests (FlexSure HP and Quick Vue) with conventional ELISA for detection of *Helicobacter pylori* infection. *Am J Gastroenterol* 1996; 91:942–948.
18 Faigel DO, Childs M, Furth EE, Alavi A, Metz DC. New noninvasive tests for *Helicobacter pylori* gastritis. Comparison with tissue-based gold standard. *Dig Dis Sci* 1996; 41:740–746.
19 Harrison JR, Bevan J, Furth EE, Metz DC. AccuStat blood finger stick test for *Helicobacter pylori* infection: a reliable screening method. *J Clin Gastroenterol* 1998; 27:50–53.
20 Kroser JA, Faigel DO, Furth EE, Metz DC. Comparison of rapid office-based serology with formal laboratory-based ELISA testing for diagnosis of *Helicobacter pylori* gastritis. *Dig Dis Sci* 1998; 43:103–108.
21 Shirin H, Bruck R, Kenet G, Krepel Z, Wardi Y, Rgif S, *et al.* Evaluation of a new immunochromatographic test for *Helicobacter pylori* IgG antibodies in elderly symptomatic patients. *J Gastroenterol* 1999; 34:7–10.
22 Chey WD, Murthy U, Shaw S, Zawadski A, Montague J, Linscheer W, *et al.* A comparison of three fingerstick, whole blood antibody tests for *Helicobacter pylori*: a United States, multicenter trial. *Am J Gastroenterol* 1999; 94:1512–1516.
23 Junical AR, Garea MT, Cid A, Penalver MD, Vazquez M, Montero E, *et al.* Assessment of 4 commercial immunoenzymatic assays for the serodiagnosis of *Helicobacter pylori* infection. *Enferm Infecc Microbiol Clin* 1998; 16:185–189.

Gastric cancer

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Helicobacter pylori (Hp) is now well established as having a role in the aetiology of gastric cancer and gastric mucosa-associated lymphoid tissue (MALT)-lymphoma. Research workers over the year have been chipping away at its role in the pathogenesis of the two conditions. Most work is focused on the virulence factors that can be shown to be associated with cancer or pre-cancerous changes, the main data derived from epidemiological, molecular biological and immunological studies. The important contributions of the year are outlined in this review, but with each advance it can be appreciated how complex and multifactorial the malignant process is. Hp is one player in a large cast of which host genetics is beginning to emerge as an important determinant.

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Abbreviations

EGF	epidermal growth factor
MALT	mucosa-associated lymphoid tissue
ODC	Ornithine decarboxylase

Introduction

This review is structured around the year's contributions concerning Hp and gastric neoplasms categorized under the broad headings of epidemiological, pathological, those related to virulence factors, molecular alterations and host genetics. The final section deals with contributions connecting Hp and MALT-lymphoma and the factors that might play a role in the transformation of Hp-associated MALT to MALT lymphoma.

Epidemiology

Epidemiological studies on gastric cancer over the year have looked at various aspects of the association with *Helicobacter pylori* (Hp). Wong *et al.* [1•] compared two populations in China, one with a high mortality from gastric cancer in Changle, versus one in Hong Kong with 10% the rate of disease. Of the symptomatic subjects in Changle 76% were positive for anti-CagA antibodies compared to 28% in Hong Kong ($P < 0.0001$). Eslick *et al.* [2••] carried out a meta-analysis on observational epidemiological studies. Amongst the 42 studies that met the criteria there was a two-fold increase in the risk of gastric cancer if patients were infected with Hp. The more competent studies supported this concept whilst in the weaker studies the association was less definitive.

Two papers by Brenner *et al.* [3••,4] investigated Hp and a family history of gastric cancer. In one [3••] a positive family history and infection with a CagA-positive strain produced a > 8-fold total risk of gastric cancer. This combination was useful for targeting high risk groups. The other study [4], based on sociodemographic-derived data from standard interviews and IgG-antibodies to Hp, suggested familial aggregation of gastric cancer might reflect the familial clustering of Hp.

The Canadian aboriginal communities were the subject of a study by Bernstein *et al.* [5] who compared the aboriginal Indian Manetoba community with the non-Indian population. They looked at demographics, CagA status and PCR for Hp in the water supply. The gastric cancer rates were similar although the peptic ulcer rates were higher in the Indian population. The local lake water was free of Hp DNA. In the prospective sero-epidemiological study by Grimley *et al.* [6] of a UK population in Coventry, in which antibodies to CagA and VacA antigens were documented in patient groups with duodenal ulcer, gastric cancer, oesophageal cancer and normal controls, those with duodenal ulcers and gastric cancer had similar profiles for these Hp-associated antibodies. This indicated virulent Hp were involved in the pathogenesis of both diseases and that antibodies to VacA could be used to identify patients at increased risk of Hp-associated disease.

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In Brouet *et al.*'s study [7] correlating cumulative gastric cancer mortality to the nationwide prevalence of Hp infection amongst 1586 patients, they found the mortality rate varied from 34.4–51.8 per 10^5 of the population. They suggested that Hp infection only explained 5% of the variability in the gastric cancer mortality and a number of biases which were difficult to control could explain the lack of association between the variables.

Through the year the majority of epidemiological work substantiated the role of Hp in the pathogenesis of gastric cancer. However, the relationship is clearly not simple and the negative studies reflect this.

Pathology

In the search to identify the factors that promote gastric carcinogenesis several pathological studies have appeared during the year. Xia *et al.*[8] in a morphological study observed antralization of the incisura was associated with an increased risk of atrophic gastritis and intestinal metaplasia. Both are important changes in the morphological multi-step process towards gastric cancer and, in intestinal-pattern gastric cancer, believed to precede dysplasia. Many studies have looked at the role of hyperproliferation in the evolution of gastric cancer. Coyle *et al.* [9] have shown that mucosal levels of epidermal growth factor (EGF), a potent epithelial mitogen and oncoprotein, with its receptor EGFR, were increased nearly two-fold in Hp-infected patients compared to controls. Fox *et al.* [10••] looked at the role of a high salt diet in a mouse gastritis model. High salt diets are known to induce gastritis and promote the effect of certain carcinogens. They concluded that an elevated salt diet enhanced Hp colonization and potentiated carcinogenesis via increased proliferation in the gastric pits and by induced glandular atrophy. Nardone *et al.* [11••] demonstrated that irrespective of Hp status in chronic gastritis there was mucosal hyperproliferation. In addition, in a subset with Hp-associated chronic atrophic gastritis, abnormalities in DNA content, c-Myc and p53 were identified along with atrophy and metaplasia. All were reversed by Hp eradication. Intestinal metaplasia type-III is considered to be a pre-cancerous lesion. However, in a study of a Saudi Arabian population, where the prevalence of gastric cancer is low, Al-Knawy *et al.* [12] showed that there was no significant relationship between a high rate of Hp infection and metaplasia in general and metaplasia type-III in particular. They postulate this absence of relationship may account for the low incidence of gastric cancer in their particular population.

Virulence factors

Contrary to many previous investigators, Kikuchi *et al.* [13] found that at least for young adults, there was little difference in the overall odds ratio for gastric cancer between Hp+/CagA– and Hp+/CagA+ groups. They concluded that both CagA+ and CagA– Hp infections were related to an increased risk of both intestinal and diffuse, early, advanced and distal gastric cancers. By contrast, although using a different study design, Rugge *et al.* [14•] studying gastric cancer in an Italian population aged < 40 years identified an actiological role for Hp infection and CagA+ status for both diffuse and intestinal pattern malignancies.

Molecular alterations

In the gastric mucosa, the size of a continuously renewed population of cells is determined by the rates of production and loss. Ornithine decarboxylase (ODC) is elevated in various gastrointestinal cancers, it being a marker of mucosal proliferative activity. Apoptosis is a marker of cell loss. Both proliferation and loss have important roles in Hp-associated gastric carcinogenesis. Hirasawa *et al.* [15] demonstrated that the eradication of Hp decreased mucosal ODC activity and increased apoptosis. This study lends support to the concepts that Hp eradication helps decrease risk factors in the development of gastric cancer.

Peek *et al.* [16•] working on human adenocarcinoma gastric cell lines cultured in the presence of certain Hp strains and CagA–, picB–, VacA– derivatives, demonstrated diminished AGS cell viability, progression of the cell cycle from G1 to G2–M and enhanced apoptosis was associated with *cagA*+ strains. This depended on expression of VacA and genes within the *cagA* pathogenicity islands.

In another molecular study Murakami *et al.* [17], via direct DNA sequencing methods of p53 exons, showed that Hp can induce p53 point mutations and is consequently likely to be involved in the dysplasia–carcinoma pathway.

Host genetic factors

It is accepted that host genetic factors are important in the development of gastric cancer and the impact of Hp infection. In the case–control study by Meining *et al.* [18••] it was shown that the grade of Hp gastritis in relatives of gastric cancer patients is significantly higher than in controls. This is indicative of a likely genetic susceptibility influencing the expression of Hp gastritis.

Genetic susceptibility was also a feature of the work by El-Omar *et al.* [19••]. They showed that host genetic factors that affect interleukin-1-beta expression increased the risk of both hypochlorhydria induced by Hp and gastric cancer. Interleukin-1-beta, is a pro-inflammatory cytokine and an inhibitor of gastric acid secretion. Interleukin-1 polymorphisms may determine why some Hp-infected individuals develop cancer and others do not.

MALT

Banerjee *et al.* [20•] investigated cell regulatory proteins and apoptosis that could be involved in the transformation of MALT to lymphoma under the influence of Hp. They investigated PCNA, Cdc2/Cdk1 and cyclin B1 as well as measuring the apoptotic index. The findings allowed them to suggest that a high labelling index for Cdc2/Cdk1 and cyclin B1 coupled with a low index of apoptosis in Hp-associated MALT may identify any at-risk group.

Chang *et al.*[21••] tried to identify specific antigens of Hp that might be associated with the transformation of MALT to lymphoma using Western blot techniques. A possible marker was truncated FldA and antibodies to this antigen might be a useful indicator of the potential for transformation.

It is known that neoplastic B-cells of Hp MALT lymphoma are T-helper cell-dependent and sensitive to T-cell withdrawal. D'Elios *et al.* [22••] compared clonal progeny of T-cells from patients with Hp-associated MALT lymphoma with those from patients having chronic gastritis associated solely with Hp. They looked at Hp specificity, cytokine profile, and perforin- or Fas-mediated regulation of B-cell proliferation. Their findings showed the genesis of low grade gastric MALT lymphoma could be related to T-cell dependent B-cell activation and deficient cytotoxic control of B-cell growth. Hp however may not be mandatory for the progression of low grade to high grade MALT lymphoma as Hp had a low frequency in advanced high grade lesions according to Bouzourene *et al.* [23].

The precursor of MALT lymphomas is Hp-induced MALT and Mazzucchelli *et al.* [24••] demonstrated the B-cell attractant cytokines BCA-1 and SLC are induced in Hp gastritis. One problem in studying MALT and the development of MALT lymphoma has been the presence of clonal B-cell populations in reactive MALT. Saxena *et al.* [25] found that in reactive MALT clonal bands were often associated with polyclonal smears and were not reproducible in deeper sections. In MALT lymphoma however there was no association with a background smear and there was reproducibility in deeper sections. This lack of reproducibility was a useful feature to help delineate a malignant from a reactive process.

The year also saw two interesting case reports related to treatment of MALT lymphoma. In one report [26], the eradication of Hp produced regression of synchronous lesions, one of which was colonic. The other report [27] was of regression of high grade MALT lymphoma following eradication of Hp organisms.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Wong BC, Lam SK, Ching CK, Hu WH, Ong LY, Chen BW, *et al.*
• Seroprevalence of cytotoxin-associated gene A positive *Helicobacter pylori* strains in Changle, an area with very high prevalence of gastric cancer in south China. *Aliment Pharmacol Ther* 1999; 13:1295–302.

An interesting study on the seroprevalence of *cagA(+) H. pylori* strains in an area with very high prevalence of gastric cancer in China.

2 Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of *Helicobacter*
•• *pylori* infection with gastric carcinoma: a meta-analysis. *Am J Gastroenterol* 1999; 94:2373–2379.

An important meta-analysis showing that *H. pylori* infection constitutes a risk factor for gastric cancer.

3 Brenner H, Arndt V, Sturmer T, Stegmaier C, Ziegler H, Dhom G. Individual
•• and joint contribution of family history and *Helicobacter pylori* infection to the risk of gastric carcinoma. *Cancer* 2000; 88:274–279.

This important study examines the significance of the individual and joint contribution of family history and *H. pylori* infection to the risk of gastric carcinoma.

4 Brenner H, Bode G, Boeing H. *Helicobacter pylori* infection among offspring of patients with stomach cancer. *Gastroenterolgy* 2000; 118:31–35.

5 Bernstein CN, McKeown I, Embil JM, Blanchard JF, Dawood M, Kaban A, *et al.* Seroprevalence of *Helicobacter pylori*, incidence of gastric cancer, and peptic ulcer-associated hospitalizations in a Canadian Indian population. *Dig Dis Sci* 1999; 44:668–674.

6 Grimley CE, Holder RL, Loft DE, Morris A, Nwokolo CU. *Helicobacter pylori*-associated antibodies in patients with duodenal ulcer, gastric and oesophageal adenocarcinoma. *Eur J Gastroenterol Hepatol* 1999; 11:503–509.

7 Broutet N, Sarasqueta AM, Cantet F, Lethuaire D, Megraud F. Is there a link between the variation in gastric cancer mortality and differences in *Helicobacter pylori* prevalence in different regions of France? *Gastroenterol Clin Biol* 1998; 23:754–760.

8 Xia HH, Kalantar JS, Talley NJ, Wyatt JM, Adams S, Chueng K, Mitchell HM. Antral-type mucosa in the gastric incisura, body, and fundus (antralization): a link between *Helicobacter pylori* infection and intestinal metaplasia? *Am J Gastroenterol* 2000; 95:114–121.

9 Coyle WJ, Sedlack RE, Nemec R, Peterson R, Duntemann T, Murphy M, Lawson JM. Eradication of *Helicobacter pylori* normalizes elevated mucosal levels of epidermal growth factor and its receptor. *Am J Gastroenterol* 1999; 94:2885–2889.

10 Fox JG, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC. High-salt diet
•• induces gastric epithelial hyperplasia and parietal cell loss, and enhances *Helicobacter pylori* colonization in C57BL/6 mice. *Cancer Res* 1999; 59:4823–4828.

An important study on the relationship between *H. pylori* infection and NaCl consumption.

11 Nardone G, Staibano S, Rocco A, Mezza E, D'armiento FP, Insabato L, *et al.*
•• Effect of *Helicobacter pylori* infection and its eradication on cell proliferation, DNA status, and oncogene expression in patients with chronic gastritis. *Gut* 1999; 44:789–799.

This significant study shows that chronic *H.pylori* infection is responsible for genomic instability in *H. pylori*-positive chronic atrophic gastritis and that eradication of *H. pylori* infection can reverse inflammation and the related atrophy, metaplasia, and genomic instability.

12 Al-Knawy B, Morad N, Jamal A, Mirdad S, Abol Fotouh M, Ahmed ME, *et al.* *Helicobacter pylori* and intestinal metaplasia with its subtypes in the gastric antrum in a Saudi population. *Scand J Gastroenterol* 1999; 34:562–565.

13 Kikuchi S, Crabtree JE, Forman D, Kurosawa M. Association between infections with CagA-positive or -negative strains of *Helicobacter pylori* and risk for gastric cancer in young adults. Research Group on Prevention of Gastric Carcinoma Among Young Adults. *Am J Gastroenterol* 1999; 94:3455–3459.

14 Rugge M, Busatto G, Cassaro M, Shiao YH, Russo V, Leandro G, *et al.*
• Patients younger than 40 years with gastric carcinoma: *Helicobacter pylori* genotype and associated gastritis phenotype. *Cancer* 1999; 85:2506–2511.

A thorough study examining *H. pylori* genotype and associated gastritis phenotype in patients younger than 40 years with gastric carcinoma.

15 Hirasawa R, Tatsuta M, Iishi H, Yano H, Baba M, Uedo N, Sakai N. Increase in apoptosis and decrease in ornithine decarboxylase activity of the gastric mucosa in patients with atrophic gastritis and gastric ulcer after successful eradication of *Helicobacter pylori*. *Am J Gastroenterol* 1999; 94:2398–2402.

16 Peek Jr RM, Blaser MJ, Mays DJ, Forsyth MH, Cover TL, Song SY, *et al.*
• *Helicobacter pylori* strain-specific genotypes and modulation of the gastric epithelial cell cycle. *Cancer Res* 1999; 59:6124–6131.

A comprehensive study, the results of which may explain heterogeneity in levels of gastric epithelial cell proliferation and apoptosis found within *H. pylori*-colonized mucosa.

17 Murakami K, Fujioka T, Okimoto T, Mitsushi Y, Oda T, Nishizono A, Nasu M. Analysis of p53 gene mutations in *Helicobacter pylori*-associated gastritis mucosa in endoscopic biopsy specimens. *Scand J Gastroenterol* 1999; 34:474–477.

18 Meining AG, Bayerdorffer E, Stolte M. *Helicobacter pylori* gastritis of the
•• gastric cancer phenotype in relatives of gastric carcinoma patients. *Eur J Gastroenterol Hepatol* 1999; 11:717–720.

An important study showing that there appears to be a genetic susceptibility influencing the expression of *H. pylori* gastritis.

19 El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, *et*
•• *al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; 404:398–402.

This significant, well-designed study shows that host genetic factors that affect interleukin-1-beta may determine why some individuals infected with *H. pylori* develop gastric cancer while others do not.

20 Banerjee SK, Weston AP, Zoubine MN, Campbell DR, Cherian R.
• Expression of cdc2 and cyclin B1 in *Helicobacter pylori*-associated gastric MALT and MALT lymphoma : relationship to cell death, proliferation, and transformation. *Am J Pathol* 2000; 156:217–225.

An interesting report which may help in identifying a population of patients with an increased risk of developing MALT lymphoma.

21 Chang CS, Chen LT, Yang JC, Lin JT, Chang KC, Wang JT. Isolation of a
•• *Helicobacter pylori* protein, FldA, associated with mucosa-associated lymphoid tissue lymphoma of the stomach. *Gastroenterology* 1999; 117:82–88.

A well-executed study showing that there are specific antigens from *H. pylori* strains associated with MALT lymphoma.

22 D'Elios MM, Amedei A, Manghetti M, Costa F, Baldari CT, Quazi AS, *et al.*
•• Impaired T-cell regulation of B-cell growth in *Helicobacter pylori*-related gastric low-grade MALT lymphoma. *Gastroenterology* 1999; 117:1105–1112.

The significance of this paper is due to the fact that it shows impaired T-cell regulation of B-cell growth in *H. pylori*-related gastric low-grade MALT lymphoma.

23 Bouzourene H, Haefliger T, Delacretaz F, Saraga E. The role of *Helicobacter pylori* in primary gastric MALT lymphoma. *Histopathology* 1999; 34:118–123.

24 Mazzucchelli L, Blaser A, Kappeler A, Scharli P, Laissue JA, Baggiolini M,
•• Uguccioni M. BCA-1 is highly expressed in *Helicobacter pylori*-induced mucosa-associated lymphoid tissue and gastric lymphoma. *J Clin Invest* 1999; 104:R49–R54.

A thorough study showing that BCA-1 is highly expressed in *H. pylori*-induced mucosa-associated lymphoid tissue and gastric lymphoma.

25 Saxena A, Moshynska O, Kanthan R, Bhutani M, Maksymiuk AW, Lukie BE. Distinct B-cell clonal bands in *Helicobacter pylori* gastritis with lymphoid hyperplasia. *J Pathol* 2000; 190:47–54.

26 Raderer M, Pfeifel F, Pohl G, Mannhalter C, Valencak J, Chott A. Regression of colonic low grade B cell lymphoma of the mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Gut* 2000; 46:133–135

27 Ng WW, Lam CP, Chau WK, Fen-Yau Li A, Huang CC, Chang FY, Lee SD. Regression of high-grade gastric mucosa-associated lymphoid tissue lymphoma with *Helicobacter pylori* after triple antibiotic therapy. *Gastrointest Endosc* 2000; 51:93–96.

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ALTERED GASTRIC EPITHELIAL CELL KINETICS IN *HELICOBACTER PYLORI*-ASSOCIATED INTESTINAL METAPLASIA: IMPLICATIONS FOR GASTRIC CARCINOGENESIS

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We have compared apoptosis and proliferation in antral epithelium from individuals not infected with *H. pylori (Hp)*, those with *Hp*-induced gastritis and those with *Hp*-induced gastritis containing areas of gastric intestinal metaplasia, the precursor lesion to gastric adenocarcinoma. Antral biopsies from 42 patients were assessed for evidence of *Hp* infection, severity of gastritis and intestinal metaplasia. Apoptosis was evaluated by the TUNEL assay and proliferation by Ki-67 immunohistochemistry and were expressed as apoptotic (AI) and proliferation (PI) indices. In the 31 *Hp*-positive (Hp^+) patients, apoptosis and proliferation were increased compared with the 11 *Hp*-negative (Hp^-) patients (AI = 1.22 ± 0.13% *vs.* 0.15 ± 0.03%, $p < 0.0001$; PI = 24 ± 1% *vs.* 13 ± 2%, $p < 0.0001$). Increases were proportional to the severity of the inflammation. Within foci of intestinal metaplasia, in 9 of the Hp^+ patients, apoptosis was significantly reduced compared with surrounding gastritis (AI = 0.20 ± 0.06% *vs.* 1.34 ± 0.23%, $p = 0.0014$), whereas proliferation was not altered (PI = 25.4 ± 4% *vs.* 24.7 ± 2%, $p = 0.87$), resulting in a lower AI/PI ratio in intestinal metaplasia than in surrounding gastritis (0.008 ± 0.005 *vs.* 0.054 ± 0.009, $p < 0.02$). *Hp*-induced gastritis is thus associated with increased epithelial apoptosis and proliferation compared with uninfected controls. In intestinal metaplasia, proliferation remains increased but apoptosis reverts to normal levels, and this perhaps contributes to *Hp*-associated gastric carcinogenesis. *Int. J. Cancer* 85:192–200, 2000.
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Identification of the pathogenetic role of *Hp* has revolutionized our approach toward peptic ulcer disease and low-grade gastric lymphomas, or MALTomas. Both entities often result from infection with *Hp* and the accompanying host inflammatory response. The host reaction, chronic gastritis, is incapable by itself of clearing the infection without antibiotic medication.

The ability of the organism to inflict injury [for review, Bodger and Crabtree (1998)] is probably related to the presence in the bacterial genome of a "pathogenicity island," for which the *cagA* gene is a marker. Evidence points to direct injury to the epithelial mucosa by cytotoxins and enzymes elaborated by the bacterium, such as lipase, protease and ammonia-producing urease. In addition, indirect injury is caused by enhanced gastric-acid secretion by the infected gastric epithelium. The host inflammatory response, with its attendant production of oxidants and reactive nitrogen intermediates, contributes to an injurious milieu. It is likely that many of these interrelated pathways play a role in the pathogenesis of chronic gastritis.

Long-standing gastritis has been known since 1981 to progress to gastric adenocarcinoma in a subset of patients. Correa (Correa, 1981) described the progression of the histologic lesion of chronic gastritis to gastric atrophy, followed by intestinal metaplasia. Intestinal metaplasia, characterized by loss of gastric glandular epithelial cells (oxyntic, chief and mucous cells) and substitution by intestinal-type epithelium containing goblet cells, is a precursor to the development of dysplasia and carcinoma. The identification of *Hp* as the most common cause of chronic gastritis prompted several epidemiologic studies linking *Hp* infection and gastric cancer (Scheiman and Cutler, 1999). The strength of the revealed association led to the classification of *Hp* as a class I carcinogen by the International Agency for Research on Cancer (1994), the first bacterial agent in this category (Anonymous, 1994). A recent meta-analysis established the increase in risk for gastric cancer in *Hp* infection to be about 2-fold (Huang *et al.*, 1998).

The recognition of *Hp* as a carcinogen has spurred the study of its effects on epithelial cell kinetics. In the rapidly dividing epithelium of the digestive tract, homeostasis is maintained by a careful balance between cell proliferation and apoptosis. Both are highly regulated processes that are also essential for tissue remodeling during development and tissue repair following injury. Disruption of the balance in favor of apoptosis results in excessive cell loss, whereas a balance favoring proliferation leads to accumulation of cells. Since apoptosis effectively removes cells with damaged DNA from the epithelium, its suppression leads to a survival advantage for mutated cells. Such disruption in the balance between apoptosis and proliferation has been implicated in carcinogenesis in other epithelial tissues.

Many investigators have demonstrated *Hp*-induced apoptosis in gastric epithelium [reviewed by Anti *et al.*, (1998)]. Other studies have shown enhanced proliferation in the gastric mucosa in *Hp*-infected patients (Anti *et al.*, 1998). This is marked not only by an increase in proliferating cells but also by an expansion of the "proliferative zone" from the neck (midportion) of the gastric pit to the surface and the deeper glands, where proliferating cells are normally absent.

To gain insight into the mechanisms of gastric carcinogenesis, we studied the processes of apoptosis and proliferation within *Hp*-infected tissue, with particular emphasis on the malignant precursor lesion of intestinal metaplasia. Biomarkers of cell turnover were measured within this lesion and compared with non-intestinalized antral epithelium with *Hp* gastritis and in non-infected controls. We now describe our findings that, coincident with progression from gastritis to intestinal metaplasia, there is a reduction in apoptosis while proliferation is maintained. This is consistent with a cell turnover state that favors further evolution into adenocarcinoma.

MATERIAL AND METHODS

Patients

We studied endoscopic gastric biopsies from 42 patients (30 male; ages 18–78 years, mean 45.4 years; 12 female; ages 28–59 years, mean 43.9 years) who underwent esophagogastroduodenos-

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copy for dyspeptic symptoms by a single endoscopist (TR) at the General Military Hospital in Athens, Greece. Gastric antral tissue biopsies were formalin-fixed and paraffin-embedded for routine histopathology. Our study was approved by the appropriate institutional oversight committees.

None of the patients took non-steroidal, anti-inflammatory medications for at least 1 week prior to the procedure. The diagnosis of *Hp* infection was established on gastric biopsies using a combination of a tissue urease test (CLO test; Tri-Med Specialties, Charlottesville, VA) and thiazine staining. CLO test was performed at the time of endoscopy and interpreted by an experienced endoscopist. Thiazine stain, a modified Diff-Quik stain (Dade International, Aguada, PR), was performed according to the directions of the manufacturer and evaluated by a pathologist (EF) blinded to the results of the CLO test. The patient was considered to be *Hp*$^+$ if both tests were positive. If only one test was positive, the infection status of that patient was considered equivocal. A patient was considered *Hp*$^-$ if both tests were negative.

Assessment of gastritis

Hematoxylin and eosin (H&E)-stained sections were examined by the same pathologist who was unaware of other experimental results. To avoid bias, a significant time interval between examination of the 2 stains elapsed, and the slides were randomly read. Samples were evaluated histologically for the following: 1, acute gastritis; 2, chronic gastritis; 3, intestinal metaplasia. Acute gastritis was defined as epithelial and stromal infiltration with neutrophils, and chronic gastritis was defined as infiltration with mononuclear cells. Each type of gastritis was scored 0–3, according to a modified Sydney classification: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe (Price, 1991). The sum of the scores for acute and chronic gastritis defined the cumulative gastritis score (CGS) used for assessment of gastritis severity: mild (CGS = 1–2); moderate (CGS = 3–4); marked (CGS = 5–6). Intestinal metaplasia was defined as the presence of foci where at least 3 neighboring gastric pits contained 2 or more goblet cells in each pit. Intestinal metaplasia was also subclassified as complete or incomplete. Complete metaplasia exhibited absorptive cells in a well-defined brush border or Paneth cells in the gastric pits (Filipe and Jass, 1986). Incomplete metaplasia displayed neither of these features.

Assessment of apoptosis and proliferation

Gastric epithelial cell apoptosis was ascertained histochemically by a modification of the terminal deoxynucleotidyltransferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) methodology. Five-micrometer tissue sections (10% neutral buffered formalin-fixed, paraffin-embedded) were mounted on charged microscope slides (Fisher, Springfield, NJ). To permit staining and scoring of separate gastric pits on a single slide, sections were cut approximately 50 μm apart. All incubations were carried out at ambient room temperature unless designated otherwise. The sections were deparaffinized in xylene. Endogenous peroxidase activity was quenched with 2% H_2O_2 solution for 15 min, and the sections were rehydrated in graded alcohols. Nuclear protein stripping was carried out with proteinase K (20 μg/ml) (Boehringer Mannheim, Indianapolis, IN) for 15 min. 3′-end DNA fragment elongation was carried out as follows: After pre-incubation with TdT buffer (30 mM Tris-HCl base, pH = 7.2/140 mM sodium cacodylate/1 mM cobalt chloride) for 5 min, sections were incubated at 37°C for 1 hr with the same buffer containing, in addition, 0.3 U/μl. TdT (Pharmacia, Piscataway, NJ) and 40 μM biotin-16-dUTP (Boehringer Mannheim). The reaction was terminated by washing for 15 min in a solution of 300 mM sodium chloride/30 mM sodium citrate, pH 7.0, and the slides were then washed with water and PBS. Staining was accomplished by incubating at 37°C for 30 min with extra avidin peroxidase (EAP) (Sigma, St. Louis, MO) diluted 1:200, to a final concentration of 0.011 mg/ml (1.32 purpurogallanin units/ml), in a buffer containing 1% BSA/0.5 M NaCl/1× PBS, followed by incubation for 2 min with freshly prepared 0.4 mg/ml diaminobenzidine (DAB, Sigma) solution. Slides were counterstained with freshly filtered Harris' hematoxylin (Shandon, Pittsburgh, PA) and 2% NH_4OH, washed, dehydrated, mounted with coverslips and examined microscopically. Positive and negative controls were included in every staining run. Positive control sections were pretreated with 1 μg/ml DNAse I (Sigma) for 10 min at room temperature prior to applying this methodology, resulting in positive (brown-colored) staining of all nuclei (Fig. 2, panel *a*, inset at upper right-hand corner). In the negative controls, TdT or biotin-16-dUTP was omitted from the histochemical procedure, resulting in uniformly negative (blue-colored) nuclear staining.

Proliferation was assayed by immunoperoxidase staining for Ki-67, a proliferation-associated antigen, using the MIB-1 monoclonal antibody (MAb). Four micrometer-thick tissue sections cut, mounted on charged slides, deparaffinized and rehydrated as described above were stained by an indirect immunoperoxidase method. Endogenous peroxidase activity was blocked with 1% H_2O_2 for 15 min at room temperature. Sections were then rinsed twice in double-distilled water, microwaved twice, 5 min each, in Citra antigen retrieval solution (BioGenex, San Ramon, CA) and allowed to cool to room temperature. Non-specific binding was blocked with 10% normal goat serum (NGS) (Pocono Rabbit Farm and Laboratory, Canadensis, PA). The primary MAb, MIB-1 (Immunotech, Westbrook, ME) diluted 1:50 (0.34 μg/ml) in 1% NGS, was incubated at 4°C in a humidified chamber overnight. Afterwards, slides were allowed to warm to room temperature, then were incubated with 1:200 dilution of horse anti-mouse secondary antibody for 30 min at room temperature (Vector, Burlingame, CA). Avidin-biotin-peroxidase complex (ABC reagent; Vector) diluted 1:100 in 1% NGS was added for 30 min at room temperature. The sections were then developed with 0.4 mg/ml DAB (Sigma), counterstained with Harris' hematoxylin (Shandon), dehydrated through graded alcohols, mounted with coverslips and examined microscopically. Only staining runs where the labeling index was comparable (within 1%) with previous runs using a single block of tissue were used for quantitative analysis. Positive controls: normal colon tissue sections, staining positively with MIB-1 at the basal portions of the crypts, stained in parallel with the gastric samples. Negative controls: IgG, diluted 1:200 in 1% NGS (final concentration) was substituted for MIB-1, resulting in uniformly negative (blue-colored) nuclear staining.

Quantification of proliferation and apoptosis

For each patient, 1,000 gastric epithelial cells in the foveolar region of longitudinally sectioned gastric pits were counted in sections stained for both apoptosis and proliferation. The apoptotic index (AI) was defined as the ratio of TUNEL-positive to total nuclei counted multiplied by 100. The proliferative index (PI) was defined as the ratio of Ki-67-positive nuclei to total nuclei counted multiplied by 100. In biopsies with intestinal metaplasia, separate measurements were obtained from the foveolar region of gastric pits with and without metaplasia. Counting was performed by a single observer (IS), who was blinded to the *Hp* status of the patient whose tissue was being examined. The apoptosis/proliferation ratio (AI/PI) was obtained by dividing the AI by the PI. Because neither is a comprehensive method that measures the proliferation or apoptosis in tissue with absolute accuracy, in normal mucosa, where apoptosis and proliferation should be in equilibrium, the AI/PI ratio is below unity. However, this ratio can be applied to compare differences in cell turnover kinetics of different groups of patients within the gastric mucosa.

The accuracy and reproducibility of the quantitative assessment was verified by repeating measurements on the first 10 specimens after all the slides had been counted. Initial and repeat measurements of AI and PI were within 0.2% of each other in each of these specimens.

TABLE I CLINICAL CHARACTERISTICS AND INDICES OF APOPTOSIS AND PROLIFERATION IN THE STUDY PATIENTS

Patient number	Age	Gender	*Hp* status	Histology	Severity of gastritis	AI (%)	PI (%)	AI/PI
1	36	F	−	N		0.08	6.9	0.011
2	20	M	−	N		0.23	14.1	0.016
3	28	F	−	N		0.28	8.1	0.034
4	46	F	−	N		0	11.7	0
5	56	F	−	N	−	0.21	17.9	0.011
6	52	M	−	N		0.33	5.7	0.057
7	29	F	−	N	−	0.10	9.2	0.011
8	48	M	−	N	−	0.10	19.0	0.005
9	20	M	−	N	−	0.19	8.7	0.022
10	36	M	−	N	−	0.10	13.0	0.008
11	37	M	−	N	−	0.08	28.2	0.003
12	50	M	+	G	Mild	1.25	20.7	0.060
13	20	M	+	G	Mild	0.87	14.2	0.061
14	45	M	+	G	Mild	0	26.0	0
15	32	M	+	G	Mild	1.31	19.7	0.066
16	37	F	+	G	Mild	0	20.7	0
17	35	M	+	G	Mild	0.12	16.9	0.007
18	20	M	+	G	Moderate	2.19	26.0	0.084
19	37	F	+	G	Moderate	1.95	19.7	0.099
20	18	M	+	G	Moderate	2.35	25.5	0.092
21	46	M	+	G	Moderate	0.93	27.4	0.034
22	38	M	+	G	Moderate	1.57	22.3	0.070
23	59	M	+	G	Moderate	1.17	37.2	0.032
24	78	M	+	G	Moderate	0.44	30.3	0.015
25	53	F	+	G	Moderate	0.59	19.1	0.031
26	58	M	+	G	Moderate	2.27	23.0	0.099
27	41	M	+	G	Moderate	1.63	22.5	0.073
28	58	F	+	G	Moderate	0.33	29.0	0.011
29	40	F	+	G	Moderate	0.64	29.9	0.021
30	50	M	+	G	Severe	1.62	35.9	0.045
31	48	F	+	G	Severe	1.18	19.1	0.062
32	76	M	+	G	Severe	1.54	24.3	0.063
33	65	M	+	G	Severe	1.81	23.6	0.077
34	40	M	+	G + IM	Mild	1.18 (0.20)	19.5 (14.7)	0.060 (0.013)
35	72	M	+	G + IM	Mild	1.19 (0.12)	21.8 (25.1)	0.055 (0.005)
36	56	M	+	G + IM	Mild	0.56 (0.20)	21.0 (14.4)	0.027 (0.014)
37	59	F	+	G + IM	Mild	0.10 (0.27)	24.8 (46.1)	0.004 (0.006)
38	31	M	+	G + IM	Moderate	1.86 (0.30)	25.1 (14.3)	0.074 (0.02)
39	56	M	+	G + IM	Moderate	1.27 (0.10)	19.3 (28.5)	0.066 (0.004)
40	65	M	+	G + IM	Moderate	1.71 (0.16)	26.3 (22.7)	0.065 (0.020)
41	62	M	+	G + IM	Moderate	2.05 (0.10)	28.4 (39.8)	0.072 (0.003)
42	36	M	+	G + IM	Severe	2.13 (0)	33.2 (22.6)	0.064 (0)

[1]As determined by CGS criteria (Price, 1991). N, normal; G, *Hp*-associated gastritis in antral epithelium without foci of metaplasia; G + IM, gastritis in antral epithelium neighboring foci of intestinal metaplasia. Values in parentheses represent staining measurements in epithelial cells within metaplastic gastric pits.

Statistical analysis

Two-tailed statistical tests were used to evaluate the data. ANOVA was used for comparisons between patient groups. Differences between foci of metaplasia and surrounding gastritis in the same patients were assessed using linear regression analysis. Statistical analysis was performed using JMP Software (SAS Institute, Cary, NC). Significance value was set at $p < 0.05$.

RESULTS

Hp *infection status*

Table I shows the clinical data of our patients. Of the 42 gastric mucosal biopsy specimens examined, 11 were *Hp*$^-$ and 31 were *Hp*$^+$. There was 100% concordance between results of the CLO test and thiazine staining test for *Hp* detection. Biopsies from all 11 *Hp*$^-$ patients were histologically normal on H&E examination. All 31 *Hp*$^+$ patients exhibited histological gastritis, with severities of mild in 10, moderate in 16 and marked in 5. Foci of intestinal metaplasia were noted in 9 of the 31 *Hp*$^+$ patients (4 with mild, 4 with moderate and 1 with marked gastritis). Intestinal metaplasia was subclassified as incomplete in 3 patients and complete in 6 patients.

Apoptosis

Table II and Figure 1 summarize the AI in this cohort of patients. Apoptosis was noted in only 0.15 ± 0.03% (this and all subsequent values are mean ± SEM) of the epithelial cells in the gastric mucosal specimens from normals. When all specimens exhibiting gastritis were examined as a group, the AI was increased 8-fold, to 1.22 ± 0.13% ($p < 0.0001$). AI increased in parallel to the severity of gastritis: 0.66 ± 0.18% in mild, 1.43 ± 0.17% in moderate and 1.66 ± 0.16% in severe ($p = 0.0084$, $p < 0.0001$, $p < 0.0001$, respectively compared with normal epithelium, Fig. 1). It is noteworthy that the AI reaches maximal intensity at the stage of moderate gastritis.

AI was also determined in the specimens with foci of intestinal metaplasia. An 85% reduction in AI was noted in the intestinalized compared with non-intestinalized pits (0.20 ± 0.06% *vs.* 1.34 ± 0.23%, respectively, $p = 0.0014$). Indeed, the AI of intestinalized epithelium was similar to that of normal controls. There was no difference between AI in the foveolar region of pits with complete or incomplete metaplasia (0.22 ± 0.06% *vs.* 0.15 ± 0.10%, respectively, $p = 0.6$). Figure 2 also illustrates these findings. This figure exhibits photomicrographs of representative

TABLE II GASTRIC EPITHELIAL CELL KINETICS IN *Hp*⁻ AND *Hp*⁺ PATIENTS

	AI (%)	PI (%)	AI/PI
Normal controls (n = 11)	0.15 ± 0.03	13 ± 2	0.012 ± 0.009
All gastritis (n = 31)	1.22 ± 0.13[1]	24 ± 1[1]	0.051 ± 0.005[1]
Mild gastritis (n = 10)	0.66 ± 0.18[6]	20 ± 2[8]	0.033 ± 0.008[6]
Moderate gastritis (n = 16)	1.43 ± 0.17[7]	25.7 ± 2[1]	0.056 ± 0.006[3]
Severe gastritis (n = 5)	1.66 ± 0.16[7]	27.2 ± 3[1]	0.061 ± 0.007[3]
Gastritis without IM (G) (n = 22)	1.17 ± 0.16[7,13]	24.2 ± 1.2[1,13]	0.050 ± 0.007[3,13]
Gastritis around IM (G + IM) (n = 9)	1.34 ± 0.23[7]	24.7 ± 2[2]	0.054 ± 0.009[4]
Intestinal metaplasia (IM) (n = 9)	0.20 ± 0.06[6,12]	25.4 ± 4[9,13]	0.008 ± 0.005[6,12]

Apoptosis (AI), proliferation (PI) and AI/PI ratio were determined in the epithelial cells of the antral mucosa of patients without *Hp* infection (normals), patients with *Hp*-associated gastritis of varying severity and within foci of intestinal metaplasia and surrounding gastritis in *Hp* gastritis.

Compared with normals (or none): [1]$p < 0.0001$, [2]$p < 0.0009$, [3]$p < 0.001$, [4]$p = 0.002$, [5]$p = 0.003$, [6]$p < 0.01$, [7]$p = 0.01$, [8]$p < 0.02$, [9]$p = 0.03$, [10]$p > 0.05$ (not significant). Compared with G + IM: [11]$p = 0.0014$, [12]$p < 0.02$, [13]$p > 0.05$ (not significant).

sections from normal *Hp*⁻ antral mucosa, *Hp*-associated gastritis, *Hp*-associated gastritis neighboring a metaplastic focus and a metaplastic focus, all stained by the TUNEL method. The relative abundance of TUNEL-positive epithelial cells in areas with gastritis is readily apparent in panels *b* and *c* (see arrows). Apoptotic cells were seen uniformly throughout the gastritic sections, thus calculated differences in AI are unlikely to be due to sampling error. Panel *c* depicts the loss of apoptotic bodies as one transits into metaplastic foci, where intestinal-type epithelium is surrounded by areas of inflammation. Panels *a* and *d* show the relative paucity of apoptotic cells in both normal mucosa and metaplastic foci, respectively. In fact, in the sections portrayed, no TUNEL-positive cells are seen.

Proliferation

Table II and Figure 3 demonstrate the PIs from the gastric biopsies of these patients. In normal antral epithelium, 13 ± 2% of epithelial cell nuclei stained positively for the Ki-67 antigen. Overall, in *Hp*-induced gastritis, the PI was increased nearly 2-fold to 24 ± 1% ($p < 0.0001$). As in apoptosis, the PI increased in proportion to the severity of gastritis: 20 ± 2% in mild, 25.7 ± 2% in moderate and 27.2 ± 3% in severe ($p < 0.02$, $p < 0.0001$ and $p < 0.0001$, respectively, compared with normal epithelium, Fig. 3). Similar to apoptosis, the increase in PI appeared to reach a maximum at the stage of moderate gastritis. In contrast to apoptosis, however, proliferative activity within the foveolae of intestinalized metaplastic pits was maintained at the elevated level seen in the surrounding epithelium (25.4 ± 4% *vs.* 24.7 ± 2%, respectively, $p = 0.87$). As was observed for the AI in the gastric pits with metaplasia, the PI measured in the foveolae of pits with incomplete metaplasia did not differ from those with complete metaplasia (27.2 ± 9% *vs.* 24.8 ± 5%, respectively, $p = 0.8$). This is illustrated in Figure 4, which shows photomicrographs of representative sections of *Hp*-infected antral mucosa and uninfected normal mucosa stained for Ki-67 expression with the MIB-1 MAb. It is noteworthy that epithelial cells in both the intestinalized (panels *c* and *d*) and non-intestinalized gastric pits (panels *b* and *c*) in the *Hp*-infected samples show similar rates of staining with MIB-1 antibody and that this is more abundant than the staining in the normal uninfected pits (panel *a*).

Apoptosis index to proliferation index ratio (AI/PI)

To assess the relative contribution of apoptosis and proliferation in a given specimen, we determined the AI/PI. As demonstrated in Table II and Figure 5, the AI/PI ratio was 4-fold higher in specimens exhibiting gastritis compared with normal samples ($p = 0.003$), reflecting the disproportionate induction of apoptosis. The AI/PI in normal epithelium was not significantly different from that found in mild gastritis ($p = 0.35$) but differed significantly from sections with moderate and marked gastritis (both, $p < 0.001$). Within intestinalized metaplastic foci, however, the AI/PI ratio was reduced 85% compared with that of the surrounding tissue with gastritis but with no evidence of metaplasia ($p < 0.02$), being similar to that of normals ($p = 0.31$). Figure 6, which shows the AI and PI of each subject, further demonstrates these changes. It is clear that the reduced AI/PI reflects the substantial reduction of AI in intestinal metaplasia. Figure 6 also shows that in addition to reduced apoptosis, the proliferation in the metaplastic mucosa tends to be slightly higher than in the normal gastric mucosa.

DISCUSSION

We investigated the effects of *Hp* infection on the epithelial cell kinetic parameters of apoptosis and proliferation in the antral mucosa of patients evaluated for dyspepsia. Both *Hp*-associated gastritis and intestinal metaplasia, the precursor lesion to gastric adenocarcinoma, were examined.

Our data demonstrate that in normal (*Hp*⁻) gastric mucosal epithelium, the baseline rate of apoptosis was low, with a mean of approximately 1 apoptotic (TUNEL⁺) cell out of every 667 cells. TUNEL⁺ staining indicates the presence of abundant broken ends of DNA and is a hallmark of apoptosis, particularly when detected in isolated cells within a tissue. Apoptosis was significantly enhanced in inflamed epithelium harboring *Hp*, with greater apoptosis in samples with moderate and severe gastritis than in those with mild grades of inflammation. Our finding of increased apoptosis associated with *Hp*-induced inflammation agrees with prior studies, which showed that augmented apoptosis is abolished by *Hp* eradication (Jones *et al.*, 1997).

The mechanisms by which *Hp* increases apoptosis in the gastric epithelium are unclear. It is possible that a bacterial factor rather than an inflammatory or other host tissue mediator is responsible for this response. For example, there is evidence that apoptosis is induced through release of reactive nitrogen intermediates by the organism (Mannick *et al.*, 1996). Enhanced apoptosis is absent in gastric inflammation due to etiologies other than *Hp*, such as non-steroidal, anti-inflammatory agents or Crohn's disease (Jones *et al.*, 1997). In addition, *Hp* stimulates apoptosis in gastric epithelial cells *in vitro* (Wagner *et al.*, 1997). Potential factors up-regulating apoptosis in *Hp* infection include cell cycle arrest associated with $p21^{WAF1}$ induction (Ashkortab *et al.*, 1998). Fas ligand expression by infiltrating CD45⁺ cells and CD95 receptor expression (Rudi *et al.*, 1998).

A major finding of our study is that apoptosis was conspicuously diminished within foci of metaplastic epithelium, approaching rates observed in non-infected tissues. Diminished presence of the

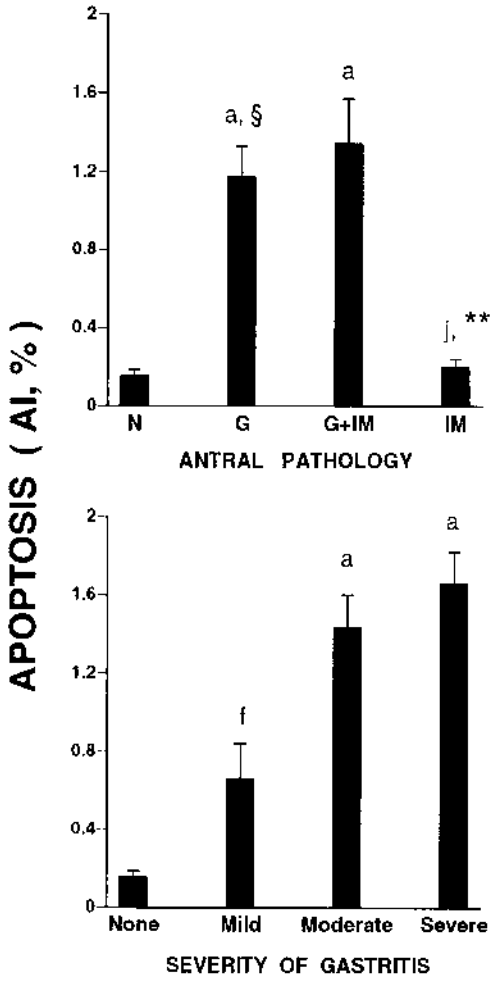

FIGURE 1 Apoptosis in antral epithelium of normal individuals and *Hp*-infected patients. Apoptosis was determined in tissue sections stained by the TUNEL technique as described in Material and Methods. AI represents the ratio of TUNEL$^+$ to total nuclei counted multiplied by 100. N: normal; G: *Hp*-associated gastritis in antral epithelium without foci of metaplasia; G + IM: gastritis in antral epithelium neighboring foci of intestinal metaplasia; IM: foci of intestinal metaplasia. Compared with normals (or none): a: $p < 0.0001$; f: $p < 0.01$; j: $p > 0.05$ (not significant). Compared with G + IM: **$p = 0.0014$; §: $p > 0.05$ (not significant).

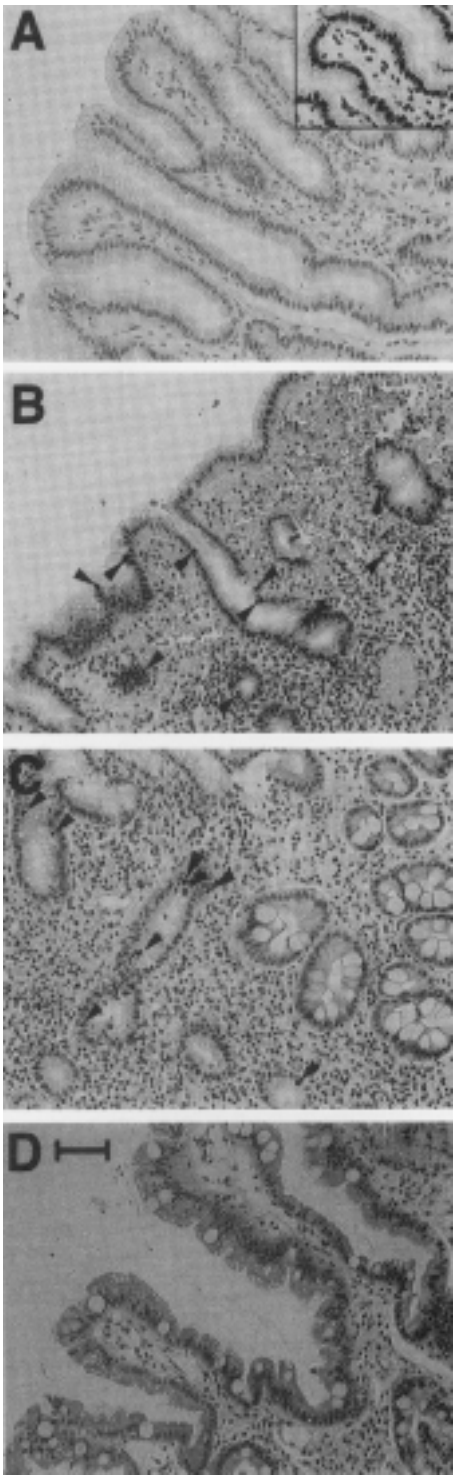

FIGURE 2 - Apoptosis in gastric epithelium. Photomicrographs of representative sections of gastric tissue stained by the TUNEL technique for the detection of apoptotic cells as described in Material and Methods. *(a) Hp*$^-$ normal mucosa; *(b)* antral gastritis in an *Hp*$^+$ patient; *(c)* transitional zone, area of gastritis neighboring a focus of intestinal metaplasia in an *Hp*$^+$ patient; *(d) Hp*-induced intestinal metaplasia. Inset in upper right-hand corner of panel *a* is a representative positive control section of *Hp*$^-$ normal mucosa pre-treated with DNAse I as described in Material and Methods. Scale bar = 0.2 mm.

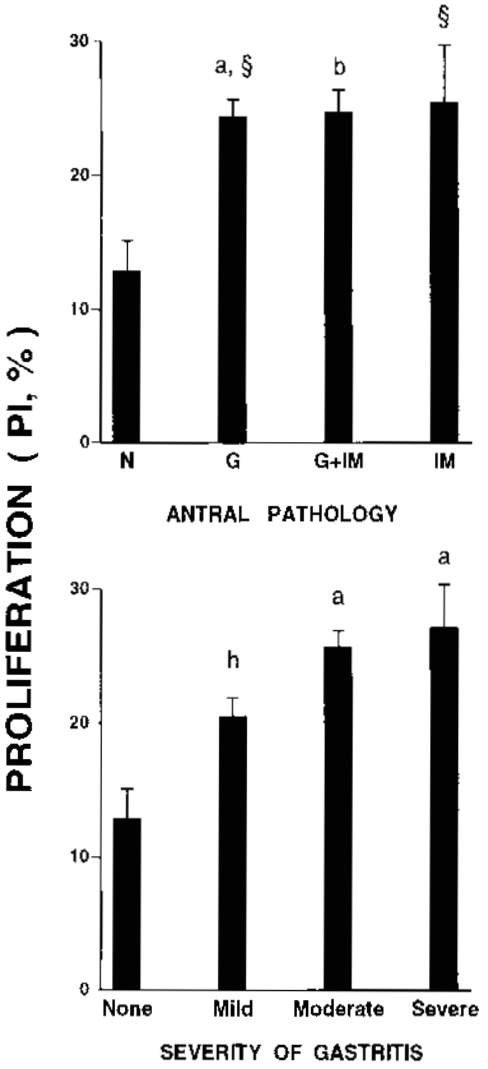

FIGURE 3 Proliferation in antral epithelium of normal individuals and *Hp*-infected patients. Proliferation was determined in tissue sections stained for expression of Ki-67 antigen as described in Material and Methods. PI represents the ratio of Ki-67-positive nuclei to total nuclei counted multiplied by 100. N: normal; G: *Hp*-associated gastritis in antral epithelium without foci of metaplasia; G + IM gastritis in antral epithelium neighboring foci of intestinal metaplasia; IM: foci of intestinal metaplasia. Compared with normals (or none): a: $p < 0.0001$; b: $p < 0.0009$; h: $p < 0.02$. Compared with G + IM: §: $p > 0.05$ (not significant).

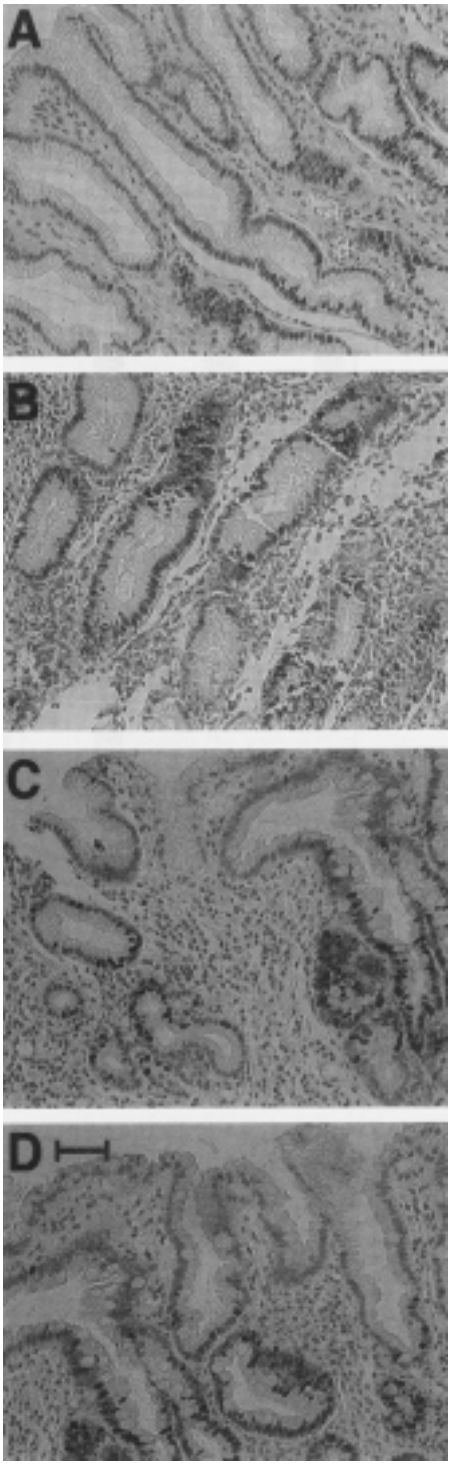

FIGURE 4 Proliferation in gastric epithelium. Photomicrographs of representative sections of gastric tissue immunohistochemically stained for expression of Ki-67 antigen as described in Material and Methods. *(a) Hp*⁻ normal mucosa; *(b)* antral gastritis in an *Hp*⁺ patient; *(c)* transitional zone, area of gastritis neighboring a focus of intestinal metaplasia in an *Hp*⁺ patient; *(d) Hp*-induced intestinal metaplasia. Scale bar 0.2 mm.

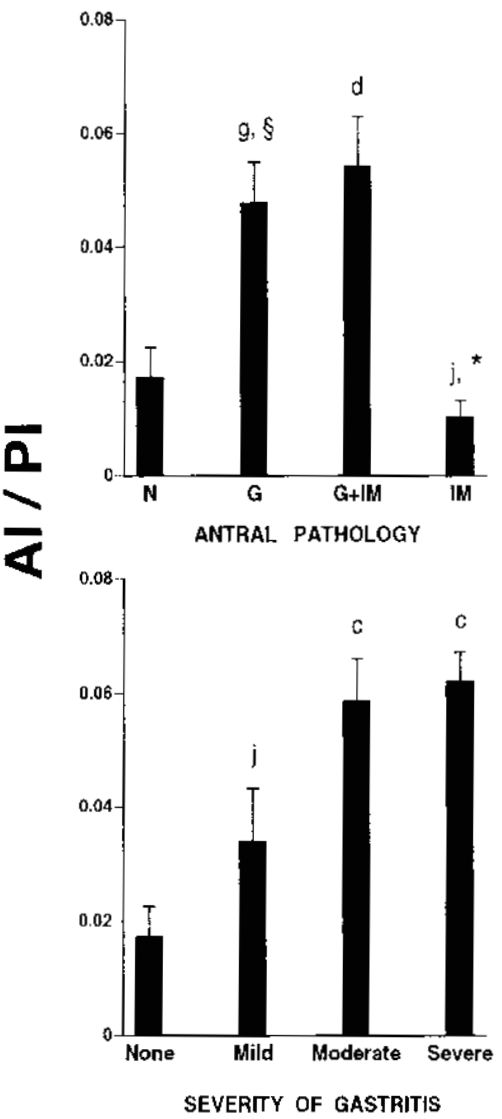

FIGURE 5 – Ratio of apoptosis to proliferation in antral epithelium. N: normal; G: *Hp*-associated gastritis in antral epithelium without foci of metaplasia; G + IM: gastritis in antral epithelium neighboring foci of intestinal metaplasia; IM: foci of intestinal metaplasia. Compared with normals (or none): c: $p < 0.001$; d: $p = 0.002$; g: $p = 0.01$; j: $p > 0.05$ (not significant). Compared with G + IM: *: $p < 0.02$; §: $p > 0.05$ (not significant).

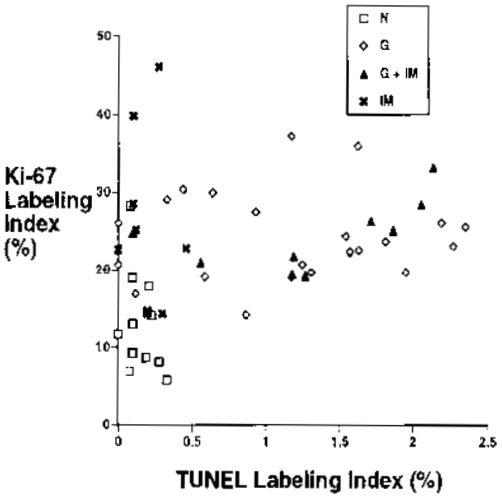

FIGURE 6 – Proliferation vs. apoptosis in the study subjects. Each point represents the PI and AI of a given subject. N: normal; G: *Hp*-associated gastritis in antral epithelium without foci of metaplasia; G + IM: gastritis in antral epithelium neighboring foci of intestinal metaplasia; IM: foci of intestinal metaplasia. Both proliferation and apoptosis are increased with *Hp*-associated gastritis, even when it contains metaplastic foci. However, within metaplastic foci, AI is diminished but PI remains relatively high.

organism in the metaplastic foci is an unlikely explanation, since *Hp* is able to adhere to intestinalized epithelial cells (Genta *et al.*, 1996). In our sections, we were able to identify areas of intestinal metaplasia closely apposing apoptosis-rich areas of inflammation (Fig. 2). It appears that the intestinalized metaplastic epithelium develops resistance to the pro-apoptotic forces applied to it by its neighboring environment. This could be mediated, for example, by aberrant expression of anti-apoptotic proteins, such as Bcl-2, which has been noted within foci of intestinal metaplasia (Lauwers *et al.*, 1994). Reduction of apoptosis was noted equally within the foveolar region of gastric pits exhibiting either incomplete or complete metaplasia. Although incomplete intestinal metaplasia has the strongest association with development of adenocarcinoma, complete intestinal metaplasia may also be a precursor lesion (Filipe and Jass, 1986). Others have reported higher rates of apoptosis and proliferation in incomplete vs. complete metaplasia (Imatani *et al.*, 1996). These studies evaluated metaplastic gastric pits in the vicinity of fully developed carcinoma in various gastric segments. We examined cell kinetics in gastric intestinal metaplasia before any malignancy could be manifest and, therefore, may be more relevant to the early events in the antral mucosa that give rise to cancer.

We also showed that there is an augmentation of cell proliferation in *Hp* infection. Fan *et al.* (1996) have previously shown that pre-incubation of cultured AGS gastric cells with supernatants from both *Hp*- and mitogen-activated peripheral blood lymphocytes increased their proliferation (Ki-67 positivity). Other *in vitro* studies, however, have shown inhibition of proliferation of gastric cell lines by soluble extract of *Hp* (Chang *et al.*, 1993). Our results agree with the *in vivo* results of Bechi *et al.* (1996) and Fraser *et al.* (1994), who have shown that *Hp* infection has a positive proliferative effect on gastric epithelium. Not only are the total numbers of proliferating cells increased but also *Hp* infection is associated with an abnormal distribution of proliferating cells along the longitudinal axis of the gastric pits (Anti *et al.*, 1998). Eradication of *Hp* is associated with return of proliferation to normal levels (Lynch *et al.*, 1995). This proliferative response to *Hp* has been linked to the development of hyperplastic gastric polyps, which were also shown to regress after *Hp* eradication (Moecek *et al.*, 1994). In our study, these relatively high rates of proliferation, measured by Ki-67 staining, were preserved within foci of intestinal metaplasia. Interestingly, Ierardi *et al.* (1997), using a BrdU proliferation assay,

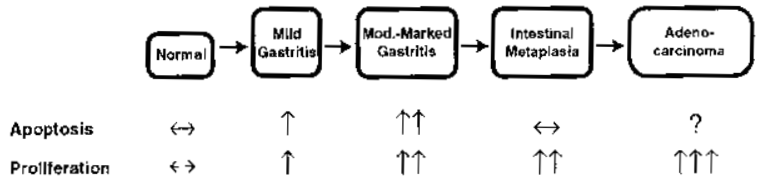

FIGURE 7 - Epithelial cell kinetics during gastric carcinogenesis. A progressive increase in proliferation is noted. Apoptosis increases in gastritis but returns to levels comparable to normal gastric mucosa (↔) in intestinal metaplasia. This cell turnover imbalance likely contributes to gastric carcinogenesis.

showed a similar pattern of augmented proliferation in foci of intestinal metaplasia compared with areas of gastritis. This is in contrast with earlier studies by this group, using PCNA immunostaining, which showed even higher levels of proliferation in areas of intestinal metaplasia compared with gastritis (Panella *et al.*, 1996). These differences are likely explained by differences in the expression of 3 different antigens during the cell cycle. Ierardi *et al.* (1997) also demonstrated that the hyperproliferation associated with intestinal metaplasia is not reversed by *Hp* eradication, suggesting that proliferation of the metaplastic cell is no longer dependent on bacterial factors. Therefore, transition to intestinal metaplasia may be a crucial and perhaps irreversible step in the chain of cellular events leading to disordered gastric cell growth and carcinogenesis.

We used the AI/PI ratio as a gauge relating the 2 processes involved in epithelial cell turnover. Moderate or severe (but not mild) gastritis was associated with a significant increase in this ratio compared with normal mucosa. Under these circumstances, the net loss of cells in a particular area may result in ulcerogenesis. However, when intestinal metaplasia appears, the ratio diminishes significantly. This is primarily due to a marked downshift in apoptosis, as proliferation within these foci is maintained (illustrated in Fig. 2*d*). Thus, the balance at this point favors increased cell numbers. This cell turnover state could sustain the accumulation of somatic mutations in gastric epithelial cells, therefore promoting progression to gastric adenocarcinoma. Since this proliferative state appears to be unaffected by eradication of *Hp*, the dysregulation of cell kinetics in intestinal metaplasia may be a critical irreversible step toward the development of gastric cancer. This sequence of events in gastric epithelial turnover is depicted in Figure 7.

In summary, our data show that *Hp*-associated gastritis is characterized by progressively increasing rates of proliferation and apoptosis in epithelial cells, whereas the development of intestinal metaplasia, a precursor to gastric carcinoma, is characterized by much lower rates of apoptosis along with relatively increased proliferation. These changes, possibly irreversible, may represent a crucial step in gastric carcinogenesis resulting from *Hp* infection.

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REFERENCES

ANONYMOUS. Schistosomes, liver flukes and *Helicobacter pylori*. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, **61**, 1–241 (1994).

ANTI, M., ARMUZZI, A., GASBARRINI, A. and GASBARRINI, G., Importance of changes in epithelial cell turnover during *Helicobacter pylori* infection in gastric carcinogenesis. *Gut*, **43**, S 27–S 32 (1998).

ASHKORTAB, H., AHMED, A., LITTLETON, G., TACKEY, R., WALTERS, C.S. and SMOOT, D.T., *H. pylori* induced G- arrest is associated with stimulation of cyclin D2/cdk2 and p21. *Gastroenterology*, **114**, A59 (1998).

BICHI, P., BALZI, M., BECCIOLINI, A., MAUGERI, A., RAGGI, C.C., AMOROSI, A. and DEL R., *Helicobacter pylori* and cell proliferation of the gastric mucosa: possible implications for gastric carcinogenesis. *Amer. J. Gastroenterol.*, **91**, 271–276 (1996).

BODGER, K. and CRABTREE, J.E., *Helicobacter pylori* and gastric inflammation. *Brit. med. Bull.*, **54**, 139–150 (1998).

CHANG, K., FUJIWARA, Y., WYLE, F. and TARNAWSKI, A., *Helicobacter pylori* toxin inhibits growth and proliferation of cultured gastric cells-Kato III. *J. Physiol. Pharmacol.*, **44**, 17–22 (1993).

CORREA, P., Epidemiological correlations between diet and cancer frequency. *Cancer Res.*, **41**, 3685–3690 (1981).

FAN, X.G., KELLEHER, D., FAN, X.J., XIA, H.X. and KELLEHER, P.W., *Helicobacter pylori* increases proliferation of gastric epithelial cells. *Gut*, **38**, 19–22 (1996).

FILIPE, M.I. and JASS, J.R., Intestinal metaplasia subtypes and cancer risk. *In:* M.I. Filipe and J.R. Jass (eds.), *Gastric carcinoma*, pp. 97–115. Churchill Livingstone, Edinburgh (1986).

FRASER, A.G., SEAL, R., SANKEY, E.A., DHILLON, A.P. and POUNDER, R.E., Effect of eradication of *Helicobacter pylori* on gastric epithelial cell proliferation. *Aliment. Pharmacol. Ther.*, **8**, 167–173 (1994).

GENTA, R.M., GRIFFER, I.E., GRAHAM, D.Y., KRISHNAN, B., SEGURA, A.M., GUTIERREZ, O., KIM, J.G. and BUCHOLTZ, J.L., Jr., Adherence of *Helicobacter pylori* to areas of incomplete intestinal metaplasia in the gastric mucosa. *Gastroenterology*, **111**, 1206–1211 (1996).

HUANG, J.Q., SRIDHAR, S., CHEN, Y. and HUNT, R.H., Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. *Gastroenterology*, **114**, 1169–1179 (1998).

IERARDI, E., FRANCAVILLA, A., BALZANO, T., TRAVERSA, A., PRINCIPI, M., MONNO, R.A., AMOROSO, A., INGROSSO, M., PISANI, A. and PANELLA, C., Effect of *Helicobacter pylori* eradication on gastric epithelial proliferation. Relationship with *ras* oncogene p21 expression. *Ital. J. Gastroenterol. Hepatol.*, **29**, 214–219 (1997).

IMAYAVI, A., SASANO, H., YABUKI, N., KATO, K., OHARA, S., ASAKI, S., TOYOTA, T. and NAGURA, H., In situ analysis of tissue dynamics and p53 expression in human gastric mucosa. *J. Pathol.*, **179**, 39–42 (1996).

JONES, N.L., SHANNON, P.T., CUTZ, E., YEGER, H. and SHERMAN, P.M., Increase in proliferation and apoptosis of gastric epithelial cells early in the natural history of *Helicobacter pylori* infection. *Amer. J. Pathol.*, **151**, 1695–1703 (1997).

LAUWERS, G.Y., SCOTT, G.V. and HENDRICKS, J., Immunohistochemical evidence of aberrant bcl-2 protein expression in gastric epithelial dysplasia. *Cancer*, **73**, 2900–2904 (1994).

LYNCH, D.A.F., MAPSTONE, N.P., CLARKE, A.M.T., SOBALA, G.M., LUCKSON, P., MORRISON, L., DIXON, M.F., QUIRKE, P. and AXON, A.T.R., Cell proliferation in *Helicobacter pylori* associated gastritis and the effect of eradication therapy. *Gut*, **36**, 346–350 (1995).

MANNICK, E.E., BRAVO, L.E., ZARAMA, G., REALPE, J.L., ZHANG, X.J., RUIZ, B., FONTHAM, E.T., MERA, R., MILLER, M.J. and CORREA, P., Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. *Cancer Res.*, **56**, 3238–3243 (1996).

MOCUK, F.W., WARD, W.W., JR., WOLFSON, S.E., RUMAGE, W.T., JR. and WILMAN, T.J., Elimination of recurrent hyperplastic polyps by eradication of *Helicobacter pylori*. *Ann. Inter. Med.*, **120**, 1007–1008 (1994).

PANELLA, C., IERARDI, E., POLIMENO, L., BALZANO, T., INGROSSO, M., AMORUSO, A., TRAVERSA, A. and FRANCAVILLA, A., Proliferative activity of gastric epithelium in progressive stages of *Helicobacter pylori* infection. *Digest. Dis. Sci.*, **41**, 1132–1138 (1996).

PRICE, A.B., The Sydney System: histological division. *J. Gastroenterol. Hepatol.*, **6**, 209–222 (1991).

RUDI, J., KUCK, D., VON HERBAY, A., KRAMMER, P.H., GALLE, P.R. and STREMMEL, W., *H. pylori*-induced gastric epithelial apoptosis is mediated by the CD95 (Fas) receptor and ligand system. *Gastroenterology*, **114**, A272 (1998).

SCHEIMAN, J.M. and CUTLER, A.F., *Helicobacter pylori* and gastric cancer. *Amer. J. Med.*, **106**, 222–226 (1999).

WAGNER, S., BEIL, W., WESTERMANN, J., LOGAN, R.P., BOCK, C.T., TRAUTWEIN, C., BLEICK, J.S. and MANNS, M.P., Regulation of gastric epithelial cell growth by *Helicobacter pylori*: evidence for a major role of apoptosis. *Gastroenterology*, **113**, 1836–1847 (1997).

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Meta-analysis of the Relationship Between *Helicobacter pylori* Seropositivity and Gastric Cancer

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Background & Aims: Reports in the literature regarding the relationship of *Helicobacter pylori* infection to gastric cancer are conflicting. The aim of this study was to identify the source of heterogeneity between studies. *Methods:* Meta-analysis of cohort or case-control studies with age- and/or sex-matched controls, providing raw data on *H. pylori* infection detected by serology, was used. *Results:* A fully recursive literature search identified 19 qualified studies with 2491 patients and 3959 controls. Test for homogeneity found a significant difference in odds ratio between patients with early and advanced gastric cancer (6.35 vs. 2.13; $P = 0.01$), patients with cardiac and noncardiac gastric cancer (1.23 vs. 3.08; $P = 0.003$), and population- and hospital-based controls (2.11 vs. 1.49; $P < 0.001$). The summary odds ratio for gastric cancer in *H. pylori*–infected patients is 1.92 (95% confidence interval [CI], 1.32–2.78), 2.24 (95% CI, 1.15–4.4), and 1.81 (95% CI, 1.16–2.84) for all studies, cohort, and case-control studies, respectively. *H. pylori*–infected younger patients have a higher relative risk for gastric cancer than older patients with odds ratios decreasing from 9.29 at age ≤29 years to 1.05 at age ≥70 years. *H. pylori* infection is equally associated with the intestinal or diffuse type of gastric cancer. *Conclusions:* *H. pylori* infection is a risk factor for gastric cancer. The heterogeneity of reported results is caused by differences in the selection of controls, patient age, and the site and stage of gastric cancer.

Gastric cancer is one of the most common malignancies in the world, although the incidence and mortality rate have been decreasing over recent decades.[1,2] Gastric carcinogenesis is a multistep and a multifactorial process. Evidence from pathological and epidemiological studies has provided us with a human model of gastric carcinogenesis with a sequential evolution. In most cases, the initial stage is chronic gastritis, followed by atrophy, intestinal metaplasia, dysplasia, and eventually carcinoma.[3] The real cause of gastric carcinogenesis is not fully understood; however, several environmental factors including *Helicobacter pylori*, excessive intake of salt, bile reflux, *N*-nitroso compounds, and a deficiency of antioxidants have been linked with different stages of gastric carcinogenesis.[3] Among these risk factors, *H. pylori* is regarded as a trigger for the sequence of carcinogenesis because there is strong evidence for *H. pylori* infection as a cause of chronic atrophic gastritis and intestinal metaplasia, two possible precancerous lesions.[4–6]

Epidemiological studies have shown that *H. pylori* infection is associated closely with the development of gastric cancer.[7–12] Based on the results from 4 cohort and 9 retrospective case-control studies, a causal relationship between *H. pylori* infection and gastric cancer was concluded by the International Agency for Research on Cancer, World Health Organization (IARC) in 1994.[13] However, the evaluation made by IARC was not quantitative, leaving the magnitude of risk of infection with *H. pylori* unassessed in patients with gastric cancer.[13] Previous analysis by Forman et al. found that the relative risk for gastric cancer increased with increasing period of time between *H. pylori* seropositivity and diagnosis of gastric cancer.[14] However, conflicting reports exist in the literature regarding the association between *H. pylori* infection and the age of patients, site, histological type, and stage of gastric cancer.[7–12,15–28] This may be the reason why many clinicians are still questioning the association between *H. pylori* infection and gastric cancer. The primary aims of this study were to review systematically the published studies, combine results statistically from each study that met predefined inclusion and exclusion criteria, and calculate a summary odds ratio (OR) for *H. pylori* infection in the development of gastric cancer and various subgroups of patients with gastric cancer. We aimed to clarify, based on the raw data from each study, why the results of the published studies are so different; what possible sources of heterogeneity might exist;

Abbreviations used in this paper: 95% CI, 95% confidence interval; IARC, International Agency for Research on Cancer, World Health Organization; OR, odds ratio.

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influence of age; differences in study design; and different characteristics of gastric cancer.

Materials and Methods

Justification of the Systematic Review

A MEDLINE search of the English language literature for human studies was performed using textword "*Campylobacter pylori*" and MeSH term "stomach neoplasms" from 1983 to 1990 and using MeSH terms "*Helicobacter pylori*, stomach neoplasms" from 1990 to April 1996. A total of 47 relevant reviews was identified. With criteria described by Cook et al.[29] and by Greenland[30] and with guidelines for the application of meta-analysis in epidemiological studies,[31] these were evaluated critically by one of the authors (J.Q.H.). No review described a systematic search strategy, and methods to include reviewed articles and assessments of study validity and appropriate statistical analyses were not performed. Only 1 review analyzed statistically the association between time intervals of *H. pylori* seropositivity and gastric cancer diagnosis, and no further analyses were performed.[14] Thus, no similar review has yet been published, and this meta-analysis was therefore justified.

Inclusion and Exclusion Criteria

The following criteria were used to include published reports and abstracts: cohort or case-control studies had raw data dealing with *H. pylori* infection and gastric cancer, with age- and/or sex-matched control groups; *H. pylori* infection was detected by serology; and the study was conducted in an adult population and published in the English language. Studies without raw data for retrieval and duplicate publications were excluded.

Identification of Primary Studies

Using the search strategy described above, the same time span was searched to identify potentially relevant studies. A fully recursive search of reference lists of all review articles and of the retrieved original studies was performed to find studies not identified by the MEDLINE search. A manual review of all abstracts from the following major international meetings held in the past 3 years (1994 to May 1996) was also performed by two reviewers (J.Q.H. and S.S.): American Digestive Disease Week (1994–1995), American College of Gastroenterology (1994–1995), British Society of Gastroenterology (1994–1996), World Congress of Gastroenterology (Los Angeles, CA, 1994), European *Helicobacter pylori* Study Group VIIth and VIIIth International Workshop (1994–1995), and 3rd and 4th United European Gastroenterology Week (1994–1995). A careful manual search was also performed by one author (J.Q.H.) in major relevant journals to find reports not yet included in the computerized database.

Study Selection

Because *H. pylori* does not colonize atrophic, metaplastic, and gastric cancer epithelium,[32,33] detection of *H. pylori* infection through histological biopsy specimens might underestimate the true infection rate and potentially bias the results.[34–36] Because our primary intent was to identify any association between patients with gastric cancer and *H. pylori* infection, the detection method should reflect, as closely as possible, the true infection rate. Thus, only studies with *H. pylori* infection tested by serology were selected for further analysis, although a false-negative result may occur in patients with severe atrophic pangastritis.[37,38]

Data Extraction

Data were extracted from each report by two independent reviewers (J.Q.H. and S.S.) using a predefined review form. Details contained ethnicity of subjects, country of study, study design, age distributions in case and control populations, histological type, site, and stage of gastric cancer, methodology of *H. pylori* detection, OR, and 95% confidence intervals (95% CIs), where applicable. Studies were excluded if lack of raw data made it impossible to derive an exact number of cases or controls with and without *H. pylori* infection.

Data were subgrouped according to study design, age, histological type (intestinal or diffuse),[39] site (noncardiac or cardiac), and stage (early or advanced) of cancer based on the information from each study.

The original investigators of each study were contacted for further information on the details of the age distribution of *H. pylori* serology in both cases and controls.

Assessment of Study Quality

To assess the validity of each study, the following important criteria, modified from the guidelines for reading case-control studies proposed by Lichtenstein et al.,[40] were applied to evaluate the quality of each study: an explicit statement of the research question, the methods for identification of cases and controls and their matching techniques, validation of *H. pylori* antigen before application for detection of *H. pylori* antibody, and sample size. An overall quality score was not generated to avoid author subjectivity,[41] but validity criteria were used to rank studies (Table 1). Disagreements were resolved by discussion and consensus between the authors.

Statistical Analysis

The following statistical techniques were used to analyze the data. First, the summary ORs and 95% CIs were calculated from the raw data of the selected studies using the Mantel–Haenszel method[42] or DerSimonian and Laird method under random effect[43]; second, regression analysis was used to assess whether a linear relationship exists between *H. pylori* seropositivity and the age of patients and controls. The Breslow–Day method was used to test for homogeneity under the null hypothesis that the ORs were consistent across studies. However, if heterogeneity was shown, subgroup or sensitivity analyses were performed using the same methods as discussed above.[42,43] An attempt was made to identify the source of heterogeneity, not simply to exclude the outliers because

Table 1. Characteristics of Studies Selected for Meta-analysis

Study (country)	Study design	Mean age (case/control) (yr)	Cases	Controls	OR (95% CI)
Nomura et al.[9] (U.S.)	Cohort	59/59	109 Histologically proven	1:1 Cohort controls matched by age at serum donation, sex, DOB, POB, and laboratory data	6.0 (2.1–17.3)
Parsonnet et al.[10] (U.S.)	Cohort	54/54	109 Histologically proven	1:1 Cohort controls matched by age, sex, race, date, and residence at serum donation	3.6 (1.8–7.3)
Forman et al.[8] (England)	Cohort	54/54	29 Verified by medical records	116 Cohort controls; 1:4 matched by age, sex, DOB, and date at serum donation	2.77 (1.04–7.97)
Asaka et al.[12] (Japan)	CC	59/60	109 Histologically proven	1:1 Health screeners matched by age, sex, and POB	2.4 (1.2–4.8)
Asaka et al.[22] (Japan)	CC	60/60	213 Histologically proven	1:1 Health screeners matched by age and sex	2.56 (1.48–4.44)
Lin et al.[28] (China)	CC	52/54	100 Histologically verified	1:1 Healthy controls matched by age and sex	1.13 (0.62–2.08)
Hu et al.[21] (China)	CC	54/54	51 Histologically proven	102 Blood donors; 1:2 matched by age and sex	5.1 (1.7–15.5)
Lin et al.[11] (China)	Cohort	63/63	29 Verified by medical record	220 Controls from a cohort matched by age, sex, and residence	1.55 (0.68–2.56)
Estevens et al.[7] (Portugal)	CC	66/66	80 Histologically proven	Blood donors; 1:1 matched by age, sex, and previous GI history	0.54 (0.24–1.2)
Archimandritis et al.[18] (Greece)	CC	62/62	47 Histologically proven	50 Healthy controls matched by age, sex, socioeconomic status, and residence	1.23 (0.51–2.95)
Webb et al.[19] (China)	Cohort	61/61	85 Histologically proven	255 From a cohort matched by age, sex, date of sample collection, and residence	0.93 (0.57–1.54)
Fukuda et al.[16] (Japan)	CC	57/57	282 Histologically proven	767 Noncancer controls; 1:1–16 matched by age, sex, and date of blood sampling	1.04 (0.73–1.49)
Hansson et al.[23] (Sweden)	CC	67/67	112 Histologically proven	103 Non-GI patients matched by age, sex, and hospital of admission	2.6 (1.35–5.02)
Kuipers et al.[15] (Denmark)	CC	67/67	116 Histologically proven	Controls without endoscopic and histological abnormalities; 1:1 matched by age and sex	0.87 (0.44–1.7)
Rudi et al.[20] (Germany)	CC	60/61	111 Histologically proven	Patients with colorectal cancer; 1:1 matched by age, race, and residence	1.39 (0.82–2.36)
Talley et al.[24] (U.S.)	CC	63/61	69 Histologically proven	76 Healthy volunteers and 176 cancer-free controls matched by age, sex, and race	1.63 (0.79–3.37)
Blaser et al.[25] (Japan)	CC	63/63	29 Histologically proven	Non-GI patients; 1:2 matched by age and sex	2.14 (0.72–6.4)
Kikuchi et al.[26] (Japan)	CC	NG	757 Cases	1005 Healthy controls matched by age	4.7 (3.6–6.1)
Sipponen et al.[27] (Finland)	CC	65/65–66	54 Histologically proven	84 Non-GC controls matched by age	2.27 (1.0–5.0)

CC, case-control; DOB, date of birth; GC, gastric cancer; GI, gastrointestinal; NG, not given; POB, place of birth.

heterogeneity has been regarded as the expectation of meta-analyses in epidemiological studies and not as the exception.[44,45] Homogeneity also was expressed graphically. Third, the t test and χ^2 test were used when necessary.

For those studies in which ORs were not given, an OR and 95% CI were calculated using raw data derived from each study according to the method described by Dawson-Saunders and Trapp.[46] All ORs and 95% CIs were expressed with D-L method unless otherwise specified. All statistical analyses were performed with SAS computer software (version 3.11, Release 6.10; SAS Institute, Inc., Cary, NC, 1994) and Statistica for Windows (Release 4.5; StatSoft, Inc., Gaithersburg, MD, 1993).

Results

Peer-Reviewed Publications

MEDLINE search generated a total of 58 peer-reviewed primary studies for retrieval. However, only 32 studies reported serological testing for *H. pylori* infection. Manual search identified another three references for a total of 35 potentially relevant articles. Sixteen studies were excluded for various reasons: no matched control group,[17,47–52] no serological data[53] or raw data,[54] and same data source.[55–61] Nineteen studies met the predeter-

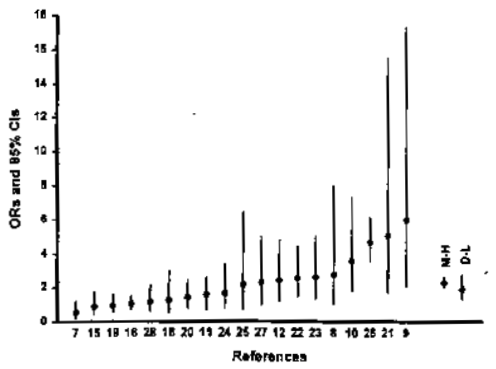

Figure 1. Graphic display of individual and summary ORs and 95% CIs of 19 studies. M-H, summary OR with Mantel–Haenszel method; D-L, summary OR with DerSimonian and Laird method.

mined inclusion criteria and were included for analysis (Table 1).

Overall Analysis

The data from 19 studies included 2491 patients with gastric cancer and 3959 controls. Overall, *H. pylori* seropositivity was 80% for patients with gastric cancer and 62.2% for controls, yielding a summary OR of 1.92 (95% CI, 1.32–2.78) (Figure 1 and Table 2).

Subgroup Analysis

Study design. Of the 19 studies, 5 were cohort (26.3%) and 14 were case control (73.7%). The summary ORs for cohort and case-control studies were 2.24 (95% CI, 1.15–4.40) and 1.81 (95% CI, 1.16–2.84), respectively. Statistical analysis did not show any significant heterogeneity in OR between study designs ($Z^2 = 1.4$; $df = 1$; $P = 0.2$), which therefore were not considered to be a source of heterogeneity.

Studies with population-based controls versus hospital-based controls. Population-based control groups were used in 13 of 19 studies consisting of 1787 patients and 2720 controls. *H. pylori* seropositivity was diagnosed in 82.4% of the patients and 58.8% of the controls, respectively, giving an estimate of OR at 2.11 (95% CI, 1.30–3.43) (Table 2).

Hospital-based control groups were used in the remaining 6 studies, including 704 patients and 1239 controls. *H. pylori* infection was detected in 74% of the patients and 69.5% of the controls, giving a summary OR of 1.49 (95% CI, 1.06–2.1) (Table 2).

H. pylori infection was significantly more common in hospital-based controls than in population-based controls ($\chi^2 = 41.19$; $df = 1$; $P < 0.0001$). There also was a significant difference in *H. pylori* infection between the two groups of patients ($\chi^2 = 22.43$; $df = 1$; $P = 0.0001$). This may be attributed to the difference in the mean age of both controls and cases in these two subgroups. The mean age was 63.2 years (n = 6; SD = 4.02) for hospital-based control groups and 58.9 years (n = 12; SD = 4.41) for population-based control groups with 1 large study excluded because mean age was not reported[26] ($t = 2.07$; $P = 0.063$).

Age difference. Original data on the age distribution in both cases and controls were obtained from the investigators of 14 original studies consisting of 2127 cases (85.4% of total cases) and 3157 controls (79.7% of total controls).

In the control group, the prevalence of *H. pylori* infection increased significantly with advancing age ($r = 0.9295$; $P = 0.0073$) (Figure 2). However, in pa-

Table 2. Summary of Results Analyzed With Two Different Models

		DerSimonian and Laird		Mantel–Haenszel				
Subgroups	n	OR	95% CI	OR	95% CI	$P_{(B-D)}$ (df = N-1)	Z^2 [a] (df = 1)	P value[a]
All studies	19	1.92	1.32–2.78	2.29	2.04–2.58	0.001		
Cohort	5	2.24	1.15–4.40	1.93	1.44–2.59	0.001	1.40	0.2
Case-control	14	1.81	1.16–2.84	2.37	2.08–2.69	0.001		
Intestinal GC	10	2.49	1.41–4.43	2.23	1.74–2.87	0.001	1.29	0.3
Diffuse GC	10	2.58	1.47–4.53	2.85	2.14–3.79	0.001		
Early GC	3	6.35	1.88–21.5	6.40	3.76–10.9	0.05	6.01	0.01
Advanced GC	3	2.13	0.42–10.7	2.20	1.51–3.21	0.001		
Cardiac GC	7	1.23	0.56–2.71	1.41	1.00–1.98	0.001	8.69	0.003
Cardiac GC[b]	6	0.92	0.61–1.38	0.93	0.62–1.38	0.4	20.4	<0.001
Noncardiac GC	10	3.08	1.78–5.31	2.77	2.20–3.50	0.001		
Population-based	13	2.11	1.30–3.43	2.89	2.51–3.43	0.001	29.9	<0.001
Hospital-based	6	1.49	1.06–2.10	1.37	1.11–1.70	0.07		

GC, gastric cancer; n, number of studies; $P_{(B-D)}$, P value of Breslow–Day test for heterogeneity with >0.10 indicating no significance in heterogeneity.

[a]Comparison between two subgroups.

[b]One study excluded with unclear definition of the site of cancer.[55]

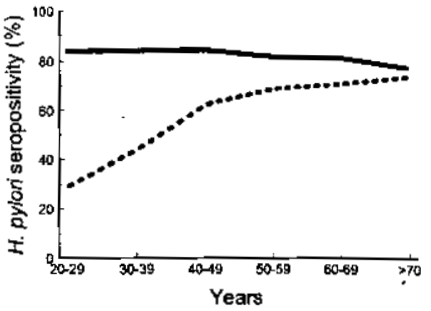

Figure 2. Age distribution of *H. pylori* seropositivity in gastric cancer cases (——) and controls (- - -) in 14 studies.

tients with gastric cancer, the *H. pylori* infection rate was >80% from age 20 to 69 years and correlated inversely with increasing age ($r = -0.874$; $P = 0.0228$) (Figure 2). The latter finding may reflect the loss of colonization by the bacteria in severely atrophic stomachs with increasing age. The ORs decreased significantly with increasing age from 9.29 (95% CI, 3.43–34.04) at age 20–29 years to 1.05 (95% CI, 0.73–1.52) at ≥70 years of age (Table 3) (trend analysis, $P = 0.005$).

Cardiac versus noncardiac gastric cancer. Of the 19 studies, 6 had information for both cardiac and noncardiac gastric cancers.[9,19,20,23,24,55] Four studies only gave data for noncardiac gastric cancer.[18,21,25,27] One study provided data for both cardiac and noncardiac gastric cancer, but the site of gastric cancer could not be determined in 38% of the patients, and this study was therefore not included in the subgroup analysis for noncardiac gastric cancer.[10] In the noncardiac group, 79.2% (467 of 590) of patients with cancer and 53.7% (594 of 1106) of controls were *H. pylori* seropositive, giving an OR estimate of 3.08 (95% CI, 1.78–5.31) (Table 2).

In the cardiac group, *H. pylori* infection was diagnosed in 57% (90 of 158) of patients and 50.3% (383 of 761) of controls, respectively, yielding a summary OR of 1.23 (95% CI, 0.56–2.71). However, 1 study did not give any information on how the proximal site of cancer was defined, and the result of this study was very different from the others. Sensitivity analysis of the other 6 studies without this outlier was performed, and the summary OR was 0.92 (95% CI, 0.61–1.38). Test of homogeneity showed no heterogeneity across these studies ($P = 0.5$) (Table 2).

There was no significant difference in *H. pylori* seropositivity between the two control groups ($\chi^2 = 2.06$; $df = 1$; $P = 0.151$). However, there was a significant difference between patients with cardiac and noncardiac gastric cancer ($\chi^2 = 32.27$; $df = 1$; $P < 0.0001$).

Early versus advanced gastric cancer. Three studies provided raw data on patients with early and advanced cancers and controls.[7,22,55] *H. pylori* seropositivity was found in 92.7% (164 of 177) of patients with early gastric cancer and 66.5% (262 of 394) of the controls, yielding an OR estimate of 6.35 (95% CI, 1.88–21.5). In patients with advanced gastric cancer compared with the controls, *H. pylori* seropositivity was detected in 80.7% (167 of 207) and 66.5% (262 of 394), respectively (Table 4), giving an OR estimate of 2.13 (95% CI, 0.42–10.7) (Table 2).

There was no difference in the mean age of these two groups. However, a significant difference in *H. pylori* seropositivity was found between patients with early and advanced gastric cancers (Table 4). The test of homogeneity between early and advanced gastric cancers was significant ($P = 0.01$). Therefore, difference in the stage of gastric cancer was considered to be a source of heterogeneity.

Diffuse- versus intestinal-type gastric cancer. Ten studies investigated the difference in *H. pylori* infection between patients with the diffuse and intestinal type of gastric cancer.[9,10,15,18,20–24,55] *H. pylori* seropositivity was diagnosed in 82% (456 of 556) of patients with intestinal-

Table 3. *H. pylori* Seroprevalence in Patients With Gastric Cancer and Matched Controls Stratified by 10-Year Intervals[7–10,12,15,16,18–20,22,24,26,27]

	Cases		Controls			
Age (yr)	HP+	HP−	HP+	HP−	OR	95% CI
<20	0	2	0	2	0	0
20–29	21	4	62	153	9.29	3.43–34.04
30–39	124	23	126	160	7.27	4.33–12.2
40–49	261	47	355	214	3.65	2.52–5.29
50–59	477	107	618	282	1.86	1.42–2.44
60–69	606	142	620	261	1.46	1.14–1.88
≥70	240	73	223	81	1.05	0.73–1.52

HP+, *H. pylori* seropositivity; HP−, *H. pylori* seronegativity; OR, odds ratio with Mantel–Haenszel method.

Table 4. Seroprevalence of *H. pylori* Infection in Patients With Early and Advanced Gastric Cancers and Matched Controls in Three Studies

	Early GC		Advanced GC		Controls	
Study	HP+	HP−	HP+	HP−	HP+	HP−
Estevens et al.[7]	8	1	39	18	65	15
Asaka et al.[22]	119	9	72	13	159	54
Kikuchi et al.[55]	37	3	56	9	38	63
Combined[a]	164	13	167	40	262	132

GC, gastric cancer; HP+, *H. pylori* seropositivity; HP−, *H. pylori* seronegativity.

[a]The combined result showed a significant difference in *H. pylori* seropositivity between patients with early and advanced gastric cancers ($\chi^2 = 11.51$; $df = 1$; $P = 0.001$).

type gastric cancer and 59.3% (718 of 1210) of controls, yielding a summary OR estimate of 2.49 (95% CI, 1.41–4.43).

In patients with the diffuse type of gastric cancer, 82.2% (310 of 377) of the cases and 58.9% (678 of 1152) of controls were seropositive, yielding an OR estimate of 2.58 (95% CI, 1.47–4.53) (Table 2).

There was no significant difference in *H. pylori* seropositivity between these two groups of patients and the controls (χ^2 test: $df = 1$; $P = 0.933$ and $P = 0.811$, respectively) (Figure 3). Furthermore, test of homogeneity showed no difference between these two subgroups ($P = 0.3$). Therefore, the histological type of gastric cancer was not considered as a source of heterogeneity.

Discussion

Since Warren and Marshall first isolated *H. pylori* from the stomachs of patients with gastritis,[62] numerous epidemiological studies and reviews have been published regarding the relationship between *H. pylori* infection and the development of gastric cancer.[13] However, none of the previous reviews has evaluated the literature systematically and statistically with the exception of 1 article by Forman et al.[14] To our knowledge, this is the first comprehensive meta-analysis addressing the relationship between *H. pylori* seropositivity and the subsequent risk of developing gastric cancer.

We have found that the overall OR of infection with *H. pylori* for developing gastric cancer is 1.92 (95% CI, 1.32–2.78). This result supports quantitatively the conclusion by IARC that infection with *H. pylori* is a risk factor for gastric cancer in humans,[13] although this might have underestimated the real attributable risk of *H. pylori* infection for gastric cancer caused by inclusion of some studies with short-term follow-up.[11,19]

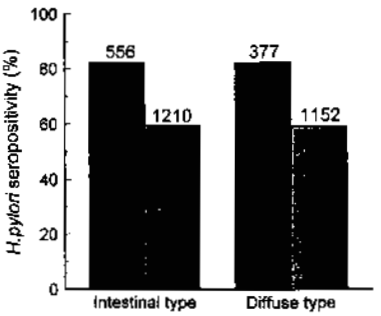

Figure 3. Comparison of *H. pylori* seropositivity between patients with intestinal- or diffuse-type gastric cancers and controls. The number above each *bar* represents the number of total subjects. ■, Gastric cancer; ■, controls.

The previous analysis reported by Forman et al.[14,63,64] combined three prospective studies and found that the relative risk increased dramatically with the duration of follow-up ranging from 2.13 within the first 5 years to 8.67 beyond 15 years. In these studies, the diagnosis of *H. pylori* infection was based on the presence of specific immunoglobulin G antibodies in serum samples collected up to 24 years before the diagnosis of cancer. Indeed, the follow-up time period is an important factor in determining the result of a cohort study because several short-term studies have failed to show a positive correlation between *H. pylori* infection and gastric cancer.[11,19] However, several other factors also may affect the result, such as the prevalence of *H. pylori* infection in the control group, the age of the study population, and the characteristics of gastric cancer.

Study design was not found to be a source of heterogeneity, and there was no significant difference in summary ORs between case-control and cohort studies, despite a wide variation of ORs among individual studies (Tables 1 and 2). The heterogeneity observed within the same study design may result from a number of differences in patient characteristics in each study, and this has been confirmed in our meta-analysis (Table 2) and will be discussed in detail below.

The selection of a control group has a major impact on the result of a case-control study. We found a significant difference in *H. pylori* seropositivity between studies with population- and hospital-based controls, although this difference may be confounded by the mean age of the subjects. Using a primary or a secondary study base as a control group is entirely dependent on the principles of comparability between cases and controls and the assumptions that the selected control is a random sample from the same study population that produced the cases.[65] Hospital-based controls are considered a nonrandom subset of the study population rather than a random sample from the study base.[65] On application of this control group, one must assume that the distribution of the exposure under study is the same as that in the control group from the study base.[66] However, this seems not to be the case in some of the studies that have used hospital-based controls[15,20] or unknown medical conditions.[16] This might be related to the absence of association between *H. pylori* infection and gastric cancer reported in these studies. Therefore, a well-defined and validated control group is essential to a case-control study to maximize the representativeness of the controls to the study base.

The difference in *H. pylori* prevalence shows a strong age dependency in the general populations in both developing and developed countries,[67] and this also is the

case in studies examining the risk of *H. pylori* infection for gastric cancer. As shown in Figure 2, *H. pylori* seroprevalence increased significantly with advancing age in the control groups but not in the cases. Thus, if gastric cancer patients of any age have a higher prevalence of the infection, then case controls would be expected to show a larger differential at younger rather than older ages. Several studies indicate that *H. pylori*–infected younger patients have a higher risk for the development of gastric cancer than older patients,[9,16,23,25,26] contrary to other studies.[11,15,18,19] In a study from Japan, Kikuchi et al. compared 105 case subjects that were younger than 40 years with different sets of well-matched controls and found an OR of 13.3 (95% CI, 5.3–35.6), the strongest association ever reported.[55] Was this a real effect or an artifact caused by a lower background of *H. pylori* infection in the younger populations? In this meta-analysis, we have shown, by obtaining raw data on the age distributions in both cases and controls from 14 studies, that *H. pylori*–infected younger patients do have a greater relative risk for gastric cancer than older age groups (Table 3). The relative risk decreased significantly with increasing age, becoming insignificant in those older than 70 years. The increasing prevalence of *H. pylori* infection in the older control groups is a principal factor determining the difference in the relative risk. The lower seroprevalence of the infection in the older patients seen in this analysis may be attributed to the underdetection of serum antibodies against *H. pylori*[37] or a result of spontaneous disappearance of the infection caused by increasing mucosal atrophy and intestinal metaplasia with advancing age.[16,38,68] However, this raises the question as to why this happened only to the patients and not to the control groups. There are several possibilities for this difference. First, patients with gastric cancer generally have more severe mucosal atrophy and intestinal metaplasia in the stomach than normal subjects,[5,69] which may increase the chance of underdetection or spontaneous loss of the infection. Atrophy and intestinal metaplasia may worsen with increasing age[38] and eventually lead to carcinogenesis of the stomach in some patients,[70] a condition in which *H. pylori* virtually cannot colonize.[33] Second, different bacterial strains may be involved in different diseases. Patients with gastric cancer are reported to be infected more frequently with toxic strains of *H. pylori* compared with their controls.[71] There is a strong association between infection with *cagA*-positive bacteria and an increased risk for intestinal-type gastric cancer.[72] Moreover, *cagA* antibodies have been associated with the progression of atrophic gastritis.[73,74]

It has been long thought that, in contrast to diffuse-type gastric cancer, intestinal-type gastric cancer is more closely linked to environmental and perhaps socioeconomic factors.[75] However, reports regarding the association of *H. pylori* infection with intestinal-type gastric cancer have been inconsistent in studies with *H. pylori* infection diagnosed by histology.[13] The histological prevalence of *H. pylori* infection has been generally greater in the intestinal-type than in diffuse-type gastric cancer,[53,76,77] although this may result from sampling error.[78] In serological studies, most of the data have not shown a difference in *H. pylori* infection between these two types of cancer, except one report by Hansson et al. who showed a positive association between *H. pylori* infection and the intestinal type but not the diffuse type of gastric cancer.[23] However, this difference has little significance because there was a large overlap of 95% CIs in OR between these two types of cancer. Another study from the same group that carefully stratified patients by age and the site of cancer did not show any difference in *H. pylori* seroprevalence between the two types.[79] In this meta-analysis, we have shown, by combining data from 10 studies, that both histological types have an almost equal association with *H. pylori* infection, suggesting that the difference between these two types of cancer may be histological but not etiologic. The hypothesis could be that simple inflammation or early chronic gastritis is prone to the development of the diffuse type, whereas the atrophic mucosa or intestinal metaplasia favors the intestinal type of gastric cancer.[27,80] Thus, *H. pylori* infection may be the trigger for subsequent histological changes in the stomach, and the evolution to certain types of gastric cancer depends on the interaction between the host and other environmental risk factors.[8,23] A recent study from Brazil provides some support for this hypothesis, showing that *H. pylori* isolates from patients with these two types of cancer expressed both *cagA* and *vacA* genes to a similar degree.[81] Furthermore, a large pathological study also has shown that both types of gastric cancer start from *H. pylori* gastritis and share many cell phenotypes such as *p53*, although the pathways leading to gastric carcinogenesis may be different.[82]

It is understandable that patients with early gastric cancer have a greater detection rate of *H. pylori* infection than those with advanced cancer by either histological or serological methods because the bacteria does not colonize severely atrophic epithelium or areas of intestinal metaplasia.[32,33] This may account for the differences in *H. pylori* prevalence detected by histology reported in the literature[78,83,84] if sampling error is avoided.[78] Serological testing for *H. pylori* infection has a better sensitivity than histology in patients with gastric cancer.[34,35] The difference in *H. pylori* positivity, if any, between patients with early and advanced gastric cancer could be shown by

serological testing. However, none of the 3 studies included in this analysis showed a significant difference in *H. pylori* seropositivity between the two groups of patients. This was caused by inadequate numbers of subjects included in each study as determined by this meta-analysis because the difference reached significance after they were combined (Table 4). This is a good example of the advantages of meta-analysis in situations in which individual studies lack the statistical power to detect a difference between two groups.[31]

The association of *H. pylori* infection with noncardiac gastric cancer has been well documented, although results vary among studies.[9,18–21,23–25,27,55] However, the overall evaluation of these studies confirmed that *H. pylori* is a risk factor for noncardiac gastric cancer but not for cancer of the cardia. This result provides further evidence that infection with *H. pylori* does not increase the risk for the development of gastric cancer at the cardia.[9,10,19,20,23,24]

Results of recent studies suggest that the clinical expressions of *H. pylori* infection depend primarily on two factors: the pathogenicity of the bacteria and the host response. We know little about the difference in host response to the infection, but extensive studies have been performed on bacterial virulence in different diseases. *cagA*-positive strains of *H. pylori* have been commonly isolated from patients with atrophic gastritis[73,74] and correlate with an increased frequency of intestinal metaplasia.[85] A study from Japan has shown that there is a correlation between the prevalence and severity of atrophic gastritis and the cytotoxic activity of the bacteria.[86] Infection with type I strains of *H. pylori* increases the risk for gastric cancer by 5.8-fold compared with noninfected patients.[71] An interesting case-control study from Rudi et al. has shown a significant difference in *cagA* and *vacA* phenotypes between patients with gastric cancer and matched controls, although no difference was found in *H. pylori* immunoglobulin G antibodies between the two groups.[87] This result provides us with an important explanation for why some studies may have failed to show a correlation between patients and controls. If further analysis of the bacterial strains could have been done in these studies, the results might have been different. Taking the above together, a possible explanation might, at least in part, be that because of the puzzling geographical differences in the contrasting incidences of duodenal ulcer and gastric cancer, which are mutually exclusive to each other,[88,89] the geographical difference of the prevalence of upper gastrointestinal diseases may be caused by infection with different strains of *H. pylori* in different populations. However, for the etiology of gastric cancer, other environmental and perhaps genetic factors cannot be ignored.[72]

In summary, our meta-analysis has confirmed quantitatively the conclusion by IARC that infection with *H. pylori* is an important risk factor for gastric cancer in humans. *H. pylori*–infected younger patients have a greater relative risk for gastric cancer than older patients. Both the intestinal and diffuse type of gastric cancer are similarly associated with the infection. *H. pylori* infection is detected more commonly in patients with early gastric cancer than in those with advanced disease, possibly because of loss of the bacterial colonization in progressively atrophic stomachs. *H. pylori* infection is not a causal factor for cardiac gastric cancer, but infection is associated strongly with noncardiac gastric cancer. Finally, we suggest for the future that a well-selected control group is essential for a case-control study. Patients with early or advanced cancer and with noncardiac or cardiac cancer should be separated for analysis. More sensitive detection methods may be required to reduce the false negativity of the current serological tests.

References

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 1993;54:594–606.
2. Coggon D, Acheson ED. The geography of cancer of the stomach. Br Med Bull 1984;40:335–341.
3. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res 1992;52:6735–6740.
4. Antonioli DA. Precursors of gastric carcinoma: a critical review with a brief description of early (curable) gastric cancer. Hum Pathol 1994;25:994–1005.
5. Sipponen P, Kekki M, Haapakoski J, Ihamäki T, Siurala M. Gastric cancer risk in chronic atrophic gastritis: statistical calculations of cross-sectional data. Int J Cancer 1985;35:173–177.
6. Correa P. Precursors of gastric and esophageal cancer. Cancer 1982;50:2554–2565.
7. Estevens J, Fidalgo P, Tendeiro T, Chagas C, Ferra A, Nobre Leitao C, Costa Mira F. Anti-*Helicobacter pylori* antibodies prevalence and gastric adenocarcinoma in Portugal: report of a case-control study. Eur J Cancer Prevent 1993;2:377–380.
8. Forman D, Newell DG, Fullerton F, Yarnell JWG, Stacey AR, Wald N, Sitas F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ 1991;302:1302–1305.
9. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325:1132–1136.
10. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–1131.
11. Lin JT, Wang LY, Wang JT, Wang TH, Yang CS, Chen CJ. A nested case-control study on the association between *Helicobacter pylori* infection and gastric cancer risk in a cohort of 9775 men in Taiwan. Anticancer Res 1995;15:603–606.
12. Asaka M, Kato M, Kudo M, Katagiri M, Nishikawa K, Yoshida J, Takeda H, Miki K. Relationship between *Helicobacter pylori*

infection, atrophic gastritis and gastric carcinoma in a Japanese population. Eur J Gastroenterol Hepatol 1995;7(Suppl 1):S7–S10.
13. International Agency for Research on Cancer. Schistosomes, liver flukes and *Helicobacter pylori* IARC monographs on the evaluation of carcinogenic risks to humans. Volume 61. Lyon, France: IARC, 1994.
14. Forman D, Webb P, Parsonnet J. *H. pylori* and gastric cancer. Lancet 1994;343:243–244.
15. Kuipers EJ, Gracia-Casanova M, Pena AS, Pals G, Van Kamp G, Kok A, Kurz-Pohlmann E, Pels NFM, Meuwissen GM. *Helicobacter pylori* serology in patients with gastric carcinoma. Scand J Gastroenterol 1993;28:433–437.
16. Fukuda H, Saito D, Hayashi S, Hisai H, Ono H, Yoshida S, Oguro Y, Noda T, Sato T, Katoh M, Terada M, Sugimura T. *Helicobacter pylori* infection, serum pepsinogen level and gastric cancer: a case-control study in Japan. Jpn J Cancer Res 1995;86:64–71.
17. Lin JT, Wang JT, Wang TH, Wu MS, Lee TK, Chen CJ. *Helicobacter pylori* infection in a randomly selected population, healthy volunteers, and patients with gastric ulcer and gastric adenocarcinoma. A seroprevalence study in Taiwan. Scand J Gastroenterol 1993;28:1067–1072.
18. Archimandritis A, Bitsikas J, Tjivras M, Anastasakou E, Tsavaris N, Kalogeras D, Davaris P, Fertakis A. Non-cardia gastric adenocarcinoma and *Helicobacter pylori* infection. Ital J Gastroenterol 1993;25:368–371.
19. Webb PM, Yu MC, Forman D, Henderson BE, Newell DG, Yuan JM, Gao YT, Ross RK. An apparent lack of association between *Helicobacter pylori* infection and risk of gastric cancer in China. Int J Cancer 1996;67:603–607.
20. Rudi J, Müller M, von Herbay A, Zuna I, Raedsch R, Stremmel W, Räth U. Lack of association of *Helicobacter pylori* seroprevalence and gastric cancer in a population with low gastric cancer incidence. Scand J Gastroenterol 1995;30:958–963.
21. Hu PJ, Micheli HM, Li YY, Zhou MH, Hanzell SL. Association of *Helicobacter pylori* with gastric cancer and observations on the detection of this bacteria in gastric cancer cases. Am J Gastroenterol 1994;89:1806–1810.
22. Asaka M, Kimura T, Kato M, Kudo M, Miki K, Ogoshi K, Kato T, Tasuta M, Graham DY. Possible role of *Helicobacter pylori* infection in early gastric cancer development. Cancer 1994;73:2691–2694.
23. Hansson LE, Engstrand L, Nyrén O, Evans DJ Jr, Lindgren A, Bergström R, Andersson B, Athlin L, Bendsten O, Tracz P. *Helicobacter pylori* infection: independent risk indicator of gastric adenocarcinoma. Gastroenterology 1993;105:1098–1103.
24. Talley NJ, Zinsmeister AR, Weaver A, DiMagno EP, Carpenter HA, Pérez-Pérez GI, Blaser MJ. Gastric adenocarcinoma and *Helicobacter pylori* infection. J Natl Cancer Inst 1991;83:1734–1739.
25. Blaser MJ, Kobayashi K, Cover TL, Cao P, Feurer ID, Pérez-Pérez GI. *Helicobacter pylori* infection in Japanese patients with adenocarcinoma of the stomach. Int J Cancer 1993;55:799–802.
26. Kikuchi S, Wada O, Kurosawa M, Nakajima T, Kobayashi O, Yamazaki T, Kikuichi M, Mori K, Oura S, Watanabe H, Nagawa H, Otani R, Inaba Y, Okamoto N, Anzai H, Kubo T, Konishi T, Futagawa S, Mizobuchi N, Kobori O, Kaise R, Sato T, Nishi T, Sato H, Ishibashi T, Ichikawa S, Hirata T, Sato N, Miki K, Myoga A. Association between gastric cancer and *H. pylori* with reference to age. Gut 1995;37(Suppl 1):A8.
27. Sipponen P, Kosunen TU, Valle J, Riihelä M, Seppälä K. *Helicobacter pylori* infection and chronic gastritis in gastric cancer. J Clin Pathol 1992;45:319–323.
28. Lin JT, Wang JT, Wu MS, Huang SC, Wang TH. Serum levels of pepsinogen I and gastrin in gastric carcinoma: the influence of *Helicobacter pylori* infection and tumor characteristics. Hepatogastroenterology 1993;40:600–603.
29. Cook DJ, Sackett DL, Spitzer WO. Methodologic guidelines for systematic reviews of randomized control trials in health care from the Potsdam consultation on meta-analysis. J Clin Epidemiol 1995;48:167–171.
30. Greenland S. Quantitative methods in the review of epidemiologic literature. Epidemiol Rev 1987;9:1–30.
31. Blair A, Burg J, Foran J, Gibb H, Greenland S, Morris R, Raabe G, Savitz D, Teta J, Watenberg D, Wong O, Zimmerman R. Guidelines for application of meta-analysis in environmental epidemiology. Regul Toxicol Pharmacol 1995;22:189–197.
32. Hanzell SL, Hennessy WB, Borody TJ, Carrick J, Ralston M, Brady L, Lee A. *Campylobacter pyloridis* gastritis II: distribution of bacteria and associated inflammation in the gastroduodenal environment. Am J Gastroenterol 1987;82:297–301.
33. Robey-Cafferty SS, Ro JY, Cleary KR. The prevalence of *Campylobacter pylori* in gastric biopsies from cancer patients. Mod Pathol 1989;2:473–476.
34. Lin JT, Wang JT, Wu MS, How SW, Wang TH. Detection of *Helicobacter pylori* infection in patients with gastric adenocarcinoma by serology, histology and polymerase chain reaction (abstr). Am J Gastroenterol 1994;89:1359.
35. Karnes WE, Jr, Samloff IM, Kekki SM, Sipponen P, Kim SWR, Walsh JH. Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. Gastroenterology 1991;101:167–174.
36. Menegatti M, Vaira D, Holton J, Miranda F, Ricci, Gusmaroli R, Ainley C, Miglioli M, Barbara L. Serological response to *Helicobacter pylori* in gastric and non-gastric cancer. Clin Sci 1996;91:219–223.
37. Crabtree JE, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN, Morgan AG. Systemic and mucosal humoral responses to *Helicobacter pylori* in gastric cancer. Gut 1993;34:1339–1343.
38. Fontham ETH, Ruiz B, Perez A, Hunter F, Correa P. Determinants of *Helicobacter pylori* infection and chronic gastritis. Am J Gastroenterol 1995;90:1094–1101.
39. Laurén P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. APMIS 1965;64:31–49.
40. Lichtenstein MJ, Mulrow CD, Elwood PC. Guidelines for reading case-control studies. J Chron Dis 1987;40:893–903.
41. Greenland S. Invited commentary: a critical look at some popular meta-analytic methods. Am J Epidemiol 1994;140:290–296.
42. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Nati Cancer Inst 1959;22:719–748.
43. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–188.
44. Colditz GA, Burdick E, Mosteller F. Heterogeneity in meta-analysis of data from epidemiologic studies: a commentary. Am J Epidemiol 1995;142:371–382.
45. Berlin JA. Invited commentary: benefits of heterogeneity in meta-analysis of data from epidemiological studies. Am J Epidemiol 1995;142:383–387.
46. Dawson-Saunders B, Trapp RG. Summarizing data in medical research. In: Dawson-Saunders B, Trapp RG, eds. Basic and clinical biostatistics. East Norwalk, CT: Appleton & Lange, 1990:43–63.
47. Menegatti M, Vaira D, Miglioli M, Holton J, Nergura M, Biasco G, Petronelli A, Ricci C, Azzarone P, Gusmaroli R, Milesi F, Maiolo P, Casadei A, Rodi M, Giorcelli W, Zambelli A, Lupinacci G, Pannuti F, Barbara L. *Helicobacter pylori* in patients with gastric and nongastric cancer. Am J Gastroenterol 1995;90:1278–1281.
48. Prabhu SR, Amrapurkar AD, Amrapurkar DN. Role of *Helicobacter pylori* in gastric carcinoma. Natl Med J India 1995;8:58–60.
49. Kato T, Saito Y, Niwa M, Inoue H, Motoyama N, Ogoshi K, Nashimoto A, Sasaki J, Sato T, Asaka M. *Helicobacter pylori*

infection in gastric carcinoma. Eur J Gastroenterol Hepatol 1994;6(Suppl 1):S93–S96.
50. Muszyński J, Dzierzanowska D, Siemińska J, Bogdanska M, Vogt E. Is *Helicobacter pylori* infection a real risk factor for gastric carcinoma? Scand J Gastroenterol 1995;30:647–651.
51. Lee WJ, Lin JT, Shun CT, Lee WC, Yu SC, Lee PH, Chang KJ, Wei TC, Chen KM. Comparison between resectable gastric adenocarcinomas seropositive and seronegative for *Helicobacter pylori*. Br J Surg 1995;82:802–805.
52. Vorobjova T, Talja I, Nilsson I, Wadström T, Tammaru M, Popova N, Uibo U. The comparison of occurrence of IgG antibodies to high molecular weight proteins of *Helicobacter pylori* antigen in gastric cancer patients and controls in Estonia. Gut 1995;37(Suppl 1):A96.
53. Loffeld RJLF, Willems I, Flendrig JA, Arends JW. *Helicobacter pylori* and gastric carcinoma. Histopathology 1990;17:537–541.
54. Hansen S, Vollset SE, Melby K, Jellum E. Strong association of previous *Helicobacter pylori* infection to distal gastric adenocarcinoma (abstr). Am J Gastroenterol 1994;89:1358.
55. Kikuchi S, Wada O, Nakajima T, Nishi T, Kobayashi O, Konishi T, Inaba Y, and the Research Group on Prevention of Gastric Carcinoma Among Young Adults. Serum anti–*Helicobacter pylori* antibody and gastric carcinoma among young adults. Cancer 1995;75:2789–2793.
56. Lin JT, Wang JT, Wang TH, Wu MS, Chen CJ. *Helicobacter pylori* infection in early and advanced gastric adenocarcinoma: a seroprevalence study in 143 Taiwanese patients. Hepatogastroenterology 1993;40:596–599.
57. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111–2115.
58. Rudi J, Müller M, von Herbay A, Meyer M, Kommerell B, Räth U. Lack of association of *Helicobacter pylori* infection and gastric cancer in an area of low gastric cancer (abstr). Gastroenterology 1994;106:A169.
59. Webb PM, Newell DG, Yuan JM, Yu M, Ross R, Gao YT, Forman D. A prospective investigation of the association between *H. pylori* infection and gastric cancer in China (abstr). Gut 1995;37(Suppl 1):308.
60. Blaser MJ, Chyou PH, Nomura A. Age at establishment of *Helicobacter pylori* infection and gastric carcinoma, gastric ulcer, and duodenal ulcer risk. Cancer Res 1995;55:562–565.
61. Parsonnet J, Samloff IM, Nelson LM, Orentreich N, Vogelman JH, Friedman GD. *Helicobacter pylori*, pepsinogen, and risk for gastric adenocarcinoma. Cancer Epidemiol Biol Prevent 1993;2:461–466.
62. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1:1273–1275.
63. Forman D. *Helicobacter pylori* and gastric cancer. Scand J Gastroenterol 1996;31(Suppl 220):23–26.
64. Forman D, Webb PM. *H. pylori* and gastric cancer: the significance of the problem. In: Hunt RH, Tytgat GNJ, eds. *Helicobacter pylori* basic mechanisms to clinical cure. London: Kluwer Academic, 1994.
65. Wacholder S, McLaughlin JK, Silverman DT, Mandel JS. Selection of controls in case-control studies. I. Principles. Am J Epidemiol 1992;135:1019–1028.
66. Miettinen OS. The "case-control" study: valid selection of subjects. J Chronic Dis 1985;38:543–548.
67. Pounder RE, Ng D. The prevalence of *Helicobacter pylori* infection in different countries. Aliment Pharmacol Ther 1995;9(Suppl 2):33–39.
68. Rugge M, Cassaro M, Leandro G, Baffa R, Avellini C, Bufo P, Stracca V, Battaglia G, Fabiano A, Guerini A, Di Mario F. *Helicobacter pylori* in promotion of gastric carcinogenesis. Dig Dis Sci 1996;41:950–955.
69. Kato S, Onda M, Matsukura N, Tokunaga A, Matsuda N, Yamashita K, Shields P. Genetic polymorphisms of the cancer related gene and *Helicobacter pylori* infection in Japanese gastric cancer patients: an age and gender matched case-control study. Cancer 1996;77:1654–1661.
70. Sakaki N, Momma K, Egawa N, Yamada Y, Kan T, Ishiwata J. The influence of *Helicobacter pylori* infection on the progression of gastric mucosal atrophy and occurrence of gastric cancer. Eur J Gastroenterol Hepatol 1995;7(Suppl 1):S59–S62.
71. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with cagA positive or cagA negative *Helicobacter pylori* infection. Gut 1997;40:297–301.
72. Forman D. Essential co-factors in gastric carcinogenesis. In: Hunt RH, Tytgat GNJ, eds. *Helicobacter pylori* basic mechanisms to clinical cure 1996. London: Kluwer Academic, 1996:255–261.
73. Beales ILP, Crabtree JE, Scunes D, Covacci A, Calam J. Antibodies to *cagA* protein are associated with gastric atrophy in *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 1996;8:645–649.
74. Kuipers EJ, Pérez-Pérez GI, Meuwissen SGM, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA*. J Natl Cancer Inst 1995;87:1777–1780.
75. Howson CP, Hiyama T, Wynder EL. The decline in gastric cancer: epidemiology of an unplanned triumph. Epidemiol Rev 1986;8:1–27.
76. Buruk F, Berberoglu U, Pak I, Aksaz E, Celen O. Gastric cancer and *Helicobacter pylori* infection. Br J Surg 1993;80:378–379.
77. Parsonnet J, Vandersteen D, Goates J, Sibley RK, Pritikin J, Chang Y. *Helicobacter pylori* infection in intestinal- and diffuse-type gastric adenocarcinomas. J Natl Cancer Inst 1991;83:640–643.
78. Endo S, Ohkusa T, Saito Y, Fujiki K, Okayasu I, Sato C. Detection of *Helicobacter pylori* infection in early stage gastric cancer. A comparison between intestinal- and diffuse-type gastric adenocarcinomas. Cancer 1995;75:2203–2208.
79. Hansson LE, Engstrand L, Nyrén O, Lindgren A. Prevalence of *Helicobacter pylori* infection in subtypes of gastric cancer. Gastroenterology 1995;109:885–888.
80. Sipponen P. *Helicobacter pylori* infection—a common worldwide environmental risk factor for gastric cancer? Endoscopy 1992;24:424–427.
81. Queiroz DMM, Mendes EN, Rocha GA, Soares TF, Lima GF, Oliveira CA. *H. pylori* strains possessing *cagA* and vacuolating cytotoxin producers are associated to both types of gastric carcinoma (abstr). Gastroenterology 1996;110:A236.
82. Solcia E, Fiocca R, Luinetti O, Villani L, Padovan L, Calistri D, Ranzani GN, Chiaravalli A, Capella C. Intestinal and diffuse gastric cancers arise in a different background of *Helicobacter pylori* gastritis through different gene involvement. Am J Surg Pathol 1996;20(Suppl 1):S8–S22.
83. Lucia Caruso M, Fucci L. Histological identification of *Helicobacter pylori* in early and advanced gastric cancer. J Clin Gastroenterol 1990;12:601–602.
84. Craanen ME, Blok P, Dekker W, Tytgat GNJ. *Helicobacter pylori* and early gastric cancer. Gut 1994;35:1372–1374.
85. Crabtree JE, Wyatt JI, Perry S, Davis GR, Covacci A, Morgan AG. *CagA* seropositive *Helicobacter pylori* infected non-ulcer patients have increased frequency of intestinal metaplasia (abstr). Gastroenterology 1996;110:A85.
86. Ito S, Murakita H, Hirai M, Azuma T, Miyaji H, Ito Y, Ohtaki Y, Yamazaki Y, Kuriyama M, Keida Y, Kohli Y. Vacuolating cytotoxin produced by *Helicobacter pylori* derived from patients living in two areas of Japan in which the prevalence of atrophic gastritis was different (abstr). Gastroenterology 1996;110:A142.

87. Rudi J, Kolb C, Maiwald M, Zuna I, Galle PR, Stremmel W. Serum antibodies against the *Helicobacter pylori* proteins *cagA* and *vacA* are associated with an increased risk for gastric carcinoma (abstr). Gastroenterology 1996;110:A245.
88. Hansson LE, Nyrén O, Hsing AW, Bergström R, Josefsson S, Chow WH, Fraumeni JF, Adami HO. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med 1996;335:242–249.
89. Correa P, Schmidt BA. The relationship between gastric cancer frequency and the ratio of gastric to duodenal ulcer. Aliment Pharmacol Ther 1995;9(Suppl 2):13–19.

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Efficacy of omeprazole in functional dyspepsia: double-blind, randomized, placebo-controlled trials (the Bond and Opera studies)

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SUMMARY

Background: The efficacy of H_2-receptor antagonists in functional dyspepsia is equivocal and the therapeutic place of proton pump inhibitors in functional dyspepsia is unknown.

Aim: To evaluate the efficacy of proton pump inhibitor therapy in functional dyspepsia.

Methods: Patients (n = 1262) with a clinical diagnosis of functional dyspepsia (persistent or recurrent epigastric pain or discomfort for at least 1 month and a normal upper gastrointestinal endoscopy) were randomized to receive omeprazole 20 mg, 10 mg or identical placebo, for 4 weeks. Symptoms were assessed using validated measures. *Helicobacter pylori* status was determined pre-entry by a ^{13}C-urea breath test.

Results: On an intention-to-treat analysis (n=1248), complete symptom relief was observed in 38% on omeprazole 20 mg, compared with 36% on omeprazole 10 mg and 28% on placebo (P = 0.002 and 0.02, respectively). Among those with ulcer-like and reflux-like dyspepsia, complete symptom relief was achieved in 40% and 54% on omeprazole 20 mg, and 35% and 45% on omeprazole 10 mg, respectively, compared with 27% and 23% on placebo (all $P < 0.05$, except omeprazole 10 mg in ulcer-like dyspepsia, P = 0.08). There was no significant benefit of omeprazole over placebo in dysmotility-like dyspepsia. Symptom relief was similar in *H. pylori*-positive and negative cases.

Conclusions: Omeprazole is modestly superior to placebo in functional dyspepsia at standard (20 mg) and low doses (10 mg) but not in patients with dysmotility-like dyspepsia.

INTRODUCTION

Pain or discomfort in the upper abdomen is a common symptom complex in the general population; approximately 25% of adults in the community report such complaints and although only a minority see a doctor for dyspepsia in the UK and USA, it accounts for up to 5% of consultations in family practice.[1–4] Less than half of these patients when appropriately investigated have an underlying structural explanation for the symptoms such as peptic ulceration or reflux oesophagitis; the remainder are classified currently as having functional or non-ulcer dyspepsia.[1, 5, 6] Functional dyspepsia is an important and costly entity because it is a significant cause of morbidity and time lost from work.[7]

The pathophysiology of functional dyspepsia remains inadequately understood. Between 30% and 60% of patients with functional dyspepsia have *Helicobacter pylori* gastritis but whether this is an incidental infection in these patients remains unclear.[1, 8, 9] Basal and peak acid outputs do not differ in patients with functional dyspepsia compared with controls.[10] but the acid response to gastrin-releasing peptide, which is considered to be a reflection of the postprandial state, may be abnormal in up to 50% of *H. pylori* infected patients with functional dyspepsia.[11] Gastroduodenal sensation is disturbed in a subset[12] but it is not clear whether the mucosa is more acid sensitive in functional dyspepsia.[13]

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Standard treatment for functional dyspepsia has included H_2-receptor antagonists:[12, 14] the majority of patients in the community on long-term H_2-receptor antagonists for dyspepsia are taking this medication for the management of functional dyspepsia rather than peptic ulcer disease.[1] However, the efficacy of this class of compounds in functional dyspepsia is controversial.[12, 14] Whether more effective acid suppression with proton pump inhibitors is efficacious in functional dyspepsia has not been adequately tested. As *H. pylori* appears to be linked to acid dysregulation in functional dyspepsia,[11] it is conceivable that infected patients would have a better response to acid suppression but this has not been tested either.

We aimed to evaluate, in two identically designed randomized controlled trials (the Bond [*B*ased on *o*meprazole in *n*onulcer *d*yspepsia] and Opera [*O*meprazole and *p*lacebo—*e*ffect on *r*elieving *a*bdominal pain/discomfort] studies), the efficacy of the proton pump inhibitor omeprazole compared with placebo, all given once daily in the morning. We hypothesized that omeprazole at a standard dose of 20 mg would, compared with placebo, provide complete relief of epigastric pain and discomfort in twice as many patients after a 4 week course of treatment. A secondary aim was to determine whether the presence or absence of *H. pylori* infection influenced the symptom response to omeprazole. We hypothesized that the symptom relief would be greater in *H. pylori* infected patients with functional dyspepsia. Finally, we aimed to evaluate whether subgroups of dyspepsia based on ranking of the most bothersome complaint would identify responders to acid suppression. We hypothesized that those with predominant epigastric pain rather than discomfort would best respond to proton pump inhibitor therapy.

METHODS

Study population

Investigators were instructed to enrol consecutive patients presenting with a clinical diagnosis of functional dyspepsia into two identical studies. The trials were approved by the local ethics committee in each centre, and informed consent was obtained from all patients. Both general practitioners and specialist gastroenterologists recruited patients from their practices.

Patients with persistent or recurrent epigastric pain and/or epigastric discomfort that was experienced on at least one of the 3 days immediately prior to randomization were eligible to be enrolled.[1] Discomfort was defined as a subjective, negative feeling that did not reach the level of pain according to the patient and which included symptoms such as postprandial fullness, bloating or early satiety.[1] Patients were also required to have at least a 1 month history of dyspeptic symptoms and symptoms had to occur on a minimum of 25% of days during that month.

Patients underwent upper endoscopy in the period 5–14 days before the inclusion visit. Those with any erosive change in the oesophagus or duodenum, oesophageal strictures, Barrett's oesophagus, duodenal deformity or chronic gastric or duodenal ulcer were excluded. Patients with five or fewer gastric erosions were considered eligible for entry if they fulfilled the other inclusion criteria, because there is no convincing evidence that gastric erosions cause a distinct symptomatic entity.[1, 3, 6] Patients with a previous history of documented peptic ulcer disease by endoscopy or radiology, or a past history of gastro-oesophageal reflux disease documented by endoscopy or 24 h oesophageal pH monitoring, were excluded. Furthermore, patients with classical heartburn or acid regurgitation as their only symptom without an epigastric component were not considered eligible for inclusion in the study, so as to minimize including patients with undiagnosed gastro-oesophageal reflux disease.[1]

The presence of any alarm symptoms (including unintentional weight loss, vomiting, dysphagia, haematemesis, melaena, fever, jaundice or other symptoms or signs suggesting serious or malignant disease) led to exclusion. Those patients who were clinically diagnosed as having irritable bowel syndrome were also not eligible: this was defined conservatively as the presence of two or more of the six standard Manning symptom criteria (pain relief by defecation, more frequent stools at the onset of pain, looser stools with the onset of pain, visible abdominal distension, rectal passage of mucus or a sensation of incomplete rectal evacuation).[15, 16] Similarly, patients with chronic severe constipation were excluded as it was considered conceivable that this group may have their upper abdominal pain or discomfort associated with their constipation. Patients who had been treated with bismuth-containing compounds, prokinetics or ulcer-healing doses of antisecretory agents for more than 7 days in the month prior to endoscopy were also considered ineligible. The remaining exclusion criteria were presence of significant medical disease that would

complicate the evaluation of outcome (e.g. unstable diabetes mellitus or malignancy), pregnancy or lactation, alcohol or drug abuse, and age below 18 or above 80 years.

Assignment

Patients entered a parallel group, double-blind, randomized, placebo-controlled trial. In the period 1 week before endoscopy and in the period between endoscopy and randomization, only antacid use on an as needed basis was allowed (in order to avoid the contaminating effects of other drugs on treatment outcome). Randomization of eligible patients was performed in the proportions 1:1:1 according to a computer-generated randomization list. This was carried out within blocks of three consecutive patients. Patients either received omeprazole 20 mg, omeprazole 10 mg or placebo, all given once daily in the morning. The patients were treated with medication for 4 weeks (±4 days) with the first doses taken the morning following the day after randomization.

Determination of H. pylori status

At baseline, a standardized ^{13}C-urea breath test was obtained using a well validated protocol.[17] An increase in the patient's breath ^{13}C of more than 5 p.p.m. over the baseline was considered positive for *H. pylori* infection.[17]

Blinding

Patients, investigators and study centres maintained strict blinding throughout the study. The centre recruitment codes were placed in opaque envelopes. They were all returned and none had been opened during the trial. The omeprazole and placebo capsules were identical in appearance.

Compliance

Patient compliance was checked by counting the returned study medication. *A priori*, non-compliance was defined as an intake of less than 75% of the medication during the study period. The administration of all drugs during the study period was recorded.

Symptom and quality of life assessment

This was undertaken at baseline and at the end of the trial, at the 4 week visit.

Symptoms. The primary outcome assessment was measurement of gastrointestinal symptoms. Epigastric pain and/or discomfort during the 3 days prior to the first and last visits in the trial was recorded by the physician. Symptoms were graded by the investigator at interview on a four-point Likert scale as follows: 0(none), no symptoms; 1(mild), awareness of the symptom but easily tolerated; 2(moderate), symptoms sufficient to cause an interference with normal activities; 3(severe), incapacitating symptoms with an inability to perform normal activities. This scale has been shown to be responsive and valid in reflux disease[18] and functional dyspepsia.[19, 20] Complete absence of epigastric pain and discomfort on each of the 3 days was the primary endpoint.

At the final visit, the patient was interviewed by a physician and asked whether the study medication had provided sufficient control of symptoms, to estimate global patient satisfaction with the treatment.

At the first visit, the patient also ranked his or her three most bothersome symptoms from the following choices: epigastric pain, heartburn, acid regurgitation, bloating, belching, rectal flatus, nausea, early satiety, and postprandial fullness, as defined by the Rome criteria.[1] The patients were then subdivided into the following *a priori* symptom subgroups based on symptom predominance:

1. Ulcer-like dyspepsia—predominant epigastric pain.
2. Dysmotility-like dyspepsia—predominant discomfort (postprandial fullness, early satiety, bloating or belching).
3. Reflux-like dyspepsia—predominant reflux symptoms (heartburn or acid regurgitation).
4. Other—predominant nausea or rectal flatus.

Patients recorded their epigastric pain or discomfort on daily diary cards during the 4 week treatment period, where symptoms were self-rated as present or absent.

Quality of life. Quality of life was assessed using two standard and validated self-report questionnaires:

(a) *The Psychological General Well Being Index (PGWB).* This measures subjective well-being or distress.[21, 22] It includes 22 items which can be combined into a global score that ranges from a maximum of 132 to a

minimum of 22. Six-point Likert scales comprise the response format, with higher values denoting better well-being. The PGWB has been applied in studies of dyspepsia and gastro-oesophageal reflux disease: there are extensive normative data available.[21, 22]

(b) *The Gastrointestinal Symptom Rating Scale (GSRS).* This has 15 items that measure gastrointestinal symptoms on seven-point Likert scales over the prior 2 weeks, and can be combined into a total score.[21, 22] The lower the score the better the symptom status. The GSRS is valid and responsive, and substantial normative data are available.[21, 22]

Statistical analysis

The primary efficacy variable, i.e. complete relief of epigastric pain/discomfort, was defined as having no symptoms during the last 3 days of the 4 weeks of treatment. The proportion of patients with complete relief of epigastric pain/discomfort was compared between omeprazole 20 mg and 10 mg vs. placebo using both an intention-to-treat (ITT) and a per protocol (PP) approach. Secondary efficacy variables were analysed using an ITT approach only.

The number needed to treat (the number of patients who need to be treated to prevent a poor outcome) and the relative risk reduction (the proportional reduction in event rates between control and experimental patients) were calculated.

A Mantel–Haenzel chi-squared test with stratification based on countries was used to analyse the difference between the two treatment groups with respect to the primary efficacy variable. The significance level was adjusted for two comparisons using the Bonferroni inequality. Confidence intervals were computed for the proportions and the differences between the treatment groups.

For the secondary efficacy variable, i.e. sufficient control of symptoms, confidence intervals were computed for the proportions and the differences between the treatment groups. The change in total score of the PGWB and mean item score of the GSRS from baseline to the 4-week visit were evaluated. Differences between treatments regarding the quality of life questionnaires were analysed with the baseline scores as a covariate.

RESULTS

The progress through the trials is summarized in Figure 1, which shows the number of eligible patients who were randomized, the numbers withdrawn, and the numbers who completed the trial in each arm.

Baseline comparisons and compliance

The three treatment groups were well balanced regarding all baseline characteristics in both trials (Table 1). Compliance was excellent: more than 90% of capsules were taken by 86, 87 and 83% of patients, respectively, in the omeprazole 20 mg, omeprazole 10 mg and placebo arms.

Relief of dyspepsia

In the intention-to-treat analysis, complete symptom relief was observed in 38.2% (161/421) on omeprazole 20 mg and 36.0% (146/405) on omeprazole 10 mg, compared with 28.2% (119/422) on placebo in the combined studies. The difference between omeprazole 20 mg and placebo, and between omeprazole 10 mg and placebo, was statistically significant (95% CI: 3.7–16.4% and 1.4–14.3%, respectively). The per protocol analysis yielded essentially the same results (Table 2). Comparing omeprazole 20 mg with placebo, the number needed to treat (NNT) was 10 (95% CI: 6–27) and the relative risk reduction was 14.0% (95% CI: 5.3–21.9%). Details from the individual studies are given in Table 2.

Sufficient control of symptoms by an intention-to-treat analysis was reported by 61% on omeprazole 20 mg (95% CI: 55.7–65.3%) compared with 59% on omeprazole 10 mg (95% CI: 54.3–64.1%) and 51% on placebo (95% CI: 46.3–56.0%) in the combined studies. Corresponding figures for the Bond study were 57% on omeprazole 20 mg (95% CI: 50.2–63.7%), 63% on omeprazole 10 mg (95% CI: 56.2–69.9%) and 44% on placebo (95% CI: 37.6–51.1%) and for the Opera study, 64% on omeprazole 20 mg (95% CI: 57.3–71.0%), 55% on omeprazole 10 mg (95% CI: 48.1–62.2%) and 59% on placebo (95% CI: 51.5–65.5%).

Omeprazole 20 mg and 10 mg provided a significantly higher proportion of symptom-free days (52.3 and 50.3%) than placebo (45.5%) over the treatment period in the combined studies. The difference between placebo and omeprazole 20 mg was 6.85% (95% CI: 2.64–11.07%) and for omeprazole 10 mg was 4.84% (95% CI: 0.59–9.10%). Symptoms improved to their maximum level by 7 days: the placebo response did not decrease over the 4 weeks of therapy.

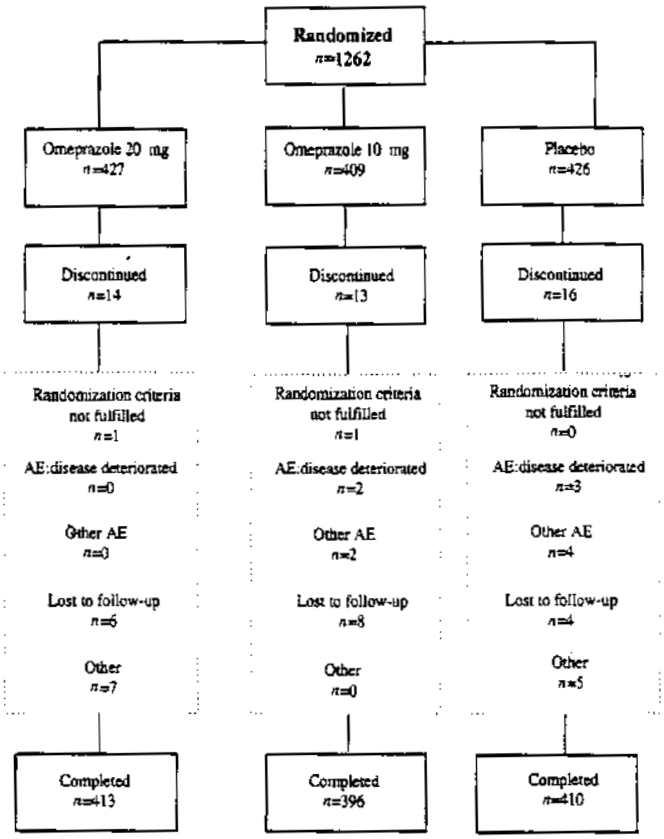

Figure 1. Flow chart of patient disposition by treatment group. Patient discontinuation = patient left the study prematurely, did not attend second visit. Numbers of patients are given in the boxes. AE = adverse events.

Symptom subgroups

In patients on omeprazole 20 mg, omeprazole 10 mg and placebo, those with ulcer-like dyspepsia (708 patients) had complete symptom relief in 40, 35 and 27% of cases, respectively ($P = 0.006$ omeprazole 20 mg vs. placebo and $P = 0.08$ omeprazole 10 mg vs. placebo), while those with reflux-like dyspepsia (143 patients) had complete relief in 54, 45 and 23% of cases, respectively ($P = 0.002$ omeprazole 20 mg vs. placebo and $P = 0.02$ omeprazole 10 mg vs. placebo). In contrast, those with dysmotility-like dyspepsia (291 patients) had complete relief on omeprazole 20 mg, omeprazole 10 mg and placebo in 32, 37 and 31% of cases, respectively ($P = 0.92$ omeprazole 20 mg vs. placebo and $P = 0.33$ omeprazole 10 mg vs. placebo) and for patients with other symptoms (i.e. nausea, flatus) (106 patients) the corresponding figures were 25, 30 and 38%, respectively ($P = 0.25$ omeprazole 20 mg vs. placebo and $P = 0.46$ omeprazole 10 mg vs. placebo).

H. pylori

A total of 41% of patients (507/1222) were *H. pylori*-positive by the ^{13}C-urea breath test. Complete relief of dyspepsia in the active treatment arms was not significantly different between the *H. pylori* infected and uninfected patients (Table 3).

Quality of life

The total GSRS score improved from 2.67 to 2.02 on omeprazole 20 mg, from 2.74 to 2.08 on omeprazole 10 mg and from 2.75 to 2.17 on placebo. The difference between omeprazole 20 mg and placebo was statistically significant ($P = 0.02$, with baseline GSRS as a

Table 1. Baseline characteristics in patients by treatment arm (ITT)

	Omeprazole 20 mg			Omeprazole 10 mg			Placebo		
	(1)	(2)	(C)	(1)	(2)	(C)	(1)	(2)	(C)
Total	219	202	421	204	201	405	219	203	422
Mean age, years (s.d.)	41(14)	42(14)	42(14)	43(14)	43(14)	43(14)	43(14)	43(15)	43(14)
Female(%)	61.2	58.9	60.1	63.7	62.7	63.2	63.9	63.5	63.7
Race									
Caucasian(%)	94.5	98.0	96.2	96.6	98.0	97.3	97.3	99.0	98.1
Black(%)	3.2	0.0	1.7	1.5	0.0	0.7	1.8	0.0	0.9
Oriental(%)	2.3	1.5	1.9	1.5	1.5	1.5	0.5	1.0	0.7
Non-smoker(%)	60.7	59.9	60.3	50.5	61.7	56.0	55.3	60.6	57.8
Previous smoker(%)	11.9	9.9	10.9	13.2	12.4	12.8	12.3	12.8	12.6
Smoker(%)	27.4	30.2	28.7	36.3	25.9	31.1	32.4	26.6	29.6
Alcohol use(%)	47.5	38.6	43.2	46.6	39.3	43.0	53.4	38.9	46.4
Duration of disease > 1 year(%)	62.6	62.9	62.7	64.2	59.7	62.0	68.5	62.1	65.4
Males (kg) Mean weight (s.d.)	77(13)	77(13)	77(13)	81(12)	76(12)	78(12)	78(10)	79(13)	78(12)
Females (kg) Mean weight (s.d.)	66(14)	63(10)	65(12)	64(11)	64(14)	64(12)	66(12)	62(10)	64(11)
H. pylori-positive (%)	39.7	36.0	38.0	45.3	38.5	41.9	44.4	44.7	44.6

(1), Bond; (2), Opera; (C), combined.

covariate), but the difference between omeprazole 10 mg and placebo was not ($P = 0.08$).

The total PGWB score improved similarly in all groups by the last visit. There was no statistically significant difference in the total score or in the subscale scores between the three treatment arms (data not shown).

Treatment response in the Bond vs. Opera studies

The difference in treatment response between active treatment and placebo was greater in the Bond than in the Opera study (Table 4). This difference was statistically significant when comparing active treatment (omeprazole 20 mg or omeprazole 10 mg) with placebo ($P = 0.006$) as well as when comparing each active drug with placebo ($P = 0.04$ for omeprazole 20 mg vs. placebo and $P = 0.005$ for omeprazole 10 mg vs. placebo). The distribution of the dyspepsia subgroups was similar in the Bond and Opera studies. However, in patients recruited by a general practitioner the difference in treatment response between active treatment and placebo was considerably greater than in patients recruited by a specialist. In the Bond study, 34% of the patients were from family practice but in the Opera study only 8% were from this setting. When adjusting for the type of investigator (family physician or specialist) the differences between the studies in treatment response between active treatment and placebo was no longer statistically significant.

Adverse events

The number of adverse events was low and was similar across the three treatment arms. A total of 34 patients discontinued treatment due to adverse events (10 on omeprazole 20 mg, 9 on omeprazole 10 mg and 15 on placebo).

DISCUSSION

Drugs that reduce gastric acid secretion are commonly prescribed for patients with functional dyspepsia, but their efficacy has been questioned.[12, 14] Randomized placebo-controlled trials of H_2-receptor antagonists in functional dyspepsia have produced conflicting results: in the positive studies the benefit over placebo was small based on the symptom scores applied and therefore of questionable clinical significance,[14, 23, 24] while the negative trials were often underpowered to detect an important difference between drug and placebo.[14, 25] A meta-analysis suggested that H_2-blockers produced a therapeutic effect of 20% over placebo but the report

Table 2. Complete relief of dyspeptic symptoms, estimates and 95% confidence intervals

Treatment	Relief of dyspepsia (estimate)	Confidence interval limits	
		Lower(%)	Upper(%)
Bond study			
Intention-to-treat			
Omeprazole 20 mg	42.5% (93/219)	35.8	49.3
Omeprazole 10 mg	43.1% (88/204)	36.2	50.2
Placebo	26.0% (57/219)	20.3	32.4
Per protocol			
Omeprazole 20 mg	45.3% (81/179)	37.8	52.8
Omeprazole 10 mg	43.9% (76/173)	36.4	51.7
Placebo	24.6% (46/187)	18.6	31.4
Opera study			
Intention-to-treat			
Omeprazole 20 mg	33.7% (68/202)	27.2	40.6
Omeprazole 10 mg	28.9% (58/201)	22.7	35.6
Placebo	30.5% (62/203)	24.3	37.4
Per protocol			
Omeprazole 20 mg	34.8% (56/161)	27.5	42.7
Omeprazole 10 mg	30.1% (52/173)	23.3	37.5
Placebo	33.5% (57/170)	26.5	41.2
Combined studies			
Intention-to-treat			
Omeprazole 20 mg	38.2% (161/421)	33.6	43.1
Omeprazole 10 mg	36.0% (146/405)	31.4	40.9
Placebo	28.2% (119/422)	24.0	32.8
Per protocol			
Omeprazole 20 mg	40.3% (137/340)	35.0	45.7
Omeprazole 10 mg	37.0% (128/346)	31.9	42.3
Placebo	28.9% (103/357)	24.2	33.9

has been criticized for only including some of the available trials.[26] Thus, it remains to be definitely established that this class of antisecretory compounds is superior to placebo in functional dyspepsia. On the other hand, the role of more potent acid suppression has not until now been adequately investigated. We found in the present study that omeprazole was superior to placebo in relieving the symptoms of functional dyspepsia, although the benefit was very modest. We combined the results of the two trials because they were of identical design, with the exception that different countries recruited the patients. Rather than relying on an arbitrary symptom score which may not translate into a clinically meaningful number, the main outcome measure in this trial chosen *a priori* was the most rigorous possible, namely absence of epigastric pain and discomfort. Importantly, all the symptom measures improved in parallel and both doses of omeprazole were superior to placebo. The evidence is consistent with the observed small therapeutic gain with omeprazole being clinically meaningful.

It has been suggested that patients with functional dyspepsia can usefully be divided into subgroups based on symptoms. In the original proposal, ulcer-like, dysmotility-like, reflux-like and non-specific dyspepsia were identified based on clusters of symptoms.[27] and subsequently the Rome criteria were developed along similar lines, although reflux-like dyspepsia was discarded.[1] However, symptom clusters have appeared to be a dismal failure because of substantial symptom overlap and a lack of correlation with pathophysiological disturbances.[2, 3, 8] Hence, in the present study we *a priori* applied a new forced choice classification based on symptom predominance. We also did not discard reflux symptoms because these are so common in patients with any upper gastrointestinal tract disease including peptic ulceration.[28] We noted that the symptom benefit with omeprazole was greatest in patients with ulcer-like or reflux-like dyspepsia, and was not observed in those with dysmotility-like symptoms. The results suggest that symptom subgrouping based on symptom predominance has clinical utility because it predicts treatment response.

There are very few other trials that have addressed the clinical benefit of proton pump inhibitor therapy in functional dyspepsia. Lauritsen and co-workers

Table 3. Number of patients and proportion (%) of patients with complete relief of dyspeptic symptoms (intention-to-treat analysis) by *H. pylori* status (^{13}C-urea breath test)

	Omeprazole 20 mg		Omeprazole 10 mg		Placebo		All	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Total	421	38	405	36	422	28	1248	34
H. pylori status								
Negative	255	35	230	37	230	24	715	32
Positive	156	43	166	36	185	34	507	37
Unknown	10	40	9	22	7	29	26	31

Table 4. Proportion of patients with complete relief of dyspeptic symptoms by type of investigator. ITT

	Omeprazole 20 mg		Omeprazole 10 mg		Placebo		All	
	n	%	*n*	%	*n*	%	*n*	%
Bond study								
Specialist	145	47	132	40	145	32	422	40
GP	74	34	72	49	74	14	220	32
All	219	42	204	43	219	26	642	37
Opera study								
Specialist	186	32	188	30	185	32	559	31
GP	16	56	13	15	18	11	47	28
All	202	34	201	29	203	31	606	31
Combined								
Specialist	331	38	320	34	330	32	981	35
GP	90	38	85	44	92	13	267	31
All	421	38	405	36	422	28	1248	34

GP, general practitioner; Specialist, gastroenterologist.

randomized 197 patients with functional dyspepsia to omeprazole 20 mg twice daily or placebo for 2 weeks in Denmark and Sweden: complete relief of dyspepsia in the last 2 days was observed in 35% on omeprazole and 13% on placebo, which was a significant difference.[19] On the other hand, a German multicentre study randomized 801 patients with functional dyspepsia to omeprazole (10 mg and 20 mg daily), ranitidine (150 mg nightly) or placebo for 2 weeks.[29] The investigator judged whether the patient required further investigation or treatment, and using this as the primary end-point there was no significant difference between the groups.

There are other studies that have evaluated proton pump inhibitor therapy in uninvestigated dyspepsia in primary care. Meineche-Schmidt & Krag reported a randomized trial of omeprazole (20 mg daily) and placebo for 2 weeks in 536 Danish patients with no documented history of ulcer or gastro-oesophageal reflux disease but who had ulcer-like or reflux-like dyspepsia: 50% on omeprazole compared with 35% on placebo had no symptoms at the end of the trial.[30] Another trial enrolled 674 patients with uninvestigated dyspepsia and/or heartburn and in an open-label study randomized them to omeprazole 10 mg daily or Gaviscon four times daily for 4 weeks: symptom abolition at 4 weeks was observed in 41% on omeprazole and 16% on Gaviscon, but although statistically significant, the results are very difficult to interpret because of the lack of blinding.[31] Jones & Baxter in a randomized trial compared lansoprazole 30 mg daily with ranitidine 150 mg twice daily: the proton pump inhibitor provided significant symptom relief compared with the H_2-antagonist in those with epigastric pain and/or heartburn, but patients with and without a history of structural disease were enrolled and no placebo control group was included.[32]

A recent systematic review of all published randomized controlled trials in functional dyspepsia evaluated a total of 52 eligible studies;[33] the majority of trials suffered from serious weaknesses in study design and execution, and only five studies used previously validated outcome measures. In this trial careful attention was given to avoiding previously identified methodological concerns. In particular, validated outcome measures were utilized, strict blinding was maintained, an adequate placebo control was included and the study was sufficiently powered to detect modest but clinically significant differences. Utilizing the hard end-point of symptom absence, the placebo response was minimized. Hence our results suggest that a subgroup of patients with functional dyspepsia are responsive to acid suppression, and we conclude that the findings are likely to be accurate and applicable in clinical practice.

If proton pump inhibitors are efficacious in some patients with functional dyspepsia, by what mechanism do they reduce symptoms? Modulation of gastric acid secretion is one consideration. Earlier reports demonstrated that both basal and peak acid output were similar in patients with functional dyspepsia compared with appropriate control groups.[10] More recent work has shown that acid secretion in response to gastrin-

releasing peptide (GRP) was significantly increased in *H. pylori* infected patients with functional dyspepsia compared to *H. pylori*-positive healthy volunteers.[11] Overall, approximately 50% of patients with functional dyspepsia had a disturbance of GRP-stimulated acid secretion and in this group the disturbance was similar to that found in patients with duodenal ulcer.[11] The data suggest that a subset of patients with functional dyspepsia and *H. pylori* infection have acid dysregulation that potentially may respond to acid suppression. However, in the present trial, *H. pylori* status did not predict response to therapy so that this mechanism appears unlikely to explain the results.

A further possible explanation for the efficacy of omeprazole in functional dyspepsia may relate to undiagnosed gastro-oesophageal reflux disease (GERD). Heartburn is a very common complaint in the general population:[34] furthermore, the majority of patients with functional dyspepsia have co-existent heartburn if specific enquiries are made.[35] It is conceivable that some patients labelled by their physician as having functional dyspepsia in this trial actually had atypical GERD and this may explain any benefit of acid suppression.[36, 37] Indeed, while the majority of patients enrolled had ulcer-like dyspepsia ($n = 708$ or 56%), 143 patients (11%) were classified at entry as having predominant reflux symptoms and it is likely that true pathological acid reflux was present in a subset of these patients. The rate of complete symptom relief observed in reflux-like dyspepsia in this trial was similar to the symptom relief of heartburn observed in patients with endoscopy-negative GERD.[38] On the other hand, in the present study patients with endoscopically diagnosed oesophagitis, a past history of documented GERD or heartburn alone were excluded. Moreover, patients had to have a diagnosis of functional dyspepsia based on the investigators' judgement in order to enter the trial. Whil 24 h oesophageal pH testing would have provided additional information to address how many patients had true gastro-oesophageal reflux, it is invasive and would have resulted in a highly selected patient population being enrolled because of refusals, and hence was considered impractical. Moreover, other work suggests that acid exposure as measured by 24 h oesophageal pH testing does not predict the response to proton pump inhibitor therapy in dyspepsia.[19] In practice this is not a crucial issue as our results show that a subset of patients with a clinical and endoscopic diagnosis of functional dyspepsia will respond to omeprazole. However, more research is required to determine if atypical GERD explains the benefit of acid suppression in some patients with functional dyspepsia.

We did observe a lower treatment effect in Study 2 compared to the Bond study. This could not be explained by baseline differences and for this reason it was justifiable to combine the studies. More general practitioners participated in the Bond study whereas a higher proportion of gastroenterologists participated in the Opera study. Interestingly, the placebo response was higher amongst those patients seeing a gastroenterologist (32%) compared to those enrolled by a family practitioner (13%), although the response to active treatment was reasonably similar in both groups (Table 4). Conceivably, those seeing a specialist may feel more reassured that they do not have a serious underlying disease, which may promote a higher placebo response.

In conclusion, our results have demonstrated that the proton pump inhibitor omeprazole at a dose of 20 mg and 10 mg was modestly superior to placebo in relieving the symptoms of functional dyspepsia, particularly in those with ulcer-like or reflux-like dyspepsia, but symptom relief was similar in those with and without *H. pylori* infection.

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REFERENCES

1 Talley NJ, Colin-Jones D, Koch KL, Kock M, Nyren O, Stanghellini V. Functional dyspepsia: A classification with guidelines for diagnosis and management. Gastroenterol Int 1991; 4: 145–60.

2 Talley NJ, Zinsmeister AR, Schleck CD, Melton LJ III. Dyspepsia and dyspepsia subgroups: A population-based study. Gastroenterology 1992; 102: 1259–68.

3 Agreus L, Svärdsudd K, Nyren O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. Gastroenterology 1995; 109: 671–80.

4 Drossman DA, Li Z, Andruzzi E, *et al.* U.S. householder survey of functional gastrointestinal disorders: Prevalence, sociodemography and health impact. Dig Dis Sci 1993; 38: 1569–80.

5 Kagevi I, Löfstedt S, Persson LG. Endoscopic findings and diagnoses in unselected dyspeptic patients at a primary health care center. Scand J Gastroenterol 1989; 24: 145–50.

6 Talley NJ, Phillips SF. Non ulcer dyspepsia: Potential causes and pathophysiology. Ann Intern Med 1988; 108: 865–79.

7 Nyrén O, Adami HO, Gustavsson S, Loof L, Nyberg A. Social and economic effects of non-ulcer dyspepsia. Scand J Gastroenterol Suppl 1985; 109: 41–7.

8 Holtmann G, Goebell H, Holtmann M, Talley NJ. Dyspepsia in healthy blood donors: Pattern of symptoms and association with *Helicobacter pylori*. Dig Dis Sci 1994; 39: 1090–8.

9 Talley NJ. A critique of therapeutic trials in *Helicobacter* pylori-positive functional dyspepsia. Gastroenterology 1994; 106: 1174–83.

10 Nyrén O, Adami HO, Gustavsson S, Lindgren PG, Loof L, Nyberg A. The 'epigastric distress syndrome'. A possible disease entity identified by history and endoscopy in patients with nonulcer dyspepsia. J Clin Gastroenterol 1987; 9: 303–9.

11 El Omar E, Penman I, Ardill JES, McColl KEL. A substantial proportion of non ulcer dyspepsia patients have the same abnormality of acid secretion as duodenal ulcer patients. Gut 1995; 36: 534–8.

12 Talley NJ. Functional dyspepsia—Should treatment be targeted on disturbed physiology? Aliment Pharmacol Ther 1995; 9: 107–15.

13 George AA, Tsuchiyose M, Dooley CP. Sensitivity of the gastric mucosa to acid and duodenal contents in patients with nonulcer dyspepsia. Gastroenterology 1991; 101: 3–6.

14 Talley NJ. Drug treatment of functional dyspepsia. Scand J Gastroenterol 1991; 26(Suppl. 182): 47–60.

15 Manning AP, Thompson WG, Heaton KW, Morris AF. Toward positive diagnosis of irritable bowel. Br Med J 1978; 2: 653–4.

16 Talley NJ, Phillips SF, Melton LJ, Mulvihill C, Wirgen C, Zinsmeister AR. Diagnostic value of the Manning criteria in irritable bowel syndrome. Gut 1990; 31: 77–81.

17 Dominguez-Munoz JE, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Gut 1997; 40: 459–62.

18 Hallerback B, Unge P, Carling L, *et al.* Omeprazole or ranitidine in long-term treatment of reflux esophagitis. Gastroenterology 1994; 107: 1305–11.

19 Lauritsen K, Aalykke C, Havelund T, *et al.* Effect of omeprazole in functional dyspepsia: A double-blind, randomized, placebo-controlled study. Gastroenterology 1996; 4: A702(Abstract).

20 Junghard O, Lauritsen K, Talley NJ, Wiklund I. Validation of seven graded diary cards for severity of dyspeptic symptoms in patients with non ulcer dyspepsia. Eur J Surg (in press).

21 Dimenas E, Glise H, Hallerback B, Hernqvist H, Svedlund J, Wiklund I. Well-being and gastrointestinal symptoms among patients referred to endoscopy owing to suspected duodenal ulcer. Scand J Gastroenterol 1995; 30: 1046–52.

22 Dimenas E, Glise H, Hallerback B, Hernquist H, Svedlund J, Wiklund I. Quality of life in patients with upper gastrointestinal disease. Improved possibility for evaluation of treatment regimens? Scand J Gastroenterol 1993; 28: 681–7.

23 Talley NJ, McNeil D, Hayden A, Piper DW. Randomized, double-blind, placebo-controlled crossover trial of cimetidine and pirenzepine in nonulcer dyspepsia. Gastroenterology 1986; 91: 149–56.

24 Saunders JHB, Oliver RJ, Higson DL. Dyspepsia: incidence of non-ulcer disease in a controlled trial of ranitidine in general practice. Br Med J 1986; 292: 665–8.

25 Nyrén O, Adami H-O, Bates S, *et al.* Absence of therapeutic benefit from antacids or cimetidine in NUD. N Engl J Med 1986; 314: 339–43.

26 Dobrilla G, Comberlato M, Steele A, Valtaperta P. Drug treatment of functional dyspepsia. A meta-analysis of randomized controlled trials. J Clin Gastroenterol 1989; 11: 169–77.

27 Anonymous. Management of dyspepsia: report of a working party. Lancet 1988; 1: 576–9.

28 Talley NJ, Weaver AL, Tesmer DL, Zinsmeister AR. Lack of discriminant value of dyspepsia subgroups in patients referred for upper endoscopy. Gastroenterology 1993; 105: 1378–86.

29 Blum AL, Arnold R, Keolz Hr, Stolte M and the FROSCH Study Group. Treatment of functional dyspepsia (FD) with omeprazole and ranitidine. Gastroenterology 1997; 112: A731(Abstract).

30 Meineche-Schmidt V, Krag E. Relief of symptoms in patients with reflux or ulcer-like dyspepsia after two weeks treatment with either omeprazole, cimetidine, or placebo—A Danish

multicenter trial in general practice. Eur J Gen Pract 1997; 3: 125–30.
31 Goves J, Oldring JK, Kerr D, Carr EP, Turbitt ML. First line treatment with omeprazole 10 mg OM is more effective strategy than gaviscon 10 mg qid in the relief of dyspepsia symptoms in primary care. Gut 1996; 39(Suppl. 1): A40(Abstract).
32 Jones RH, Baxter G. Lansoprazole 30 mg daily versus ranitidine 150 mg b.d. in the treatment of acid-related dyspepsia in general practice. Aliment Pharmacol Ther 1997; 11: 541–6.
33 Veldhuyzen van Zanten SJO, Cleary C, Talley NJ, *et al.* Drug treatment of functional dyspepsia: A systematic analysis of trial methodology with recommendations for design of future trials. Am J Gastroenterol 1996; 91: 660–73.
34 Locke GR III, Talley NJ, Fett S, Zinsmeister AR, Melton LJ III. Prevalence and clinical spectrum of gastroesophageal reflux in the community. Gastroenterology 1997; 112: 1448–56.
35 Talley NJ. *H. pylori* and dyspepsia. Yale J Biol Med (in press).
36 Farup PG, Hovde O, Breder O. Are frequent short gastro-oesophageal reflux episodes the cause of symptoms in patients with non-ulcer dyspepsia responding to treatment with ranitidine? Scand J Gastroenterol 1995; 30: 829–32.
37 Small PK, Loudon MA, Waldron B, Smith D, Campbell FC. Importance of reflux symptoms in functional dyspepsia. Gut 1995; 36: 189–92.
38 Carlsson R, Dent J, Watts R, *et al.* Gastro-oesophageal reflux disease in primary care: an international study of different treatment strategies with omeprazole. Eur J Gastroenterol Hepatol 1998; 10: 119–24.

