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Expression of HLA-DR, costimulatory molecules B7-1, B7-2, intercellular adhesion molecule-1 (ICAM-1) and Fas ligand (FasL) on gastric epithelial cells in *Helicobacter pylori* gastritis; influence of *H. pylori* eradication

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SUMMARY

There is evidence that *Helicobacter pylori* infection up-regulates the expression of HLA class II molecules by gastric epithelial cells (GEC). In this study we evaluated whether GEC are capable of expression of costimulatory molecules in *H. pylori* gastritis. The expression of FasL by GEC, before and after eradication of *H. pylori*, was also studied. Thirty patients (23 men) aged 27–81 years (53·67 ± 13·99 years (mean ± s.d.)) with dyspepsia were studied. Upper gastrointestinal endoscopy was performed and six biopsies were obtained (antrum, $n = 3$; corpus, $n = 3$) for Campylobacter-Like Organisms (CLO) test and histology; 23 (16 men) were *H. pylori*$^+$ and seven (all men) were *H. pylori*$^-$ by both methods and served as controls. *Helicobacter pylori* eradication therapy was given to *H. pylori*$^+$ patients and all patients were re-endoscoped after 116 ± 9 days. Formalin-fixed paraffin-embedded tissue sections were stained by the ABC immunoalkaline phosphatase method. In *H. pylori* gastritis HLA-DR was expressed and correlated with disease activity ($P < 0{\cdot}01$). No HLA-DR was observed in controls. In *H. pylori*-eradicated patients significant decrease of HLA-DR was found (antrum, $P < 0{\cdot}001$). ICAM-1 was expressed by GEC in 80% of *H. pylori*$^+$ patients; ICAM-1 expression did not correlate with gastritis parameters and decreased significantly after eradication (antrum, $P < 0{\cdot}01$). B7-1 and B7-2 were expressed on *H. pylori*$^+$ samples and their expression decreased after eradication, albeit not significantly. Weak epithelial expression of both B7 molecules was observed in all the controls. FasL was steadily expressed by GEC in both *H. pylori*$^+$ and *H. pylori*$^-$ patients and remained almost unchanged after eradication. These findings suggest that GEC may acquire antigen-presenting cell properties in *H. pylori* infection through *de novo* expression of HLA-DR and costimulatory molecules. This seems to be attenuated after eradication and resolution of mucosal inflammation. The same cells exhibit the capacity to control the inflammatory process, probably by inducing apoptotic cell death to Fas-bearing infiltrating lymphocytes.

Keywords *Helicobacter pylori* gastritis epithelial cell antigen-presenting cell costimulatory molecules FasL

INTRODUCTION

The relationship between *Helicobacter pylori* infection, chronic antral gastritis and peptic ulcer is established [1,2]; *H. pylori* infection also seems to be a predisposing factor in the development of gastric carcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [3–8]. On the other hand, there is evidence that successful eradication of the microorganism leads to a great reduction of the peptic ulcer recurrence rate [9,10], and in some cases to MALT lymphoma regression [11]. Thus, defining the factors that influence the pathophysiology of *H. pylori* gastritis is clearly of considerable importance.

On a histological level, erosion of the surface epithelium of the gastric mucosa is commonly observed, accompanied by infiltration of the lamina propria by neutrophils, lymphocytes—often organized in lymphoid follicles—plasma cells and mononuclear cells [12,13]. The intensity of the inflammatory process seems to be affected by the interaction of bacterial (bacterial enzymes, CagA, lipopolysaccharide (LPS)) and host (secretor gene, Lewis b antigen) factors [14–16].

The detection of chemokines (C-C and C-X-C) and neutrophil

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infiltration in gastric mucosa indicates participation of innate immune mechanisms [17], whereas infiltration of the mucosa by *H. pylori*-specific T and B lymphocytes and the detection of *H. pylori*-specific immune cells and antibodies in peripheral blood show activation of the adaptive immune response [18–21]. Mounting of an effective adaptive-type immune response requires the presence of a functional antigen-presenting cell (APC), which must have the ability both to process and to present *H. pylori* peptides (signal 1), as well as to express costimulatory molecules (signal 2) necessary for the activation of specific T lymphocytes [22]. Foreign antigenic peptides are presented to their corresponding T cell receptors by HLA class II molecules, whilst other molecules such as CD80 (B7-1), CD86 (B7-2) and CD54 (ICAM-1) function as costimulatory ones [23,24].

Besides the above mentioned requirements for the initiation and evolution of the immune response, it is also critical to know how the immune response is turned off and immune cell homeostasis is maintained. Taking into consideration that immune reactions may lead to potentially dangerous alterations in normal physiology, it is essential that they be carefully controlled or extinguished if the antigenic stimulus either becomes too great or is successfully eliminated [25]. One important mechanism of lymphocyte population size control is programmed cell death (apoptosis). T cells become apoptotic by at least two independent mechanisms: first, active death, which is antigen-driven in the context of persistent antigenic stimulus, with the Fas–FasL system being a major contributing factor; and second, passive death, which occurs at the conclusion of the immune response where no further antigenic and/or lymphokine stimulation is present [26].

In this study we investigated the immunohistochemical expression of HLA-DR, B7-1, B7-2, ICAM-1 and FasL in *H. pylori* gastritis and in normal gastric mucosa. We also searched for possible correlations between the gastritis parameters according to Sydney classification and the expression of the above mentioned molecules, as well as for alterations in histopathology and immunohistochemistry after eradication of the microorganism.

PATIENTS AND METHODS

Patients

In this prospective study a total of 30 patients (23 males and seven females) aged between 27 and 81 years (53·67 ± 13·99 years (mean ± s.d.)) with dyspeptic symptoms were included.

Inclusion criteria

Patients were included if they were tested *H. pylori*$^+$ or *H. pylori*$^-$ by both methods used, namely Campylobacter-Like Organisms (CLO) test and histological identification of the microorganism. Patients with peptic ulcer who tested *H. pylori*$^+$ were excluded. Patients who reported any eradication therapy or any previous gastric surgery were excluded. Patients who reported any anti-ulcer therapy or any antibiotic or bismuth salt therapy during the previous 4 weeks were also excluded. Non steroidal anti-inflammatory drug (NSAID) or aspirin users, patients on corticosteroids or any immunosuppressive therapy, pregnant women, women of reproductive age who did not take efficient contraception and patients suffering from severe disease of any kind were not included in the study.

The study was done in accordance with the declaration of Helsinki. Informed consent was given by all the patients.

Study design

All patients underwent upper GI endoscopy and six biopsies were taken as follows: two biopsies, one from the antrum and one from the corpus for rapid urease test (CLO test), two from the antrum (anterior and posterior wall) and two from the corpus (anterior and posterior wall) that were immediately fixed in formalin 10% and sent to the Pathology Department for histological and immunohistochemical evaluation. The patients who were tested positive for *H. pylori* were given an appropriate triple eradication therapy consisting of omeprazole 20 mg bid, plus amoxycillin 1 g bid, plus clarithromycin 500 mg bid for 7 days. Omeprazole 20 mg qd was given for an additional 3 weeks after eradication therapy. No anti-ulcer therapy was given to the patients who were tested negative for *H. pylori*. A second endoscopy was performed on all patients [16 ± 9·5 days after the initial one. Tissue samples were taken again according to the above mentioned protocol and were evaluated in the same way. It must be pointed out that biopsy specimens from the initial and follow-up endoscopies were assessed simultaneously for the immunohistochemical parameters, in order to ensure validity of the results, i.e. to avoid discrepancies in evaluation due to different laboratory conditions.

Histopathology

Formalin-fixed, paraffin-embedded tissue samples were routinely cut at 3–4 μm and stained with haematoxylin and eosin (H–E), alcian blue (pH 2·5) and Giemsa.

Specimens were classified independently by two expert pathologists (P.G.F., P.D.) who were unaware of the corresponding clinical and endoscopic findings. Differences in their independent reports were resolved by re-examination and consensus.

Gastritis parameters (chronic inflammation, activity, intestinal metaplasia, gland atrophy, *H. pylori* density) were graded semiquantitatively in a scale of 0–3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe) based on previously reported criteria [27,28].

Immunohistochemistry

Immunohistochemistry was performed on paraffin sections on poly-L-lysine (Sigma Chemical Co., St Louis, MO)-pretreated slides, by applying the three-stage avidin–biotin complex (ABC) method. In sort, deparaffinized and rehydrated sections were incubated for 20 min at room temperature with 1% bovine serum albumin (BSA) in order to prevent non-specific binding, followed by overnight incubation at 4°C with the primary antibody (Table 1). Whenever necessary, antigen retrieval techniques were used before blocking serum application. For negative control the same procedure was followed, replacing the primary antibody with Tris-buffered saline (TBS) or with an irrelevant antibody of the same animal and isotype. Biotin-conjugated secondary antibodies (Dako, Glostrup, Denmark) were added at a 1:500 dilution for 45 min at room temperature. Next, the sections were incubated with streptavidin–biotin–alkaline phosphatase complex (Dako) for 30 min at room temperature. All incubations were stopped by three 5-min washes in TBS.

For colour development fast red (Sigma) was used as the chromogen. Endogenous alkaline phosphatase activity was excluded by the addition of levamizole in the substrate buffer, which can block endogenous alkaline phosphatase, and by the use of appropriate negative controls. Slides were counterstained with Mayer's haematoxylin.

Table 1. Studied molecules and antigen retrieval techniques

Specificity	Dilution (overnight incubation)	Antigen retrieval technique	Source
Mouse, anti-HLA-DR	1:250	None	Dako
Goat, anti-B7 1 (CD80)	1:30	Heat, citrate buffer, pH 6, 20 min	Santa Cruz
Goat, anti-B7 2 (CD86)	1:30	Heat, citrate buffer, pH 6, 20 min	Santa Cruz
Mouse, anti-ICAM-1 (CD54)	1:30	Heat, citrate buffer, pH 6, 20 min	Zymed
Rabbit, anti-FasL (CD95L)	1:100	Heat, citrate buffer, pH 6, 20 min	Santa Cruz

Table 2. The immunohistochemical expression of the studied molecules in *Helicobacter pylori* infected patients before and after eradication ($n = 20$)

	H. pylori-infected		After *H. pylori* eradication	
Molecules	Antrum*	Corpus	Antrum*	Corpus
HLA-DR	20	6	5	0
ICAM-1	16	5	3	0
B7-1	13	10	13	8
B7-2	7	4	8	2
FasL	20	20	20	20

HLA DR: *$P < 0.001$; ICAM 1: *$P < 0.01$.

Statistical analysis

χ^2 test (Fisher's exact test) was used; the non-parametric Wilcoxon tests for paired and unpaired measurements and Spearman's rank correlation coefficient test were used as appropriate. (Computer program: Graphpad prism Version 2.01; Graphpad Software Inc., San Diego, CA).

RESULTS

Of the 30 patients, 23 (16 men) were *H. pylori*$^+$. Of them, 19 had duodenal ulcer (DU), three duodenal and gastric ulcer (GU) and one had only gastritis. Seven patients were *H. pylori*$^-$ and served as controls. Patients and controls did not differ in age.

Helicobacter pylori was successfully eradicated in 20/23 (87%) patients.

Histopathology

In all, *H. pylori*$^+$ patients' chronic inflammation, activity and *H. pylori* density were significantly greater in the gastric antrum than corpus (Wilcoxon: $P < 0.001$, $P < 0.01$, $P < 0.001$, respectively). In the antrum lymphoid follicles were observed in 17/23 (74%) patients; atrophy was detected in 14/23 (61%) and intestinal metaplasia in 9/23 (39%). In the corpus, only two patients had mild atrophy and one had intestinal metaplasia.

After eradication, significant reduction of chronic inflammation both in antrum and corpus was observed (Wilcoxon: $P < 0.001$, $P < 0.01$, respectively). Activity was also significantly decreased (Wilcoxon; antrum, $P = 0.001$; corpus, no activity after eradication). Lymphoid follicles were significantly reduced (Fisher's exact test: $P < 0.05$). No changes in incidence of atrophy and intestinal metaplasia were observed during the follow-up period.

Immunohistochemistry

Immunopositivity for the studied molecules, except FasL, was more prevalent in gastric antrum than in corpus in all cases of *H. pylori* gastritis, probably following the intensity of inflammatory response to the microorganism (Fig. 1). The immunohistochemical expression of the studied molecules in *H. pylori*-infected patients before and after eradication is presented in Table 2.

HLA-DR expression (Fig. 2a,b)

In all cases ($n = 23$) of *H. pylori* gastritis, immunopositivity for HLA-DR by the epithelial cells was observed; the stain was more intense on the lower portions of the gastric pits, i.e. on the

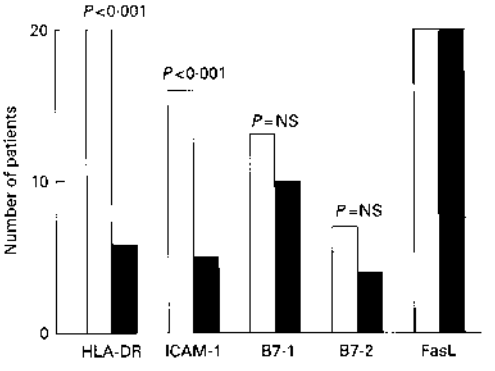

Fig. 1. Immunopositivity for the studied molecules in gastric antrum (□) and corpus (■) in the 20 patients in whom the microorganism was subsequently eradicated.

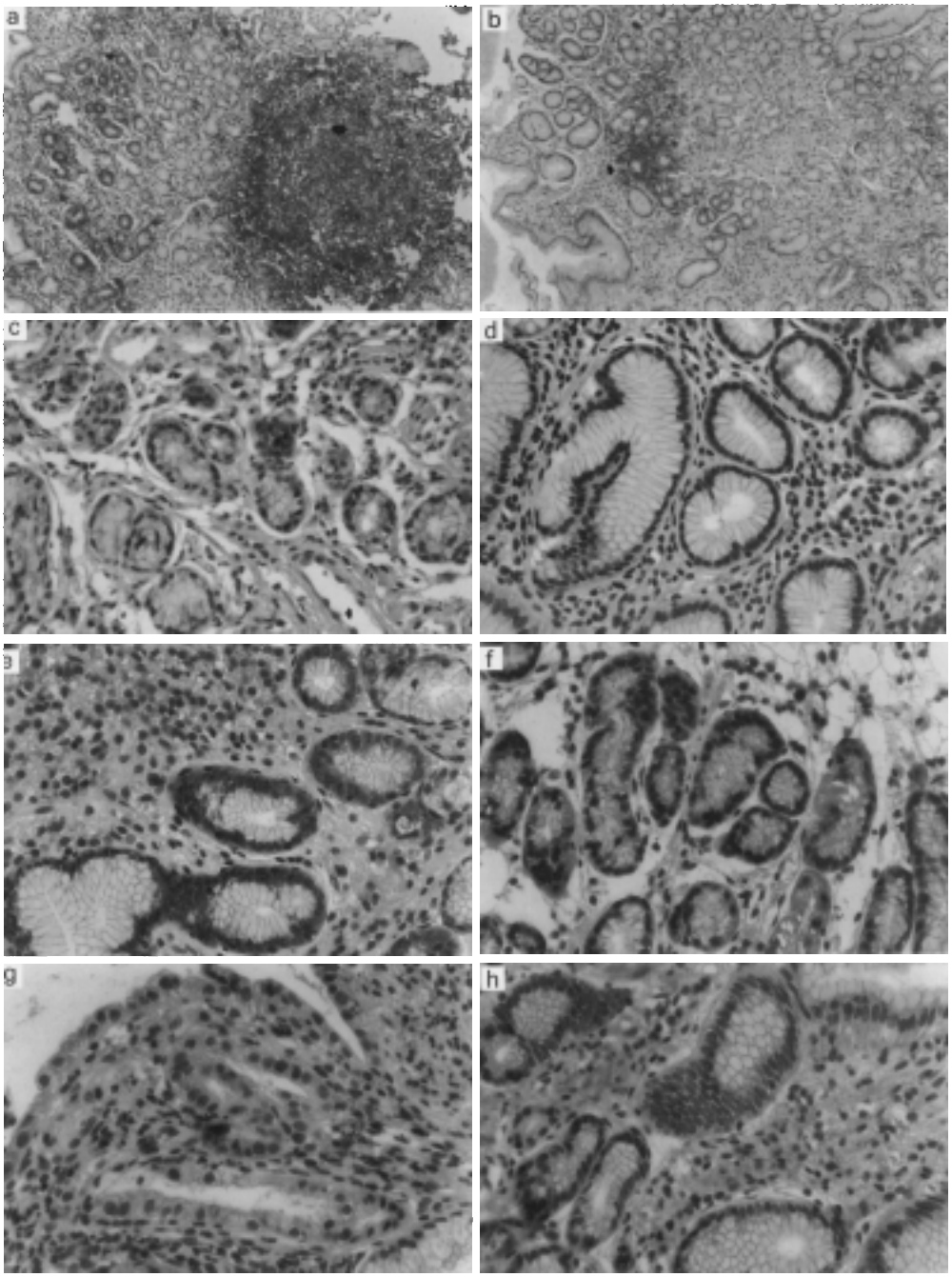

Fig. 2. (See next page for caption)

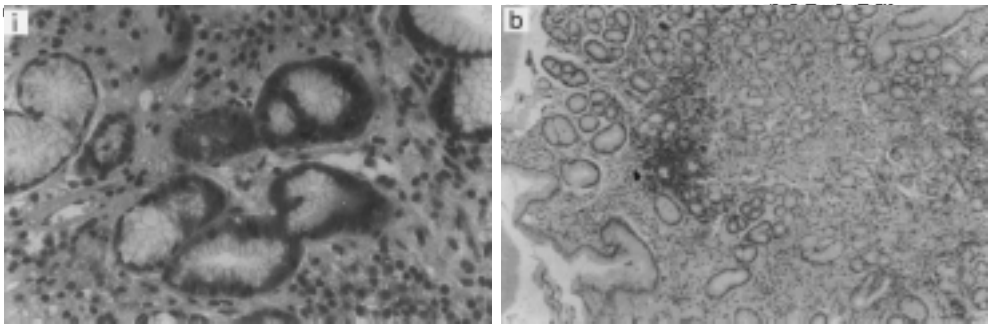

Fig. 2. (See previous page) In *Helicobacter pylori* gastritis (a) anti-HLA-DR staining of epithelial cells is observed mainly on the proliferating zone (small arrowhead) and of lymphocytes on a secondary follicular structure (large arrowhead). After successful eradication (b) only some lymphocytes of a lymphoid nodule remnant (arrowhead) and some scattered lymphocytes remain HLA-DR$^+$ (mag. × 100). Anti-ICAM-1 staining of epithelial cells, lymphocytes and endothelial cells (arrowhead) in a case of *H. pylori* gastritis (c). After successful eradication (d) only some endothelial cells (arrow) and some mononuclear cells remain ICAM-1$^+$ (mag. × 400). Anti-B7-1 and anti-B7-2 staining of epithelial cells and infiltrating mononuclear cells in *H. pylori* gastritis before (e,g) and after eradication (f,h); anti-FasL staining of epithelial cells and infiltrating mononuclear cells before (i) and after (j) *H. pylori* eradication (mag. × 400).

proliferating zone. The intensity of staining correlated with the activity of gastritis according to the Sydney classification (Spearman, $P < 0{\cdot}01$). In *H. pylori*$^-$ patients no HLA-DR immunostaining of epithelial cells was observed. In the 20 *H. pylori*$^+$ patients in whom eradication occurred, a statistically significant decrease of HLA-DR expression was observed (Wilcoxon: antrum, $P < 0{\cdot}001$; corpus, no HLA-DR expression after eradication).

ICAM-1 expression (Fig. 2c,d)

Immunopositivity for ICAM-1 was observed on epithelial cells of the gastric antral mucosa in 80% of *H. pylori*$^+$ patients and did not correlate with any of the gastritis parameters. However, it was marginally correlated with HLA-DR expression (Spearman: $P = 0{\cdot}08$). ICAM-1 was expressed focally on the gastric mucosa and the positive stained epithelial cells were restricted in the proliferating zone. After eradication the number of patients expressing ICAM-1 decreased significantly (Wilcoxon: antrum, $P < 0{\cdot}01$; corpus, no ICAM-1 expression after eradication).

B7-1(Fig. 2e,f) and B7-2 (Fig. 2g,h) expression

Weak epithelial positivity for both B7 molecules was observed in all control tissues. In all *H. pylori*-infected tissues (except for two cases, one remained infected after eradication therapy), epithelial cells were stained for B7 proteins. The staining for B7-1 and B7-2 was stronger on surface and proliferating zone epithelial cells compared with glandular cells and mid-foveolar regions, whereas in four cases the expression of B7-1 was significantly more intense than that of B7-2. The cells of intestinal metaplasia were stained more intensely for both molecules, but no evident relationship between the localization of mononuclear cells and neutrophils and B7 molecule expression was documented. After eradication, reduction in the intensity of expression of both B7 molecules was observed.

FasL expression (Fig. 2i,j)

Unexpectedly, FasL immunopositivity was constantly observed on glandular and foveolar epithelial cells of gastric antrum and corpus, both in normal and in *H. pylori*-infected mucosa. The intensity of stain was greater on the proliferating zone of the gastric pits and the surface epithelium, irrespective of inflammation. However, FasL expression was greater in sites of intestinal metaplasia and focally in sites of excessive active and/or chronic inflammation. The pattern of staining was cytoplasmic and membranous. No significant difference in intensity and distribution of stain before and after eradication was noted.

DISCUSSION

MHC class II molecules are normally expressed by a subset of cells with antigen-presenting capacity, i.e. B lymphocytes, macrophages and dendritic cells [29]. Non-haematopoietic cells, such as epithelial cells, may acquire the capacity to deliver antigen-specific signals to CD4$^+$ T lymphocytes, through the inducible *de novo* expression of MHC class II molecules [30,31]; this has been documented *in vitro* [32] but also *in vivo*, in inflammatory conditions [33–35]. MHC class II expression alone, in these non-professional APC, is a poor and rather tolerogenic stimulus for naive T cells, and this has been mainly attributed to their inability to provide costimulatory signals [22,36]. As has been shown in many T cell proliferation assay models, costimulatory signals are provided by B7 and ICAM-1 molecules after binding to their ligands, CD28/CTLA-4 and LFA-1, respectively, on the surface of T cells [24,37].

Normally, gastric epithelial cells, as was also shown by this study, do not express HLA class II molecules [38]. Only the HLA-DR molecule was studied, because its expression is dominant against DP and DQ and it is indicative of the expression of HLA class II molecules on non-lymphoid tissues [32,39]. In all cases of *H. pylori* gastritis, expression of the HLA-DR molecule on the epithelial cells, mainly in the proliferation zone, was observed. This was probably due to increased excretion of interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) by activated T lymphocytes of the lamina propria [40–42], the density of which is greater in this area. On the other hand, the action of these two cytokines in the induction of HLA-DR expression seems to be synergistic in immature cells, such as the cells of the proliferating

zone, and antagonistic in more differentiated ones [43]. HLA-DR expression correlates significantly with the activity of gastritis and it is significantly decreased after successful *H. pylori* eradication. This may well be the result of a reduction in the excretion of cytokines, due to the removal of foreign antigens. The observed correlation of HLA-DR expression with the intensity of the mucosal inflammatory reaction suggests that the epithelial cell may acquire APC properties.

As has already been mentioned, a second signal is required for the effective activation of T lymphocytes [44]. In our study, immunopositivity for B7-1 and B7-2 was observed on gastric epithelial cells, with the B7-1 molecule showing greater intensity than the B7-2, both before and after eradication. A reduction in the expression of both molecules was noted after eradication.

To the best of our knowledge the expression of B7 molecules by gastric epithelial cells has been previously studied only by Ye *et al.* [45], who found B7-2 expression to be higher than B7-1, at both protein and mRNA levels. The results of our immunohistochemical study are not fully compatible with the above mentioned findings, since we found higher expression of B7-1 molecule in *H. pylori*-infected subjects. This may be due to the different detection methods used in the two studies. On the other hand, there is experimental evidence considering the immune regulating function of B7 molecules in the resulting Th1- or Th2 type of immunity. It has been proposed that B7-1 expression drives the immune response toward the Th1 phenotype, as is the case in *H. pylori*-inflamed mucosa, while B7-2 expression drives toward Th2 phenotype [46,47].

We have also observed immunopositivity for both B7 family molecules in normal mucosa, and this finding suggests that their role is probably not limited to costimulation and activation of T lymphocytes, but they may exhibit other, as yet unknown, functions. Indeed, *in vitro* experiments have shown that B7 molecules on the surface of keratinocytes do not necessarily provide costimulatory signals to support T cell proliferation [32].

ICAM-1/CD54 is an adhesion/costimulatory molecule of the immunoglobulin superfamily, that is expressed on endothelial cells in inflamed tissues and some APC (dendritic cells, epidermal Langerhans cells). Coupling of T cell LFA-1 (CD11a/CD18) with ICAM-1 has been shown to provide a costimulatory signal for complete T cell activation [24]. ICAM-1 is also a ligand for the neutrophil integrin Mac-1 (CD11b/CD18) molecule [48].

In our study, ICAM-1 was immunohistochemically detected on epithelial cells in 80% of *H. pylori* gastritis cases and this immunopositivity was not correlated with the histological parameters of gastritis, as they were quantified according to the Sydney system, while it was correlated only marginally with HLA-DR expression. After eradication, a decrease of ICAM-1 expression was observed, probably due to the accompanying reduction of inflammation and cytokines (TNF-α, IFN-γ) [32]. Scheynius & Engstrand [49,50] and Hatz *et al.* [51] have shown no expression of ICAM-1 by gastric epithelial cells, while El Kaissouni *et al.* [39] observed ICAM-1 immunopositivity on epithelial cells in a case of *H. pylori* gastritis. Furthermore, Crowe *et al.* [52] demonstrated up-regulation of ICAM-1 expression on a human gastric epithelial cell line after *H. pylori* infection. The explanation of these conflicting results may be related to different forms (monomeric, dimeric) of ICAM-1 in various cells [53] and the observation that MoAbs specific for the monomeric form may not recognize the dimeric form [54]. Epithelial ICAM-1 expression may contibute to the pathophysiology of *H. pylori* inflammation by three ways. First, by acting as costimulatory factor to T cells. Second, through the interaction with Mac-1 molecule on neutrophils, providing anchoring signals to them; indeed, in all cases with ICAM-1 expression by epithelial cells, neutrophils have been observed infiltrating the epithelium. Third, based on recent experimental data that ICAM-1 and MHC class II co-expression selects toward Th1 immunity by down-regulating the production of Th2-type cytokines [55], it may contribute to the Th1 immune response that seems to predominate in *H. pylori* inflammation.

An interesting finding of the present study is that gastric epithelial cells constantly express FasL protein, i.e. irrespective of *H. pylori*-related inflammation. FasL (CD95L) is a type II transmembrane protein that belongs to the TNF family and is expressed mainly on activated $CD4^+$ and $CD8^+$ T cells in lymphoid organs such as thymus, spleen and lymph nodes [56]. In non lymphoid tissues its expression is restricted to the so-called immunologically privileged sites, i.e. eye (corneal endothelium and epithelium) and testis (Sertoli cells) [57,58]. It should also be mentioned that normal thyrocytes and thyrocytes in Hashimoto's thyroiditis [59], as well as epithelial cells in Sjögren's syndrome [60], express FasL, and this expression has been implicated in the pathogenesis of those diseases. Furthermore, expression of FasL by tumour cells has been observed in colon [61] and gastric [62] cancers, and thus these may escape the immune attack.

FasL interacts with its receptor, Fas (CD95), and its known function is to induce apoptotic cell death to Fas-bearing cells [63]. FasL expression by gastric epithelial cells may contribute to gastric mucosa physiology by two ways. First, the Fas–FasL system may be implicated in epithelial homeostasis in normal, as well as in *H. pylori*-infected mucosa, by inducing apoptosis to Fas-bearing epithelial cells (unpublished data, [64]). Second, it is possible that the interaction of FasL on epithelial cells with Fas on the surface of activated lamina propria T lymphocytes contributes to down regulation of inflammation by inducing apoptosis in Fas^+ mucosa-infiltrating activated lymphocytes.

In conclusion, our data suggest that gastric epithelial cells acquire features of the APC in the context of *H. pylori* infection, orchestrating a specific immune response against the microorganism.

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"Reappearance" of *Helicobacter pylori* After Eradication: Implications on Duodenal Ulcer Recurrence

A Prospective 6 Year Study

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Abstract

We estimated the rate of *Helicobacter pylori* "reappearance" and of duodenal ulcer relapse up to 6 years after eradication of *H. pylori*. Of 220 patients in whom *H. pylori* was eradicated, 165 were eligible at 12 months to follow-up. Endoscopy was scheduled every 12 months or whenever symptoms appeared. Baseline *H. pylori* eradication was confirmed by CLO test, histology (hematoxylin-eosin and Giemsa stain), and culture. *H. pylori* was tested for by the three methods at 12 months and subsequently by 2 methods (CLO, histology) on biopsies obtained from the gastric antrum and body. We reviewed 90 patients after 1 year, 32 after 2 years, 13 after 3 years, 12 after 4 years, 2 after 5 years, and 16 after 6 years (range, 12 to 72 months; average, 25.23 months; patient-years, 347). At 12 months after eradication, 16 of 165 patients (9.7%) were *H. pylori* positive and 5 had ulcer relapse. Of 75 patients evaluated at 24 months, 7 (9.3%) were *H. pylori* positive and 1 (1.3%) had ulcer relapse. At 36 months, 43 patients were seen and 1 (2.3%) was *H. pylori* positive and had ulcer relapse (2.3%). Thirty, 18, and 16 patients were seen at 48, 60, and 72 months, respectively. None was *H. pylori* positive and none had ulcer relapse. Overall, 24 *H. pylori*-positive patients were found, two thirds of them in the first year after eradication. In 7 of 24 (29%, 6 smokers), ulcer recurred. None of the *H. pylori*-negative patients had ulcer relapse. The *H. pylori* reappearance rate was 7% and the ulcer relapse rate was 2% per patient-year. If the 16 *H. pylori*-positive patients who were found the first year are considered as recrudescence, then the reinfection rate will be 2.3% per patient-year.

Key Words: Eradication—Duodenal ulcer—Follow-up—*Helicobacter pylori*—Omeprazole—Ranitidine—Reappearance of *H. pylori*—Recrudescence—Recurrence—Reinfection—Classic triple eradication therapy—Ulcer relapse.

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Some data document the association of *Helicobacter pylori* eradication with a dramatic reduction in peptic ulcer recurrence for a significant period of time after the eradication; reinfection rate seems to be negligible. Thus, in 141 patients with duodenal ulcer (DU) and in 45 patients with gastric ulcer in whom *H. pylori* was successfully eradicated, none had an ulcer relapse after follow-up of 367 and 113 patient-years, respectively; no reinfection was observed.[1] Others[2] who studied 175 patients for 360 patient-years reported a reinfection rate of 2.2% and an ulcer recurrence rate of 1.6%. Abu-Mahfouz et al.[3] in the United States studied 58 patients 5 years after successful eradication of *H. pylori* and, defining *H. pylori* recurrence as reinfection occurring 1 year after the therapy, reported 1% *H. pylori* recurrence per year post-treatment.

It has been suggested[4] that under optimal conditions and if reinfection is distinguished from recrudescence, the acquisition rate and the reinfection rate will be identical. *H. pylori* acquisition rate varies depending on various factors such as age, geographic area, and socioeconomic status. Here we report results concerning 165 patients with DU who were followed up for up to 6 years after *H. pylori* eradication in a country with roughly 60% seroprevalence of *H. pylori*-positive people among healthy adults.[5]

METHODS

Patients

We followed, for up to 6 years, 165 of 220 patients with healed DU in whom *H. pylori* was successfully eradicated by a

triple-drug regimen of tripotassium-dicitrato-bismuthate 125 mg four times a day and tetracycline hydrochloride 500 mg four times a day plus metronidazole 500 mg three times a day for 15 days. Patients were pretreated with omeprazole 20 mg daily or ranitidine 150 mg twice daily for 4 to 8 weeks. Results concerning the first 132 patients, who were seen at the end of a 12-month follow-up, are presented elsewhere.[6] Patients were excluded if they used corticosteroid drugs, aspirin, nonsteroidal anti-inflammatory drugs, or any antiulcer therapy systematically. In addition, patients with previous gastric surgery or severe disease of any kind were excluded.

Endoscopy

Endoscopy was scheduled every 12 months or whenever symptoms reappeared. The endoscopes used (Olympus GIF 1T-20 fiberoptic and GIF-100 videoendoscope EVIS 100) were disinfected with an automatic washing machine (CIR-CLEAN MC-II) or manually by immersing them for 20 minutes in a solution containing 2% glutaraldehyde. All channels of the instruments were rinsed with this solution. Biopsy forceps were disinfected as was described for the endoscopes but the immersion time was at least 30 minutes.

Eradication of *H. pylori*

At baseline, *H. pylori* eradication was confirmed on multiple gastric biopsies[6] by three methods: CLO test (Delta West, Australia), histologically after hematoxylin-eosin stain, and culture. As common practice in our pathology department, all patients who were *H. pylori* dubious or negative after hematoxylin-eosin stain were reviewed using Giemsa stain. *H. pylori* was considered eradicated if all three tests were negative at least 4 weeks after the triple therapy ended. Search for *H. pylori* was done by all the three methods at the first 12-month follow-up and subsequently by two methods (CLO and histology after hematoxylin-eosin and Giemsa stains). Biopsies were obtained from the gastric antrum and body as follows: CLO, one biopsy from the antrum and one from the lesser curvature of the stomach at the gastric angle; histology, two biopsies from the antrum and two from the body (anterior and posterior wall).

Criteria for Completing the Study

Patients were considered to have completed the study if they were found *H. pylori* positive or if the ulcer recurred.

RESULTS

So far, we reviewed 165 patients as follows: 90 patients after 1 year, 32 after 2 years, 13 after 3 years, 12 after 4 years, 2 after 5 years, and 16 after 6 years (range, 12 to 72 months; average, 25.23 months). Analytically, 165 patients (113 men, ages 18 to 65 years, 80 smokers) were eligible for evaluation 12 months after eradication. Eighty-one (54 men, 37 smokers) were pretreated with ranitidine and 84 (59 men, 43 smokers) with omeprazole. Sixteen (9.7%, 10 men) *H. pylori*-positive patients were found. Of them, nine were pretreated with ranitidine and seven with omeprazole: five (3%, four men) had asymptomatic ulcer relapse. Seventy-five (75) patients were evaluated at the end of 24 months after eradication. Seven patients (9.3%, six men) were *H. pylori* positive three were treated with ranitidine and four with omeprazole and one woman (1.3%, nonsmoker) had asymptomatic ulcer relapse. Forty-three patients were examined at the end of 36 months and only one man (2.3%, smoker who was pretreated with ranitidine was *H. pylori* positive and had ulcer relapse. Thirty, 18, and 16 patients were evaluated at the end of 48, 60, and 72 months, respectively. None was *H. pylori* positive and none had ulcer relapse (Fig. 1).

Overall, we followed 165 patients for 347 patient-years. Twenty-four *H. pylori*-positive patients were found, 16 of them in the first year after eradication; 1 were pretreated with ranitidine and 11 with omeprazole. In 7 of 24 patients (29%, six smokers), ulcer recurred. However, none of the *H. pylori*-negative patients had ulcer relapse. None of the patients needed any systematic antiulcer therapy during the follow-up and no patient was endoscoped because of appearance of symptoms.

It can be calculated that the *H. pylori* "reappearance" rate was 7% and the ulcer relapse rate was 2% per patient-year.

DISCUSSION

In this study we found that in a country with roughly 60% seroprevalence of *H. pylori*-positive people among healthy adults,[5] at least 14.5% of 165 successfully *H. pylori*-eradicated patients were *H. pylori* positive 3 years after eradication. Overall, the annual *H. pylori* reappearance rate and the annual ulcer relapse rate were calculated as 7% per patient-year and 2% per patient-year, respectively. Because each year we followed only a part of the *H. pylori*-eradicated patients, it is possible to underestimate the real number of *H. pylori*-positive ones. However, this is an inevitable limitation of all similar studies.

We do not know if we are dealing with recrudescence of *H. pylori* or reinfection with *H. pylori*. The reported

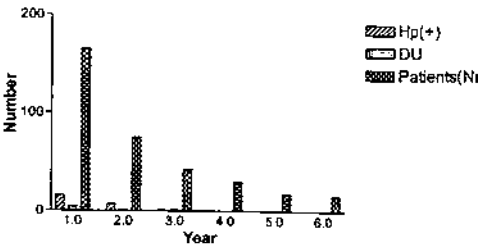

FIG. 1. The total number of patients, *H. pylori*-positive patients, and patients with ulcer relapse each year of follow-up.

reinfection rate varies from 0.36% to 13.7%[4] but decreases as the follow-up period progresses. Most reinfections (80 to 95%) occur within the first year of follow-up, and it has been suggested[4] that they really represent recrudescence. If *H. pylori* reappearance within the first year is excluded, there was a dramatic reduction in the annual reinfection rate: from 4.2% to 0.44%.[7] Reinfection has been defined as the *H. pylori* infection that occurs at least 1 year after the completion of therapy.[7–9] Under this definition we are dealing with recrudescence when *H. pylori* infection is documented within 1 year after treatment. If we consider as recrudescence the 16 *H. pylori*-positive patients who were found in the first year and exclude them, then the reinfection rate is 2.3% per patient-year, about twice that reported in the United States, under the same definition, by Abu-Mahfouz et al.[3] However, it is impossible to differentiate between recrudescence and reinfection without DNA fingerprinting of *H. pylori*.[1,4] It is suggested[4] that even so, results from the DNA fingerprinting must be interpreted with caution because the infection could be due to a heterogeneous *H. pylori* population. For this reason, we believe that the term "reappearance" of *H. pylori* is more suitable, describing more accurately the state of affairs.

Irrespective of the terminology, the fact is that practically all reappearances of *H. pylori* we observed were in the first 2 years of follow-up, that is, in a period within which recrudescence can occur.[4,10–12] This is compatible with the hypothesis that eradication, contrary to what is widely held, is not successful all the time.

A practical point compatible with the results of Van Der Hulst[1] is that no *H. pylori*-negative patient had ulcer relapse. Thus, recurrence of DU seems to be completely prevented after successful eradication of *H. pylori*. However, the problem is how to achieve successful eradication in all patients we treat and how "successful eradication" should be defined in routine clinical practice.

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The CLO test is unreliable in diagnosing *H. pylori* infection in post-surgical stomach; is there any role of *H. pylori* in peptic ulcer recurrence?

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Aim To evaluate the validity of the CLO test in detecting *Helicobacter pylori* in patients with gastric operation and to investigate the relationship of *H. pylori* with peptic ulcer recurrence in these patients.

Methods In this prospective study, 110 consecutive patients, the majority of whom had undergone gastric operation for benign disease ($n = 102$), were included. Eighty patients (62 males), aged 38–87 years, had had a gastrectomy (10 Billroth I, 70 Billroth II), and 30 patients (27 males), aged 36–73 years, had had a vagotomy (13 vagotomy plus gastroenterostomy, 17 vagotomy plus pyloroplasty). *H. pylori* was sought on multiple biopsy specimens, using CLO test and histology (modified Giemsa stain). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the CLO test were estimated using histology as 'gold standard'.

Results Overall, 21 gastrectomy patients (26%) were *H. pylori*-positive by CLO and 25 (31%) were *H. pylori*-positive by histology. The estimated sensitivity, specificity, PPV and NPV of the CLO test, using histology as 'gold standard', were 68%, 91%, 77% and 86%, respectively. The CLO test was positive in 67% of vagotomy patients (20 of 30), while 50% (15 of 30) were *H. pylori*-positive by histology. The estimated sensitivity, specificity, PPV and NPV of the CLO test were 87%, 53%, 65% and 80%, respectively. *H. pylori* prevalence by histology was 50% in patients with vagotomy and 31% in those with gastrectomy ($P = 0.0787$). Recurrent ulcers were observed in 8/30 patients (27%) after vagotomy and in 10/72 patients (14%) after gastrectomy. Recurrent ulcer was documented in 6/15 *H. pylori*-positive patients with vagotomy (40%), and in one of 25 *H. pylori*-positive patients with gastrectomy (4%). This difference was significant (Fisher's exact test, $P = 0.007$, relative risk 5.091, 95% CI 0.819–31.64).

Conclusion The CLO test seems to be unreliable in diagnosing *H. pylori* in post-surgical stomach. The *H. pylori* prevalence is higher, although not significantly, in vagotomized patients compared with gastrectomized patients, and in this group is closely related to the presence of recurrent ulcer. So, at least in this group of patients, it is strongly recommended to look for and eradicate *H. pylori*. *Eur J Gastroenterol Hepatol* 12:93–96

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Keywords: CLO test, gastrectomy, gastric operation, gastric surgery, *Helicobacter pylori*, post-surgical stomach, rapid urease test, ulcer recurrence, ulcer relapse, vagotomy

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Introduction

The significance of *Helicobacter pylori* infection in the non-operated stomach is well established, and the roles of *H. pylori* infection in the pathogenesis of peptic ulcer [1], gastric cancer [2,3] and gastric malt lymphoma [4] are widely accepted. By contrast, in the post-operative stomach, the exact pathogenic role of *H. pylori* is unclear. Thus, the implication of *H. pylori* infection in the pathogenesis of recurrent peptic ulceration [5–9] and the carcinogenesis of the gastric stump [10–13] is still controversial. Nevertheless, the European Maastricht Consensus advises that patients should have *H. pylori* eradicated post-operatively [14].

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There are a number of reliable methods to diagnose *H. pylori* infection in patients with intact stomach. Among these, the rapid urease test (RUT) – CLO test in particular, is one of the most commonly used in daily clinical practice worldwide; it is easy to perform and is considered quite reliable, with sensitivity 89–98% and specificity 93–98% [15]. However, the validity of the CLO test to diagnose *H. pylori* infection after gastric operation has been questioned [16].

The aim of the present study was twofold. First, to evaluate in these patients the validity of the CLO test in diagnosing *H. pylori* infection using histology as the

'gold standard', and second, to investigate the relationship, if any, of *H. pylori* infection with the recurrence of peptic ulcer.

Patients and methods

This prospective study was performed over a 12-month period (May 1996 to April 1997). One hundred and ten consecutive patients of both sexes (89 men, 21 women), aged 36–87 years (mean 63 ± 12), were included. One hundred and two patients were operated on for peptic ulcer disease and eight patients for gastric malignancies (two gastric cancers, six gastric lymphomas). The mean time interval between gastric operation and the study was 17.4 years (range 1–51). Patients who reported recent (less than 2 months) treatment with proton-pump inhibitors, H_2-receptor antagonists, bismuth salts, sucralfate and antibiotics, as well as those who had taken any *H. pylori* eradication therapy any time before, were excluded. In patients with active gastrointestinal bleeding, biopsies and CLO test were performed during a second endoscopy which was performed after the bleeding cessation.

The patients were classified in two groups according to the type of the gastric operation, as follows. The gastrectomy group (group A) comprised 80 patients (62 men), aged 38–87 years (mean 64), who had had gastrectomy (Billroth I $n = 10$, Billroth II $n = 70$) during the last 51 years (from 1945 to 1996, mean time interval between the gastric resection and the study 19 years). The vagotomy group (group B) comprised 30 patients, aged 36–73 years (27 men), including 13 patients (11 men) (mean age 61.5 years) who had undergone vagotomy and gastroenterostomy (V+G) during a 22-year period (1970–1992, mean time interval 17 years), and 17 patients (16 men) (mean age 58 years) who had undergone vagotomy and pyloroplasty (V+P) during a 22-year period (1973–1995, mean time interval 9.4 years). No patient with highly selective vagotomy was included among the patients (Table 1).

All patients were endoscoped (upper gastrointestinal endoscopy). Peptic ulcer was defined as a loss of mucosal surface, at least 5 mm in diameter, as measured by the opened tip of the biopsy forceps. *H. pylori* was sought in tissue specimens of gastric mucosa, using the CLO test (Delta West, Australia) and histology (modified Giemsa stain), in pairs. The tissue specimens were taken from the following areas in each group of patients: group A – two specimens from the peri-anastomotic area and two from the fundo-corporal segment; group B – two specimens from the antrum, two from the peri-anastomotic area and two from the corpus in patients with V+G, and two specimens from the antrum and two from the corpus in patients with V+P.

Each specimen was examined blindly by two expert pathologists, who were unaware of the endoscopic findings and the results of the CLO test. Those patients who were found to be *H. pylori*-positive at *any* site of the stomach were considered *H. pylori*-positive. The CLO test was evaluated 2 and 12 h after endoscopy.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the CLO test were estimated using histology as the 'gold standard'. Fisher's exact test was used for the statistical analysis.

Results

Overall, of the 110 patients studied, *H. pylori* was found by CLO test in 42 (38%), and by histology in 40 (36%) (Table 2).

Gastrectomy group (80 patients)

Overall, 21 (26%) were *H. pylori*-positive by CLO and 25 (31%) were *H. pylori*-positive by histology. The estimated sensitivity, specificity, PPV and NPV of the CLO test, using histology as the 'gold standard', were 68%, 91%, 77% and 86%, respectively.

Of the 70 patients who underwent the Billroth II operation, 19 (27%) were positive by the CLO test and 21 (30%) were positive by histology. Of the 10 patients who underwent a Billroth I operation, three (30%) were *H. pylori*-positive by the CLO test and four (40%) by histology. There was no significant difference in *H. pylori* prevalence between the two groups for either the CLO test ($P = 0.553$) or histology ($P = 0.380$).

Table 1 **Patient characteristics**

		Gastrectomy		Vagotomy	
	Total	BI	BII	V-G	V-P
Number of patients	110	10	70	13	17
Mean age (± SD) (years)	63 ± 12	63 ± 14	65 ± 11	62 ± 14	58 ± 14
Male to female ratio	4.2:1	1:1	4.4:1	5.5:1	16:1
Time interval from operation (± SD) (years)	17 ± 12	14 ± 10	20 ± 13	17 ± 5	9 ± 8

BI, Billroth I; BII, Billroth II; V-G, vagotomy and gastroenterostomy; V-P, vagotomy and pyloroplasty.

Table 2 ***H. pylori* prevalence and endoscopic findings**

	Total	Gastrectomy	Vagotomy
Number of patients with *H. pylori* infection diagnosed by histology (%)	40 (36)	25 (31)*	15 (50)*
Number of patients with *H. pylori* infection diagnosed by CLO test (%)	42 (38)	21 (26)**	20 (67)**
Number of patients with ulcer during endoscopy (%)	18 (16)	10 (14)†	8 (27)

*P 0.0787, **P – 0.0001.
†Eight patients who were operated on for gastric malignancy were excluded from the analysis.

In this group, 19 patients had been operated on during the last five years and 61 patients more than five years before: the numbers *H. pylori*-positive by histology were 3/19 (16%) and 22/61 (36%) respectively (difference not significant ($P = 0.155$), Fisher's exact test, 95% CI 0.087–1.269).

Vagotomy group (30 patients)

The CLO test was positive in 67% (20 of 30), whereas 50% (15 of 30) were *H. pylori*-positive by histology. The estimated sensitivity, specificity, PPV and NPV of the CLO test were 87%, 53%, 65% and 80%, respectively.

Eight of the 13 patients (61%) with V+G were *H. pylori*-positive by the CLO test and 6 of the 13 patients (46%) were positive by histology; in the V+P group, 12 of the 17 patients (70%) were *H. pylori*-positive by the CLO test and 9 of 17 (53%) were positive by histology. There was no significant difference in *H. pylori* prevalence between the two groups for either the CLO test ($P = 0.446$) or histology ($P = 0.500$). In this group of patients, six were operated on during the last five years and 24 more than five years before; the numbers *H. pylori*-positive by histology were 3/6 (50%) and 12/24 (50%), respectively (Fisher's exact test, $P = 1.3487$, difference not significant, 95% CI 0.167–5.99).

Comparison of the two groups

The prevalence of *H. pylori*-positivity by the CLO test differed significantly ($P = 0.0001$, 95% CI 0.071–0.441) between patients with gastrectomy (group A) and those with vagotomy (group B); however, no difference was found by histology ($P = 0.0787$, 95% CI 0.191–1.072).

There was also an obvious discrepancy in the groups A and B concerning the sensitivity and the specificity of the CLO test: sensitivity was 68% and 87% and specificity 91% and 53%, respectively.

H. pylori and ulcer recurrence

The eight patients who were operated on for gastric cancer or lymphoma were not included in this analysis. Thus, 30 patients with vagotomy and 72 patients with gastrectomy were analysed. Recurrent ulcer was documented in 8/30 (27%) patients after vagotomy and in 10/72 patients (14%) after gastrectomy ($P = 0.123$). Of the eight patients with ulcer after vagotomy and the ten patients with ulcer after gastrectomy, *H. pylori* was documented histologically in seven patients (87.5%) and one patient (10%), respectively. This difference was significant (Fisher's exact test, P 0.0029, relative risk = 8.75, 95% CI 3.320–1196). Recurrent ulcer was observed in 1/25 *H. pylori*-positive patients with gastrectomy (4%) and in 6/15 *H. pylori*-positive patients with vagotomy (40%). This difference was also significant (Fisher's exact test, $P = 0.007$, relative risk = 5.091, 95% CI 0.819–31.64).

Discussion

The prevalence of *H. pylori* infection in patients who have undergone gastric operation varies between studies [5,7–9,16–20]. These studies were performed in a number of countries, with a different time interval from the operation and different methods used to diagnose *H. pylori* infection.

Estimating the validity of the CLO test in the post-surgical stomach, using histology as the 'gold standard', a method that is easy to perform in every country all over the world, we found that the CLO test is a non-acceptable method for the detection of *H. pylori* infection in the gastrectomized patients, with sensitivity, specificity and PPV 68%, 91% and 77%, respectively. This is also true for the vagotomized patients in whom the sensitivity was 87%, but with a very low specificity (53%) and PPV (65%). As factors which may affect the validity of CLO test, such as antibiotics, proton-pump inhibitors and active gastrointestinal bleeding [21], had already been excluded from the present study, our results suggest that the CLO test is unreliable to diagnose *H. pylori* infection in the post-surgical stomach. This is in keeping with the results of Leung *et al.* [16].

Using histology, we found a higher prevalence, although not significantly, of *H. pylori* infection in the vagotomy patients (50%) than in the gastrectomy group (31%). The relatively low prevalence of *H. pylori* in patients with vagotomy has been reported by others

[8,16,19], and is explained as a result of the bile reflux that is observed in these patients. Danesh *et al.* [22], in a review of 36 studies, found a higher prevalence of *H. pylori* after vagotomy (83%), but most of the data concerned were collected after highly selective vagotomy, which is associated with no increase in bile reflux [23]. No patient with highly selective vagotomy was included among our patients. Furthermore, in the gastrectomy group, *H. pylori* prevalence was higher in the Billroth I group (40%) than in the Billroth II group (30%). Although the difference is not significant, perhaps because of the small number of patients in the Billroth I group, a role for bile acid reflux, which is more common in Billroth II than Billroth I, in *H. pylori* colonization of the gastric remnant could be suggested [24]. Leivonen *et al.* [7] and Tomtitchong *et al.* [20] found that Billroth II anastomosis was followed by a significantly lower rate of *H. pylori* infection than Billroth I anastomosis.

Regarding the ulcer recurrence in post-surgical stomach, which was more common in vagotomy (27%) than in gastrectomy (14%), we found that *H. pylori* infection was closely related with recurrent ulcer disease in the vagotomized patients (relative risk 5.091). Therefore, *H. pylori* eradication is strongly recommended in this group of patients. In the gastrectomized group, there was no such relation, and it seems that some other factors, such as inadequate gastric resection, play a more predominant role [25].

In conclusion, *H. pylori* infection is relatively common in patients after gastric operation, but the CLO test seems to be unreliable in diagnosing *H. pylori* in the post-surgical stomach. *H. pylori* prevalence is higher, although not significantly, in vagotomized patients, and in this group is closely related with the presence of recurrent ulcer. So, at least in this group of patients, it is strongly recommended to look for and eradicate *H. pylori*.

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Factors that May Affect Treatment Outcome of Triple *Helicobacter pylori* Eradication Therapy with Omeprazole, Amoxicillin, and Clarithromycin

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Factors affecting *Helicobacter pylori* eradication rate with omeprazole (OME), clarithromycin (CL), and amoxicillin (AMO) have not been extensively studied. We have investigated the effect of age, sex, smoking, ulcer disease, compliance with therapy, *H. pylori* colonization density, degree and activity of antral gastritis, the coexistence of corpus gastritis, and the presence of lymphoid follicles on *H. pylori* eradication rate. We studied 80 consecutive *H. pylori*-positive patients, with duodenal ulcer ($N = 35$) or nonulcer dyspepsia ($N = 45$) treated with OME 20 mg, CL 500 mg, and AMO 1 g, each given twice daily for 10 days. *H. pylori* was eradicated in 71/80 (88.8%, 95% CI 82–96%) patients. The regimen failed to eradicate the only strain (1.8%, 95% CI 0–5.2%) that was clarithromycin resistant. Multivariate discriminant analysis showed that two histological variables (Wilks $\lambda = 0.74$, $\chi^2 = 23.41$, $df = 2$, $P < 0.001$), absence of lymphoid follicles in routine gastric biopsies ($F = 13.63$, $P < 0.001$) and coexistence of antral and body gastritis ($F = 13.68$, $P < 0.001$), significantly increased *H. pylori* eradication rate. No other factor examined predicted *H. pylori* eradication with this regimen. Our data suggest that body gastritis is a positive and presence of lymphoid follicles in routine gastric biopsies is a negative predictive factor of treatment outcome with the omeprazole, clarithromycin, and amoxicillin regime.

KEY WORDS: *Helicobacter pylori*; follicular gastritis; omeprazole; clarithromycin; amoxicillin; *Helicobacter pylori* gastritis.

Helicobacter pylori is the causative agent of antral gastritis (1) and a key factor in the pathogenesis of peptic ulcer disease (2). Eradication of *H. pylori* is now the treatment of choice to cure peptic ulcers and prevent ulcer complications (3, 4). Recent studies have shown that triple therapy with omeprazole, clarithromycin, and amoxicillin is an effective regimen to eradicate *H. pylori* (5). Several studies have examined the optimal dose and duration of therapy (6, 7), but only a few have evaluated factors affecting *H. pylori* eradication rate with this regimen (5, 8). Therefore, the objective of the present study was to investigate whether clinical, demographic, or histological factors

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may affect *H. pylori* eradication rate in patients treated with omeprazole 20 mg, clarithromycin 500 mg, and amoxicillin 1 g, each given twice daily for 10 days.

MATERIALS AND METHODS

Patients. Eighty consecutive, symptomatic *H. pylori*-positive patients with active duodenal ulcer or nonulcer dyspepsia participated to this open, prospective study. They all gave written consent after full explanation by the investigator. The study protocol was approved by the Ethical Committee on Human Studies of the Department of Internal Medicine, Medical School, Athens University. Patients with malignancy, gastric surgery, and chronic renal, hepatic, or cardiorespiratory diseases were excluded from the study. No one patient had received *H. pylori* eradication therapy at any time or antibiotics, bismuth, proton-pump inhibitors or nonsteroidal antiinflammatory drugs during the last month preceding the study. Pregnant or lactating women were not included in the study.

Diagnosis and Treatment of *H. pylori* Infection. Upon admission to the study each patient had upper gastrointestinal endoscopy with biopsies ($N = 8$) to define endoscopic and histologic diagnosis, and *H. pylori* infection. Two antral and two corpus biopsy specimens were sent for histology, two antral specimens were used for a rapid urease test (CLO-test, Delta West, Bentley, Western Australia), and two biopsies taken from the lesser curvature of the antrum were sent for culture and antibiotic sensitivity tests. Patients with a positive culture or a combination of positive CLO-test and histology were defined as *H. pylori* infected. All patients were treated with a 10-day regimen of omeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg each twice daily. Endoscopy was repeated at four to six weeks after treatment to confirm ulcer healing and *H. pylori* eradication. Successful eradication was defined by both negative histology and culture. Compliance with therapy was evaluated by the number of tablets (total 60) used by each patient.

All biopsies were evaluated by one senior pathologist, who was blinded to the treatment results. A modified Giemsa staining was used to detect *H. pylori* in biopsy specimens. The Warthin-Starry technique was employed for negative cases. Degree and activity of gastritis were assessed by hematoxylin–eosin stain and classified according to the Sydney System as none, mild, moderate, or severe (9). The following four-point scale was used to grade *H. pylori* colonization density (absent = 0, few = 1, dense = 2, heavy = 3). Follicular gastritis was evident by prominent lymphoid follicles with surrounding mantle zone and plasma cells, with no lymphoepithelial lesion (10).

***Helicobacter pylori* Culture and Antibiotic Sensitivity Tests.** Biopsy specimens were cultured on Wilkins-Chalgren anaerobe agar (Oxoid CM619) supplemented with 7% horse blood, 10% horse serum, and antibiotics (Dent's supplement, SR147E, Oxoid) at 37°C under microaerobic conditions for up to seven days. Colonies of typical appearance, Gram stain, and positive for both catalase and urease production were identified as *H. pylori*. Minimal inhibitory concentrations (MICs) of metronidazole and clarithromycin were determined by the agar dilution method, using Mueller-Hinton agar supplemented with 7% sheep blood and containing the antibiotics to be tested at the designated concentrations as previously described (11). The minimal inhibitory concentration was defined as the lowest antibiotic concentration at which no *H. pylori* growth could be observed. The cutoff concentrations used to define resistance were: >1 mg/liter for clarithromycin and >8 mg/liter for metronidazole.

Statistical Analysis. All data were stored in dBASE software (Microsoft Access 97, Microsoft Corp.). Analysis of the results was performed with the Statgraphics Plus 2.1 statistical package (Manugistics Inc., Statistical Graphics Corp., Rockville, Maryland). Qualitative data were assessed by χ^2 test with Yates' correction, as appropriate. Numerical data were examined for normality by the goodness-of-fit tests and the Kolmogorov-Smirnov one-sample test. Non-normally distributed data were analyzed by the nonparametric Mann-Whitney (Wilcoxon) two-sided W test. Multivariate discriminant analysis with a forward stepwise selection was used to identify variables with the strongest influence on treatment failure or success. $P < 0.05$ was regarded as significant.

RESULTS

Eighty consecutive *H. pylori*-positive patients, 51 men and 29 women, median age 46, (range 23–79) years, 35 with active duodenal ulcer and 45 with nonulcer dyspepsia were studied. Thirty-eight of the 80 patients were smokers. *H. pylori* was eradicated in 71/80 (88.8%, 95% CI 82–96%) patients. Five (6.3%, 95% CI 1–11.6%) patients reported minor treatment side effects (mild diarrhea = 2, stomatitis = 3), but there were no dropouts from the study. No patient was lost to follow-up. Compliance with therapy, ie, number of tablets taken, was not significantly different between patients who had and had not successfully eradicated *H. pylori* (60, 56–60 vs 60, 58–60 tablets, $W = 250$, $P = 0.25$). Eradication rate was not significantly different in patients with duodenal ulcer or nonulcer dyspepsia (29/35 vs 42/45, $\chi^2 = 3.04$, $P = 0.08$). The effect of age on *H. pylori* eradication rate was studied after categorizing all 80 patients into age groups (21–30, 31–40, 41–50, 51–60, 61–70, 71–80 years). There was no statistically significant difference in *H. pylori* eradication rate among the six age groups ($\chi^2 = 5.44$, $df = 5$, $P = 0.36$). Gender and smoking status did not significantly affect *H. pylori* eradication rate. The results of the histological parameters studied are presented in Table 1. Degree and activity of antral gastritis and *H. pylori* colonization density were not significantly related to treatment outcome. However, *H. pylori* eradication rate was significantly different (lower) in pa-

TABLE 1. COMPARISON OF GRADED (MEDIAN, RANGE) HISTOLOGICAL PARAMETERS STUDIED BETWEEN SUCCESSFULLY AND UNSUCCESSFULLY TREATED PATIENTS

	Eradicated (N = 71)	*Noneradicated* (N = 9)	*W test**
Grade of degree	2, 1–3	2, 1–3	$P = 0.80$
Grade of activity	2, 1–3	2, 1–3	$P = 0.32$
Grade of atrophy	2, 0–3	1, 0–3	$P = 0.28$
H. pylori colonization density	2, 1–3	2, 1–3	$P = 0.62$
Degree of body gastritis	2, 0–3	0, 0–2	$P = 0.013$

* Mann-Whitney (Wilcoxon) two-sided W test.

tients with lymphoid follicles than in those without (16/23 vs 55/57 $\chi^2 = 5.4$, $P = 0.02$). Patients who had both antral and any degree of body gastritis had a significantly higher *H. pylori* eradication rate as compared with those who had only antral gastritis (40/41 vs 31/39 $\chi^2 = 4.85$, $P = 0.03$) (Table 2).

Helicobacter pylori strains were isolated by culture of the gastric biopsy specimens from 68 of the 80 patients studied. Fifty-seven isolates were available for susceptibility testing. Twenty-five of 57 (43.9%, 95% CI 31.0–56.7%) *H. pylori* strains were metronidazole resistant and 1/57 (1.8%, 95% CI 0–5.2%) was clarithromycin resistant. The regimen failed to eradicate the strain that was resistant to clarithromycin.

Eighty complete cases were used to develop a model to discriminate among two levels of *H. pylori* eradication, ie, success and failure. Using a stepwise selection algorithm, it was found that two variables (Wilks $\lambda = 0.74$, $\chi^2 = 23.41$, $df = 2$, $P < 0.001$), ie, absence of lymphoid follicles from routine gastric biopsies ($F = 13.63$, $P < 0.001$) and coexistence of antral and body gastritis ($F = 13.68$, $P < 0.001$), were both significantly related to *H. pylori* eradication rate.

TABLE 2. PRESENCE OF FOLLICULAR GASTRITIS, GASTRITIS LIMITED TO ANTRUM AND BOTH ANTRAL AND ANY DEGREE OF CORPUS GASTRITIS*

	Eradicated (N = 71)	*Noneradicated* (N = 9)	*Total*
Follicular gastritis†	16	7	23
Absence of lymphoid follicles†	55	2	57
Antral gastritis‡	31	8	39
Antral plus body gastritis‡	40	1	41

* The results are presented separately for successfully and unsuccessfully treated patients.
† $P = 0.02$.
‡ $P = 0.03$.

DISCUSSION

It has recently been suggested that *H. pylori* eradication rates lower than 80% are not accepted nowadays (12). Therefore, factors affecting treatment failure should be identified. Overall, it has consistently been shown that patients' compliance and antimicrobial resistance are key factors of treatment outcome, whatever the regimen used (13–15). In this study we have evaluated factors underlying treatment failure of *H. pylori* therapy other than insufficient compliance and microbial resistance to antibiotics. This was possible because our patients had excellent compliance with the omeprazole–clarithromycin–amoxicillin 10-day therapy and we encountered only one *H. pylori* strain resistant to clarithromycin.

Our data show that gender, smoking, and age of the patient do not affect treatment success. Advanced age has been associated with improved bacterial eradication in certain studies (14, 15). Theoretically, advanced age could favorably affect treatment outcome by reducing acid output (16, 17), which may facilitate the bactericidal effect of antibiotics. Our data do not support such a relationship, possibly because of the relatively small number of elderly patients included in our study.

The influence of pretherapeutic histological parameters on *H. pylori* eradication rate is still controversial. Certain studies have shown that patients' gastric histology may affect *H. pylori* eradication rate (14, 15). It has been suggested that high scores of degree and activity of antral gastritis may favorably affect treatment outcome, supporting the idea of facilitated diffusion of antibiotics into the inflamed mucosa. In the present study neither the degree and activity of antral gastritis nor *H. pylori* colonization density of the gastric mucosa predicted treatment outcome. However, we encountered only nine treatment failures, and the statistical analysis of factors related to failure of the three-drug regimen have a marginally significant probability level.

Our data suggest that the coexistence of antral gastritis with any degree of corpus gastritis is a favorable predictive factor of eradication therapy. Corpus gastritis prevails in gastric ulcer patients and compromises gastric acid output (18, 19). It therefore increases intragastric pH, facilitating the bactericidal effects of antibiotics (20). In contrast, the presence of lymphoid follicles was the only factor adversely affecting *H. pylori* eradication rate with our regimen. The normal gastric mucosa contains scant lymphocytes. The development of lymphoid follicles in gastric mu-

cosa indicates B-lymphocyte proliferation and represents an immune response to common *H. pylori*-associated antigens (21). The importance of follicular gastritis is that it may progress to overt MALT lymphoma. The reported wide variation (14–72%) of the prevalence of follicular gastritis (22–24) probably implies differences in the degree and extension of a commonly observed immune phenomenon, which rather represents host- than strain-dependent processes (25). The low prevalence of follicular gastritis we have found (28.8%) is probably related to the following factors. First, we evaluated only lymphoid follicles and not lymphoid aggregates. Secondly, biopsies were processed "routinely" regarding the number of tissue sections, and finally no special staining was employed. We have therefore probably detected only cases with dense follicle formation in the antral mucosa, ie, severe follicular gastritis. Severe follicular gastritis, therefore, might represent a marker of some yet unknown host factors, ie, host genetics, HLA haplotypes, possibly interfering with *H. pylori* eradication (26).

In conclusion, our data suggest that patients with body gastritis may have a more favorable treatment outcome with the omeprazole, clarithromycin, and amoxicillin 10-day regimen. In contrast, the presence of lymphoid follicles in routine gastric biopsies indicating severe follicular gastritis may be a histological marker predicting poor treatment response. Since follicular gastritis has been recently implicated in the development of gastric MALT lymphoma (27) and molecular techniques could define follicular gastritis patients at risk for subsequent development of this tumor (28), it is very important further to define follicular gastritis related factors, which could prevent *H. pylori* eradication.

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CASE REPORT

Helicobacter pylori-Associated Protein-Losing Hypertrophic Gastropathy with Hypercholesterolemia

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KEY WORDS: protein-losing gastropathy; Menetrier's disease; *Helicobacter pylori*; hypoproteinaemia; hypercholesterolemia; ^{99m}Tc-labeled albumin.

Hypertrophic gastropathy (HG) is a rare clinical entity of unknown etiology. There are numerous criteria, classifications, and names given to entities associated with thickened gastric folds. However, the firm diagnosis of Menetrier's disease should be reserved for patients with giant gastric folds, hypoalbuminemia, and characteristic histological features (1). Bayerdorffer et al, in a retrospective study of 138 patients, detected *Helicobacter pylori* (HP) infection in more than 90% of cases with hypertrophic gastropathy, and a possible etiological role in the pathogenesis of the disease has been proposed (2). It has been suggested that Menetrier's disease may represent a hypersensitivity reaction to an HP antigen (3). Another study concludes that giant-fold gastritis is indeed a special form of HP gastritis and eradication of the organism in these patients helps to distinguish it from other conditions with giant folds (4). According to several reports (1, 2, 4–9), effective HP eradication induces remission of protein-losing HG, and it seems rational to apply such treatment in these patients.

We present here two patients with HG, gastric protein loss documented by radionuclide studies, hypercholesterolemia, and HP infection who experienced clinical, biochemical and scintigraphic normalization after eradication of the HP infection.

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CASE REPORTS

Case 1. A 43-year-old man, was admitted to our department in December 1995 with epigastric pain, postprandial fullness, and facial and lower extremity edema of at least 5 years duration. He also had β-thalassemia minor. On examination he had symmetrical lower limb pitting edema and signs of right pleural effusion without evidence of right heart failure. Hepatic and renal function were normal, and there was no protein in the urine. Laboratory investigations showed hemoglobin level and red blood cell count compatible with a β-thalassemia trait, total serum protein 4.1 g/dl (normal: 6.2–8.5 g/dl) with albumin 1.5 g/dl (normal: 3.5–5 g/dl), and a low calcium level at 6.9 mg/dl (normal: 8.2–10.6 mg/dl). Ceruloplasmin, transferrin, C3 component of complement, and immunoglobulin G (IgG) levels were all reduced at 79%, 69.3%, 82.4% and 44.5% of the lower limits of normal, respectively, whereas α_1-antitrypsin, C4, IgA, and IgM levels remained normal. He had an increased serum cholesterol level at 410 mg/dl (normal: 140–200 mg/dl). Serum gastrin was normal. Other tests and an extensive investigation for malabsorption or an occult malignancy were negative.

Chest x-ray revealed a right pleural effusion; abdominal ultrasound and computed tomography showed minimal free peritoneal fluid and gross thickening of the gastric wall. Gastroscopy revealed diffuse mucosal edema and umbilicated lesions throughout the stomach. Examination of the biopsy specimens showed hypertrophic gastritis with the presence of HP organisms and normal counts of intraepithelial lymphocytes. Serial scans performed after ^{99m}Tc-labeled albumin injection (human serum albumin-Sn-^{99m}Tc injectable solution, CIS Bio International) demonstrated isotope migration into the stomach lumen (Figure 1A).

The patient was treated with omeprazole, 20 mg plus amoxicillin, 1 g, and clarithromycin, 500 mg, twice daily for 10 days. On examination after two months, the edema was reduced but protein levels were still abnormal. Gastroscopy revealed no macroscopic improvement and HP infection, although limited, was still histologically present. Therefore,

A B

Fig 1. (A) ^{99m}Tc-labeled albumin extravasation in the site of the stomach (before treatment), (s: stomach). (B) No evidence of the ^{99m}Tc-labeled albumin extravasation (after treatment).

the patient started therapy with bismuth subcitrate 120 mg four times daily, in combination with omeprazole, clarithromycin and ornidazole for 14 days. Two months later the edema had disappeared, biochemical tests became normal, endoscopy revealed only mild erythematous gastritis, and histology showed complete eradication of HP. Repetition of serial scans after ^{99m}Tc-labeled albumin injection did not demonstrate isotope migration into the gastric lumen (Figure 1B). Two years after the diagnosis, he remains symptom free, with normal serum albumin and cholesterol.

Case 2. A 21-year-old female, without relevant previous medical history, was admitted in September 1996 with bilateral lower limb edema and weight loss. She also complained of fatigue and dizziness. She denied any abdominal discomfort and had normal bowel movements. Preliminary laboratory investigations showed hypoproteinemia without proteinuria and normal liver function tests. Total protein was 4.4 g/dl, with albumin 2.1 g/dl, β_2-globulins 0.3 g/dl (normal: 0.7–1.1 g dl) and γ-globulins 0.4 g/dl (normal: 0.8–1.6 g/dl). The IgG level was reduced to 79% of the lower limit of normal, whereas IgA and IgM were within normal range. Gastrin, α_1-antitrypsin, ceruloplasmin, transferrin, and components of complement were also within normal limits. Cholesterol was elevated (290 mg/dl), while triglycerides were normal. Other malabsorption tests did not show any abnormality. Abdominal computed tomography showed asymmetrical thickening of the gastric wall. Serial scans after ^{99m}Tc-labeled albumin injection revealed isotope migration into the stomach lumen. On endoscopic examination and barium meal, markedly thickened and edematous folds were noted in the fundus and body of the stomach without erosions or active peptic ulcer. The esophagus, antrum, and duodenum were normal, and snare biopsies of the gastric folds were obtained. Histology of the biopsies was compatible with Menetrier's disease with the presence of HP (Figure 2).

The patient was treated with omeprazole, 20 mg, amoxicillin, 1 g, and clarithromycin, 500 mg, twice daily plus bismuth subcitrate 120 mg four times daily for two weeks. Two months later, there was no evidence of the lower extremity edema. The serum total protein was 7.2 g/dl, albumin 5 g/dl, and cholesterol 182 mg dl. Endoscopic and upper gastrointestinal x-ray examinations revealed normal gastric folds and there was no ^{99m}Tc-labeled albumin secretion into the gastric lumen. Microscopically, there was only residual superficial gastritis with absence of HP infection. One year later the patient remains asymptomatic with normal laboratory values.

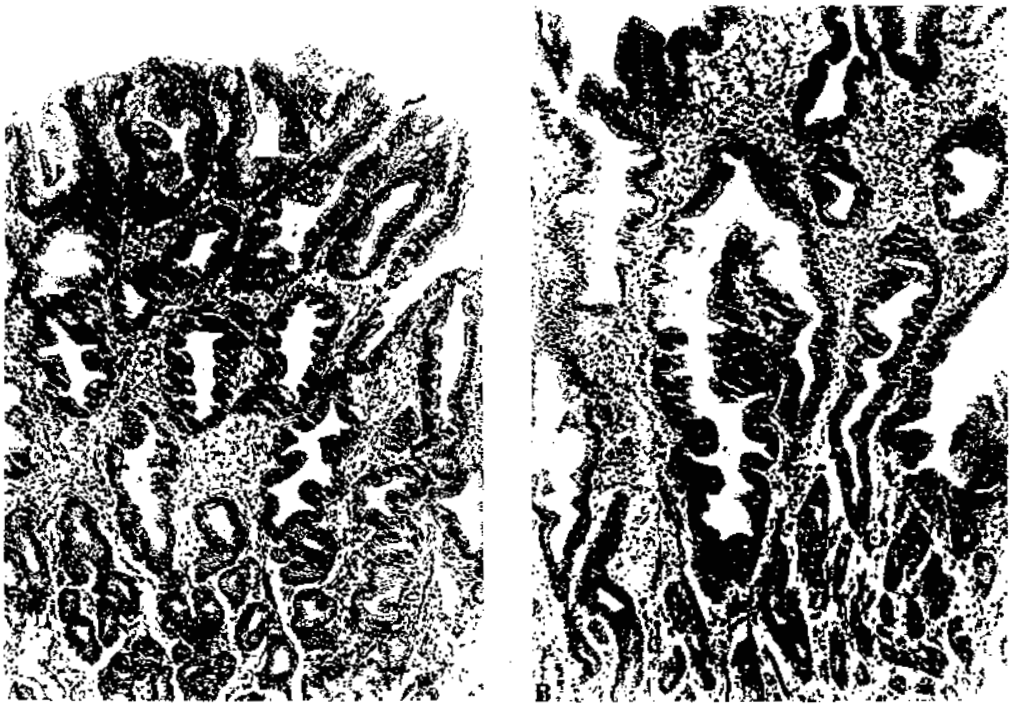

Fig 2. Body mucosa with foveolar hyperplasia. (A) H&E. ×100; (B) H&E. ×160.

DISCUSSION

Protein-losing gastropathy is a life-threatening condition characterized by intractable hypoproteinemia. Benign gastric disorders associated with excessive protein loss into the stomach, except Menetrier's disease, include hypertrophic hypersecretory gastropathy (10), allergic gastropathy (11) and lymphocytic gastritis (7, 12). Several mechanisms are incriminated in this loss of protein, including increased mucosal permeability secondary to disrupted mucosal architecture, mucosal inflammation, and lymphatic obstruction due to dilated mucosal lymph vessels or increased lymphatic pressure (12). The leakage of serum proteins appears to be independent of their molecular weights, and reduced levels of serum γ-globulins, transferrin, and ceruloplasmin develop during active disease (13). Protein loss is not associated with either increased fecal fat excretion or carbohydrate malabsorption.

In the first patient, there was no typical endoscopic or histological evidence of Menetrier's disease. The generic term hypertrophic protein-losing gastropathy should be preferred for this case. Alternatively, the case could be presented as one of giant fold gastritis secondary to HP infection, which mimics Menetrier's disease, as has already been described (6). The diagnosis of authentic Menetrier's disease should be reserved for the second patient with typical giant gastric folds, especially in the fundus and body, hypoproteinemia, and characteristic histological features of foveolar hyperplasia, atrophy of the glands and increased mucosal thickness.

In both cases peripheral edema was the prominent symptom and other digestive and extragastrointestinal manifestations were largely overshadowed. Hypoalbuminemia was also responsible for the free peritoneal fluid and pleural effusion in the first patient. Dyspepsia and epigastric pain are common complaints in patients with hypertrophic protein-losing gastropathy (12). Loss of substantial quantities of albumin into the gastric lumen is not usually accompanied by diarrhea in protein-losing gastropathy be-

cause hypoalbuminemia does not develop rapidly and intestinal mucosal edema as a result of decreased submucosal oncotic pressure does not occur. The decrease in serum proteins and hypocalcemia seldom cause clinical problems; thus hypogammaglobulinemia is not associated with an increased susceptibility to infections and frank tetany is not observed.

A remarkable increase in serum cholesterol levels during the hypoalbuminemia was noted in our patients, which regressed with normalization of the albumin value after treatment. The exact mechanism by which the albumin reduction determines the increase of cholesterol is obscure, it has been attributed to a compensatory phenomenon leading to increased synthesis of low-molecular-weight proteins by the liver, including LDH and high-density lipoproteins, in order to compensate for the fall in the colloid osmotic pressure that results from the reduction of albumin (14).

The α_1-antitrypsin is degraded in the gastric lumen and α_1-antitrypsin clearance can not serve as a measure of albumin loss into the gastric lumen, whereas it is a sensitive marker of protein leakage occurring beyond the pylorus (12). Intravenously administered radiolabeled proteins allowed accurate determination of the site of abnormal protein extravasation in the gastric lumen in our patients. Radioisotopic tests using ^{99m}Tc-labeled albumin have been performed in patients with protein-losing gastropathy (4, 5, 12), and it seems that this scintigraphic technique is best for detection of protein leakage into the stomach.

According to recent data, more than 90% of cases of HG are associated with HP infection (2). This encouraged treatment regimens aiming at eradication of the organism with clinical, endoscopic, and histological remission of the disease (2, 7, 9, 12). In the above studies the patients were treated successfully with H_2-receptor antagonists, proton pump inhibitors, and/or antibiotics. In our first patient triple therapy proved unsuccessful, but the addition of bismuth subcitrate had the desired effect. Further studies are needed to assess a possible superiority of bismuth in this condition.

The resolution of protein-losing gastropathy in our patients after eradication of HP strongly suggests the possibility for an etiological role of this organism. On the other hand, according to some authors, the prevalence of HP infection in HG has been reported to be rather low, ranging between 30% and 40% (1, 15). In these studies the detection of HP was mainly based on histology. If serology tests to detect past infection were used, the association of HP infection might have proved stronger. In addition, some authors (3, 16) suggest that HG might represent an abnormal response to a local antigen to which the patient has become sensitized, and this antigen could well be HP. Animal studies provide further evidence for this hypothesis. It has been shown that infected transgenic mice developed sustained anti-*H. felis* serum immunoglobulin G antibody responses, and one year after infection severe adenomatous and cystic hyperplasia of the surface foveolar epithelium was observed (17). Although the exact pathogenetic role of HP infection remains unclear, the dramatic therapeutic response of our patients to eradication therapy indicates that this treatment should be strongly recommended to patients with HP-positive hypertrophic gastropathy.

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Predictive Factors and Prevalence of Follicular Gastritis in Adults with Peptic Ulcer and Nonulcer Dyspepsia

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Follicular gastritis is an important histological entity, because it may progress to overt gastric MALT lymphoma. However, there is no universal agreement on whether there is any correlation of follicular gastritis with histological features of the antral mucosa or on the prevalence of follicular gastritis. To shed further light on these issues, we studied antral biopsies obtained from 735 adult patients, who had participated in six consecutive clinical trials. They included 348 patients with duodenal ulcer, 82 with gastric ulcer, and 305 with nonulcer dyspepsia. The Sydney classification system of gastritis was used, using a score of 0–3 to grade degree and activity of inflammation, gland atrophy, intestinal metaplasia, and *H. pylori* colonization density. Follicular gastritis was defined as prominent lymphoid follicles with no lymphoepithelial lesion. None of the *H. pylori*-negative patients ($N = 159$) had follicular gastritis. Among *H. pylori*-positive patients, 80/340 (23.5%) with duodenal ulcer, 5/77 (6.5%) with gastric ulcer, and 20/159 (12.6%) with nonulcer dyspepsia had follicular gastritis ($P < 0.001$). Multivariate discriminant analysis selected the following four significant predictor variables for follicular gastritis (Wilks $\lambda = 0.91$, $x^2 = 70.6$, $df = 4$, $P < 0.001$): gastritis sum score, atrophic gastritis, age of the patient, and disease. The prevalence of follicular gastritis was linearly correlated ($y = 24.55 - 0.98x$, $r = -0.62$, $F_{1,11} = 6.12$, $P = 0.03$) with the age groups of the 576 *H. pylori*-positive patients studied. In conclusion, follicular gastritis is highly correlated with *H. pylori*-caused severe, active gastritis. It is mostly prevalent in the young *H. pylori*-infected patients with duodenal ulcer.

KEY WORDS: follicular gastritis; *Helicobacter pylori*; duodenal ulcer; gastric ulcer; nonulcer dyspepsia; MALT lymphoma.

Follicular gastritis is characterized by hyperplasia of lymphoid follicles in the gastric mucosa. The close association of *Helicobacter pylori* infection with follicular gastritis (1, 2) suggests that *H. pylori* has antigenic properties that condition lymphoid tissue hyperplasia in gastric mucosa. Indeed, regression of follicular gastritis has been observed following *H. pylori* eradication (3, 4). Furthermore, it has recently been shown that follicular gastritis harbors the clonal B cell that may give rise to mucosa-associated lymphoid tissue (MALT) lymphoma (5). Therefore, diagnosing severe follicular gastritis may be of clinical importance, since it could be the first step in identi-

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fying a group of patients at risk of developing gastric low-grade MALT lymphoma.

The prevalence of follicular gastritis has been studied in adult patients with duodenal or gastric ulcer or gastritis (1, 6–10) but not in nonulcer dyspepsia. In addition, there is no universal agreement that the histological features of the antral mucosa or the colonization density by *H. pylori* are correlated with follicular gastritis (4, 6–10). To shed further light on these issues, we have studied antral biopsies taken from adults with duodenal ulcer (DU), gastric ulcer (GU), and nonulcer dyspepsia (NUD). These patients are known to have a high prevalence of *H. pylori* infection of the gastric mucosa.

MATERIALS AND METHODS

We used antral mucosa paraffin tissue blocks and slides from six consecutive clinical trials of patients with duodenal ulcer, gastric ulcer, and nonulcer dyspepsia performed in our institutions during 1994–1997. For the present study only biopsies at the entry of patients to the trials were used. No patient had recently received *H. pylori* eradication or nonsteroidal antiinflammatory drug therapy.

One or two antral biopsies were available from each patient. All biopsies had been obtained by using the Olympus FB-24Q biopsy forceps, which provide adequate gastric mucosa biopsy specimens (11). Biopsies were studied in sections stained with hematoxylin-eosin and Giemsa by two experienced pathologists. No special techniques were employed. For each biopsy, results were written in specially designed charts, including patients code number, age, sex, and disease (DU, GU, NUD).

Histological parameters, such as degree of gastritis (evident by infiltration of the gastric mucosa by lymphocytes and plasma cells), activity of inflammation (shown by infiltration with polymorphonuclear neutrophils), glandular atrophy, and *H. pylori* colonization density were also graded according to the Sydney classification system (12) and recorded in the data chart. The following four point scales were used to grade gastritis: normal = 0, mild = 1, moderate = 2, severe = 3; and *H. pylori* colonization density: absent = 0, few = 1, dense = 2, heavy = 3. The two main forms of intestinal metaplasia (complete and incomplete) were pooled and graded as present or absent. Follicular gastritis was defined as prominent lymphoid follicles with surrounding mantle zone and plasma cells, with no lymphoepithelial lesion (13).

Data Analysis and Statistics. All data were stored in a dBASE software (Microsoft Access 97, Microsoft Corp.) and analyzed by the statistical package Statgraphics Plus 2.1 (Manugistics Inc., Statistical Graphics Corp.). Qualitative data were assessed by chi-square test with Yates' correction, as appropriate. Numerical data were examined for normality. Nonnormally distributed data were analyzed by the nonparametric Kruskal-Wallis test.

Multivariate discriminant analysis with a forward stepwise selection algorithm was used to investigate whether age, sex, disease (DU, GU, NUD), *H. pylori* colonization density, and histological parameters (predictor variables) may discriminate between two levels (present, absent) of follicular gastritis (dependent variable). The objective of this analysis is to predict the group into which a new case is most likely to fall or to obtain a small number of predictor variables for follicular gastritis. To simplify statistical analysis, a sum gastritis score was obtained by adding the grade of degree and activity of gastritis in each case. Discriminant analysis and not regression analysis was as regarded most appropriate for our data, because follicular gastritis (dependent variable) is categorical and not metric.

Simple regression analysis with the best-fitting curvilinear model to our data was used to evaluate correlation of follicular gastritis prevalence with patients' age groups, as well as the grade of *H. pylori* colonization of the antral mucosa with gastritis score. $P < 5\%$ was regarded significant.

RESULTS

Baseline Data Analysis. Antral biopsies from 735 (99.0%) out of a total of 742 patients who had participated in six consecutive clinical trials were available for study. Three hundred forty-eight patients (266 men, 82 women) had duodenal ulcer, 82 patients (55 men, 27 women) had gastric ulcer, and 305 patients (222 men, 83 women) had nonulcer dyspepsia. The median age of the patients with gastric ulcer (55, range 28–78 years) was significantly different from that of patients with duodenal ulcer (47, range 18–90 years) or nonulcer dyspepsia (45, range 18–85 years)(Kruskal-Wallis T statistic = 27.9, $P < 0.001$).

The gastric mucosa of patients with duodenal ulcer or gastric ulcer was more often colonized by *H. pylori* (97.7 and 93.9% respectively) as compared with patients with nonulcer dyspepsia (52.1%, $x^2 = 212$, $df = 2$, $P < 0.001$). Gastritis sum score (y axis) was strongly correlated ($r = 0.88$) with the grade of colonization of the antral mucosa by *H. pylori* (x axis) ($y = 0.37 + 2.61\ x$, $F_{1,734} = 2581$, $P < 0.001$). None of the *H. pylori*-negative ($N = 159$), but 105/576 *H. pylori*-positive patients had follicular gastritis (18.2, 95% CI 15.1–21.4%). Among *H. pylori*-positive patients, follicular gastritis was found in 80/340 (23.5%, 95% CI 19.0–28.1%) patients with duodenal ulcer, 5/77 (6.5%, 95% CI 1.0–12.0%) patients with gastric ulcer, and 20/159 (12.6%, 95% CI 7.4–17.8%) patients with nonulcer dyspepsia ($x^2 = 16.9$, $df = 2$, $P < 0.001$).

Factors Predicting Follicular Gastritis. Seven hundred thirty-five cases were used to develop a model to discriminate among two levels of follicular gastritis (present, absent). Using a stepwise selection algorithm, it was found that four variables (Wilks $\lambda = 0.91$, $x^2 = 70.6$, $df = 4$, $P < 0.001$), ie, sum score of gastritis (severity plus activity score),

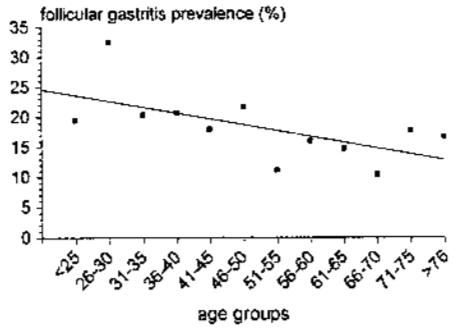

Fig 1. Correlation of prevalence follicular gastritis (*y* axis) with age (*x* axis) in 576 *Helicobacter pylori* positive patients (age range 18–90 years) categorized into 12 age groups. There is an inverse ($y = 24.55 - 0.98x$) moderately strong correlation ($r = -0.98$, $F_{1,11} = 6.12$, $P = 0.033$) between the two variables.

atrophic gastritis, age of the patient, and disease (DU, GU, NUD) were significant predictors of follicular gastritis ($F = 45.8$, 21.4, 18.5 and 29.3, respectively; $P < 0.001$).

Prevalence of Follicular Gastritis. Since it has been shown above that the age of the patients studied is a predictive factor for follicular gastritis, all 576 *H. pylori*-positive patients were categorized into 12 age groups (≤25, 26–30, 31–35, 36–40, 41–45, 46–50, 51–55, 56–60, 61–65, 66–70, 71–75, ≥76 years). Prevalence (%) of follicular gastritis (*y* axis) for each age group (*x* axis) was then calculated. The best fitting model of the regression analysis was linear ($y = 24.55 - 0.98x$), suggesting an inverse moderately strong correlation ($r = -0.62, F_{1,11} = 6.12, P = 0.033$) between the two variables (Figure 1).

DISCUSSION

The normal gastric mucosa contains scanty if any B lymphocytes. The presence of lymphoid follicles and aggregates in the gastric mucosa indicates B-lymphocyte proliferation, and it is now understood as an immune response to *Helicobacter pylori* infection (1, 2, 6). The term follicular gastritis is used to denote chronic active gastritis with florid lymphoid follicle formation, but no lymphoepithelial lesion (13). The importance of follicular gastritis is that it may progress to overt MALT lymphoma (14). Recently, it has been shown that biopsies taken from certain patients with active *H. pylori*-associated chronic gastritis have detectable clonal populations, but no histologic proof of MALT lymphoma (15). It has also been suggested that in patients with *H. pylori* gastritis analysis of B-cell clonality by polymerase chain reaction may identify certain patients with monoclonal B-cell population at an early reversible step in the development of MALT-oma (5). These patients may benefit from *H. pylori* eradication therapy.

It may be argued that because of the close association of *H. pylori* chronic active gastritis with presence of lymphoid follicles in the gastric mucosa, there is no need for reporting or grading follicular gastritis, because its prevalence is determined by the number of biopsies taken (16). In this context, the term follicular gastritis has not been included in the Updated Sydney System for classification of gastritis (17). However, recent studies have shown a close relationship of follicular gastritis with low-grade MALT lymphoma (5, 14), suggesting that dense lymphoid follicles shown in routine biopsies should be reported.

These recent developments on the relationship between *H. pylori* follicular gastritis and MALT lymphoma stress the need for a better understanding of the epidemiology of follicular gastritis. In the present study we did not find lymphocyte follicles in any of the 159 *H. pylori*-negative patients with nonulcer dyspepsia. Our data, therefore, agree with published reports suggesting that follicular gastritis is exclusively found in *H. pylori*-infected patients (6, 10). We have also shown that follicular gastritis is most prevalent in patients with duodenal ulcer and nonulcer dyspepsia as compared with gastric ulcer. These results are comparable to those of Eidt and Stolte (9), who found a 46% prevalence of lymphoid follicles in the antral mucosa of patients with duodenal ulcer, but 31% in patients with gastric ulcer. Similar to our results, their patients with gastric ulcer were almost a decade older than the age of patients with duodenal ulcer. Multivariate discriminant analysis of our data has shown that, among other factors, age is related to follicular gastritis. This was confirmed by regression analysis, which showed a slow but significant decrease in the prevalence of follicular gastritis with increasing age. This could explain in part the lower incidence of follicular gastritis found in patients with gastric ulcer, but other factors, such as host humoral and cellular immune response may be involved.

To date, published studies have shown that in *H. pylori*-infected patients, the prevalence of follicular gastritis is at the range of 24–72% (1, 3, 6, 8–10, 14). However, Genta and Hamner suggested that most *H. pylori*-infected patients have lymphoid follicles and aggregates in the gastric mucosa (10), but they had evaluated multiple gastric mucosal biopsies with ad-

ditional tissue sectioning and employed special staining techniques. This variation in the prevalence of follicular gastritis is probably due to differences in the degree and extension of a commonly observed immune phenomenon. The relatively low prevalence of follicular gastritis we have found may be explained by the following factors. First, we have evaluated only lymphoid follicles and not lymphoid aggregates, as was the case in some published studies (9, 10). Secondly, in our study biopsies were processed "in routine," regarding the number of tissue sections and, furthermore, no special staining was employed. We have therefore probably detected only cases with dense follicle formation in the antral mucosa.

Our results suggest that there is a significant negative correlation of follicular gastritis prevalence with increasing age of *H. pylori* infected patients. To date published studies have shown a similar but not statistically significant trend (8), or no correlation of follicular gastritis with patients' age (9, 10, 16). However, they had evaluated less than 200 *H. pylori*-positive cases (10, 17) and, therefore, allocation of patients into age groups could have resulted in small numbers, invalidating further statistical analysis.

Another important, contradictory issue is the relationship of follicular gastritis with the activity of gastritis and the colonization density of the antral mucosa by *H. pylori*. Certain studies have shown positive (6, 9), but others no, correlation (7, 8, 16). Our data lend support to earlier publications (6, 9) and suggest that a high gastritis score, ie, gastritis severity and activity, are, first, correlated with dense *H. pylori* colonization and, second, that a high gastritis score is a predictive factor of follicular gastritis. Since activity and degree of gastritis and *H. pylori* colonization density are graded according to four-point scales, we suggest that, as in our study, large numbers of cases should be evaluated (6, 9) to provide valid information.

Since we have established that follicular gastritis is correlated with severe, active *H. pylori* gastritis, it may be argued that there is no rationale for seeking factors that predict follicular gastritis. However, since follicular gastritis may be a prerequisite for the development of gastric low-grade MALT lymphoma (5), looking for factors that predict follicular gastritis may be of major interest because certain of these factors may be related to the development of gastric MALT lymphoma. It will be possible, therefore, to identify patients at risk. In this context, it has been suggested that MALT lymphoma patients express a significantly higher summed gastritis score in the gastric corpus as compared to the gastritis patients (18) and that the *H. pylori* CagA status may be related to MALT lymphoma (19, 20).

In conclusion, our data suggest that follicular gastritis is highly correlated with *H. pylori*-induced severe, active gastritis and that the prevalence of follicular gastritis is slowly reduced with increasing age. Since follicular gastritis may give rise to MALT lymphoma, studies on the evolution of follicular gastritis following *H. pylori* eradication therapy (3, 4, 16) are imperative.

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REVIEW ARTICLE

Non–Gastrointestinal Tract Associations of *Helicobacter pylori* Infection

What Is the Evidence?

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Helicobacter pylori infection is linked to conditions of the upper gastrointestinal tract, including peptic ulcer and gastric adenocarcinoma. It has also been associated with a wide variety of non–gastrointestinal tract conditions. However, the evidence in support of *H pylori* infection as a cause of the non–gastrointestinal tract conditions is not widely understood. We reviewed the medical literature for publications and abstracts dealing with putative non–gastrointestinal tract associations of *H pylori* infection. We appraised the level of evidence and applied it to an established set of 9 criteria for determining causation. We found that many studies examining a possible causal relationship have been uncontrolled or inadequately controlled. Studies have often failed to control for socioeconomic status. Studies of treating *H pylori* infection in patients with these disorders have been poorly designed and inappropriately controlled, and therefore add little to the evidence base. Attention should be focused on appropriate testing for and treatment of *H pylori* infection in patients with conditions that are of proven association, notably peptic ulcer disease. *Arch Intern Med. 1999;159:925-940*

Helicobacter pylori has been conclusively linked to different forms of gastritis, as well as to peptic ulcer disease of the stomach and duodenum,[1,2] gastric adenocarcinoma,[3] and low-grade gastric lymphoma arising from mucosa-associated lymphoid tissue.[4] *Helicobacter pylori* may also have a role in dyspepsia and nonulcer dyspepsia, although this role is currently unresolved.[5,6]

In view of the excitement and interest generated by the link between *H pylori* and gastric abnormalities, different investigators have sought to determine a role for the infection in a variety of non–gastrointestinal tract disorders. This is despite our current understanding that *H pylori* infection is confined to gastric mucosa. Although the infection is noninvasive, it triggers a marked local inflammatory response and a systemic immune response.[10,11] *Helicobacter pylori* infection of the stomach could conceivably produce effects elsewhere by altering levels of systemic inflammatory mediators.[12,13] Our aim is to review critically the evidence that *H pylori* infection causes various other disorders outside the alimentary tract.

Since *H pylori* infection is so common throughout the world,[14,15] it is not surprising that it has been found in patients with other diagnoses. Such findings may have been because of chance alone. Therefore, assumptions that certain conditions are caused by *H pylori* infection might be spurious: association need not necessarily imply causation. Evidence in support of causation comes in different forms. The strongest evidence comes from randomized, controlled trials, which are seldom available. After those, in decreasing levels of strength, come cohort studies, case-control studies, and case series or single-case reports.[16] Other forms of evidence reviewed herein include experimental studies and observational, cross-sectional studies (controlled or uncontrolled).

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Table 1. Application of 9 Diagnostic Tests for Causes of Certain Non–Gastrointestinal Tract Conditions Proposed to Be Related to *Helicobacter pylori* Infection*

Tests	Coronary Heart Disease	Cerebrovascular Disease	Hypertension	Raynaud Phenomenon	Migraine
Is there evidence from true experiments in humans?	No	No	No	No	No
Is the association strong?	No	No	No	No	No
Is the association consistent from study to study?	No	No	No	No	?
Is the temporal relationship correct?	?	?	?	?	?
Is there a dose-response relationship?	?	?	?	?	?
Does the association make epidemiological sense?	?	?	?	?	?
Does the association make biological sense?	?	?	No	?	?
Is the association specific?	No	No	No	No	No
Is the association analogous to a previously proven causal association?	No	No	No	No	No

* *Question mark indicates absence of sufficient evidence for answer.*

MATERIALS AND METHODS

We conducted a fully recursive MEDLINE search for published articles dealing with *H pylori* infection and conditions outside the gastrointestinal tract. We supplemented this with a review of abstracts from recent national and international gastroenterology conferences and conferences specifically devoted to *H pylori*. We deliberately chose to include abstracts, since research on *H pylori* is developing rapidly, and we wanted to identify any recent important developments or trends. We reviewed in full those articles published in English. For articles published in other languages, we reviewed an English abstract.

DETERMINING THE PLAUSIBILITY FOR CAUSATION

The following 9 questions were conceived by Sackett et al[16] for considering whether a possible association is causal:

1. Is there evidence from true experiments in humans?
2. Is the association strong?
3. Is the association consistent from study to study?
4. Is the temporal relationship correct?
5. Is there a dose-response relationship?
6. Does the association make epidemiological sense?
7. Does the association make biological sense?
8. Is the association specific?
9. Is the association analogous to a previously proven causal association?

These are listed in decreasing order of importance. We have attempted to answer these 9 questions for each of the conditions under review (**Table 1**). However, when considering a possible role for *H pylori* infection in a condition outside the gastrointestinal tract, it is not possible to answer each of these questions conclusively. For example, apart from 2 well-described case studies of self-inoculation,[17-19] there have been no direct experiments of *H pylori* infection in humans.

The means of testing for *H pylori* infection might influence the strength of association with any of the non–gastrointestinal tract conditions under review. Some studies have used highly sensitive and specific means for determining the presence of the infection, such as a carbon-labeled urea breath test (UBT) using carbon 13 or carbon 14. Such tests are highly accurate in determining active *H pylori* infection.[20-21] Other studies have determined *H pylori* status by serological means. Generally, serological tests have lower sensitivity and specificity than UBT or endoscopic tests; their results may be false-positive in some patients successfully treated for *H pylori* infection in the past.[21,22]

It is seldom possible to assess the temporal relationship between the proposed cause (namely, *H pylori* infection) and the proposed outcome (viz, the non–gastrointestinal tract condition in question). However, our present understanding is that *H pylori* infection is generally acquired in childhood.[23-25] Therefore, it might be reasonable to assume that acquisition of the infection would antedate the development of any condition presenting for the first time in adulthood.

It is generally impossible to know if a dose-response relationship exists between *H pylori* infection and another condition. The diagnosis of *H pylori* infection is essentially qualitative rather than quantitative. Since the infection is present or absent, its "dose" is unknown. Also, the duration of *H pylori* infection is usually unknown and cannot otherwise be assessed, although most infections are probably acquired in childhood. We have attempted to evaluate the scientific validity of the biological rationale for any proposed association. However, for many of the putative associations, there is no obvious biological rationale or pathogenetic mechanism.

POSSIBLE MECHANISMS THAT MIGHT EXPLAIN NON–GASTROINTESTINAL TRACT ASSOCIATIONS OF *H pylori* INFECTION

Helicobacter pylori typically infects and is confined to gastric mucosa. Such mucosa is customarily restricted to the stomach but may also occur elsewhere in the alimentary tract. Heterotopic or metaplastic gastric mucosa infected with *H pylori* has been documented in the proximal esophagus,[23,24] the distal esophagus,[25,26] the duodenum,[27,28] Meckel diverticulum,[29,30] and the rectum.[31]

Rosacea	Chronic Urticaria	Iron Deficiency Anemia	Autoimmune Thrombocytopenic Purpura	Hyperammonemia	Sudden Infant Death Syndrome	Growth Retardation	Anorexia of Aging
No	No	No	No	No	No	No	No
No	No	?	?	No	No	No	No
No	No	?	?	No	No	No	?
?	?	?	?	?	Yes	Yes	No
?	?	?	?	?	?	?	?
?	No	?	No	No	?	?	No
No	No	?	?	Yes	?	?	No
No	No	No	No	No	No	No	No
No	No	No	No	No	No	No	No

Changes in systemic inflammatory mediators could conceivably produce effects elsewhere in the body.[32,43] *Helicobacter pylori* infection may also be associated with altered coagulation.

Effects of *H pylori* Infection on Coagulation

Coagulation Factors. In England, Patel and colleagues[32] studied 72 middle-aged white men without a known history of coronary heart disease (CHD). They found a higher mean serum fibrinogen concentration in the men with serological evidence of *H pylori* infection compared with those who were uninfected ($P = .007$). In a case-control study of 388 men in England, their group confirmed a significantly raised mean serum fibrinogen concentration associated with *H pylori* infection.[33] In a controlled study of patients recovering from acute myocardial infarction (MI), Rajput-Williams and colleagues[34] found a higher mean serum fibrinogen level in their *H pylori*–infected healthy control subjects than in their noninfected controls ($P = .04$).

A case-control study involving more than 2000 men and women in Northern Ireland failed to demonstrate any association between *H pylori* infection and serum fibrinogen or plasma viscosity.[35] In a study of patients with dyspepsia in England, no association was found between *H pylori* status and serum fibrinogen concentration or levels of other coagulation factors, including factors VIIc and VIIIc and von Willebrand factor.[36] In 292 patients with CHD, *H pylori* status was not significantly associated with serum levels of fibrinogen, factor VII or von Willebrand factor.[37]

In Italy, Bierti and colleagues[38] found a significantly higher mean serum fibrinogen level in 64 *H pylori*–infected patients compared with 66 noninfected patients ($P = .04$). They also found higher antigen levels of von Willebrand factor in the *H pylori*–infected group ($P < .01$). Levels of plasminogen activator inhibitor were no different between *H pylori*–infected and noninfected patients.

In 300 healthy blood donors from Italy, 53% were seropositive for *H pylori* infection.[39] Levels of fibrinogen were no different between those who were seropositive and seronegative. Those who were seropositive for *H pylori* had higher concentrations of factor VIIc and prothrombin cleavage fragment. However, these differences disappeared after adjustment for age, sex, and social class.

No significant association was demonstrated between *H pylori* status and serum fibrinogen levels in almost 1500 male and female patients with CHD in Scotland.[40] In a prospective study, Wald et al[41] observed 21 520 professional men in England for a mean of 15.6 years. They found no association between *H pylori* status and serum fibrinogen levels. A recent meta-analysis found no significant association between *H pylori* status and serum fibrinogen level.[42]

The best evidence for an association between *H pylori* infection and hyperfibrinogenemia comes from cross-sectional or case-control studies. However, the association between *H pylori* infection and increased levels of fibrinogen or other coagulation factors is inconsistent. The largest and most robust studies and a meta-analysis failed to demonstrate any association.

Platelets. Increased levels of circulating platelet aggregates were demonstrated in 5 patients with upper gastrointestinal tract complaints who were seropositive for *H pylori*, compared with 5 similar patients who were seronegative.[44] There is no epidemiological evidence of an effect of *H pylori* infection on platelet function.

Effects of *H pylori* Infection on Markers of Systemic Inflammation

Leukocyte Counts. A raised whole blood leukocyte count is associated with an increased risk for CHD.[44] Patel et al[33] found a significantly higher leukocyte count in 191 middle-aged white men who were seropositive for *H pylori* infection than in 197 who were seronegative. Karttunen et al[45] from Finland studied 96 patients with dyspepsia, of whom 58 were seropositive for *H pylori* infection. Total whole blood leukocyte counts were significantly higher in the *H pylori*–positive group, as were absolute counts of lymphocytes and basophils. Three studies[36,37,46] and a meta-analysis[42] found no association between *H pylori* status and leukocyte counts.

The best evidence of an association between *H pylori* infection and a raised leukocyte count comes from case-control studies, but is inconsistent. Prospective studies and a meta-analysis do not confirm the effect.

C-reactive Protein and Tumor Necrosis Factor. In a population-based, cross-sectional study of 388 middle-aged, white men in England, *H pylori* status was one of many variables found to correlate with serum levels of C-reactive protein (CRP).[47] In turn, raised CRP levels were associated with elevated levels of serum fibrinogen, total cholesterol, triglyceride, and glucose. They were negatively associated with high-density lipoprotein (HDL) cholesterol concentration. Concentration of CRP is strongly associated with a propensity to CHD. Serum concentrations of tumor necrosis factor α are positively related to *H pylori* status.[48] Tumor necrosis factor α is one of the cytokines that regulate CRP production by the liver. Higher circulating levels of soluble tumor necrosis factor receptor 1 are reported in patients with CHD who are seropositive for *H pylori* infection.[49]

Helicobacter pylori infection may elevate circulating levels of CRP. However, this is a very nonspecific finding.

Effects of *H pylori* Infection on Other Risk Factors for Cardiovascular Disease

Cholesterol, Triglyceride, and Glucose Levels. Patel et al[33] found no association between *H pylori* status and serum levels of cholesterol, triglyceride, apolipoproteins A or B, or glucose. Murray et al[35] found no significant association between *H pylori* status and levels of total or HDL cholesterol. McDonagh et al[36] found no association between *H pylori* status and total cholesterol levels.

Scragg et al[50] studied a large group of asymptomatic, nondiabetic workers in New Zealand. They found no association between *H pylori* status and levels of total cholesterol, triglyceride, or glucose. Levels of HDL cholesterol were slightly lower in individuals who were seropositive for *H pylori* infection than in those who were seronegative (mean difference, 0.07 mmol/L [2.7 mg/dL]; $P = .03$). Infection with *H pylori* was also associated with a lower HDL cholesterol concentration in a cross-sectional study of the elderly in Finland.[51]

In a Spanish study of 112 patients with CHD admitted to a coronary care unit (CCU), there was no association seen between *H pylori* status and the presence of hypercholesterolemia or diabetes.[52] In a cross-sectional study of 1756 Danish women, *H pylori* serological status did not correlate with serum triglyceride or cholesterol levels.[53] In a United States–based study, *H pylori* serological status was determined in 103 patients undergoing coronary angiography for suspected CHD.[54] Fifty-two patients (50%) were seropositive for *H pylori*. Serological status was not related to total cholesterol levels or to the presence of diabetes.

Among 91 elderly, dyspeptic patients in Italy, results of gastric histological and rapid urease tests were positive for *H pylori* infection in 60.[55] There were no significant differences between the patients with positive and negative results with respect to mean levels of glucose, total cholesterol, HDL cholesterol, or triglyceride.

In a nested case-control study, Whincup et al[56] studied 95 middle-aged British men who had survived an MI, 93 who had survived a stroke, and a similar number of matched controls. *Helicobacter pylori* status was not significantly associated with total cholesterol, HDL cholesterol, or triglyceride levels. However, serum glucose levels were significantly higher in the patients who were seropositive for *H pylori* ($P = .006$). In their prospective study of healthy English men, Wald et al[41] found no association between *H pylori* status and total cholesterol or triglyceride levels.

In South Korea, Kang and colleagues[36] studied 274 patients with suspected CHD who were undergoing coronary angiography. Of these, 64.1% were seropositive for *H pylori* infection. They found no association between *H pylori* status and hyperlipidemia or diabetes. However, the same group identified a significantly higher ($P = .006$) mean serum cholesterol level in association with *H pylori* infection in a study of 3274 healthy Korean adults.[57] Although statistically significant, this difference was small and unlikely to be clinically meaningful.

The seroprevalence of *H pylori* infection was 53% in 381 healthy Spanish subjects without a history of peptic ulcer disease or hyperlipidemia.[58] Although the *H pylori*–infected subjects had a higher mean total serum cholesterol level than those who were not infected, this difference was not apparent after adjustment for age and sex.

A meta-analysis found no significant association between *H pylori* status and serum cholesterol levels.[42] However, it found a highly statistically significant, although quantitatively small, difference in HDL cholesterol levels (mean difference, −0.032 mmol/L [−1.2 mg/dL]; $P<.001$) between *H pylori*–infected and noninfected individuals. It found a small, statistically significant association between *H pylori* infection and serum or plasma glucose levels (mean difference, 0.14 mmol/L; $P<.05$), but no association with serum triglyceride levels.

The best evidence of an association between *H pylori* infection and hypercholesterolemia comes from cross-sectional studies. There is little consistency in results between different studies. The magnitude of effect, if any, is low. There may be a small, negative effect on HDL cholesterol levels.

Homocysteine. A raised serum level of homocysteine is an independent risk factor for CHD.[59-61] Sung and Sanderson[61] have proposed that *H pylori* infection predisposes to hyperhomocysteinemia through nutritional deficiencies of folate and vitamins B_6 and B_{12}. However, B_{12} malabsorption might only occur in few infected patients, and only after many years of *H pylori* infection associated with extensive atrophic gastritis and loss of parietal cell mass. Even then, body stores of vitamin B_{12} should be sufficient to delay the development of true deficiency for years. There is no particular reason why *H pylori* infection per se should

be associated with dietary deficiencies of folate or vitamin B_{12}.[62]

Whincup et al[63] found no difference in serum homocysteine levels between 63 *H pylori*–infected and 35 noninfected individuals without CHD. Saxena et al[64] found no significant difference in mean serum homocysteine levels between 122 patients seropositive for *H pylori* and 98 seronegative patients. Markus and Mendall[65] found no association between *H pylori* status and serum levels of homocysteine or folate in a case-control study of patients with ischemic stroke. Two studies[64,65] found a nonsignificant trend toward lower homocysteine levels in association with *H pylori* infection.

There is no evidence to suggest that *H pylori* infection causes hyperhomocysteinemia.

Heat-Shock Proteins. Heat-shock protein (hsp) 65 is a stress protein that is expressed in high concentrations in atherosclerotic tissue.[66] Immunization of animals with hsp65 can induce atherosclerotic lesions in the absence of hypercholesterolemia. Levels of serum antibodies to hsp65 are significantly higher in patients with carotid atherosclerosis than in matched controls.[66] An immune reaction to hsps might play a role in the pathogenesis of atherosclerosis.[67] As a group, hsps have high homology among different species from bacteria to man.[66] *Helicobacter pylori* expresses an hsp[10] known as hsp62 that is highly homologous with hsp65 and endogenously produced hsp60.[68] Among 136 men consecutively investigated using cardiac catheterization for chest pain or valve abnormalities, there was a highly significant correlation (r = 0.39; P<.001) between the presence of antibodies to hsp65 and *H pylori*.[68] The patients who were seropositive for *H pylori* had significantly higher levels of antibodies to hsp65 than those who were seronegative.

There may be an association between *H pylori* infection and the presence of antibodies to hsps. The relevance of this to the pathogenesis of atherosclerosis is not fully understood.

POSSIBLE DISEASE ASSOCIATIONS WITH *H pylori* INFECTION

Cardiovascular Disorders

Coronary Heart Disease. Mendall and colleagues[69] in England found a 59% seroprevalence of *H pylori* infection in 111 consecutive white men with established CHD and a 39% seroprevalence in 74 community-based controls (P = .007). After adjustment for socioeconomic status and other cardiovascular risk factors, the odds ratio (OR) for CHD in the presence of *H pylori* infection was 2.15 (P = .03). However, others thought this association was not causal.[70] In a cross-sectional study of a random sample of 388 white men in England, Patel and colleagues[33] found an association between *H pylori* seropositivity and the presence of abnormal results of electrocardiography consistent with underlying CHD (OR = 3.82; P<.01).

In the cross-sectional, population-based study from Northern Ireland by Murray and colleagues,[35] there was only a weak association between *H pylori* status and CHD that did not reach statistical significance (OR = 1.31; P = .10). McDonagh and colleagues[49] in Scotland found no significant association between *H pylori* seropositivity and any measure of CHD among 1428 randomly selected men and women. In Finland, Niemala and colleagues[71] performed a case-control study in 116 patients with angiographic evidence of CHD and 116 age- and sex-matched community controls. Seroprevalence of *H pylori* infection was similar in both groups (64% vs 53%; OR = 1.5). In a nested case-control study in the United Kingdom, Whincup and colleagues[63] found a seroprevalence of *H pylori* infection of 70% in 95 middle-aged men who survived an MI and 57% of 78 age-matched controls (OR = 1.77; P = .03). An analysis of the Eurogast database[71] found that deaths due to CHD were negatively correlated with seropositivity for *H pylori* infection (r = –0.73; P<.05).

Morgando and colleagues[72] from Italy studied the seropositivity of *H pylori* infection in 42 patients admitted to a CCU with acute MI and 198 hospital-based controls. The rates of seroprevalence were 85.7% and 57.1%, respectively. The controls were not well-matched for age, sex, or the absence of cardiac disease. In Spain, Martín-de-Argila and colleagues[73] reported a 84.1% seroprevalence of *H pylori* infection in 101 patients with CHD admitted to the CCU, and a 58.8% prevalence in 68 healthy controls (P<.001) who were not well-matched for age or sex. In a similar study of 112 patients with CHD admitted to the CCU, 81.3% were seropositive for *H pylori* compared with 63.8% of 53 healthy controls (P<.01) matched roughly for age but not for sex.[32]

In another poorly controlled study, Ponzetto and colleagues[74] reported a seroprevalence of 89% among 27 patients with acute MI and 47% among 619 blood donors (OR = 4.4). Rathbone and colleagues[75] performed a case-control study in 342 consecutive patients admitted to a CCU with acute MI and 236 population-based controls. Seroprevalence of *H pylori* infection was 60.2% in the patients and 55.9% in the controls (age- and sex-stratified OR = 1.05; P = .87). In Italy, Parravicini and colleagues[76] performed ^{13}C-UBT on 137 patients with acute MI and 312 healthy blood donors, not well-matched for age. *Helicobacter pylori* infection was found in 86.1% of the patients and 58.6% of the blood donors, who were younger.

In Germany, Maier and colleagues[77] performed a prospective serological study of 87 consecutive patients with known or suspected CHD who were undergoing coronary angiography. The seroprevalence of *H pylori* infection was no different in those with or without angiographic evidence of CHD (68.4% vs 80.0%; P = .04). Ossei-Gerning[37] in England studied 292 patients having coronary angiography for suspected CHD; 204 had angiographic evidence of CHD, and the seroprevalence of *H pylori* infection in these 204 was 68%. Of the 88 patients without evidence of CHD, the seroprevalence was 50% (P = .003). However, a similar angiographic study of 70 patients in Germany found no significant dif-

ference in rates of *H pylori* infection, determined using endoscopy, in patients with and without CHD (36% vs 44%; $P>.05$).[78] Furthermore, a study in Korea of 274 patients undergoing coronary angiography for suspected CHD found no difference in *H pylori* seroprevalence between patients without visible lesions and those with disease in 1, 2, or 3 coronary arteries.[36] In a United States–based angiographic study, *H pylori* serologic studies were performed in 179 patients with suspected CHD[79]; 121 had angiographic evidence of CHD and 58 did not. Seroprevalence of *H pylori* infection was 45% in those with CHD and 47% in those without ($P=.96$).

Balaban and colleagues[80] in the United States studied the seroprevalence of *H pylori* infection in 201 consecutive patients referred for noninvasive cardiac investigation because of suspected CHD. Adjusted ORs for an association with *H pylori* infection were not significant for CHD (OR = 0.54; $P=.10$) or a previous MI (OR = 2.14; $P=.10$). However, they found a significant relationship between *H pylori* infection and a history of MI in women (OR = 10.9; $P=.03$), although the sample size was only 34.

In a prospective cohort study of elderly people in Finland, Strandberg and colleagues[81] found no significant association between *H pylori* seropositivity and evidence of vascular disease. Cardiovascular and total mortality were not related to *H pylori* status. Similarly, Wald and colleagues in England found no association between *H pylori* seropositivity and death due to CHD in their prospective study (nested case-control design) of more than 21 000 professional men (OR = 1.06).[43]

The best evidence of an association between *H pylori* infection and CHD comes from a nested case-control study. Larger prospective studies did not find a significant association. There is no consistent conclusion among different studies. In general, large controlled studies have not confirmed the findings of earlier smaller studies. Those that used appropriate controls were less likely to report a significant association.[82]

Cerebrovascular and Peripheral Vascular Disease. In a nested case-control study, Whincup et al[46] studied results of *H pylori* serologic testing in 137 middle-aged British men in whom a stroke developed before December 1991 and in 136 age-matched and geographically matched controls. Of the patients with stroke, 93 (67.9%) were seropositive for *H pylori* compared with 78 (57.4%) of the controls. The OR for stroke associated with *H pylori* infection was 1.57 ($P=.07$). After adjustment for socioeconomic status, smoking, and blood pressure, the OR was 0.96 ($P=.92$).

In a study of 91 elderly dyspeptic patients in Italy, 60 had results of gastric histological examination and rapid urease testing that were positive for *H pylori*.[75] All patients underwent echodoppler ultrasonography of extracranial and peripheral arteries. Those with *H pylori* infection had a similar number of detectable atherosclerotic plaques as the uninfected patients and a similar number of arteries with detectable plaques. The prevalence of concomitant risk factors for atherosclerosis, including hypertension, diabetes, and hypercholesterolemia, was no different between *H pylori*–infected and noninfected patients.

In a case-control study, *H pylori* seropositivity was significantly more common in 238 patients with nonhemorrhagic stroke or transient ischemic attack (38.8%) than in a control group of the spouses of 119 patients (44.5%; OR = 1.78; $P<.01$).[83] This association held when controlled for other risk factors, including socioeconomic status. In subgroup analyses, the association was only true for large-vessel disease and lacunar stroke rather than for embolic stroke or stroke of unknown causes. Patients with stroke and those with transient ischemic attacks had similar rates of *H pylori* seropositivity (59.6% and 58.6%, respectively; $P=.90$).

In an Italian study of 45 patients with carotid or femoral arterial obstruction, 39 (86.7%) were seropositive for *H pylori* infection,[83] compared with 111 (60.7%) of 183 blood donors who acted as controls. It is unclear how well matched the control group was.

The best evidence of an association between *H pylori* infection and stroke comes from a case-control study; the magnitude of effect was small.

Hypertension. Barnes and colleagues[84] from England observed 103 patients who had been investigated for dyspepsia between 1973 and 1980 and had normal upper endoscopic results. To look for evidence of *H pylori* infection, they reexamined gastric mucosal biopsy specimens that had been collected from all patients. They were unable to establish a link between *H pylori* status and the nature, severity, or progression of dyspeptic symptoms. However, they noticed an unexpected significant association between *H pylori* infection and hypertension.

Lip et al[85] reported a significantly higher seroprevalence of *H pylori* infection in patients with hypertension compared with healthy controls. Of 124 hypertensive patients, 85% were seropositive compared with 66% of 38 healthy controls ($P=.007$). The seroprevalence of *H pylori* infection was not further increased in patients with malignant-phase hypertension.

Whincup et al[46] found a nonsignificant association between *H pylori* positivity and systolic blood pressure (SBP) in their nested case-control study in England. Mean SBP was 143.7 mm Hg in 78 men with and 138.3 mm Hg in 58 men without *H pylori* infection ($P=.06$). Mean diastolic blood pressure (DBP) was 81.5 mm Hg in infected and 79.5 mm Hg in noninfected patients ($P=.37$).

At least 7 studies have failed to find an association between *H pylori* infection and hypertension.[33,35,40,41,50,52,63] A meta-analysis[42] found a statistically significant, although quantitatively small, difference in SBP between *H pylori*–infected and noninfected individuals (mean difference, 0.9 mm Hg; $P<.05$), but no association between *H pylori* status and DBP.

The best evidence of an association between *H pylori* infection

and hypertension comes from a cross-sectional study. No consistent relationship was demonstrated. Most evidence points toward no association.

Idiopathic Arrhythmia. In an uncontrolled study, the prevalence of *H pylori* infection among 54 patients with idiopathic arrhythmia was 42%.[85] The rationale for this study was unclear. The prevalence was similar between those with supraventricular and ventricular arrhythmia.

There is no evidence to support an association between *H pylori* infection and idiopathic arrhythmia, and no apparent biological rationale for such an association.

Raynaud Phenomenon. Investigators have examined a role of *H pylori* infection in other vascular conditions, based presumably on results of early studies in CHD. In an Italian study, the prevalence of *H pylori* infection using results of ^{13}C-UBT was 81% in 26 patients with primary Raynaud phenomenon (PRP) and 20% in 10 age- and sex-matched controls.[87] The same group of investigators also studied the effects of treatment of *H pylori* infection on the symptoms of PRP.[88] Of 46 patients with PRP, 36 (78%) were infected with *H pylori* as judged using results of ^{13}C-UBT. There was no difference in the frequency or severity of attacks of PRP between infected and noninfected patients. The infected patients were treated for *H pylori* infection, and successful eradication was achieved in 30 of 36. Up to 12 weeks after stopping treatment, 5 had complete resolution of the symptoms of PRP, and 18 others reported a marked improvement. In the noninfected patients who did not receive treatment for *H pylori* infection, there was no change in the frequency or severity of attacks of PRP. This study was not randomized or blinded and was not appropriately controlled.

There is little biological rationale for an association between *H pylori* infection and Raynaud phenomenon. The best evidence of such an association comes from an uncontrolled case series. Supportive evidence from a treatment study is very weak.

Migraine. In an Italian study, the prevalence of *H pylori* infection assessed using results of ^{13}C-UBT was 48% in 225 patients with primary migraine.[89] These patients were treated for *H pylori* infection, and successful eradication was achieved in 84%. Of the patients with successful eradication, 23% had complete resolution of migraine for up to 24 weeks. Patients in whom eradication of *H pylori* infection failed did not improve. This study was not randomized, not blinded, and not appropriately controlled. The same investigators reported a higher prevalence of *H pylori* infection in patients with migraine (47% of 300) than in patients with tension headache (31% of 162; $P<.05$).[90]

There is no obvious biological rationale for an association between *H pylori* infection and migraine. The strongest evidence of such an association is from an uncontrolled case series. Supportive evidence from a treatment study is very weak.

Endocrine and Metabolic Disorders

Diabetes Mellitus. Since diabetes mellitus may be associated with a variety of upper gastrointestinal tract complaints, investigators have sought to determine whether *H pylori* infection is linked to different forms of diabetes.

In a study from the Netherlands, Oldenburg and colleagues[91] examined the seroprevalence of *H pylori* infection in 45 patients with type 1 diabetes, 98 with type 2 diabetes, and 159 outpatient controls. Seroprevalence was higher in some age groups of diabetic patients. However, the 3 groups were not adequately matched for age or socioeconomic status. Multiple statistical comparisons between groups increased the chance of a type I statistical error.[92]

In Italy, Pocecco and colleagues[93] studied the seroprevalence of *H pylori* infection in 69 children and adolescents with type 1 diabetes and 310 age-matched controls without evidence of diabetes or gastrointestinal tract complaints. Controls were matched for age, geographic location, and socioeconomic status. There was a significantly higher seroprevalence in the diabetic patients than the controls ($P<.001$). Among the diabetic patients, *H pylori* infection did not influence diabetic control, insulin dose, height, or weight. In a US-based study, Begue and colleagues[94] studied the prevalence of *H pylori* infection in 69 young diabetic patients (mean age, 11.2 years). Sixty-three of the patients had type 1 diabetes and 6 had type 2. Overall, 16% had serologic evidence of *H pylori* infection that was confirmed with results of UBT. Of the patients with type 1 diabetes, insulin requirements were significantly higher in the infected than in the noninfected patients ($P = .03$). There was a significantly higher mean glycosylated hemoglobin level among infected patients with type 2 diabetes than in the noninfected patients ($P = .04$). However, only 6 patients with type 2 diabetes were included.

In Spain, Martin-de-Argila and colleagues[95] studied the seroprevalence of *H pylori* infection in 101 diabetic patients and 100 controls. Of the patients, 80 had type 1 and 21 had type 2 diabetes. The controls were roughly matched for age. The seroprevalence of infection was not significantly different between diabetic patients and controls. However, patients younger than 24 years and with type 1 diabetes had a higher seroprevalence than age-matched controls ($P<.05$). Among patients older than 24 years and with type 1 diabetes, there was a significantly lower seroprevalence of *H pylori* infection than in age-matched controls ($P<.05$).

Ojetti and colleagues[96] in Italy studied the effects of treating *H pylori* infection on insulin requirements in patients with type 1 diabetes. They recruited 119 patients with type 1 diabetes, 42 of whom had *H pylori* infection determined by a ^{13}C-UBT. There was no significant difference between infected and noninfected patients regarding mean daily insulin requirements. Of the 42 infected patients, 20 had successful eradication determined by repeated ^{13}C-UBT. Eradication of *H py-*

lori infection did not influence diabetic control.

The best evidence of an association between *H pylori* infection and diabetes comes from case-control studies. There is, however, inconsistency among different studies. There is no substantial evidence that *H pylori* infection affects diabetic control or insulin requirements.

Thyroiditis. Although there is no obvious link between *H pylori* and thyroid disease, the infection was found endoscopically in 16 of 30 patients with various autoimmune thyroid disorders and in 16 of 30 control subjects with dyspepsia but without a history of thyroid disease.[97] In a separate study, serologic testing detected *H pylori* infection in 34 (71%) of 48 women with thyroid disease and antibodies to thyroglobulin and in 16 (48%) of 33 women who served as age-matched controls ($P<.05$).[98] In infected patients with thyroid disease, levels of antibodies to thyroglobulin were no higher than in uninfected patients. Similarly, *H pylori* status did not appear to influence levels of thyroid hormones.

The best evidence of an association between *H pylori* infection and thyroid disease comes from a case-control study. There is no obvious biological rationale for such an association. There is no evidence that *H pylori* infection influences thyroid function.

Acromegaly. Ten patients with acromegaly who had received octreotide acetate treatment for longer than 2 years complained of a variety of gastrointestinal tract problems.[99] Upper endoscopy was performed in 9 patients, which showed evidence of *H pylori* infection in 8.

There was histological evidence of gastritis and *H pylori* infection in 10 of 33 untreated patients with acromegaly.[100] Of patients treated for acromegaly with octreotide, 17 of 36 had evidence of *H pylori* infection. Of 21 patients studied before and during octreotide therapy, *H pylori*–related gastritis appeared to have developed in 3.

Helicobacter pylori infection was probably a chance finding in these acromegalic patients. There is no credible evidence of an association between *H pylori* infection and acromegaly.

Dermatological Disorders

Rosacea. In the past, rosacea may have been erroneously linked to gastritis. However, based on a study published in 1967, gastritis was found in 11 of 18 patients with rosacea compared with 9 of 16 controls,[101] suggesting no such association. An Italian group considered that rosacea and peptic ulcer disease showed seasonal variation, and that rosacea may be ameliorated by some of the antibiotics commonly used to treat *H pylori* infection, such as metronidazole and tetracycline.[102] In an uncontrolled study, they determined that 85% of 31 patients with rosacea had some evidence of *H pylori* infection. The investigators then treated 5 patients with rosacea with metronidazole and observed them serologically, reporting a reduction in anti–*H pylori* IgG levels.

Some dermatologists have greeted the observations of Rebora and colleagues with enthusiasm.[103] Anecdotal case reports suggest improvement in rosacea after systemic antibiotic treatment for *H pylori* infection.[104]

An uncontrolled study from Ireland reported a 95% seroprevalence of *H pylori* infection in a small group of patients with rosacea.[105] However, 2 controlled studies do not support any association between *H pylori* infection and rosacea. In the first of these,[106] the seroprevalence of *H pylori* infection in 94 patients with rosacea was 49%, compared with 53% in a control group of 32 patients with dermatitis. In the second,[107] 27% of 45 patients with rosacea were seropositive for *H pylori* infection compared with 35% of age-comparable healthy subjects without chronic skin disease.

The proposed biological rationale for an association between *H pylori* infection and rosacea is weak and probably based on erroneous assumptions. The only evidence of any association is from uncontrolled case series. Studies are inconsistent. Controlled studies of seroprevalence show no association.

Psoriasis. A number of bacterial and fungal pathogens have been proposed as causal for psoriasis.[108] *Helicobacter pylori* is among the list of putative bacterial agents because of anecdotal case reports of improvement in psoriasis following treatment for this infection.[108] Schneider et al[106] found a seroprevalence of *H pylori* infection of 46.9% in 32 patients with psoriasis, compared with 53.1% of 32 patients with chronic dermatitis and 35.7% of 14 patients with other forms of skin disease.

In an uncontrolled study of 33 patients with psoriasis and without any history of chronic gastrointestinal tract complaints, the seroprevalence of *H pylori* infection was 27%.[109] Three patients were treated for *H pylori* infection without apparent improvement in their psoriasis.

There is no evidence of an association between *H pylori* infection and psoriasis, and no obvious biological rationale for any association.

Chronic Urticaria. Circulating immune complexes may trigger urticaria. Investigators have considered that *H pylori* infection might be a source of such complexes.

An uncontrolled study of 10 patients with urticaria in Germany found histological evidence of *H pylori* infection in 8.[110] The authors reported improvement in features of cutaneous urticaria within days of starting treatment for *H pylori* infection. However, a study of 104 patients was unable to identify an association between *H pylori* infection and any 1 of 7 varieties of chronic urticaria.[111]

Tebbe and colleagues[112] identified 25 patients with chronic urticaria. They assessed *H pylori* status by ^{13}C-UBT and serological testing. Of the 25, they found *H pylori* infection in 17, each of whom was then treated for the infection. Results of repeated ^{13}C-UBT verified eradication of infection in 14. Each of these 14 reported remission (>75% improvement) or partial remission (50%-75% improvement) in symptoms of urticaria for up to 10 weeks

after treatment. There was no improvement in the 3 patients with failed eradication or in the uninfected patients who did not receive treatment for *H pylori* infection. This study was not randomized or blinded, and it is unclear if the patients were informed of their *H pylori* status or of the success or failure of eradication treatment.

Among 85 patients with chronic urticaria in Austria, 26 were seropositive for *H pylori* infection on results of endoscopy and biopsy.[113] These patients were then randomized into 1 of 2 groups. Patients received standard treatment, along with ranitidine, for chronic urticaria and placebo or a combination of amoxicillin and metronidazole for *H pylori* infection. Chronic urticaria was unaffected by this relatively ineffective antimicrobial regimen for *H pylori* infection.

In a study from Italy, 22 of 32 consecutive patients with chronic urticaria were infected with *H pylori* as determined by serologic examination and ^{13}C-UBT.[114] The infected patients were randomized to treatment for *H pylori* infection or to no treatment. It is unclear if randomization was blinded or if concealed allocation was used. Despite successful eradication of *H pylori* infection in 10 of the 11 treated patients, their chronic urticaria was not improved. There was no significant difference among the untreated control patients.

In a separate study from Italy, *H pylori* infection was present in 23 of 42 patients with chronic urticaria.[115] Eighteen patients completed treatment for *H pylori* infection, of whom 16 had successful eradication. Of these, 13 showed an apparent complete resolution of symptoms of chronic urticaria for up to 3 months after treatment. No such improvement was seen in untreated patients. This study was unblinded and not randomized.

There is no obvious biological rationale for an association between *H pylori* infection and urticaria. The best evidence of any association comes from uncontrolled case series. Supportive evidence from studies of treating *H pylori* infection in patients with urticaria is weak.

Schönlein-Henoch Purpura. Schönlein-Henoch purpura associated with *H pylori* infection has been described in a 21-year-old woman. The condition regressed after initial, unsuccessful treatment for *H pylori* infection but later recurred. After a second course of the same treatment for the infection, the disease again went into clinical remission.[116]

The only evidence of an association between *H pylori* infection and Schönlein-Henoch purpura is from a single case report with incomplete follow-up.

Other Dermatological Disorders. In a group of 68 consecutive patients with alopecia areata, the seroprevalence of *H pylori* infection was higher than in age-matched controls.[117] There are isolated case reports linking *H pylori* infection to atopic dermatitis[118] and Sweet syndrome.[119]

The best evidence of an association between *H pylori* infection and alopecia areata is from a case-control study. Evidence of other dermatological conditions is based on individual case reports. A biological rationale for such associations is lacking.

Rheumatological Disorders

Rheumatoid Arthritis. Seventeen of 54 patients with rheumatoid arthritis (RA) had cultures from gastric biopsy specimens that yielded *H pylori*.[120] Eight of these patients were treated for *H pylori* infection and observed for 18 weeks. Their serum concentrations of anti–*H pylori* IgG fell significantly, but there was no discernible effect of treatment for *H pylori* infection on the course of their RA. The mean titer of anti–*H pylori* antibodies was not different between 14 patients with RA and 24 age-matched controls with chronic pulmonary disease.[121]

There is no evidence of a causal association between *H pylori* infection and RA, and no biological rationale for any such association.

Scleroderma. In an uncontrolled study, 5 of 12 patients with scleroderma had evidence of *H pylori* infection on ^{13}C-UBT.[122] Scleroderma did not improve after treatment for *H pylori* infection. The mean titer of anti–*H pylori* antibodies was not different between 11 patients with scleroderma and 24 age-matched controls with chronic pulmonary disease.[121]

There is no evidence of and no obvious biological rationale for an association between *H pylori* infection and scleroderma.

Sjögren Syndrome. In the Japanese study already referred to,[121] the mean titer of anti–*H pylori* antibodies was significantly higher ($P<.05$) in 7 patients with Sjögren syndrome than in 24 age-matched controls with chronic pulmonary disease.

In an Italian study, *H pylori* infection was present in 71% of 21 patients with primary Sjögren syndrome and 63% of 80 controls with dyspepsia.[123] In Finland, Collin and colleagues[124] studied 32 consecutive patients with primary Sjögren syndrome and 64 age- and sex-matched controls using endoscopy. Although atrophic gastritis of the antrum was found more frequently in the patients with Sjögren syndrome (25% vs 4%; $P = .01$), there was no significant difference in the prevalence of *H pylori* infection (31% vs 39%; $P > .05$).

There is no evidence of and no obvious biological rationale for an association between *H pylori* infection and Sjögren syndrome.

Hematological Disorders

Iron Deficiency Anemia. Certain bacteria, including *H pylori*, are able to acquire iron from their host.[125] The elucidation of the genome of *H pylori*[126] identified a number of genes that encode for iron-scavenging functions.[127] By this mechanism, *H pylori* infection might lead to anemia from iron deficiency without blood loss. *Helicobacter pylori* infection acquired early in life might also lead to iron deficiency in adulthood by producing chronic atrophic gastritis, which impairs the absorption of dietary iron.

Isolated case reports have suggested an association between *H pylori* infection and iron deficiency anemia in children and young adults.[128-132] In at least 2 of these

cases,[129,131] iron deficiency anemia was not accompanied by detectable gastrointestinal tract blood loss. In all cases, anemia resolved with successful eradication of *H pylori* infection.

A cross-sectional study of 103 children in Bangladesh aged 6 months to 2 years found a significantly lower ($P = .04$) mean hemoglobin level in infected than in noninfected children.[133] The anemia was presumed to have been due to iron deficiency.

If the association between iron deficiency anemia and *H pylori* infection is true, it may be confined to children. In a study of more than 2000 adults in Denmark, *H pylori* serologic status did not affect a number of red blood cell indices.[134]

The best evidence of an association between *H pylori* infection and iron deficiency anemia comes from a population-based cross-sectional study in children. There is a plausible biological rationale for such an association. Alternatively, *H pylori* infection might simply be a surrogate marker for poverty and malnutrition in childhood.

Autoimmune Thrombocytopenic Purpura. Among 15 patients with autoimmune thrombocytopenic purpura and in whom other causes of thrombocytopenia had been excluded, the prevalence of *H pylori* infection assessed by the ^{13}C-UBT was 67%.[135] The 10 patients with *H pylori* infection were treated for it, and 7 had successful eradication. In these patients, platelet counts increased from a mean of 90 200/mm^3 to a mean of 148 800/mm^3 ($P<.05$). Antiplatelet antibodies became undetectable 6 weeks after treatment in 7 patients. In infected patients who were treated for *H pylori* infection but in whom eradication was unsuccessful, there was no change in platelet count or in the levels of antiplatelet antibodies.

The evidence of an association between *H pylori* infection and autoimmune thrombocytopenic purpura is from an uncontrolled case series. Objective evidence from an uncontrolled study of an increase in platelet count following treatment of *H pylori* infection in autoimmune thrombocytopenic purpura should be confirmed prospectively in a randomized controlled trial.

Hyperammonemia. Plasma ammonia levels are generally increased in patients with hepatic encephalopathy,[136] although this does not fully explain its clinical manifestations. Hyperammonemia in patients with hepatic encephalopathy is thought to be derived predominantly from bacterial activity in the colon. *Helicobacter pylori* has potent urease activity[10] that may be a source of ammonia in circulating blood. Some investigators have studied the influence of *H pylori* infection on plasma ammonia levels and the risk for hepatic encephalopathy in patients with liver disease.

In a prospective, multicenter, Veterans Affairs–based study, Gubbins and colleagues[137] studied 188 patients with moderate or severe alcoholic hepatitis. Of these, 117 had hepatic encephalopathy. There was a higher seroprevalence of *H pylori* infection in these patients with than in those without encephalopathy (79% vs 62%; $P = .01$). Using stepwise linear regression, *H pylori* infection was identified as an independent risk factor for hepatic encephalopathy.

Ito and colleagues[138] from Japan described 2 patients with recurrent hepatic encephalopathy due to cirrhosis from chronic hepatitis C virus infection. Both had evidence of *H pylori* infection. After successful eradication of infection, plasma ammonia levels were reduced, and hepatic encephalopathy did not recur for at least 3 months. Both patients were subsequently observed for more than 2 years[139]; plasma ammonia levels stayed below those seen before treatment for *H pylori* infection.

In an experimental study in 20 patients with cirrhosis, Plevris and colleagues[140] determined *H pylori* status by ^{14}C-UBT and administered urea by mouth. Plasma ammonia levels rose in all patients irrespective of *H pylori* status, and there was no difference between the *H pylori*–infected and noninfected patients.

Kirchner and colleagues[141] from Germany studied plasma ammonia levels and the *H pylori* status of 132 patients with cirrhosis, 38 patients with chronic viral hepatitis but without cirrhosis, and 39 age-matched controls. The controls had cardiovascular or cerebrovascular disease but no gastrointestinal tract complaints or evidence of liver disease. Patients with cirrhosis had higher seropositivity for *H pylori* infection (81%) than those with chronic viral hepatitis (62%) or controls (34%). However, there was no association between plasma ammonia levels and *H pylori* status.

Miyagi and colleagues[142] in Japan studied 18 patients with cirrhosis and persistent hyperammonemia despite treatment with low-protein diet, kanamycin sulfate, lactulose, and branched-chain amino acids. They divided these 18 patients into 3 groups of 6 patients according to *H pylori* status as assessed endoscopically. One group had diffuse gastric involvement with *H pylori*, the second had more patchy involvement, and the third had no *H pylori* infection. Patients in all 3 groups received treatment for *H pylori* infection. The mean plasma ammonia level was initially reduced in all patients following the standard treatment for hepatic encephalopathy. However, there was a further reduction in plasma ammonia level in the 6 patients with diffuse gastric involvement and *H pylori* infection following eradication treatment. There was no further reduction in plasma ammonia levels in the other groups after similar treatment. In the patients who had diffuse gastric involvement with *H pylori* infection, plasma ammonia levels remained low for up to 12 weeks after eradication treatment.

Llach and colleagues[143] from Spain found no difference in plasma ammonia concentration or in hepatic encephalopathy scores between 32 *H pylori*–infected and 30 noninfected patients with cirrhosis. Furthermore, treatment of *H pylori* infection did not produce any significant change in plasma ammonia concentrations or encephalopathy scores.

Cho and colleagues[144] in South Korea studied levels of ammonia in plasma and gastric juice in 31 patients with cirrhosis and 34 controls. They determined *H pylori* sta-

tus in all patients by serologic examination and endoscopic biopsy. Little information was provided on the control group, but they were not particularly well matched for age. Plasma ammonia levels were higher in the cirrhotic patients than the controls. Levels of ammonia in gastric juice were higher in *H pylori*–infected than in noninfected patients in both groups. *Helicobacter pylori* infection did not affect plasma ammonia concentrations in the patients with cirrhosis.

Among 55 patients with cirrhosis, 37 had clinical or neurophysiological evidence of chronic hepatic encephalopathy.[145] The prevalence of *H pylori* infection determined by gastric mucosal biopsy and rapid urease testing was 67% in the encephalopathic group and 33% in the nonencephalopathic group ($P = .002$). Levels of ammonia in gastric juice were higher in the encephalopathic than in the nonencephalopathic patients ($P = .05$). Plasma ammonia levels were not reported. Seventeen encephalopathic patients, including 13 with and 4 without *H pylori* infection, had treatment with a relatively ineffective regimen for *H pylori* infection. Results of testing for encephalopathy improved in each of the *H pylori*–infected patients. The test results did not change appreciably in the 4 uninfected patients. Eradication of infection was not confirmed.

In an experimental study, Zullo and colleagues[146] from Italy examined the effects of acetohydroxamic acid on plasma ammonia levels in 16 cirrhotic patients. Acetohydroxamic acid is a direct inhibitor of bacterial urease.[147] Eight patients had *H pylori* infection determined by histological examination and a rapid urease test. Plasma ammonia levels did not change after acetohydroxamic acid administration in the 8 patients without *H pylori* infection. However, plasma ammonia concentration at 15 to 30 minutes after acetohydroxamic acid administration fell by a mean of 27% in the 8 patients with *H pylori* infection.

Patients with chronic renal insufficiency have high levels of urea in their gastric lumen.[148] Those with *H pylori* infection might, therefore, have elevated plasma ammonia levels. However, comparison of 9 *H pylori*–infected and 7 noninfected uremic patients found similar plasma and intragastric concentrations of both urea and ammonia.[149]

Observational studies suggest a relationship between *H pylori* infection and raised plasma ammonia levels in patients with chronic liver disease. However, there is inconsistency among different studies. There is a plausible biological rationale for the association (Table 1). Experimental studies investigating the possible association have been inconsistent.

Miscellaneous Disorders

Sudden Infant Death Syndrome. There are some similarities between the epidemiological features of sudden infant death syndrome (SIDS) and those of *H pylori* infection. Both are more common in families of lower socioeconomic status and from nonwhite ethnic groups. Furthermore, the incidence of both appears to be decreasing in parallel.[149,150] Possible links between *H pylori* infection and SIDS include raised systemic levels of cytokines such as interleukin 1 that may promote fever, immune activation, and deep sleep.[150] Alternatively, *H pylori* might be aspirated from the stomach into the airways, where the generation of ammonia through the action of *H pylori* urease might promote respiratory arrest.[149]

Helicobacter pylori was reported at autopsy in the gastric antrum and trachea in 7 infants who died of SIDS.[151] However, the same investigators were subsequently unable to confirm this observation when examining autopsy material from 22 consecutive infants with a postmortem diagnosis of SIDS using histological or polymerase chain reaction testing.[150] In a separate study, polymerase chain reaction testing on antral biopsy specimens from 11 infants who died of SIDS identified *H pylori* in 8 of 9 specimens when it was apparent histologically, and in 1 of 2 when it was not.[152] Primary tracheal colonization by *H pylori* without gastric antral involvement has been reported in 3 of 12 infants with SIDS,[151] suggesting possible transmission of *H pylori* by the respiratory route. *Helicobacter pylori* may have been isolated from tracheal secretions of adults in an intensive care unit.[153]

In autopsies of 37 infants with SIDS in 2 metropolitan areas in the United States, there was evidence of gastric antral *H pylori* infection in 20 (54%). Organisms compatible with *H pylori* were identified in tracheal specimens in 22 (59%).[155]

The best evidence of an association between *H pylori* infection and SIDS comes from uncontrolled case series. The temporal relationship for an association is correct, and there is a plausible biological rationale (Table 1). There is unconfirmed evidence of colonization of the upper airways of infants with SIDS by bacterial organisms that may be *H pylori*.

Growth Retardation in Childhood. Patel and colleagues[156] studied 554 11-year-old children in Scotland. Of these, 62 had evidence of *H pylori* infection since 7 years of age. On average, these children had grown 1.1 cm less than noninfected children from the ages of 7 through 11 years. Girls had grown 1.6 cm less than their noninfected peers had.

Raymond et al[157] compared 77 French children infected with *H pylori* and 74 age-matched children without the infection. Compared with 23% of the controls, 27% of the infected group were of short stature. Of the children with short stature, there was no evidence of hypoproteinemia or malabsorption.

In a cross-sectional study from southern Italy, 49 of 216 children aged 3 through 14 years were infected with *H pylori* as determined by the ^{13}C-UBT.[158] Of the 49 infected children, 8 were below the 25th percentile for height, compared with 13 of 167 uninfected children. In the subgroup of children aged 8.5 through 14 years, 8 of 31 infected children were below the 25th percentile for height compared with 8 of 96 uninfected children ($P = .02$).

Oderda and colleagues[159] in Italy found serologic evidence of *H pylori* infection in 20% of 134 con-

secutive children with short stature and in 13% of matched controls ($P = .19$). The authors concluded that *H pylori* infection was not an independent risk factor for short stature in childhood but that low socioeconomic status was of more importance.

In a population-based survey of more than 4700 people in Northern Ireland, the mean height of women older than 25 years who were infected with *H pylori* was 1.6 cm lower than in uninfected women ($P < .01$ after adjustment for age and socioeconomic status).[160] Among 1756 Danish women in a random sample, those in the upper quartile for height were significantly less likely to be infected with *H pylori* than those in the lower quartile.[53] The likelihood of *H pylori* infection was also related to late menarche, leading the investigators to speculate that an impaired pubertal growth spurt may have explained the finding. The Eurogast Study Group conducted a cross-sectional survey of the seroprevalence of *H pylori* infection in more than 3000 subjects in 2 age groups in various countries.[161] In subjects aged 55 through 64 years, infection with *H pylori* tended to be associated with a low body mass index and short stature. However, this was not statistically significant after adjustment for other variables.

The best evidence of an association between *H pylori* infection and growth retardation in childhood comes from a cohort study. The temporal relationship for any association is correct (Table 1). There is some inconsistency between different studies. *Helicobacter pylori* infection might be a marker for low socioeconomic status and relative malnutrition in childhood.

Anorexia of Aging. One report described 3 institutionalized, elderly patients with a variety of medical complaints that included anorexia.[162] Each patient had evidence of *H pylori* infection. Treatment for the infection resulted in improvement in anorexia. Treatment regimens were suboptimal, and eradication of infection was not confirmed. *Helicobacter pylori* infection was not proven as the cause of the anorexia in any of the 3 patients.[163]

There is no evidence of an association between *H pylori* infection and the anorexia of aging.

COMMENT

Numerous different conditions have been linked preliminarily to *H pylori* infection. However, many of these associations are based on uncontrolled or inappropriately controlled observations. A biological rationale for an association with *H pylori* infection is often lacking.

Further research on the role of *H pylori* as a possible causal or contributory factor may be warranted for some conditions. Examples might include autoimmune thrombocytopenic purpura[135] and SIDS.[152,153,155] However, any such research should be conducted in a responsible, planned, and cautious manner.

Unfortunately, weak evidence of causation by *H pylori* infection in some conditions has already led to poor-quality studies of the effects of treatment for the infection.[88,89,113] Sackett[26] has proposed a 5-level system of grading evidence from treatment trials to help determine whether a treatment should be recommended. The highest level of evidence comes from large randomized controlled trials and carries a strong recommendation for adoption of the treatment into routine practice. Studies on the treatment of *H pylori* infection in the conditions reviewed herein have been nonrandomized, unblinded, and uncontrolled or inappropriately controlled.[88,89,113] Therefore, these studies present evidence that is, at best, weak. Furthermore, treatment regimens have often been ineffective, and eradication of infection has not been confirmed.[113,145,161]

Rather than treating *H pylori* infection in all infected patients identified with a specific diagnosis, it would be of more value to randomize infected patients to receive active or placebo treatment in a double-blinded manner. Only then could investigators adequately assess any effects of treating *H pylori* infection on the underlying condition. Conceivably, antimicrobial therapy might improve 1 of the conditions in question through a mechanism unrelated to eradication of *H pylori* infection. For example, apparent improvement in patients with hepatic encephalopathy after antimicrobial treatment of *H pylori* infection could have been explained by a reduction in levels of colonic bacteria. Therefore, there may be a case for antibiotic treatment, as if for *H pylori* infection, in uninfected patients to eliminate any unrelated, beneficial effect of antimicrobial therapy.[142,145]

Although *H pylori* infection has been linked to a wide variety of non–gastrointestinal tract conditions, the level of supporting evidence is low (Table 1). Conversely, ample evidence links *H pylori* infection to various conditions of the upper gastrointestinal tract (**Table 2**). Limited experiments in humans have established a specific and direct relationship with gastritis, with consistent temporality.[17–19] Although there are no direct experimental data in humans that link *H pylori* infection with peptic ulcer disease, there is a mass of highly consistent and strong circumstantial evidence.[1,28] Nested case-control studies[166,167] and a meta-analysis[5] have established a strong and temporally correct relationship between *H pylori* infection and gastric adenocarcinoma. There is also a strong biological rationale for this association.[1] Similarly, there is a strong, temporally correct association between *H pylori* infection and low-grade gastric lymphoma arising from mucosa-associated lymphoid tissue.[8,168] However, even in these conditions of the upper gastrointestinal tract, about which there is broad agreement regarding causation by *H pylori*, it is not possible to answer affirmatively all 9 of the questions proposed by Sackett (Table 2).

Demonstration of *H pylori* infection would be unhelpful at present in an individual patient with any of the non–gastrointestinal tract conditions proposed to be associated with it. Since the infection is highly prevalent, it will be found by chance in many patients who seek medical attention for another condition. Demonstrating the infection in a patient with another disorder does not

Table 2. Application of 9 Diagnostic Tests for Causes of Upper Gastrointestinal Tract Conditions Generally Accepted to Be Related to *Helicobacter pylori* Infection*

Tests	Gastritis	Peptic Ulcer	Gastric Cancer	Gastric MALT Lymphoma
Is there evidence from true experiments in humans?	Yes	No	No	No
Is the association strong?	Yes	Yes	Yes	Yes
Is the association consistent from study to study?	Yes	Yes	Yes	Yes
Is the temporal relationship correct?	Yes	Yes	Yes	Yes
Is there a dose-response relationship?	?	?	?	?
Does the association make epidemiological sense?	?	?	?	?
Does the association make biological sense?	Yes	Yes	Yes	Yes
Is the association specific?	Yes	No	No	No
Is the association analogous to a previously proven causal association?	No	No	No	No

*MALT indicates mucosa-associated lymphoid tissue; question mark, absence of sufficient evidence for answer.

prove a causal link. Furthermore, the finding of an unexpected chronic bacterial infection might promote anxiety in a patient presenting with another disorder. Alternatively, it could lead to unwarranted optimism of a possible explanation for or cure of the primary condition. There is no current need for such patients to undergo testing for *H pylori* infection. Testing is only indicated in patients with present or past peptic ulcer disease or gastric lymphoma arising from mucosa-associated lymphoid tissue.[22]

Practitioners need to be aware of the proven and important association between *H pylori* infection and peptic ulcer disease.[2,21] Patients with ulcer disease should undergo appropriate testing for *H pylori* infection. Those with positive test results should receive an effective combination drug regimen for eradication of the infection.[2,21] These patients will derive the greatest benefit from testing for and treating *H pylori* infection. Many of the possible associations of *H pylori* infection discussed herein are speculative at best. Unfortunately, the excessive attention and publicity paid to these may detract from the proven and important role of *H pylori* in ulcer disease.

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Complete regression of Barrett's esophagus with heat probe thermocoagulation: mid-term results

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Background: **Barrett's esophagus is a premalignant condition. It has been reported that several methods of endoscopic ablation in combination with acid suppression result in replacement of specialized columnar epithelium by squamous epithelium. The aim of this study was to assess whether ablation of Barrett's mucosa by means of heat probe and acid suppression restores normal esophageal mucosa.**

Methods: **Thirteen patients with Barrett's epithelium but not dysplasia were enrolled in the study. *Helicobacter pylori* was eradicated when discovered. Thermal energy was applied using a heat probe (pulses of 5 to 10 joules). Four-quadrant biopsies were obtained at 1 to 2 cm intervals 1 to 3 months after the last treatment session. All patients continuously took omeprazole, 40 mg/day.**

Results: **Macroscopically, ablation of Barrett's mucosa was achieved in all patients after 1 to 5 sessions. Three of the 13 patients had residual specialized columnar epithelium beneath the restored mucosa but not overexpression of p53 and c-erbB-2. During follow-up (6 to 36 months) two patients in whom the length of Barrett's mucosa was greater than 2.5 cm relapsed after omeprazole discontinuation, whereas another two with length of less than 2.5 cm did not. One patient with residual Barrett's islands developed low-grade dysplasia.**

Conclusions: **Heat probe is an effective and inexpensive method for Barrett's ablation. Islands of residual specialized columnar epithelium were found in 23% of patients. The length of Barrett's epithelium determines relapse after omeprazole discontinuation. (Gastrointest Endosc 1999;50:165-72).**

Barrett's esophagus (BE) is a relatively common condition wherein distal squamous epithelium is replaced by specialized columnar epithelium (SCE). It is a premalignant condition.[1,2] The incidence of esophageal adenocarcinoma is rapidly increasing in the United States and other Western countries.[3-6]

The currently recommended surveillance procedure includes multiple random biopsies at regular intervals, but the efficacy of such surveillance is not unanimously accepted.[7] The importance of attempting to reverse BE lies in the potential to diminish the probability of development of esophageal adenocarcinoma, a goal until recently unattainable. Acid suppression and antireflux surgical procedures have failed to completely reverse BE.[8,9] Eradication of the metaplastic epithelium by laser, photodynamic therapy, multipolar electrocoagulation, and argon plasma coagulation has been reported. However, islands of residual SCE often remain beneath the regenerated squamous epithelium.[10-16]

The heat probe is an inexpensive modality that, to our knowledge, has not been applied to the treatment of BE except for a case of upper esophageal stricture caused by heterotopic gastric mucosa.[17] Although comparative data on the methods used for ablation of Barrett's mucosa in humans do not exist, a study in a canine model of photodynamic therapy, neodymium-yttrium aluminum garnet laser, KTP laser, heat probe and multipolar probe concluded that the best methods were the last two mentioned.[18]

The aim of this study was to evaluate ablation of SCE by means of thermocoagulation (heat probe) including the long-term outcomes of this therapy.

PATIENTS AND METHODS

Patients

Thirteen consecutive patients with an endoscopic diagnosis of BE and histologic confirmation of intestinal metaplasia type III without dysplasia were enrolled. Exclusion criteria included high-grade dysplasia, esophageal adenocarcinoma, esophageal ulcer or stricture, pregnancy or lactation, known allergy to omeprazole or poor general medical condition. Patients with SCE identified on biopsies taken from an endoscopically normal gastroesophageal junction were not included. No patient had undergone previous antireflux or gastric surgery. There were 8 men and 5 women, mean age of 54.6 ± 10.7 years.

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Figure 1. Regenerated nonkeratinized stratified squamous epithelium replacing Barrett's epithelium after heat probe coagulation *(big arrow)*. Underlying cardiac-type glands *(small arrows)* (H&E, orig. mag. ×100).

All patients included in the study gave informed consent. The protocol was approved by the ethics committee of our institution.

Endoscopic protocol

In all sessions, lidocaine spray for oropharyngeal anesthesia and midazolam at a dose of 0.05 mg/kg intravenously for sedation were used. Endoscopies were performed with a fiberscope (GIF-IT20 or GIF IT-140; Olympus Co. Ltd., Tokyo, Japan). The length of BE was determined at the index endoscopic examination by means of a Teflon-covered guidewire (Protector guide plus 480; Wilson Cook Medical Inc., Winston-Salem, N.C.). On the unmarked part of the guidewire marks were added with a black water-resistant marker every 1 cm. Measurements were made beginning 2 cm proximal to the most proximal fundic fold and terminated at the most proximal extent of Barrett's mucosa. If narrow, linear extensions of Barrett's mucosa were present, the length of Barrett's mucosa was taken as that of the longest extension. In patients with coexistent esophagitis, the length of Barrett's epithelium was measured after treatment of the esophagitis. BE was considered circular, if there were no bridges of normal mucosa between extensions of Barrett's epithelium and there was no predominance of any extension. One antral biopsy was obtained for a rapid urease test (CLO-test; Tri-Med Sp Inc., Ballard Medical Products, Osborne Park, Western Australia) and four additional biopsies (two from the antrum and two from the gastric body) were taken at the baseline endoscopy to check for *Helicobacter pylori* gastritis.

Ablation of the SCE was performed with a 2.4 mm heat probe (Olympus). The generator was set to deliver 5 to 10 J of energy. Thermocoagulation was applied at multiple sites starting from the proximal limit of the SCE and proceeding distally to the gastroesophageal junction or to a level 2 cm above the most proximal fundic fold when a hiatal hernia was present. In each session, an attempt was made to coagulate almost all of the Barrett's surface. Coagulation was thought to be complete when the salmon pink colored SCE was replaced by a white coagulum. Two

Figure 2. Persisting glands lined by SCE under the regenerated squamous epithelium characterized by the presence of acid sialomucins and sulfomucins in goblet and columnar cells. (**A**) Neutral mucins, red *(small arrows)*; acid mucins, blue *(big arrow)*; (PAS-alcian blue stain, orig. mag. ×100). (**B**) Sialomucins, blue *(small arrow)*; sulfomucins, brown-black *(big arrow)*; (alcian blue stain, high-iron diamin, orig. mag. ×200).

endoscopists assessed the result. The patient then underwent endoscopy at 4-week intervals to treat any remaining columnar-appearing mucosa. During repetitive endoscopic sessions, methylene blue staining was used to guide thermoablation. Five to 10 mL 0.5% methylene blue solution (Merck Co. Inc., Darmstadt, Germany) was sprayed on the distal esophagus using a 2.8 mm spraying catheter (PWSL-1; Olympus), so that the entire area of preexisting Barrett's epithelium was covered. Immediately thereafter, 120 to 240 mL tap water was sprayed on the esophageal mucosa to wash off excess dye. Positive staining was defined as blue staining of endoscopically normal esophageal mucosa that persisted despite vigorous water irrigation. Barrett's epithelium was thought to be ablated when salmon pink colored epithelium had totally disappeared and no area of preexisting BE was stained with methylene blue.

One to 3 months after complete macroscopic reversal of BE, endoscopy was performed and biopsies were taken. Methylene blue was used to guide the placement of biopsies. These were obtained with a jumbo biopsy forceps (FP13K, Olympus; or 2.4 160-2, Mill Rose Lab Inc.

Table 1. Demographic data and endoscopic findings for all patients included in the study

Patient No.	Gender	Age (yr)	Smoke cigarettes/ day	Alcohol (gm/wk)	Length of Barrett's epithelium (cm)	Other diagnoses	*H pylori*	HH (cm)	No. of sessions	Total energy (J)	Medications
1	M	56	40	300	2.5 T (1)	DU	Positive	2.5	1	135	S/N
2	F	32	15	150	6 T (3)	DU	Positive	0	4	1610	
3	F	53	45	20	5 T (3)	DU/E	Positive	3	5	1240	
4	M	50	20	25	5 C	DU	Positive	2.5	5	545	S/N
5	M	63	10	10	2.5 T (1)	DU/E	Positive	2.5	1	150	Et
6	M	71	0	40	2.5 T (2)		Positive	0	1	100	S/N
7	F	70	0	20	4 T (2)		Positive	6	4	1750	
8	M	59	0	50	6 C	DU	Positive	2.5	5	3300	
9	M	41	25	400	2.5 T (2)	GU	Positive	2.5	1	150	
10	F	53	15	80	2.5 T (2)	GU	Positive	0	2	230	
11	F	51	0	10	2 T (1)		Positive	0	1	1300	
12	M	59	0	40	2 I (5)	E	Positive	4	3	375	S/N
13	M	51	0	400	2.5 T (4)	E	Negative	5	3	1255	

C, Circular; *T*, tongues (long, narrow extensions) or *I*, islands (no. of T or I is shown in parentheses); *DU*, duodenal ulcer; *GU*, gastric ulcer; *E*, esophagitis; *HH*, length of hiatal hernia; *No. of sessions*, no. of sessions to achieve complete macroscopic disappearance of BE; *Total energy*, utilized in each person; *S*, sphincter relaxing drugs; *N*, nonsteroidal anti-inflammatory drugs; *Et*, etidronate.

Mentor, Ohio) at 1 to 2 cm intervals within the initially measured area of SCE (four quadrants) for pathologic and immunohistochemical examination.

Thereafter, endoscopy with placement of biopsies was performed every 6 months. Any reappearance of BE was treated with additional sessions of thermocoagulation. After each session of coagulation, patients were advised to take only liquid or semiliquid food for 3 days.

Medical therapy

All patients started omeprazole 20 mg twice a day 15 minutes before breakfast and dinner. Treatment to eradicate *H pylori* was prescribed in all *H pylori*–positive patients. One or more eradication regimens were given until both the rapid urease test and histology were negative for *H pylori*. Maintenance treatment with omeprazole was stopped for 4 weeks to assess eradication success and restarted after successful eradication. Only antacids were allowed on an as needed basis while the patient was receiving the eradication regimen. For *H pylori*–positive patients, ablation therapy was initiated only after *H pylori* eradication. After the initiation of endoscopic treatment and during the entire follow-up period, patients were instructed to communicate the reappearance of any symptom and particularly symptoms of gastroesophageal reflux. Continuous treatment with omeprazole (20 mg twice a day) was prescribed after successful thermoablation as specified in the protocol.

Histology

Biopsy specimens were fixed in 10% formalin, embedded in paraffin, serially sectioned and then stained with H&E. The presence of distended, barrel-shaped goblet cells on routine H&E-stained slides was considered indicative of intestinal metaplasia.[19] Metaplasia was confirmed using alcian blue pH 2.5/PAS and alcian blue pH1/HID and periodic acid-Schiff stains.[20] Diagnoses of low- and high-grade dysplasia (LGD and HGD, respectively) were made according to previously established criteria.[21]

Immunostaining for p53 and c-erbB-2 was performed using immunoalkaline phosphatase with fast red (Kwik-Kit; Lipshaw, Pittsburgh, Pa.) according to manufacturer's instructions. DO-7 (Ylem, Rome, Italy) and NCL-CB-11 (Ylem) were used respectively as specific monoclonal antibodies and were applied in 1:50 dilutions for 60 minutes on formalin-fixed tissue. Sections were considered to overexpress c-erbB-2 when at least 10% of cells showed unequivocal red staining. Cytoplasmic and membranous staining was recorded separately. Only membranous staining was considered indicative of overexpression whereas cytoplasmic staining was reported as c-erbB-2 cytoplasmic expression. The p53 was considered positive in the presence of red nuclear staining. To increase sensitivity, weak pink immunostaining, instead of red, was recorded as weakly positive, even if less than 10% of the cells were stained. If immunostaining was positive at the initial endoscopy, endoscopy was repeated within 1 month and extra biopsies were taken to exclude dysplasia.

Statistical analysis

Values are expressed as the mean ± standard deviation (mean ± SD).

RESULTS

Thirteen patients with BE with a length between 2 and 6 cm (circular in 2, with long, narrow extensions in 10 and large islands in 1) were enrolled in the study (Table 1). *H pylori* was positive in all but 1 patient and was successfully eradicated with one (in 10 patients) or two (in 2 patients) eradication regimens. No patient had a family history of malignancy. Cholecystectomy had not been performed in any patient. Four patients were taking medications that lower esophagus sphincter pressure (nitrates or calcium channel blockers) and nonsteroid anti-inflamma-

Table 2. Endoscopic and histologic findings at postablation and follow-up endoscopies

Patient No.	E0	H0	E6	H6	E12	H12	E18	H18	E24	H24	E30	H30	E36	H36	Follow-up (mo)
1	N	SC													
2	N	SC	2	III	N	SC									12
3	N	SC	N	SC	N	SC	N	SC	N	SC	N	SC	N	SC	35
4	N	SC	N	SC	N	SC	2.5	III	N	SC					25
5	N	III	N	LGD	N	LGD	N	LGD							17
6	N	III	N	SC	N	SC	N	SC							14
7	N	III	N	SC	N	SC	N	SC							15
8	N	SC	N	SC	N	SC	N	SC							14
9	N	SC	N	SC	N	SC	N	SC							15
10	N	SC	N	SC	N	SC	N	SC	N	SC					26
11	N	SC	N	SC											6
12	N	SC	N	SC											6
13	N	SC	N	SC											6

E0, E6, E12, E18, E24, E30, E36: results of postablation and follow-up esophagoduodenoscopies after 6, 12, 18, 24, 30, 36 months, respectively (time limits are not strict); *N*, macroscopically normal mucosa. Numeric values represent the length of recurrent Barrett's epithelium in cm.

H0, H6, H12, H18, H24, H30, H36: histologic results of postablation and follow-up esophagoduodenoscopies after 6, 12, 18, 24, 30, 36 months, respectively; *SC*, squamous cell epithelium; *III*, SCE.

tory drugs and one was taking etidronate (400 mg/day). Four patients had a positive cytoplasmic expression of c-erbB-2, whereas none was positive for p53 at the initial endoscopy. No patient had dysplasia, either on the entry or the reevaluation endoscopy.

A total of 36 heat probe ablation sessions were performed. The mean number of sessions was 2.77 ± 1.69 (range 1 to 5) per patient. The initial session lasted 20 to 30 minutes depending on the area of SCE to be ablated. The following sessions were of shorter duration. A mean of 933.8 ± 938.7 J (range 100 to 3300 J) was transferred to achieve macroscopic reversal. Our initial endoscopic impression of macroscopically complete ablation was changed in 2 patients when methylene blue was used.

After treatment, squamous epithelial regeneration was confirmed in all patients, both endoscopically and histologically (Fig. 1) (Table 2). However, in 3 patients and in 4 of 28 post-treatment biopsy specimens, persisting glands lined by SCE under areas of squamous regeneration were found (Fig. 2). No evidence of dysplasia was encountered at the initial endoscopy after successful treatment. The p53, evaluated on biopsies taken at the same period of the study, was weakly positive in 1 of 3 patients (patient No. 6) with persisting Barrett's mucosa under areas of regenerated squamous epithelium. The c-erbB-2 was positive (cytoplasmic expression) in 2 of 3 patients (Nos. 5 and 6).

All but 1 patient (No. 11) experienced no symptoms or only mild retrosternal discomfort during the first 24 hours after the ablation. Patient No. 11 complained of acute retrosternal pain after receiving 1300 J during one session. Endoscopy performed the following day revealed an esophageal ulcer. Symptoms resolved within 48 hours while on treatment and the esophageal ulcer was found to be healed at endoscopy performed 1 month later.

One patient was lost in the follow-up. During follow-up of 15.92 ± 8.89 months (range 6 to 36 months), two patients (Nos. 2 and 4) relapsed 3 and 9 months, respectively, after discontinuation of maintenance treatment with omeprazole in violation of the protocol. Both had relatively long BE (6 and 5 cm, respectively). Recurrent Barrett's epithelium was ablated after one and two additional sessions, respectively, of thermocoagulation. None of the other patients had any evidence of recurrence of Barrett's mucosa. Among them, 2 patients (Nos. 9 and 10) with BE 2.5 cm in length at initial evaluation had neither macroscopic nor histologic recurrence 12 months after discontinuation of omeprazole in violation of the protocol. Islands of persisting SCE beneath areas of squamous regeneration were reconfirmed in only 1 of 3 patients (No. 5), who had SCE islands after macroscopically successful ablation. This patient developed LGD at 17 months' follow-up without macroscopic evidence of Barrett's relapse. Persistence of p53 was not identified during follow-up, whereas c-erbB-2 was reconfirmed only in this patient (No. 5). Results of immunohistochemistry for c-erbB-2 and p53 are summarized in Table 3.

DISCUSSION

This prospective uncontrolled trial assessed the ability to eradicate BE by means of acid suppression and heat probe thermocoagulation. In all 13 patients,

Table 3. Results of immunohistochemistry for c-erbB-2 and p53

Patient No.	Ci	Pi	C0	P0	C6	P6	C12	P12	C18	P18	C24	P24	C30	P30	C36	P36
1	+	–	–	–												
2	–	–		–	–	–	–	–								
3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
4	+	–		–	–	–	–	–	–	–	–	–				
5	–	–	+	–	+	–	+	–	+	–						
6	+	–	+	$+_w$	–	–	–	–	–	–						
7	+	–		–	–	–	–		–	–						
8	–	–	–	–	–	–	–	–	–	–						
9	–		–	–	–	–	–	–	–	–						
10	–	–	–	–	–	–	–	–	–	–	–	–				
11	–	–	–	–	–	–										
12	–		–	–	–	–										
13	–	–	–	–	–	–										

Ci, C0, C6, C12, C18, C24, C30, C36: results of immunohistochemistry for c-erbB-2 cytoplasmic expression in initial (*i*), postablation (*0*) and follow-up esophagoduodenoscopies after 6, 12, 18, 24, 30, 36 months, respectively (time limits are not strict); –, negative; +, positive.

Pi, P0, P6, P12, P18, P24, P30, P36: results of immunohistochemistry for p53 in initial (*i*), postablation (*0*) and follow-up esophagoduodenoscopies after 6, 12, 18, 24, 30, 36 months, respectively (time limits are not strict); –, negative; $+_w$, weakly positive.

None of the examined samples were positive for membranous c-erbB-2 or p53 (nuclear staining > 10% of the cells).

BE was eliminated macroscopically although glands lined by SCE persisted under areas of squamous epithelial regeneration in three patients. Reversal of Barrett's epithelium was sustained during follow-up under continuous acid suppression. Barrett's mucosa under areas of squamous epithelial regeneration was not present during the later phases of follow-up in 2 of the 3 patients. LGD was identified in the third patient with persisting SCE in biopsies taken later during follow-up. Discontinuation of omeprazole, despite instructions, resulted in relapse in 2 patients with initial length of BE of 5 and 6 cm but not in 2 others with 2.5 cm lengths of BE.

This and other studies of ablation methods are based on the hypothesis that putative esophageal stem cells can undergo differentiation to a squamous epithelium in a low acid environment. This hypothesis derives from the observation that squamous cells regenerate in denuded dog lower esophagus in acid suppressed conditions whereas columnar mucosa develops in the presence of an acid environment.[22]

Successful eradication of BE has been reported in humans using a wide variety of endoscopic modalities in combination with acid suppression.[10-16] The heat probe has not, to our knowledge, been tested for Barrett's ablation, although it has been shown to be the best method for ablation of esophageal mucosa in a canine model.[18]

Our endoscopic results seem to confirm the aforementioned experimental data because complete macroscopic restoration of normal esophageal mucosa occurred in all patients. Methylene blue staining can identify SCE in 70% of patients with BE.[23] Methylene blue staining altered endoscopic assessment and therapy in 2 of our patients. We did not use a mucolytic agent, which may have reduced the accuracy of staining.

Both pluripotential cell differentiation and regrowth of adjacent squamous cells are thought to contribute to squamous regeneration.[24,25] None of the ablative methods used to date have prevented the persistence of Barrett's mucosa beneath regenerated squamous mucosa.[11-16,24-26] Neither of the aforementioned mechanisms could guarantee replacement of nonablated Barrett's mucosa by squamous epithelium. Therefore, the greater the destruction of BE, the less the probability of remaining SCE islands under the regenerated squamous epithelium. Deep thermal ablation is thought to reduce this possibility. Nevertheless, deeply buried metaplastic epithelial tissue can escape ablation.[26] However, the greater the depth of thermal destruction, the greater the risk of fibrosis.[24] This could reduce regenerative capacity of stem cells. Total energy transfer in a predetermined surface area during each session seems to affect the final result. In one of our patients (No. 11), the application of high total energy in a relatively small area during one session in an attempt to accelerate treatment and avoid the persistence of SCE resulted in patient discomfort and an esophageal ulcer.

Pharmacologic control of acid alone can reduce the length of Barrett's mucosa.[8,27] Thus, failure to reconfirm persisting Barrett's mucosa under areas of squamous regeneration in 2 of 3 patients who initially had this finding could be due to sampling error. However, it could also be the result of elimination of these islands in an acid suppression environment.

Persistence of SCE islands in patient No. 5 and possible progression to LGD could be attributed to a

variety of factors. If dysplasia had existed since the initial examination, acid suppression could not prevent its progression.[28] Noxious agents, in addition to acid, such as chemotherapeutic drugs[29] and caustic agents,[30] have also been incriminated in the development of BE. The patient mentioned above was under continuous treatment with etidronate, a biphosphonic salt known for its deleterious effects on esophageal mucosa.[31]

Relapse with reappearance of BE developed in 2 patients with long segment Barrett's mucosa (5 and 6 cm) at initial evaluation. The length of relapsed Barrett's mucosa was less than the length of the initial Barrett's epithelium (2 and 3 cm, respectively). Patients with shorter segment BE (2.5 cm) did not relapse 1 year after discontinuation of acid suppressive therapy.

Overexpression (membranous) of c-erbB-2 has been noted in 26% to 60% of esophageal adenocarcinomas and 13% to 75% of dysplastic but not metaplastic Barrett's mucosa,[32,33] suggesting that the c-erbB-2 mutation is an early event in esophageal carcinogenesis. However, some investigators consider it a late event.[34] C-erbB-2 immunostaining is membranous in adenocarcinomas and cytoplasmic in dysplastic mucosa.[33] Cytoplasmic expression but not overexpression of c-erbB-2 can be identified in low-grade dysplastic and even nondysplastic Barrett's mucosa.[35] The p53 mutation has been identified in 40% to 60% of Barrett's adenocarcinomas,[36,37] 31% of dysplastic Barrett's mucosa,[38] and in even 57% of metaplastic epithelium adjacent to adenocarcinomas.[33]

Despite the low sensitivity and positive predictive value of immunostaining for dysplasia in BE, we attempted to use it as an additional method to identify a possible subgroup of patients who would require a closer follow-up.[39] Dysplasia was not identified during either initial or reconfirmation endoscopy 1 month later in any patient with positive immunostaining (cytoplasmic c-erbB-2) at entry into the study. Only 1 patient (No. 6) with positive cytoplasmic c-erbB-2 before treatment remained positive at the first endoscopy after successful ablation. He became negative for cytoplasmic c-erbB-2 when SCE islands disappeared. In addition, 1 patient (No. 5) who was negative at the initial endoscopy became positive for cytoplasmic c-erbB-2 after ablation. Cytoplasmic c-erbB-2 expression was confirmed in this patient on four consecutive occasions where instead of a progression to LGD, p53 was continuously negative. The p53 immunostaining was weakly positive in only 1 patient (No. 6) who did not have dysplasia. As the signal was faint and involved less than 10% of the epithelial cells, it is questionable whether it should be considered positive. In three consecutive examinations during follow-up (14 months), its presence could not be reconfirmed. Although we expanded our cutoff for c-erbB-2 and p53 positivity, our immunostaining results predicted neither ablation treatment success nor presence of dysplasia.

There are few data on the coexistence of BE and duodenal ulcer. Duodenal ulcers and unspecified site peptic ulcers have been reported as protective factors for the development of adenocarcinoma of the gastric cardia[40]; others have reported an increased risk for adenocarcinoma of the esophagus and gastroesophageal junction when there is a history of ulcer, especially of the duodenum. Five patients in our study had duodenal ulcers but 3 were taking nonsteroidal anti-inflammatory drugs or etidronate.

Although *H pylori* seems not to predispose to the development or the progression of BE,[42] we eradicated the infection because most of our patients had ulcers and we intended to use omeprazole long term. More recent data suggest an inverse relationship between infection with Cag A–positive *H pylori* strains and the development of BE.[43] There are no data on Cag A positivity of the *H pylori* strains in this study. When the study was initiated it was not known that eradication of *H pylori* could worsen GERD,[44] although the existence of such a relationship is not universally accepted.[45] Moreover, because all of our patients were taking 40 mg omeprazole daily, a dose thought to be sufficient for most patients with GERD, a slight increase in acid reflux due to *H pylori* eradication is not likely to be a significant aggravating factor.[46] However, *H pylori* eradication reduces the antisecretory efficacy of omeprazole in certain patients, a factor that requires further study in patients with BE.[47]

The heat probe is a relatively inexpensive, readily available treatment modality compared with laser and photodynamic therapy. The complications of this form of therapy may also be less severe than the potential complications of neodymium-yttrium aluminum garnet laser and photodynamic therapy.[24] Adverse effects can be attributed to greater depths of thermal injury. We encountered no strictures despite almost circumferential coagulation in a single session.

The significance and the evolution of residual SCE islands are a crucial issue. The reduction in the length of BE is expected to diminish the probability of dysplasia and development of cancer because the probability of the latter depends on the total length of BE.[48,49] However, the biologic behavior and evolution of SCE islands under the regenerated mucosa are not known. Confirmation of our hypothesis that some islands could regress in a high pH environ-

ment would be advantageous for ablative methods, particularly the heat probe.

Another interesting finding in this study is the possible existence of a subgroup of patients in whom both maintenance treatment and follow-up are unnecessary. This raises the question of whether both could be stopped when certain conditions are fulfilled.

Until all of these issues are clarified by future studies with a larger number of patients and longer follow-up, it must be emphasized that this technique is still experimental and not applicable to practice.

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Serologic detection of CagA positive *Helicobacter pylori* strains predicts the presence of peptic ulcer in young dyspeptic patients

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Background: *Helicobacter pylori* infection has been strongly associated with upper gastrointestinal (GI) disease, especially duodenal ulcer. Endoscopy or contrast radiography is needed to diagnose and appropriately manage peptic ulcer disease. These diagnostic procedures, however, are time consuming and expensive; endoscopy is invasive and contrast radiography cannot help in the diagnosis of *H pylori* infection. Our aim was to examine in a prospective study the relation between serologic detection of cytotoxic associated gene (CagA) *H pylori* strains and endoscopic findings among young dyspeptic patients to determine whether this noninvasive test can help differentiate patients with from those without ulcers.

Methods: One hundred patients younger than 45 years with dyspepsia referred for upper GI endoscopy were included in the study. During endoscopy antral biopsy specimens were obtained for the rapid urease test and histologic examination. At histologic examination gastritis was graded from 0 (normal histologic features) to 3 (severe gastritis). After endoscopy blood was obtained for serologic determination of CagA status.

Results: Among the 100 patients 56 were *H pylori* positive and 44 were *H pylori* negative. In the group of 56 *H pylori*–positive patients 36 (64.3%) had peptic ulcers and 20 (35.7%) did not. Among patients with peptic ulcer 34 of 36 (94.4%) were CagA positive and 2 (5.6%) were CagA negative. The respective values for the group of patients without ulcers were 9 of 20 (45%) and 11 of 20 (55%). The difference in the proportion of CagA-positive subjects between the group with and that without peptic ulcer was highly significant ($p < 0.0001$).

Conclusions: Among young patients with dyspepsia, CagA seropositivity is highly associated with duodenal ulcer at endoscopy. (Gastrointest Endosc 1999;50:511-5.)

Beyond doubt *Helicobacter pylori* infection plays a central role in chronic active gastritis and peptic ulcer disease.[1-4] Eradication of this infection dramatically alters the natural history of peptic ulcer disease as evidenced by reduced ulcer recurrences and rates of recurrent bleeding.[5-10] Some years ago *H pylori* strains with particular ulcerogenic potential and bearing the cytotoxic associated gene (cagA) were associated with gastroduodenal pathogenesis.[11-17]

Apart from endoscopy and contrast radiography, no current diagnostic method can help differentiate patients with dyspepsia and an ulcer from those with dyspepsia but no ulcer to provide appropriate treatment. However, the use of a suitable noninvasive serology-based test should help to reduce the number of unnecessary endoscopic procedures and maximize the use of endoscopy-saving schemes. In this study we prospectively examined the hypothesis that among young (less than 45 years) patients with dyspepsia, the serologic detection of CagA-positive *H pylori* strains differentiates patients with from those without ulcers.

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PATIENTS AND METHODS

Subjects and samples

One hundred consecutive patients with dyspepsia younger than 45 years (70 men and 30 women; median age 38 years, range 18 to 43 years) with findings of peptic ulcer or no ulcer at upper endoscopy participated in the study. Dyspepsia was defined as upper abdominal discomfort other than reflux that had lasted longer than 4 weeks. None of the patients had undergone upper GI operations or had used nonsteroidal anti-inflammatory drugs, antibiotics, bismuth, H2 receptor antagonists, or proton pump inhibitors during the previous 4 weeks. None of the patients in the study had undergone upper endoscopy in the past, and all endoscopies were performed by the same experienced endoscopist (T.R.). An ulcer was defined as a circumscribed break in the mucosa larger than 5 mm in diameter with

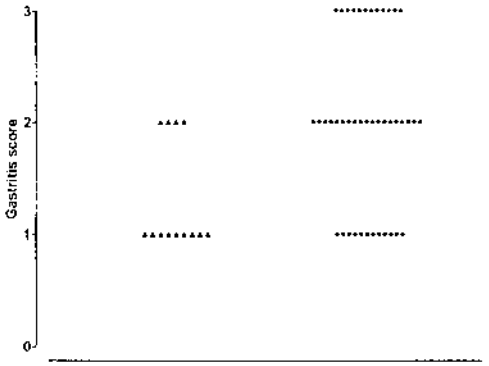

Figure 1. Gastritis score for CagA-negative (n = 13) and CagA-positive (n = 43) groups of *H pylori*–positive patients.

apparent depth. During endoscopy antral biopsy specimens were obtained for *H pylori* detection and histologic examination. Immediately after endoscopy a blood sample was drawn from each patient. After centrifugation at 3000*g* for 10 minutes, sera were collected and stored at –20°C. All determinations were performed in a coded and blinded manner. The study had the approval of the institutional review board of our institution. All patients gave informed consent to participation in the study.

Helicobacter pylori detection

In each subject *H pylori* was sought in two ways: the rapid urease test and histologic examination. For the rapid urease test (CLOtest; Delta Ltd., Perth, W. Australia), a biopsy specimen was immediately inserted into a yellow gel containing urea and a pH indicator and held at room temperature for up to 24 hours. Urease was present if the gel turned pink.[18,19]

Histologic examination

Antral mucosal biopsy specimens were immediately fixed in buffered neutral Formalin and embedded in paraffin sections 5 mm thick. They were stained with H&E and Giemsa stain modified for *H pylori* detection[20] and evaluated by means of microscopy for the presence of *H pylori* organisms. Patients were considered to be *H pylori* positive when the bacterium was identified on both tests. Negative results on both tests were considered to confirm the absence of *H pylori* infection.

The diagnosis and evaluation of gastritis in sections stained with H&E were based on accepted criteria.[21] Severity of gastritis was estimated by means of scoring the inflammatory infiltrate in the lamina propria. This infiltrate was semiquantitively scored for polymorphonuclear leukocytes and mononuclear cells as follows: 0, absence of any inflammatory infiltrate; 1, mild inflammation (polymorphonuclear leukocytes occasionally infiltrating the glandular structures, mainly associated with sparse mononuclear cells detected throughout the lamina propria); 2, the findings in grade 1 in moderate degree; and 3, severe infiltration (polymorphonuclear leukocytes infiltrating the glandular lumen and associated with diffuse and severe mononuclear cell infiltrate). All histologic slides were examined by the same experienced pathologist (A.K.), who was unaware of the patient's condition.

Cytotoxic associated gene serologic testing

H pylori CagA status was determined serologically by means of immunoblotting the sera of patients against *H pylori* antigens.[22] This was performed with a commercial immunoblot (AID; GmbH, Strasberg, Germany). In this test, antigens of *H pylori* are electrophoretically separated by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Antigens migrate through the gel as fine bands according to their molecular weights. After electrophoresis the bands are transferred to nitrocellulose membranes, and the band pattern is analyzed with a kit-specific template. Antibodies against 120, 87, 67, 66, 29, 25, 19, 17, 14 kd *H pylori* antigens can be detected. The 120 kd antigen (cagA gene product) is highly specific for *H pylori*. The 87 kd vacuolating toxin antigen (vacA gene product) often is associated with cagA and also is highly specific for *H pylori*.

Statistical analysis

All statistics were computed with a suitable program (GraphPad Version 2.0; Prism, San Diego, Calif.). Data are expressed as median values with ranges. Because most data showed skewing, comparisons between groups were performed with the nonparametric Mann-Whitney *U* test. Comparisons between proportions were made with the Fisher's exact test. A p value less than 0.05 was considered significant. Positive and negative predictive values for the CagA tests in relation to the presence of ulcer and sensitivity and specificity were calculated as follows[23]: positive predictive value = true positives ÷ (true positives + false positives); negative predictive value = true negatives ÷ (true negatives + false negatives); sensitivity = true positives ÷ (true positives + false negatives); specificity = true negatives ÷ (true negatives + false positives). True positive describes a positive test result and an ulcer present; false positive, test result positive and ulcer absent; false negative, test result negative and ulcer present; true negative, test result negative and ulcer absent.

RESULTS

Among the 100 patients in the study 56 were *H pylori* positive and 44 *H pylori* negative. None of the 44 *H pylori*–negative patients had peptic ulcer or other significant findings at either endoscopy or histologic examination, and none was CagA positive at serologic testing. In the group of 56 *H pylori*–positive patients 43 (76.8%) were CagA positive and 13 (23.2%) CagA negative. Also in this group were 36 (64.3%) patients with and 20 without (35.7%) peptic ulcer. All ulcers observed were *H pylori*–positive, nonbleeding ulcers of the duodenum. There were no statistically significant differences between gender

in terms of *H pylori* and CagA-positive status. *H pylori* positive to *H pylori* negative ratio for the men was 40:30 versus 16:14 for the women. CagA positive to CagA negative ratio was 29:41 for the men versus 14:16 for the women. Among *H pylori*–positive patients, the gastritis median score in the CagA-positive group was 2 (range 1 to 3), significantly higher (Mann-Whitney U 140; $p = 0.0067$) than the respective value in the CagA-negative group (1.5) (Fig. 1).

The results analyzed on the basis of endoscopic findings are shown in Table 1. There were no statistically significant differences with regard to age, gender, or smoking and drinking habits. There was a highly significant statistical difference ($p < 0.0001$) with respect to *H pylori* status between patients with and those without peptic ulcer. In the peptic ulcer group 34 of 36 (94.4%) patients were CagA positive and 2 of 36 (5.6%) were CagA negative. The respective values in the group of patients without ulcers were 9 of 64 (14.1%) and 55 of 64 (85.9%) (Table 2). The difference between the two groups in terms of number of positive CagA results was highly significant ($p < 0.0001$, Fisher's exact test). The prevalence of ulcers among CagA-positive patients was 34 of 43 (79.1%) and among CagA-negative patients was 2 of 57 (3.5%). According to these results, the sensitivity of a positive serologic result for CagA in the detection of peptic ulcer was 94.4%, the specificity 85.9%, positive predictive value 79.1%, and negative predictive value 96.5%.

Table 1. Age, gender, *H pylori* serologic status, smoking and drinking analyzed in relation to endoscopic findings

Factor analyzed	Peptic ulcer (n = 36)	No ulcer (n = 64)	p Value
Age (yr, median and range)	38 (18–43)	38 (20–43)	NS
Gender (M/F)	28/8	42/22	NS
H pylori positive (%)	100 (36/36)	31.2 (20/64)	$p < 0.0001$
Smoking (%)	55.5 (20/36)	65.6 (42/64)	NS
Drinking (%)	13.8 (5/36)	15.6 (10/64)	NS

NS, Not significant.

Table 2. Relation between CagA status and endoscopic findings

Endoscopic finding	CagA positive	CagA negative	Total
Peptic ulcer	34	2	36
No ulcer	9	55	64
Total	43	57	100

$p < 0.0001$, Fisher's exact test.

DISCUSSION

Peptic ulcer disease is a common clinical problem, and conventional treatment with antisecretory drugs represents a socioeconomic burden.[24] Contemporary concepts of management of peptic ulcer disease have radically changed, *H pylori* eradication being the mainstay of therapy. According to consensus reports,[25,26] all patients with peptic ulcers should be treated to eradicate the bacterium. Eradication of this infection dramatically alters the natural history of peptic ulcer disease evidenced in reduced ulcer recurrence and bleeding rates.[5-10] It is apparent that the proper diagnosis of both peptic ulcer and *H pylori* infection is crucial in daily clinical practice.

Endoscopy or contrast radiography currently is needed to diagnose and appropriately manage peptic ulcer disease. However, these diagnostic procedures are time consuming and expensive; endoscopy is invasive and contrast radiography cannot help in the diagnosis of *H pylori* infection. Heath care resources are limited. Because endoscopy units have been facing increased demand, the result has been increased waiting times, which in many places exceed several weeks. Because of the large number of patients with dyspepsia and finite endoscopic resources, some form of screening is mandatory. Several treatment strategies for patients with dyspepsia have been tested over the years. Predictive scoring models in which multiple demographic and clinical criteria are used to screen for risk for dyspepsia caused by the presence of underlying pathologic conditions have not been useful in selecting candidates for endoscopy.[27,28]

The advent of noninvasive *H pylori* testing renewed the hope that a test would be available to screen patients with dyspepsia before endoscopy. The urea breath test is noninvasive and easy to perform, and the results are quantifiable. However, Sharma et al.[29] were unable to establish a correlation between ^{13}C urea breath test results and endoscopic diagnosis among patients with symptoms of peptic ulcer disease.

In our study we examined the hypothesis that detection of CagA-positive *H pylori* infection by means of serologic testing differentiates patients with from those without ulcers. We found that patients with peptic ulcer were infected with cagA-positive *H pylori* strains significantly more frequently than patients without ulcers. The serologic CagA test has good sensitivity, specificity, and positive and negative predictive values. These results apply to duodenal ulcer only. All ulcers found in young patients with dyspepsia in this study were duodenal ulcers; no patient had gastric ulcers. The significant difference in the prevalence of CagA-bearing *H pylori* strains between the patients with

and those without ulcers strongly supports the virulent nature of this particular strain, which induces more profound inflammation than *H pylori* CagA-negative strains.[30,31] We found that *H pylori* CagA-positive patients had a statistically significantly higher gastritis score than the *H pylori* CagA-negative patients. From the results of our study it appears that a noninvasive test to help detect "ulcerogenic *H pylori* strains" can be used to predict the presence or absence of peptic ulcer disease and therefore CagA serology might contribute greatly to averting unnecessary endoscopy in the care of young patients with dyspepsia. The serologic test used in this study offers the advantage of helping to detect antibodies to multiple *H pylori* antigens. Other serologic tests for CagA antibodies, such as enzyme-linked immunosorbent assay, have been used in other studies.[32]

Our findings are in agreement with those of several studies that found a close relation between *H pylori* CagA-positive strains and peptic ulcer disease.[16,17,22,33-35] However, in another study[32] a positive association between the presence of peptic ulcer and the titer of serum CagA immunoglobulin G antibodies could not be shown in a North American population. The reason for this discrepancy is not clear. Geographic differences in the prevalence of circulating *H pylori* strains may be responsible for these results. Genetic analysis of cagA in *H pylori* strains isolated from Taiwanese patients showed that there was a very high percentage of cagA positivity in *H pylori* strains, which means that the CagA-positive phenotype cannot be used as a single marker for ulcer in Taiwan. Sequence analysis indicates that *H pylori* strains in Taiwan contain different genetic sequences than those from other geographic regions.[36] Allelic variation, compared with the gene in the United States, in the cagA gene of *H pylori* was obtained in Korea.[37]

Previous studies[38-40] have shown that serologic testing for *H pylori* and subsequent gastroscopy only in the care of patients with positive *H pylori* serologic results reduces the number of endoscopies. Among our 100 patients with dyspepsia, 44 (44%) endoscopies would have been avoided with use of this serologic test to screen for anti–*H pylori* antibodies because 56 patients were found to be *H pylori* positive. However, an additional 13 endoscopies (for a total of 57 [57%]) would have been avoided with use of a test for CagA antibodies because 43 patients were found to have CagA antibodies in their serum, and only these would have undergone endoscopy. In another study[41] this percentage among young patients with dyspepsia was even higher (73.6%). It can be suggested that if such a policy were adopted, major abnormalities would have been missed. It can be argued, however, that serious diseases such as malignant tumors are rare among young persons and that a very low percentage of ulcers would have been missed. Among 36 peptic ulcer patients we found only 2 (5.6%) patients to have negative serologic results for CagA antibodies who would not have undergone endoscopy.

In summary, among young patients with dyspepsia, *H pylori* CagA seropositivity is strongly associated with findings of duodenal ulcer at endoscopy. This noninvasive test might be of value in differentiating patients with from those without ulcers.

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Relationship of *Helicobacter pylori* CagA(+) status to gastric juice vitamin C levels

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Abstract

Background To date it is not known whether gastric juice vitamin C levels are influenced by *Helicobacter pylori* CagA(+) strains. The aim of the present study, therefore, was to study the impact of *H. pylori* CagA status on gastric juice vitamin C levels.

Materials and methods We studied 30 *H. pylori*(+) patients, and the results were compared with 10 endoscopically and histologically normal *H. pylori*(−) subjects (control group) who were similar to the *H. pylori*(+) group in terms of age and sex. In all patients, gastric juice vitamin C levels were determined and the severity of gastritis was graded on a scale of 0 (absent) to 3 (severe). CagA was determined by immunoblotting the sera from patients against *H. pylori* antigens.

Results Among 30 *H. pylori*(+) patients, 20 were CagA(+) and 10 CagA(−). In the entire group of *H. pylori*(+) patients, the median gastric juice vitamin C levels (mg L^{-1}) were 16·35 (range 3·5–33·6) and were significantly lower ($P<0·001$) than in the control group of *H. pylori*(−) patients [35·5 (23·1–50·2)]. In addition, in the entire group of *H. pylori*(+) patients there was a highly significant ($P<0·0001$) inverse correlation between the gastritis activity score and the gastric juice vitamin C levels. In the group of *H. pylori* CagA(+) patients, the median levels of gastric juice vitamin C were 13·8 (3·5–31·2) and were significantly lower than the corresponding levels in both the *H. pylori* CagA(−) group [24·8 (22–33·6), $P<0·01$] and the *H. pylori*(−) control group [35·5 (23·1–50·2), $P<0·0001$], the last groups being similar. Furthermore, the gastritis activity median score in the *H. pylori* CagA(+) group [2 (1–3)] was significantly higher ($P<0·05$) than in the *H. pylori* CagA(−) group [1 (1–2)].

Conclusion These data indicate that infection with CagA(+) *H. pylori* strains significantly lowers the gastric juice vitamin C levels in comparison with CagA(−) *H. pylori* strains, which might have a significant impact on gastric carcinogenesis.

Keywords Antioxidant factors, CagA serology, gastritis, *Helicobacter pylori* infection.
Eur J Clin Invest 1999; 29 (1): 56–62

Introduction

The relationship between vitamin C and cancer is based on the fact that vitamin C is a powerful antioxidant and is potentially important in the prevention of gastric cancer by scavenging nitrite and preventing the nitrosation of dietary substances to potentially carcinogenic N-nitrosamines [1–4]. In addition, it is capable of eliminating oxygen radicals, which may also damage the gastric epithelium [5,6].

Infection with *Helicobacter pylori* (*H. pylori*) has been associated with gastritis, duodenal ulcer and gastric ulcer [7–13]. Furthermore, *H. pylori* infection has been recognized as a risk factor for gastric cancer [14–19]. However, its precise role in gastric carcinogenesis is as yet unknown. Previous studies [20–22] have shown that *H. pylori* infected

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patients have lower gastric juice vitamin C levels in comparison with *H. pylori*(−) patients, and that *H. pylori* eradication restores these levels, which may prove potentially important in the prevention of gastric cancer.

In recent years, research has been focused on the clinical relevance of differences in virulence factors among *H. pylori* strains. In particular, the cytotoxin-associated gene (cagA)-bearing *H. pylori* strains were associated with increased pathogenicity [23], and in addition it has been shown [24] that *H. pylori* strains that express CagA protein are associated with increased risk for development of gastric cancer. However, it is not known whether gastric juice vitamin C levels are influenced by *H. pylori* CagA status.

In this prospective study, therefore, we examined whether gastric juice vitamin C levels are influenced by *H. pylori* CagA status. CagA status was determined by Western blot analysis to detect serum antibodies against the *H. pylori* CagA protein.

Materials and methods

Subjects and samples

During September 1996, 57 dyspeptic patients underwent upper gastrointestinal (GI) endoscopy. Forty patients fulfilled the inclusion criteria and constituted the study groups: 30 consecutive *H. pylori*(+) patients and 10 consecutive *H. pylori*(−) patients who had no significant findings, either endoscopically or histologically (control group), and matched to the *H. pylori*(+) group for age and sex. The exclusion criteria were previous upper GI surgery, use of antibiotics, bismuth salts or proton pump inhibitors (PPIs) over the previous 2 months or refusal to sign the consent form for study inclusion. Thus, out of 57 dyspeptic patients, 17 [12 *H. pylori*(+) and five *H. pylori*(−)] were excluded. In the group of 30 *H. pylori*(+) patients there were 14 with no ulcer (NU) and 16 with peptic ulcer (PU). All patients had the endoscopy after overnight fasting. After inserting the scope in the stomach and before biopsies were taken, a special gastric juice trap was connected to the suction tube to avoid mixing with blood. Immediately after gastric juice sampling, pH determination was made, and then the gastric juice was analysed for vitamin C. During endoscopy, antral biopsies for the rapid urease test and histology were taken, and immediately after endoscopy a blood sample was drawn from each patient directly into lithium heparin for measurement of plasma vitamin C concentration. For CagA determination after blood centrifugation at $3000 \times g$ for 10 min, sera were collected and stored at −20 °C before use. All determinations were carried out in a coded and blinded manner.

Helicobacter pylori detection

In each subject, *H. pylori* was sought in two ways, i.e. the rapid urease test (CLO-test, Delta, Perth, W. Australia) [25,26] and histology (Giemsa stain modified for *H. pylori*) [27]. Patients were considered to be *H. pylori*(+) when the bacterium was identified in both tests.

Histology

For histology, antral mucosal biopsy specimens were immediately fixed in buffered neutral formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin and Giemsa modified for *H. pylori* detection. In haematoxylin–eosin sections, the diagnosis of gastritis, atrophy and intestinal metaplasia was based on accepted criteria [28]. Gastritis activity was estimated by the inflammatory infiltrate in the lamina propria, and in addition the presence of atrophy and intestinal metaplasia was reported. The inflammatory infiltrate was semiquantitively scored for polymorphonuclear leucocytes and mononuclear cells as follows: 0 = absence of any inflammatory infiltrate; 1 = mild inflammation (polymorphonuclear leucocytes occasionally infiltrate the glandular structures, mainly associated with sparse mononuclear cells detected through the lamina propria); 2 = the above in moderate degree; and 3 = severe inflammation (polymorphonuclear leucocyte infiltrating the glandular lumen and associated with diffuse and severe mononuclear cell infiltrate). All histological slides were examined by the same experienced pathologist unaware of the patient's condition.

CagA status

Helicobacter pylori CagA status was determined serologically by immunoblotting the sera from patients against *H. pylori* antigens [29]. We used the commercial immunoblot manufactured by AID, Strassberg, Germany. The principle of it is as follows: *H. pylori* antigens are electrophoretically separated by SDS-PAGE, and according to their molecular weights migrate through the gel as fine bands. After electrophoresis, the bands are transferred to nitrocellulose membranes. After putting a number of strips into their respective channels of an incubation tray, free unspecific binding sites are blocked with proteins from the blocking buffer during a first incubation. After discarding the blocking buffer, membrane strips are incubated with sera, and if antibodies against *H. pylori* are present in the specimen, they will bind to their respective antigens on the strips during the second incubation. Unbound serum components are rinsed away. Specifically bound IgG/IgA is traced during a third incubation with a highly specific anti-human IgA/IgG antibody labelled with alkaline phosphatase. After a second rinse cycle, the specifically bound enzyme is detected by reaction with substrate (BCIP), then labelled antigens appear as blue bands on the strip, and using a kit-specific template these antigens can be identified (Fig. 1).

Vitamin C and pH measurements

Gastric juice and plasma vitamin C levels were measured

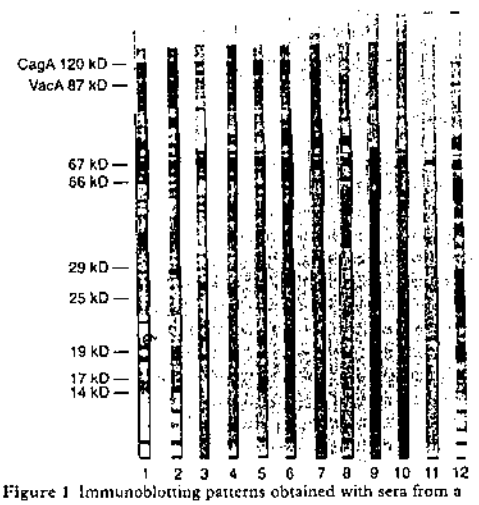

Figure 1 Immunoblotting patterns obtained with sera from a sample of 12 patients studied. The 120-kD band is the CagA gene product. Numbers on the horizontal axis represent patients. Patients 1–6 and 12 were CagA(+) and patients 7–11 were CagA(−). The remaining bands are 87 kD (VacA gene product), 67 kD (flagellin protein), 66 kD and 29 kD (subunits of urease enzyme), 25 kD, 19 kD, 17 kD and 14 kDa (*H. pylori* proteins of which little is known).

spectrophotometrically as previously described in detail [22]. Briefly, a 0·5-mL aliquot of gastric juice or plasma was mixed with 1·5 mL of 5% trichloroacetic acid to precipitate protein, which was then removed by centrifugation at 2500 × *g* for 10 min. Then, 50 mg of activated charcoal was added to the supernatant and agitated for 15 min to oxidize the ascorbic acid to dehydroascorbic acid and to clarify the solution. This was followed by new centrifugation at 2000 × *g* for 10 min, and in the resulting extract 2,4-dinitrophenylhydrazine solution in H_2SO_4 (2 g dL^{-1} in 4·5 mol L^{-1} H_2SO_4) was added to form phenylhydrazone products that absorb at 520 nm. The intra- and interassay coefficients of variation were tested by measuring vitamin C from the same individual on the same day or different days and were 2·8% and 3·5% respectively (accepted values < 5%).

The pH of the gastric aspirate was measured immediately after sampling with an electronic pH meter (DL-25, Mettler Instrument, Switzerland) equipped with a combined glass electrode. The electrode was calibrated at pH 4 and pH 7 before each measurement with appropriate buffers (Titrisol, Merck, Germany). All vitamin C and pH measurements were performed 'blind' by the same experienced biochemist.

Statistical analysis

All statistics were computed using a suitable program (GraphPad, PRISM, Version 2·0). The results are represented graphically as boxes and whiskers, whereas data in the text are expressed as median values with ranges. As most data showed skewing, comparisons between the multiple groups were performed using non-parametric Kruskal–Wallis analysis of variance. If the result of this was significant, then simple comparisons between pairs of groups were performed with the non-parametric Mann–Whitney *U*-test [30]. Comparisons between proportions were made using Fisher's exact test. A *P*-value < 0·05 was considered significant.

Results

There were no statistically significant differences between the entire group of *H. pylori*(+) patients, the control group of *H. pylori*(−) subjects, the subgroup of *H. pylori* CagA(+) and the subgroup of *H. pylori* CagA(−) patients, as far as demographic characteristics (age, sex, etc.) were concerned (Table 1).

Among 30 *H. pylori*(+) patients, 20 (66·7%) were CagA(+) and 10 (33·3%) CagA(−).

In the entire group of 30 *H. pylori*(+) patients, the median levels of gastric juice vitamin C (mg L^{-1}) were 16·35 (range 3·5–33·6) and were significantly lower

Table 1 Characteristics of subjects studied.

	H. pylori(−)	*H. pylori*(+)	CagA (−)	CagA (+)	Statistical significance
Patients (*n*)	10	30	10	20	NS
Sex (M/F)	6/4	17/13	6/4	11/9	NS
Age (years) [median (ranges)]	42·5 (20–51)	42 (18–53)	40·5 (28–53)	42[18–50]	NS
Smokers	6/10 (60%)	19/30 (63·3%)	6/10 (60%)	13/20 (65%)	NS
Daily alcohol consumers	1/10 (10%)	4/30 (13·3%)	1/10(10%)	3/20(15%)	NS
NSAID users	0/10 (0%)	1/30 (3·3%)	1/10 (10%)	0/20 (0%)	NS

NSAID, non-steroidal anti-inflammatory drug.

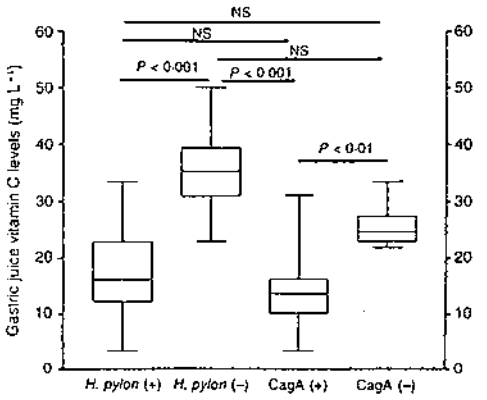

Figure 2 Gastric juice vitamin C levels in the entire group of *H. pylori*(+), *H. pylori*(−), *H. pylori* CagA(+) and *H. pylori* CagA(−) patients. Results are expressed as boxes and whiskers. Central vertical lines indicate median values, boxes represent interquartiles. Whiskers represent upper and lower range.

($P < 0{\cdot}001$) than the control group [35·5 (23·1–50·2)]. The corresponding numbers for the *H. pylori* CagA(+) patients ($n = 20$) were 13·8 (3·5–31·2): significantly lower than the corresponding levels in both the *H. pylori* CagA(−) group ($n = 10$) [24·8 (22–33·6), $P < 0{\cdot}01$] and the *H. pylori*(−) control group ($n = 10$) [35·5 (23·1–50·2), $P < 0{\cdot}001$] ($n = 10$) (Fig. 2). There were no significant differences between the entire group of *H. pylori*(+) patients and the subgroup of CagA(+) patients or between the group of *H. pylori*(−) and the subgroup of CagA(−) patients.

On histology of gastric antral biopsies, all *H. pylori*(+) patients had findings of chronic active gastritis, whereas none of the *H. pylori*(−) control subjects showed pathological findings. In the entire group of *H. pylori*(+) patients, there was a significant ($P < 0{\cdot}0001$) inverse correlation between the gastritis activity score and the gastric juice vitamin C levels. The gastritis activity median score in the *H. pylori* CagA(+) group was 2 (1–3) and was significantly higher ($P < 0{\cdot}05$) than in the *H. pylori* CagA(−) group [1 (1–2)]. Among the *H. pylori* CagA(+) patients, there were 8/20 (40%) with atrophy as opposed to 0/10 (0%) in the *H. pylori* CagA(−) group ($P < 0{\cdot}05$). When we split the CagA(+) group into two subgroups, i.e. atrophy(+) ($n = 8$) and atrophy(−) ($n = 12$) patients, we found that the respective gastric juice vitamin C levels were 9·8 (3·5–16·7) and 14·7 (9·4–31·2), and the difference was significant ($P < 0{\cdot}01$). Neither *H. pylori* CagA(+) nor *H. pylori* CagA(−) patients showed findings of intestinal metaplasia.

Plasma vitamin C median levels were similar in all groups studied, i.e. in the entire group of *H. pylori*(+) patients [18·7(3·7–35·6)], *H. pylori*(−) patients [18·15 (14·1–30·3)], *H. pylori* CagA(+) patients [18·8 (3·7–22·8)] and *H. pylori* CagA(−) patients [19·45 (16·3–35·6)].

Similarly, the gastric juice median pH did not differ in the four groups studied, i.e. in the entire group of *H. pylori*(+) patients [2·1 (1·2–3·5)], in the *H. pylori*(−) patients [2·05 (1·4–3)], in *H. pylori* CagA(+) [2 (1·4–3·5)] and *H. pylori* CagA(−) group [1·95 (1·2–2·2)].

Discussion

Recently, evidence has accumulated to support a role for *H. pylori* in the aetiology of gastric cancer [14–19]. There is some evidence that the bacteria may itself, or via the stimulation of tissue macrophages [31], produce reactive oxygen, which could function as a DNA oxidant in the gastric epithelia. Nevertheless, the suggested association between gastric cancer and *H. pylori* infection is mainly based on the fact that *H. pylori* is the major causative agent of chronic gastritis, a condition that over the years develops into atrophic gastritis and intestinal metaplasia, a precursor lesion of intestinal-type gastric cancer [32–34]. In this development, dietary factors must be taken into account, and indeed others [35,36] believe in a multifactorial causality for this type of cancer when dietary factors play an important role, both hostile (nitrates, salt, carbohydrates) or protective (dietary antioxidants). For the above reasons, it has been suggested that eradication of *H. pylori* gains particular importance by reversing inflammation and restoring gastric antioxidant defences [20–22]. However, these studies did not examine the relationship between *H. pylori* Cag A status and gastric juice vitamin C levels, to see whether *H. pylori* Cag A seropositivity influences vitamin C gastric juice levels.

In the present study, we confirmed that in the entire group of *H. pylori*(+) patients the gastric juice vitamin C levels were significantly lower than the *H. pylori*(−) group, and that there was a striking inverse correlation between gastric juice vitamin C levels and the chronic gastritis activity score. Furthermore, when we split the entire group of *H. pylori*(+) patients, the gastric juice vitamin C levels were significantly lower than the *H. pylori*(−) group and that there was a striking inverse correlation between gastric juice vitamin C levels and the chronic gastritis activity score. Furthermore, when we split the entire group of *H. pylori*(+) patients into Cag A(+) and Cag A(−), we found that the significant difference between the entire group of *H. pylori*(+) patients and the *H. pylori*(−) control group was almost exclusively due to the *H. pylori* CagA(+) subgroup because only this group had significantly lower gastric juice vitamin C levels than the control group as opposed to the CagA(−) group, which did not differ significantly from the control group of *H. pylori*(−) patients. These data therefore clearly indicate that CagA(+) *H. pylori* strains negatively influence the gastric juice vitamin C levels in comparison with the CagA(−) strains. The mechanism by which CagA(+) *H. pylori* strains exert this function is unknown. However, according to our findings there are at least two mechanisms that could be relevant. The first possible mechanism could involve more intense inflammation, as it is believed that CagA(+) strains are more virulent and induce more profound

inflammation than the CagA(−) strains [37–39], perhaps through an enhanced production of proinflammatory cytokine interleukin 8 (IL-8), interleukin 1α (IL-1α) and interleukin 1β (IL-1β) [38,39]. Our results are consistent with this notion, as we found that CagA(+) patients had a statistically significantly higher gastritis activity score than the CagA(−) strains. The second possible mechanism might involve the presence of atrophy, as on one hand we found that *H. pylori* CagA seropositivity was significantly associated with gastric atrophy histologically, which is in concordance with the findings of previous studies [40,41], and on the other hand among CagA(+) patients, those with atrophy had significantly lower levels than those without atrophy. Indeed, this could be the case even though Sobala *et al.* [42] found that in patients with antral *H. pylori* chronic gastritis atrophy did not negatively influence gastric juice vitamin C levels. It seems possible, therefore, from all the above, that the combined negative influence of at least these two mechanisms on gastric juice vitamin C levels could be responsible for our findings.

In this study we used Western blotting to determine CagA status, and we did not determine cagA genotype of the *H. pylori* isolates. However, Cover *et al.* [43], studying the relationship between CagA seropositivity and cagA genotype of *H. pylori* isolates, described anti-CagA antibodies in 26·7% of their patients from whom a strain lacking cagA was isolated and, in contrast, no antibodies in 7·6% of patients with cagA(+) isolates. Based on this, therefore, it could be suggested that serology alone may not be the most appropriate test in determining differences in virulence among *H. pylori* strains. However, *H. pylori* CagA status, as determined by immunoblotting, has been used in recent relevant papers in the literature [40,44]. In addition, this test has been found to have greater sensitivity than other relevant serological tests such as enzyme-linked immunosorbent assay [45].

To date, the main reason for determining *H. pylori* strains using potential virulence factors has been to explore pathogenetic mechanisms. However, once the relationship between *H. pylori* virulence factors and gastric cancer has been better clarified, testing for such factors could form a part of the management strategy in this situation. In this direction, Ponzetto *et al.* [46] from Italy claimed that it would be more cost-effective to test for anti-CagA and cure those testing positive than to test for any *H. pylori* infection. They calculated that treatment of CagA(+) *H. pylori* infection prevents 30% of the attributable gastric cancer, and that they would need to treat only about 25 such individuals for each case of cancer prevented. For all the above reasons, the eradication of *H. pylori* CagA(+) strains takes on a significant dimension, especially in areas with high incidence of gastric cancer. This notion is supported by the fact that, in a high-risk population for gastric cancer in Venezuela, oral vitamin C supplementation alone is insufficient to increase gastric juice ascorbic acid concentration in the presence of *H. pylori* infection [47].

Previous studies [42,48] have shown that gastric juice vitamin C concentrations are influenced by gastric pH, with significantly lower values in patients with high gastric juice pH (pH > 4) than in those with low pH (pH < 4). Therefore, it can be suggested that pH differences might be responsible for the observed results in *H. pylori*-positive and -negative patients. However, we have shown that gastric juice pH was similar, with median values < 4 in infected and non-infected patients, and, furthermore, that the pH results in *H. pylori* CagA(+) and CagA(−) groups were also similar. The possibility that gastric juice pH might be responsible for the results therefore seems out of the question.

The methodology used in this and other studies [22] to assess vitamin C levels measured total vitamin C levels and did not distinguish between total vitamin C and ascorbic acid levels, the latter being biologically active as far as nitrosamine, nitrite and oxygen radical scavenging is concerned. It could be suggested, therefore, that the method used is not precise enough. However, studies in man [49] have shown that about 80–90% of the measured material is in the form of ascorbic acid. In addition, it has been shown [48] that there is a highly significant linear relationship between gastric juice vitamin C and ascorbic acid levels.

We did not estimate daily intake of vitamin C, and therefore one might suggest that dietary vitamin C variations were responsible for the observed differences in gastric juice vitamin C levels between *H. pylori*(+) and (−) patients and also between subjects infected with CagA(+) and CagA(−) *H. pylori* strains. However this possibility seems unlikely because plasma vitamin C levels in patients infected with different *H. pylori* strains were similar, as were levels between *H. pylori*(+) patients and control subjects, and it has been found by others [42] that there is a close relationship between plasma vitamin C concentrations and vitamin C daily intake assessed by dietary recall questionnaires. One might also suggest that the gastric juice vitamin C levels found were due to residual vitamin C from the last meal. However, this also seems unlikely because all subjects had been fasting overnight when the gastric juice was collected.

In conclusion, these data indicate that in comparison with CagA(−), CagA(+) *H. pylori* strains negatively influence the gastric juice vitamin C levels and this might be of significant importance in gastric carcinogenesis.

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Upper gastrointestinal disease, *Helicobacter pylori* and recurrent abdominal pain

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Over a 5-y period, 396 children complaining of recurrent abdominal pain (RAP) underwent upper gastrointestinal endoscopy in order to identify any underlying organic pathology and determine the prevalence of *Helicobacter pylori* (*H. pylori*) infection. Histologically confirmed mucosal inflammation was found in 338 out of 396 children (85.4%); in 113 of 396 patients (28.5%), *H. pylori* was identified on the gastric mucosa. Significant discriminating factors between *H. pylori* positive and negative children with RAP included age (mean age for positive 11 y vs. 8.1 y for negative, $p < 0.01$) and gender (male gender predominance in the *H. pylori* positive, $p < 0.001$). No significant difference was found between *H. pylori* positive and negative groups regarding incidence and character of the presenting symptoms. All *H. pylori* positive children (100%) had abnormal histology compared with 225 out of 283 negative ones (79.5%). Histologically confirmed gastritis was the most prominent finding in *H. pylori* positive children compared with *H. pylori* negative (98.2% vs. 19%, $p < 0.001$). Conversely, oesophagitis was more common in *H. pylori* negative children (47.7% vs. 27.4%, $p < 0.001$). The incidence of peptic ulcer was higher in *H. pylori* infected patients than in the *H. pylori* negative group (5.3% vs. 1%, $p < 0.05$). Our data suggest that gastrointestinal pathology is more common than previously thought in children with RAP, while *H. pylori* infection is a relatively important factor in the etiology of upper gastrointestinal inflammation in RAP syndrome. □ *Helicobacter pylori, recurrent abdominal pain, upper gastrointestinal endoscopy*

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Recurrent abdominal pain (RAP) is one of the most common complaints in childhood, but still frustrates parents and paediatricians. The prevalence of RAP varies from 9% to 15% in different studies, however its aetiology has remained for the most part obscure (1–3). Only 10% of children with RAP have been considered as having an organic background (4). This knowledge has served paediatricians well for many years, however development in the field of paediatric gastroenterology and endoscopy techniques has provided a new perspective and diagnostic approach in children with RAP.

Helicobacter pylori (*H. pylori*) has been shown to be a major cause of upper gastrointestinal symptoms in adults and children (5–7). However, the definitive role of *H. pylori* in the pathogenesis of RAP remains controversial, since the results of different studies are either conflicting or inconsistent (8, 9).

The aim of this study was firstly to identify any underlying histological abnormality of the upper gastrointestinal tract in children with RAP and, secondly, to determine the prevalence of *H. pylori* infection in this group of children.

Patients and methods

Over a period of 5 y between Jan. 1991 and Jan. 1996 a total of 402 children aged from 3 to 15 y (mean 10.5 y) underwent upper gastrointestinal endoscopy for investigation of RAP. These were children who were seen in the Outpatient Department of the 1st Paediatric University Clinic in Aghia Sophia Children's Hospital in Athens (250 children) or were admitted in the paediatric ward (152 children). We included children with RAP when unexplained abdominal pain severe enough to interfere with daily activities had occurred at least 3 times in the preceding 3 mo. The children enrolled in the study had previously undergone a series of investigations that did not reveal any organic cause of abdominal pain and had been referred for upper gastrointestinal endoscopy. Laboratory investigation included a full blood count, erythrocyte sedimentation rate, aspartate aminotransferase and gamma glutamyl transferase, urea and creatinine, serum amylase, microscopy and culture of urine and faeces and sickling test. Patients who had been on antibiotics, H_2 antagonists or

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Table 1 Characteristics and symptoms of 396 children with RAP with respect to *H. pylori* infection.

Characteristic	*H. pylori* positive	*H. pylori* negative	*p*
Number (%)	113 (28.5)	283 (71.5)	
Mean age (SD) (y)	11 (2.05)	8.1 (4.3)	0.003
Sex (M/F)	75/38	123/160	0.001
Symptoms[a]			
periumbilical pain	23 (20.4)	64 (22.7)	0.620
epigastric pain	90 (79.6)	219 (77.3)	0.620
vomiting	21 (18.5)	45 (15.9)	0.520
nausea	4 (3.5)	14 (4.9)	0.540
belching	2 (1.8)	5 (1.8)	0.670
Mean duration (SD) (mo)	21.5 (7.5)	17.9 (6.2)	0.090

[a] Results given in number (%).

omeprazole for <6 wk before the investigation were not included in the study. Upper gastrointestinal endoscopy was performed by 2 paediatric gastroenterologists using an Olympus GIF-XP20 endoscope. Children were examined under general anaesthesia or sedation with intravenous diazepam or midazolam.

Biopsies were taken for routine histology and identification of *H. pylori*. These included 1 duodenal, 2 antral and 2 oesophageal biopsies. Both the endoscope and the biopsy forceps were cleaned and disinfected with glutaraldehyde after each use. Informed consent was obtained from the patients' parents in all cases.

Histology

The biopsy samples were fixed in 10% neutral formalin, embedded in paraffin and sections were stained with haematoxylin-eosin and modified Giemsa or Masson trichrome and assessed under light microscopy. All histological analysis was performed by a single histopathologist, who assessed the presence and the degree of inflammation and the presence or absence of *H. pylori* in a blind manner. Classification of gastritis was according to that of the Sydney system (10). The grading of oesophagitis, gastritis or duodenitis was classified as mild, moderate or severe, according to the severity of inflammation using the Carrick codification (11). Only moderate or severe inflammations were validated for the purposes of the study.

Statistics

Bivariate analysis was done either by the χ^2 or the two sample *t*-test for nominal or continuous variables, respectively. Values of $p < 0.05$ were accepted as significant.

Results

From 402 children studied, 396 had suitable biopsy specimens and were finally included in the study. Their age varied from 3 to 15 y (mean: 10.5 y) and the mean duration of their symptoms was 19.6 mo (range 3–98 mo). The abdominal pain was accompanied with vomiting in 66 children (14%), nausea in 18 (4.5%) and belching in 7 (1.8%).

Upper gastrointestinal endoscopy showed histological changes in 338 of 396 children studied (85.4%). In 58 children (14.6%) no organic pathology was revealed.

H. pylori was detected on the antral mucosa of 113 out of 396 patients (28.5%) by histological analysis. The characteristics and the clinical findings in *H. pylori* positive and negative children are shown in Table 1. *H. pylori* positive children were significantly older than *H. pylori* negative ($p < 0.01$). Male gender was significantly more common in *H. pylori* positive than in *H. pylori* negative patients ($p < 0.001$). There was no significant difference between the 2 groups regarding the incidence or character of the presenting symptoms.

All *H. pylori* infected children (100%) had abnormal histological findings compared with 225 of 283 *H. pylori* negative children (79.5%). The histological findings for both patient populations are presented in Table 2. Almost all *H. pylori* positive children (98.2%) had gastritis with or without concomminant oesophagitis or duodenitis, compared with 19% of *H. pylori* negative patients ($\chi^2 = 208$, $p < 0.001$). Conversely, histologically proved oesophagitis with or without gastritis or duodenitis was more common in the *H. pylori* negative children (47.7% vs. 27.4%, $p < 0.001$).

Although duodenitis as single diagnostic lesion was significantly higher in the *H. pylori* negative group (22.3% vs 0.9%, $p < 0.001$), the incidence of duodenitis with or without gastritis or oesophagitis was not found to differ between the 2 populations (44.2% vs. 53%, $p = 0.11$).

Peptic ulcer was found in 9 of 396 children studied (2.2%). Six (2 gastric, 4 duodenal) in the *H. pylori* positive group (5.3%) and 3 (1 gastric, 2 duodenal) in the *H. pylori* negative group (1%), the difference being statistically significant ($p = 0.028$)

Discussion

The results of this study suggest that abnormal pathology is more common than previously considered

Table 2. Histological findings in 396 children with RAP with respect to *H. pylori* infection.

Histological finding	*H. pylori* positive n (%)	*H. pylori* negative n (%)
Normal	0	58 (20.5)
Gastritis	56 (49.6)	14 (4.9)
Gastritis + duodenitis	25 (22.1)	13 (4.6)
Gastritis + oesophagitis	6 (5.3)	7 (2.5)
Oesophagitis	1 (0.9)	54 (19.1)
Oesophagitis + duodenitis	0	54 (19.1)
Duodenitis	1 (0.9)	63 (22.3)
G + O + D	24 (21.2)	20 (7.0)

in children with RAP (2, 4). We followed the recruitment criteria mentioned in our study design with the intention of investigating whether there was any underlying pathology in children considered to have "non-organic" RAP according to Apley (2). We studied children with RAP who had a normal physical and laboratory investigation and were referred for upper GI endoscopy. Indeed, 85.4% of the children studied were found to have an underlying cause for their symptoms. This finding is in agreement with more recent studies based on relatively smaller number of patients, which showed prevalence of organic disease in 58.5–93% of children with RAP investigated with oesophago-gastro-duodenoscopy (12, 13).

On the basis of our study design we believe that we studied a relatively unselected group of children with RAP, since they were not recruited from the gastroenterology out-patient clinic but from the general out-patient department of our hospital, which offers mainly primary healthcare. It could be pointed out that the long-lasting symptoms in our population might reflect either an underlying organic pathology or selection of severe cases. But as others described about 25% of patients with RAP may have continuing and persisting symptoms for several years (14).

Great emphasis has been given to the apparent role of psychological factors in the aetiology of RAP (2). Nevertheless, it is difficult to distinguish the effect of stress on the gastrointestinal system or the impact of an organic disease on child's behaviour. There is little doubt that emotional factors are of great importance, but in evaluating the child it is of course necessary to consider that psychological disturbances and organic pathology may coexist. (15)

H. pylori infection is widely distributed geographically. Its prevalence is related to demographic factors, socioeconomic status and ethnic background (8, 16). Several studies have tried to investigate the association of *H. pylori* infection and RAP in children (17–23). Their findings were inconsistent since the reported prevalence of *H. pylori* infection ranged from 9 to 81% in patients with RAP. This is due either to different study design or variation of *H. pylori* infection between different ethnic background. Furthermore, the majority of them were based on the detection of serum antibodies and not on histological findings, which are considered a more reliable indicator of active *H. pylori* infection (24).

In Greece, the prevalence of *H. pylori* in the general paediatric population based on serum antibodies ranges between 15% and 39.9% according to 2 different studies, while in adults, 67.1% of recruits (20–27 y) and 70% of blood donors (20–50 y) had positive antibodies (25, 26).

In the present study, 28.5% of children with RAP were diagnosed as having histologically proved *H. pylori* infection. Blecker et al., in a study of 143 children with RAP found that 25.2% were infected with *H. pylori*, diagnosed either by histology or culture (27). Our results are also in agreement with those of a Finnish study, which showed that 22% of children with RAP had *H. pylori* gastritis (13). Lower rates (7–10%) were found in 2 other studies based on smaller study groups (12, 28). Conversely, Heldenberg et al. found a higher prevalence of *H. pylori* infection (54%) among Israeli children with RAP investigated with upper gastrointestinal endoscopy (29).

The mean age of *H. pylori* positive patients was significantly higher than that of *H. pylori* negative ones, a finding also previously reported by other studies (16, 25).

It is generally accepted that *H. pylori* infection is not gender related (16, 30). In this study we observed that there was a significantly higher proportion of boys in the *H. pylori* positive group than in the *H. pylori* negative, this being in agreement with the study of Chong et al. (18).

It seems that there are no clinical symptoms that could differentiate the *H. pylori* positive from the *H. pylori* negative children with RAP (27). It is well known that *H. pylori* demonstrates a unique affinity for gastric mucosa; therefore gastric inflammation was the prominent finding in the *H. pylori* positive children. On the other hand, our study revealed that oesophagitis was the principal histological result in the *H. pylori* negative children.

There is evidence that *H. pylori* infection is strongly associated with duodenal ulcer disease in children (8). An association of *H. pylori* and antral gastritis with gastric ulcer disease has not been clearly demonstrated, as primary gastric ulceration is rare in childhood (24). We found that peptic ulcer was more common in *H.*

pylori infected children, but the small number of children with ulcer in the study do not allow for further comparisons or definite conclusions. Nevertheless, it is clear that peptic ulcer is not a common underlying cause of RAP in children as it is in adults (31).

In conclusion, the results of this study indicate a high incidence of upper gastrointestinal mucosal inflammation in children with RAP and provide further evidence that careful examination may reveal previously unsuspected abnormalities of the gastrointestinal tract. It is also suggested that *H. pylori* could be regarded as a possible somatic cause of RAP syndrome, although it is well known that it can be detected in asymptomatic children. Finally, there is increasing body of evidence in the literature, that long-standing RAP syndrome might be considered an indication for endoscopic investigation, even though it is an invasive procedure.

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Diagnosis of *Helicobacter pylori* infection by HpSA test

Sir D Vaira and colleagues[1] report on a *Helicobacter pylori* diagnostic test with a new stool antigen, the HpSA enzyme immunoassay (Premier Platinum HpSA, Meridian Diagnostics, Cincinnati, OH, USA). They conclude that this stool assay is a reliable and simple diagnostic tool. Patients were considered *H pylori* positive if histology and urease test were positive or if culture was positive. In clinical practice, however, two invasive tests are usually used to diagnose *H pylori* infection (a rapid urease test and histology with Giemsa staining), and patients are considered *H pylori* positive if either test is positive; both need to be negative for the patient to be classified as *H pylori* negative. Using these definitions, we have evaluated the accuracy of the HpSA assay.

49 patients (28 men, 21 women aged 23 68) with dyspepsia with or without peptic ulcer were studied. 43 were examined for the first time whereas six had had an *H pylori* eradication treatment previously. Exclusion criteria were previous treatment for *H pylori* or treatment with any antibiotic in the previous 6 weeks, active upper gastrointestinal bleeding, previous gastric surgery, current treatment with corticosteroids or non-steroidal anti-inflammatory drugs, and treatment with a proton pump inhibitor or bismuth compound during the previous 3 months. At endoscopy two biopsy samples were taken from the antrum and two from the corpus for histology and rapid urease test. Stool samples were collected during the first 3–4 days after endoscopy and before any treatment and were tested by HpSA (450 nm spectrophotometry cut-off 0·140), all results being interpreted blind.

35 of the 43 patients examined for the first time were *H pylori* positive as defined, and 31 of 35 tested positive by the stool assay. All eight *H pylori* negative patients tested negative by the stool assay. The sensitivity, specificity, and positive and negative predictive values of the stool assay in these patients were 89%, 100%, 100%, and 67%, respectively. Of the six patients who had received eradication treatment, three were *H pylori* positive and three *H pylori* negative by definition; no positive tested positive on the stool assay but all the negatives were negative. No equivocal HpSA results were observed.

HpSA is a reliable, non-invasive, simple test with an impressive specificity and positive predictive value in patients who have not received any *H pylori* eradication treatment. Patients testing *H pylori* positive on this stool assay really are *H pylori* positive. The number of patients tested after eradication treatment is too small to permit any conclusion, and the results obtained by others in such patients are controversial.[2–4] Also remaining to be studied is whether HpSA can be used to assess *H pylori* status in bleeding patients or patients after gastric surgery in whom the reliability of rapid urease test seems questionable.[4,5]

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Letter to the Editors

Nitroimidazoles for *H. pylori* eradication

SIRS, We read with interest the article of Goddard *et al.*[1] concerning the comparison of nitroimidazoles for the eradication of *Helicobacter pylori* and the relief of ulcer-associated and nonulcer dyspepsia. The authors compared two 1-week courses of twice daily omeprazole 20 mg, clarithromycin 250 mg and either metronidazole 400 mg (OCM) or tinidazole 500 mg (OCT) in a double-blind fashion and found that OCM was as effective as OCT at eradicating *H. pylori*. The authors concluded that 'metronidazole can be used in place of tinidazole in combination with omeprazole and clarithromycin'.

In 1997 we published a prospective, open, two-centre study[2] that is not mentioned by Goddard *et al.* comparing metronidazole with another nitroimidazole, namely ornidazole, at eradicating *H. pylori* in patients with duodenal ulcer. In brief, we compared three 1-week courses of: twice daily omeprazole 20 mg, clarithromycin 250 mg and either metronidazole 500 mg (OCM) or ornidazole 500 mg (OCOr) and once daily omeprazole 20 mg plus twice daily clarithromycin 250 mg and ornidazole 500 mg (O1COr). Two hundred and three *H. pylori* positive patients (modified Giemsa stain and CLO test, Delta West, Australia) older than 18 years, with active duodenal ulcer were included in the study, randomly assigned as follows: 50 patients on O1COr, 47 patients on OCOr and 106 patients on OCM. *H. pylori* eradication was assessed endoscopically, 8–9 weeks after the start of treatment.

Eleven patients were lost to follow up; thus 192 patients were analysed 'per protocol' (48 patients on O1COr, 44 patients on OCOr, and 100 patients on OCM). Results of 'per protocol' and of 'intention-to-treat' analysis are shown in Table 1.

No differences were observed in the *H. pylori* eradication.

In conclusion, our data show that ornidazole is also as effective as metronidazole in the used regimens. Therefore, all the nitroimidazoles seem to be equally effective in triple regimens containing omeprazole and clarithromycin at eradicating *H. pylori*.

Table 1. *Helicobacter pylori* eradication: 'per protocol' and 'intention-to-treat' analysis

H. pylori eradication	O1COr	OCOr	OCM
'Per protocol'*	44/48 (91.7%)	40/44 (90.9%)	93/100 (93.0%)
95% CI	80.0–97.7	78.3–97.5	86.1–97.1
'Intention-to-treat'†	44/50 (88.0%)	40/47 (85.1%)	93/106 (87.7%)
95% CI	75.7–95.5	71.7–93.8	81.5–94.0

* $P = 0.901$
† $P = 0.887$

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C3 Phenotypes and Peptic Ulcer: Association of C3 with Peptic Ulcer but No Association of C3 with Helicobacter pylori

TO THE EDITOR: We read with interest the article by Hein et al. (1) on the genetic markers for peptic ulcer published in *Scandinavian Journal of Gastroenterology* in 1997. The authors found no association between the three major C3 complement phenotypes and peptic ulcer history and stated that this interesting negative association had not previously been reported.

In 1992 we published a study (2) of C3 complement phenotypes (C3S, C3F, and C3FS) in patients with peptic ulcer which is not mentioned by Hein et al., and our results are quite different. Indeed, we studied 232 consecutive Greek patients with endoscopically proven active peptic ulcer (173 patients aged 20–80 years with duodenal ulcer (DU) and 65 patients aged 23–75 years with gastric ulcer (GU)). As controls served 1052 healthy Greeks. C3 phenotyping was performed by electrophoretic immunofixation on suitable cellulose acetate strips using specific antiserum. Phenotypes did not differ between patients with ulcer ($P = 0.933$), but their distribution differed significantly from that of healthy controls (DU, $P = 10^{-8}$; GU, $P = 10^{-8}$). More specific, there was an overpresentation of both C3FS and C3F phenotype in both patients with DU (55% and 14%, respectively) and patients with GU (55.4% and 15%) compared with healthy people (32% and 5%). It is interesting that the C3*F gene was almost twice as common in patients as in healthy controls. The relative risk of C3F and C3FS phenotype was 2.87 and 2.63 for DU and 3.24 and 2.68 for GU, respectively.

As is well known, C3 complement is of major importance in the inflammatory process. In particular, C3F phenotype had an enhanced capacity to bind with the respective receptor on mononuclear cells in vitro compared with C3S phenotype (3). In view of the crucial role of *Helicobacter pylori* in the pathogenesis of peptic ulcer, an association of C3 phenotypes with peptic ulcer would be reasonable. Thus we studied further another 100 consecutive Greek patients aged 19–84 years with endoscopically diagnosed DU in comparison with 100 completely matched healthy people. Among other factors, we determined the *H. pylori* status by serology, using a validated enzyme-linked immunosorbent assay (anti-*H. pylori* IgG antibodies) and the C3 phenotypes (4), as previously reported.

The significant difference in the distribution of C3 phenotypes between patients and controls was confirmed ($P = 0.007$), as was the overpresentation of C3F + C3FS phenotypes in DU patients (78% versus 60%, $P = 0.005$, RR = 2.61). As was expected, there was a significant predominance of *H. pylori*-positive people among DU patients (88% versus 65%, $P = 0.0006$, RR = 4.31), but the prevalence of *H. pylori*-positive people did not differ among patients ($P = 1.0$) and controls ($P = 0.68$) with different C3 phenotypes. Thus, although there is an association of C3 phenotypes with DU, there is no association between *H. pylori* status and C3 phenotypes.

In conclusion, it seems that there is an association of C3F and C3FS phenotypes with peptic ulcer in Greek people. However, no association of C3 with *H. pylori* status was documented. Therefore, *H. pylori* and C3 seem to act independently in the pathogenesis of peptic ulcer. Provided that genetic polymorphism varies in different populations, further studies in other populations are needed to confirm our results.

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IN REPLY: Inspired by the quite strong associations found by Archimandritis et al. (1) between the C3 complement phenotypes and peptic ulcer (duodenal or gastric), we looked at our own data again. In our cohort of more than 3000 men with a mean age of 63 years and an age range of ±10 years there was no significant association between C3 complement phenotypes and lifetime prevalence of peptic ulcer or operation due to peptic ulcer. Lifetime prevalences of peptic ulcer were, for men with phenotypes F, FS, and S, 11.9%, 12.9%, and 10.8%, respectively ($P > 0.10$); the corresponding prevalences with regard to operation history were 0.7%, 4.1%, and 3.6% ($P > 0.10$).

Why were the two studies inconsistent? One reason might be a much wider age range in the study by Archimandritis et al., which included many young people. To clarify whether such an age relation exists in the Copenhagen Male Study, we reanalysed our data, examining the association between C3 complement and the peptic ulcer history reported when the men in The Copenhagen Male Study were on average 48 years old (range, 40 to 59 years (data from the first base line established in the study in 1970/71). There was a tendency for men with either the F or FS phenotype to have a higher lifetime prevalence at that time: the prevalence of peptic ulcer was 9.9% as compared with 7.8% among subjects with the S phenotype ($P = 0.058$).

Thus, the results of the study by Archimandritis et al. and the results of The Copenhagen Male Study suggest that manifestation of the disease in younger age groups is necessary for the C3 complement to be strongly associated with peptic ulcer. As reviewed by Lam, the importance of genetic factors for early or late onset of duodenal ulcer has been shown repeatedly (2).

In the light of our findings recently published in this journal (3), we considered the interaction between the complement phenotypes and the two main lifestyle factors associated with peptic ulcer risk: sugar intake and smoking. No interaction between smoking and the complement phenotypes was found; however, the strength of the association of C3 complement with peptic ulcer risk seemed to depend on sugar intake in hot beverages. Thus, only among sugar users (one-third of the population) was the association significant between C3 complement phenotypes and lifetime prevalence of peptic ulcer at age 48 years. C3 F/FS had a lifetime prevalence of 15.4%, C3 S of 10.0% ($P = 0.009$). Corresponding figures among those who did not use sugar were 6.8% and 6.6%, respectively ($P = 0.96$).

In conclusion, the discrepancy between the results of the two studies may at least in part be explained by differences in age and perhaps lifestyle. The results suggest that age-stratified analyses may be important when investigating the association of genetic and other potential risk factors for peptic ulcer.

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The Simultaneous Treatment of Inflammatory Bowel Diseases and Associated Pyoderma Gangrenosum with Oral Cyclosporin A

TO THE EDITOR: Patients with ulcerative colitis or Crohn disease associated with pyoderma gangrenosum (PG) represent a definite subset belonging to the diversified inflammatory bowel disease spectrum. PG occurs in up to 5% of patients with ulcerative colitis (1) and 1.2% of patients with Crohn disease (1). The severity of PG parallels the activity of the underlying inflammatory bowel disease in many cases (1), and it is possible that PG is 'driven' by the presence of non-dermatologic suppuration (2). PG is often associated with active and severe inflammatory bowel disease and is viewed as an adverse prognostic event in ulcerative colitis (3). Cyclosporin A (CsA) has been shown to be able to 'salvage' from surgery patients with ulcerative colitis who otherwise would have undergone colectomy (4) and to enable substantial steroid sparing in active Crohn disease (5). Meanwhile, CsA has emerged as an effective drug for the treatment of PG (6). To our knowledge, this is the first study expressly devoted to the *combined* medical treatment of this peculiar constellation of diseases.

We report on five women (Table I) with PG associated with moderate to severe inflammatory bowel disease, diagnosed clinically, radiologically, endoscopically, and histologically, whose inflammatory bowel disease–PG complex could not be controlled with standard oral and rectal codified medical treatments. The severity of inflammatory bowel disease was quantified by means of the modified Truelove–Witts criteria, following the example of other authors (7). Crohn disease was colonic in both cases; thus the quoted criteria, which had originally been designed for evaluation of ulcerative colitis, were applied also to Crohn disease patients. A commercially available microemulsion formulation of CsA was administered orally at a low dosage (that is, 4–5 mg/kg/day). The oral methylprednisolone dosage was reduced (from 40–75 to 8–25 mg/day) and, in one case (Patient 5), withdrawn from the beginning of CsA administration. All other medications were suspended. CsA serum levels reached appropriate equilibrium values in all the patients, and clinical results were very rapid and impressive. The mean reepithelialization time of PG lesions was 67.9 cm^2 per week. By the 8th week all PG ulcers had completely healed, and a dramatic improvement in

Table. In-vitro activities of ampicillin and ciprofloxacin, alone or in combination, against *E. faecium* strain 662 and mutant derivatives of this isolate with varying MICs of ampicillin

Strain (MIC; mg/L)	Change in $\log_{10}$ cfu/L after incubation for 24 h[a]			
	control	ampicillin	ciprofloxacin	ampicillin/ciprofloxacin combination
662 (32)	+3.15	+1.76	–0.98	–3.92
662.1 (64)	+3.33	+2.51	–1.22	–2.10
662.2 (128)	+2.61	+2.02	–1.37	–1.39
662.3 (150)	+3.65	+2.17	–0.85	–0.45

[a] +, increase in growth; –, decrease in growth compared with the initial suspension.

of the inoculum used (data not shown). The combination, however, was bactericidal against all five strains when the lower inoculum was used (–4.07 ± 0.32 change in $\log_{10}$ cfu/L after 24 h), but only bacteriostatic at the higher inoculum (–0.43 ± 0.19 change in $\log_{10}$ cfu/L).

The present study demonstrates that the level of ampicillin resistance in the study strain and the size of the inoculum have profound effects on the in-vitro activity of the ampicillin/ciprofloxacin combination against a HLGR strain of *E. faecium*. The poor activity of this combination in the endocarditis animal model[2] may therefore be accounted for by both the high level of ampicillin resistance exhibited by the strain used in the study and the high density of bacteria in the valvular vegetations.

The study has also defined the strengths and limitations of the ampicillin/ciprofloxacin combination in terms of its activity against multidrug-resistant enterococci. Regardless of the limitations, we believe that the combination might be effective therapy for some patients with severe infections caused by HLGR strains for which the MICs of ampicillin and ciprofloxacin are ≤32 and ≤4 mg/L, respectively. Two elderly patients with endocarditis caused by HLGR enterococci that were susceptible to ampicillin and exhibited low-level resistance or susceptibility to ciprofloxacin were recently treated successfully by us with combinations of ampicillin and a fluoroquinolone; one of the strains was also resistant to glycopeptides (VanA phenotype).[5] Further studies to evaluate the efficacies of combinations of newer quinolones and β-lactams as treatment of patients with infections caused by multidrug-resistant enterococci are warranted.

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Susceptibilities of *Helicobacter pylori* strains isolated from children with gastritis to selected antibiotics

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Sir,

High percentages of children with gastritis or peptic ulcer disease are infected with *Helicobacter pylori* and, in common with adults, eradication of this pathogen results in

long-term cure.[1] However, the increasing frequencies with which metronidazole and clarithromycin, both constituents of commonly used treatment regimens, are administered, either for the eradication of *H. pylori* or as therapy of other infections, may lead to high rates of resistance to these antibiotics amongst *H. pylori* strains: primary or acquired resistance is predictive of treatment failure in adults[2] and children[1] with infections caused by this bacterium. Periodic monitoring of the antibiotic susceptibilities of isolates is therefore of considerable importance. The purpose of the present study was to assess the in-vitro activities of selected antibiotics against *H. pylori* strains isolated from children in Greece.

Thirty-six *H. pylori* isolates were studied. The strains were recovered from gastric antral biopsies obtained from children (20 males and 16 females) 6–15 years of age (mean age 10.5 ± 3.2 years) with symptoms of gastritis, i.e. recurrent abdominal pain for at least 3 months, with or without nausea, vomiting, upper gastrointestinal tract bleeding or weight loss. None of the children had received antibiotics for at least 2 months before undergoing endoscopy. The isolates were identified by routine laboratory methods and stored in Brain Heart Infusion broth (Oxoid Ltd, Basingstoke, UK) containing glycerol at −80°C until just before testing. The antibiotics evaluated were as follows: amoxycillin, clarithromycin and ciprofloxacin, which were provided by SmithKline Beecham Hellas S.A. (Athens, Greece), Abbott Laboratories Hellas S.A. (Athens) and Bayer Hellas S.A. (Athens), respectively; and tetracycline, minocycline, metronidazole and tinidazole, all of which were purchased from Sigma–Aldrich OM Ltd (Athens). MICs were determined by an agar dilution method as described previously, but with slight modifications;[3] the medium used was Mueller–Hinton (Oxoid) supplemented with 7% horse blood. Inocula of 10^4–10^5 cfu were applied with a multipoint inoculator and *H. pylori* strain CGUT 17874 and three clinical isolates of *H. pylori*, the susceptibilities of which had been determined in previous studies, were included as controls. The MIC was taken as the lowest concentration of each antibiotic that inhibited visible growth after incubation at 37°C in a microaerophilic atmosphere for 72 h.

The susceptibility test results for the 36 isolates are shown in the Table. All of the strains were susceptible to amoxycillin, tetracycline, minocycline and ciprofloxacin. Ten (28%) of the isolates were resistant to metronidazole and tinidazole, cross-resistance being observed in all resistant isolates. Two (5.5%) isolates were resistant to clarithromycin (MICs > 8 mg/L).

At the time the study was undertaken, there was no standardized method of determining the antibiotic susceptibilities of *H. pylori* isolates. The agar dilution method used here is time-consuming, but the results are both reliable and reproducible and the correlation with clinical outcome is greater than those of other methods. It should therefore be regarded as the reference method when evaluating other susceptibility testing techniques.[4]

The incidence of resistance to metronidazole amongst *H. pylori* strains isolated from adults in Greece is 49%,[5] while that to clarithromycin is 6% (unpublished data). We have detected a lower incidence of resistance to metronidazole, but a comparable incidence of resistance to clarithromycin, in strains recovered from children. The lower incidence of resistance to metronidazole amongst isolates from children probably reflects the less frequent use of this drug in childhood. In general, data relating to the incidences of antibiotic resistance amongst *H. pylori* isolates from children are limited; in France, rates of resistance to metronidazole and clarithromycin have been reported to be between 26 and 40% and between 4.3 and 7%, respectively[6]—figures that are comparable to those described here. Knowledge of antibiotic resistance rates is important to the successful treatment of patients with *H. pylori* infections, as eradication rates are reduced when

Table. In-vitro susceptibilities of 36 *H. pylori* strains isolated from children to selected antibiotics

	MICs (mg/L)			
Antibiotic	MIC_{50}	MIC_{90}	range	No. (%) of resistant strains[a]
Amoxycillin	0.03	0.125	<0.015–0.125	0
Tetracycline	0.125	0.5	0.015–0.5	0
Minocycline	0.125	0.5	0.03–0.5	0
Metronidazole	2	32	0.25–>64	10 (28)
Tinidazole	1	32	0.125–>64	10 (28)
Clarithromycin	0.03	0.125	<0.015- >8	2 (5.5)
Ciprofloxacin	0.06	0.25	0.03–0.25	0

[a]According to the following breakpoints for resistance recommended by the NCCLS:[7] tetracycline, minocycline and amoxycillin, >4 mg/L; clarithromycin, >2 mg/L; and ciprofloxacin, >1 mg/L. The breakpoints used to define resistance to metronidazole and tinidazole were >8 mg/L. For the purpose of data analysis, the intermediate susceptibility and resistance categories have been combined.

children are infected with resistant strains.[7] In order to facilitate optimal therapy, the susceptibilities of isolates from children (and adults) should therefore be monitored locally at periodic intervals.

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Alimentary tract and pancreas
Alimentarni trakt i pankreas

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Functional dyspepsia in the era of Helicobacter pylori infection

Funkcionalna dispepsija i infekcija sa Helicobacterom

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Key words:
Functional dyspepsia, helicobacter pylori.

Abstract
Functional dyspepsia is considered as a heterogenous disorder characterized by non-specific upper abdominal symptoms for which no organic cause can be documented. The etiology is unknown and the pathophysiological mechanism(s) is obscure; heightened visceral nocioception has been incriminated as a crucial factor. The role of H. pylori as a causative agent has been extensively studied but the results are inconclusive. Eradication therapy has been attempted with conflicting results; more studies are needed to clarify the contribution of the microorganism in the pathogenesis of functional dyspepsia, if any.

Ključne reči:
funkcionalna dispepsija, Helicobacter pylori.

Sažetak
Funkcionalna dispepsija je heterogeni poremećaj koji odlikuju nespecifične tegobe u abdomenu, a u čijoj osnovi nije organski poremećaj. Etiologija ovoga stanja nije u potpunosti razjašnjena, a njeni patofiziološki mehanizmi nisu potpuno jasni. Povišenje stepena visceralne senzitivnosti se smatra da je ključni činilac. Uloga bakterije Helicobacter pylori u ovome sindromu je veoma mnogo proučavana, ali do sada bez jasno odredjenih zaključaka. Primena mera eradikacije bakterije je pokušana, ali sa veoma različitim rezultatima. Potrebna su dodatna ispitivanja u cilju razjašnjenja uloge ovoga mikroorganizma u patogenezi funkcionalne dispepsije.

INTRODUCTION

Functional dyspepsia (FD) is a poorly defined disorder that is characterized by chronic, persistent or intermittent abdominal symptoms, such as discomfort, nausea, eructations, flatulence, and a feeling of fullness, with or without pain, at the epigastrium, for which no organic cause can be found by the conventional clinical and laboratory investigation. The above mentioned symptoms may be related with food ingestion (1).

Functional dyspepsia is a common health problem; it is estimated that 40% of the adults will have symptoms consistent with FD somewhen in their lives. However, the reported prevalence of this disorder depends on various factors such as, the population studied, the methodology used, the duration of the study and, of course, the definition used to describe FD. The disorder does not seem to be an acute one and symptoms are usually present for some weeks; 70% of those affected continue complaining even 5 years after the onset of symptoms (2).

To clarify the underlying pathophysiological mechanisms and facilitate causative therapy, the following classification of FD according to salient symptoms has been

Abbreviations used in this article:
FD, Functional Dyspepsia.
H. Pylori, Helicobacter pylori;
CNS, central Nervous System;
GRP, Gastrin Releasing Peptide.
NIH, National Institutes of Health;
MMC, Migrating Motor Complex.

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proposed: ulcer like, reflux like, dysmotility like and non specific FD.

However, this classification does not seem to be useful in every day clinical practice, since there is significant overlap of the reported symptoms; in addition, 1/3 of patients with FD manifest symptoms of irritable bowel syndrome as well (3,4).

The etiopathogenesis and the pathophysiology of FD have not been clarified, yet. Various scenaria have been proposed trying to explain the underlying mechanism(s) of this disorder (5). More specific, some people believe that FD is a part of a generalized functional disorder of the gastrointestinal tract. Diet and other factors such as smoking and alcohol (6) do not seem to play any significant role; however, the role of coffee is questionable. Gastric motility disorders, such as delay in the solid phase of gastric emptying or postprandial antroduodenal hypomotility, have been observed in 20-60% and up to 50% of patients, respectively. Neither basal nor maximal acid output are elevated in FD. Many investigators claim that patients with FD have heightened visceral nocioception, that is, they react abnormally even to normal stimuli. Thus, patients report pain after intragastric infusion of 0.1N HCL or normal saline; pentagastrin induced acid secretion causes pain that is not relieved by H2 receptor antagonists; significantly lower levels of intragastric balloon inflation causes pain in patients than in controls. It is suggested, this sensory hypersensitivity may result from abnormal processing of afferent stimuli from the gut to the spinal and CNS.

Although higher degrees of anxiety and depression have been observed in patients with FD and it is quite common for the patients to report a relationship between symptoms and stress, a causal relationship has not been established. It seems that stress is a co-factor to pain development, since it is reported among others, to modify splachnic motility, perhaps by autonomic overactivity or by decreasing the threshold of splachnic pain (7,8).

Hormonal abnormalities have been described in patients with FD; decreased levels of motilin and slightly increased basal serum gastrin levels have been reported. Other peptides and hormones such as cholecystokinin, prolactin, estradiol, and endogenous opioids have been studied as well. It should be pointed out that the observed hypersensitivity of the CNS 5-HT receptors after buspirone intake (9,10) may have therapeutic implications. The influence of H. pylori infection on gastrin release and possibly on gastric motility, along with, the reported by some authors, increased prevalence of infection among patients with FD as compared with controls, have given rise to the hypothesis that H.pylori infection may have a causal relationship with FD. However, published evidence on this subject is sometimes conflicting and by no means conclusive, till now (11,12). In particular, motility disorders have been observed in patients with FD; however, patients with FD and H. pylori infection report no improvement after successful eradication therapy (13). Others (14,15) found that H. pylori infection in patients with FD affects neither gastric emptying of solid or liquid meal nor gallbladder emptying. Gilia et al (16) studied antral volume variation and found no difference between dyspeptics and controls; however fasting antral volumes were smaller in H. pylori(+) than H. pylori(-) patients.

El Omar et al (17) studied dyspeptic patients with H. pylori infection and found increased hydrochloric acid release after GRP (Gastrin-Releasing Peptide) infusion as compared with that in controls, irrespective of their H. pylori status. These findings were not confirmed by others (18). Parente et al (19) reported that H. pylori infection with CagA(+) strains has no effect on gastrin release, gastric emptying or symptom severity of dyspeptic patients. On the other hand, Konturek et al (20) studied a small number of H. pylori (+) dyspeptic patients and found improvement in symptom severity, improvement in gastric motility and 40% decrease in postprandial gastrin levels, 6 weeks after succesful eradication therapy.

Holtmann et al (21) reported lower duodenal sensory thresholds in patients with functional dyspepsia as compared with age- and sex- matched controls; however, no influence of H. pylori status on this finding was documented. Testoni et al (22), studied 100 consecutive dyspeptic patients and found that patients with absence of gastric phase of MMC (Migrating Motor Complex) had higher prevalence of H. pylori colonization. Qvist et al (23), on the other hand, reported that the duration of phase III and the whole MMC cycle was similar in patients with FD, and gastritis, irrespective of H. pylori colonization. In that study, however, shorter duration of phase I and longer duration of phase II of the MMC was observed in dyspeptics irrespective of H. pylori status, as compared with normal controls. H. pylori eradication therapy normalized the motility disorders in H. pylori (+) dyspeptics. All the above information makes it clear that the possible relationship of H. pylori with disorders of gastrointestinal motility and in particular with FD, is not conclusive. More data from clinical trials dealing with the effectiveness of H. pylori eradication therapy in patients with FD may clarify the issue. It should be pointed out that a lot of clinical trials concerning the efficacy of H. pylori eradication in dyspeptics exist in the literature, but the results are conflicting, possibly due to design weakness. This is the conclusion that Veldhuyzen et al (24) and Talley et al (25) have reached after reviewing this subject. Talley et al, in particular, reviewed 16 studies and found that, in a short term after eradication, 50% of them considered H. pylori eradication as efficacious therapy and the others as treatment failure. Possible explanations proposed for these conflicting data include non-randomization, non-placebo controlled design, lack of maintenance of blindedness and application of inadequate outcome measures.

More recently, Elta et al (26) did not find any difference in symptom severity of patients with H. pylori(+) or H. pylori(-) FD after eradication treatment; Greenberg et al (27), in a double blind study of 33 H. pylori(+) dyspeptics, found that eradication treatment did not improve symptom severity. On the contrary, Laheij et al (28), after reviewing 10 studies dealing with H. pylori and dyspepsia, found improvement in 73% of patients after succesful eradication and in 43% of patients who remained H. pylori (+).

Interesting information has been provided by McCarthy et al (29) and Gilvarry et al (30), who claim that eradication therapy in H. pylori (+) dyspeptic patients may have a substantial delay for the good results to appear. In particular, McCarthy et al assessed the effect of eradicating H. pylori infection on the symptoms of FD, 2 months and one year after treatment; one year after they found symptom improvement in patients in whom H. pylori eradication was successful. Similar results were reported by Sheu et al (31) and by Lazzaroni et al (32). Gilvarry et al (30) claim that the effectiveness of eradication treatment depends on the type of FD. Patients with ulcer-like FD showed significant symptomatic response 2, 6 and 12 months after eradication therapy, whereas those with reflux like and dysmotility like FD showed improvement only at 6 months after eradication.

It seems probable, then, that eradication therapy is not effective in treating FD and it is not recommended by the NIH consensus conference (33). In the Maastricht Consensus (34), the establishment of the relationship between H. pylori and FD was ranked as equivocal, and eradication therapy is advised but not strongly recommended, under the condition that all forms of organic dyspepsia have been excluded.

The question remains open. More, carefully designed studies are needed to be safely determined whether those infected with H. pylori will benefit - even only in the long run - from H. pylori eradication therapy.

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