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Identifying dyspepsia in the Greek population: translation and validation of a questionnaire

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Abstract

Background: Studies on clinical issues, including diagnostic strategies, are considered to be the core content of general practice research. The use of standardised instruments is regarded as an important component for the development of Primary Health Care research capacity. Demand for epidemiological cross-cultural comparisons in the international setting and the use of common instruments and definitions valid to each culture is bigger than ever. Dyspepsia is a common complaint in primary practice but little is known with respect to its incidence in Greece. There are some references about the Helicobacter Pylori infection in patients with functional dyspepsia or gastric ulcer in Greece but there is no specific instrument for the identification of dyspepsia. This paper reports on the validation and translation into Greek, of an English questionnaire for the identification of dyspepsia in the general population and discusses several possibilities of its use in the Greek primary care.

Methods: The selected English postal questionnaire for the identification of people with dyspepsia in the general population consists of 30 items and was developed in 1995. The translation and cultural adaptation of the questionnaire has been performed according to international standards. For the validation of the instrument the internal consistency of the items was established using the alpha coefficient of Chronbach, the reproducibility (test – retest reliability) was measured by kappa correlation coefficient and the criterion validity was calculated against the diagnosis of the patients' records using also kappa correlation coefficient.

Results: The final Greek version of the postal questionnaire for the identification of dyspepsia in the general population was reliably translated. The internal consistency of the questionnaire was good, Chronbach's alpha was found to be 0.88 (95% CI: 0.81–0.93), suggesting that all items were appropriate to measure. Kappa coefficient for reproducibility (test – retest reliability) was found 0.66 (95% CI: 0.62–0.71), whereas the kappa analysis for criterion validity was 0.63 (95% CI: 0.36–0.89).

Conclusion: This study indicates that the Greek translation is comparable with the English-language version in terms of validity and reliability, and is suitable for epidemiological research within the Greek primary health care setting.

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Background

Dyspepsia is a common complaint in primary health care (PHC) in most western countries, accounting for 5% of all consultations in general practice [1]. Studies in Europe have reported incidence rates for functional dyspepsia between 8 per 1000 person-years [2] to 13 per 1000 person-years [3]. In Greece there are some hospital-based data on the prevalence of Helicobacter Pylori infection [4,5] but primary care data are lacking. A project on measuring the frequencies of functional gastrointestinal disorders was established on Crete in 2001 and the need of an instrument practical for researchers and PHC physicians for the identification of dyspepsia in Greece was considered a priority. A thorough literature search did not reveal any specific instrument in the Greek language, with the exception of one that refers predominantly to the identification of functional bowel disease [6].

Several instruments have been developed for the identification of dyspepsia [7-10] and its impact on quality of life [11,12]. The English postal questionnaire for the Identification of Dyspepsia in the General Population (IDGP), which was developed and standardised in 1995 by T. Kennedy and R. Jones [10] was considered as appropriate for our purpose for certain reasons; it was developed for the general population; it was short in length and easy to answer (Yes/ No); that meant practical for use in everyday practice. According to the developers it was proved to be accurate and reliable in identifying people with dyspeptic symptoms. The questionnaire had been successfully used in a UK population study for the prevalence of gastro-esophageal reflux disease (GERD) symptoms [13].

This paper reports on the translation and validation of the IDGP and discusses several possibilities of its use in the Greek primary care.

Methods

Questionnaire

The original questionnaire consists of 8 short questions on demographics and a core part of 30 items, 29 of which are answered by Yes or No. An open question at the end of the questionnaire gives an opportunity for the patient to refer to what ever seems important for the matter and was not asked (Additional file 1). The IDGP classifies the symptoms into clinical subgroups namely dyspepsia, GERD like symptoms, past diagnosis of peptic ulcer. According to the questionnaire dyspepsia is diagnosed by the presence of "any of the symptoms of dyspepsia in the last year" [10]. GERD is likely when either heartburn or acid regurgitation is present also in the last year. Furthermore, the IDGP seeks the frequency of the dyspeptic and GERD like symptoms along with patients' consultation behaviour. The questionnaire proved to have a good internal consistency (an overall kappa coefficient 0.92) [10].

Translation

The translation and cultural adaptation of IDGP were performed according to "The Minimal Translation Criteria" [14]. Two independent bilingual physicians forward translated the questionnaire; two other physicians, native English speakers, then back translated the agreed Greek version. The agreed back translation was sent to the authors of the original questionnaire for comparison and their suggestions were incorporated into the final Greek version.

A cognitive debriefing process was then used for the cultural adaptation of the questionnaire [14]. This process was carried out in order to identify any areas presenting problematic language, and to assess the patient's level of understanding.

The questionnaire was administered to five attendants of a PHC centre, and comments made by them were discussed and included to the final Greek version.

Validation

Reliability was assessed by measuring internal consistency and reproducibility (test- retest reliability) [15,16]. Internal consistency was determined by checking the components of a questionnaire against each other, using Chronbach's alpha [17-19].

A minimum value of 0.70 for group and 0.90 for individual comparisons is generally desirable [19,20].

Reproducibility (test- retest reliability) is a measure of strength of association for determining stability of the questionnaire's results over time because it corrects for lack of independence between measurement intervals [15]. Forty consecutive PHC attendants visiting one rural PHC unit in Crete over a period of two months were recruited and asked to complete the questionnaire twice with an interval of 3 weeks. All participants had a record of upper abdominal symptoms during the past year; no one refused to complete the questionnaire. The overall Cohen's kappa coefficient was estimated [16].

Criterion validity refers to the extent to which the instrument correlates with a gold standard [21]. To define the criterion validity of the questionnaire, the diagnoses available in medical records of a fully qualified General Practitioner (GP) of the rural PHC unit were used as a gold standard to which we compared the outcome of the questionnaire given on the first visit. Kappa analysis was used in order to assess agreement between the diagnoses (dyspepsia / GERD or ulcer) as they were confirmed by the questionnaires and the GP. The diagnose of dyspepsia in our validation process was established according to the Rome II definition [22] by the positive answer to one or

Table 1: IDGP: Reproducibility (test- retest reliability).

DIAGNOSTIC CATEGORIES	K*	ITEM	K*
Dyspepsia	0.67	1	0.724
		4	0.609
		18	0.603
Frequent dyspepsia	0.61	2	0.358
		5	0.694
		19	0.691
GERD like sympcoms	0.69	7	0.746
		10	0.694
		13	0.314
		14	0.730
		15	0.652
		21	0.658
		24	0.749
Frequent GERD like symptoms	0.71	8	0.700
		11	0.742
		16	0.698
		22	0.444
Consultation behaviour	0.49	3	0.413
		6	0.405
		9	0.336
		12	0.481
		17	0.688
		20	0.306
		23	0.278
		26	0.722
Investigation for organic gastric disease	0.80	28	0.653
		29	0.950
Past diagnosis of stomach or duodenal ulcer		27	0.688
Open question		30	0.615

*: Kappa coefficient

more of items 1, 4 or 18, (pain or discomfort, feeling of excess wind or fullness, nausea) combined with negative response on the items referring to GERD like symptoms. The diagnosis of GERD was made by the positive response to one of the items 7, 10, 13 and 15 (heartburn, heart burn when lying in bed, heartburn only when lying in bed, acid tasting fluid at the back of the throat). Ulcer was diagnosed when there was a positive answer to item 27 (past diagnosis of stomach or duodenal ulcer).

A factor analysis was performed in order to identify the separate factors, which make-up this questionnaire and highlight how the items group together [23]. Factor structure was studied by Principal Component Analysis using Varimax with Keiser Normalization as Rotation Method. Both Kaiser criteria for applicability were fulfilled [24]. An analysis on the patients' symptoms (items 1, 4, 7, 10, 13, 14, 15, 18, 21, 24) was performed and a factor was considered as important if its eigenvalue value exceeded 1.0 [23].

Ethics

The scientific committee of the University Hospital of Heraklion, Crete has approved this study (number of protocol: 7173/ 12/7/2000). All participants in the cultural adaptation and reproducibility (test- retest reliability) procedure were informed about the scope and the purpose of the study and provided their oral consent.

Results

Translation

The authors suggested changes to the demographic data section of the questionnaire and added questions regarding employment. They further suggested making all items referring to the duration of the symptom(s) more specific by replacing the phrase "the past year" with the phrase "the last 12 months" in accordance with the latest definitions of Rome II [22]. The concept of discomfort was also taken into account, and the word "discomfort" was added also to the second question according to the same criteria.

During the process of cultural adaptation only one of the five patients reported problems in comprehension of the questionnaire in the total. Problems were focused mostly

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Table 2: Factor analysis for the symptoms: Rotated Component Matrix for 3 factors.

	Component		
SYMPTOMS	1	2	3
(Item 1) Pain or discomfort			0.870
(Item 4) Feeling of excess wind or fullness in the upper abdomen	0.566		
(Item 7) Heartburn	0.777		
(Item 10) Heartburn when lying in bed	0.882		
(Item 13) Heartburn only when lying in bed	0.483		
(Item 14) Awakened by the heartburn	0.861		
(Item 15) Acid taste at the back of the throat	0.555		
(Item 18) Nausea		0.816	
(Item 21) Vomiting		0.876	
(Item 24) Difficulty in swallowing	0.651		
Eigenvalues	3.60	1.40	1.13
Degree of explanation (%)	36.00	14.03	11.32

in expressions used and less in the understanding of the actual questions.

The two older and less educated participants reported some problems but any misunderstanding was solved after they read again the troubling question. No external help was given to the participants regarding the meaning of any of the questions. The suggestion of a bigger picture was accepted as well as the suggestion to explain in parenthesis the areas shown in the picture (Additional file 2).

Validation

The IDGP questionnaire showed a high overall internal consistency (alpha value: 0.88, 95% CI: 0.81–0.93) for individual comparison. Each diagnostic group also showed acceptable alpha values: 0.81 for dyspepsia; 0.76 for frequent dyspepsia; 0.82 for GERD like symptoms; 0.75 for frequent GERD like symptoms; 0.89 for investigation for organic gastric disease; 0.82 for past diagnosis of stomach or duodenal ulcer, while internal consistency was relatively low for consultation behaviour: 0.66 and for the open question: 0.72.

The overall Cohen's kappa coefficient for the reproducibility (test – retest reliability) of the questionnaire was found "substantial" (0.66, 95% CI: 0.62–0.71) [16]. Twenty-five of the 30 items had good reproducibility (Cohen's kappa coefficient>0.40), while the remaining five items had a fair reproducibility (Cohen's kappa coefficient<0.40). These results are illustrated in Table 1.

The kappa coefficient for criterion validity was also "substantial" (0.63, 95% CI: 0.36–0.89) and the overall agreement between the results of the questionnaire and the doctor's diagnose was 85%.

The performed factor analysis indicated three factors with eigenvalue over 1.0. Those factors were responsible for 61,34 % of variance and rotation converged in 4 iterations. (Table 2).

Discussion

The development of academic general practice within the Mediterranean setting does not only need support and funds but also research capacity [25]. Studies on "clinical issues", including diagnostic strategies, are considered to be the core content of general practice research as a recent publication reported [26]. Thus, the use of standardised instruments is considered as an important component for the development of PHC research capability and some questionnaires measuring the frequency of health problems in primary care and the impact of ill conditions in quality of life of Greek patients have been already published [27,28]. Moreover, the increasing demand for epidemiological cross-cultural comparisons in the international setting and the use of common instruments and definitions valid to each culture is stronger than ever [21].

We focused on dyspepsia because it is a symptom with which patients frequently present to PHC services worldwide. In addition, no data regarding the prevalence of dyspepsia in primary care population in Greece have been reported. We followed international criteria for the translation, and the Greek version was well perceived by the participants in the pilot study. The validation process revealed a "substantial" Cohen's kappa coefficient for the questionnaire and the satisfactory Chronbach's alpha suggests that the instrument is reliable for the Greek setting. The criterion validity was also good supporting that our instrument was valid when we judged it with the diagno-

sis of the GP as a gold standard. The factor analysis of the symptoms revealed the shared variance of 3 separate factors.

However, there are some concerns in terms of its validation into Greek language and particularly: (a) in some questions reproducibility (test – retest reliability) was found to be fair to moderate. Those questions referred mostly to consultation behaviour and did not change the outcome of the questionnaire, thus they were not considered as a strong limitation for the use of the instrument.

(b) during the reproducibility (test – retest reliability) process patients were informed that they would be invited sometime in the future to answer the questionnaire for a second time. It was unavoidable for us to not disclosure this issue when we were seeking for permission and making aware the respondent about the scope of the study. However patients did not know when they would be asked again.

(c) the original questionnaire was developed prior to the Rome II consensus. Nevertheless it is approaching the Rome II definition of dyspepsia and the modified Greek version is much more closer to Rome II consensus.

(d) overlap with IBS is potential since there is no question referring to the bowel habits. The simultaneous use with an IBS specific instrument or a combined questionnaire for both diseases [29] is recommended.

(e) item 4 that refers to the "feeling of excess wind or fullness" is generally accepted as a symptom which is included in the dyspepsia definition, however in the factor analysis a potential overlap with the GERD like symptoms is indicated.

The translated and validated questionnaire is anticipated to be a practical instrument for primary care physicians in Greece; it can be applied in daily practice for identifying patients with dyspepsia. Greek speaking doctors who are practicing in Cyprus and other countries may find it helpful and the questionnaire could be used in epidemiological studies highlighting some of the missing information from Greece.

Conclusion

In conclusion, the Greek translated questionnaire appears to be a reliable and valid tool for the identification of dyspepsia in clinical practice. Due to its short length and consumption of time it seems to be a practical instrument in the Greek primary care.

List of Abbreviations

PHC: Primary Health Care.

IDGP: Identification of Dyspepsia in the General Population questionnaire.

GERD: Gastro-esophageal reflux disease.

GP: General Practitioner.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

CL conceived the study design, participated in the translation of the questionnaire, formed the layout of the manuscript and wrote the final draft of the manuscript.

FA participated in the translation of the questionnaire, contributed in the data collection, carried out the analysis and co- wrote the final manuscript.

NA carried out the statistical analysis and co- wrote the final manuscript.

GH participated in the data collection and interpretation.

PNT contributed in the data interpretation and the final manuscript.

All authors approved the final manuscript.

Additional material

Additional File 1
The original English questionnaire. *The original English questionnaire.*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2458-6-56-S1.doc]

Additional File 2
The Greek version of the questionnaire. *The final Greek version of the questionnaire.*
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[http://www.biomedcentral.com/content/supplementary/1471-2458-6-56-S2.doc]

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Hypergastrinemia Is Associated with Increased Risk of Distal Colon Adenomas

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Key Words
Helicobacter pylori · Colon adenomas · CagA · Gastrin · Hypergastrinemia

Abstract
Background/Aims: *Helicobacter pylori* infection is a recognized cause of hypergastrinemia, but the association of blood gastrin levels with colonic adenomas (CAs) is controversial. The aim of this study is to investigate if hypergastrinemia, *H. pylori* infection and/or cagA protein are risk factors for CAs. ***Methods:*** In this prospective case-control study, fasting serum samples from 78 consecutive patients with CAs and 78 demographically matched colonoscopy-negative controls were assayed for anti-*H. pylori* immunoglobulin G, cagA protein and serum gastrin levels. Multivariate analysis was performed to identify risk factors for colon adenomas. ***Results:*** Though prevalence of *H. pylori* antibodies was not significantly different, the prevalence of cagA protein was significantly higher in patients with adenomas (42.3%) as compared with controls (25.6%, $p < 0.03$). Median gastrin levels were significantly higher in patients with CAs (55, 20–975 pg/ml) than in controls (45.2, 23–529 pg/ml) ($p < 0.001$). Hypergastrinemia (>110 pg/ml) was commoner in patients with CAs than in controls (29.5 vs. 11.5%, $p = 0.006$) and was the only independent risk factor for adenomas (odds ratio 3.2, 95% CI 1.4–7.5) by multivariate analysis, but not *H. pylori* infection or cagA positivity. There was a significant association of hypergastrinemia and distal distribution of adenomas ($p < 0.002$). ***Conclusions:*** Our study shows that hypergastrinemia is a risk factor for CAs, especially of the distal colon.

Introduction

Gastrin is one of the factors that have been implicated in adenoma formation and colorectal cancer (CRC) development. It has been suggested that hypergastrinemia is mitogenic to human intestinal mucosa possibly leading to increased risk of carcinogenesis [1, 2], and that subjects with gastrin levels above normal limits have a 3.9-fold risk for CRC development [3]. The proliferative effect of gastrin is, apparently, through activation of specific receptors (gastrin/CCK_B/CCK2), expressed early in the adenoma-carcinoma sequence [4], suggesting increased rate of cell proliferation as the underlying mechanism for adenoma formation [5]. The effect of gastrin precursors, i.e. progastrin and glycine-extended gastrin, in colonic neoplastic changes has been extensively investigated and is nicely reviewed by Aly et al. [6]. However, the role of the circulating amidated form is still unclarified. Several studies have shown a positive association of plasma gas-

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trin levels with colonic neoplasia [7–12], whereas in others this association has not been confirmed [13–16].

Helicobacter pylori infection causes increased basal and stimulated gastrin secretion that is reversed after the eradication of the organism [17–19]. Therefore, several studies investigated the prevalence of *H. pylori* in patients with colonic adenomas (CAs) and controls, but the results have been conflicting [20–29]. Interestingly, a recent meta-analysis of 11 human studies found positive association of *H. pylori* infection and risk of colorectal neoplasia in general (both adenomas and cancer) [30]. Cytotoxic cagA+ *H. pylori* strains carry a more marked proinflammatory activity, cause more severe gastritis, may be related to higher gastrin levels [31, 32] and have been previously associated with CRC. Indeed, Hartwich et al. [33] found higher cagA seropositivity in CRC patients than in controls, whereas Shmuely et al. [34] reported that cagA+ *H. pylori* strains are associated with 10.6-fold risk for CRC. To our knowledge, the role of cytotoxic strains in the adenoma stage of colorectal tumorigenesis has not been studied so far. In the present prospective case-control study, we have investigated whether hypergastrinemia, *H. pylori* infection and/or cagA protein are risk factors for CAs.

Materials and Methods

This prospective case-control study was carried out in two collaborating centers, one in a major hospital of Athens (Evangelismos) and one of Piraeus (Tzaneion). All patients and controls were recruited from the daily colonoscopy list of our Endoscopy Units. The study protocol was conducted according to the Helsinki Declaration for Human Rights, and approved by the ethics committees of our institutions.

Patients

From January 2000 to December 2001, 1,347 consecutive patients were referred for colonoscopy to the two Endoscopy Units. 245 polyps were detected in 180 patients. For the purpose of our study, subjects with incomplete colonoscopy, inadequate bowel preparation, hyperplastic polyps and inflammatory bowel disease were excluded. Additional exclusion criteria were any condition associated with elevated gastrin levels, namely, peptic ulcer disease, previous gastric surgery, pernicious anemia, Zollinger-Ellison's syndrome, acromegaly, myeloid cancer of the thyroid, hypercalcemia, renal failure and recent treatment (<1 month before) with H_2 antagonists and/or proton pump inhibitors. We also excluded subjects with history of CRC, family history of CRC and/or colonic polyps, chronic use of NSAIDs, previous *H. pylori* eradication therapy, current serious illness (e.g. cancer) and pregnant or breast-feeding women. Finally, we excluded patients with polyps of <5 mm in size as well as patients harboring more than three adenomas, since the latter group have been linked with inherited mutations syndromes [35, 36]. The recruitment stopped when the required number of eligible patients was reached, based on sample size calculations (see statistical analysis), that is 78 out of the 180 patients with discovered polyps. All subjects had colonoscopy to the cecum and found to harbor one to three CAs of at least 0.5 cm in size. In addition, using the same criteria, we studied 78 matched controls with similar demographic (age, sex) and socioeconomic characteristics, selected from the same series of consecutive patients who had been referred for colonoscopy for lower abdominal pain with or without change of bowel habits or to exclude colonic disease because they had seen blood on their stools. Eligible controls for the study were those who had normal total colonoscopy or uncomplicated diverticular disease. The order of factors used for matching was as follows: age ±2 years, sex, and finally socioeconomic status. There were no double matching controls. The investigator in charge for the matching process during the study (K.T.) was unaware of the *H. pylori* and cagA status and the gastrin levels of adenoma patients and of the subjects selected as controls.

Patients' and controls' demographic characteristics were recorded in special datasheets as well as the characteristics of the detected adenomas (number, location, histological type, grade of dysplasia). The socioeconomic status of the participants was estimated as high or low based on an evaluation questionnaire, which has been validated for *H. pylori* transmission during childhood (sanitation, number of households, use of common bed, hot water supply and family income) [37].

Informed consent was obtained from study participants to agree in using serum and part of the bioptic material for the purpose of our investigation.

Serological Testing

A 10-ml fasting blood sample was taken from patients and controls at least 48 h before bowel preparation for colonoscopy. Serum samples were stored in –70°C until the time of the assays.

A non-competitive heterogeneous enzyme-linked immunosorbent assay (ELISA) that has been developed and validated by the Hellenic 'Pasteur' Institute was used to detect IgG serum antibodies against *H. pylori*. It has a sensitivity, specificity, positive and negative predictive value for the Greek population of 96.7, 90.9, 93.5 and 95.2%, respectively [38]. A commercially available immunoenzymatic assay (RADIM, *H. pylori* cagA IgG EIA Well, Radim, Liège, Belgium) was used to detect serum antibodies against cagA protein, according to manufacturer's instructions. Serum gastrin levels were measured by a radioimmunoassay (ICN, Costa Mesa, Calif., USA) specific for the end product of gastrin biosynthetic pathway, i.e. the amidated gastrin. The assay has been validated in the local population by the reference laboratory ('Vioiatriki') and the value of 110 pg/ml has been defined as the upper limit of normal.

Statistical Analysis

Sample size calculations were undertaken based on the seroprevalence of *H. pylori* in the Greek population and specifically in the relevant to the study age range [39]. To detect an increased *H. pylori* seroprevalence by 20% in the CAs group with 80% power and a 5% significance level, 78 patients were required in the control and the adenoma group.

Table 1. Demographic characteristics in patients with CAs and controls

Parameter	Patients with CAs (n = 78)	Controls (n = 78)
Age, years (median, range)	64 (37–80)	64.5 (38–80)
Male/female	42/36	42/36
Socioeconomic status, high/low	30/48	30/48
Smoking, % smokers	46.1	47.4

Qualitative parameters were analyzed with the χ^2 test and Fisher's exact two-sided tests. Gastrin values did not show normal distribution and analyzed with the non-parametric Mann-Whitney U-test. Multivariate analysis was performed using a logistic regression model, to identify factors associated with an increased risk of CAs (dependent variable). Independent variables were hypergastrinemia (gastrin value >110 pg/ml) (yes: 1, no: 0), smoking habit (yes: 1, no: 0), socioeconomic status (low: 1, high: 0), *H. pylori* status (+: 1, –: 0) and cagA protein (+: 1, –: 0). A p value <0.05 was considered statistically significant. Data were analyzed with Statistical Package SPSS 11.5 (SPSS Inc., Chicago, Ill., USA).

Table 2. Location, number, size and histological features of CAs[1] in patients with and without hypergastrinemia

Variable	Gastrin values >110 pg/ml	Normal gastrin values (20–110 pg/ml)
Site		
Distal to splenic flexure	23[2]	39
Proximal to splenic flexure	0	16
Number		
1	19	42
>1	4	13
Size[1]		
<1 cm	6	26
≥1 cm	17	29
Histological type[1]		
Tubular	10	29
Tubulovillous	13	23
Villous	0	3
Dysplasia[1]		
Low grade	4	22
Intermediate grade	18	28
High grade	1	5

[1] Concerning the largest adenoma.
[2] $p < 0.002$ for the relation of hypergastrinemia and location of CAs distally to the splenic flexure.

Results

Among the 180 patients with polyps detected in colonoscopy, 102 were excluded from analysis, namely, 43 patients with hyperplastic polyps, 13 patients with diminutive adenomas (size <0.5 cm), 5 with previous *H. pylori* eradication therapy, 5 with more than 3 adenomas, 11 with recent proton pump inhibitors/H2 antagonist treatment, 9 with family history of CRC, 5 with chronic use of NSAIDs, 7 because of incomplete colonoscopy, 3 with past history of peptic ulcer, 2 with previous gastric surgery, 1 with pernicious anemia and 2 with chronic renal failure. 78 patients with adenomas were eligible for the study.

Demographic characteristics did not differ significantly between patients with CAs and controls (table 1). The prevalence of antibodies against *H. pylori* was higher in patients with CAs (79.5%) than in controls (67.9%), but this difference was not statistically significant ($\chi^2 = 2.7$, $p = 0.1$). However, the prevalence of cagA protein was significantly higher in patients with CAs than in controls (42.3 vs. 25.6%, $\chi^2 = 4.83$, $p = 0.03$).

The median value of serum gastrin was significantly higher in patients with CAs (55, 20–975 pg/ml) in comparison with controls (45.2, 23–529 pg/ml) ($p < 0.001$). A higher percentage of patients with CAs had hypergastrinemia (>110 pg/ml) in comparison with controls (29.5 vs. 11.5%, $\chi^2 = 7.7$, $p = 0.006$). Logistic regression analysis (backward conditional model) showed that only hypergastrinemia was related with an increased risk of CAs (OR 3.2, 95% CI 1.4–7.5, $p < 0.007$). *H. pylori* status ($p = 0.44$) and cagA protein ($p = 0.12$) were removed from the model at steps 2 and 3, respectively. Of note, hypergastrinemia was associated with left colon distribution of adenomas, i.e. distally to the splenic flexure (Fisher's exact test, $p < 0.002$), but it was not related with the number, size or histological features of the adenomas (table 2).

Discussion

This prospective case-control study showed that patients with CAs have higher serum gastrin levels than adenoma-free controls. Serum gastrin levels above normal (>110 pg/ml) were associated with a 3.2-fold risk of CAs. We also noted a significant association between hypergastrinemia and distal distribution of CAs. In all patients with hypergastrinemia, the location of adenomas was distal to the splenic flexure and their majority with-

in the rectosigmoid area (20 out of 23) (table 2). This finding is supported by published evidence in animal models suggesting that the mitogenic action of gastrin is limited to the left colon [40, 41].

The proliferative effect of chronic hypergastrinemia in the normal colon may increase the mutation susceptibility and lead to development of adenomas. Studies showed that there is genetic expression of both gastrin and its specific receptor (gastrin/CCK_B/CCK2) in quite early stages of adenoma formation [4], but also in advanced tumors [42]. There is significant amount of experimental evidence that gastrin precursors (i.e. progastrin, glycine-extended gastrin) produced locally by CRC cells, enhance tumor growth (autocrine action) [43–45]. In fact, very recent data suggest that amidated gastrin (G17) has antiproliferative effects in CRC cell lines expressing CCK2 receptor [44]. However, the role of amidated gastrin in early neoplastic stages or in normal colon cells is not clarified yet. In recent animal work, both the amidated and glycine-extended gastrin-releasing peptide induced neoplastic changes in normal rat recta [40]. Colonic adenomatous tissue is deficient in processing progastrin to amidated gastrin, and therefore, the latter is presumably of gastric origin [6, 45].

Our data is in accordance with that of Thorburn et al. [3] who reported a 3.9-fold risk of CRC in subjects with hypergastrinemia. A significantly higher proportion of our patients with CAs as compared with controls had hypergastrinemia (>110 pg/ml). These results are supported by published reports [8–10, 12], but disputed by others [13–16]. This discordance of results may reflect differences in study design, lack of strict exclusion criteria, such as a family history of colon cancer, and inappropriate selection of controls.

There have been reports of higher *H. pylori* seroprevalence in patients with CAs [20–25] than in controls, but this has not been confirmed by others [28, 29]. In some studies, investigators fail to achieve optimal matching between patients and controls regarding basic parameters concerning *H. pylori* infection (e.g. age, socioeconomic status) [22, 28], while other studies are characterized by small numbers of patients [29] or have been published in abstract form [20, 21], making their evaluation difficult. Furthermore, in no one of these studies a multivariate analysis comprising the parameter of hypergastrinemia has been applied, bearing in mind that *H. pylori* infection and specifically from cytotoxic cagA$^+$ strains is often accompanied by hypergastrinemia [19, 31]. The results of our study showed that the prevalence of cagA protein was significantly higher in patients with CAs than in controls but this was not an independent factor in multivariate analysis. This might reflect a sample dilution phenomenon since only about half of the subjects with hypergastrinemia were cagA positive. However, it is possible that the subgroup of patients with hypergastrinemia secondary to *H. pylori* cagA$^+$ infection might benefit from *H. pylori* eradication.

In conclusion, the findings of the present study are strongly linking high gastrin levels with the presence of colorectal adenomas, specifically distal adenomas. This link requires definite confirmation in the future, as this concept might be the target of prevention and therapy.

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Editorial

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Gastrin and Colorectal Neoplasia: Cause and Effect

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Gastrin is a peptide hormone, synthesized and released from gastric antral G cells. Hypergastrinemia is a pathological state where gastrin concentration, usually gastrin 17, is increased in the circulation. There are four main reasons for this phenomenon: *Helicobacter pylori* infection; treatment with proton pump inhibitors (PPIs); autonomic secretion of gastrin from tumor, gastrinoma, in Zollinger Ellison Syndrome (ZES) or in multiple endocrine neoplasm type I (MEN I), and atrophic gastropathy, where the physiological negative feedback on gastrin secretion by G cells is not functioning. Eradication of *H. pylori*, stopping PPIs, resection of the tumor in ZES and the gastric antrum in atrophic gastropathy may return gastrin levels to within the normal range. In *H. pylori* infection and in ZES, hypergastrinemia may cause severe peptic disease because of the high rate of gastric secretion by the parietal cells. In PPI therapy and atrophic gastropathy, when parietal cells are inhibited or lost, acid secretion is not a clinical problem but the high gastrin concentration in the peripheral blood may be dangerous for other reasons. Gastric endocrine cells (ECL) may proliferate because of gastric trophic effect, and ECLomas and carcinoid tumors may develop [1].

Gastrin has a trophic effect on epithelial cell growth and proliferation not only in the stomach, and may have a role in the development of colonic adenomas and the polyp-carcinoma sequence [1, 2]. There are several lines of evidence to support this role. Gastrin effects are mediated by CCKB (CCK-2) receptors, which have been detected in colon cancer tissues [2]. Furthermore, gastrin stimulates cell line and xenograft growth [3], and hypergastrinemia has been associated with an increased risk of colorectal cancer [4]. Altered colonic proliferation of the normal mucosa, with a movement of the proliferative zone to the upper crypt, has been demonstrated in patients with hypergastrinemia due to pernicious anemia or in patients with a hereditary predisposition to colorectal cancer [6, 7]. Colucci et al. [5] demonstrated increased transcriptional activity of COX-2 gene followed by prostaglandin E2 production in HT-29 (a human colonic cancer cell line) after CCKB receptor activation by gastrin-17. Prostaglandin E2 stimulates growth and proliferation of epithelial cells, and may be the final common pathway by which gastrin exerts its activity. Other possible mechanisms are by enhancing angiogenesis or inhibition of apoptosis, as recently described [8–10]. Amidated gastrin-17, glycine-extended gastrin-17 and other precursors, as well as CCKB receptor isoforms, CCKC and glycine-extended gastrin receptor may all play an important role in colonic epithelial cell proliferation and adenoma formation in endocrine, paracrine or autocrine pathways [3].

In this issue of *Digestion*, Georgopoulos et al. [11] demonstrate a positive correlation between hypergastrinemia and colonic adenomas. Comparing a group of 78 consecutive patients with colonic adenomas with matched colonoscopy negative controls, hypergastrin-

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emia was the only independent risk factor for adenomas, especially of the distal colon, by multivariate analysis, but not *H. pylori* infection or cagA positivity. This study joins many others that established the role of gastrin in adenoma formation and colorectal cancer development. Since hypergastrinemia due to *H. pylori* infection, PPI therapy or atrophic gastropathy is common, the danger of developing colonic adenomas should be taken into account by the medical community and a preventive strategy is needed. *H. pylori* eradication, PPI dose reduction and screening colonoscopy should be more aggressively applied to these patients. A new approach for prevention of colorectal cancer by developing monoclonal antibodies to glycine-extended gastrin-17 and carboxy-amidated gastrin-17 has been recently described and may have an important role in this regard [12].

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Relationship between *Helicobacter pylori* infection and Alzheimer disease

Abstract—The authors investigated the association between *Helicobacter pylori* infection (*Hp*-I) and Alzheimer disease (AD) by using histology for diagnosis of *Hp*-I. Fifty patients with AD and 30 iron deficiency anemic control participants without AD were included. The histologic prevalence of *Hp*-I was 88% in patients with AD and 46.7% in controls ($p < 0.001$).

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Helicobacter pylori infection (*Hp*-I) is associated with upper gastrointestinal (GI) and non-GI conditions, including peripheral neuropathies[1,2] where autoantibodies to specific neural targets may impair native neural function by inducing nerve tissue damage possibly by apoptosis. With respect to the CNS, recent evidence suggests the presence of antineuronal antibodies and autoimmunity-induced cell death in Alzheimer disease (AD).[3] Correspondingly, *Hp*-I has been implicated in extradigestive vascular conditions including functional vascular disorders caused by vascular dysregulation, hypertension, atherosclerotic disease, ischemic heart disease, and ischemic cerebrovascular disorders,[4] conditions that are also more often detected in AD and contribute to its clinical manifestations and worsening.[5]

Recently, a higher seropositivity for anti-*Hp* immunoglobulin (Ig) G antibodies was reported in 30 patients with AD than in age-matched controls.[6] Although this serologic test establishes the presence of antecedent *Hp*-I, it does not discriminate between current and old infections. Such a distinction is crucial because current *Hp*-I induces humoral and cellular immune responses that, owing to the sharing of homologous epitopes (molecular mimicry), cross-react with components of nerves,[2] thereby contributing and possibly perpetuating neural tissue damage. Moreover, eradicating *Hp*-I might delay AD progression, particularly at early disease stages.

Based on the histologic analysis of gastric mucosa biopsy for the documentation of current *Hp*-I, we investigated whether *Hp*-I is associated with AD using histology, recognized as the standard for the diagnosis of active *Hp*-I.[1,2]

Methods. We studied 50 patients (Group A) who fulfilled the diagnostic criteria for AD. Details in patient selection have been described previously.[7] Control subjects (Group B) consisted of 30 patients without AD who underwent upper GI endoscopy for investigation of mild iron deficiency anemia but in whom endoscopy was normal. Given that weight loss is common and may precede a decade before diagnosis of AD,[8] both our patients and controls tended to be thinner than the age-matched healthy individuals in our community.

All patients and controls underwent diagnostic upper GI endoscopy after informed consent. Exclusion criteria were described previously.[1]

H pylori detection methods were reported previously,[1,2] except for the total serum homocysteine (Hcy) concentration that was measured with a fluorescence polarization immunoassay on the IMx® analyzer (Abbott Laboratories, Abbott Park, IL). The mean intraassay imprecision (CV) of this method is 2.1%, with a range of 0.7% to 8.3%, and the mean interassay CV over a course of 20 days is 3.2%, with a range of 1.0% to 8.3%. Biopsy urease test and histopathology process were also described previously.[1,2] Notably, the pathologist (I.V.) who assessed all specimens was unaware of the rapid urease test result and the clinical diagnosis.

The Mann–Whitney *U* test, χ^2, odds ratios, 95% CI, and two-tailed *t* test were used. Significance was set at $p < 0.05$.

Results. Mean age, sex ratio, and socioeconomic status did not differ between the two groups (table). In patients with AD, the mean Mini Mental State Examination score was 17.4 ± 7.1, the mean Neuropsychiatric Inventory score was 10.9 ± 8.6, the mean Hamilton Depression Rating Scale score was 10.4 ± 5.9, the mean Cambridge Cognitive score was 57.4 ± 20.8, the mean Geriatric Depression Scale score was 4.0 ± 2.3, and the mean Functional Rating Scale for Symptoms of Dementia score was 13.6 ± 7.3.

The prevalence of *Hp*-I was 88% (44 of 50) in patients with AD and 46.7% (14 of 30) in the controls, as confirmed by the presence of *Hp* bacteria histologically (χ^2 14.1, $p < 0.001$, odds ratio 8.4, 95% CI 2.4 to 28.7). When compared with the control values, the mean serum anti-*Hp* IgG concentration was higher in patients with AD (34.0 ± 40.1 vs 17.0 ± 18.1 U/mL; $p = 0.016$). Mean total serum Hcy concentration was also higher in patients with AD than in controls (17.7 ± 4.9 vs 13.5 ± 4.0 μmol/L; $p < 0.001$; see table).

When compared with the anemic participants, demented patients exhibited more often multifocal (body and antral) gastritis (98% vs 70%; $p < 0.001$). According to Sydney classification, Grade 3 gastritis was noted in 9 of 50 patients with AD (18%) and in none of the anemic control participants ($p = 0.03$); Grades 0 and 2 gastritis did not differ significantly between the two groups.

Discussion. The current series suggests a link between *Hp*-I and AD. In our cohort of Greek patients, 88% of the patients with AD exhibited histologically proven *Hp*-I, whereas the rate of infection was significantly lower in the anemic control group (46.7%).

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Table Helicobacter pylori *positivity, total Hcy concentrations, and socioeconomic status in patients with AD and anemic controls*

Characteristic	Patients with AD, n = 30	Anemic controls, n = 30	Odds ratio (95% CI)	*p* Value
Age, mean ± SD (range), y	65.0 ± 6.9 (53–80)	62.2 ± 8.6 (44–70)	NA	0.07
No. of men/No. of women	18/32	14/16	NA	0.48
Positive urease test result (gastric mucosa)	30 (60%)	14 (46.7%)	1.7 (0.7–4.3)	0.35
Mean serum anti-*H pylori* IgG concentration, U/mL	34.0 ± 40.1	17.0 ± 18.1	NA	0.016
Anti-*H pylori* IgG >10 U/mL	31 (62%)	14 (46.7%)	1.9 (0.7–4.7)	0.24
Histologically confirmed presence of *H pylori*	44 (88%)	14 (46.7%)	8.4 (2.4–28.7)	<0.001
Mean serum total Hcy concentration, μmol/L	17.7 ± 4.9	13.5 ± 4.0	NA	0.001
Socioeconomic status*				
Low	9 (18%)	6 (20%)	NA	>0.50
Medium	35 (70%)	22 (73.3%)	NA	>0.50
High	6 (12%)	2 (6.7%)	NA	>0.50

* Socioeconomic status of the patients was evaluated according to the following variables: 1) social class (manual, nonmanual), 2) household income (<national average, >national average), 3) education (primary, secondary, higher), and 4) household crowding (persons/room; low, high). The patients were then classified to three categories: low, medium, and high socioeconomic status. Low and high socioeconomic status was determined if the patients had scored either low or high in all four categories. The rest of the subjects who had variable scores among the four categories were regarded as of medium socioeconomic status.

Hcy = homocysteine; AD = Alzheimer disease; NA = not applicable; Ig = immunoglobulin.

It is important to consider whether the rate of *Hp*-I in the control group has been negatively influenced by the coexistence of anemia. There is no evidence to suggest that anemia protects against the development of *Hp*-I. Anemic controls have been used before,[1] and the frequency of *Hp*-I in the anemic control group is similar to that reported by other investigators when using serodiagnostic assays to evaluate Greek cohorts and other ethnic populations, showing a frequency distribution of 34% to 62%.[1,2]

Our study relied on histologic analysis for the documentation of *Hp*-I. Although culture is the theoretic accepted standard for detection of the bacterium, it has been established that there is an excellent correlation with histologic identification.[1,2] Therefore, for most studies, mucosal biopsy and histologic examination of the specimen for the presence of *Hp* and gastritis is the actual standard for diagnosis of *Hp*-I.[1,2]

In this study, multifocal chronic gastritis (body and antrum atrophy) was observed in the majority of our patients compared with controls. Moreover, an increased total serum Hcy concentration has been shown in our patients with AD, a finding also reported by others.[6] Chronic gastritis owing to *Hp*-I can lead to malabsorption of vitamins (B_{12}) and folate, which results in failure of methylation by 5-methyl-tetrahydrofolic acid and hence accumulation of Hcy.[6,9] The increased Hcy, in turn, could trigger endothelial damage and result in atherothrombotic disorders and AD. In this respect, investigators reported that *Hp*-induced chronic atrophic gastritis or atrophic gastritis per se decreases serum vitamin B_{12} and folate concentrations, thereby increasing the Hcy, a potent contributor to vascular disorders. Considering the above-mentioned data, we can speculate that *Hp*-I might contribute, at least in part, to the pathogenesis of AD through induction of chronic atrophic gastritis and Hcy sequence. However, further studies are needed to elucidate this field.

We emphasize that the current study did not establish causality, because this would require showing that eradication of *Hp* alters the course of AD.

H pylori infection may influence the pathophysiology of AD by one of the following mechanisms: 1) Promoting platelet and platelet–leukocyte aggregation.[1] Platelet activation and aggregation have also been proposed to play pathophysiologic roles in the development of AD; platelets are a source of β-amyloid, the major constituent of senile plaques, considered to be the primary and central event in the etiology and pathogenesis of AD, and both increased platelet activation and increased circulating β-amyloid have been identified in AD. 2) Releasing large amounts of proinflammatory and vasoactive substances, such as cytokines (interleukin [IL]-1, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor-α, interferon-γ), eicosanoids (leukotrienes, prostaglandins catalyzed by cyclooxygenase enzymes), and acute phase proteins (fibrinogen, C-reactive protein)[1] involved in a number of vascular disorders including AD[6] and other AD-related neuropathies such as glaucoma.[1] 3) Stimulating mononuclear cells to produce a tissue factor–like procoagulant that converts fibrinogen into fibrin.[1] 4) Causing the development of cross mimicry between endothelial and *Hp* antigens.[1] 5) Producing reactive oxygen metabolites and circulating lipid peroxides that have also been involved in the pathophysiology of AD.[6] 6) Influencing the apoptotic process that may also be an important form of cell death in many relative neurodegenerative dis-

eases including AD,[9] or Down syndrome that predisposes to the early onset of the neurodegeneration of AD.[10]

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Helicobacter pylori Infection is Probably the Cause of Chronic Idiopathic Neutropenia (CIN)-Associated Splenomegaly

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Splenic volume and *Helicobacter pylori* (*H. pylori*) infection were evaluated in 67 patients with chronic idiopathic neutropenia (CIN) and 39 healthy individuals. Using ultrasound, splenomegaly was found in 61.7% of *H. pylori*-infected subjects compared to only 8.7% noted in the group of *H. pylori*-non-infected individuals ($P < 0.0001$). Splenomegaly was also found in 47.8% of CIN patients compared to 12.8% in the group of non-CIN subjects ($P = 0.0003$). However, applying the two-way ANOVA test, a statistically significant effect on splenic volume was documented for "factor *H. pylori*" ($F^1_{102} = 16.800$, $P < 0.0001$) but not for "factor CIN" ($F^1_{102} = 3.213$, $P = 0.0760$), suggesting that CIN-associated splenomegaly is probably due to *H. pylori* infection. Am. J. Hematol. 81:142–144, 2006. © 2006 Wiley-Liss, Inc.

Key words: chronic idiopathic neutropenia (CIN); *Helicobacter pylori* infection; splenomegaly

INTRODUCTION

It has been reported that patients with chronic idiopathic neutropenia (CIN) have increased splenic volume in ultrasonography [1] and increased serum pro-inflammatory cytokines and chemokines [2,3], suggesting that a low-grade chronic inflammatory process may underlie the disease and affect splenic size [3]. The prevalence of *Helicobacter pylori* infection has also been found to be significantly increased in CIN patients [4]. It was then conceivable to investigate the possible role of *H. pylori* infection in the determination of splenic volume in these patients.

SUBJECTS AND METHODS

One hundred six subjects—16 men of age 28–75 years (median 48 years) and 90 women of age 21–77 years (median 50 years)—were studied. Of these, 67 fulfilled the diagnostic criteria of CIN [3]. The remaining 39 subjects were used as healthy controls.

Diagnosis of *H. pylori* infection was based on the positivity of at least two out of five diagnostic tests, i.e., ^{13}C-urease breath test, *Campylobacter*-like organism (CLO) test, histologic detection of the bacterium in gastric mucosa biopsies, and increased serum titers of anti-*H. pylori* IgG or IgA antibodies detected with ELISA (Pyloriset EIA-GIII and EIA-AIII, Orion Diagnostica, Espoo, Finland) [4]. ELISA was also used for the detection of anti-*H. pylori* CagA IgG antibodies.

Splenic volume was assessed by determining the "corrected splenic index" (CSI) using ultrasound. The product of the length, width, and thickness of the spleen was calculated and normalized by dividing

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the obtained value (in cm^3) by the body surface area (in m^2) [5]. Splenomegaly was defined as any rise in CSI above 207.7 cm^3, representing the upper 95% limit of distribution of values seen in healthy non-infected control subjects.

Data were analyzed using the Mann–Whitney test to compare two mean values and the Fischer's exact test to compare two percentages. The two-way ANOVA test was applied to assess the roles of "factor CIN" and "factor *H. pylori*" on the values of CSI.

RESULTS

CSI values are presented in Table I. In *H. pylori*-positive subjects ($n = 60$), the mean CSI value was 209.8 ± 55.6 cm^3, while the respective mean in the group of *H. pylori*-negative individuals was 152.7 ± 37.3 cm^3 ($n = 46$) ($P < 0.0001$). Splenomegaly was observed in 37 *H. pylori*-positive (61.7%) but in only 4 *H. pylori*-negative (8.7%) subjects ($P < 0.0001$). Interestingly, increased CSI was found in *H. pylori*-positive compared to *H. pylori*-negative individuals in both CIN patients ($P = 0.0007$) and in healthy controls ($P = 0.0338$).

The mean CSI value in CIN patients ($n = 67$) was 200.6 ± 59.7 cm^3 compared to 158.3 ± 36.6 cm^3 in healthy controls ($n = 39$) ($P = 0.0002$). Splenomegaly was noted in 47.8% of CIN patients and in 12.8% of healthy controls ($P = 0.0003$). These data show that CSI values are significantly higher in CIN patients compared to healthy controls. However, using the two-way ANOVA test, a "factor CIN" affecting CSI could not be proved ($F^1_{102} = 3.213$, $P = 0.0760$), while a highly significant effect was noted for the "factor *H. pylori*" ($F^1_{102} = 16.800$, $P < 0.0001$). These findings clearly show that the increased CSI in CIN patients [1] is probably due to *H. pylori* infection.

Anti-CagA IgG seropositivity was evaluated in 29 CIN and 4 non-CIN *H. pylori*-positive subjects with splenomegaly. Elevated titers of anti-CagA IgG were found in 13 of 29 CIN patients (44.8%) and in 1 of 4 non-CIN subjects (25%) ($P = 0.6197$). These data indicate that CagA antigenicity has no effect on the splenic size of *H. pylori*-infected subjects.

TABLE I. CSI (cm^3) in *H. pylori*-Infected Individuals ("Factor *H. pylori*") in Relation to the Underlying CIN ("Factor CIN")[a]

	H. pylori (+) subjects	*H. pylori* (−) subjects	*P* value[a]
CIN patients	215.1 ± 56.2[b] ($n = 50$)	157.8 ± 49.0 ($n = 17$)	$P = 0.0007$
Healthy controls	183.4 ± 46.0 ($n = 10$)	149.7 ± 29.0 ($n = 29$)	$P = 0.0338$
P value[a]	$P < .0001$	$P = 0.9546$	

[a]"Factor *H. pylori*", $F^1_{102} = 16.800$, $P < 0.0001$; "factor CIN," $F^1_{102} = 3.213$, $P = 0.0760$ (NS).
[b]Values are expressed as means ± 1 SD. The number of subjects studied is indicated in parentheses. NS, non-significant.

DISCUSSION

The data presented in the current study show that *H. pylori* infection plays a role in the determination of splenic size. This is true not only for CIN patients but also for healthy subjects infected with the bacterium. Indeed, CSI values were significantly increased in *H. pylori*-positive compared to *H. pylori*-negative individuals, while the proportion of subjects with splenomegaly was significantly higher in *H. pylori*-positive compared to *H. pylori*-negative individuals irrespectively of the underlying CIN.

The mechanism by which *H. pylori* infection may affect splenic volume is unknown. *H. pylori* causes damage in the gastric mucosal cells [6,7], which respond to challenge by releasing chemokines accelerating chemotaxis of neutrophils and other inflammatory cells to enter the locally produced gastric inflammation [7,8]. *H. pylori*-derived proteins and even other bacterial products may activate innate and host immune responses leading to a variety of gastric and extragastric manifestations [9,10]. It is then possible that bacterial products or molecules related to the inflammatory process enter the circulation and affect the spleen either by direct antigenic stimulation or by inducing alterations in leukocyte trafficking. There is no evidence that CagA antigenicity may play a role in the determination of splenic size in *H. pylori*-infected subjects.

In conclusion, *H. pylori* infection may be accompanied by increased splenic volume. Such an effect of the bacterium is more pronounced in patients with CIN.

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Effects of *Helicobacter pylori* and Nonsteroidal Anti-Inflammatory Drugs on Peptic Ulcer Disease: A Systematic Review

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Background & Aims: **The aim was to systematically review the interactions between *Helicobacter pylori* (HP) infection and NSAID use on the risk of uncomplicated or bleeding peptic ulcer.** ***Methods:*** **All relevant full articles published in MEDLINE from January 1989–June 2004 were included. Sensitivity analyses for type of controls or use of aspirin or non-aspirin NSAIDs were performed.** ***Results:*** **In 21 studies involving 10,146 patients, uncomplicated peptic ulcer was more common in HP-positive than HP-negative patients (pooled odds ratio [OR], 2.17) or in HP-positive than HP-negative NSAID users (OR, 1.81). In 6 age-matched controlled studies, ulcer was more common in HP-positive than HP-negative patients (OR, 4.03), irrespective of NSAID use, and in NSAID users than non-users (OR, 3.10), irrespective of HP status; the risk of ulcer was 17.54-fold higher in HP-positive NSAID users than HP-negative non-users. The use of aspirin or non-aspirin NSAIDs did not affect the results. Ulcer bleeding was evaluated in 17 studies involving 4084 patients. NSAID use was more frequent in bleeding patients than control subjects (OR, 5.13), irrespective of HP status and type of controls. In contrast, HP infection in bleeding patients compared with control subjects was less frequent in the 8 studies with ulcer cases as control subjects (OR, 0.40) and more frequent in the 9 studies with uninvestigated subjects as controls (OR, 2.56). In the latter studies, presence compared with the absence of both HP and NSAIDs increased the risk of bleeding 20.83-fold.** ***Conclusion:*** **HP infection and NSAID use represent independent and synergistic risk factors for uncomplicated and bleeding peptic ulcer.**

Aspirin and non-aspirin NSAIDs are widely used agents,[1,2] although their consumption is often associated with the development of serious gastrointestinal complications, with the most common being acute bleeding from peptic ulcers.[3,4] Both uncomplicated and complicated peptic ulcers mostly develop in NSAID users with certain risk factors, such as older age, history of peptic ulcer with or without complications, recent dyspepsia, or use of anticoagulants.[2] However, none of these factors can be modified or removed to reduce the risk of NSAID gastrotoxicity.

Helicobacter pylori (HP) infection is also a documented risk factor for peptic ulcer disease.[5] Because HP infects almost 50% of the population worldwide and is more prevalent in older individuals,[6] the establishment of a synergistic or additive effect of HP infection and NSAID use in peptic ulcer development would be of great clinical importance, because eradication of the bacterium would likely reduce the risk of upper gastrointestinal complications in infected NSAID users. Although the presence of 2 factors that might damage the gastric mucosa, such as HP and NSAIDs, would be reasonably considered to increase the risk of peptic ulcer, data from several, mainly epidemiologic studies appeared to be controversial and did not always confirm such an assumption.[7] In a systematic review published in 2002, the combined analysis of the data available up to October 2000 showed that HP infection and NSAID use act synergistically for the development of peptic ulcer and ulcer bleeding.[8] However, several relevant studies have been published after 2000, and the interactions between HP infection and NSAID use in several patient subgroups have not been entirely clarified.[6] Thus, the aim of our systematic review was to evaluate in detail the relations between HP infection and use of NSAIDs on the risk of developing uncomplicated or bleeding peptic ulcer.

Methods

Data Identification

We searched the MEDLINE/PUBMED database from January 1989–June 2004 to identify all medical literature included under the search text terms *aspirin* or *NSAID* and *pylori* and *ulcer* or *bleeding* or *complication*. We also performed a

Abbreviations used in this paper: CI, confidence interval; HP, *Helicobacter pylori*; OR, odds ratio.

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full manual search of all review articles and of the retrieved original studies.

Inclusion Criteria

Studies published as full articles were included in our systematic review if they met all the following criteria: (1) to be observational studies (case-control, cross-sectional, or cohort) or randomized trials; (2) to investigate endoscopically the presence or absence of uncomplicated or bleeding peptic ulcer; (3) to include adults (>16 years old) taking NSAIDs and, in case of ulcer bleeding, include both patients with bleeding and nonbleeding control subjects; (4) to provide data on the prevalence of HP infection and NSAID use; and (5) to exclude patients with recent (within the last 4 weeks) antibiotic use or anti-ulcer drugs or a history of gastric surgery as well as patients with non-ulcer gastrointestinal bleeding (unless they provided data for ulcer and non-ulcer bleeding separately).

Data Extraction

Data were extracted independently from each study (by G.V.P. and S.S.) by using a predefined form, and disagreement was resolved by consensus.

Events for Analysis

The events selected for analysis were (1) endoscopically documented, uncomplicated peptic ulcer with a diameter ≥3 mm and (2) acute bleeding from peptic ulcer documented by endoscopy.

Statistical Analysis

The pooled odds ratio (OR) and 95% confidence interval (CI) were calculated from the raw study data by using the Mantel-Haenszel (fixed effect model) or the DerSimonian and Laird method (random effect model). The χ^2 test was used to assess heterogeneity, which was considered to be present if P value was less than .05. In the absence of statistically significant heterogeneity, pooled OR and 95% CI by the fixed effect model are given in the results, whereas in the case of significant heterogeneity, pooled OR and 95% CI by the random effect model are given. In the presence of significant statistical heterogeneity, we searched for the sources of any possible clinically important (methodologic or biologic) heterogeneity. Agreement in the selection of studies between the 2 reviewers was evaluated by the κ coefficient.

Because of the lack of statistical power for heterogeneity testing for both the detection and extent of clinically significant heterogeneity, we performed separate sensitivity analyses according to the following parameters. First, because two thirds of the studies selected for the evaluation of uncomplicated peptic ulcer included control subjects (non-users of NSAIDs) unmatched for age, which influences both the prevalence of HP infection[3] and the risk of NSAID-induced peptic ulcer,[9] we performed separate analyses for the effect of HP infection on the risk of uncomplicated peptic ulcer according to the study design (age-matched or unmatched controls).

Second, because almost half of the studies selected for evaluation of peptic ulcer bleeding included cases with uncomplicated ulcers as nonbleeding controls and the prevalence of HP infection is expected to be rather high in such patients,[3] separate analyses for ulcer bleeding were performed for studies including patients with uncomplicated ulcers and for studies including endoscopically uninvestigated subjects as nonbleeding controls. These analyses are only provided in the Results.

Third, all analyses for uncomplicated peptic ulcer were performed separately for aspirin or non-aspirin NSAID users, because it is still controversial whether these 2 types of agents have the same ulcerogenic potential.[6] Such a sensitivity analysis was not performed for ulcer bleeding because of limited available data.[10–13]

Results

Descriptive Assessment

There were 626 citations generated by the literature searches. Of those, 37 were found to meet our inclusion criteria. In particular, the presence of uncomplicated ulcer was reported in 21 studies[14–34] and of ulcer bleeding in 17 studies[10–13,26,35–46]; one study evaluated patients with both uncomplicated and bleeding ulcers.[26] Initial agreement between the reviewers for the selection of relevant articles was high (κ = 0.94).

Uncomplicated Peptic Ulcer

In the 21 studies that evaluated the presence of uncomplicated ulcer, raw data on the HP status were provided for 10,146 cases, 3938 users and 6208 non-users of NSAIDs.[14–34] The main characteristics of these studies are shown in Table 1 of Appendix.

The overall pooled prevalence of ulcer was significantly higher in HP-positive (40%, 2468/6214) than HP-negative (29%, 1126/3932) subjects, irrespective of NSAID use (heterogeneity, $P < .001$; pooled OR, 2.17; 95% CI, 1.69–2.79; $P < .001$). In particular among NSAID users, the pooled prevalence of ulcer was significantly higher in HP-positive than HP-negative cases (47% vs 39%; heterogeneity, $P < .001$; pooled OR, 1.81; 95% CI, 1.40–2.36; $P < .001$) (Figure 1*A*; Table 2 of Appendix).[14–34] Similarly, the pooled prevalence of ulcer was significantly higher in HP-positive than HP-negative NSAID non-users (36% vs 19%; heterogeneity, $P < .001$; pooled OR, 6.02; 95% CI, 2.72–13.33; $P < .001$) in the 9 studies providing raw data for both users and non-users of NSAIDs (Figure 1*B*; Table 2 of Appendix).[15,16,19,20,22,27,28,30,32]

In the latter 9 studies,[15,16,19,20,22,27,28,30,32] the prevalence of ulcer was not significantly different between users (31%, 431/1331) and non-users of NSAIDs (30%, 1891/6208) (heterogeneity, $P < .001$;

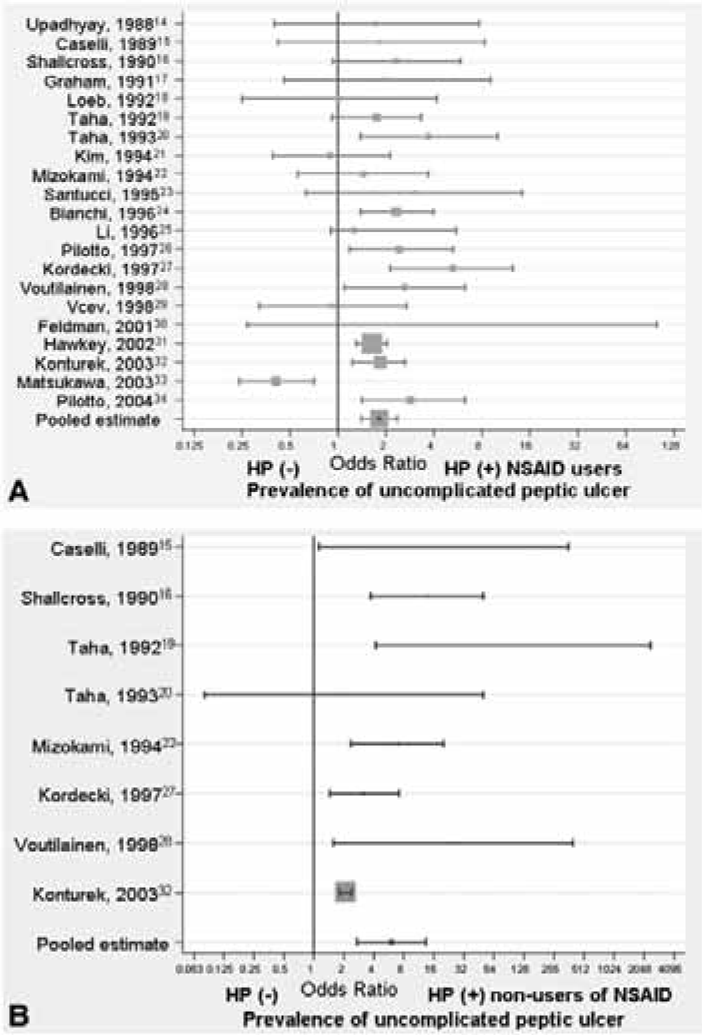
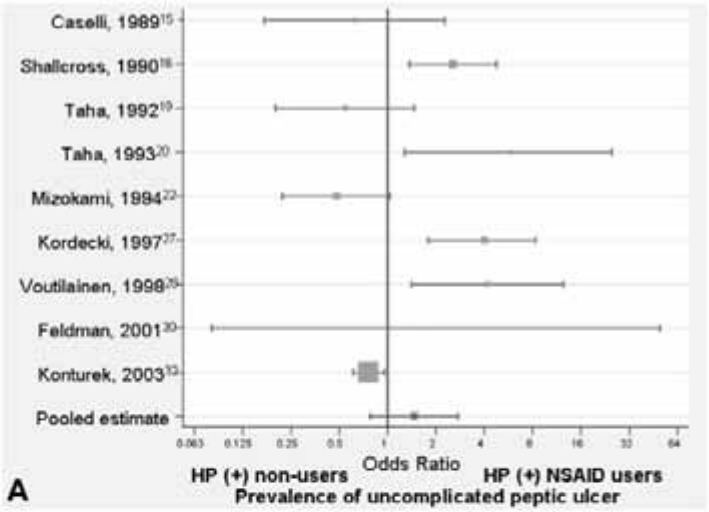


Figure 1. Risk of uncomplicated peptic ulcer in users (*A*)[14–34] or non-users (*B*)[15,16,19,20,22,27,28,30,32] of NSAIDs in relation to the presence of HP infection. Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs; area of symbol inverse proportional to estimate's variance. For both (*A*) and (*B*), significant heterogeneity ($P < .001$) and pooled estimate by random effect model ($P < .001$).

pooled OR, 1.87; 95% CI, 0.97–3.58; $P = .06$). However, the effect of NSAID use was found to be significantly affected by the HP status. In particular, the pooled prevalence of ulcer did not significantly differ between HP-positive users and HP-positive non-users of NSAIDs (38% vs 36%; heterogeneity, $P < .001$; pooled OR, 1.47; 95% CI, 0.78–2.75; $P = .24$), but it was significantly higher in HP-negative users than HP-negative non-users of NSAIDs (26% vs 19%; heterogeneity, $P < .001$; pooled OR, 5.00; 95% CI, 1.71–14.71; $P = .003$) (Figure 2*A* and *B*; Table 3 of Appendix). The pooled prevalence of ulcer was also significantly higher in HP-positive NSAID users than HP-negative NSAID non-users (38% vs 19%; heter-



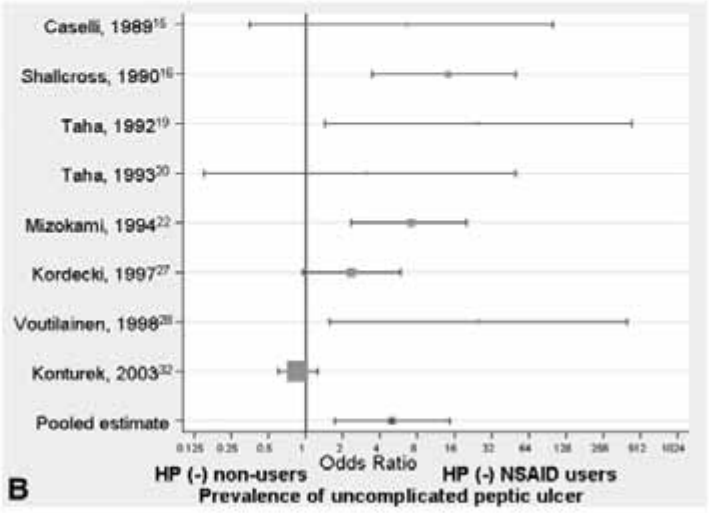


Figure 2. Risk of uncomplicated peptic ulcer in HP-positive *(A)* or HP-negative *(B)* subjects in relation to the use of NSAIDs.[15,16,19,20,22,27,28,30,32] Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs; area of symbol inverse proportional to estimate's variance. For both *(A)* and *(B)*, significant heterogeneity ($P < .001$) and pooled estimate by random effect model ($P = .06$ for [*A*] and $P = .003$ for [*B*]).

ogeneity, $P < .001$; pooled OR, 9.80; 95% CI, 3.11–30.30; $P < .001$).

The pooled results of the subgroup analyses for the effect of HP infection and/or NSAID use on the risk of uncomplicated ulcer in 6 of the 7 age-matched controlled studies[15,20,25,27,28,30] and in the remaining 14 unmatched studies[14,16–19,21,22,24–26,29,31,32,34] are shown in Table 1. One age-matched controlled study was excluded from this analysis, because it was the only one including exclusively patients with gastric ulcers in the group of NSAID non-users.[33] The pooled prevalence of HP infection was significantly higher in patients with ulcers than control subjects, irrespective of NSAID use in the analyses of both types of studies, whereas the NSAID

Table 1. Pooled Effects of HP Infection and/or Use of NSAIDs on the Risk of Uncomplicated Peptic Ulcer in Age-Matched Controlled or Unmatched Studies

	Age-matched controlled studies		Unmatched studies	
	OR (95% CI) Peptic ulcers/total (%)	References	OR (95% CI) Peptic ulcers/total (%)	References
HP effect	4.05 (2.80–5.88)[a,b]	15,20,23,27,28,30	2.06 (1.87–2.28)[a,b]	14,16–19,21,22,24–26,29, 31,32,34
HP-positive cases	138/366 (38)		2275/5714 (40)	
HP-negative cases	58/423 (14)		1013/3422 (30)	
HP effect in NSAID users	3.60 (2.23–5.78)[a,b]	15,20,23,27,28,30	1.78 (1.53–2.08)[a,b]	14,16–19,21,22,24–26,29, 31,32,34
HP-positive users	97/202 (48)		801/1681 (48)	
HP-negative users	43/229 (19)		625/1605 (41)	
HP effect in NSAID non-users	5.03 (2.53–10.00)[a,b]	15,20,23,27,28,30	7.41 (2.01–27.78)[c,d]	16,19,22,32
HP-positive non-users	41/164 (25)		1474/4033 (37)	
HP-negative non-users	15/194 (8)		361/1817 (20)	
NSAID effect	2.99 (1.26–7.09)[c,e]	15,20,27,28,30	1.22 (0.59–2.53)[e]	16,19,22,32
NSAID users	129/393 (33)		284/938 (30)	
Non-users of NSAID	56/358 (16)		1835/5850 (31)	
NSAID effect in HP-positive subjects	3.03 (1.82–5.03)[a,b]	15,20,27,28,30	0.88 (0.45–1.74)[e]	16,19,22,32
HP-positive NSAID users	88/178 (49)		187/550 (34)	
HP-positive non-users of NSAID	41/164 (25)		1474/4033 (37)	
NSAID effect in HP-negative subjects	3.86 (1.79–8.33)[c,f]	15,20,27,28,30	5.59 (0.95–33.33)[c,d]	16,19,22,32
HP-negative NSAID users	31/188 (16)		126/423 (30)	
HP-negative non-users of NSAID	15/194 (8)		361/1817 (20)	
NSAID plus HP effect	15.38 (7.69–31.25)[a,b]	15,20,27,28,30	7.30 (1.44–37.04)[c,d]	16,19,22,32
HP-positive NSAID users	88/178 (49)		187/550 (34)	
HP-negative non-users of NSAID	15/194 (8)		361/1817 (20)	

[a]$P < .001$.
[b]Nonsignificant heterogeneity.
[c]$P = .003$.
[d]Significant heterogeneity.
[e]$P = .015$.
[f]$P = .001$.
[g]$P = .057$.

use was significantly associated with the presence of peptic ulcer only in the analyses of age-matched controlled studies but not of unmatched studies (Table 1). The risks of ulcer in relation to the presence of HP infection or NSAID use in the age-matched controlled studies appear in Figures 3 and 4.

The effect of HP infection and/or NSAID use on the risk of gastric or duodenal ulcer was also evaluated. In the 5 age-matched controlled studies, which provided data on the site of peptic ulcer,[15,20,23,28,30] presence of HP infection significantly increased the risk of both duodenal and gastric ulcers, irrespective of NSAID use, but its effect was stronger on the risk of duodenal ulcer (pooled OR, 5.05; 95% CI, 2.32–10.99; $P < .001$) than that of gastric ulcer (pooled OR, 1.74; 95% CI, 1.06–3.16; $P = .03$). In the same studies,[15,20,23,28,30] NSAID use was found to significantly increase the risk of gastric ulcer (pooled OR, 7.87; 95% CI, 3.28–18.87; $P < .001$) but not the risk of duodenal ulcer, irrespective of presence of HP infection (Table 2).

The effect of HP infection and/or NSAID use on the risk of ulcer was also evaluated separately in 4 studies with subjects taking aspirin alone[27,30,33,34] and in 13 studies with subjects taking non-aspirin NSAIDs alone[14,16,17,19–24,28,29,31,33] (Table 3). The overall effect of HP infection or the effect of HP infection in NSAID users did not differ significantly between these 2 subgroups of studies. The effect of aspirin could be evaluated in 2 of the 4 studies[27,30] and the effect of non-aspirin NSAIDs in 5 of the 13 studies.[16,19,20,22,28] In HP-positive subjects, the risk of ulcer was found to increase 3.8-fold by aspirin use ($P < .001$) and only 1.6-fold by non-aspirin NSAID use without reaching statistical significance ($P = .32$). In contrast, in HP-negative subjects,

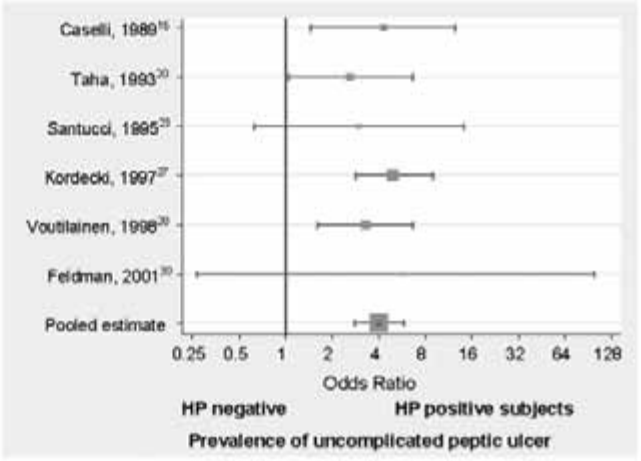


Figure 3. Risk of uncomplicated peptic ulcer in relation to the presence of HP infection in 6 age-matched controlled studies.[15,20,23,27,28,30] Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs; area of symbol inverse proportional to estimate's variance. Nonsignificant heterogeneity ($P = .88$). Pooled estimate by fixed effect model ($P < .001$).

the risk of ulcer was found to increase 2.5-fold by aspirin use ($P = .068$) and 10-fold by non-aspirin NSAID use ($P < .001$) (Table 3).

Peptic Ulcer Bleeding

In the 17 studies that evaluated the development of ulcer bleeding, raw data on HP status were provided for 4084 cases, 1588 patients with ulcer bleeding and 2496 nonbleeding control subjects.[10–15,26,35–46] The main characteristics of these studies are shown in Table 4 of Appendix.

In the 9 studies with uninvestigated subjects as nonbleeding controls,[10–15,36,38–43] HP infection was detected significantly more frequently in patients with ulcer bleeding (76%, 798/1055) than in control subjects (56%, 587/1043) (heterogeneity, $P = .52$; OR, 2.56; 95% CI, 2.11–3.11; $P < .001$) (Figure 5). In these studies, a similar effect of HP infection was observed in both users (397/532 or 75% vs 252/438 or 56%; heterogeneity, $P = .15$; OR, 2.35; 95% CI, 1.75–3.14; $P < .001$) and non-users of NSAIDs (174/209 or 83% vs

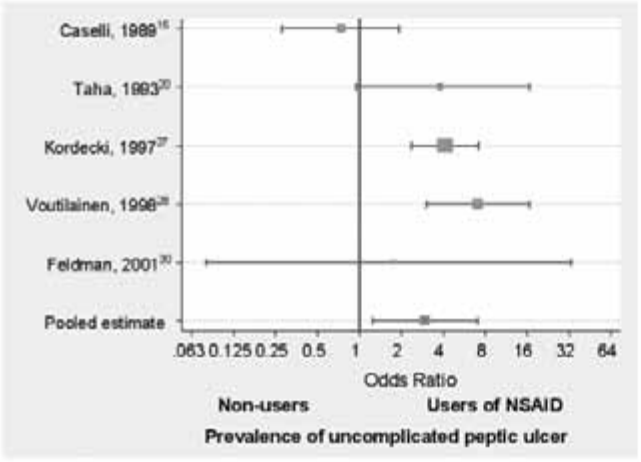


Figure 4. Risk of uncomplicated peptic ulcer in relation to the use of NSAIDs in 5 age-matched controlled studies.[15,20,27,28,30] Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs; area of symbol inverse proportional to estimate's variance. Significant heterogeneity ($P = .02$). Pooled estimate by random effect model ($P = .01$).

Table 2. Pooled Effects of HP Infection and/or Use of NSAIDs on the Risk of Uncomplicated Gastric or Duodenal Ulcer in Age-Matched Controlled Studies

	OR (95% CI)		OR (95% CI)	
	Gastric ulcers/total (%)	References	Duodenal ulcers/total (%)	References
HP effect	1.74 (1.06–3.16)[a,b]	15,20,23,28,30	5.05 (2.32–10.99)[b,c]	15,20,23,28,30
HP-positive cases	34/250 (14)		33/250 (13)	
HP-negative cases	27/313 (9)		8/313 (3)	
HP effect in NSAID users	1.84 (1.00–3.38)[b,d]	15,20,23,28,30	3.34 (1.42–7.87)[b,d]	15,20,23,28,30
HP-positive users	29/137 (21)		19/137 (14)	
HP-negative users	27/198 (14)		8/198 (4)	
HP effect in NSAID non-users	7.35 (0.88–62.50)[b,f]	15,20,28,30	9.43 (1.70–52.63)[b,c]	15,20,28,30
HP-positive non-users	5/113 (4)		14/113 (12)	
HP-negative non-users	0/115		0/115	
NSAID effect	7.87 (3.28–18.87)[c,g]	15,20,28,30	0.97 (0.29–3.27)[b,h]	15,20,28,30
NSAID users	51/297 (17)		21/297 (7)	
Non-users of NSAID	5/228 (2)		14/228 (6)	
NSAID effect in HP-positive subjects	4.68 (1.82–12.99)[b,g]	15,20,28,30	0.96 (0.39–2.34)[b,h]	15,20,28,30
HP-positive NSAID users	26/113 (23)		13/113 (12)	
HP-positive non-users of NSAID	5/113 (4)		14/113 (12)	
NSAID effect in HP-negative subjects	8.20 (3.37–20.00)[b,g]	15,20,28,30	3.69 (0.45–30.30)[b,h]	15,20,28,30
HP-negative NSAID users	25/184 (14)		8/184 (4)	
HP-negative non-users of NSAID	0/115		0/115	
NSAID plus HP effect	12.66 (3.11–50.00)[b,c]	15,20,28,30	7.94 (1.41–45.45)[c,h]	15,20,28,30
HP-positive NSAID users	26/113 (23)		13/113 (12)	
HP-negative non-users of NSAID	0/115		0/115	

[a]$P = .03$.
[b]Nonsignificant heterogeneity.
[c]$P < .001$.
[d]$P = .049$.
[e]$P = .005$.
[f]$P = .07$.
[g]Significant heterogeneity.
[h]$P > .20$.

202/343 or 59%; heterogeneity, $P = .60$; OR, 4.03; 95% CI, 2.59–6.29; $P < .001$) (Figure 6*A* and *B*; Table 5 of Appendix).

On the contrary, in the 8 studies with cases with endoscopically documented peptic ulcers as nonbleeding controls,[26,35,37,42–46] HP infection was detected significantly less frequently in patients with ulcer bleeding (74%, 368/494) than in control subjects (92%, 1249/1363) (heterogeneity, $P = .001$; OR, 0.40; 95% CI, 0.23–0.68; $P = .001$). In these studies, the difference in the pooled prevalence of HP infection between patients with ulcer bleeding and control subjects maintained statistical significance in non-users (166/201 or 83% vs 1016/1070 or 95%; heterogeneity, $P = .04$; OR, 0.44; 95% CI, 0.20–0.66; $P = .001$) but not in users of NSAIDs (202/293 or 69% vs 233/295 or 79%; heterogeneity, $P < .001$; OR, 0.65; 95% CI, 0.23–1.84; $P = .42$) (Table 6 of Appendix).

The overall effect of NSAID use could be evaluated in 12 studies,[10,11,35–37,39,40,42–46] because 4 studies did not include NSAID non-users,[12,13,26,41] and 1 study did not include NSAID users among the control subjects.[38] NSAID use significantly increased the risk of ulcer bleeding in both studies with uninvestigated subjects (heterogeneity, $P = .16$; pooled OR, 4.85; 95% CI, 3.77–6.25; $P < .001$)[10,11,36,39,40] (Figure 7) and studies with cases with ulcers as controls (heterogeneity, $P = .04$; pooled OR, 5.59; 95% CI, 4.29–7.30; $P < .001$).[35,37,42–46]

The effect of NSAID use in relation to the HP status could be evaluated in 9[11,35,37,40,42–46] of the latter 12 studies, because the HP status was not provided separately for patients and/or control subjects in 3 of them.[10,36,39] In the 2 of these 9 studies with uninvestigated subjects as nonbleeding controls,[11,40] NSAID use was reported significantly more frequently by bleeding patients than control subjects in both HP-positive patients (171/312 or 55% vs 46/232 or 20%; heterogeneity, $P = .08$; pooled OR, 5.21; 95% CI, 3.48–7.75; $P < .001$) and HP-negative patients (51/76 or 67% vs 29/156 or 19%; heterogeneity, $P = .51$; pooled OR, 11.49; 95% CI, 5.78–22.73; $P < .001$) (Table 7 of Appendix).

Table 3. Pooled Effects of HP Infection and/or Use of NSAIDs on the Risk of Peptic Ulcer in Users of Aspirin or Non-Aspirin NSAID Alone

	Aspirin users			Non-aspirin NSAID users		
	Peptic ulcers/ total (%)	OR (95% CI)	References	Peptic ulcers/ total (%)	OR (95% CI)	References
Total HP effect		1.81 (0.28–8.62)[a]	27,30,33,34		2.06 (1.52–2.80)[a,f]	14,16,17,19–24,28,29,31,33
HP-positive	120/288 (42)			766/1580 (48)		
HP-negative	59/236 (23)			621/1630 (38)		
HP effect in users		1.83 (0.35–9.61)[a]	27,30,33,34		1.68 (1.42–1.99)[b,f]	14,16,17,19–24,28,29,31,33
HP-positive users	98/230 (43)			668/1261 (53)		
HP-negative users	40/149 (27)			615/1374 (45)		
HP effect in non-users		3.25 (1.49–7.04)[c,f]	27,30		10.53 (4.95–22.73)[a,f]	16,19,20,22,28
HP-positive non-users	22/58 (38)			185/515 (36)		
HP-negative non-users	15/87 (17)			104/585 (18)		
NSAID effect		3.98 (2.36–6.71)[a,f]	27,30		2.31 (0.97–5.49)[a,g]	16,19,20,22,28
Users	62/142 (44)			185/515 (36)		
Non-users	37/145 (26)			104/585 (18)		
NSAID effect in HP-positive subjects		3.82 (1.49–8.00)[a,f]	27,30		1.63 (0.62–4.31)[a]	16,19,20,22,28
HP-positive users	51/87 (59)			100/223 (45)		
HP-positive non-users	22/58 (38)			98/329 (30)		
NSAID effect in HP-negative subjects		2.48 (0.93–6.54)[c,f]	27,30		10.10 (4.59–22.22)[a,f]	16,19,20,22,28
HP-negative users	11/55 (20)			104/300 (35)		
HP-negative non-users	15/87 (17)			6/256 (2)		
NSAID plus HP effect		9.39 (4.90–17.86)[a,f]	27,30		18.52 (4.52–76.92)[a,f]	16,19,20,22,28
HP-positive users	51/87 (59)			100/223 (45)		
HP-negative non-users	15/87 (17)			6/256 (2)		

[a]Significant heterogeneity.
[b]$P < .001$.
[c]Non significant heterogeneity.
[d]$P = .003$.
[e]$P = .057$.
[f]$P = .068$.

Similarly, in the 7 studies with ulcer cases as nonbleeding controls,[35,37,42–46] NSAID use was also reported more frequently by bleeding patients than control subjects in HP-positive patients (184/380 or 48% vs 191/1292 or 15%; heterogeneity, $P = .012$; pooled OR, 5.43; 95% CI, 3.88–7.63; $P < .001$) and HP-negative patients (68/103 or 66% vs 31/83 or 37%; heterogeneity, $P = .014$; pooled OR, 3.51; 95% CI, 0.85–14.49, $P = .082$), although the difference did not reach statistical significance in the latter cases (Table 8 of Appendix).

In the comparison between subjects with or without both HP infection and NSAID use, presence of both factors was detected significantly more frequently in patients with ulcer bleeding than in nonbleeding control subjects, but such an effect was greater in the studies with uninvestigated subjects (87% or 171/196 vs 27% or 46/173, respectively; heterogeneity, $P = .02$; pooled OR, 20.83; 95% CI, 7.94–55.55; $P < .001$)[11,40] compared with the studies with ulcer cases as controls (83% or 175/210 vs 78% or 186/238, respectively; heterogeneity, $P = .15$; pooled OR, 1.91; 95% CI, 1.10–3.31; $P = .02$).[35,37,42,44–46]

Discussion

The overall results of our systematic review suggest that HP infection and NSAID use have at least additive effect on the risk of developing uncomplicated peptic ulcer. The effect of each factor might be seen more clearly when it acts alone. In our study, the risk of ulcer was found to increase 6-fold by HP infection in non-users and 5-fold by NSAID use in HP-negative subjects, whereas it increased 10-fold by the simultaneous presence compared with the absence of both factors. It should be noted that there was significant heterogeneity in all analyses for the risk of ulcer, which is probably related to variations in the inclusion and exclusion criteria as well as the design and differences among the study populations.

The background prevalence of HP infection might be an important factor in interpreting the findings of studies evaluating its effect on the risk of peptic ulcer, but such data are not available for most studies. Because both the prevalence of HP infection and the risk of NSAID-induced peptic ulcer are age-dependent,[3,9] the analyses of

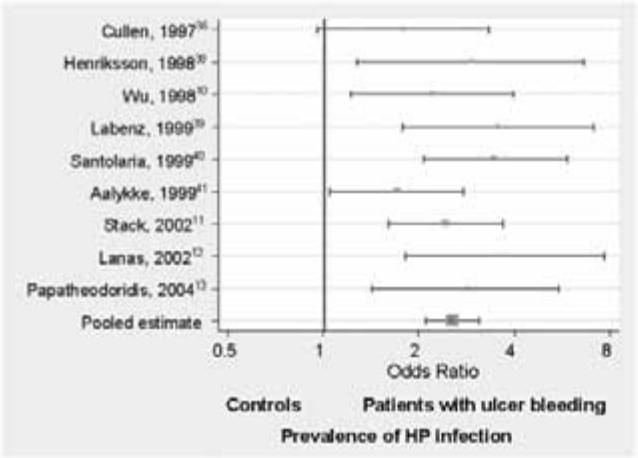


Figure 5. Effect of HP infection on the risk of peptic ulcer bleeding in 9 studies with uninvestigated subjects as controls.[10–13,36,38–41] Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs; area of symbol inverse proportional to estimate's variance. Nonsignificant heterogeneity ($P = .52$). Pooled estimate by fixed effect model ($P < .001$).

data of age-matched controlled studies are expected to provide more meaningful results. In fact, except for the overall NSAID effect, there was no significant heterogeneity in any other analysis of the age-matched controlled studies, in which the risk of ulcer increased 3.5-fold to 5-fold by HP infection irrespective of NSAID use, 3- to 4-fold by NSAID use irrespective of HP infection, and 15-fold by presence compared with absence of both factors (Table 1, Figure 3).

In contrast, there was significant heterogeneity in almost all analyses of the unmatched studies, which tended to underestimate the effects of HP infection and particularly of NSAID use. In the latter studies, the risk of ulcer was found to increase 2-fold by HP infection and not to be affected by NSAID use. It should be noted that the absence of NSAID effect was mostly due to the findings of a recent, large (n = 5967) Polish study,[12] in which ulcers were detected in 22% of HP-negative subjects not taking NSAIDs (20% of all ulcers), and there was a negative interaction between HP infection and NSAID use on the development of duodenal ulcers (the majority of ulcers in this study). Whether the development of ulcers in HP-negative subjects not taking NSAIDs is an isolated phenomenon in certain populations or whether it is increasing in recent years as suggested by Konturek et al[12] cannot be easily answered. A similar proportion of ulcers in HP-negative non-users of NSAIDs was also reported in an older, small Polish study,[27] which did not strongly influence the results of our meta-analysis. Nevertheless, the prevalence of ulcers unrelated to HP and NSAIDs has been found recently to increase in some reports (without exceeding 10%)[47] but not in others.[48] It should be noted that the relative proportion of non-HP, non-NSAID ulcers is expected to increase following the progressively decreasing prevalence of HP infection, whereas underreporting of NSAID use and false-positive endoscopic findings should also be taken into account.

Whether aspirin and non-aspirin NSAIDs are associated with a similar risk of ulcer and particularly whether they have similar interactions with HP infection are not clear. It has been suggested that the damaging effect of aspirin on the gastric mucosa might be less potent than the effect of non-aspirin NSAIDs,[49] but even low doses of aspirin, such as 75 mg per day, have been shown to increase the risk of gastroduodenal ulcerations.[50,51] According to our meta-analysis, HP infection had a similar effect on the risk of ulcer (increase of 1.7- to 1.8-fold) in users of aspirin and non-aspirin NSAIDs. On the other hand, the use of aspirin compared with the use of non-aspirin NSAIDs was associated with a greater increase of the risk of ulcer in HP-positive subjects (3.8-fold vs 1.6-fold) and a lower increase of the risk of ulcer in HP-negative subjects (2.5-fold vs 10-fold) (Table 2). Such findings might have been influenced by the heterogeneity among studies, whereas their validity for aspirin users might be limited by the small sample size. However, they might also suggest that the ulcerogenic activity of aspirin, which is lower than that of non-aspirin NSAIDs in the absence of HP (prevalence of ulcers: 20% in HP-negative aspirin users and 35% in HP-negative non-aspirin NSAID users), is greatly increased in the

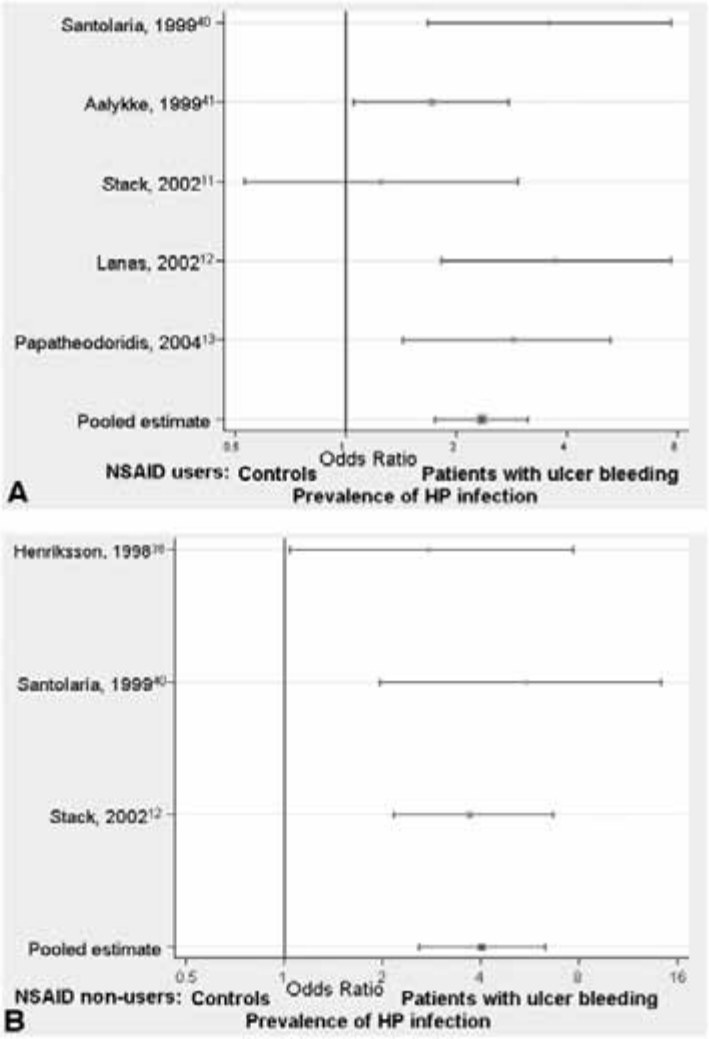


Figure 6. Effect of HP infection on the risk of peptic ulcer bleeding in users *(A)* or non-users *(B)* of NSAIDs in 9 studies with uninvestigated subjects as nonbleeding controls.[10–13,38,39–41] Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs; area of symbol inverse proportional to estimate's variance. For both *(A)* and *(B)*, nonsignificant heterogeneity ($P = .15$ for *(A)* and $P = .60$ for *(B)*) and pooled estimate by fixed effect model ($P < .001$).

presence of HP infection (prevalence of ulcers: 59% in HP-positive aspirin users and 45% in HP-positive non-aspirin NSAID users). This is compatible with the results of a randomized therapeutic trial in HP-positive NSAID users with recent ulcer bleeding, according to which HP eradication was associated with significant reduction of the risk of rebleeding similar to that achieved by long-term omeprazole therapy only in aspirin but not in non-aspirin NSAID users.[52]

The majority of ulcers in NSAID users are completely asymptomatic because they are incidentally found at endoscopy in more than 20% of cases, whereas ulcer complications develop in only 2%–5% of them.[4,53–57] Thus, the evaluation of the effects of NSAIDs on the risk

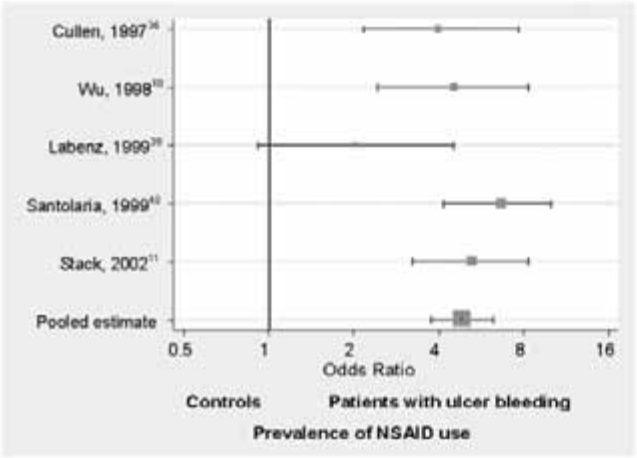


Figure 7. Effect of NSAID use on the risk of peptic ulcer bleeding in 5 studies with uninvestigated subjects as controls.[10,14,36,39,40] Plot standard graphic representation of ORs (logarithmic scale) and 95% CIs. Nonsignificant heterogeneity ($P = .16$); area of symbol inverse proportional to estimate's variance. Pooled estimate by fixed effect model ($P < .001$).

of ulcer complication is more important for clinical practice. To define better the roles of HP and NSAIDs on the risk of ulcer bleeding, we performed subanalyses according to the nonbleeding control group. Specifically, we detected 2 main types of studies, those that included patients with ulcers and those that included endoscopically uninvestigated subjects as controls. In studies with ulcer patients as control subjects, HP infection was more common in nonbleeding controls than in bleeding patients (the difference reached statistical significance in the total analysis and in the analysis of NSAID nonusers). We speculate that this is probably related to the strong association between uncomplicated ulcer and HP infection, particularly in patients with chronic dyspeptic symptoms undergoing endoscopy.[5]

The risk of ulcer bleeding might be more meaningful to be evaluated in studies with uninvestigated subjects as controls to avoid the strong association between HP infection and peptic ulcer. All these studies included age-matched nonbleeding control subjects.[10–13,35,38–41] The results of these analyses suggest that HP infection and NSAID use have a synergistic effect on the risk of ulcer bleeding. In particular, HP infection was more common (OR, 4.0) in bleeding patients than in control subjects not taking NSAIDs, and NSAID use was more common (OR, 11.5) in HP-negative bleeding patients than in control subjects, whereas the presence compared with the absence of both factors significantly increased the risk of bleeding (OR, 20.8).

In conclusion, HP infection and NSAID use represent independent, synergistic risk factors for uncomplicated and complicated peptic ulcers. Thus, HP eradication will have a beneficial effect in NSAID users. However, whether HP testing and subsequent HP eradication must be recommended to all NSAID users cannot be answered directly by such data, and this question should be examined by prospective randomized controlled trials of HP eradication in several subgroups of NSAID users. In current clinical practice, taking into consideration that guidelines are usually influenced by cost-benefit analysis data, HP testing and eradication should probably be individualized,[6,7,58] taking into account the presence of other risk factors such as history of complicated or uncomplicated peptic ulcer, old age, recent-onset dyspepsia, treatment with anticoagulants,[2,9,59–61] and the duration[59–64] and perhaps the type (aspirin or non-aspirin) of NSAID use.[5,7]

Appendix: Supplementary Data

To access the supplementary materials accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org.

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Review

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Respiratory Diseases and *Helicobacter pylori* Infection: Is There a Link?

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Key Words
Helicobacter pylori · Respiratory diseases · Chronic obstructive pulmonary disease · Bronchiectasis · Tuberculosis · Lung cancer · Bronchial asthma

Abstract
Recent studies suggest an epidemiological association between *Helicobacter pylori* infection and several extragastroduodenal pathologies, including cardiovascular, rheumatic, skin and liver diseases. The observed associations might be explained by a role of *H. pylori* infection in the pathogenesis of certain extradigestive disorders, as a variety of inflammatory mediators are activated by *H. pylori* infection. The present review summarizes the current literature, including our own studies, concerning the association between respiratory diseases and *H. pylori* infection. A small number of epidemiological and serologic case-control studies suggest that patients with chronic obstructive pulmonary disease have an increased seroprevalence of *H. pylori*. A frequent coexistence of bronchiectasis and *H. pylori* infection has also been found. Moreover, recent studies have shown an increased prevalence of *H. pylori* infection in patients with pulmonary tuberculosis and in those with lung cancer. On the other hand, bronchial asthma does not seem to be related to *H. pylori* infection. At present, there is no definite proof of a causal relationship between *H. pylori* and respiratory diseases. The primary evidence rests on case-control studies, concerning relatively small numbers of patients. Future studies should be large enough for moderate-sized effects to be assessed or registered reliably. The activation of inflammatory mediators by *H. pylori* infection might be the pathogenetic mechanism underlying the observed associations. Therefore, the role of genetic predisposition of the infected host, the presence of strain-specific virulence factors and the serum concentration of proinflammatory markers in *H. pylori*-infected patients with respiratory diseases need further evaluation.

Introduction

Helicobacter pylori is a spiral-shaped, microaerophilic and Gram-negative bacterium. *H. pylori* infection affects approximately 50% of the world population [1]. It is well known that this bacterium possesses a well-defined battery of virulence factors. These factors allow the organism to colonize the gastric mucosa, evade host defense and, finally, damage host tissue [2, 3]. Extensive clinical trials, carried out in the past few years, have proved the role of *H. pylori* as the main cause of both chronic gastritis [4] and peptic ulcer disease [5]. This bacterium is also causally related to low-grade B-cell lymphoma of gastric

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Table 1. Extradigestive nonrespiratory disorders for which an association with *H. pylori* infection has been discussed

Skin diseases
Prurigo nodularis
Pruritus cutaneous
Idiopathic chronic urticaria
Vascular diseases
Coronary artery disease
Stroke
Primary Raynaud's phenomenon
Autoimmune diseases
Behcet's disease
Rheumatoid arthritis
Autoimmune thrombocytopenia
Schoenlein-Henoch purpura
Other diseases
Diabetes mellitus
Growth retardation
Chronic idiopathic sideropenia
Pancreatic cancer

mucosa-associated lumphoid tissue [6]. Moreover, *H. pylori* infection has been established as a risk factor for the development of gastric adenocarcinoma [7]. Finally, recent studies indicate that *H. pylori* might be related to nonulcer dyspepsia [8].

Recent studies suggest an increased *H. pylori* prevalence in patients with various extragastrointestinal disorders, including skin, cardiovascular, rheumatic and liver diseases. Table 1 summarizes those extradigestive pathologies, characterized by a high prevalence of *H. pylori* infection [9, 10]. At present, there is no definite proof of a causal relationship between *H. pylori* and these diseases. The observed associations might be explained by a potential etiopathogenetic role of *H. pylori* infection in these disorders. It is well known that *H. pylori* colonization of the gastric mucosa stimulates the release of a variety of proinflammatory cytokines, including interleukin (IL)-1, IL-8 and tumor necrosis factor-α. Moreover, a crossmolecular mimicry between bacterial and host antigens exists in *H. pylori*-infected patients. Therefore, *H. pylori* might have a pathogenetic role in diseases characterized by abnormal activation of inflammatory mediators and/or induction of autoimmunity [11, 12].

Chronic inflammation and increased immune response have been observed in a variety of respiratory disorders, including chronic obstructive pulmonary disease (COPD) and bronchiectasis [13–15]. Moreover, active pulmonary tuberculosis (TB) is frequent among patients with partial gastrectomy for peptic ulcer disease [16]. Finally, the prevalence of chronic bronchitis in peptic ulcer patients is increased two- to three-fold compared with findings in ulcer-free controls [17]. Based on these observations, many recent studies have evaluated the relation between various respiratory disorders and *H. pylori* infection. Table 2 summarizes those respiratory diseases whose association with *H. pylori* infection has been studied in the literature [18].

Table 2. Respiratory diseases studied for a relationship with *H. pylori* infection

COPD
Bronchiectasis
Lung cancer
Pulmonary tuberculosis
Bronchial asthma

The aim of the present report is to provide a critical review of the current literature, including our own studies, as regards the association between respiratory diseases and *H. pylori* infection.

H. pylori Infection and COPD

COPD is a chronic disorder, characterized by not fully reversible and usually progressive airflow limitation. This limitation is thought to be associated with an abnormal inflammatory response of the lungs to noxious particles and/or gases [19]. COPD represents a leading cause of morbidity and mortality worldwide. Moreover, it results in an economic and social burden that is both substantial and increasing [20].

COPD had been associated with peptic ulcer disease many years ago. Three epidemiological studies, published between 1968 and 1986, showed that the prevalence of COPD in peptic ulcer patients was increased two- to three-fold compared with that in ulcer-free controls [17, 21, 22]. Moreover, a follow-up study, concerning a large population, demonstrated that chronic bronchitis was a major cause of death among patients with peptic ulcer disease [23]. The impact of cigarette smoking on development of both disorders was originally thought to be the major factor underlying the reported association. However, recent studies showed that the role of tobacco consumption in ulcerogenesis is minor and *H. pylori* infec-

tion seems to be the main cause of peptic ulcer disease [24, 25].

Therefore, in 1998, Caselli et al. [26] carried out a prospective pilot study in a sample of 60 bronchitic patients and found an increased *H. pylori* seroprevalence (81.6 vs. 57.9% in controls). Moreover, for the first time, they showed that *H. pylori* infection per se might be related to an increased risk of developing chronic bronchitis. Two years later, a large epidemiological study in a Danish adult population showed that COPD might be much more prevalent in *H. pylori* immunoglobulin (Ig)G seropositive women than in uninfected ones [27]. In order to further investigate the reported association, we performed two case-control studies in the Greek population. In the first, we studied a cohort of 144 patients with chronic bronchitis and 120 control subjects. We found that *H. pylori* seroposivity in patients was significantly higher than that in controls [28]. More recently, we assessed the seroprevalence of *H. pylori* and especially of the high-virulent cytotoxin-associated gene A (CagA)-positive strains in patients with COPD. An increased prevalence of these strains have previously been found in several other extragastroduodenal pathologies, characterized by activation of inflammatory mediators (i.e. ischemic heart disease, rosacea) [29, 30]. According to our results, both anti-*H. pylori* and anti-CagA seropositivity were significantly higher in patients than in control subjects, whereas no statistically significant difference, as regards the spirometric values, was detected between *H. pylori*-infected COPD patients and uninfected ones [31].

A more recent study by Kanbay et al. [32] concerning the *H. pylori* seroprevalence in a subgroup of COPD patients (those with chronic bronchitis) confirmed our results. They found that *H. pylori* seropositivity in bronchitic patients was significantly higher than that in controls (66.1 vs. 57.7%, respectively). Moreover, Gencer et al. [33] showed that *H. pylori* IgG levels might be correlated with the severity of COPD.

The mechanisms underlying the suggested association between COPD and *H. pylori* infection are unclear. Both *H. pylori* colonization of gastric mucosa and COPD development are related to old age, male sex and low socioeconomic status [20, 34]. In all reviewed studies, COPD patients were well matched with control subjects for all these parameters. However, as *H. pylori* infection is usually acquired during childhood, matching for socioeconomic status should be performed for childhood and not for the time of study. Therefore, inappropriate matching for socioeconomic status should be regarded as a limitation of all mentioned studies. Cigarette smoking could be another confounding factor. It is well known that tobacco use represents the major cause of COPD [19]. On the other hand, data on the relation between *H. pylori* infection and smoking habits are controversial. A low [35], normal [36] and high [37] *H. pylori* prevalence in smokers has been reported in the literature. Therefore, and as the relation between tobacco use and *H. pylori* remains unclear, the possible impact of cigarette smoking on both COPD development and *H. pylori* infection should be regarded as a limitation of all reviewed studies.

There are no studies in the literature focused on the potential etiopathogenetic role of *H. pylori* infection in COPD. It is well known that *H. pylori* and particularly CagA-positive strains, whose prevalence in COPD patients is extremely increased, stimulate the release of a variety of proinflammatory cytokines, including IL-1, IL-8 and tumor necrosis factor-α [38, 39]. Moreover, the eradication of *H. pylori* leads to normalization of serum cytokine levels [40]. Inflammation is a prominent feature of COPD, as shown by the presence in the airway of activated neutrophils and macrophages and the increased number of inflammatory mediators [41–43]. Recent studies showed that cytokines identical to those stimulated by *H. pylori* are released during the course and exacerbations of COPD, and especially IL-8 might also be implicated in the pathogenesis of the disease [44–46]. The underlying mechanisms, which induce and control this inflammatory process in COPD, are still unclear. Therefore, we could hypothesize that *H. pylori* infection might play a proinflammatory role and cotrigger COPD with other more specific environmental, genetic and yet unknown factors.

In conclusion, the primary evidence for an association between *H. pylori* infection and COPD rests on serologic case-control studies. Future studies should be focused on estimating the relative risk of developing COPD for *H. pylori*-infected patients. The effect of *H. pylori* eradication on the natural history of the disease needs further evaluation as well. Finally, the pathogenetic mechanisms underlying a possible link between *H. pylori* infection and COPD must be clarified.

H. pylori Infection and Active Bronchiectasis

Bronchiectasis is an abnormal and permanent dilation of bronchi, due to chronic inflammation and destruction of the structural components of the bronchial wall. High levels of proinflammatory cytokines are present in air-

way secretions, and neutrophils are the predominate cells in the airway lumen. In patients with active bronchiectasis, bronchial damage is thought to exist due to neutrophil inflammatory products, released in response to bacterial infection [47, 48].

In 1998, Tsang et al. [49] found an increased *H. pylori* seroprevalence (76 vs. 54.3% in controls) in patients with active bronchiectasis. A positive association between 24-hour sputum volume and *H. pylori* seropositivity in those patients was also detected. The authors hypothesized that the inhalation of the bacterium into the respiratory tract might lead to a chronic bronchial inflammatory disorder such as bronchiectasis. However, neither identification of *H. pylori* in human bronchial tissue nor isolation from bronchoalveolar lavage fluid have yet been achieved [34]. Moreover, it has not been identified in culture and histopathological examination of protected catheter brush and biopsy specimens from the bronchiectatic site in patients with active bronchiectasis [50].

According to recent studies, chronic airway inflammation in bronchiectasis seems to be primarily cytokine mediated [51, 52]. Therefore, the activation of systemic inflammatory mediators by chronic *H. pylori* infection and not the spilling or inhalation of *H. pylori* into the airway lumen could explain the increased prevalence of *H. pylori* infection in patients with active bronchiectasis.

In conclusion, the possible association between *H. pylori* and bronchiectasis seems intriguing and might have a pathogenetic basis. However, studies in larger series are needed to confirm this association and to clarify the underlying mechanisms.

H. pylori Infection and Lung Cancer

Primary carcinoma of the lung represents a major health problem. In 2003, 171,900 new estimated diagnoses and 157,200 deaths from lung cancer occurred in the United States. The environmental causes of lung cancer have been the focus of intense epidemiologic and experimental research for more than 50 years. The resulting evidence associates lung cancer development with active and passive smoking, a variety of occupational agents and indoor and outdoor air pollution [53, 54].

In a recent study, Gocyk et al. [55] showed an increased *H. pylori* seroprevalence (89.5 vs. 64% in controls) in a cohort of 50 patients with lung cancer. Moreover, anti-CagA seropositivity was significantly higher in patients than in control subjects (63 vs. 21.5%, respectively). An extremely high gastrin concentration in both serum and bronchoalveolar lavage was detected. Tumors were also characterized by an enhanced mRNA expression for gastrin and its receptor, as well as for cyclooxygenase 2. Therefore, the authors hypothesized that *H. pylori* might contribute to lung carcinogenesis via induction of gastrin synthesis. Gastrin might induce increased mucosal cell proliferation of bronchial epithelium and lead to atrophy and induction of cyclooxygenase 2. The same mechanism has been proposed for the development of gastric cancer in *H. pylori*-infected patients [56]. However, although some authors have also shown an increased gastrin concentration in serum and bronchoalveolar lavage fluid in lung cancer patients [57, 58], others did not confirm this finding [59].

Therefore, in order to further investigate the observed relation, we recently assessed the *H. pylori* seroprevalence in a cohort of Greek patients with lung cancer. In our study, the *H. pylori* seropositivity did not differ significantly between lung cancer patients and controls (61.1 vs. 55.9%, respectively). Concerning the mean serum concentration of IgG antibodies against *H. pylori*, no significant difference between the two groups had been detected [60].

However, a more recent study including 43 patients with nonsmall cell carcinoma and 28 control subjects showed that seropositivity for *H. pylori* was significantly higher in patients than in control subjects. Moreover, the high-virulent vacuolating toxin-associated positive strains were more prevalent in patients with lung cancer [61].

In conclusion and as the association between *H. pylori* infection and lung cancer remains a matter of debate, we believe that further studies are needed to confirm the existing results in a larger number of patients. Moreover, as it was mentioned previously, the possible impact of cigarette smoking on both lung cancer and *H. pylori* infection should be regarded as a limitation of all reviewed studies. Finally, the pathogenetic mechanisms underlying a possible association between these two diseases must be clarified.

H. pylori Infection and Pulmonary TB

Although there is a lack of epidemiological evidence concerning the worldwide prevalence of TB, it has been estimated that one third of the world population is infected with *Mycobacterium tuberculosis*, and there are ten million new cases of active TB each year. The vast major-

ity of them occur in the developing countries, where TB remains a common health problem [62].

In 1992, Mitchell et al. [63] examined the epidemiological factors predisposing to *H. pylori* colonization of the gastric mucosa in a southern China population. They found that *H. pylori* infection might be associated with a previous history of active pulmonary TB. More recently, Woeltje et al. [64] found that a history of peptic ulcer disease was one of the identified risk factors for a positive tuberculin skin test in newly hospitalized patients. Unfortunately, there were no data concerning the *H. pylori* seroprevalence in the studied population.

In order to further investigate the possible association between pulmonary TB and *H. pylori* infection, in 1998, Sanaka et al. [65] performed a serologic case-control study in a hospitalized population. No difference in *H. pylori* seroprevalence among 40 inpatients on antituberculosis chemotherapy for less than 3 months, 43 TB patients on chemotherapy for more than 3 months and 60 control subjects was detected (73.3, 65 and 69.8%, respectively). However, the possible eradication of *H. pylori* by antituberculosis drugs represented a potential confounding factor of the study. It is well known that both rifampicin and streptomycin are effective against *H. pylori*, and eradication of *H. pylori* infection during antituberculosis therapy has been reported previously [66, 67].

We recently examined the seroprevalence of *H. pylori* in a cohort of TB patients before the initiation of antituberculosis treatment. A total of 80 TB patients and 70 control subjects, well matched for age, sex and social status, were recruited into this study. The *H. pylori* seroposivity in the TB group was significantly higher than that of controls (87.5 vs. 61.4%). The mean serum concentration of IgG antibodies against *H. pylori* was also significantly higher in patients than in control subjects [68].

In conclusion, data in the literature on the relationship between *H. pylori* infection and pulmonary TB are conflicting, although a frequent coexistence of these infections has been reported. Poor socioeconomic and sanitary conditions during childhood could be a factor responsible for this coexistence. It is well known that in developing countries, acquisition of both *H. pylori* and *M. tuberculosis* occurs early in life. On the other hand, susceptibility to both bacteria induced by common host genetic factors might be responsible for the association of these two infections. It has been suggested that HLA-DQ serotype may be associated with increased susceptibility to *H. pylori* infection [69]. Recent studies showed that the same serotype contributes to enhanced mycobacterial survival and replication [70]. Therefore, we believe that studies focused on the common, either genetic or environmental, predisposition to both bacteria are needed.

H. pylori Infection and Bronchial Asthma

In 2000, Tsang et al. [71] assessed the seroprevalence of *H. pylori* in asthmatic patients. They found that *H. pylori* seroprevalence did not differ significantly between patients with bronchial asthma and control subjects (47.3 vs. 38.1%). Moreover, serum concentration of IgG antibodies against *H. pylori* did not correlate with spirometric values and duration of asthma. The authors concluded that bronchial asthma might not be associated with *H. pylori* infection. A more recent study showed no significant association between mild asthma and *H. pylori* infection [72]. Moreover, there is the lack of a theoretical hypothesis that might explain a possible link between these two diseases.

Therefore, we believe that our knowledge on the association between *H. pylori* infection and respiratory diseases is unlikely to be advanced by more studies concerning the prevalence of *H. pylori* infection in patients with bronchial asthma.

Conclusions – Future Challenges

At present, there is no definite proof of a causal relationship between *H. pylori* and respiratory diseases. The primary evidence for an association between a variety of respiratory diseases (COPD, bronchiectasis, lung cancer and pulmonary tuberculosis) and *H. pylori* infection rests on case-control studies. Case-control studies could never prove a causal relationship. Moreover, there is a lack of studies focused on the pathogenetic link between respiratory diseases and *H. pylori*.

We believe that larger studies should be undertaken to confirm the observed results. The activation of inflammatory mediators by *H. pylori* infection might be the pathogenetic mechanism underlying the observed associations. The role of genetic predisposition of the infected host, the presence of strain-specific virulence factors (CagA and vacuolating toxin) and the serum concentration of proinflammatory markers in *H. pylori*-infected patients with respiratory diseases need further evaluation as well. Finally, randomized control studies should be undertaken in order to clarify the effect of the *H. pylori* eradication to the prevention, development and natural history of these disorders.

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REVIEW

Clinical outcome of patients with *Helicobacter pylori* infection: the bug, the host, or the environment?

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It is well established that only a minority of patients with *Helicobacter pylori* infection develop severe inflammation leading to peptic ulcer or gastric cancer. Recent evidence suggests that the virulence factors of the organism do not seem crucial in the progression of inflammation towards a more severe disease. It seems probable that other host derived and environmental factors are more significant in determining clinical outcome but additional studies are needed to clarify the underlying mechanisms involved in the pathogenesis of infection.

Helicobacter pylori is a micro-acrophilic, Gram negative, slow growing, spiral shaped, and flagellated organism. Its most characteristic enzyme is a potent multi-subunit urease that is crucial for its survival at acidic pH and for its successful colonisation of the gastric environment, an area that few other microbes can colonise. *H pylori* infection is probably the most common chronic bacterial infection of humans, present in almost half of the world population.[1] The presence of the bacterium in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases, including chronic atrophic gastritis (a precursor of gastric carcinomas), peptic ulceration, and mucosa associated lymphoid tissue (MALT) lymphomas.

Because of its importance as a human pathogen investigators have sequenced the complete genome of two representatives *H pylori* strains (*26695* and *J99*) by the whole genome random sequencing method.[2 3] Comparing *H pylori* genes with genes of known function in other bacteria gave immediate insights into *H pylori* metabolism, structure, adaptive mechanisms, and virulence. In addition, comparison of the genomic sequence of the two independent clinical isolates has shown that they are highly conserved, with only 7% of the proteins being strain specific.

The pathogenesis of *H pylori* associated gastroduodenal disease remains poorly understood. It is clear that only a minority of infected people develop severe inflammation leading to peptic ulcer or gastric cancer. What are the factors that decide if an infected person will develop severe disease?

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H PYLORI RELATED FACTORS

Virulence factors of *H pylori* may be divided into colonisation factors, factors that allow it to evade host defence, and factors that are responsible for tissue injury.

Colonisation factors

Colonisation factors are attributes of an organism that allow it to establish its presence and to persist despite the host's attempts to rid himself of infection.

Flagella and motility

H pylori has been shown to require flagella for infection of the stomach. Flagella allow the bacterium to swim across the viscous gastric mucus and reach the more neutral pH below the mucus. To analyse whether flagella themselves or motility is needed by these pathogens, investigators constructed flagellated non-motile mutants. Their results support a model in which motility is used for the initial colonisation of the stomach and also to attain full infection levels.[4]

Urease system

H pylori synthesises urease constitutively. As urease hydrolyses urea to form ammonia and carbon dioxide, and ammonia can absorb acid to form ammonium, it is natural to suspect that this dedication to make urease has a relation to survival and growth in the acidic environment of human stomach. This suspicion has been confirmed in animal models but it is not certain that the requirement for urease is for colonisation as well as for infection.[5]

There are data showing that the organisms do buffer their periplasm that lies between their inner and outer membrane, in acidic pH, using their intrabacterial urease activity.[6] In contrast with surface or free urease, measurement of intrabacterial urease activity at different pH values, shows low urease activity at neutral pH, rapid increase between pH 6.0 and 5.0, and steady activity down to a pH of 2.5 but still present at pH 2.0.[7] Expression of the *H pylori* ureI gene is required for acidic pH activation of cytoplasmic urease.[8]

Adhesins

H pylori selectively binds to gastric epithelial cells and its colonisation of the digestive tract is limited to areas lined by gastric type epithelial cells. On adhesion, tyrosine phosphorylation and cytoskeletal rearrangement occurs, leading to a remodelling of the apical surface of the epithelial cells.[9] Several epithelial structures have been

Abbreviations: MALT, mucosa associated lymphoid tissue; IL, interleukin; Hsp, heat shock protein; LPS, lipopolysaccharides; Hp-NAP, neutrophil activating protein of *H pylori*; cagA, cytotoxin associated gene A

implicated in adhesion, including lipids, gangliosides, and sulphated carbohydrates, but to date the adhesins on the bacterial surface that bind to the epithelium are poorly understood.

As of yet, no adhesions have been confirmed as being important for in vivo survival of *H pylori*. With the sequence of the *H pylori* genome in hand, it should be possible to more easily determine the role of specific genes in virulence. Genes of immediate interest are the OMPs, which may undergo phase and antigenic variation and may represent adhesions.[10]

Adhesion is necessary for the initiation of the inflammatory cascade. In particular adhesion is a prerequisite for interleukin 8 (IL8) secretion by gastric epithelial cells.[11] Adherence also promotes the development of more severe disease. BabA adhesin binds the Lewis b blood group antigen on the gastric epithelium and is associated with duodenal ulcer, distal gastric cancer and more severe gastritis.[12]

Other factors that may also participate in *H pylori* adhesion are AlpA and AlpB. Both of these are required for adhesion to human gastric tissue sections.[13] BabA and Alp proteins are members of the large family of related outer membrane proteins (Hop proteins). These proteins are not present in all strains of *H pylori* and thus may represent means by which the pathogen gains control of the host response.

A recent described adhesin is a sialic acid binding adhesin (SabA). The ability of many *H pylori* strains to adhere to sialylated glycoconjugates expressed during chronic inflammation might contribute to virulence and the extraordinary chronicity of *H pylori* infection.[14]

Heat shock proteins (Hsp)

H pylori expresses two Hsp, A and B. They are highly antigenic. The clinical outcomes of *Hpylori* infection are not related to HspA antigenicity or to sequence variation.[15] Recent data suggest that a common epitope is present in human hsp60 and its bacterial homologue hspB.[16] Thus infection with *H pylori* may induce antibodies against bacterial hspB that cross react with human hsp60, through the molecular mimicry of these proteins. On the other hand, it is well established that the immune response to hsp60 is closely associated with MALT lymphoma.[17] Currently patients with gastric disease other than MALT lymphoma and increased IgG titres to hsp60 are under careful follow up to see whether they will develop gastric MALT lymphoma.[16] If this occurs it seems reasonable to hypothesise that hspB is closely associated to pathogenesis of MALT lymphoma.

Metal acquisition proteins

Adaptation of *H pylori* to the conditions in the gastric mucosa includes acquisition mechanisms that overcome a temporary lack of the metals iron, nickel, and zinc. Iron is essential for maintaining the basic energy and redox metabolism, whereas nickel is an essential cofactor of urease, an important virulence determinant of *H pylori*. However, as overacquisition of iron, nickel, and other metals is deleterious, the control mechanisms regulating the intracellular availability of these metals are of crucial importance.

Iron responsive regulation in prokaryotes is usually mediated through the ferric uptake regulator (Fur) protein. Fur homologs downregulate the expression of genes involved in iron uptake when the cytoplasmatic ferrous iron concentration increases, thus abolishing iron acquisition. Iron responsive regulation has been seen in *H pylori*, and genetic analysis showed that *H pylori* possesses a Fur homologue.[18] The *H pylori* ferritin protein Pfr is a member of the non-heme ferritin subfamily, all of which store iron in the inner space of a multimeric protein shell consisting of 24 identical subunits. The protein plays a substantial part in the storage of iron and protects the bacteria from metal toxicity.[19] Ferritins thus catalyse a function that is the exact opposite of that of iron uptake systems, which increase the cytoplasmic iron concentration.

Induction of hypochlorhydria

It is well established that acute infection is accompanied by transient hypochlorhydria.[20] Suggestions for the mechanism by which *H pylori* increases the gastric pH include: (1) presence of acid neutralising substances (ammonia) in the infected gastric mucosa, (2) increased levels of cytokines such as IL1b, which is known to inhibit gastric acid secretion, (3) exposure of parietal cells to acid inhibitory substances released by *H pylori*.

H pylori interferes with parietal cell acid production by two mechanisms: (1) the bacterium increases proton permeability at the secretory membrane of the parietal cell (it causes back diffusion of protons from the secretory canaliculus into the cytosol of the parietal cell), and (2) in addition inhibits H^+/K^+-ATPase activity.[21]

Factors that allow organism to evade host defence

The bacterium possesses a well defined battery of virulence factors that allow it to evade host defence. These are shedding of surface proteins, catalase, superoxide dismutase, and poorly reactive lipopolysaccharide.

It has been shown that after successful colonisation some bacteria are killed by host defensive mechanisms resulting in shedding of their surface proteins. These proteins are connected to receptors on the surface of other bacteria and bind cytokines and immunoglobulins. This has been interpreted as an indirect defensive mechanism of *H pylori* to evade host defence.

Despite the fact that the organism is an obligate aerobe, it is unable to grow in atmospheric concentrations of oxygen. Microaerophilic organisms, like *H pylori*, are particularly vulnerable to the detrimental effects of oxygen and oxidative stress. Nevertheless, they do possess some of the enzymatic machinery needed to eliminate or minimise toxic oxygen derived products. These enzymes are superoxide dismutase, catalase, and several putative peroxidases.[22]

It is well known that bacterial lipopolysaccharides (LPS) may induce both strong local and systemic inflammation in animals as well as humans, and therefore, *H pylori* LPS is one of the factors that could potentially influence local gastric inflammation and the clinical outcome during an *H pylori* infection. In general, *H pylori* LPS is much less potent in activation of inflammatory cells than LPS from members of the family enterobacteriaceae, for example, *Escherichia coli* and *Salmonella spp*. Despite its comparatively low toxic activity, *H pylori* LPS has been shown to activate inflammatory cells to produce different cytokines and chemokines, such as TNFα, IL8, IL1, and monocyte chemotactic protein-1.[23] In addition, the LPS of some strains contains structures identical to the fucosylated Lewis x and Lewis y blood group antigens expressed on the gastric mucosa. The antigenic mimicry may result in immune tolerance against antigens of the pathogen or in induction of autoantibodies that recognise gastric epithelial cells, frequently seen in patients with chronic active gastritis.[24]

Key points 1

H pylori colonisation factors do not seem crucial in the pathogenesis of infection. Adhesins (mainly BabA) are associated with more severe inflammation but this requires further research.

Key points 2

Bacterial lipopolysaccharides might induce autoantibodies that are implicated in the pathogenesis of chronic active gastritis.

Factors that induce tissue injury

The vacuolating cytotoxin A (vacA)

The vacuolating toxin (VacA) is an important determinant of *H pylori* associated gastric disease. The association of vacA with peptic ulcer disease, MALT lymphoma, and gastric cancer has been well validated, at least in Europe where the background population has a low incidence of type I strains (defined as cagA and vacA positive).[29] *H pylori* vacA s1 strains have been associated with the occurrence of peptic ulcer disease[30] and vacA m2 allele is also associated with peptic ulcer disease and gastric cancer.[31] The original hypothesis was that the s1 genotype was associated with duodenal ulcer disease and the s2 genotype had low ulcerogenic potential. Data are now overwhelming that vacA genotyping is not useful to predict symptoms, presentation, response to therapy, or degree of inflammation. VacA genotyping is useful to predict cagA status.[29]

The mechanism of action of vacA has recently been further described. Binding of free or membrane bound vacA to epithelial cells is receptor mediated. VacA forms pores in lysosomal membranes, increasing anion permeability and generating vacuoles.[29] In addition, vacA has been shown to reduce transepithelial resistance by loosening tight junctions.[30] Finally, vacA inhibits de novo antigen binding by MHC class II receptor, a mechanism that can contribute to a down regulation of the host immune response, which has been correlated in mice with increased gastritis and atrophy.[31]

The neutrophil activating protein of *H pylori* (*H pylori*-NAP)[32]

H pylori-NAP has been shown to be chemotactic for neutrophils and monocytes. It induces the production of oxygen radicals in human neutrophils via a cascade of intracellular activation events that may contribute to the damage of the stomach mucosa. This protein has recently been shown to be an important antigen in the human immune response to *H pylori* infection, making it a strong vaccine candidate. In addition, mice vaccinated with recombinant *H pylori*-NAP were protected against *H pylori* challenge. A number of other reports have proposed that *H pylori*-NAP acts as an adhesin being capable of binding several different compounds in vitro.

The cytotoxin associated gene A (cagA) and the cag associated pathogenicity island (cag-PAI)

CagA is the product of one gene from cag-PAI and is involved in the cytoskeletal changes and host proteins dephosphorylation that occur when a cagA positive strain adheres to host cell. The cag-PAI is a type IV secretory apparatus that injects cagA into the host cell and is involved in the induction of cytokine expression in gastric epithelial cells, which is seen as a pronounced increase in IL8 expression.[33] Cytokine induction associated with the cag-PAI is independent of cagA. The signal transduction pathways are thought to be through nuclear factor kB (NF-kB) and activator protein 1 (AP1). Before activation, NF-Kb, resides in the cytoplasm and upon activation it translocates to the nucleus, where it binds to DNA at kB sites and upregulates IL8 gene production.[34]

People infected with *H pylori* who have a functional cag-PAI have increased mucosal concentrations of IL8, pronounced neutrophilic infiltration into the gastric mucosa, and a theoretically increased risk of developing peptic ulcer and gastric cancer. However, in East Asia where more than 90% of isolates possess the cag-PAI, a relation of the cag-PAI and clinical outcome has not been reported. Conversely, in Western countries, where *H pylori* strains lacking cag-PAI are found in higher percentage, there are data showing increased likelihood of symptomatic outcome.[35] Nevertheless, the presence of a functional cag-PAI has no predictive value regarding current or future clinical presentation. *H pylori* strains lacking a functional cag-PAI are not commensal as they are also found in patients with peptic ulcer disease or gastric cancer, only at lower frequency.

IceA

IceA is a gene that is induced by contact with epithelium. The gene product is unknown but it seems to be a bacterial restriction enzyme. There are two variants of the iceA gene, iceA1 and iceA2. The initial studies suggested that iceA1 was correlated with duodenal ulcer.[36] More recent studies are conflicting. In a large study involving four different countries (USA, Colombia, Japan, and Korea), to avoid the regional variation of *H pylori* genes, the results failed to confirm an association between iceA1 and clinical outcome,[37] but a more recent large study in Japan showed that the iceA1 allele is associated with increased gastric inflammation.[38]

HOST RELATED FACTORS

Several laboratories have provided evidence that the host response is an important determinant in *H pylori* associated disease progress. An alternative model of *H pylori* associated disease is the *H felis* mouse model, which has been extensively used to examine how the host response prevents and/or exacerbates *H pylori* induced gastroduodenal disease. In the mouse *H felis* infection model, several inbred strains of mice, exhibit severe inflammation/gastric atrophy ("high responders"), in contrast with others that are low gastritis/atrophy responders to *H felis* infection.[39] These results suggest that the nature of the host immune or inflammatory response to *H pylori* infection in humans might be more important in determining disease outcome than *H pylori* virulence factors.

In concordance to this hypothesis is the fact of rapid change worldwide in the incidence of gastric cancer and duodenal ulcer disease. This might be explained from a similar decrease in the prevalence of a particular virulence factor. However, several studies evaluating the prevalence of putative virulence factors in different birth cohorts have shown that this is not the case.[40]

Genetic susceptibility to infection has been reported from large epidemiological studies, which implies that the host response may be regulated from genetically determined factors. There are data from developed countries such as USA, which exhibit different prevalence among different ethnic groups of similar socioeconomic status.[40] Similar findings come from South East Asian countries in which the Malays have been shown to have consistently low prevalence compared with the Indians and Chinese.[41] These data show a racial linked genetic susceptibility to infection. Genetic susceptibility has been confirmed also, in studies showing that monozygotic twins reared apart or together had a higher rate of concordance of infection than did age matched dizygotic twins.[42]

Key points 3

Despite the results of initial studies from developed countries, *H pylori* factors that induce tissue injury do not correlate with a more severe clinical outcome.

One small study has shown significant association between the prevalence of the HLA-DQ5 genotype and *H pylori* infection with accompanying atrophic gastritis or intestinal metaplasia while investigations of the HLA-DQA1*0102 genotype noted a lower prevalence of DQA1*0102 among patients with gastric cancer and coexisting *H pylori* infection. This work in HLA may be pointing in an interesting direction but requires much larger studies, adjusted appropriately for the multiple comparisons being made, before any conclusions can be drawn.[41]

A host related factor that has been shown to predict disease progression is the size of parietal mass at the time of exposure to *H pylori*.[42] Those with a large cell mass and high acid output have an infection confined to the antrum, where the environment is less acidic and favours *H pylori* colonisation. These patients have antrum predominant gastritis and are likely to develop duodenal ulcers. To date this unique response to *H pylori* infection has not been linked to a distinct cytokine response; duodenal ulcer disease seems to require both hypersecretion of gastric acid and the activity of proinflammatory cytokines.[43]

On the other hand, for those with a small cell mass, acid production is insufficient to protect the corpus from infection and subsequent cellular degeneration compromises acid output still further. This favours the loss of specialised glandular cell types such as parietal and chief cells and the development of corpus predominant atrophy, which seems to be a critical initiating step in the progression towards gastric cancer.[43]

Other host related factors that have been shown to predict disease progression towards gastric cancer, are increased gastrin levels at the time of exposure to *H pylori*,[44] and single nucleotide polymorphisms in the gene encoding IL1b.[45] It is probable that single nucleotide polymorphisms in other genes encoding cytokines or cytokine receptors that influence the risk of gastric atrophy and cancer will be found.

ENVIRONMENTAL FACTORS

It is well established that environmental factors may also affect clinical outcome of *H pylori* infection. For example, migrating from a region with high prevalence of gastric cancer to a region with low prevalence did not reduce the rate of cancer in the migrants but resulted in an important reduction in risk for their offspring, suggesting that the environment is more important than genetics in determining the clinical outcome of an *H pylori* infection.

The environmental factors that seem most important in determining the pattern of gastritis (and thus the risk of any of the different *H pylori* outcomes) are the presence of childhood febrile illnesses and diet.

Childhood infections such as tonsillitis, infectious diarrhoeas, and diphtheria are associated with a pronounced decrease in acid secretion. Low acid secretion in childhood occurs also in malnutrition. Thus, regions where childhood infections and malnutrition are common would provide the ideal environment for *H pylori* colonisation and the development of corpus predominant atrophy, as discussed above. Diphtheria is especially prone to cause gastric damage and may even be a cause of gastric atrophy. Indeed, there are speculations considering that immunisation against diphtheria played an important part in the prevention of early onset atrophic gastritis and therefore, of gastric cancer.[46]

Key points 4

Genetic susceptibility to infection and single nucleotide polymorphisms in genes encoding cytokines or cytokine receptors might influence the clinical outcome of *H pylori* infection.

Key points 5

Epidemiological data suggest that dietary habits might influence the severity and clinical outcome of *H pylori* infection.

However, in regions where childhood infectious diseases, malnutrition and *H pylori* infection are all common, one would expect a high frequency of an accelerated development of corpus gastritis. This is not a universal finding suggesting that a number of other factors may also be important in determining whether atrophic gastritis develops after *H pylori* infection. In these regions there is a year round availability of fresh fruits and vegetables. Investigators speculated that ingestion of fresh fruits and vegetables might retard the development of gastric atrophy (the "banana hypothesis").

There is some evidence that establishes a long suspected correlation between salt intake, *H pylori*, and gastric cancer risk. In the Intersalt study,[47] authors note that, where measured appropriately, salt intake levels in African countries are considerably lower than in most other countries and they suggest that salt might be the permissive cofactor that is required for *H pylori* infection to act as a cancer risk factor.

Recent data suggest that some dietary habits might have antihelicobacter activity such as mastic gum (1 mg per day for two weeks)[48] or Chinese tea.[49]

CONCLUSIONS

H pylori is a common bacterial pathogen that colonises the gastric mucosa of over 50% of the world's population. All infected people exhibit chronic gastric inflammation, and about 1% of patients develop gastric cancers, including adenocarcinomas and MALT lymphomas. In 1994, the World Health Organisation International Agency for Research on Cancer classified *H pylori* as a type I, or definite carcinogen. Because the prevalence of gastric inflammation among *H pylori* infected patients varies between persons, countries, and geographical areas, *H pylori* disease related outcomes are believed to be determined by an interplay between bacterial factors, host factors, and their interaction with the environment.

Through novel techniques and experimental approaches, a great deal of progress has been made in our understanding of *H pylori* induced gastric inflammation. Although infection with *H pylori* is known to be a prerequisite for promoting peptic ulcer disease and gastric cancers, it has become increasingly clear that, in addition to the bacteria, host and environmental factors are involved. Elucidating these factors and delineating how they work together may ultimately lead to the development of novel therapeutic targets to combat these diseases.

Initially, there were data showing a clear predominance of *H pylori* virulence factors on human's disease outcome but additional studies, mainly from East Asia, failed to support this model. It seems probable that other host derived and environmental factors are more significant in determining clinical outcome, but additional studies are needed to evaluate the underlying pathophysiological mechanisms involved in the clinical outcome of infection.

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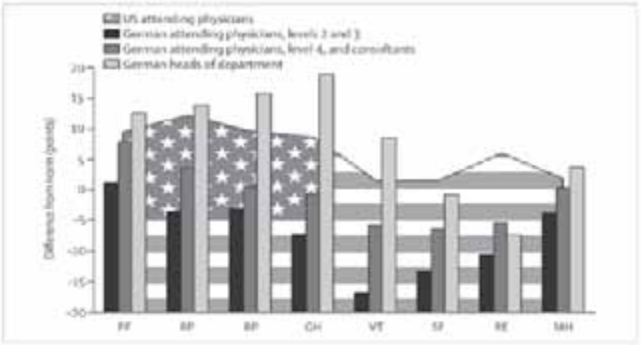

Figure: Extent to which average SF-36 scores of German and American physicians depart from respective norm values
Physical scales: PF=physical functioning, RP=role (physical), BP=bodily pain, GH=general health. Mental scales: VT=vitality, SF=social functioning, RE=role (emotional), MH=mental health.

We are not against hierarchies in hospitals per se. We believe that complex organisations, including hospitals, need a well defined command structure to allow processes to run smoothly. However, organisational structures have to fulfil organisational needs. When they lead to a negative working atmosphere and reduced health-related quality of life among employees, they are probably not serving organisational needs, let alone employees'. Thus, the chairman of the medical labour union Marburger Bund, Frank Ulrich Montgomery, has heartfelt support for demanding a cutback of hierarchical structures in German hospitals.[1]

We declare that we have no conflict of interest.

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Helicobacter pylori and gastro-oesophageal reflux disease

In their Seminar (June 24, p 2086),[1] Paul Moayyedi and Nicholas J Talley state that "A negative association with *Helicobacter pylori* exists, but eradication of *H pylori* does not seem to cause reflux disease". This statement rather creates confusion. For instance, if *H pylori* "protects" against gastro-oesophageal reflux disease (GORD) by inducing corpus gastritis associated with reduced acidity, then corpus gastritis also protects against duodenal ulcer disease.[2] Therefore, using the same argument, one could state that *H pylori* protects against duodenal ulcer disease, which is clearly irrational.

The increased incidence of GORD in the developed world might be explained not just by the declining prevalence of *H pylori* infection, as Moayyedi and Talley propose, but by healing of *H pylori*-associated peptic ulcer disease, which unmasks coexisting GORD.[2] Our data show that *H pylori* is frequent in GORD and even in non-endoscopic reflux disease,[3,4] and that *H pylori* eradication leads to better control of GORD symptoms and improves oesophagitis.[3] Others[5] have also reported improvement in reflux symptoms after *H pylori* treatment. Much evidence further potentiates the concern that the *H pylori* is not "protective" against GORD.[2]

H pylori could contribute to the pathogenesis of GORD via several mechanisms including release of several mediators (cytokines and nitric oxide) which could adversely affect the lower oesophageal sphincter; direct damage of the oesophageal mucosa by bacterial products; increased production of prostaglandins that sensitise afferent nerves and reduce lower oesophageal sphincter pressure; and increased acidity through gastrin release.

We declare that we have no conflict of interest.

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Authors' reply

Jannis Kountouras and colleagues present data to suggest that *H pylori* could contribute to the pathogenesis of GORD, and outline a plausible hypothesis. Unfortunately, the history

of medicine is littered with plausible hypotheses that have turned out to be false. The data they present are mainly mechanistic and epidemiological, which are potentially valuable but not conclusive.

Only well designed, double-blind, randomised controlled trials (RCTs) provide evidence that minimises bias and confounding. We based our conclusions on systematic reviews of RCTs wherever possible. We summarised one such systematic review[1] that indicated that *H pylori* eradication did not cause or protect against GORD in patients with peptic ulcer disease. That review did not find enough RCT evidence to determine whether *H pylori* eradication affects patients with pre-existing reflux disease. Since this systematic review, we have identified three further RCTs,[2–4] giving a total of 586 GORD patients infected with *H pylori* with evaluable data randomised to eradication therapy or no antibiotics. Patients were treated with proton pump inhibitors and followed up for 8–24 months. There was no significant effect of *H pylori* eradication on the relapse of symptoms: those allocated to active therapy had a relative risk of symptomatic relapse of 1·13 (95% CI 0·92–1·39). There was also no significant effect of *H pylori* eradication on the relapse of oesophagitis (1·95, 0·93–4·07). Other RCT data suggest that this conclusion probably extends to functional dyspepsia.[5]

Although we cannot exclude the possibility that *H pylori* eradication could have a small positive or negative effect on reflux disease, all the available data suggest that the infection is unlikely to have a major effect in either protecting against or causing GORD.

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Altruistic kidney donation

The report by Robert Montgomery and colleagues (July 29, p 419),[1] in which an altruistic kidney donor made two transplantations possible by a domino-paired donation procedure, describes further possibilities by which to expand the number of kidney transplants derived from living donors.

In the Netherlands, we run a national paired kidney exchange programme for ABO-incompatible and cross-match-positive donor-acceptor combinations under supervision of the Dutch Transplant Foundation.[2] From January, 2004, we enrolled 158 couples and found compatible donors for 82 recipients. Although our programme is efficient, we cannot match all patients and therefore alternative strategies are justified.

Living donor list exchange is an option but has met with ethical objections since it will increase the waiting time for blood type O recipients. However, utilitarian arguments hold that benefits for an entire patient group can outweigh disadvantages for a subgroup. Recently, the Dutch Health Council installed a committee to advise on list exchange restricted to unsuccessful pairs in our direct exchange programme.

Altruistic kidney donation to non-emotionally related people is another alternative.[3] At the Erasmus Medical Center, 23 individuals were assessed for this procedure, resulting in five directed, five non-directed single, and nine domino-paired donations—ie, 28 transplantations. Non-directed donor kidneys were allocated according to the criteria applied for cadaveric kidneys. Couples participating in domino-paired exchange had been unsuccessful in the direct-paired exchange programme.[4]

Alternatives by which to expand the living kidney donor pool should be integrated in a national exchange programme under supervision of an independent allocation authority.

We declare that we have no conflict of interest.

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Letter to the Editor

Helicobacter Pylori Infection in Dialysis Patients Undergoing Kidney Transplantation

Dear editor,

We have read with interest the letter to the editor of Dr Mukhtar and colleagues, and their suggestion that all patients awaiting a renal transplant should be preemptively screened and treated for *Helicobacter pylori* (*H. pylori*) infection. [1] The authors reported a high prevalence of up to 80% of *H. pylori* colonization in renal transplant recipients; However, recent data shows that the incidence of peptic ulcer in these patients is only 3%,[2] and that *H. pylori* infection seems to be less frequent than in the general population.[3-5] Potential reasons for this low prevalence include spontaneous seroconversion of *H. pylori* in up to 29% owing to long-standing immunosuppression,[3] or due to a defect in humoral immunity and a decrease in antibody response caused by concurrent medications or the high urea concentration observed in renal transplant recipients.[4,5]

We agree with the authors' comment that upper gastrointestinal mucosal lesions are common in patients with renal transplants with *H. pylori* being an important factor contributing to peptic ulcer disease. However, it should be noted that the case presented herein was under immunosuppressive medications that included mycophenolate mofetil shown to display a similar side-effect profile to non-steroidal anti-inflammatory drugs (NSAIDs) such as development of gastritis, duodenitis, esophagitis or ulcers,[6] with 3–8% cases of ulcer perforation or bleeding within 6 months.[7] Moreover, the patient received corticosteroids whose role in directly causing peptic ulcer disease may be controversial, but when combined with NSAIDs (in the present case with mycophenolate mofetil possessing a similar profile) they delay the healing of lesions caused by NSAIDs.[8] Therefore, we propose that the severe erosive antral gastritis, erosive duodenitis and anterior wall duodenal ulcer revealed by the second upper gastrointestinal (GI) endoscopy should not only be attributed to the existent *H. pylori* infection but also to the concurrent immunosuppressive medications.

From a further point of view, during the period between the two upper GI endoscopies, the patient was treated with omeprazole, an agent found to inhibit cyclosporine A metabolism, thereby increasing its serum concentration.[9] It would be interesting to know if the authors had monitored carefully the trough level of cyclosporine A during the initial omeprazole and the later *H. pylori* eradication therapies.

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Sometimes, the lentigines are arranged in a segmental pattern and unfrequently cross the midline. Two cases showing bilateral involvement have been signalled.[10,11] Histological examination of lentigines reveals a lentiginous pattern in most specimens but five cases, in which a jentigo pattern was found.[8,9,11,12,13] PUL has to be distinguished from segmental speckled lentigious naevus (SSLN), a zosteriform lentiginous lesion containing darker macules and/or papules (junctional naevi, compound naevi, blue naevi and/or Spitz naevi). Differentiation between PUL and SSLN, which may be difficult in patients with a dark phototype, appears to be essential because onset of malignant melanoma is reported in several cases of SSLN.[14]

The present case of PUL was characterized by extensive involvement of left hemisoma from C3 to L4 dermatomes. Lentigines trespassed midline in the lower back.

Histological evaluation demonstrated both lentigo simplex and jentigo. The combination of these two patterns has never been described before in patients with PUL. We suggest that the two biopsied lesions were in a different evolutive phase, according to the hypothesis of the biological 'continuum' from lentigines to melanocytic naevi. In particular, since melanocytic naevi would originate with a lentiginous pattern of growth and the nesting phenomenon would represent nevus cells entering a quiescent phase,[15] the lentiginous junctional naevus may represent the second stage in the natural history of lentigo.

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Primary cutaneous MALT-type lymphoma and *Helicobacter pylori*: a possible relationship

Marginal zone B-cell lymphoma (MZBCL) account for approximately 5% of primary B-cell lymphomas of skin.[1] Some authors believe that MZBCL affects only a few patients;[2] others consider that this entity accounts for the majority if not all primary cutaneous B-cell lymphomas (CBCL).[3]

A 53-year-old woman presented at our clinic with pruritic skin lesions on the trunk and upper arms, consisting of infiltrated deep red violaceus nodules with a brain-like surface surrounded by annular erythema and measuring 1–3 cm (fig. 1). Histopathological examination showed non-epidermotropic, dense lymphocytic infiltrate. Cytologically the infiltrate was mainly of B-cell phenotype with marginal zone B cells, CD20+ and CD45+ and a limited number of T cells CD45Ro and CD8.

A 32-year-old man with a 7-year history of hepatitis B presented multiple infiltrated pruritic nodules, which measured 1.5 × 1 cm and were located on the neck and trunk. The lesions had appeared 6 months before. Histopathological examination and immunohistochemistry

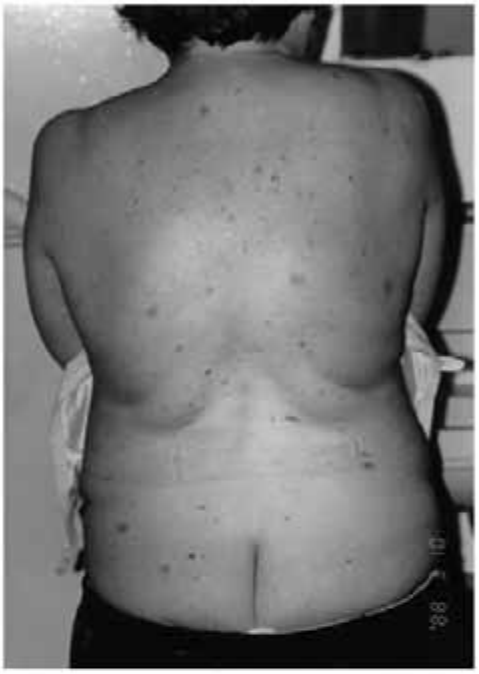

fig. 1 Multiple infiltrated deep red violaceus nodules measuring 1–3 cm on the trunk of the first patient.

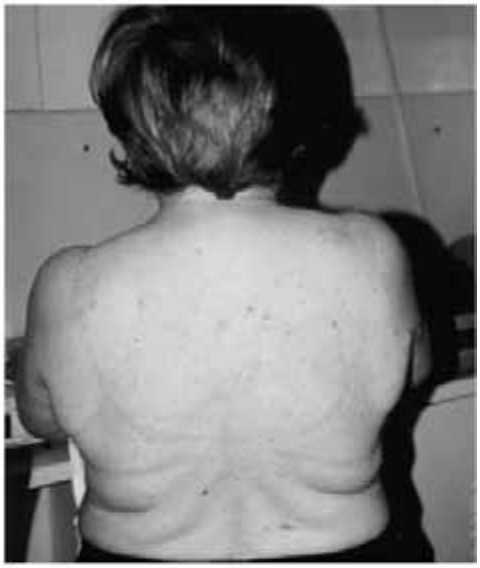

fig. 2 Clinical picture of the first patient 3 months after the third eradication treatment for *Helicobacter pylori*

study confirmed the clinical MZBCL. Staining for Bcl-6, CD10, CD21 and CD45Ra were positive. A small number of follicular and extrafollicular B cells showed expression of Bcl-2 protein. The t(14;18) translocation with reverse-transcriptase quantitative-polymerase chain reaction (TaqMan) method was not found. Laboratory tests were all normal, apart from confirming the previously diagnosed hepatitis B.

In both cases the investigations for MALT (mucosa-associated lymphoid tissue) lymphoma of the gastrointestinal tract, lung, salivary and thyroid glands were negative. We used molecular analysis for detecting if there existed clonal proliferation of lymphoid cells and it had showed to be negative. Other investigations including CT scan of the chest, abdomen and pelvis showed no abnormalities. The patients were advised to consult a gastroenterologist. A gastric mucosa biopsy revealed the presence of *Helicobacter pylori* and the patients were treated with clarithromycin, amoxicilin and omeprazole for 14 days, repeated three times a year. Three months after the end of this treatment the patients were free of skin lesions (fig. 2).

Santucci *et al.* in 1991 emphasized the clinical similarities (such as localized disease, low-tendency to disseminate and favourable response to local therapy) between primary CBCL and extracutaneous extranodal B-cell lymphomas.[3] Their study suggested that all cases of primary CBCL represent a distinct and unique type of extranodal B-cell lymphomas, and have proposed use the term 'skin-associated lymphoid tissue (SALT)-related B-cell lymphomas'.[3] The exact reason for the favourable prognosis remains uncertain, but it possibly included the antigenic dependency of the lymphoma. In a recent study of 32 patients, no one developed lymph node or internal involvement after a mean follow-up of more than 4 years.[4]

There is at present no consensus among pathologists regarding the definition of the term marginal zone B-cell lymphoma.[5] Cutaneous marginal zone lymphoma shares cytological and architectural features with extracutaneous extranodal marginal zone lymphomas. This is further emphasized by a case reported by Isaacson and Norton about a patient with synchronous low-grade B-cell lymphoma of marginal zone in the lung and a cutaneous lymphoma of identical pattern.[6]

Most marginal zone cell lymphomas express bcl-2 protein, but only molecular studies searching for t(14; 18) (q32: 21) are helpful in this differential diagnosis.[7,8] MALT lymphomas do not express CD5 and CD10 antigens, and is not associated with the interchromosomal 14 :18 translocation.[7,9]

The stomach is the most common site for MALT lymphoma, but it may also occur in the lungs, the thyroid gland, salivary glands and other organs such as the intestine. Approximately two-thirds of MALT lymphoma affecting the stomach is caused by infection of *H. pylori* and it has been shown that eradication of the organism can lead to regression of the lymphoma.[10] The response of gastric MALT lymphoma to *H. pylori* is dependent on the presence of T cells, and it is possible that T-cell component of cutaneous lymphoid hyperplasia and CBCL had a similar function.[10]

As with gastric lymphoma, the development of primary CBCL is regarded as being dependent on the acquisition of B-cell lymphoid tissue. The fact that in our two cases skin lesions progressively regressed after repeated eradication therapy for *H. pylori* makes us believe that there exists a close relation between CMZL and *H. pylori* colonization of the stomach. This observation has been poorly emphasized in the literature, although the skin is the second most common site for extranodal B-cell lymphoma after the gastrointestinal tract.

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Eccrine squamous syringometaplasia in a patient with systemic lupus erythematosus

Eccrine squamous syringometaplasia (SEE) was first defined in 1979[1] by King and Barr who provided histological criteria to differentiate it from squamous cell carcinoma.[1] SEE is a histopathologic process in which the cuboidal epithelium of the eccrine sweat ducts undergoes some metaplastic changes developing into mature squamous cells with keratinization, similar to the stratum spinosum of the epidermis.[2] SEE is an unusual dermatosis described mainly in chemotherapy-treated diseases. These metaplastic changes have also been reported in inflammatory, neoplastic and infectious skin diseases.[2] We report a patient diagnosed with systemic lupus erythematosus with cutaneous lesions characterized by SEE.

A 24-year-old woman diagnosed with epilepsy at the age of 13 and treated with carbamazepine for the first 10 years and gabapentin and clonacepam for another 3 years. She came to our department presenting a 2- to 3-month history of weakness, low-grade fever and dyspnea. One month prior to the visit she had developed multiple skin lesions on her face which had gradually extended to

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LETTERS TO THE EDITOR

Triple Levofloxacin-Based Rescue Therapy is an Accepted Empirical Third-Line Treatment

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TO THE EDITOR: We read with great interest the paper by Gisbert *et al.* (1) on third-line rescue therapy with levofloxacin after two *H. pylori* treatment failures, in which they concluded that levofloxacin-based rescue therapy constitutes an encouraging empirical third-line strategy after multiple previous *H. pylori* eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, and tetracycline. Indeed, first-line eradication therapy with proton pump inhibitor, clarithromycin, and amoxicillin fails in a considerable number of cases and also a second-line treatment with omeprazole-bismuth-tetracycline-metronidazole (or ranitidine-bismuth-citrate with these antibiotics) fails in a substantial proportion of treated patients. So far a standard third-line therapy is lacking and European guidelines (2) recommend culture, after second-line treatment failure, to determine microbial sensitivity to antibiotics. Nevertheless, endoscopy with biopsies for culture is expensive and therefore an effective empirical third-line regimen would be welcome. In this sense the above paper from Spain clearly proposes such a third-line rescue therapy. However, more data from different countries are needed. In this letter we would like to briefly report our experience on this matter. Thirty consecutive patients received the 10 day regimen omeprazole 20 mg b.i.d., ampicillin 1 g b.i.d., and levofloxacin 500 mg b.i.d., as a third-line empirical strategy after two previous *H. pylori* eradication failures, initially with first-line eradication triple regimen (omeprazole, amoxicillin, clarithromycin) and subsequently with second-line quadruple regimen (omeprazole, bismuth, metronidazole, and tetracycline). Eradication was confirmed with ^{13}C-urea breath test 4–10 wk after therapy. All patients took all the medications correctly and six (20%) reported mild-to-moderate adverse effects (mainly metallic taste, nausea, and diarrhea). Eradication was achieved in 21 of 30 (70%). In the nine (30%) patients in whom the levofloxacin-based third-line therapy failed, endoscopy with biopsies for culture was performed to determine microbial sensitivity to antibiotics. Our results are similar to those reported by Gisbert *et al.* (1) and it seems therefore, that indeed the triple levofloxacin-based rescue therapy can be introduced as empirical third-line treatment in cases in which recommended first- and second-line therapies have failed. In this manner a substantial number of cultures that determine the microbial sensitivity to antibiotics can be avoided with all the beneficial consequences concerning cost.

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Levofloxacin-Based Regimens: An Alternative for Second- and Third-Line Eradication Treatment After *H. pylori* Eradication Failures: Reply to Dr. Rokkas and Gisbert

TO THE EDITOR: *H. pylori* eradication therapy with proton pump inhibitor (PPI), clarithromycin, and amoxicillin fails in a considerable number of cases, and a rescue therapy still fails in more than 20% of these last patients. In a previous study, we evaluated the efficacy of a third-line levofloxacin-based regimen in patients with two consecutive *H. pylori* eradication failures, achieving eradication rates of 60–65%, thus suggesting that levofloxacin-based rescue therapy constitutes an encouraging empirical third-line strategy after multiple previous *H. pylori* eradication failures with key antibiotics such as amoxicillin, clarithromycin, metronidazole, and tetracycline (1). These encouraging results have been confirmed in the study by Rokkas *et al.*, in which eradication of *H. pylori* was achieved in 70% of the patients in similar circumstances and with a similar regimen (2).

These favorable results suggest that, perhaps, the levofloxacin-based regimen could be also prescribed for patients when only one previous eradication regimen has failed. After failure of a combination of a PPI-based triple regimen, the use of the so called quadruple therapy (that is, PPI, bismuth, tetracycline, and metronidazole) has been generally used as the optimal second-line therapy (3). However, this regimen requires the administration of four drugs with a complex scheme (bismuth and tetracycline usually prescribed every 6 h, and metronidazole every 8 h) and is associated with a relatively high incidence of adverse effects (3). Furthermore, this quadruple regimen still fails to eradicate *H. pylori* in approximately 20–30% of the patients, and these cases constitute a therapeutic dilemma, as patients who are not cured

with two consecutive treatments including clarithromycin and metronidazole will have at least single, and usually double, resistance (4).

Recently, some studies have evaluated the efficacy of new fluoroquinolones, such as levofloxacin, as second-line rescue regimens. In this context, we have performed a meta-analysis of studies comparing levofloxacin-based regimen with the traditional quadruple therapy for eradication failures, showing better results with the first combination (81% *vs.* 70%) (5). Furthermore, levofloxacin-based regimen had less adverse effects in general (19% *vs.* 44%), and less severe adverse effects in particular (0.8% *vs.* 8.4%), than the quadruple regimen (5). In summary, we believe that, although more homogeneous clinical trials are awaited providing more robust data, and the results of our meta-analysis should be undertaken and interpreted with caution, the available data suggest that after *H. pylori* eradication failure, levofloxacin-based rescue regimen is more effective and better tolerated than the generally recommended quadruple therapy as second-line rescue regimen.

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Alpha-Fetoprotein and Hepatocellular Carcinoma

TO THE EDITOR: In their large multicentric series, including 1,158 Italian patients with hepatocellular carcinoma (HCC) diagnosed according to the accepted criteria, Farinati *et al.* (1) have reported an α -fetoprotein (AFP) concentration >20 ng/mL in only 54% of the cases at diagnosis, thus ruling out any value of AFP in this relevant setting of patients.

The strength of the above conclusion, however, should be attenuated on the basis of different methodological concerns. First of all, the study design, (i.e., a case series) prevents an actual estimation of the test specificity; secondly, the selection of patients who clearly had the target disease could have affected the estimation of sensitivity leading to an overestimation (2, 3).

Moreover, as stated by the authors themselves, 61% of patients lacked histological confirmation of HCC and, in these cases, HCC was diagnosed according to the EASL criteria based on either the agreement of two imaging procedures or findings consistent with HCC at one imaging technique plus increased AFP levels. Accordingly, in their series, Farinati *et al.* included the index test (AFP) in the reference standard, thus leading to a distortion in both the design and conduct of the study, called "incorporation bias," whose effects on the results are not defined (3). Overall, the above methodological flaws do not permit a real assessment of AFP accuracy in HCC.

Again, in two recent systematic reviews (4, 5), the pooled estimation of sensitivity and specificity of AFP was precluded by the poor quality of the examined studies. The present huge case series cannot add more evidence, being still possible that dissonant voices can claim a role for AFP in diagnosing HCC. High quality prospective studies are still needed for a definitive obituary of AFP.

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Letter to the Editor

Seropositivity to *Chlamydia pneumoniae* or *Helicobacter pylori* and coronary artery disease

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Abstract

Our aim was to investigate the relationship between the serologic status concerning *Chlamydia pneumoniae* and *Helicobacter pylori* with the presence of coronary artery disease (CAD), which remain a controversial issue in literature. We studied 208 patients with CAD and 94 controls with no evidence of obstructive CAD; all of them angiographically confirmed. The seropositivity to *C. pneumoniae* was 91% in patients with CAD vs 86% in controls ($P>0.05$). The *H. pylori* seroprevalence rates were 77% and 68%, respectively ($P>0.05$). The multivariate analysis, adjusting for age, sex, educational level, diabetes, hypertension, obesity, smoking, family history of CAD and lipids, confirmed the results of univariate analysis. Therefore, this study adds evidence against the association of seropositivity to *C. pneumoniae* and *H. pylori* with angiographically documented CAD.

After perusing the report of Vijayvergiya et al. [1] on correlation of *Chlamydia pneumoniae* and *Helicobacter pylori* with CAD, we thought that would be of interest to your readers to demonstrate our findings of a similar study. The population of the latter consisted of 208 patients (mean age: 63 ± 10 years) with angiographically confirmed coronary artery disease (CAD) and 94 controls (mean age: 59 ± 12 years) with no evidence of obstructive CAD. *H. pylori* and *C. pneumoniae* IgG antibodies were detected by ELISA and an indirect microimmunofluorescence method, respectively. The Pearson's chi-square test, student's *t*-test and logistic regression analysis were used for statistical analyses.

The seroprevalence of *H. pylori* infection was 77% (161/208) among patients with CAD and 68% (64/94) among controls ($P=0.09$). No difference was found in mean value of IgG titre between patients and controls (132 ± 82 vs. 116 ± 82, $P=0.12$). The rate of seropositivity (IgG titre ≥ 1:80) for *C. pneumoniae* was 91% (184/203) in patients and 86% (79/92) in controls ($P=0.22$). Moreover, the *H. pylori* or *C. pneumoniae* seropositivity did not differ significantly between subgroups of patients with single, double or triple vessel CAD in comparison with controls. Logistic regression analysis adjusted for age, sex, educational level, diabetes, hypertension, obesity, smoking, family history of CAD and lipids did not reveal any significant relation between *C. pneumoniae* or *H. pylori* and CAD.

Although the methodology of both studies was similar, our results are in disagreement with the data from Vijayvergiya et al. [1], who contend that *C. pneumoniae* and *H. pylori* are significantly associated with CAD. The only differences between the two studies are concerning the sample's size ($n=302$ and $n=120$, respectively) and the differences in seropositivity rates to *C. pneumoniae* and *H. pylori*. In regard with the latter, it should be mentioned that the seropositivity rates in the study of Vijayvergiya et al. would be expected to be higher, which are characteristic to a developing country

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such as India. Nevertheless, it is unclear if these apparent differences can explain the disagreement in results.

However, this is reflecting the situation on the relevant literature where the association of *C. pneumoniae* and *H. pylori* with CAD is a controversial issue: some studies show evidence in favour of this association [2,3] while some others failed to demonstrate such association [3,4]. Furthermore, the data concerning possible relation of the *C. pneumoniae* and *H. pylori* with cardiovascular risk factors remain controversial [4–6]. In this context, our investigation is adding to the evidences against association of seropositivity to *C. pneumoniae* and *H. pylori* with CAD.

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