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Short Communication

The effect of mastic gum on *Helicobacter pylori*: A randomized pilot study

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A R T I C L E   I N F O

A B S T R A C T

Our aim was to study the effect of pure mastic gum on *Helicobacter pylori (H. pylori)* eradication in patients suffering from an *H. pylori* infection

Fifty two patients were randomized to receive either 350 mg three times a day (tid) of pure mastic gum for 14 days (Group A), or 1,05 g tid of pure mastic gum (Group B) for 14 days, or pantoprazole 20 mg twice a day (bd) plus pure mastic gum 350 mg tid for 14 days (Group C) or pantoprazole 20 mg bd plus amoxicillin 1 g bd plus clarithromycin 500 mg bd for 10 days (Group D). All patients harboured *H. pylori* before entering the study and that was confirmed by a ^{13}C urea breath test (UBT). *H. pylori* eradication was tested by a UBT 5 weeks after completion of the eradication regime.

Eradication of *H. pylori* was confirmed in 4/13 patients in Group A and in 5/13 in Grour B. No patient in Group C achieved eradication whereas 10/13 patients in Group D had a negative UBT. There were no statistically significant differences in mean UBT values in Groups A, B, C although there was a trend in Group A (p=0.08) and in Group B (p=0.064). The difference was significant in Group D (p=0.01). All patients tolerated mastic gum well and no serious adverse events were reported. Mastic gum has bactericidal activity on *H. pylori* in vivo.

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Introduction

Helicobacter pylori (H. pylori) is a Gram-negative spiral bacterium that colonises the stomach. Its prevalence in Europe is in the range of 10-25% and has been falling during the last decades while in the developing world it is estimated that its prevalence is much higher (Magalhaes-Queiroz and Luzza 2006). Infection with *H. pylori* is etiologically linked to gastritis, peptic ulcer disease, primary B cell gastric lymphoma and adenocarcinoma of the stomach (Lai and Sung 2007; Eslick 2006). *H. pylori* can be eradicated but this is difficult to achieve and at least two antibiotics and an acid suppressant are required to achieve eradication (Malfetrheiner et al. 2007). Side effects for these regimes are common and a major concern is the development of antimicrobial resistance. Development and testing of new safe alternatives to those regimes is therefore warranted.

Mastic gum is a natural resin that is excreted from the trunk and branches of the mastic bush (*Pistacia Lentiscus* var. Chia). This excretion is produced by incising the bark with a sharp instrument. Mastic gum appears in the incisions in the form of tears and exudes in droplets onto the soil. While it is flowing, it is a gummy, clear liquid; it solidifies in irregular shapes after 15-20 days.

Collection is completed in September. Then it is cleaned first by hand and then with mechanized means. Finally mastic gum is sorted, classified and graded according to the color and size of the granule.

The clean mastic gum granules were milled to fine powder (particle size < 200 μm) by using a Hosokawa Alpine Fine Impact Mill 100 UP2 (Hosokawa Alpine, Augsburg, Germany). The encapsulation of powder was performed using the Profill Capsule Filling System (Torpac Inc, Mumbai, India). Capsule shells (Capsulegel, V caps, size 0) were made of Hypromellose (Hydroxypropyl methylcellulose) and each contained 0.35 (± 0,002) g of mastic powder.

There have been references to mastic gum as a medicinal product since ancient times. It has successfully been used in gastrointestinal upsets (Kaliora et al. 2007).

Previous studies have shown some effect of mastic gum on the healing of peptic ulcers in humans (Al-Habbal et al. 1984; Al Said et al. 1986). Those studies were conducted before the discovery of *H. pylori*. A recent case study has shown no effect of mastic gum on *H. pylori* (Bebb et al. 2003).

The aim of our randomised controlled trial was to assess the efficacy of mastic gum monotherapy or in combination with a proton pump inhibitor on *H. pylori* eradication and to compare this efficacy with the standard treatment regime.

Patients and methods

This prospective randomized controlled trial study was conducted at the Gastroenterology Department of Chios General

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Hospital Skylitsion. The study was approved by the Local Ethics Committee and the Greek Medicines Agency.

All eligible patients had an upper gastrointestinal endoscopy and were found to harbour *H. pylori* by a rapid urease test (CLO test, Kimberky Clark, Draper, Utah, USA).They were then asked to participate in the study and gave their written informed consent. Patients were excluded if they had a gastric or duodenal ulcer, were pregnant women or had used in the previous 4 weeks, non steroidal anti-inflammatory drugs, anticoagulants, steroids, proton pump inhibitors or antibiotics. Patients who chewed mastic gum more than once a week were also excluded from the study.

Patients had confirmation of their *H. pylori* infection by a ^{13}C urea breath test (UBT) (INFAI, York, UK). They were then randomized to receive either a low dose mastic gum monotherapy 350 mg three times a day (tid) for 14 days, a high dose mastic gum monotherapy 1 g tid for 14 days, a dual regime of mastic gum 350 mg tid and pantoprazole 20 mg bd for 14 days, or a standard triple therapy which consisted of pantoprazole 20 mg bd, amoxicillin 1 g bd and clarithromycin 500 mg bd for 10 days.

They were asked to keep a log of adverse events. One week into the study patients received a telephone call, in which their compliance was assessed and an enquiry on possible adverse events was made. At the end of the study a physical examination was performed as well as routine laboratory tests.

Five weeks after the end of treatment, eradication was tested with a second UBT.

Pure mastic gum was dispensed in capsule form. It contained no additives or flavourings that could have an effect on its activity.

The randomization was generated using Proc random (SAS version 6.9). The randomization code was kept at the Central Pharmacy of Chios General Hopsital Skylitsion. The technician who performed the UBTs and the operator who analysed the UBTs were blinded as to which treatment each patient had received.

Data record and statistical analysis

Data were recorded prospectively in case report forms for all participating patients. All analysis was conducted by intention to treat. Results are shown as mean and SEM.

Comparisons between UBT values before and after the intervention were made using the paired t-test. A *p* value of < 0.05 was taken as significant (two-tail test of significance).

Results and discussion

We enrolled fifty two patients from the Endoscopy unit: 13 received low mastic gum monotherapy 350 mg tid for 14 days (Group A), 13 received high mastic gum monotherapy 1 g tid for 14 days (Group B), 13 received mastic gum 350 mg tid and pantoprazole 20 mg bd for 14 days (Group C) and 13 patients received triple therapy with pantoprazole 20 mg bd, amoxicillin 1 g bd and clarithromycin 500 mg bd for 10 days (Group D).

There were no statistically significant differences between groups with regards to age, sex, previous use of antibiotics or proton pump inhibitors.

One patient from Group A completed the study but did not return for his follow up UBT 5 weeks later. Two patients from Group C completed the study but did not return for their UBT 5 weeks later. One patient in Group D stopped because of side effects (diarrhoea and abdominal cramps).

Table 1 shows an overview of the results. Four patients in group A achieved eradication (30,8%), whereas five in Group B (38,5%) none in Group C and ten patients in Group D (76,92%) achieved eradication.

Table 1

Patients in Group A received low dose mastic gum monotherapy for 14 days. Group B patients received high dose mastic gum monotherapy for 14 days. Group C patients received mastic gum and pantoprazole for 14 days. Group D received triple therapy with pantoprazole, amoxicillin and clarithromycin for 10 days. Results are shown as mean ± SEM

	Eradication	UBT pre	UBT post	p
Group A	4/13 (30.8%)	28.86 ± 4.4	18,76 ± 3.1	0.08
Group B	5/13(38.5%)	27.11 ± 5.2	17.68 ± 4.8	0.064
Group C	0/13	25.56 ± 3.8	23.67 ± 4.7	NS
Group D	10/13 (76,92%)	26.73 ± 3.9	7.85 ± 2.8	0.01

Abbreviations: UBT=Urea breath test values.

UBT was performed a mean of 8 days (4-14 days) before starting treatment. It was repeated a mean of 39 days (33-61 days) after completion of the study medication. Fig. 1 shows mean UBT values before and after treatment. There was a trend towards significance in Group A (28.86 ± 4.4 vs 18.76 ± 3.1) (p=0.08), and in Group B (27.11 ± 5.2 vs 17.68 ± 4.8)(p=0.064). Group C showed no difference (25.56 ± 3.8 vs 23.67 ± 4.7) (p=NS) There was a statistically significant difference in UBT values in Group D (26.73 ± 3.9 vs 7.85 ± 2.8) (p < 0.01).In nine patients in Group A and ten patients in Group B the UBT value post treatment decreased compared with the UBT value before treatment.

Patients who received mastic gum tolerated it well. One patient in Group A complained of diarrhoea and another in Group B complained of nausea. Both completed the therapy as per protocol. Three patients in Group D complained of abdominal cramping and diarrhoea, one stopped the treatment on day 4.

This study was designed to evaluate the effect of Mastic gum on *H. pylori in vivo*. Our results showed that mastic gum has some effect on *H. pylori in vivo*. Nine patients in the monotherapy groups achieved eradication while in ten more patients the UBT value decreased compared to the pre-treatment reading. UBT values have been shown to provide rather accurate estimation of the *in vivo H. pylori* load (Perri et al. 1998).

We have also shown that the combination of mastic gum and pantoprazole was ineffective in eradicating *H. pylori* but it had no effect on bacterial load either. Our control group with standard triple therapy achieved an acceptable eradication rate.

The constituents which might contribute to the therapeutic effects of mastic gum belong to the class of mono- and sesquiterpenoids (essential oils) (Barra et al. 2007) and triterpenoids (e.g. masticadienonic acid) (Assimopoulou and Papageorgiou 2005). Previous *in vitro* studies have shown that crude mastic gum possesses antibacterial properties against *H. pylori* (Huwez et al. 1998; Marone et al. 2001). A recent study has shown that the acid fraction of mastic gum which contains triterpenic acids and constitutes about 50% of its total weight has bactericidal activity against *H. pylori* (Paraschos et al. 2007). In particular, moronic acid seems to be a powerful antibiotic not only against *H. pylori* but also other bacteria (Hostettmann-Kaldas and Nakanishi 1979).

The activity of mastic gum against *H. pylori in vivo* was the subject of studies with conflicting results. Early studies showed bactericidal activity on *H. pylori in vitro* and it was hypothesized that mastic gum killed *H. pylori* and thus helped ulcer healing (Huwez et al. 1998; Marone et al. 2001). Unfortunately, two recent *in vivo* studies, one in mice and the other in humans showed no eradication of *H. pylori* and only a modest antibacterial activity (Bebb et al. 2003; Loughlin et al. 2003).

Mastic gum is a herbal remedy and all studies that used mastic gum on patients showed minimal side effects (Al-Habbal et al. 1984; Al Said et al. 1986; Bebb et al. 2003). Also a recent study on animals showed that long term use of mastic gum was not
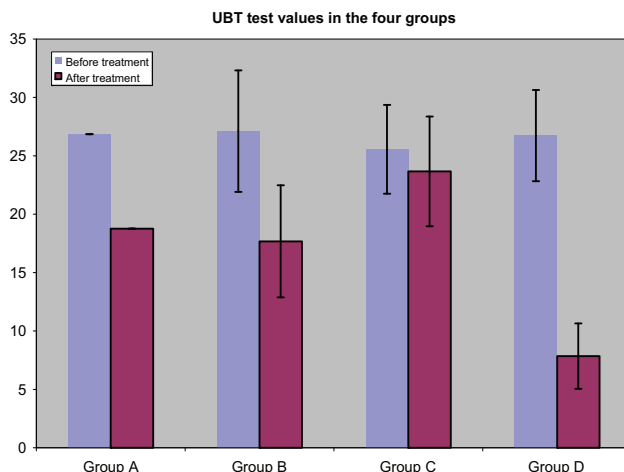
UBT test values in the four groups

Fig. 1. Urea breath test (UBT) was reformed a mean of 8 days (4-14 days) before starting treatment. It was repeated a mean of 39 days (33-61 days) after completion of the study medication. Mean values of UBT before and after treatment are shown There was a trend towards significance in Group A (28.86 ± 4.4 vs 18.76 ± 3.1) (p=0.08), and in Group B (27.11 ± 5.2 vs 17.68 ± 4.8) (p=0.064). Group C showed no difference (25.56 ± 3.8 vs 23.67 ± 4.7) (p=NS) There was a statistically significant difference in UBT values in Group D (26.73 ± 3.9 vs 7.85 ± 2.8) (p < 0.01).

associated with serious side effects (Kang et al. 2007). As a considerable fraction of patients harbouring the bacterium are unable to tolerate triple therapy due to side effects mastic gum might provide a reasonable alternative in the future.

The fact that the combination of mastic gum and pantoprazole showed no effect on *H. pylori* is somewhat surprising. Most active substances of mastic gum belong to its acidic fraction. They possibly require an acidic environment in the stomach to successfully kill *H. pylori*. Proton pump inhibitors block the hydrogen – potassium ATP enzyme system on the gastric parietal cells. In that way, they increase the intragastric pH. Buffering the acidity of the stomach by the proton pump inhibitors could result in a hostile environment for mastic gum. This hypothesis needs to be tested in further studies.

We have used two doses of mastic gum in our study the main reason being that a low dose mastic gum monotherapy has shown some activity in a previous study (Al-Habbal et al. 1984) while a high dose monotherapy has been shown to be ineffective in another study (Bebb et al. 2003).

Our study has limitations, the main one being its small size. We also did not use two different methods of confirming *H. pylori* status after treatment as a second endoscopy was deemed inappropriate.

In conclusion, this proof of principle study showed that mastic gum possesses antibacterial activity against *H. pylori in vivo* and is able to eradicate it from patients. Although even the high dose monotherapy did not achieve acceptable eradication rates it could be used as an alternative regime in patients unwilling to undergo eradication with the triple therapy regime.

Acknowledgements

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Original Paper

High Prevalence of *Helicobacter pylori* Infection in Greek Patients with Myelodysplastic Syndromes

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Abstract

Background/Aims/Methods: To determine the frequency of *Helicobacter pylori* infection (*Hp*-I) in 73 patients with myelodysplastic syndromes (MDS) and 40 controls, serologic analyses of *Hp* and ^{13}C-urease breath tests (INFAI) were performed. Gastric mucosal biopsy specimens were obtained to determine the presence of *Hp*-I using a rapid urease test, i.e. the Campylobacter-like organism (CLO) test, and cresyl violet staining. Peripheral blood (PB) flow cytometry for CD3, CD4, CD8, CD14, CD19 and CD34 was conducted in 35 patients and in controls. ***Results:*** *Hp*-I was detected by: (a) serology in 75.34% of patients (p = 0.000), (b) INFAI in 57.69% of patients, (c) CLO in 60.71% of patients and (d) histological confirmation in 80.36% of patients (p = 0.001). No correlation between *Hp*-I and CD3, CD4, CD8, CD14, CD19 expression, leukemic transformation or death was observed. However, in 20 cases, significant variation in the PB lymphocytic proportion possibly attributable to *Hp*-I was ascertained, in contrast to the expected MDS ratio. ***Conclusion:*** Although there is no evidence for a causal relationship between *Hp*-I and MDS, the increased prevalence of *Hp*-I among the MDS patients is an interesting finding that deserves further investigation as it may indicate a common factor causing susceptibilities to both MDS and *Hp*-I or that *Hp* might influence the pathophysiology of MDS.

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Introduction

Myelodysplastic syndromes (MDS) are defined as heterogeneous clonal disorders caused by intrinsic defects in hematopoietic stem cells; they include a diverse group of diseases in which the bone marrow production of blood

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cells is disrupted. Immunophenotypic aberrations, along with disorders in the number of peripheral blood (PB) cells, are common in this disease. Patients suffer from associated cytopenia leading to an increased risk of infection, transfusion-dependent anemia and bleeding. In spite of the wealth of information on therapeutic options, little is known about the epidemiology and risk factors of MDS [1].

Helicobacter pylori (Hp) is a curved spiral Gram-negative bacterium that colonizes the gastric mucosa of most humans worldwide, mainly affecting older adults in the developed world including Greece [2, 3]. More than half of the world's population is infected with this bacterium. It is listed as a class I carcinogen according to the World Health Organization, being the leading cause of gastric cancer worldwide [4–6]. It is associated with various upper-gastrointestinal diseases and nondigestive conditions including idiopathic thrombocytopenic purpura or iron-deficiency anemia [5, 7]. The large number of important studies revealing such associations suggests that pathogenic mechanisms may link this infection with many diseases of unknown etiology.

Although MDS also affect mostly the elderly [1], their association with *Hp* infection (*Hp*-I) has not yet been thoroughly researched. Therefore, we investigated a possible causal relationship between *Hp*-I and MDS by evaluating the presence of *Hp*-I and studying variations in lymphocytic antigen expression in the PB of MDS patients (either positive or negative for *Hp*-I) compared with healthy individuals.

Materials and Methods

Patients and Controls
A total of 73 MDS patients [36 men, 37 women, median age 73 (range 53–88 years)] and 40 healthy volunteers [19 men, 21 women, median age 68 (range 58–76 years)] were evaluated for *Hp*-I. Moreover, PB flow cytometry for CD3, CD4, CD8, CD14, CD19 and CD34 evaluation was conducted in 35 patients and in the total control group. All participants gave their informed consent and the study was approved by the local ethics committee.

All patients fulfilled the diagnostic criteria for MDS. They were categorized according to the WHO classification of MDS. Patients were excluded if they had taken H_2-receptor antagonists, proton pump inhibitors, antibiotics, bismuth compounds, or nonsteroidal anti-inflammatory drugs in the preceding 4 weeks. Patients were also excluded if they had undergone previous gastric surgery, were on anticoagulant therapy, were alcohol abusers, were allergic to penicillin or macrolides, had gastric cancer or other neoplasms, or had severe cardiac, pulmonary, kidney, or liver diseases.

The control group was matched for socioeconomic status, geographical distribution, residential area, smoking, alcohol use, age and sex. The socioeconomic status was determined using the World Bank 2007 report. Thus, two groups were defined both in the patients and the control group: low income (less than USD 3,705 per year) and medium or high income (more than USD 3,706 per year). The patients and the healthy volunteers were inhabitants of either the prefecture of Thessaloniki (an area in Northern Greece consisting of about 1,000,000 residents) or the proximal Northern Greece prefectures of Kilkis, Pella, Imathia, Pieria, Serres and Chalkidiki (700,000 residents). The population of the study (patients and controls) was rural or urban.

Detection of Hp-I
Hp detection methods were reported previously [2, 8]. Serum anti-*Hp* IgG antibody titers were assessed by the enzyme-linked immunosorbent assay (ELISA) method in all patients and healthy individuals. The manufacturer's recommended cut-off value of 10 U/ml was used to define each patient's serological analysis as positive or negative. Diagnostic upper-gastrointestinal endoscopy was performed and gastric mucosal biopsy specimens were obtained in 56 patients and in the entire control group to detect the presence of *Hp* by histology and a rapid urease test [Campylobacter-like organism (CLO test)]. The remaining 17 patients were not assessed endoscopically due to the severity of their condition; they were evaluated with the ^{13}C-urease breath test (INFAI test).

White Blood Cell Count, Immunofluorescence Staining and Flow Cytometry
PB flow cytometry was conducted in 35 patients and in the entire control group. Anti-CD3, -CD4, -CD8, -CD14, -CD19 and -CD34 antibodies were used. Venous blood samples (3 ml) were collected in sterile test tubes containing K_3 ethylenediamine tetraacetic acid as anticoagulant. Blood cell enumeration and white blood cell differential counts were performed in a Coulter hemocytometer (Coulter Electronics, UK). Values were expressed in absolute numbers of cells per microliter of blood. The samples were studied within 2–3 h following blood collection. The percentages of lymphocyte subsets were determined by direct immunofluorescence using a panel of fluorochrome-conjugated monoclonal antibodies.

One hundred microliters of the blood sample were mixed into tubes with the appropriate amount of fluoroisothiocyanate (FITC)- and phycoerythrin (PE)-labeled monoclonal antibody. An FITC- or PE-conjugated mouse isotypic IgG served as negative control for each monoclonal antibody used. The stained and labeled cells were vortexed at low speed and incubated at room temperature (25°C) for 30 min. All erythrocytes were lysed with 2 ml FACS lysing solution, vortexed at low speed for 3 s and incubated for 10 min at room temperature. Afterwards, the tubes were centrifuged at 300 *g* for 5 min and the supernatant was aspirated. The cells were washed twice with 2 ml of PBS containing 0.1% sodium azide and then centrifuged at 200 *g* for 5 min. Finally, 500 µl of 1% paraformaldehyde-containing PBS was added to each tube. All samples were processed in a FACSorter within 8 h after venipuncture.

Two-color flow cytometry was performed using a FACSorter cytometer. LeucoGate (CD45/CD14) was used to create a lymphocyte acquisition gate. The lymphocyte analysis gate parameters included 98% of the normal mature lymphocytes. The pheno-

typic panel used in this experiment consisted of: anti-Murine-IgG1 (FITC)/anti-IgG2a (PE); anti-CD45HLeu1 (FITC)/anti-CD14LeuM3 (PE); anti-CD3Leu4 (FITC)/anti-CD4Leu3A (PE); anti-CD3Leu4 (FITC)/anti-CD8Leu2A (PE); anti-CD45HLeu1 (FITC)/anti-CD19LeuI2 (PE); anti-FL1Height (FITC)/anti-CD34

(PE). The monoclonal antibodies were from Coulter and Immunotech Laboratories. In each stained blood sample 5,000 events were acquired. The results of flow cytometry were analyzed and elaborated with the Windows Flow Cytometry program WinMDI Version 2.5.

Statistical Analysis

Statistical analysis was conducted with SPSS 16.0. The Mann-Whitney nonparametric U test, χ^2 test with Yates' correction, odds ratios, 95% confidence intervals (95% CIs) and Student's t test were performed where necessary. Significance was set at $p < 0.05$.

Table 1. Patients and controls characteristics, according to socio-economic status, geographical distribution, residential area, smoking, alcohol use, age and sex

Characteristics	Patients		Controls		p value
	n	%	n	%	
Socioeconomic status					
Low	29	39.7	12	30	
Medium or high	44	60.3	28	70	0.41
Geographical distribution					
Thessaloniki area	31	42.5	16	40	
Proximal prefectures	42	57.5	24	60	0.956
Residential area					
Rural	25	34.2	10	25	
Urban	48	65.8	30	75	0.421
Smoking status					
Smokers	32	43.8	21	52.5	
Nonsmokers	41	56.2	19	47.5	0.493
Alcohol use					
Yes	64	87.7	32	80	
No	9	12.3	8	20	0.415
Age, years					
<70	14	19.2	10	25	
≥70	59	80.8	30	75	0.629
Sex					
Female	37	50.7	21	52.5	
Male	36	49.3	19	47.5	1

Results

There was no difference between the MDS patients and the controls for socioeconomic status, geographical distribution, residential area, smoking, alcohol use, mean age and sex (table 1). Three MDS patients were lost to follow-up and were thus excluded from our study. *Hp* was detected in 79.45% (58/73) of MDS patients and in 45% (18/40) of controls, by the presence of *Hp* bacteria histologically or with the INFAI test or by *Hp* serologic analysis ($\chi^2 = 12.407$, $p = 0.000$, odds ratio = 0.21, 95% CI = 0.09–0.49, table 2). Similarly, *Hp* was detected in 75.34% (55/73) of MDS patients and in 45% (18/40) of the controls, by the presence of *Hp* bacteria histologically or with the INFAI test ($\chi^2 = 9.119$, $p = 0.003$, odds ratio = 0.27, 95% CI = 0.12–0.61, table 2). Specifically, the severity of the condition of 17 patients did not allow endoscopy and their evaluation regarding *Hp*-I was thus conducted with the INFAI test, along with the determination of the anti-*Hp* IgG antibody titers.

Table 2. *H. pylori* positivity in patients with MDS (n = 73) and controls (n = 40)

	MDS patients	Controls	Odds ratio[1]	p value
Age, years				
Median	73	68		>0.05
Range	53–88	58–76		
Sex (male/female)	36/37	19/21		>0.05
INFAI test positive	30/52 (57.69)	14/40 (35)	0.4 (0.17–0.93)	0.051
CLO test positive	34/56 (60.71)	16/40 (40)	0.43 (0.19–0.99)	0.073
Anti-*Hp* IgG >10 U/ml	55/73 (75.34)	16/40 (40)	0.22 (0.1–0.5)	0.000
Histological presence of *Hp*	45/56 (80.36)	18/40 (45)	0.2 (0.08–0.5)	0.001
Histological presence of *Hp* or INFAI test positive	55/73 (75.34)	18/40 (45)	0.27 (0.12–0.61)	0.003
Histological presence of *Hp*, INFAI test positive or anti-*Hp* IgG >10 U/ml	58/73 (79.45)	18/40 (45)	0.21 (0.09–0.49)	0.000

Unless otherwise indicated, data are number and percentage (shown in parentheses) of patients.
[1] 95% CI are shown in parentheses.

The prevalence of Hp-I was 80.36% (45/56) in the MDS patients and 45% (18/40) in the controls as histologically confirmed by the presence of Hp bacteria (χ^2 = 11.411, p = 0.001 < 0.05, table 2). The odds ratio for the association of Hp with MDS was 0.2 and the 95% CI ranged between 0.08 and 0.5. Moreover, 75.34% (55/73) of the patients and 40% (16/40) of the controls were seropositive for Hp (χ^2 = 12.349, p = 0.000 < 0.05, odds ratio = 0.22, 95% CI = 0.1–0.5, table 2). Likewise, positive INFAI tests were obtained in 57.69% (30/52) of the patients and only in 35% (14/40) of the controls (p = 0.051), whereas positive CLO tests were obtained in 60.71% (34/56) of the patients and in 40% (16/40) of the controls (p = 0.073) (table 2).

The 35 MDS patients evaluated by flow cytometry were classified according to the WHO classification: refractory anemia (RA, n = 7), refractory anemia with ringed sideroblasts (RARS, n = 3), refractory anemia with multilineage dysplasia (RCMD, n =5), refractory anemia with multilineage dysplasia and ringed sideroblasts (RCMD-RS, n = 2), refractory anemia with excess blasts 1 (RAEB1, n = 1), refractory anemia with excess blasts 2 (RAEB2, n = 14), myelodysplastic syndromes/chronic myeloproliferative diseases (MDS/MPD, n = 3). All these patients were followed up for at least 3 years (36 months) with the exception of those who died in a shorter interval after diagnosis. Nine patients developed leukemic transformation (25.7%) in a median time of 12.75 months (range 3–28 months) after diagnosis. These patients were previously classified as RAEB2 and all eventually died from leukemic evolution within a median time of 5 months (range 2–8 months). Overall, 22 patients are alive today and 13 have died, 9 from leukemic transformation after an initial RAEB2 diagnosis and 4 classified as: RA (n = 1), RAEB2 (n = 2) and MDS/MPD (n = 1). These 4 patients died because of complications, including infections or hemorrhagic manifestations related to severe cytopenia, which is common in MDS. Median overall survival was 38.44 months (range 3–63 months).

There was no correlation between the overall expression of CD3, CD4, CD8, CD19, leukemic transformation or death and Hp-I (tables 3, 4). CD19 expression, reflecting the proportion of B lymphocytes in the PB, was decreased in the MDS patients compared with normal individuals (p < 0.05). In contrast to the expected decreased proportion of B lymphocytes in MDS [9, 10], 6/35 patients (17.14%) with Hp-I (detected histologically or with the INFAI test) had normal or increased CD19 expression. Moreover, contrary to the expected low proportion of total T lymphocytes in MDS [10], 19/35 patients (54.3%) had normal CD3 expression. Of these 19 patients, 14 were positive for Hp-I (detected histologically or with the INFAI test, p < 0.05). Furthermore, in contrast to the expected low proportion of CD4 T-helper cells in MDS [10], a high prevalence of Hp-I (detected histologically, 15/20, p < 0.05) was observed among the 20 patients who had normal CD4 expression. Eight of 35 patients (22.86%) lacked CD4, CD8 or CD19 expression. Five of them were positive for Hp-I (detected histologically or with the INFAI test). CD14 expression was not within the normal range in 14/35 cases (40%). Of these 14 patients, 9 were positive for Hp-I (detected histologically). Besides, CD34 expression was within the normal range, apart from the 9/35 cases (25.7%) in whom leukemic evolution was noted. CD34 expression was increased in these 9 cases, as expected. Seven of them (77.8%) were positive for Hp-I (detected histologically or with the INFAI test). Finally, contrary to the expected low number of CD8 T-cytotoxic cells in early MDS stages [10, 11], 5/35 (14.29%) patients with Hp-I (detected histologically) and early MDS stage (RA, RARS) had normal CD8 expression.

Discussion

Since its original description by Warren and Marshall in 1983 [12], Hp has been associated with various upper-gastrointestinal diseases and nondigestive disorders including idiopathic thrombocytopenic purpura, iron-deficiency anemia or vitamin B_{12} deficiency anemia [5, 7].

In this study, we established, for the first time, a higher prevalence of Hp in a Greek ethnic MDS cohort than in a control group matched for socioeconomic status, geographical distribution, residential area, smoking, alcohol use, mean age and sex. The cause of this finding is unknown. This result may indicate either a common factor that causes susceptibilities to both MDS and Hp-I or that Hp might influence the pathophysiology of MDS. It could be hypothesized that Hp might be a causal agent for MDS. Another possibility is that Hp-I could be the result of neutropenia that is frequently noted in MDS. Moreover, the present study does not establish causality as this requires showing that eradication of Hp alters the course of MDS.

Hp-I was determined by histological detection of organisms in mucosal biopsy specimens, which is considered as the gold standard for the diagnosis of this infection [3], as well as by the ^{13}C-urease breath test (INFAI test) and serology. The Hp-I prevalence in our control group is similar to that reported by other investigators for Greek cohorts and other ethnic populations by using a serodiagnostic assay [13, 14].

Table 3. PB flow cytometric results of our cohort of MDS patients

Patient No.	MDS	Antibodies to *Hp*	CD4/CD3[1] (NR 28–59%)	CD8/CD3[1] (NR 18–49%)	CD19/total[2] (NR 8–20%)	CD3/total[2] (NR 59–85%)	CD14/CD45[3] (NR 2–11%)	CD34[4] <5%
1	RAEB2/AML	3.6 (−)	42	37	4	76	1	35
2	RAEB1	12 (+)	43.6	24.5	3	68	4.4	0
3	RAEB2/AML	3 (−)	59.3	23.6	2	87	6.5	35
4	RARS	11 (+)	55	12.5	5.6	69	6	0
5	RCMD	14.8 (+)	44	21	4	68	7	2
6	RAEB2/AML	178 (+)	61	8.5	4	70	3.5	7
7	RAEB2/AML	84.3 (+)	2	2	1	4	1	60
8	RAEB2	99 (+)	47	22	5	70	5	4
9	RA	147 (+)	31	31	2	65	7	5
10	MDS/MPD	125 (+)	54	20	8	76	0.2	10
11	RAEB2/AML	150 (+)	33	12	11	50	12	8
12	RAEB2/AML	7.9 (−)	0.3	0.1	0.1	50.5	0.3	8
13	RCMD	32 (+)	15	0.1	1	45	48	0
14	RA	8.2 (−)	55	8.9	7.1	62.6	9.9	7
15	RA	200 (+)	29.8	0.1	2	35.7	4.8	4
16	RAEB2/AML	61 (+)	45.5	51.6	2.9	87	1.4	5
17	RAEB2	150 (+)	52.5	19.1	9.2	75.1	0.3	2
18	RCMD RS	135 (+)	13	0.4	0.1	37	0.4	1
19	RAEB2	3.6 (−)	0.1	50.4	3.2	51.8	0.6	9
20	RAEB2/AML	102.5 (+)	17.3	7.9	48.7	27.2	7.2	22
21	RCMD	6 (−)	46.7	15.5	18.29	59	0.8	4
22	RARS	28 (+)	39.4	35.3	1.3	72	15.24	2
23	RAEB2/AML	81 (+)	32.25	24.3	0.1	58	10.9	38
24	RCMD	150 (+)	42.56	27	10.7	73.2	0.1	1
25	MDS/MPD	4.6 (−)	0.1	0.1	5.57	35	15.3	1
26	MDS/MPD	150 (+)	30	59	2	61	7	6
27	RA	47.2 (+)	23	0.1	1	57	6	5
28	RAEB2	6 (−)	36	21	1	80	10	5
29	RARS	16 (+)	49	23.6	6	68	5	4
30	RAEB2	3.8 (−)	17	13	17	25	11	11
31	RA	3.8 (−)	46	13	2	60	4	18
32	RCMD RS	43 (+)	47	24	11	69	3	2
33	RA	150 (+)	36	21	8	54	10	28
34	RCMD	124 (+)	13	18	4	29	43	33
35	RA	10.8 (+)	57	15	3	76	8	13

'AML' applies to those patients who underwent leukemic transformation after an initial MDS diagnosis. RA = Refractory anemia; RARS = refractory anemia with ringed sideroblasts; RCMD = refractory anemia with multilineage dysplasia; RCMD-RS = refractory anemia with multilineage dysplasia and ringed sideroblasts; RAEB1 = refractory anemia with excess blasts 1; RAEB2 = refractory anemia with excess blasts 2; MDS/MPD = myelodysplastic syndromes/chronic myeloproliferative diseases. The serum anti-*Hp* IgG antibody titer is specified with a cut-off value of 10 U/ml in order to define positivity or negativity. NR = Normal range.

[1] CD4 T-helper lymphocytes and CD8 T-cytotoxic lymphocytes are expressed as percentages of CD3, which reflects the total number of T lymphocytes.
[2] B lymphocytes (CD19) and T lymphocytes (CD3) are expressed as percentages of the total number of lymphocytes (CD19/total).
[3] Monocytes/macrophages (CD14) are expressed as percentages of the total number of leukocytes (CD45).
[4] The precursors of hematopoietic cells (CD34) in PB were considered to be lower than 5% of the total cells.

Table 4. PB flow cytometric results of our normal controls

Control No.	Antibodies to Hp	CD4/CD3 (NR = 28–59%)	CD8/CD3 (NR = 18–49%)	CD19/total (NR = 8–20%)	CD3/total (NR = 59–85%)	CD14/CD45 (NR = 2–11%)	CD34 <5%
1	19.2 (+)	44.2	22.8	16.2	68.3	12.6	2
2	0.8 (–)	44	37	10	75	4	4
3	3 (–)	49	19	17	61	6	5
4	4.2 (–)	52	42	8	83	10	4
5	55 (+)	35	23	12	65	3	2
6	65 (+)	29	40	15	64	5	2
7	1 (–)	47	25	14	67	4	1
8	6.7 (–)	42	23	17	78	9	1
9	150 (+)	43	28	12	72	3	4
10	3 (–)	38	28	11	80	2	5
11	100 (+)	52	20	9	62	2	3
12	2 (–)	54	20	10	65	6	3
13	2 (–)	50	26	13	68	7	4
14	47 (+)	35	36	18	71	2	1
15	7 (–)	41	38	9	64	5	2
16	104 (+)	29	17.4	13	67	6	2
17	2 (–)	42	16	9	71	3.5	3
18	4.1 (–)	35	23	9.5	70	4.9	4
19	3 (–)	33	30	16.4	64	3	4
20	39 (+)	40	24	17	69	6	1
21	2.5 (–)	51	26	11	68	6	2
22	123 (+)	37	29	12	63	8	2
23	0.4 (–)	32	33	12.7	72	7.1	3
24	1.2 (–)	38	36	12.9	70	6	2
25	101 (+)	47	32	15	68.5	8	1
26	121 (+)	43	23	15	72	5	1
27	98 (+)	37.6	25	14.3	74.8	6.5	3
28	45 (+)	37.2	27	18.7	70	6.8	2
29	33 (+)	39	29	9	65.3	4	2
30	42 (+)	45	19.8	12.3	68.2	5	3
31	47 (+)	46.3	26	14.3	69.4	5	2
32	5 (–)	49.1	32	13.9	65.5	5	3
33	3 (–)	39.9	33.1	16.9	62	8	1
34	5 (–)	38.5	30	17.2	80	7.5	1
35	7 (–)	37	31	13.1	79.5	3.5	4
36	2.5 (–)	35.5	22.3	12.8	78.2	2.5	1
37	1.5 (–)	40	24.6	15.5	77.5	6.5	1
38	3.5 (–)	41	27.6	16.2	76	7	1
39	3 (–)	36.8	22.8	18	80	7	0
40	5 (–)	37.2	25	19	82.5	4	1

For detailed explanations, see footnotes to table 3.

Our study has primarily relied upon histology for the documentation of Hp-I. Although culture is the theoretical gold standard for detection of the bacterium, it has been shown that there is an excellent correlation with histological identification [15]. Therefore, for most studies, mucosal biopsy and histological examination of the specimen for the presence of Hp and gastritis are the actual gold standard for the diagnosis of Hp-I [16–18], whereas gastric mucosa urease testing is insensitive, especially in the elderly [19]. However, an upper-gastrointestinal endoscopy is required to obtain specimens for histology or culture of Hp, and this method is costly and may lead to complications, particularly cardiopulmonary problems in elderly patients as they usually have a higher preva-

lence of comorbidities such as chronic heart, lung disease or malignancies including MDS [20, 21]; most patients with MDS are elderly individuals [22]. Therefore, noninvasive tests, such as breath tests or serological detection of antibodies to Hp, are widely recommended in primary-care settings for screening purposes and/or follow-up of the efficacy of Hp treatment.

The ^{13}C-urease breath test is noninvasive and reliable. Although it requires fasting, false-negative results may occur if antibiotics have been used within the previous 4 weeks and false-positive results can occur from urease present in the mouth [23]. At present, it is considered the test of choice for Hp-I [24]. Therefore, we introduced this test in our 17 MDS patients in whom the severity of their condition did not allow endoscopy. On the other hand, although the serological test establishes the presence of previous Hp-I, it does not discriminate between current and old infections. However, such a distinction is crucial because current Hp-I infection induces humoral and cellular immune responses that cross-react with host components owing to the sharing of homologous epitopes (molecular mimicry), thereby contributing to (and possibly perpetuating) tissue damage [25], and perhaps the development of MDS; immunological (cellular/humoral) responses are increasingly recognized as being essential in the initiation and progression of MDS [26–28]. Moreover, eradicating Hp-I might delay the progression of such diseases, particularly at early disease stages.

In this respect, Hp-I is strongly associated with idiopathic thrombocytopenic purpura [29], and long-term platelet responses to Hp eradication have been reported in Canadian patients with idiopathic thrombocytopenic purpura [30]. Hp urease B antibody could cross-react with human platelet glycoprotein IIIa and partly inhibit platelet aggregation [31]. In addition, Hp-I is implicated in the pathophysiology of mucosa-associated lymphoid tissue lymphomas by suppressing the apoptosis of B lymphocytes, rendering its eradication essential in such cases [32, 33]. Moreover, in a proportion of patients with monoclonal gammopathy of undetermined significance, Hp-I is involved through chronic antigenic stimulation [34]. However, no evidence of resolution of monoclonal gammopathy of undetermined significance after Hp eradication has been noted [35]. Besides, a high prevalence of the infection along with monoclonal gammopathy of undetermined significance has been reported in patients with chronic idiopathic neutropenia [36]. These patients have splenomegaly, attributed to Hp-I [37]. Finally, complete regression of a primary gastric plasmacytoma [38] and of a primary extragastric thyroid mucosa-associated lym-

phoid tissue lymphoma [39] after eradication of Hp-I have been described.

Regarding B lymphocytes and T lymphocytes in the PB of MDS patients, our relative results showed no correlation between CD3, CD4, CD8, CD14 and CD19 expression, leukemic transformation or death and Hp-I. We have previously reported an association of age (<72 years), bone marrow blast percentage (>5%) and pancytopenia with leukemic evolution of MDS [40]. However, a decreased proportion of B lymphocytes and T lymphocytes, as well as of CD4 T-helper cells in MDS patients, in parallel with a low number of CD8 T-cytotoxic cells in early MDS stages, as stated by Marisavljevic et al. [10], was not observed in a proportion of patients in our study. Interestingly, a significant prevalence of Hp-I was found among the MDS patients who had normal CD3, CD4, CD8, CD14 and CD19 expression, suggesting that the infection might be responsible for such discrepancies and be implicated in the pathogenesis of MDS with a mechanism yet to be identified [41]. Overall, a significant variation in the proportion of lymphocytes in PB was ascertained, possibly attributable to Hp-I, in contrast to the expected lymphocytic proportion in PB, due to MDS.

CD34 expression was within the normal range in this series, apart from the cases in whom leukemic transformation was noted. CD34 expression was increased in such cases. There was no fever or indications of an active bacterial (i.e. pneumonia), viral or fungal infection during the conduction of the flow cytometry. Previous flow cytometric studies examining the number of lymphocytes in PB of MDS have not taken into account the fact that a chronic infection (such as Hp-I) might modify the results.

A variety of molecules, cells and mechanisms tightly controlled in time and space are involved in the immune system. Therefore, full elucidation of the immunological defects in MDS in combination with the perturbations attributed to Hp-I is challenging. Various immunological abnormalities have been described in patients with MDS, including a decrease in total T lymphocytes, mainly due to a decrease in the number of CD4 helper T cells, a low ratio of helper to suppressor T cells (CD4/CD8 ratio), impaired natural-killer cell activity and production of cytokines, such as interferon-α and interleukin (IL)-2, defective cytotoxicity of T lymphocytes, impaired interactions between T and B cells, reduced T cell responses to mitogens and immunoglobulin abnormalities [10, 42]. Moreover, enhanced apoptosis is observed in bone marrow myeloid, erythroid and megakaryocytic cells in MDS, leading to ineffective hematopoiesis [43]. Although lym-

phocytes are not involved in the malignant clone in most MDS cases, lymphopenia is a common finding in MDS [9]. Apoptosis in the bone marrow of MDS patients has been reported to be increased in B lymphocytes, but not in T lymphocytes [43]. Interestingly, T cell apoptosis in PB has been described to be increased, especially in high-risk MDS patients [9]. Moreover, patients with MDS display several T cell expansions, which are polyclonal in the CD4 subset and oligoclonal in the CD8 subset. All the findings described above could reflect the selective involvement of cytotoxic T cells either in the antitumor immune surveillance or in an autoreactive aggression towards hematopoietic precursors [44].

In view of the aforementioned data, we first reported that *Hp*-I could protect from or delay the leukemic transformation of MDS by retaining increased apoptotic rates in the bone marrow via stimulation of the production of tumor necrosis factor-α in PB [45]. Although this notion might be correct, it possibly reflects only one side of the coin. Even though the infection exerts a degree of pro-apoptotic activity in early MDS stages, its antiapoptotic properties will more likely prevail in the long run, suggesting a possible dual role of *Hp*-I in MDS [46].

The majority of the patients who underwent leukemic transformation (7/9, 77.8%) were positive for *Hp*-I, detect-ed histologically or with the INFAI test. Thus, the findings of the present study do not support the hypothesis of a possible protective role of the bacterium in the leukemic transformation of MDS, but rather imply that *Hp*-I might be implicated in the leukemic transformation of MDS. Larger studies are needed for safe conclusions since the number of patients assessed in the present study was small.

In conclusion, the increased prevalence of *Hp*-I among MDS patients is an interesting finding, suggesting that there might be a link between *Hp*-I and MDS and deserving further investigation. However, there is no evidence of a causal relationship between *Hp*-I and MDS, although such a possibility cannot be entirely ruled out. The prospect of a chronic *Hp*-I causing continuous stimulation, fatigue and distress of the bone marrow deposits, thereby leading to alterations facilitating the induction of MDS, is intriguing.

Acknowledgments

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Original article

Helicobacter pylori is a major public health priority in western Balkans: An endoscopy referral center experience

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A R T I C L E   I N F O

A B S T R A C T

Background: Helicobacter pylori infection is a highly prevalent community infection. The prevalence of *H. pylori* infection has been reported to vary worldwide by geographical area and by social and economic conditions.
Aim: To investigate possible differences regarding the prevalence of *H. pylori* infection and related gastritis in Greek and Albanian patients undergoing routine endoscopy.
Materials and methods: Single referral endoscopy center retrospective analysis for the period of 2005–2008. For each of the first 101 consecutive Albanian patients, one age and sex matched Greek patient was included. No patient was previously treated for *H. pylori*. Endoscopic and pathology findings were recorded for *H. pylori* infection and the presence of active gastritis.
Results: In total 101 Albanians and 101 Greek patients were analyzed. A significantly higher prevalence of *H. pylori* in Albanians compared to Greeks was observed (54% vs 34%, $p = 0.005$, OR 2.3, 95%CI 1.3–4.0). There were no differences in *H. pylori* prevalence among sex or age groups. Active gastritis was significantly more frequent in Albanians compared to Greeks (48% vs 32%, $p = 0.02$, OR 2.0, 95%CI 1.3–2.6).
Conclusion: This is the first attempt in western Balkans to demonstrate by routine gastroscopy and biopsy that there is a significantly higher prevalence of *H. pylori* and active gastritis in Albanians as compared to Greeks.

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1. Introduction

Helicobacter pylori (*H. pylori*) infection is a major public health problem and remains an important and highly prevalent community infection. *H. pylori* infection is usually acquired in early childhood and possibly family members are the main source of infection while the prevalence of *H. pylori* infection is significantly higher among families of infected index children [1].

The prevalence of *H. pylori* infection has been reported to vary worldwide and by geographical area. Low and high prevalence areas have been recognized for *H. pylori*. High endemic areas are represented by developing countries while in the developed countries the prevalence of *H. pylori* infection is lower [2]. However, there is emerging data suggesting that the prevalence of *H. pylori* infection is also diminishing by time in developing countries especially in patients from the medium and upper socioeconomic strata [3]. An influence of social and economic conditions on the frequency of *H. pylori* infection has been also suggested in children [4].

In 1991, a serious political and socioeconomic crisis in Albania caused a massive migration of immigrants to northwestern Greece and to the Apulia region of southern Italy. Northwest Greece represents an area of an intermediate prevalence of *H. pylori* infection [5,6]. During the last fifteen years northwestern Greece that is bordering to southern Albania has received a larger number of Albanian immigrants, mainly belonging to young age groups with a previously reported *H. pylori* prevalence up to 80% [7].

We aimed to investigate differences regarding the prevalence of *H. pylori* infection and related gastritis in Greek and Albanian patients undergoing routine endoscopy in a single referral center in Northwestern Greece.

2. Materials and methods

2.1. Retrospective single referral center study

Our Department is a referral center for Hepatology and Gastroenterology in northwestern Greece and is offering endoscopy facilities and treatment in Albanian patients on hospital and outpatient clinic basis.

A retrospective analysis of our records of upper gastrointestinal tract endoscopies was performed for the period of 2005–2008.

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2.2. The Greek and the Albanian cohort

No patient was previously treated for *H. pylori*. The Greek and the Albanian cohort of patients were matched for age and sex with the following procedure.

We initially identified the first 101 Albanian patients and then for each Albanian patient we matched the first subsequent Greek patient of the same sex and age (± 5 years).

2.3. Endoscopy protocol and biopsy testing

The indication of endoscopy was symptom based and all endoscopic findings were recorded. Gastric biopsies from all patients were obtained during upper gastrointestinal endoscopy in a 4-year period. Two biopsies from antrum and corpus were histologically assessed.

H. pylori testing in biopsies is routinely performed in the pathology department of our hospital. All sections are stained with haematoxylin and eosin for histopathological details and Giemsa stain for the presence of *H. pylori*. All biopsy reports from the corresponding endoscopies were collected and were coded according to *H. pylori* status (positive–negative) and also according to the pathology conclusion related to the basic type of gastritis (active or non-active) or other important pathology findings.

2.4. Ethical considerations

All participants gave informed consent during endoscopy and every procedure was according to the rules of good clinical practice. All patients with *H. pylori* positive infection were subsequently treated and followed up accordingly.

2.5. Statistical analysis

Percentages were calculated for binary and categorical variables while continuous variables were described with median and interquartile range (IQR). For each binary outcome, odds ratios were calculated with 95% confidence intervals. Comparison between groups was performed using chi-square and Fisher's exact test for binary variables and Kruskal–Wallis for categorical variables. A two tailed *p* value <0.05 was considered to be significant and for calculations we used the SPSS 13.0 (SPSS Inc., Chicago, IL) and StatXact 3.0.

3. Results

In total 101 Albanians and 101 age and sex matched Greek patients were analyzed. The demographic, endoscopic and histologic characteristics of the Greek and the Albanian cohort of patients are presented in Table 1.

3.1. Prevalence of H. pylori

H. pylori testing showed a significantly higher prevalence in the Albanian compared to the Greek cohort (54% vs 34%, $p = 0.005$, OR 2.3

Table 1
Overview of the Albanian and age–sex matched Greek cohort with endoscopy biopsies for *Helicobacter pylori*.

Parameter	Greek cohort ($n = 101$)	Albanian cohort ($n = 101$)	*p* value OR (95%CI)
Male/female	60/41	60/41	NS
Age in years (median, IQR)	49 (36, 58)	43 (33.5, 54)	NS
H. pylori (+)/*H. pylori* (−)	34 (33.7%)/67	54 (53.5%)/47	0.005 2.3 (1.3–4.0)
Male/female *H. pylori* (+)	20 (33.3%)/14	35 (58.3%)/19	NS
Active/non-active gastritis	32 (31.7%)/69	46 (48.4%)/49	0.02 2.0 (1.3–2.6)

95% CI 1.3–4.0) (Fig. 1). There were no differences in *H. pylori* prevalence among sex or age groups.

3.2. H. pylori related gastric pathology

Active gastritis in biopsies of the Albanians was significantly more frequent compared to Greeks (46% vs 32%, $p = 0.02$, OR 2.0, 95%CI 1.3–2.6) (Fig. 2). The odds for an *H. pylori* positive test were 4-folds higher in patients with active gastritis than in patients without active gastritis (OR 4.0, 95% CI 2.2–7.3) (Fig. 3). There were no differences in active gastritis among sex or different age groups. Two patients (one Greek and one Albanian) were diagnosed with acute erosive gastritis.

Besides those patients with gastritis, two *H. pylori* positive Albanians were diagnosed with adenocarcinoma and one with non-Hodgkin lymphoma. None of the Greek patients was diagnosed with malignancy.

4. Discussion

Our study was the first attempt to compare pathology findings after endoscopy between Albanian and Greek patients in a referral center in Northwestern Greece. Our results suggested that Albanians who had an endoscopy had a higher *H. pylori* prevalence than the Greek age and sex matched individuals. Active gastritis was also significantly higher in Albanians as compared to Greek patients. An *H. pylori* positive test in biopsy was associated with the presence of active gastritis.

The proportion of *H. pylori* positive test was in accordance with the one that was reported in previous studies. Specifically, the prevalence of *H. pylori* infection in Greece has been reported to vary from 27% in Navy recruits [8] and 44% in children [1] to 70% in blood donors [9]. A study evaluating changes in *H. pylori* infection prevalence in Greece during a ten-year period showed a significant decrease of *H. pylori* infection in Greece [10]. According to previous unpublished data from our Department the prevalence of *H. pylori* in northwestern Greek population as assessed by rapid urease detection tests was 48% and there was no difference between sexes [5,6].

H. pylori infection is highly prevalent among Albanian population. The seroprevalence of *H. pylori* infection in Albanian healthy volunteers was 70% and increased by age, from 60% in individuals younger than 20 years to 81% among those of more than 50 years with a significant trend of increase by age [11]. In Albanian immigrants to southern Italy, the seroprevalence of *H. pylori* infection was 78% [12] and another study in unselected Albanian individuals demonstrated a 70% *H. pylori* prevalence irrespective of age [7]. Our results showed a lower prevalence for *H. pylori* in Albanian patients. This may be
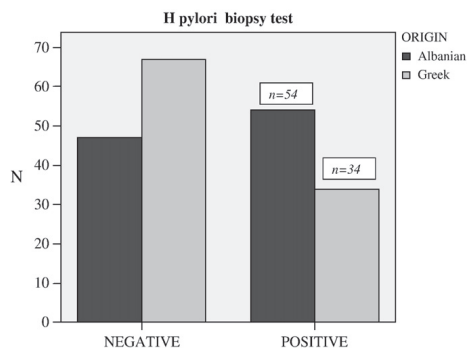

Fig. 1. Results of the *Helicobacter pylori* testing in the Greek and the Albanian cohort ($p = 0.005$).
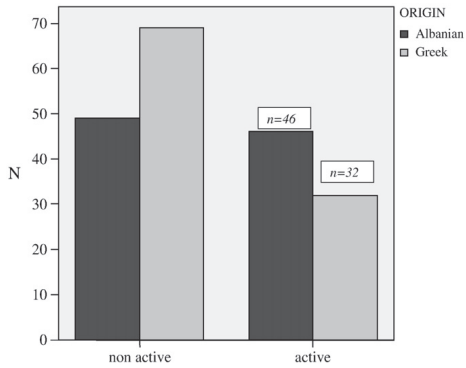
Fig. 2. Results of active gastritis in biopsies of the Greek and the Albanian cohort ($p = 0.02$).

potentially explained by a decrease in *H. pylori* prevalence in Albanian population over the years. Another potential explanation for the difference might be the fact that different diagnostic tests have been used in previous studies as compared to our report (serum vs. biopsy detection for *H. pylori*).

Untreated *H. pylori* infection may be related to significant upper gastrointestinal tract morbidities and increased health care expenditures. Specifically, *H. pylori* infection has been related to chronic gastritis [13], gastric precancerous lesions and gastric carcinoma [14] and accumulating data have shown that *H. pylori* may be a primary pathogenic factor for chronic nonspecific gastritis [15]. In our study there was a higher prevalence of active gastritis in Albanian individuals than in Greek patients. A possible explanation may be the fact that *H. pylori* infection was acquired earlier in life in the Albanian population due to high prevalence and thus, the infection was long enough to induce more severe or active changes in gastric mucosa. Another possible explanation could include the fact that the perception of symptom severity and the indication or access to endoscopy may differ between the two population groups. Therefore, Greek patients may be diagnosed with a less severe disease
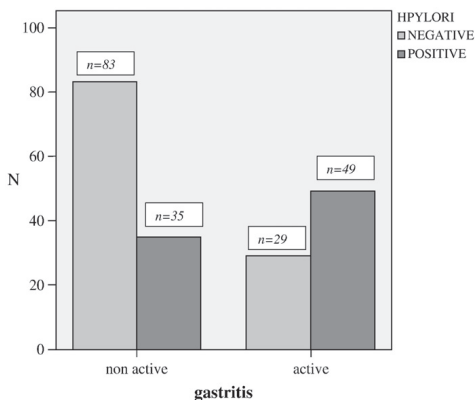

Fig. 3. *H. pylori* positive in patients with active gastritis and without active gastritis ($p < 0.001$).

than the Albanian peers. Of note, in this study only Albanians were diagnosed with underlying malignancies. The possibility that other genetic or/and environmental factors including diet could play an important role cannot be excluded as well as probable differences in the infectivity of *H. pylori* per se.

It has been previously demonstrated that *H. pylori* may differ [16,17] or not [18] in patients with chronic diseases compared to background healthy population. Of interest, *H. pylori* was related to the grade of gastritis but not to the tumor grade or intestinal metaplasia [19] or gastroduodenal lesions observed in Crohn's disease [20].

To the best of our knowledge, there is no study comparing *H. pylori* related gastric pathology in immigrants and native population. Our study suggested that the differences in the prevalence of active gastritis between the Albanian and Greek patients might be strongly related to the higher prevalence of *H. pylori* infection in the Albanian participants. Of note, pathologists were blinded to the results of *H. pylori* test in biopsies.

Testing for *H. pylori* implies many methodological considerations and it has been suggested that at least two invasive diagnostic methods should be performed. Furthermore, the performance of a non-invasive diagnostic method such as a 13C-urea breath test before the exclusion of *H. pylori* infection could also be considered [21]. Although we did not compare results from different methods used for *H. pylori* detection, *H. pylori* biopsy test sensitivity depends on the observer's experience and the extent of biopsy sampling. In general, the histological method has a sensitivity and specificity of 90–95% [22].

Albanian immigrants in Greece comprise a highly mobile population with unknown health care profile. Socioeconomic, cultural and language parameters underlying health care inequalities in highly mobile immigrant populations need better study [23]. Regional European policy on prevention of transmittable diseases resides a lot in information of large scale epidemiologic and well designed comparative studies. Public health measures to improve living conditions, provide education on hygiene, and to supply running water could prevent the transmission of *H. pylori* infection and other infections spread by the fecal-oral route in Albania [24].

Our study had several limitations. First, we included only the first 101 Albanian patients who underwent an endoscopy in our referral center. Although this may give information for the first working period, it would be difficult to capture the potential change in *H. pylori* prevalence over time for the Albanian population as compared to the Greek participants. Therefore, whether Albanians are still presented with a higher proportion of *H. pylori* infection and active gastritis than Greeks remains unclear. However, the endoscopy database in our referral center is enriched by additional Albanian and Greek patients, and in the future, we will be able to perform a comparative study with larger number of patients, which will also show the change in *H. pylori* infection and active gastritis over time. In addition, we should clarify that pathologists were not blinded to the names of the patients and therefore, a higher proportion of *H. pylori* infection among Albanians that may be due to bias cannot be excluded.

Our study was the first attempt to compare the *H. pylori* infection prevalence, and the presence of active gastritis between Albanian and Greek patients in a referral endoscopy center in northwestern Greece. The study supported that *H. pylori* infection may be a major public health problem for Western Balkans. Larger studies would be needed to identify the exact size of this problem so that organized prevention policies may eliminate it.

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ORIGINAL STUDY

Five-year Survival After *Helicobacter pylori* Eradication in Alzheimer Disease Patients

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Background: Alzheimer disease (AD) is a progressive, fatal neurodegenerative condition.

Objective: We tested the hypothesis that eradication of *Helicobacter pylori* infection (*Hp*-I) could improve survival in a Greek cohort of AD patients, in a 5-year follow-up.

Method: Forty-six patients diagnosed with probable AD were enrolled in the analysis. Study population was classified into 3 groups: patients for whom *Hp* eradication treatment was successful; those for whom eradication of *Hp* had failed, they refused, and/or were noncompliant with eradication therapy; and those who were *Hp* negative at baseline. Cox proportional hazards model was built with all-cause mortality as the dichotomous outcome.

Results: During the 5-year follow-up [47.19 ± 15.11 mo (range 12 to 60)], overall 21 patients died and 25 patients remained alive. Patients who died were older and exhibited lower mean MMSE score compared with the patients still alive. Successful eradication of *Hp*-I was associated with a significantly lower mortality risk [HR (95% CI) = 0.287 (0.114-0.725), $P = 0.008$]. The results were similar in adjusted and unadjusted models, for age and MMSE at baseline.

Conclusion: *Hp* eradication regimen in AD patients is associated with a higher 5-year survival rate.

Key Words: Alzheimer disease, dementia, *Helicobacter pylori*

(*Cog Behav Neurol* 2010;23:199–204)

Alzheimer disease (AD) is a known neurodegenerative disease of the elderly, with devastating effects on patients' functional abilities and personality; dementia is a progressive, fatal neurodegenerative condition characterized by impairment in cognition and memory, progressive deterioration in the ability to carry out activities of daily living, and a number of neuropsychiatric symptoms.[1] Estimates of median survival from the onset of symptoms of dementia revealed 3.1 years for patients with probable AD, 3.5 years for patients with possible AD, and 3.3 years for patients with vascular dementia,[2] and the 14-year survival rate for AD is 2.4% versus an expected rate of 16.6%.[3]

Although the early events underlying AD remain uncertain, the consideration that microorganisms can cause AD has recently been addressed[4–6]; brain infiltration by pathogens acts as a trigger or cofactor for AD, with *Herpes simplex virus type* 1 and *Chlamydophila* being most frequently implicated.[5,6] The association of *Helicobacter pylori (Hp)* infection (*Hp*-I) and AD has also only recently been addressed.[7–9] A higher seropositivity for anti-*Hp* IgG antibodies was reported in AD patients, but this serologic test has limitations because it does not discriminate between current and old infections.[7] Such a distinction is essential, 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,[9,10] thereby affecting or perpetuating neural tissue damage. Moreover, eradication of *Hp*-I might delay AD progression, particularly at early disease stages including mild cognitive impairment (MCI). On the basis of the histologic analysis of gastric mucosa biopsy for the documentation of *Hp*-I, Kountouras et al[8] reported a higher prevalence of *Hp*-I in AD patients accompanied with increased homocysteine (Hcy) concentration, an independent risk factor for dementia and AD, thereby suggesting an association between these 2 diseases. They also reported comparable data in MCI patients than in age-matched controls.[9] Quite recently, a French group of investigators also reported an association between *Hp*-I and AD.[11] Moreover, the same group of investigators (Kountouras et al)[12,13] suggested that *Hp* eradication may positively influence AD manifestations at 2-year clinical endpoint, and the observed increased concentrations of *Hp*-specific IgG antibody levels in the cerebrospinal fluid might reflect the AD severity, thereby supporting a role for this common infection in the pathobiology of the disease.

To our knowledge, whether *Hp* eradication is associated with long-term improved AD prognosis outcomes, has not been investigated. Given the fact that *Hp*-I confers susceptibility in a number of digestive and extradigestive conditions with negative impact on general population's health and life quality in terms of morbidity and mortality,[14] we tested the hypothesis that eradication of *Hp*-I would also improve survival rate, thereby decreasing mortality in a Greek cohort of AD patients, in a 5-year follow-up period.

MATERIALS AND METHODS

Patients

Fifty-six patients diagnosed with probable AD were included in this series. However, for 10 patients there were no data available concerning survival outcome. Therefore, 46 patients were eventually enrolled in the analysis. Recruitment and baseline assessment of patients and neuropsychologic 2-year follow-up evaluation were described earlier.[8,12] All patients had been referred to the Memory and Dementia Outpatient Clinic by their caregivers, mainly relatives, who certified a cognitive deterioration and/or other cognitive functional disturbances in the participants for at least a period of 6 months. Patients were diagnosed with probable AD according to the NINCS-ADRDA and DSM-IV criteria.[12,15] Screening procedure for their evaluation was conducted at their first visit to the Memory and Dementia Outpatient Clinic. Patients and controls underwent neuropsychologic assessment that included measurement for cognitive deterioration (MMSE: Mini Mental State Examination and CAM COG: Cambridge Cognitive Examination for the Elderly), functional disorders (FUCAS: Functional Cognitive Assessment Scale), neuropsychologic disorders (NPI: Neuropsychiatric Inventory), and depression (GDS: Geriatric Depression Scale).

Apart from the aforementioned assessment, MRI tomography was conducted as a diagnostic neuroimaging technique to confirm temporal lobe and hippocampal formation atrophy. It was also used to exclude other causes of dementia (stroke, tumor, fronto-temporal dementia, etc). Patients with vascular, Lewy-body, fronto-temporal, and other types of dementia were excluded from the study. We also excluded patients with known or subclinical thyroid disorders, and patients with depression. None of the patients had been treated with cholinesterase inhibitors (ChEIs), memantine, or any other pharmacologic treatment for dementia.

All patients and controls underwent diagnostic upper gastrointestinal (GI) endoscopy after informed consent. Participants were excluded if they had taken H_2-receptor antagonists, proton pump inhibitors, antibiotics, bismuth compounds, or nonsteroidal antiinflammatory drugs in the preceding 4 weeks. Participants were also excluded if they had undergone earlier gastric surgery; received anticoagulant therapy; were alcohol abusers; had allergy to penicillin or macrolides; had gastric cancer or other

neoplasms; or had severe cardiac, pulmonary, kidney, or liver disease.

All participants and/or their relatives signed a consent form before enrollment, and the study protocol was approved by the local ethics committee. All patients received the same ChEI during the 5-year follow-up period of the study. None of the participants in this study received oral drugs that could influence cognitive state, other than the medication prescribed by the researchers.

Study Design

Hp detection and total serum Hcy concentration measurement methods were described earlier.[8,16] Biopsy urease test and histopathology process were also described earlier.[16] Success of *Hp* eradication regimen was evaluated by control endoscopy at least 8 weeks after cessation of therapy and patients were considered as *Hp* negative if both histology and the rapid urease test proved negative. The follow-up study population was classified into 3 AD groups: patients in whom *Hp* eradication treatment was successful (group A); those in whom eradication of *Hp* had failed, they refused and/or were noncompliant with their eradication therapy (group B); and those who were *Hp* negative at baseline (group C).

For the 5-year follow-up, data on patients' survival were obtained through telephone interviews conducted by a neuropsychologist. In most cases of the surviving patients, cognitive and functional deterioration, reported by the caregivers, indicated final stage of the disease, with rare verbal communication, time and place disorientation, and failure to recognize close relatives. Therefore, efficacy and reliability of neuropsychologic assessment at this stage was considered questionable, and neuropsychologic data are not presented. Whether death was owing to AD or other medical condition was clarified through the telephone interview. The neuropsychologist conducting telephone interviews in this study was masked to the *Hp* status of the patients.

Statistical Analysis

For comparison of baseline characteristics and *Hp* positivity status between patients' groups and between patients who did and did not die during the follow-up, χ^2, independent samples' 2-tailed *t*-test and 1-way analysis of variance were used. Comparison of serum anti-*Hp* IgG antibody level between Group A and Group B patients was conducted by the use of Mann-Whitney *U* test. Significance was set at $P < 0.05$.

Cox proportional hazards model was built with all-cause mortality as the dichotomous outcome. The time-to-event variable was time from baseline evaluation to death. Patients who did not die were censored at the time of their last follow-up. Patients' group according to *Hp* eradication status was the main predictor. Group C patients were not included into the analysis owing to their small absolute number, thus only Group A and Group B patients were included. In subsequent Cox proportional hazards models results were adjusted for these variables: age at baseline, sex, and MMSE score at baseline.

The analysis was done by using the statistical software package SPSS (Statistical Package for Social Sciences, version 14.0; SPSS Inc, Chicago, IL).

RESULTS

Demographic, Clinical, and *Hp*-positivity Characteristics

Groups A, B, and C consisted of 23, 19, and 4 patients, respectively. Patients with (Groups A and B) and without (Group C) *Hp*-I did not differ in terms of age at baseline (73.47 ± 6.1 vs. 74.25 ± 3.86 respectively, $t = 0.247$, $P = 0.806$). Serum T3, T4, Free T4, and TSH concentration were within normal range in all patients included. All patients exhibited brain cortical and hippocampal atrophy and ventricle enlargement; volumetric measurements were not conducted. Patients' use of donepezil, rivastigmine, and galantamine was equally distributed among the 3 groups, at a mean daily dose of 10, 9, and 16 mg, respectively. In addition, 6 patients received memantine at a daily dose of 10 to 15 mg. Patients' treatment with ChEIs was constant during the follow-up and none of the patients switched to another ChEI. Mean values of baseline characteristics did not differ between Goup A and B patients at baseline (Table 1). The same values did not differ among the 3 patients' groups either, although comparison's value remains considerable, given the small size of Group C (Table 1).

All Group A and B patients had *Hp* infection, as verified by the histologically confirmed presence of *Hp*.

Distribution of Sydney score values did not differ between Group A and B patients ($\chi^2 = 1.802$, $P = 0.406$). The mean serum IgG anti-*Hp* level also did not differ between the 2 groups: 55.9 ± 54.64 and 48.04 ± 66.71, respectively ($Z = -0.47$, $P = 0.982$).

Outcome of *Hp*-I Eradication on 5-year Survival

For 1 patient in Group A and 2 patients in Group B, an ischemic stroke was reported by the caregivers, at least 6 months before the patient's death. However, owing to prior overall condition (severe physical deterioration that did not permit patients' transportation) examination and subsequent diagnosis was conducted at home by a physician and was not verified by a CT or MRI-scan. Caregivers did not report other sources of mortality for the rest of the patients, thereby attributing patients' death to the terminal stage of AD. During the 5-year follow-up [47.19 ± 15.11 mo (range 12 to 60)] overall 21 patients died who were of older mean age and exhibited lower mean MMSE score compared with the 25 patients still alive (Table 2). These results are consistent with the known association of the disease progression with age and cognitive status at baseline evaluation, therefore, interaction of age and MMSE score at baseline was taken into account on the subsequent analysis. Successful eradication of *Hp*-I was associated with significantly lower mortality risk (Table 3, Figure 1). The results were similar in adjusted and unadjusted models (Table 3).

TABLE 1. Baseline Characteristics and *Helicobacter pylori* (*Hp*) Positivity Status of Alzheimer Disease Patients, According to the Status of *Hp* Eradication Therapy

	Group A (N = 23)	Group B (N = 19)	Group C (N = 4)	*P*†	*P*‡
Baseline characteristics					
Age	72.86 ± 6.85	74.21 ± 5.12	74.25 ± 3.86	$t = -0.705$, $P = 0.485$	$F = 0.289$, $P = 0.75$
Sex (male/female)*	10/13	5/14	1/3	$\chi^2 = 1.536$, $P = 0.464$	$\chi^2 = 1.536$, $P = 0.464$
MMSE	18.43 ± 5.89	15.78 ± 6.44	20.75 ± 6.39	$t = 1.388$, $P = 0.173$	$F = 1.551$, $P = 0.224$
CAMCOG	58.87 ± 44.54	22.69 ± 15.78	71 ± 14.17	$t = 1.81$, $P = 0.082$	$F = 2.809$, $P = 0.078$
FUCAS	68.56 ± 21.08	70.16 ± 15.94	59.75 ± 8.61	$t = -0.22$, $P = 0.827$	$F = 0.499$, $P = 0.612$
NPI	9.6 ± 8.47	10.5 ± 10.25	7 ± 4.54	$t = -0.274$, $P = 0.807$	$F = 0.228$, $P = 0.797$
GDS	2.31 ± 1.74	3.18 ± 1.94	2.66 ± 2.3	$t = -1.192$, $P = 0.247$	$F = 0.709$, $P = 0.501$
B12, pg/mL	393 ± 388.44	516.44 ± 291.32	310.2 ± 83.4	$t = -0.836$, $P = 0.395$	$F = 0.665$, $P = 0.521$
Folate, ng/mL	8.48 ± 5.74	10.07 ± 8.23	7 ± 2.64	$t = -0.629$, $P = 0.534$	$F = 0.429$, $P = 0.655$
Hcy, μmol/L	18.27 ± 5.24	17.34 ± 3.66	17 ± 3.79	$t = -0.484$, $P = 0.632$	$F = 0.222$, $P = 0.802$
Hp positivity status					
Positive urease test result (gastric mucosa)*	12	10	0	$\chi2 = 4.017$, $P = 0.134$	$\chi2 = 4.017$, $P = 0.134$
Sydney score*				$\chi2 = 1.802$, $P = 0.406$	$\chi2 = 47.974$, $P < 0.001$
0	0	0	4		
1	8	4	0		
2	11	13	0		
3	4	2	0		
Mean serum anti-*Hp* IgG concentration, U/mL	55.9 ± 54.64	48.04 ± 66.71	6.54 ± 7.35	$Z = -0.47$, $P = 0.982$	$F = 1.359$, $P = 0.272$

Unless otherwise indicated, cells indicate mean values ± standard deviation.
*Numbers indicate No. of cases.
†Group A and B included in the analysis.
‡Group A, B, and C included in the analysis.
CAMCOG indicates Cambridge cognitive examination for the elderly; FUCAS, functional cognitive assessment scale; GDS, geriatric depression scale; Group A, patients in whom *Hp* was successfully eradicated; group B, patients in whom eradication of *Hp* had failed, they refused and/or were noncompliant with eradication therapy; group C, *Hp*-negative patients at baseline; Hcy, homocysteine; MMSE, mini mental state examination; NPI, neuropsychiatric inventory.

TABLE 2. Baseline Characteristics and *Helicobacter pylori* (*Hp*) Positivity Status of Alzheimer Disease Patients who Did and Did not Die During the Follow-up Period

	Alive (N = 25)	Dead (N = 21)	P
Baseline characteristics			
Age	71.96 ± 6.65	75.42 ± 4.31	$t = -2.051, P = 0.046$
Sex (male/female)*	8/17	8/13	$\chi^2 = 0.187, P = 0.665$
MMSE	19.24 ± 6.58	15.52 ± 5.25	$t = 2.086, P = 0.043$
CAMCOG	59.22 ± 23.94	48.25 ± 14.18	$t = 1.424, P = 0.165$
FUCAS	65.87 ± 20.27	73.75 ± 16.2	$t = -1.105, P = 0.279$
NPI	9.15 ± 9.92	10.15 ± 6.33	$t = -0.319, P = 0.752$
GDS	2.27 ± 1.67	3.25 ± 2	$t = -1.44, P = 0.161$
B12, pg/mL	363.95 ± 190.36	387.83 ± 286.41	$t = -0.295, P = 0.77$
Folate,ng/mL	9.54 ± 6.43	8.1 ± 5.74	$t = 0.626, P = 0.535$
Hcy, μmol/L	17.29 ± 4.5	17.54 ± 4.22	$t = -0.156, P = 0.877$
Hp positivity status			
Positive urease test result (gastric mucosa)	9	13	$\chi^2 = 2.438, P = 0.118$
Sydney score			$\chi^2 = 4.352, P = 0.226$
0	3	1	
1	9	3	
2	10	14	
3	3	3	
Sydney score change at 3 mo			$\chi^2 = 0.038, P = 0.845$
0	10	9	
−1	15	12	
Mean serum anti-*Hp* IgG concentration, U/mL	51.98 ± 61.96	40.44 ± 41.09	$Z = -0.092, P = 0.927$

Unless otherwise indicated, cells indicate mean values ± standard deviation.
*Numbers indicate No. of cases.
CAMCOG indicates Cambridge cognitive examination for the elderly; FUCAS, functional cognitive assessment scale; GDS, geriatric depression scale; Group A, patients in whom *Hp* was successfully eradicated; group B, patients in whom eradication of *Hp* had failed, they refused and/or were noncompliant with eradication therapy; group C, *Hp*-negative patients at baseline; Hcy, homocysteine; MMSE, mini mental state examination; NPI, neuropsychiatric inventory.

DISCUSSION

In this series, we documented for the first time a beneficial effect of *Hp* eradication upon progression of probable AD. Indeed, we provided evidence of impact of *Hp* eradication on AD patients' survival in a 5-year follow-up period. Regarding the mean survival time, AD patients in whom *Hp* eradication was successful lived 57.28 months on average, compared with 46.66 months survival in the patients, in whom *Hp* eradication was unsuccessful, that is, 10.62 months longer.

Age and level of cognitive deterioration at baseline have been reported to be major contributors to the disease progression.[17,18] In our study population, patients who died during the follow-up period were at an older age and carried out worse in the MMSE scale at baseline, compared with the patients still alive. When these differences were taken into account in the adjusted proportional hazard model, the effect of the *Hp* eradication status remained statistically significant.

Hp may confer mortality risk to AD through various pathways. We have earlier focused on the finding, also reported by others, that AD patients show increased serum Hcy levels, an independent confounding factor of AD.[8] In our series, mean total serum Hcy, folate, and B12 concentration did not differ at baseline, neither among

FIGURE 1. Survival curves based on Cox analyses comparing Alzheimer disease mortality in patients in whom *Helicobacter pylori* (*Hp*) eradication therapy succeeded or failed. Group A: patients in whom *Hp* was successfully eradicated; group B: patients in whom eradication of *Hp* had failed, they refused and/or were noncompliant with eradication therapy.

TABLE 3. Hazard Ratios (HRs) for Mortality by *Helicobacter pylori* Eradication Group

Model	All	Dead	HR (95% CI)	P
1	42	20	0.287 (0.114-0.725)	0.008
2	42	20	0.29 (0.11-0.765)	0.012

Model 1 is unadjusted. Model 2 is adjusted for age at baseline, gender, and mini-mental scale examination score at baseline.

Five-year Survival After Helicobacter pylori Eradication

the 3 groups, nor between surviving and nonsurviving patients. However, we did not include data concerning the effect of *Hp* eradication in Hcy, folate, or B12 over time. Elevated plasma Hcy levels are independently associated with increased rate of cerebrovascular disease mortality in the elderly.[19] In this respect, chronic gastritis observed in the vast majority of our patients, as a result of *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.[10] The elevated Hcy, in turn, could trigger endothelial damage and result in atherothrombotic disorders and AD. Hcy is thought to be implicated in endothelial damage and neurodegeneration through oxidative injury in AD,[10–20] and oxidative damage has also been described in the brain of subjects with mild cognitive impairment, suggesting that oxidative damage may be one of the earliest events in the onset and progression of AD. It has been shown that the serum Hcy concentration correlates with the severity of dementia, and it is a significant predictor of the severity of dementia.[10]

Apart from *Hp*-induced chronic gastritis-decreased B12/folate-increased Hcy sequence, *Hp* may further confer mortality risk to AD through its implication in a variety of extradigestive vascular conditions including ischemic heart disease,[21] ischemic cerebrovascular,[15] and functional vascular disorders caused by vascular dysregulation, frequently detected in AD; these conditions are the most common causes of death in AD patients.[22,23]

In particular, *Hp* may be involved in the pathophysiology of the aforementioned cardiovascular and cerebrovascular disorders, including AD, thereby contributing to the decline of AD by: (1). Promoting platelet and platelet–leukocyte aggregation.[8,10] Platelet activation and aggregation have also been proposed to play pathophysiologic roles in the development and deterioration of AD.[24,25] (2). Releasing large amounts of proinflammatory and vasoactive substances, such as cytokines [interleukin (IL) − 1, − 6, − 8, − 10, − 12, tumor necrosis factor (TNF)-α, interferon-γ] eicosanoids (leukotrienes, prostaglandins catalyzed by cyclo-oxygenase enzymes), and acute phase proteins (fibrinogen, C-reactive protein)[8,10] involved in a number of vascular disorders, including AD, which can lead to long-term neurologic deficits.[26,27] As in the case of *Hp*-I, recent data also indicate that systemic inflammation, associated with the increased systemic inflammatory marker TNF-α, are related to disease progression in AD[28] (3). Inducing the chronic atrophic gastritis with concomitant decrease in vitamin B12 and folate concentrations, thereby increasing the Hcy. (4). Stimulating mononuclear cells to produce a tissue factor-like procoagulant that converts fibrinogen into fibrin[29]; fibrin is a mediator of inflammation and may accelerate neurovascular damage in AD.[30] (5). Producing reactive oxygen metabolites and circulating lipid peroxides[8,10] that have also been involved in the pathophysiology and prognosis of AD[31,32]; oxidative/nitrosative stress plays a potential role in the prognosis of the disease.[32] (6). Influencing the apoptotic process that may also be an important form of cell death in many neurodegenerative diseases including AD[8,10]; the apoptotic process contributes to the damage of brain cells and progression of the disease.[33]

Finally, adherence to the Mediterranean diet might affect not only risk for AD but also the subsequent disease course; higher adherence to the Mediterranean diet is associated with lower mortality in AD.[34] As our Greek cohort usually ingested this diet, further prospective relative studies with large number of Greek AD patients associated with or without *Hp*-I are needed to elucidate this field.

A limitation of this series is the small number of patients studied. Therefore, our findings are considered rather preliminary, thereby requiring future confirmation.

In conclusion, *Hp*-I seems to contribute to AD decline by several mechanisms. Therefore, *Hp* eradication regimen may improve the long-term survival rate of the disease.

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Original Paper

Audiology & Neurotology

Eradication of *Helicobacter pylori* Infection Aids with the Outcome of Motion Sickness Adaptation

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Abstract

Introduction: Airsickness affects many aviators, especially at the beginning of their flight training. From the symptoms of airsickness, stomach awareness and nausea are among the most common and unpleasant. *Helicobacter pylori* infection of the gastric mucosa is a common cause of gastrointestinal symptoms in the general population, although it has seldom been associated with motion sickness in the scientific literature. *Methods:* A retrospective review was conducted in all cases of pilot trainees taking basic flight training who were referred to the Hellenic Air Force Aeromedical Center due to airsickness and tested for *H. pylori,* for the time period 1996–2005. We compared the *H.-pylori*-positive pilots with the uninfected ones according to their responses to the habituation sorties and subsequent completion of the basic flight training as a whole. A statistical analysis was performed using Fisher's exact test. *Results:* The findings of the study suggest that diagnosing *H. pylori* infection and treating it with eradication therapy increases the possibility of a pilot trainee successfully completing the habituation flights, while it does not affect success in the basic flight training as a whole. *Conclusions:* Eradication therapy for *H. pylori* may provide a temporary reduction in reported nausea during flight training. The findings are not conclusive, but highly suggestive of a pathophysiologic link between *H. pylori* and motion sickness, needing further clarification through targeted studies.

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Introduction

Airsickness is a clinical syndrome characterized by nausea and vomiting during an aircraft flight. It is a subset of the 'motion sickness syndrome' which results from exposure to an unfamiliar apparent or perceived motion [Reason and Brand, 1975]. The syndrome presents with a variety of signs and symptoms such as malaise, pallor, sweating, increased salivation, headache and dizziness, but the most unpleasant symptoms include nausea, vomiting, retching and abdominal discomfort.

Airsickness affects many aviators, especially at the beginning of their flight training. The incidence ranges from 11 to 35% [Dobie, 1974; Lucertini et al., 2008] in basic flight training, but the majority of pilot cadets quickly adapt to the novel motion environment and continue

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their flight training. A minority of trainees encounter difficulties in adaptation and are offered a number of habituation sorties in between the planned sorties of the usual training syllabus. In the Hellenic Air Force (HAF), a pilot trainee who completes 5 sorties with overt airsickness symptoms is referred to the Aeromedical Center, where he undergoes a thorough examination for any pathologic condition which may affect the flight performance. The medical examination routinely includes, among other diagnostic procedures, a gastroendoscopy and *Helicobacter pylori* testing by biopsy. If the referred pilot is diagnosed with *H. pylori* infection, he is treated with eradication therapy according to the international guidelines [Chey and Wong, 2007; Malfertheiner et al., 2007], and is grounded during the course of the therapy. The success of the treatment is determined by a negative urea breath test 1 month after the initial diagnosis. The pilot is then offered up to 3 habituation flights, success in which, defined as the ability to perform a complete training sortie without manifesting overt motion sickness symptoms, leads to continuation of the training syllabus. If airsickness reappears in 2 flights before the end of the training, the trainee is excluded from flight training as 'unfit to fly'.

More than 20 years after its discovery, *H. pylori* is considered the main cause of a variety of gastric and duodenal diseases, including dyspepsia, chronic gastritis, peptic ulcer disease and gastric lymphoma [Kandulski et al., 2008; Marshall and Warren, 1984]. Chronic gastritis is by far the most common, almost universal, histopathologic finding in the infected patients, even without symptoms of gastric disease. Eradication of *H. pylori* after medical treatment restores to a great extent the histopathologic findings [Arkkila et al., 2006; Kokkola et al., 2002]. However, limited research has been done on the potential relationship of *H. pylori* infection and motion sickness. Golan et al. [2007] tried to find a correlation between seasickness and *H. pylori* by comparing a group of sailors who were susceptible to seasickness with a group of nonsusceptible control subjects. However, this study did not show a statistically significant difference in *H. pylori* seropositivity, measured by the urea breath test, between the two groups [Golan et al., 2007].

Methods

A retrospective review was conducted in all cases of male pilot trainees taking basic flight training (T-37 or T-6 aircrafts) who were referred to the Aeromedical Center due to airsickness according to the HAF protocol, during the time period 1996–2005.

The HAF airsickness management protocol has been approved by the Supreme Medical Committee of the HAF Directorate of Medicine, independently of the study. The retrospective use of the archived medical records of the pilot cadets for the purpose of this study was approved by the Scientific Committee of the Hellenic Air Force General Hospital (HAFGH). As is common practice before every invasive or unpleasant procedure in the HAFGH, all pilots provided written informed consent before undergoing gastroendoscopy.

Data were collected from hospitalization records from the HAFGH, the records from the Department of Gastroenterology and the Department of Pathology of the HAFGH and the medical records from the Medical Service of the 120 Flight Training Wing which provides basic flight training for HAF.

All cases of pilot trainees who had undergone gastroendoscopy and had been tested for *H. pylori* by biopsy were included in the study. Cases with incomplete or questionable data were excluded from the analysis. Moreover, pilots who, after the evaluation in the HAFGH, did not complete training for medical reasons other than motion sickness or discontinued training due to loss of motivation to fly were also excluded from the study.

The sample was divided into two groups. The positive group included pilot trainees who were found positive for *H. pylori* and were treated with eradication therapy according to the international guidelines. The negative group included airsick pilots with negative *H. pylori* testing. The instructor pilots were officially blinded to the trainee's diagnosis, but no attempt was made to blind the trainee subjects or to prevent them from disclosing their medical condition to their instructors.

Outcome measures of the study were: (1) success in completing the habituation flights without overt airsickness, and (2) success in completing the basic flight training as a whole without relapse of airsickness. Fisher's exact test was used to compare the two groups and $p < 0.05$ was considered statistically significant.
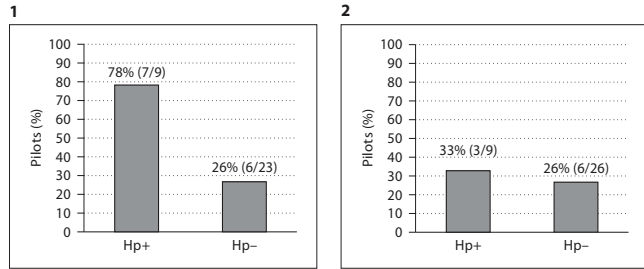
Results

Ultimately, 32 pilot trainees were included in this study. Nine trainees with incomplete or questionable medical records, 6 trainees who were medically disqualified for reasons other than airsickness and 5 trainees who voluntarily discontinued flight training due to loss of motivation were excluded. The positive group consisted of 9 pilots, the biopsy samples of whom had evidence of chronic gastritis and presence of *H. pylori*. Seven pilots (7/9, 78%) from the positive group successfully completed the habituation flights, and 3 pilots (3/9, 33%) successfully completed the basic flight training. Twenty-three pilots without pathologic findings on gastric biopsy were included in the negative group. Six of them (6/23, 26%) were successful in finishing the habituation flights, and the same pilots (26%) completed the basic flight training.

Statistical comparison of the two groups revealed that the positive group was significantly more successful (p =

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Fig. 1. The pilots of the *H. pylori* (Hp)-positive group were more successful in habituation flights than the pilots of the negative group (p = 0.014).
Fig. 2. No statistical significance was found between the *H. pylori* (Hp)-positive and the negative group for the completion of basic flight training (p = 0.68).

0.014) in completing the habituation flights than the negative group. No statistical significance was found between the two groups (p = 0.68) for the completion of the basic flight training as a whole (fig. 1, 2).

Discussion

The results of the present study suggest that diagnosing *H. pylori* infection and treating it with eradication therapy increases the possibility that an airsick pilot trainee will successfully complete the habituation flights, while it may not affect success in the basic flight training as a whole. It is possible that the benefit of *H. pylori* eradication is temporary and may be attributed to a placebo effect. Interpretation of the findings is challenging due to the limited past use of gastroendoscopy in the investigation of motion sickness and the paucity of research on the relationship between motion sickness and *H. pylori* infection. For example, Afonin et al. [1991] used gastroendoscopy to study the effect of motion sickness to the stomach, comparing a group of susceptible and nonsusceptible subjects. They reported that in the susceptible group, motion sickness, provoked by Coriolis cross-coupling stimulation, resulted in the opening of antral and pyloric sphincters, while it did not affect the nonsusceptible group. On the other hand, Golan et al. [2007] hypothesized that *H. pylori* infection may play a role in vomiting resulting from seasickness. However, they found no statistically significant difference in *H. pylori* seropositivity, when they compared a group of sailors susceptible to seasickness with nonsusceptible control subjects.

During the habituation flights, the infected pilots were initially expected to be more susceptible to airsickness than the other airsick cadets, contrary to our results. According to Reason and Graybiel [1973], the individual variation in susceptibility can be attributed to the strength

or weakness of three factors: receptivity, adaptability and the retention of adaptation. All the pilots in our study had achieved about the same amount of flight adaptation before their referral to the Aeromedical Center. The infected pilots were not allowed to fly during the 1-month-long eradication therapy, unlike the negative group who returned to flying status within a week after referral. However, the 'adaptation-deprived' group of *H.-pylori*-positive cadets was not more susceptible to motion sickness upon returning to the habituation flights. It is not known whether this is due to the length of retention of adaptation, the benefits of *H. pylori* treatment or other factors.

It is unknown how *H. pylori* might fit into the pathophysiology of motion sickness. The most broadly accepted theory for the development of motion sickness is the 'sensory conflict theory' [Reason and Brand, 1975]. According to this theory, the motion sickness syndrome arises when conflicting information is processed within the different human sensory modalities. The conflict may be either intersensory between visual and inertial (vestibular and nonvestibular) signals or intrasensory between canal and otolith signals. The conflict is believed to trigger the cascade of reactions leading to the development of the syndrome. Under this concept, it is unreasonable to believe that *H. pylori* infection and chronic gastritis influence the sensory input procedure. On the other hand, motion sickness is a syndrome in which a variety of other symptoms coexist, although dominated by symptoms such as nausea and emesis. Gianaros et al. [2001] illustrated the multidimensionality of motion sickness through the development of a Motion Sickness Assessment Questionnaire in which the list of symptoms were divided into four groups: gastrointestinal, central, peripheral and sopite-related. *H. pylori* infection seems more likely to interact with, or at least be comorbid with, the gastrointestinal symptoms of motion sickness, rather than having a direct etiologic connection.

The present study was not designed to answer whether *H. pylori* infection makes a pilot more susceptible to motion sickness, and the findings were rather incidental. However, one possible explanation could be that *H. pylori* causes baseline stomach symptoms, similar to the symptoms caused by motion sickness, but via different mechanisms. The interaction of the two challenges cumulatively leads to greater symptom reports. According to a questionnaire-based study on motion sickness, individuals with gastrointestinal disorders had a higher incidence of motion sickness (27%) compared to individuals who reported no gastrointestinal problems (20%) [Sharma and Aparna, 1997]. Since *H. pylori* infection is a major cause for several gastrointestinal symptoms, a link between infection and motion sickness susceptibility might actually exist.

Conclusion

The present study presents a possible explanation for the incidental finding that motion sickness-susceptible pilots with an *H. pylori* gastric infection and therefore treated with eradication therapy showed increased tolerance in motion sickness during the habituation flights, compared to a group of uninfected but otherwise similarly susceptible pilots. Our findings may serve as an incentive for future studies which try to answer specific questions on the possible relationship between *H. pylori* and motion sickness. Should a pathophysiologic link actually exist, eradication of *H. pylori* prior to the initiation of flight training is feasible and may increase individual tolerance in motion sickness. Future studies are recommended to employ full blinding and a larger sample size of *H.-pylori*-infected subjects, and to possibly include comparison data concerning the proportion of successful and unsuccessful trainees who are infected with *H. pylori* but do not get referred for additional airsickness habituation. Such research would help to rule out placebo effects and errors associated with small samples, and would aid in the interpretation of the findings relative to baseline failure rates among *H.-pylori*-infected pilots not susceptible to airsickness.

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ORIGINAL ARTICLE

Clinical and laboratory study of rosacea in northern Greece

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Abstract

Background   Numerous factors have been implicated in the pathogenesis of rosacea, which remains obscure.
Objectives   To examine the epidemiological characteristics of rosacea patients, the histopathological alterations, the prevalence of gastric *Helicobacter pylori* infection and the role of ultraviolet radiation, to detect the presence of Demodex folliculorum on affected skin and to elucidate the immunological nature of this disorder.
Methods   The study included 100 patients with rosacea. Each patient was assessed with a clinical, haematological, biochemical and histological examination; serology test for the detection of antibodies against *H. pylori*; direct immunofluorescence on perilesional, sun exposed skin and indirect immunofluorescence with monkey oesophagus as a substrate; antinuclear antibody titre and a skin surface biopsy to search for Demodex folliculorum.
Results   Women were more frequently affected. Half of our patients were 51–70 years old. About two-thirds were phototypes I and II and 73% complained of worsening of conditions after sun exposure. An almost permanent histopathological feature was solar elastosis. Higher prevalence of *H. pylori* was not established. Prevalence and mean density of Demodex folliculorum were significantly increased in rosacea patients. Direct and indirect immunofluorescence tests were positive in 6.4% and 6.7% respectively. Antinuclear antibody titres were found in 21.1%.
Conclusions   Our results suggest the pivotal role of chronic sun exposure in the pathogenesis of rosacea. Demodex folliculorum represents a significant cofactor that may contribute to the transition of the disease from a vascular to an inflammatory stage. The low positive results of direct and indirect immunofluorescence do not support a potential autoimmune role in the development of rosacea.
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Conflict of interest

None declared.

Introduction

Rosacea is a chronic skin disorder, usually affecting middle-aged individuals. Its classic form is characterized by papules, papulopustules, erythema and telangiectasias, usually preceded by episodes of flushing.[1,2] At times the eyes are also involved, resulting in ophthalmic complications such as blepharitis, conjunctivitis and iritis.[3]

The precise aetiopathogenesis of rosacea remains unknown and is generally considered to be multifactorial.[4] Various factors have been mentioned as inducing the disease or contributing to its manifestations. Proposed aetiological mechanisms can be grouped into the following categories: vasculature,[5] climatic exposure,

matrix degeneration,[6] chemicals and ingested agents, pilosebaceous unit abnormalities[7] and microbial organisms.[8] It is likely that rosacea's distinct nosological subtypes represent heterogeneous responses to a combination of these purported factors.[9,10]

This study was designed to: (i) examine the epidemiological characteristics of rosacea patients, (ii) observe the histopathological alterations and estimate their role in certain stages of rosacea, (iii) establish the prevalence of gastric *Helicobacter pylori* infection based on standard Hp detection tests, (iv) evaluate the role of ultraviolet radiation in correlation with the patients' phototype, (v) detect the presence of Demodex folliculorum and assess the relation between this colonization and distinct rosacea symptoms

and (vi) investigate specific immunological characteristics of these patients to elucidate a potential link between them and the aetiological mechanism.

Patients and methods

The study was conducted during a 4-year period and included 100 patients with rosacea, who attended the First Department of Dermatology of the Aristotle University of Thessaloniki, and three control groups; one of 100 healthy individuals matched for age and gender, one of 100 acne patients and one of 50 patients suffering from discoid lupus erythematosus (DLE).

Inclusion criteria were (i) written informed consent provided by all four studied groups, (ii) diagnosis of rosacea, based on the clinical criteria stated by the Expert National Rosacea Society Committee[11] (presence of one or more of the following primary features concentrated on the convex areas of the face: flushing, permanent erythema, papules and pustules and telangiectasia) and the histopathological findings.

At first visit, the gender, age, personal and family history, occupation, phototype, habits and the clinical stage of patients and controls were recorded. Staging was made according to the standard classification system of rosacea reported by the Expert National Rosacea Society Committee.[12]

Punch technique skin biopsy was performed in the patient group. The histological specimens were stained with haematoxylin–eosin dye and examined with an Ernst Leitz microscope.

Human sera were obtained from all patients and from 100 age- and gender-matched, healthy control subjects. The level of immunoglobulin G (IgG) antibodies against *H. pylori* in the serum samples was measured using the enzyme-linked immunosorbent assay method (Milenia *H. pylori* IgG; DPC Biermann GmbH, Bad Nauheim, Germany). The titre was considered to be positive if the IgG value was equal or greater than 44 U/mL and negative if the value was lower than 36 U/mL. In case of the value being between 36 and 44 U/mL, the result was characterized as equivocal.

Demodex folliculorum mites were extracted from designated facial sites using the standardized skin surface biopsy technique with a cyanoacrylate glue and glass slides. The total number of specimens received from each individual was 6. The samples were studied microscopically at standard magnifications (×40, ×100). The mite count was the mean value of the six standard facial sites, calculated as the sum of mites/cm^2 of all samples divided by six, in our rosacea patients and 250 controls (100 acne and 50 DLE patients and 100 healthy controls).

Statistical analysis of all results was carried out using the chi-squared, Fisher's exact, Kruskal–Wallis and Wilcoxon tests.

Antinuclear antibody (ANA) screening was performed in 90 of 100 patients using the ANA/Hep-2 test system (Bega), which is a prestandardized kit designed for the qualitative and semi-quantitative detection of ANA. The indirect immunofluorescence technique was used on snap-frozen sections of monkey oesophagus (Medical Diagnostics Laboratories, Santa Ana, CA, USA) for the detection of circulating antibodies. Skin biopsy specimens from perilesional uninvolved skin were examined for the presence of bound IgG, IgM, IgA, C3 and fibrinogen using direct immunofluorescence staining.

Results

A total of 100 patients, 37 men and 63 women, with a mean age of 58 years, were studied. With regard to gender, women were more frequently affected than men, with a ratio of 1.7 over 1. However, in patients older than 71 years of age, men predominated at a ratio of 1.6 to 1, which suggests that men may develop the disease at an older age (Fig. 1). Approximately half of our patients were 51–70 years old. The mean age of disease onset was 54.2 years and the mean disease duration on first examination was 3.82 years.

According to the standard classification system of rosacea, 38% of our patients belonged to the erythematotelangiectatic subtype, whereas 56% and 8% of them were classified into the papulopustular and phymatous subtype respectively (Table 1). Ophthalmic symptoms and signs were observed in 33% of our patients. The ocular manifestations included blepharitis, conjunctivitis, inflammation of the lids and meibomian glands, interpalpebral conjunctival hyperaemia and conjunctival telangiectasias.

Although the majority of our patients lived in the countryside, we could not identify any obvious correlation between patients' profession and rosacea. Furthermore, there was no association between the socioeconomic level and the disease.

According to the Fitzpatrick skin phototypes, 63% of the patients were classified into phototype II, and 30%, 4% and 3%
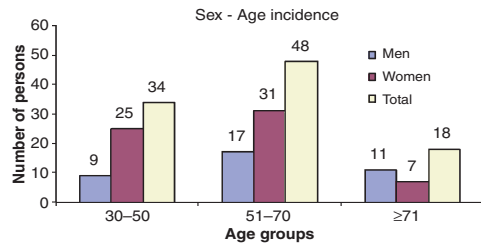

Figure 1 Number of patients with age and gender distribution.

Table 1 Classification of the patients according to the rosacea standard classification system

Rosacea subtype	Number of patients
Erythematotelangiectatic	38 (38)
Papulopustular	56 (56)
Phymatous	6 (6)

Values in parentheses are percentages.

into phototype III, I and IV respectively. In addition, an impressively high percentage of our patients (73%) experienced a severe exacerbation in their rosacea symptoms after sun exposure. Other exacerbating factors included alcohol intake (24%), heat (10%), stress (8%), hot beverages (5%), spicy food (1%), smoking (1%) and abrupt changes in temperature (1%).

At least one coexisting dermatological disease was present in 32% of the patient group, with dermatitis being the commonest one (6%). Basal cell epithelioma (4%), toenail onychomycosis (4%), psoriasis (3%), common warts (2%), melasma (2%), alopecia areata (2%) and pemphigus (2%) were diagnosed during the clinical evaluation. Concomitant systemic diseases included hypertension (12%), gastritis (12%), diabetes (6%), arthritis (6%) and hyperlipidaemia (6%). A positive family history of rosacea was established only in 18% of our patients.

Histological features varied according to the subtype and quality of the disease. Erythematotelangiectatic type was characterized mainly by ectatic venules and lymphatics, perivascular and perifollicular lymphohistiocytic infiltration, moderate elastic tissue hyperplasia, slight oedema and actinic elastosis. On the other hand, in the papulopustular type, there was evidence of intrafollicular collections of neutrophils, increased perifollicular infiltration, diffuse expansion of connective tissue and distorted follicular canals, accompanied by hyperplasia of sebaceous follicles. Complementarily, skin biopsies performed on phymatous lesions revealed sebaceous hyperplasia, dilated follicular infudibula, telangiectases, perifollicular infiltrates of plasma cells, lymphocytes and histiocytes, suppuration and severe elastosis. Granulomas were present in 13% of the specimens. Demodex folliculorum mites were detected in 32 of the 100 patients (32%) and in eight of the 13 patients with granulomatous findings (61.5%).

Immunoglobulin G antibodies against *H. pylori* were detected in 16 of 37 male patients (43.2%), as well as in 26 of 63 female patients (41.3%) (Table 2). No difference was found in the prevalence of antibodies to *H. pylori* in the total number of patients (42%), compared with that in the total number of controls (46%), even when patients and controls were divided into two different age groups. According to Fisher's exact test analysis, the statistical difference noticed between rosacea and healthy subjects was insignificant ($P = 0.6692$). Similar results were obtained when male and female rosacea patients were compared ($P = 1$). After segregation of rosacea and controls into two distinct subgroups, one with, and one without previous use of antibiotics, the comparison of the seroprevalence of IgG *H. pylori* antibodies revealed that previous use of antibiotics modified the relationship between the factors studied and the outcome. A strong association was found between *H. pylori* and rosacea in the group of patients who had not taken any antibiotics.

Using skin surface biopsies, mite prevalence was found to be significantly higher (χ^2 test) among patients with rosacea, compared with that of the control groups and is shown in Table 3. By using Fisher's exact test, statistically significant differences were

Table 2 Prevalence of IgG antibodies against *Helicobacter pylori* in rosacea patients and healthy controls

	H. pylori IgG	Age groups	
		30–60 years	61–90 years
Men with rosacea	16/37 (43.2)	6/15 (40)	10/22 (45.5)
Women with rosacea	26/63 (41.3)	13/34 (38.2)	13/29 (44.8)
All patients with rosacea	42/100 (42)	19/49 (38.8)	23/51 (45.1)
Men healthy controls	18/40 (45)	5/18 (27.8)	13/22 (59.1)
Women healthy controls	28/60 (46.7)	10/31 (32.3)	18/29 (62.1)
All healthy controls	46/100 (46)	15/49 (30.6)	31/51 (60.8)

Values in parentheses are percentages.

Table 3 Prevalence and mean density of Demodex folliculorum in rosacea patients and control groups

Study groups	Number of individuals	Mite prevalence	Mean mite density	
			Average ± SD	Fluctuation
Rosacea patients	100	84	2.20 ± 1.61	0–6.83
Healthy control group	100	17	0.28 ± 0.66	0–2.83
Acne control group	100	20	0.37 ± 0.83	0–3.50
DLE control group	50	18	0.28 ± 0.67	0–2.67

observed between rosacea patients and healthy individuals ($P < 0.0001$), rosacea and DLE patients ($P < 0.0001$) and rosacea and acne groups ($P < 0.0001$) regarding mite prevalence (Table 3). On the contrary, statistically insignificant differences were recorded among healthy, DLE and acne groups.

The mean mite density was higher in rosacea patients than in the control groups (Table 3). Kruskal–Wallis test was used to confirm the presence of significant differences among the four subject groups. Subsequently, Wilcoxon test was used to compare the results for each of the two categories. With regard to the mite density, statistically significant differences were observed between the rosacea and healthy group ($P < 0.0001$), as well as between the rosacea and DLE ($P < 0.0001$) and rosacea and acne group ($P < 0.0001$), although no such results were recorded among control groups.

Antinuclear antibody screening was positive in 19 of 90 (21.1%) rosacea patients. Detected titres fluctuated from 1/40 to 1/160. The indirect immunofluorescence assay was performed in 90 of 100 rosacea patients and was found to be positive in six patients (6.7%), while titres were between 1/20 and 1/40. Direct immunofluorescence staining was positive in five of 78 (6.4%) specimens studied. Binding immunoglobulin was of IgG type in three of them and of IgM and CPH type in three and two others respectively.

Discussion

Approximately half of our patients were 51–70 years old, which confirms the literature data, where an increased incidence is described between 50 and 60 years.[13,14] The gender ratio of studied patients, as well as the mean age recorded, is in agreement with previously reported epidemiological data.[14] However, we noticed that over the age of 71 years, male patients preceded female patients. These results suggest that men may develop the disease at an older age.

The mean age of disease onset was 54.2 years. Our patients had complained of rosacea for approximately 3 months to 10 years. Most of them were classified in the papulopustular subtype, whereas only 6% belonged to the phymatous stage. The majority lived in the countryside. However, we were unable to find any association of patients' profession with rosacea. Furthermore, there was no correlation between the socioeconomic level and the disease, as has been stated in other studies.[15]

The significant role of skin phototype in the development of the disease is underlined by the fact that the vast majority of our patients were classified into phototype II. This is in contrast with the predominant phototypes in the general population in northern Greece, where the vast majority of inhabitants belong to phototypes III–IV. Moreover, the disease exacerbation by sunlight in 73% in combination with the professional exposure to sun in 19%, the biopsy elastosis findings and the predominant facial distribution confirm the important role of solar exposure in rosacea pathogenesis. These results are in contrast with previous epidemiological data demonstrating that only 17–31% of rosacea patients report worsening of symptoms by sunlight.[14,16] However, other authors advocate for the pathogenetic role of solar radiation, reporting that there is an association of the disease with fair skin and light eyes and a predilection for disease flares in early spring.[13]

Other exacerbating factors, also mentioned by many other authors earlier,[17–19] included alcohol intake, heat, stress, hot beverages, spicy food, smoking and abrupt changes in temperature. No strong evidence of a family history was detected, as only 18% of our patients had family members with this disorder, which is lower than the number reported in previous studies.[15]

There was no correlation with any other skin disease. Our data show that there was a weak association with hypertension, gastritis and peptic ulcer. However, after comparing the incidence of these diseases with their incidence in our general population with the same age and gender distribution, no difference could be identified.

Our histological findings did not differ from the ones reported in the literature. The pathogenetic role of the pilosebaceous unit abnormalities in the development of the disease is still under research.[20] However, it is documented that the glandular type of rhinophyma is a follicle-based inflammatory process.[21]

Although no difference was found in the prevalence of antibodies to H. pylori in the total number of patients (42%), compared with that of the total number of controls (46%), a strong associa-tion was found between H. pylori and rosacea in the group of patients who had not taken any antibiotics. Prolonged repeated use of antibiotics in rosacea could diminish the antigenic effect of H. pylori without completely eradicating the organism, resulting in low levels of reactivity, which would be considered negative by the usual kits. Despite exhaustive studies,[22–27] the controversy concerning the possible role of H. pylori in rosacea still goes on.

Our results support a strong aetiopathogenetic correlation between the mite D. folliculorum and rosacea. The mite prevalence and mean density were both significantly higher between patients' group and controls. These observations, together with the findings of other investigators,[28–33] suggest that Demodex probably represents a significant cofactor, which along with others is responsible for an inflammatory reaction at the site of the lesions, thus contributing to a transition from the purely vascular (erythematotelangiectatic) to the papulopustular subtype.

The results of direct and indirect immunofluorescence were insignificant and antibodies to extractable nuclear antigens were detected in a very small number of patients. These are in agreement with previous literature data.[34] However, there are studies which report immunofluorescence percentage in rosacea, similar to the one observed in DLE.[35–39] ANAs were found in 21.1% of cases and their detection was attributed to sun exposure, as has been suggested by other authors.[40] None of our patients developed any evidence of lupus erythematosus so far.

Patients with rosacea demonstrate a broad spectrum of possible findings. Thus rosacea may essentially be a syndrome with a multifactorial aetiology and chronic course. Initially, there may be an abnormal cutaneous vascular response to multiple factors in persons with a genetic predisposition, which results in the appearance of recurrent flushing, followed by permanent erythema and telangiectasia. On such a background, specific aetiological factors like the skin changes of solar elastosis or the mite D. folliculorum may contribute to the appearance of the late inflammatory stages of the disease. Many aspects of rosacea require further investigation and more future studies are needed to elucidate the role of certain factors in the pathogenesis of this disease.

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CagA and VacA Polymorphisms Are Associated with Distinct Pathological Features in *Helicobacter pylori*-Infected Adults with Peptic Ulcer and Non-Peptic Ulcer Disease[▽]

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Polymorphic variability in *Helicobacter pylori* factors CagA and VacA contributes to bacterial virulence. The presence of one CagA EPIYA-C site is an independent risk factor for gastroduodenal ulceration (odds ratio [OR], 4.647; 95% confidence interval [CI], 2.037 to 10.602), while the presence of the *vacA* i1 allele is a risk factor for increased activity (OR, 5.310; 95% CI, 2.295 to 12.287) and severity of gastritis (OR, 3.862; 95% CI, 1.728 to 8.632).

Helicobacter pylori, colonizing the gastric mucosa of 35 to 70% of people worldwide, is the etiologic factor for peptic ulcer development and increases the risk for gastric cancer. *H. pylori* pathogenesis is exerted via distinct virulent factors such as the secreted cytotoxin VacA (vacuolating cytotoxin A), the *cag* pathogenicity island (*cag*PAI) encoding the type IV secretion system (T4SS), and the cytotoxin-associated gene A (CagA) protein (6). We analyzed *H. pylori* clinical isolates from the antrum of 144 Greek adults (mean age ± standard deviation [SD], 52.6 ± 13.7 years; 78 male) diagnosed with peptic ulcer (gastric, $n = 21$; duodenal, $n = 44$) and non-peptic ulcer disease (nonulcer dyspepsia, $n = 61$; esophagitis, $n = 18$) on the basis of functional CagA EPIYA motifs as well as *vacA* alleles for signal, intermediate, and middle regions, as described previously (13, 15), and assessed putative associations with disease parameters and gastric inflammatory response.

Approximately 27% of the strains were found to be *cagA* negative with complete absence of the *cag*PAI. Among the 96 *cagA*-positive isolates, 15 (10.4%) lacked a functional T4SS as they induced minimal interleukin-8 (IL-8) levels (Fig. 1A) and no phosphorylated CagA was detected (Fig. 1B) following infection of gastric epithelial AGS cells (15). Infection with strains possessing a functional T4SS led to significantly higher IL-8 secretion, irrespective of the number of EPIYA-C sites, and to CagA phosphorylation (Fig. 1A and B). Hence, for univariate and multivariate logistic regression analysis, *cagA*-positive isolates with a nonfunctional T4SS were grouped together with *cagA*-negative cases, comprising the "None" category. In single *H. pylori* strain infections, the majority of isolates ($n = 59$, 41.0%) were of the ABC EPIYA type (15), with a second EPIYA-C repeat observed in 19 (13.2%) strains,

while ABCCC strains were also identified ($n = 2$, 1.4%). In 11 cases (7.7%), the presence of mixed infection by isogenic strains differing solely with regard to the number of EPIYA-C repeats was identified as shown before (13).

The dominant *vacA* polymorphisms for the signal, intermediate, and middle regions were s1, i1, and m2, respectively, as reported for Western-type *H. pylori* strains (5, 17). More specifically, 102 (70.8%) isolates were identified as *vacA* s1, with 57 (39.6%) carrying the *vacA* m1 allele simultaneously. No strain with *vacA* s2/m1 was recorded. Of the 91 *vacA* i1 strains, 54 (59.3%) were also typed as *vacA* s1/m1, whereas 38/53 (71.7%) *vacA* i2 strains were s2/m2 ($P < 0.001$). Depending on the *vacA* genotype, strains were further classified into three categories (7, 14), namely, nonvacuolation (s2/i2/m2, s1/i2/m1, s1/i2/m2, and s2/i1/m2), low vacuolation (s1/i1/m2), and high vacuolation (s1/i1/m1). Vacuolating *vacA* s1/i1/m1 or s1/i1/m2 types were present in strains harboring functional CagA variants with more EPIYA-C phosphorylation repeats, with frequencies reaching approximately 90% in cases of multiple infections, whereas *cag*PAI-defective strains were almost exclusively related to a nonvacuolating *vacA* genotype ($P < 0.001$).

vacA s1 and i1 polymorphisms were found to be associated with marked chronic inflammatory infiltration and activity of chronic gastritis in the antrum (Table 1). No association was observed with the density of *H. pylori* colonization or the presence of gastric atrophy and intestinal metaplasia (IM), even though in 41/57 (71.9%) of recorded IM the strain carried the *vacA* i1 allele ($P = 0.111$). However, the risk for IM development increased 2-fold upon infection with *vacA* m1 strains (odds ratio [OR], 2.182; 95% confidence interval [CI], 1.098 to 4.338; $P = 0.026$). Heavy *H. pylori* colonization (OR of 6.866 and 95% CI of 3.072 to 15.344 [$P < 0.001$] and OR of 8.476 and 95% CI of 3.633 to 19.777 [$P < 0.001$], respectively) and infection with *vacA* i1 strains (OR of 3.862 and 95% CI of 1.728 to 8.632 [$P = 0.001$] and OR of 5.310 and 95% CI of 2.295 to

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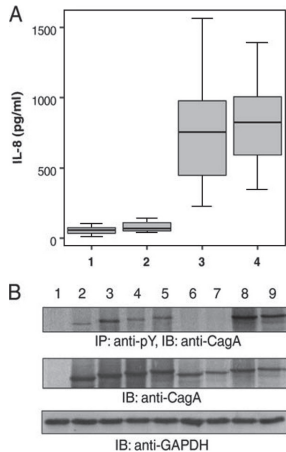
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FIG. 1. (A) Levels of secreted IL-8 following infection of gastric ep-ithelial AGS cells with *H. pylori* clinical strains (1, *cagA* negative; 2, *cag*PAI defective; 3, 1 EPIYA-C repeat; 4, ≥2 EPIYA-C repeats). No difference was observed between *cagA*-negative and *cag*PAI-defective strains (U = 32.500 and $P = 0.201$ by the Mann-Whitney U test). *cagA*- and *cag*PAI-positive strains induced higher levels of IL-8 than *cag*PAI-defective strains (U = 0.000 and $P < 0.0001$ by the Mann-Whitney U test), irrespective of the number of EPIYA-C sites (U = 224.500 and $P = 0.627$ by the Mann-Whitney U test). (B) Tyrosine phosphorylation and expression patterns of CagA protein following infection of AGS cells with representative *H. pylori* clinical strains. CagA tyrosine phosphorylation was de-tected by immunoblotting (IB) following immunoprecipitation (IP) with PY20 antiphosphotyrosine antibody. The expression of GAPDH (glycer-aldehyde-3-phosphate dehydrogenase) was utilized as a total protein load-ing control. Lanes: 1, CagA-negative clinical isolate; 2 to 5, CagA-positive isolates with functional *cag*PAI harboring 2 (AB), 3 (ABC), 4 (ABCC), and 5 (ABCCC) motifs in CagA, respectively; 6 and 7, CagA-positive *H. pylori* strains carrying 3 (ABC) and 4 (ABCC) EPIYA motifs with defec-tive *cag*PAI, respectively, as depicted by the absence of phosphorylated CagA; 8 and 9, CagA-positive strains with 5 (ABCCC) EPIYA motifs.

12.287 [$P = 0.001$], respectively) were recognized as indepen-dent risk factors for the development of severe chronic inflam-matory infiltration and marked activity of chronic gastritis in the antrum. This is the first report associating *vacA* intermediate region polymorphisms with increased activity of antral gastritis. *vacA* s1 strains of Western origin have previously been associated with more-severe gastric inflammation (5, 11, 16). The only re-ports relating specific *cagA* polymorphisms with histological le-sions involve strains of East Asian origin carrying the ABD EPIYA sites, which present distinct biological properties com-pared to Western-type ABC EPIYA motifs (4).

The development of gastric ulcers (GU) or duodenal ulcers (DU) was associated with the occurrence of *H. pylori* strains harboring functional EPIYA-C repeats in CagA ($P = 0.001$) as well as with the *vacA* s1 allele ($P = 0.004$) and marked activity of chronic gastritis ($P = 0.014$). Despite that, CagA EPIYA polymorphisms were found to be the only independent risk factor for ulcer disease (Table 2), with over 50% of strains with 1 (57.6%) or 2 or more (52.4%) EPIYA-C repeats found to be isolated from ulcer cases (8 GU/26 DU and 4 GU/7 DU, respectively) and the majority (8/11, 72.8%) of multiple infec-tions with isogenic strains (4 GU and 4 DU) (Table 2). To date, infection with *cagA*-positive *H. pylori* has been well associated with gastroduodenal ulcers (2, 6, 12), whereas variability in the EPIYA phosphorylation sites and in particular CagA variants with an increased number of EPIYA-C repeats or of East Asian origin have been reported to augment the risk for gastric adenocarcinoma (1, 4, 8–10, 20). In our study, we observed that the presence of single or multiple infecting strains rather than the number of EPIYA-C sites in CagA *per se* is probably crucial in determining the type of gastric disease, since the majority of mixed infections with isogenic strains expressing CagA with various numbers of EPIYA-C repeats were isolated from peptic ulcer patients. Previous reports relate ulcer lesions with the presence of *vacA* s1 and i1 types (3, 5, 14), although in our sample, the association of VacA determinants with peptic

TABLE 1. Univariate logistic regression analysis showing association of *vacA* and *cagA* polymorphisms with severity and activity of chronic gastritis in the antrum of 144 Greek adults

Risk factor	Chronic inflammatory infiltration				Activity of chronic gastritis			
	No. (%) of isolates with mild/moderate severity	No. (%) of isolates with marked severity	OR (95% CI)[a]	P	No. (%) of isolates with mild/moderate activity	No. (%) of isolates with marked activity	OR (95% CI)[a]	P
vacA alleles								
s2	25 (17.4)	17 (11.8)	Reference		27 (18.8)	15 (10.4)	Reference	
s1	34 (23.6)	68 (47.2)	2.941 (1.402–6.171)	0.004	38 (26.4)	64 (44.4)	3.032 (1.435–6.405)	0.004
i2	30 (20.8)	23 (16.0)	Reference		34 (23.6)	19 (13.2)	Reference	
i1	29 (20.1)	62 (43.1)	2.789 (1.385–5.614)	0.004	31 (21.5)	60 (41.7)	3.463 (1.704–7.040)	0.001
cagA EPIYA status								
None	32 (22.2)	21 (14.6)	Reference		33 (22.9)	20 (13.9)	Reference	
1 EPIYA-C repeat	18 (12.5)	41 (28.5)	3.471 (1.589–7.58)	0.002	23 (16.0)	36 (25.0)	2.583 (1.204–5.539)	0.015
≥2 EPIYA-C repeat	6 (4.2)	15 (10.4)	3.810 (1.274–11.389)	0.017	6 (4.2)	15 (10.4)	4.125 (1.376–12.363)	0.011
Mixed infections	3 (2.1)	8 (5.6)	4.063 (0.966–17.091)	0.056	3 (2.1)	8 (5.6)	4.4 (1.044–18.542)	0.044
Vacuolation potential								
None	32 (22.2)	24 (16.7)	Reference		35 (24.3)	21 (14.6)	Reference	
Low	12 (8.3)	22 (15.3)	2.444 (1.014–5.895)	0.047	11 (7.6)	23 (16.0)	3.485 (1.418–8.566)	0.007
High	15 (10.4)	39 (27.1)	3.467 (1.563–7.690)	0.002	19 (13.2)	35 (24.3)	3.070 (1.411–6.681)	0.005

[a] Reference, used as the reference category for the calculation of risk in each case.

TABLE 2. Multivariate logistic regression model depicting parameters relating to the development of peptic ulcers

Risk factor	No. (%) of cases with non-peptic ulcers	No. (%) of cases with peptic ulcers	OR (95% CI)[a]	P
cagA EPIYA status				
Defective *cag*PAI	41 (28.5)	12 (8.3)	Reference	<0.001
1 EPIYA-C repeat	25 (17.4)	34 (23.6)	4.647 (2.037–10.602)	0.015
≥2 EPIYA-C repeat	10 (6.9)	11 (7.6)	3.758 (1.288–10.969)	0.003
Mixed infections	3 (2.1)	8 (5.6)	9.111 (2.085–39.810)	<0.001
vacA alleles				
s2	30 (20.8)	11 (7.6)	Reference	
s1	49 (34.0)	54 (37.5)		0.24
i2	34 (23.6)	19 (13.2)	Reference	
i1	45 (31.3)	46 (31.9)		0.76
Activity of chronic gastritis				
Mild	11 (7.6)	1 (0.7)	Reference	
Moderate	32 (22.2)	21 (14.6)		0.383
Marked	36 (25.0)	43 (29.9)		0.129

[a] Reference, used as the reference category for the calculation of risk in each case.

ulcer disease was not sustained through multivariate analysis, possibly reflecting geographical differences in the prevalence of the various genotypes (17–19).

Collectively, our data indicate a distinct yet coordinated activity of virulence factors associated with *H. pylori* pathogenesis, with CagA contributing to the development of particular disease phenotypes, such as peptic ulcer, and VacA differentially affecting the inflammatory process. Our findings emphasize the necessity to meticulously assess the functionality of virulence factors in *H. pylori* clinical strains so as to discern the true biological significance that lies beneath the plasticity of the *H. pylori* genome.

Nucleotide sequence accession numbers. Partial *cagA* nucleotide sequences were submitted to the GenBank/EMBL/DDBJ databases under accession numbers FN561978 to FN562025.

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Helicobacter pylori infection and gastric histology in first-degree relatives of gastric cancer patients: a meta-analysis

Theodore Rokkas, Panos Sechopoulos, Dimitrios Pistiolas, Georgios Margantinis and Georgios Koukoulis

Objectives **Helicobacter pylori (H. pylori) is believed to predispose to gastric cancer by inducing the precancerous changes, that is, atrophy and intestinal metaplasia (IM). First-degree relatives of patients with gastric cancer might be at an increased risk of developing gastric cancer. However, this evidence is based on the scattered individual studies. The aim of this study was to examine the risk of first-degree relatives developing gastric cancer, in comparison with controls that have no family history of gastric cancer, by meta-analyzing all relevant studies.**

Methods **Extensive English language medical literature searches for human studies were performed up to the end of November 2009, using suitable keywords. Inclusion and exclusion criteria were identified and in eligible studies data on three parameters, that is, H. pylori prevalence, atrophy and IM, were extracted. Pooled estimates (odds ratio with 95% confidence intervals) were obtained using either the fixed or random-effects model as appropriate. Heterogeneity between studies was evaluated with the Cochran Q test, whereas the likelihood of publication bias was assessed by constructing funnel plots. Their symmetry was estimated by the Egger's regression asymmetry test.**

Results **Out of 155 initially identified studies, 11 studies, from various countries, fulfilling the inclusion criteria, examined the risk of first-degree relatives developing gastric cancer (n=1500) in comparison with controls**

(n=2638). For H. pylori prevalence, the pooled odds ratio with 95% confidence interval was 1.925 (1.419–2.611) and the test for overall effect Z was 4.211 (P=0.000). The respective values for atrophy and IM were 2.200 (1.266–3.824), Z=2.797, (P=0.005) and 1.982 (1.363–2.881), Z=3.582 (P=0.000) respectively.

Conclusion **The results of this meta-analysis showed that first-degree relatives of patients with gastric cancer might be at an increased risk of developing gastric cancer, as judged by significantly higher prevalence of H. pylori, gastric atrophy and IM, in comparison with controls. Consequently, H. pylori detection and prophylactic eradication of the infection should be offered to such individuals. However, follow-up studies are required to prove the above.** *Eur J Gastroenterol Hepatol* **22:1128–1133 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Introduction

Gastric cancer is the second most common cause of cancer deaths worldwide [1,2]. It comprises two major types [3,4], that is, first the intestinal, which is the more common variant and which has a strong association with environmental factors including cigarette smoking, diet (particularly salted foods), and *Helicobacter pylori* (*H. pylori*) and second diffuse gastric cancer, which is less common than the intestinal type but is more likely to be attributed to host-factor effects, such as mutations of the *E-cadherin* gene [5,6].

H. pylori is believed to predispose to gastric cancer by inducing precancerous changes, that is, atrophy and intestinal metaplasia (IM) [7]. First-degree relatives (siblings or offspring) of patients with gastric cancer might be at an increased risk of developing gastric cancer, as judged by studies that examined the prevalence of *H. pylori* infection and the development of gastric atrophy and IM in relatives and controls. However, this evidence is based on the scattered individual studies. The aim of this study was to estimate the risk of first-degree relatives developing gastric cancer by meta-analyzing all relevant studies.

Materials and methods
Data identification and extraction
We searched the Pubmed, Medline and Embase databases from November 2009 to identify all relevant English language medical literature for human studies

under the search text terms *gastric cancer AND first-degree relatives AND increased risk*. We also performed a full manual search of all review articles, recently published editorials and all retrieved original studies. The data were extracted independently from each study by two of the authors (G.M. and G.K.) by using a predefined form, and disagreements were resolved by discussion with a third investigator and consensus.

Selection criteria
Inclusion and exclusion criteria were delineated before the commencement of the literature search. Thus, eligible studies were included in this meta-analysis if they met the following criteria: (a) published as full article or abstract and (b) contained raw data, on comparison of *H. pylori* infection and/or gastric atrophy and/or IM in first-degree relatives versus controls who were matched to relatives for age and sex and had no family history of gastric cancer. Studies that did not meet the aforementioned criteria, studies without data for retrieval and duplicate publications were excluded. When two papers reported the same study, the publication that was more informative was selected.
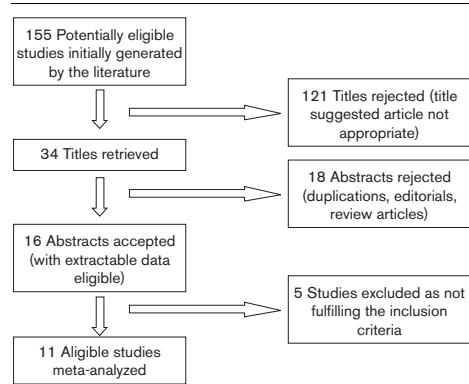
Statistical analysis
Agreement on the selection of studies between the two reviewers was evaluated by the κ coefficient. We calculated the pooled odds ratios (ORs) and 95% confidence intervals (CIs) and compared the outcomes of individual studies by using the fixed-effects model (Mantel and Haenszel method) [8], unless significant heterogeneity was present, where the random-effects model was applied (DerSimonian and Laird method) [9]. Heterogeneity between studies was evaluated with the Cochran Q test and the I^2 statistic [10–12]. The forest plots were constructed for visual display of ORs of individual studies. The likelihood of publication bias was assessed by constructing funnel plots, which were obtained by plotting the log ORs versus precision (1/SE) of individual studies [13]. Their symmetry was estimated by the Egger's regression asymmetry test [14]. Data were meta-analyzed by using suitable meta-analysis software (Comprehensive Meta Analysis – Version 2, BIOSTAT Inc., Englewood, New Jersey, USA).

Results
Descriptive assessment and study characteristics
A flow chart describing the process of study selection is shown in Fig. 1. Out of 155 titles initially generated by the literature searches, 11 studies, fulfilling the inclusion criteria, were eligible for meta-analysis [15–25]. These were case–control [16,21,22] or cohort studies [15,17–20,23–25] and were published as full papers. Initial agreement between the reviewers for the selection of relevant articles was high [$\kappa = 0.923$, 95% CI (0.849–0.997)].

Fig. 1

Flow diagram of the studies identified in this meta-analysis.

The main characteristics of the studies eligible for meta-analysis are shown in Table 1. In brief, 11 studies were selected of which 10 applied to the prevalence of *H. pylori*, six to the prevalence of gastric atrophy and eight to the prevalence of IM. They were conducted in different parts of the world and included 1500 first-degree relatives and 2638 controls.

H. pylori prevalence
Ten studies [15,17–25] examined the *H. pylori* prevalence in 1263 first-degree relatives of gastric cancer patients in comparison with 2401 controls. In all studies, *H. pylori* infection was determined by histology. There was significant heterogeneity between studies [heterogeneity Q value = 22.984, d.f. (Q) = 9, $I^2 = 60.842$, $P = 0.006$] and therefore the random-effects model was used. The group of first-degree relatives of gastric cancer patients was at an increased risk of harbouring *H. pylori* infection [pooled OR with 95% CI = 1.925 (1.419–2.611) and test for overall effect $Z = 4.211$ ($P = 0.000$)]. The forest plot of these results is presented in Fig. 2. There was no significant publication bias (Egger's asymmetry regression test, two-tailed P value = 0.866) (Fig. 3).

Atrophy
Six studies [15,18–21,24] examined the atrophy prevalence in 825 first-degree relatives of gastric cancer patients in comparison with 978 controls. There was significant heterogeneity between studies [heterogeneity Q value = 18.943, d.f. (Q) = 5, $I^2 = 73.605$, $P = 0.002$] and therefore the random-effects model was used. The group of first-degree relatives of gastric cancer patients was at an increased risk of developing atrophy [pooled OR with 95% CI = 2.200 (1.266–3.824) and test for overall

Table 1　The main characteristics of studies selected for meta-analysis

Study/Year	Country	Type of publication	Type of study	No. of relatives involved	No. of controls involved	Prevalence evaluated HP	Atrophy	IM
Carneiro et al. [15]	Portugal	Full paper	Cohort study	63	151	Yes	Yes	Yes
Meining et al. [16]	Germany	Full paper	Case–control study	237	237	No	No	Yes
Brenner et al. [17]	Germany	Full paper	Cohort study	39	1021	Yes	No	No
El-Omar et al. [18]	UK	Full paper	Cohort study	100	60	Yes	Yes	Yes
Jablonská and Chlumská [19]	Czech Republic	Full paper	Cohort study	108	73	Yes	Yes	Yes
Chang et al. [20]	Korea	Full paper	Cohort study	300	426	Yes	Yes	Yes
Sheu et al. [21]	Taiwan	Full paper	Case–control study	150	150	Yes	Yes	Yes
Romiti et al. [22]	Italy	Full paper	Case–control study	39	39	Yes	No	Yes
Leung et al. [23]	China	Full paper	Cohort study	270	330	Yes	No	No
Motta et al. [24]	Brazil	Full paper	Cohort study	104	118	Yes	Yes	Yes
Shin et al. [25]	Korea	Full paper	Cohort study	90	33	Yes	No	No
				Total number of patients involved = 1500	Total number of controls involved = 2638			

HP, *Helicobacter pylori*; IM, intestinal metaplasia.

Fig. 2

Study name	Odds ratio	Lower limit	Upper limit	Z value	P value
Carneiro et al. [15]	5.202	1.185	22.832	2.185	0.029
Brenner et al. [17]	2.901	1.453	5.790	3.020	0.003
El-Omar et al. [18]	0.851	0.434	1.669	−0.469	0.639
Jablonská and Chlumská [19]	1.105	0.610	2.004	0.330	0.741
Chang et al. [20]	2.028	1.463	2.811	4.246	0.000
Sheu et al. [21]	4.000	2.396	6.677	5.303	0.000
Romiti et al. [22]	1.108	0.456	2.697	0.227	0.821
Leung et al. [23]	1.772	1.280	2.455	3.445	0.001
Motta et al. [24]	1.711	0.866	3.381	1.546	0.122
Shin et al. [25]	3.375	1.205	9.455	2.314	0.021
Pooled data	1.925	1.419	2.611	4.211	0.000

Random effects model

The forest plot (random-effects model), concerning *H. pylori* prevalence in first-degree relatives of gastric cancer patients and controls.

effect $Z = 2.797$ ($P = 0.005$)]. The forest plot of these results is presented in Fig. 4. There was no significant publication bias (the Egger's asymmetry regression test, two-tailed P value = 0.521).

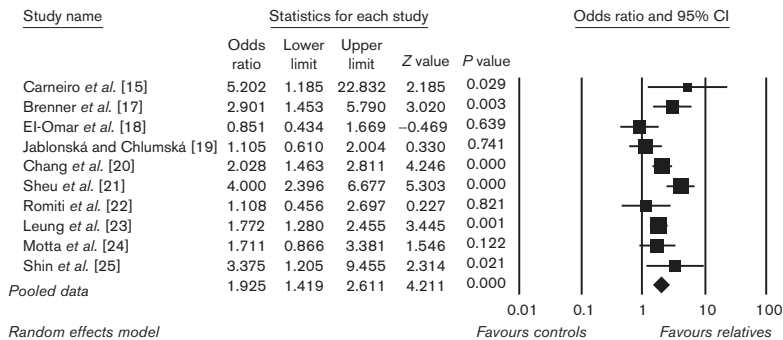### Intestinal metaplasia

Eight studies [15,16,18–22,24] examined the IM prevalence in 1101 first-degree relatives of gastric cancer patients in comparison with 1254 controls. There was significant heterogeneity between studies [heterogeneity Q value = 17.843, d.f. (Q) = 7, $I^2 = 60.768$, $P = 0.013$] and therefore the random-effects model was used. The group of first-degree relatives of gastric cancer patients was at an increased risk of developing IM [pooled OR

with 95% CI = 1.982 (1.763–2.881) and test for overall effect $Z = 3.582$ ($P = 0.000$)]. The forest plot of these results is presented in Fig. 5. There was no significant publication bias (Egger's asymmetry regression test, two-tailed P value = 0.757).

Discussion

About 50% of the world population is infected by *H. pylori* [26] and a positive correlation has been found between *H. pylori* seropositivity and gastric cancer mortality rates [27]. According to meta-analyses, *H. pylori* infection is associated with a two-fold increased risk of developing gastric adenocarcinoma [28,29] and the World Health Organization has classified *H. pylori* in the group of

Fig. 3

Funnel plot of precision by log odds ratio

The funnel plot concerning *H. pylori* prevalence. No evidence of publication bias.

number one carcinogens [30]. Despite the fact that the rates of both *H. pylori* infection and gastric cancer are decreasing in developed countries, 900 000 deaths from gastric cancer are expected in the year 2010 [26,31].

H. pylori is believed to predispose to gastric cancer by inducing precancerous changes, such as atrophy and IM [7]. The results of this meta-analysis showed that the first-degree relatives of gastric cancer patients had a significantly higher risk of harbouring *H. pylori* and this was paralleled by statistically significant higher risks for developing the precancerous lesions of atrophy and IM, in comparison with controls. All of the above means that the first-degree relatives of gastric cancer patients might be at a high risk for developing gastric cancer. However, it must be stressed that follow-up studies are required to prove this hypothesis.

The familial clustering of gastric cancer and the significance of family history has been studied in the

Fig. 4

Study name	Odds ratio	Lower limit	Upper limit	Z value	P value
Carneiro *et al.* [15]	1.544	0.796	2.994	1.286	0.198
El-Omar *et al.* [18]	12.667	3.710	43.248	4.052	0.000
Jablonská and Chlumská [19]	4.521	1.880	10.867	3.371	0.001
Chang *et al.* [20]	2.280	1.535	3.386	4.083	0.000
Sheu *et al.* [21]	1.192	0.666	2.134	0.592	0.554
Motta *et al.* [24]	0.955	0.408	2.235	−0.105	0.916
Pooled data	2.200	1.266	3.824	2.797	0.005

Statistics for each study

Odds ratio and 95% CI

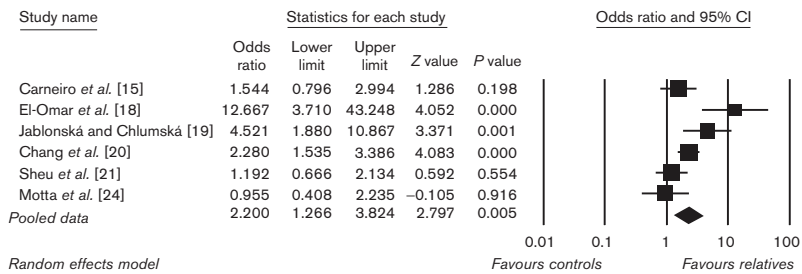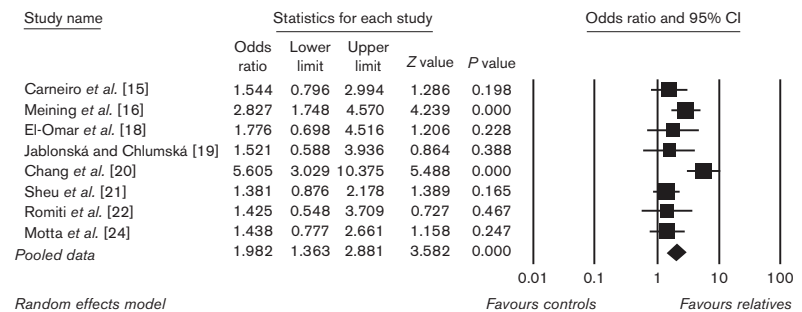

Random effects model   Favours controls   Favours relatives

The forest plot (random-effects model), concerning gastric atrophy prevalence in first-degree relatives of gastric cancer patients and controls.

Fig. 5

Study name	Odds ratio	Lower limit	Upper limit	Z value	P value
Carneiro *et al.* [15]	1.544	0.796	2.994	1.286	0.198
Meining *et al.* [16]	2.827	1.748	4.570	4.239	0.000
El-Omar *et al.* [18]	1.776	0.698	4.516	1.206	0.228
Jablonská and Chlumská [19]	1.521	0.588	3.936	0.864	0.388
Chang *et al.* [20]	5.605	3.029	10.375	5.488	0.000
Sheu *et al.* [21]	1.381	0.876	2.178	1.389	0.165
Romiti *et al.* [22]	1.425	0.548	3.709	0.727	0.467
Motta *et al.* [24]	1.438	0.777	2.661	1.158	0.247
Pooled data	1.982	1.363	2.881	3.582	0.000

Statistics for each study

Odds ratio and 95% CI

Random effects model   Favours controls   Favours relatives

The forest plot (random-effects model), concerning intestinal metaplasia (IM) prevalence in first-degree relatives of gastric cancer patients and controls.

past [32,33] and the increased risk had been explained by the exposure to similar environmental factors within a family, such as *H. pylori* infection. Towards this notion, Brenner *et al.* [34] showed that a family history increased the risk of gastric carcinoma (OR = 3.2, 95% CI = 1.3–8.0). They also showed that a positive family history and infection with a *CagA* positive *H. pylori* strain further increased the risk of gastric carcinoma (OR = 8.2, 95% CI = 3.9–66.4). In addition, very recently, researchers from Korea [25] found a synergistic interaction between *H. pylori* infection and family history, which further increased the risk of gastric cancer. All these findings stress the fact that the host-bacterial interaction might play a crucial role in the development of gastric cancer.

As for colorectal cancer, the population may be stratified according to the risk for gastric cancer, and the stratification can be applied to subgroups with a high, average, or low prevalence of the disease with regard to gastric cancer. The mortality rate of gastric cancer has decreased in Western societies, along with the eradication of *H. pylori* infection; so, it would probably not be cost–effective to test for *H. pylori* and treat the whole population. Therefore, separating out only relatives of gastric cancer patients (high risk) from the general population (average or low risk) for *H. pylori* screening would be more feasible. This approach has already been recommended by the European *Helicobacter pylori* Study Group [35]. However, the timing of the eradication therapy should be determined cautiously. Thus one randomized trial, conducted in China, has assessed the benefit of *H. pylori* eradication in preventing gastric cancer [36]. Even though they failed to show a benefit statistically, the subgroup analysis showed a substantial reduction in the incidence of gastric cancer for patients without premalignant changes such as atrophic gastritis, IM, and dysplasia, supporting the concept that these changes represent a 'point of no return' [7,37]. In addition, another study showed that eradication of *H. pylori* did not prevent the development of gastric cancer once IM had developed in the stomach [38]. Taking into account that IM begins in the decade between 30 and 40 years of age [39], all these results could mean that screening and treatment for *H. pylori* infection may be more beneficial 10–20 years before the risk for gastric cancer starts to increase [40]. Indeed, it was estimated that in Korea *H. pylori* eradication treatment might be most effective in the 20–29 years age group [25].

In conclusion, the results of this meta-analysis showed that first-degree relatives of patients with gastric cancer might be at an increased risk of developing gastric cancer, as judged by significantly higher prevalence of *H. pylori*, gastric atrophy and IM, in comparison with controls. Consequently, *H. pylori* detection and prophylactic eradication of the infection should be offered to such individuals. However, follow-up studies are required to prove the above.

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Helicobacter

Endoscopic Tests for the Diagnosis of *Helicobacter pylori* Infection in Children: Validation of Rapid Urease Test

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Abstract

Background: Rapid urease test (CLO-test) is an inexpensive and quick method for diagnosis of *Helicobacter pylori* infection with controversial results in children. We evaluated the performance of CLO-test in relation to endoscopic and histological findings in children with *H. pylori* infection.

Materials and methods: We studied the medical records of children with *H. pylori* infection who were diagnosed between 1989 and 2009. Non-infected children were used as controls. *H. pylori* infection was defined by positive culture or by two other positive tests (histology and CLO-test, or urea breath test when a single test was positive). All children had histology together with CLO-test. Tissue culture was performed whenever possible.

Results: Five hundred thirty infected children (10.4 ± 3.0 years) and 1060 controls (7.3 ± 4.4 years) were studied. Sensitivity of CLO-test was 83.4% (95% CI, 79.9–86.3%), of culture 84.6% (95% CI, 78.7–89.1%), of histology 93.2% (95% CI, 90.7–95.1%), and specificity 99% (95% CI, 98.2–99.4%), 100%, and 100% respectively. CLO-test positivity was correlated with higher bacterial density ($p < .001$), activity ($p < .001$) and severity of gastritis ($p < .01$), older age ($p < .01$), and the presence of antral nodularity ($p < .001$). When CLO-test was positive, the concordance with histology and culture was high (95.5 and 89.2% respectively), whereas low concordance was observed when CLO-test was negative (17.05 and 45.83% respectively).

Conclusions: CLO-test had lower sensitivity and comparable specificity with histology. Both tests should be performed concurrently to accurately diagnose *H. pylori* infection in children.

Current knowledge indicates that most often *Helicobacter pylori* (*H. pylori*) infection is acquired during childhood or adolescence [1], and this infection with this common pathogen at a young age increases the risk of *H. pylori*-related complications later in life. Thus, an accurate early diagnosis of the infection during childhood is important. Diagnosis of *H. pylori* infection includes a combination of both invasive and noninvasive methods such as urea breath test (UBT), *H. pylori* IgG antibodies, and *H. pylori* stool antigen (HpSA). All recommendations for diagnosis and treatment of *H. pylori* infection in children, published for Europe [2] and USA [3] in 2000 and Canada [4] in 2005 consider endoscopy with biopsy, the only reliable method for diagnosis of the patient's symptoms including *H. pylori* infection. In addition, the 2000 European and 2005 Canadian

recommendations considered C13-UBT, the best noninvasive test. The most widely used biopsy-based tests are histological determination of *H. pylori*, bacterial isolation by culture, and rapid urease tests (CLO-test). Positive culture for *H. pylori* can be used alone for the diagnosis, has 100% specificity, but it is not available in every hospital, and its sensitivity varies from high to very low [5–8]. Biopsy urease tests can determine the presence or absence of urease activity in a gastric biopsy, produced by *H. pylori*. The presence of urease activity in a biopsy could therefore be considered as proof of the presence of this infection [9]. The CLO-test is the most widely used commercial biopsy urease test because it is rapid and economical. Moreover, CLO-test is not dependent on the experience and accuracy of individual laboratories as is the case for histological

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examinations or culture. In adults CLO-test is widely used, with a sensitivity range from 70 to 90% [10], while other studies report a high sensitivity and specificity [11]. Studies concerning the sensitivity of CLO-test in children are controversial [5,6,9,12–15] and results may not be comparable to those obtained in adult patients.

We report the results of a retrospective study with reference to the sensitivity and specificity of the CLO-test and histology in detecting *H. pylori* infection in a large cohort of *H. pylori*-infected children diagnosed at a single center. Moreover, we aimed to assess whether the probability of CLO-positivity varied according to age, bacterial density, activity and severity of gastritis, gastrointestinal (GI) bleeding, and presence of nodularity at the antral mucosa.

Materials and Methods

Patient Groups and Sample Collection

The study was conducted at the Gastroenterology Unit of the First Department of Pediatrics of Athens University, Aghia Sophia Children's Hospital. Following approval of the local ethical committee, we retrospectively reviewed the files of 530 consecutive children proved to be infected with *H. pylori* by upper GI endoscopy performed by the same gastroenterologists (ER and JP) during the period January 1989 to April 2009. For each *H. pylori* positive patient, two *H. pylori* negative children who immediately followed the index case were selected as controls (total 1060 children). Inclusion criteria were the performance of CLO-test (Delta West Limited, Bentley, Western Australia) together with antral biopsy, and culture whenever was available. An Olympus XP20 gastroscope was used. Three antral biopsies were taken for histology, rapid urease test (CLO-test), and culture.

H. pylori Detection

Histological analysis was performed by the same histopathologist. Staining with hematoxylin and eosin for evaluation of gastric inflammation and with May-Grünwald Giemsa for assessment of *H pylori* colonization were used. Bacterial density and gastric inflammation were scored according to the updated Sydney System [16].

One biopsy was inoculated on CLO-test slides according to the manufacturer's recommendations and the results were read up to 24 hours by the gastroenterologist who had performed the endoscopy. For the purpose of *H. pylori* isolation by culture, antral mucosa biopsies

were aseptically placed in thioglycollate medium (Oxoid, Basingstoke, UK) and were processed within 2–4 hours after endoscopy as described before [17].

H. pylori infection was defined by positive culture or by positive histology and CLO-test. Those children with negative or not available culture and only one positive test (histology or CLO) were further evaluated by UBT (^{13}C-UBT, INFAI GmbH Bochum, Germany) and the positives were also included in the infected group.

Antral and duodenal nodularity, were defined upon endoscopic observation, as a cobblestone-like mucosa with nodules that varied in size from 2 to 4 mm, covered with smooth, nonulcerated mucosa.

Statistical Analysis

In order to compare percentages or proportions, the binomial distribution was used with null hypothesis for difference of proportions equal to 0.0 and alpha = 0.05. Chi-square test according to equidistribution of the samples was applied when appropriate. Yates correction was applied when necessary. Age stratification was performed namely, children of ≤5-years-old, 6 to 10 years, and 11 to 15 years.

For the estimation of sensitivity and specificity (with 95% confidence interval (CI)) the positive *H. pylori* status – according to our definition – was used as gold standard. Sensitivity and specificity of CLO-test and histology were also determined by using the positive culture, as the sole reference. Elaboration of data was accomplished using the Statgraphics Statistical Package (Manugistics, Inc., Rockville, MD, USA). *P* values of less than 5% were considered statistically significant.

Results

Five hundred and thirty children (254 males), aged 0.5 to 15 years (mean 10.4 ± 3.0 SD years) were classified as *H. pylori*-infected according to the selection criteria and 1060 children (526 males), aged 0.1 to 16.2 years (mean 7.3 ± 4.4 SD years) as negative for *H. pylori* infection were used as controls. Among children classified as having *H. pylori* infection, 80 had a single positive test (CLO-test or histology). In these cases *H. pylori* positive status was confirmed by a positive UBT, therefore providing a second test for the confirmation of *H. pylori* infection.

Among the 530 infected children with *H. pylori*, CLO-test was positive in 442 (sensitivity 83.4%, 95% CI 79.9–86.3) and histology in 494 (sensitivity 93.2%, 95% CI 90.7–95.1, *p* < .001). No difference in specificity between the two tests was found (CLO-test 99%, 95% CI 98.2–99.4 and histology 100%). Culture

Roma-Giannikou et al.

Endoscopic Tests for Diagnosis of *H. pylori* Infection in Children

was performed in 182 children of whom 154 (84.6%) yielded positive results. The sensitivity and specificity of CLO-test were 91.5% (95% CI 86.1–95) and 99% (95% CI 98.2–99.4) respectively, when the culture results were used as the reference method. Similarly, performance of histology was found to be the same with sensitivity 90.3 (95% CI 84.6–94) and specificity 100% when we used the same reference method. Figure 1 (panel A) depicts the concordance of CLO-test with histology and culture, and panel B the concordance of the two above methods with the CLO-test. When culture was positive, CLO-test was also found positive in 91.5% and histology in 90.3% of the cases, but when histology was positive the CLO-test was positive in 85.2%, indicating therefore a higher concordance of CLO-test positivity with positive culture ($p < .01$). However, when we selected cases with positive CLO-test the concordance of histology was higher compared with that of culture (95.5 and 89.2% respectively, $p < .01$). Positive CLO-test had a high concordance with histology and culture, whereas low concordance was observed when CLO-test was negative (17.05 and 45.83% respectively).

In children with negative culture, CLO-test was also negative in 39.3% and histology only in 7.1% ($p < .01$), indicating the need to perform histological

assessment in addition to the above two methods. Concordance of CLO-test with those that were both culture- and histology-negative was 99.1%.

The majority (n = 459, 86.6%) of infected children presented with moderate to marked chronic active gastritis, while only three children were found not having gastritis. Positivity of CLO-test was correlated with higher activity of chronic gastritis ($p < .001$) as well as chronic inflammatory infiltration ($p < .01$) (Table 1). Antral nodularity was recorded in 75.8% of the *H. pylori* infected children. A positive correlation of CLO-test with the presence of antral ($p < .001$), but not duodenal nodularity was found (Table 2). Both antral nodularity and positivity of CLO-test were significantly increased in children above the age of 5 years, compared with those below 5 years (Table 3). False negative CLO-test result was identified in 88 (16.6%) children mainly in those below the age of 5 years (<5 years 31.7%, 6–10 years 19.5%, 10–15 years 12.25%, $p = .0025$), while false positive result was found in 9 (0.85%) among 1060 controls. Bacterial density was positively correlated with CLO-test positivity ($p < .001$), antral nodularity ($p < .001$), activity of chronic gastritis ($p < .001$), and chronic inflammatory infiltration ($p < .001$), but not with age (Table 4). Cases with marked chronic gastritis without detection of
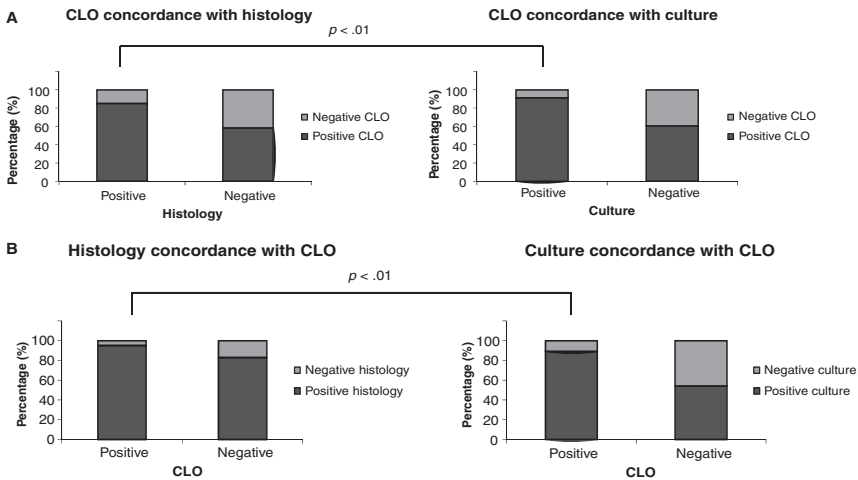

Figure 1 Concordance of CLO-test, histology and culture. (*A*) Comparison of CLO-test in relation to histology and culture status. (*B*) Comparison of histology and culture results in relation to CLO-test status.

Table 1 CLO test with relation to gastric pathology

| | CLO test result | | | | |
| | Negative | | Positive | | |
	n	(%)	n	(%)	p value
Activity of chronic gastritis					
Mild	28	41.2	40	58.8	<.001
Moderate	55	13.7	347	86.3	
Marked	5	8.8	52	91.2	
Chronic inflammatory infiltration					
Mild	16	32.0	34	68.0	<.01
Moderate	48	17.6	225	82.4	
Marked	24	11.6	182	88.4	

Table 2 Correlation of CLO test with antral and duodenal nodularity

| | | Presence of nodularity | | | | |
| | | Yes | | No | | |
Nodularity	Test result	n	(%)	n	(%)	p value
Antral	CLO positive	358	89.0	84	65.6	<.001
	CLO negative	44	11.0	44	34.4	
Duodenal	CLO positive	38	84.4	404	83.3	NS
	CLO negative	7	15.6	81	16.7	

NS, nonsignificant.

Table 3 Age in relation to CLO-test result and presence of antral nodularity

| | Age (years) | | | | | |
| | ≤5 | | 6–10 | | 11–15 | |
	n	%	n	%	n	%
CLO						
Positive	28	68.3[a]	169	80.5[b]	245	87.8[c]
Negative	13	31.7	41	19.5	34	12.2
Antral nodularity						
Present	22	53.6	158	75.2	222	79.6[d]

[a] For age ≤5, $p < .05$.
[b] For 5 < age <10, $p < .001$.
[c] For age >10, $p < .05$.
[d] Nodularity, $p < .01$.

H. pylori in histology were much less prevalent (34.3%) among the infected children, compared to those with moderate and marked bacterial density (95.3 and 98.7% respectively). Fifty-eight children presented with upper GI bleeding and the positivity of CLO-test did not

Table 4 Bacterial density in relation to CLO-test, nodularity, activity, chronicity, and age of children with *H. pylori* positive status

| | Grades of bacterial density | | | |
	[a]*H.pylori* (n = 36) n (%)	Mild (n = 76) n (%)	Moderate (n = 344) n (%)	Marked (n = 74) n (%)
CLO-test				
Positive	21 (58.3)	47 (61.8)	302 (87.8)	72 (97.3)[b]
Nodularity				
Present	21 (58.3)	42 (55.3)	278 (80.8)	61 (82.4)[c]
Activity				
Mild	23 (65.7)	28 (37.3)	16 (4.7)	1 (1.3)
Moderate/marked	12 (34.3)	47 (62.7)	327 (95.3)	73 (98.7)[d]
Chronicity				
Mild	10 (27.8)	22 (29.0)	15 (4.4)	3 (4)
Moderate/marked	26 (72.2)	54 (71.0)	328 (95.6)	71 (96)[e]
Age groups				
≤5	4 (11.1)	5 (6.6)	29 (8.4)	3 (4.0)[f]
6–10	13 (36.1)	34 (44.7)	138 (40.1)	25 (33.8)
11–15	19 (52.8)	37 (48.7)	177 (51.5)	46 (63.2)

[a] No isolation of *H. pylori* on histology.
[b] $p < .001$.
[c] $p < .001$.
[d] $p < .001$.
[e] $p < .001$.
[f] $p > .5$.

differ compared to the nonbleeding group (81 and 83.7% respectively, data not shown).

Discussion

In the present study we evaluated the sensitivity and specificity of CLO-test in comparison with histology in a large number of children with positive *H. pylori* status. The sensitivity of CLO-test was significantly lower compared to histology when all infected children were included, whereas when culture was used as the gold standard the sensitivity of the two tests was comparable regarding the 154 children with positive culture. Data concerning sensitivity of CLO-test in children are controversial with rates between 75 and 100%. Madani et al. [13] found a lower sensitivity (75%) in 67 children with *H. pylori* gastritis, while Oderda et al. found a 90% positivity of CLO-test after 24 hours in 42 infected children as shown by Giemsa staining of antral specimens [18]. Guarner et al. [19] in a review of the literature from 1999 to 2009 found that CLO-test has a better sensitivity than histology to detect the presence of *H. pylori*. In the present study, the sensitivity of CLO-test was found 91.5% using culture as gold standard, whereas it was found 83.4% when we used as gold

Roma-Giannikou et al.

standard the positive status according to our definition. Thus, an additional reason for the wide range of sensitivity in different studies could be the different gold standards used for this estimation. Positive CLO-test had a high concordance with histology, as also found in adults, where it has been recommended that antral biopsies for routine histology not be processed for reasons of cost containment, when CLO-test was positive on the day of endoscopy. However, histopathological study using hematoxylin and eosin is the only method that can detect other *H. pylori*-associated lesions (atrophy and intestinal metaplasia) [9].

In the present study the bacterial density seems to be strongly correlated with the positivity of CLO-test. Such lack of reliability of tissue urease test in detecting *H. pylori* infection in patients with a low degree of *H. pylori* colonization in children has been mentioned in a pediatric review [20]. Probably the CLO-test positivity with higher grades of bacterial density results from the abundance of urease enzyme. The significant association between CLO-test with the density and severity of gastritis is in line with a previous study on 67 children [13]. Thus, the accuracy of CLO-test depends mainly on the *H. pylori* density in the gastric sample, which is usually lower in children than in adolescents and in adults [20].

Antral nodularity was found in a large proportion of children and was increasing with age. Antral nodularity is common in children [21,22], rarely seen in adults [23,24] and has a high positive predictive value for the presence of *H. pylori* infection [22]. We noted a statistically significant correlation between CLO positive result with the presence of antral nodular gastritis, as well as with the age of the patients. We did not observe a significant difference in CLO-test positivity in children with or without upper GI bleeding. On the contrary, studies in adults have shown a lower sensitivity of CLO-test in the presence of blood in the stomach [25].

According to our findings, CLO-test was not reliable as a sole diagnostic test for *H. pylori* gastritis because of a significant number of false negative results, especially in young children, and is therefore, inferior to histology. A possible factor leading to such a significant discrepancy between the two tests could be the lower grade of colonization in childhood compared to adults [13]. However, in the present study, although CLO-test sensitivity was increasing in line with age, no statistically significant difference of bacterial density among the different age groups was noticed.

The advantage of this retrospective study is the large cohort of children examined by the same gastroenterologists and histopathologist over the study period. However, as it also happens in several other retrospective studies, the drawback is the fact that in both groups (*H. pylori* infected and uninfected controls) information for a possible previous antibiotic [26] or anti-acid agents consumption [27,28] was not available. This consumption could decrease the sensitivity of all three tests used, but it is obvious that this effect regards both index patients and controls accordingly.

In conclusion, sensitivity of CLO-test was inferior compared to histology to detect the presence of *H. pylori* when positive status was used as reference, but had similar sensitivity when culture was used as gold standard. The sensitivity of CLO-test was increasing according to age, bacterial density, severity of gastritis, and the presence of antral nodularity. It must be used combined with histology in order to increase the accuracy of *H. pylori* detection in children.

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Chapter 8

CHRONIC PERSISTENT INFECTIONS AS A RISK FACTOR FOR ISCHEMIC HEART DISEASE: THE *HELICOBACTER PYLORI* MODEL

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ABSTRACT

A variety of pathogens have been implicated in the induction of atherosclerosis, a fundamental process for the onset of ischemic heart disease (IHD). This atherogenic potential of microorganisms may become evident either acutely (ie Coxsackie B4, HSV, CMV) or on a more chronic basis, as it has been proposed for *Chlamydia pneumoniae*, *Helicobacter pylori* (*H. pylori*) and several bacteria of the oral cavity. Although in the case of IHD, caused by acute infection, a well-established model exists, this is not the case for chronic persistent infection-dependent IHD, partly due to the publication of controversial results.

In order to highlight the role of pathogens as risk factors for IHD, we have chosen *H. pylori* infection to serve as a model, since it involves all mechanisms proposed for the causation of IHD by chronic persistent infection: chronic inflammation, molecular mimicry, oxidative modifications, endothelial dysfunction, direct effect of the microorganism on atherosclerotic plaques and platelet-*H. pylori* interactions.

Through a classified presentation of key evidence-epidemiologic, dyslipidemic alterations, upregulation of inflammatory markers or homocysteine levels, induction of hypercoagulability, causation of impaired endothelial function, detection of *H. pylori* DNA in atherosclerotic plaques, participation of certain antigens and antibodies in a cross-reactivity model, interactions between *H. pylori* and host's genotype, oxidative stress, vascular remodeling, vascular calcification and vasomotor activity- "atherogenic" changes on traditional or novel risk factors for IHD will be examined under the light of *H. pylori*

2   C. Anastassios Manolakis, N. Andreas Kapsoritakis, K. Elisavet Tiaka et al.

infection. The "model" *H. pylori* infection is also used not only to clarify the role of individual pathogens but also to elucidate their contribution in the so called pathogen burden, a parameter involved in the onset and progression of atherosclerosis and eventually IHD.

Keywords: chronic infections, ischemic heart disease, *Helicobacter pylori*, pathogen burden

INTRODUCTION

The possible link between infectious agents and the atherosclerotic process, leading to ischemic heart disease (IHD), has been debated for more than a hundred years [1–3]. A variety of pathogens have been implicated in the induction of atherosclerosis, a fundamental process for the onset of ischemic heart disease (IHD). This atherogenic potential of microorganisms may become evident either acutely-ie Coxsackie B4, herpes simplex virus (HSV), cytomegalovirus (CMV)- or on a more chronic basis, as it has been proposed for *Chlamydia pneumoniae* (*C.pneumoniae*), *Helicobacter pylori* (*H. pylori*), several bacteria of the oral cavity even fungi (Table 1) [4-16]. Although in the case of IHD, caused by acute infection, a well-established model exists, this is not the case for chronic persistent infection-dependent IHD, partly due to the publication of controversial results.

In order to highlight the role of pathogens as risk factors for IHD, *H. pylori* infection has been chosen to serve as a model, since it involves all mechanisms proposed for the causation of IHD by chronic persistent infection: chronic inflammation, molecular mimicry, oxidative modifications, endothelial dysfunction, direct effect of the microorganism on atherosclerotic plaques and platelet-*H. pylori* interactions.

Table 1. Pathogens with a possible contribution in atherosclerosis

1. Coxsackie viruses ie B4
2. HSV-1
3. Epstein-Barr virus (EBV)
4. CMV
5. Hepatitis A&B viruses (HAV, HBV)
6. Influenza viruses, groups A and B
7. *Mycobacterium tuberculosis*
8. Bacillus Calmette-Guerin (BCG)
9. *Chlamydia pneumoniae*
10. *Helicobacter pylori*
11. Oral bacteria ie *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum-periodonticum-simiae group, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia*
12. *Escherichia coli* (*E.coli*)
13. Fungi

Through a classified presentation of key evidence-epidemiologic, dyslipidemic alterations, upregulation of inflammatory markers or homocysteine levels, induction of hypercoagulability, causation of impaired endothelial function, detection of *H. pylori* DNA in atherosclerotic plaques, participation of certain antigens and antibodies in a cross-reactivity model, interactions between *H. pylori* and host's genotype, oxidative stress, vascular calcification and vasomotor activity- "atherogenic" changes on traditional or novel risk factors for IHD will be examined under the light of *H. pylori* infection. The "model" *H. pylori* infection is also used not only to clarify the role of individual pathogens but also to elucidate their contribution in the so-called pathogen burden, a parameter involved in the onset and progression of atherosclerosis and eventually IHD.

PERSISTENT INFECTION AND IHD

The process of atherosclerosis starts in childhood and proceeds in steps, whereby episodes of vascular alteration are followed by incomplete healing [17]. Intimal thickening may be associated with systemic infections already during the first years of life while in adults, progression of atherosclerotic disease is associated with an increase of inflammatory markers in serum such as C-reactive protein (CRP), interleukin-6 (IL-6), and cell adhesion molecules [18-20]. This state of chronic inflammation is most likely maintained by repeated and/or chronic infection [21]. When considering chronic infections, higher rates of infection with individual pathogens, including *H. pylori*, have been recorded in epidemiologic studies focusing on patients with IHD, while others showed a beneficial effect of antibiotic treatment, targeting at these pathogens, in atheroslerosis progression as well as in the mortality during acute coronary events [22-34]. The role of chronic infections in IHD was further supported by the observation that microorganisms of greater virulence, ie cytotoxin asssociated gene A or CagA-bearing *H. pylori* strains, were associated with greater risk for coronary events as well as atherosclerotic plaque instability [35-42].

POSTULATED CAUSATIVE MECHANISMS FOR INFECTION-INDUCED IHD

The way in which microorganisms could induce or accelerate atherosclerosis in the coronary arteries, leading to IHD, still remains in dispute. As far as *H. pylori* is concerned, there are numerous reports implicating the bacterium in the development of digestive and a number of extradigestive diseases [43]. Although the list of extradigestive diseases is quite long, most reports in the literature are focused on two specific vascular diseases: stroke [44] and IHD. The postulated mechanisms for the onset of an *H. pylori*-and chronic persistent infection, in general-induced IHD are summarised in Table 2.

Summing up we could say that the atherogenic actions of *H.pylori* are either direct, through interactions with "ingredients" of the plaque- or indirect, in which the chronic persistent infection induces changes in parameters and risk factors that can predispose to IHD.

Table 2 Postulated mechanisms for the onset of an
***H. pylori*-induced ischemic heart disease**

1. Induction of metabolic dysregulation (dyslipidemia, insulin resistance)
2. Systemic increase of inflammatory markers and mediators, associated with atherosclerosis
3. Establishment of a hypercoagulable state
4. *H. pylori*-induction of platelet aggregation
5. Molecular mimicry
6. Oxidative stress
7. Hyperhomocysteinemia
8. Endothelial dysfunction and increase of vasoconstrictor factors
9. Direct effect of *H. pylori* on the progression and instability of atherosclerotic plaques

Dyslipidemia

Chronic inflammatory processes have been shown to favor a modified dyslipidemic condition with an atherogenic potential: decrease in HDL and increase in total cholesterol, LDL and/or triglyceride levels. Based on this notion, studies have been performed so as to record potential changes in lipoprotein or triglyceride concentrations.

Lower HDL cholesterol along with a lower HDL/total cholesterol ratio have been repeatedly recorded in *H. pylori*-infected individuals [45-49]. Upregulation of LDL and total cholesterol in *H. pylori*-infected individuals has also been described [49-51]. Elevated serum triglyceride levels were also reported in subjects, who were seropositive for both anti-*H. pylori* IgG and anti-*H. pylori* IgA. These associations remained significant even after adjustment for smoking, age, body-mass index and social class. It has also been shown that *H. pylori* infection correlated with lower apolipoprotein AI (ApoAI) and higher apolipoprotein B concentrations [48]. The association between infection with *H. pylori* and an altered lipid profile remained significant in a group of *H. pylori*-infected diabetic patients with regard to HDL-cholesterol and triglyceride levels [52].

Succesful eradication of *H. pylori* has led to beneficial changes in lipids, in patients previously infected with the bacterium: HDL, ApoAI and ApoAII concentrations increased, Lp(a), LDL and total cholesterol levels decreased [50, 52-54].

Homocysteinemia

It has been proposed that infection with *H. pylori* could lead to an elevation of homocysteine concentration in serum or plasma, as a result of reduced folate and/or poor B12 absorption [55, 56]. This hypothesis is mainly supported by two studies in which lower serum levels of vitamin B12 and/or folate as well as higher homocysteine levels were found in *H. pylori*-infected subjects compared to individuals negative for *H. pylori* [57, 58]. The vast majority of the associated literature, however, supports the case against. In four separate

studies no significant differences in homocysteine levels between *H. pylori*-positive and negative individuals were found [59-62]. These results were in agreement with reports that *H. pylori* eradication had no effect on homocysteine levels in patients who had undergone percutaneous transluminal coronary angiography (PTCA) [63].

Changes in pro- and Atherogenic Proinflammatory Cytokines

It is suggested that a chronic low-grade inflammatory process leading to atherosclerosis could be a possible mechanism for the onset of an *H. pylori*-induced IHD. This hypothesis has gained more ground since evidence of increased concentrations of markers of inflammation as a result of *H. pylori* infection emerged. Elevated concentrations of IL-6, tumor necrosis factor-a (TNF-a), IL-1b, and IL-8 in the *H.pylori*-colonised gastric mucosa have been reported [64–68]. Higher levels of circulating TNF-a have also been found in *H. pylori*-infected individuals [69, 70]. Moreover, the levels of circulating TNF-a, IL-1b, and IL-8 decreased significantly after successful eradication of *H. pylori* , in patients who had undergone PTCA [63, 70]. The assessment of adhesive molecules has shown the presence of higher soluble vascular cell adhesion molecule-1 (sVCAM-1) levels in children and of soluble intercellular adhesion molecule-1 (sICAM-1) in adults [71, 72]. The association between infection with *H. pylori* and IHD has also been investigated with focus on white blood cell count and CRP. Higher levels of CRP in *H. pylori*-infected patients with or without IHD compared to controls have been observed [37, 72, 73]. A significant positive correlation between the anti-*H. pylori* IgG titer and CRP has been found in patients with vasospastic angina [74]. These upregulated CRP levels seem to decrease after successful *H. pylori* eradication treatment [54].

Oxidative Stress

The presence of *H. pylori* has been shown to be related to a series of adverse oxidative phenomena that are not isolated within the boundaries of the gastrointestinal mucosa. A lower serum total antioxidant capacity (TAC) along with higher total oxidant status (TOS) and oxidative stress index (OSI) have been reported in *H. pylori* carriers. Interestingly, these disorders were also associated with the increased insulin resistance, found in the same *H. pylori*-positive group [75, 76]. Further studies in the same direction revealed that specific determinants in the oxidative-antioxidative pathways were compromised in *H. pylori*-infected individuals since lower serum total thiol (SH) levels, paraoxonase and arylesterase activities and higher lipid hydroperoxide (LOOH) levels were recorded, compared to individuals without *H. pylori* infection [77].

Induction of Hypercoagulability

The hypothesis that *H. pylori* infection could induce changes in coagulation parameters has been investigated in a number of studies with focus on fibrinogen, prothrombin

fragments, plasminogen-activating inhibitor 1 (PAI-1), factor VII and von Willebrand factor (vWF). An association between *H. pylori* infection and fibrinogen levels in patients with or without IHD was initially found [78, 79]. These higher levels of fibrinogen fell significantly after successful eradication of *H. pylori* in different studies performed on patients with IHD, stroke or otherwise healthy individuals [50, 80, 81]. It has also been reported that individuals with *H. pylori*-positive gastritis had higher levels of prothrombin fragment 1+2, compared to *H. pylori*-negative gastritis patients and *H. pylori*-negative individuals with a normal gastric mucosa. Moreover, the prothrombin fragment 1+2 levels fell significantly during a period of 2 months, following successful eradication of *H. pylori* [70]. In animal models, *H. pylori* infection has been shown to induce the formation of platelet aggregates [82]. Moreover, a significant increase in the number of platelet emboli and the duration of embolization in mice chronically infected with *H. pylori*, was observed [83]. This platelet-aggregation phenomenon, was also confirmed in children and it was shown to be reversed after succesful eradication [84]. Possible mechanisms for the *H. pylori*-induced platelet aggregation are the binding of vWF on *H. pylori,* followed by interaction with glucose phosphate isomerase-b (GPIb) or the activation of platelets by *H.pylori* urease, through a lipoxygenase-mediated pathway [85, 86].

Metabolic Dysregulation

H. pylori has been linked to various alterations in the levels of substances, involved in the metabolic syndrome. An impact of *H. pylori*-infection on circulating adipokine profile, similar to that observed in obese individuals, has been described with respect to adiponectin, obestatin, ghrelin as well as leptin levels [87-91]. In addition, infection with *H. pylori* and/or *C. pneumoniae* was associated with obesity and metabolic syndrome , in large cohort studies [91-93]. A specific association between *H. pylori* and increased insulin resistance, as expressed through HOMA-IR score, was also established in a large population of more than 1000 men and women [49].

Direct Impact on Atheromas

The *H. pylori*'s implication in the process of atherogenesis has been investigated by means of polymerase chain reaction (PCR) techniques, aiming at a potential detection of *H. pylori* DNA in atheromatic lesions. PCR was performed on 46 endarterectomy specimens obtained from atherosclerotic lesions, serving as cases, and 39 samples excised from healthy regions of the ascending aorta, accepted as controls. *H. pylori* DNA was found in 17 of the 46 cases and none of the controls [94]. In two different studies it was reported that 22 of 46 IHD patients were tested positive for *H. pylori* DNA in atheroclerotic plaques, whereas none of the 19 controls showed evidence of *H. pylori* DNA. Moreover, 11 of 14 anti-CagA positive patients from the IHD group showed positive detection of *H. pylori* DNA [63, 95]. A correlation between DNA presence and prior myocardial infarction and unstable angina was also found in the IHD group [95]. Likewise, *H. pylori* DNA in 20 of 38 atherosclerotic plaques, along with immunohistochemical evidence of *H. pylori* infection in 10 of these 20 *H.*

pylori DNA-positive plaques were succesfully detected [96]. In another study, *H. pylori* DNA was detected in nine out of 52 atheromatic specimens, but in none of the macroscopically healthy ascending aorta wall specimens [97]. Finally, evidence of *H. pylori* DNA were detected in three out of 14 coronary endarterectomy atherosclerotic specimens and in one out of the 15 left internal mammary artery specimens [98].

Molecular Mimicry

Based on the hypothesis that an immune response mounted against antigens on *H. pylori* can cross-react with homologous host proteins in a form of molecular mimicry, some authors investigated the role of heat-shock proteins (hsps) that are expressed and sometimes secreted by several pathogens, principally *C. pneumoniae* and *H. pylori* and are also present in human vascular cells and cells within atherosclerotic plaques [99]. The presence of antibodies against hsp60 and mycobacterial hsp65 (mHSP65) has been shown to correlate with atherosclerosis in carotid and coronary arteries [100, 101]. Elevated serum soluble hsp60 levels have been shown to correlate with *H. pylori* infection, while an association between serum antibodies to mHSP65 and seropositivity to *H. pylori* has been reported in a series of studies, even after adjustment for IHD risk factors and seropositivities to other pathogens [102-105]. Similarly, succesful eradication in *H. pylori*-infected patients led to a significant fall in anti-hsp65 titers [106].

Interestingly, anti-CagA antibodies have been shown to react with the cytoplasm and nuclei of smooth muscle cells, cytoplasm of fibroblast-like cells, and the cell membranes of endothelial cells. Anti-CagA antibodies also specifically immunoprecipitated two high molecular weight antigens of 160 and 180 kDa from artery lysates [107].

Miscelaneous Effects

A higher systolic blood pressure was recorded in *H. pylori*-seropositive individuals in a large cohort of more than 7000 patients [108]. Successful eradication of *H. pylori* led to a significant decrease in the levels of endothelin-1, and a significant elevation of nitrate/nitrite levels [109]. The possible role of *H. pylori* in the induction of endothelial dysfunction has also been studied, leading to the observation that microvascular and epicardial dilation with acetylcholine tended to be lower in subjects who were seropositive for *H. pylori* [110].

Flow-mediated vasodilation was significantly lower in *H. pylori*-seropositive than in seronegative subjects [72]. More specifically, an *H. pylori*, vacuolating toxin A (VacA)-dependent reduction in endothelial NO was confirmed experimentally, in bovine aortic endothelial cells [111]. An upregulation of an endogenous inhibitor of nitric oxide synthase, asymmetric dimethylarginine, was also asscociated with *H.pylori* infection [112].

An indirect link between *H. pylori* infection and elevated coronary calcification levels, independent of IHD risk factors after multivariate adjustment, has been reported, a link, however, that was not verified in the Multi-Ethnic Study of Atherosclerosis [105, 113].

THE ROLE OF HOST'S PREDISPOSITION

The impact of several gene polymorphisms of key components in the host-pathogen interactions such as CD14 receptor and Toll-like receptor 4 (TLR 4) has been studied. The C(2260)T polymorphism in the promoter of the CD14 receptor gene is associated with enhanced transcriptional activity, increased CD14 expression and acute coronary events [114-116]. The 299Gly TLR4 polymorphism, which is associated with attenuated receptor signalling, is related to the risk of developing acute severe infections but has been associated with low concentrations of circulating mediators of inflammation and a decreased risk of atherogenesis and acute coronary syndromes [117-119]. IL-6 polymorphic alleles, on the contrary, were implicated in an increased risk for IHD in the presence of pathogens [120]. It is possible that a host predisposition to respond to Lewis determinants or other surface antigens present in microbial LPS, including *H. pylori*, by IgG could be held accountable for the progression of atherosclerosis [121-123].

COEXISTENCE OF CHRONIC PERSISTENT INFECTIONS-THE UBIQUITOUS PATHOGEN "BURDEN" AND THE MULTIPLE HITS THEORY

The idea of a synergistic effect of different infectious agents, with respect to the onset and progression of atherosclerosis, can be identified in the IHD-related literature. This hypothesis is based on the notion that the atherogenic potential of individual pathogens can add up to an enhanced, prolonged response, capable of afflicting arterial wall damage while at the same time triggering and sustaining atherosclerotic plaque formation. The quantity or sum of pathogens for which the host is found to be positive is referred to as pathogen burden. Common constituents of this infectious burden are bacteria and viruses, either causing chronic persistent infections-*C. pneumoniae*, *H. pylori*, CMV, EBV, HSV- or acting acutely-influenza viruses, HAV, *E.coli*- while even vaccines such as BCG have also been included, in this heterogenic pathogenic repertoire [99]. The pathogen burden has been associated with the presence and severity of coronary atherosclerosis as well as with the presence of vulnerable plaques [124, 125]. Moreover, the simultaneous presence of more than one pathogens has been confirmed immunohistochemically in coronary atheromas and pathogen burden has been linked with a profound expression of atherogenic hsp60 in the same lesions [126]. The co-existence of pathogens has also been verified for oral microbes, as well as different fungal phylotypes, whose DNA was detected in coronary atherosclerotic plaques [15, 16]. The recorded variety of pathogens with a surprising proximity to atheromas seems to have led to the formation of the theory of multiple hits: the individual immune history as determined by sequential infectious/pathogenic events ("multiple hits") during a lifetime, contributes significantly to the cardiac ageing process [127].

CONCLUSION-ANTIMICROBIAL THERAPY FOR IHD?

All the evidence mentioned above seem to underline the importance of microorganisms and especially of their accumulative multifactorial atherogenic effect. There is, however, great controversy in the literature regarding this topic, mainly due to the publication of conflicting results. For each and everyone of the mechanisms of an infection-induced IHD there is the case against. A large series of studies with negative results in the field of pathogen-triggered atherosclerosis have been published so far, raising doubt whether individual microorganisms, their more virulent strains as well as total pathogen burden can cause dyslipidemia, a generalised metabolic dysregulation, hypercoagulability, systemic inflammatory responses and oxidative stress, the genesis of cross-reacting antibodies or whether they are present in arterial atherosclerotic plaques [128-154]. In addition, the data originating from studies investigating the potential merit of antibiotics in decreasing IHD risk were not all in favor of such a beneficial effect [33, 34, 155-157]. Notwithstanding the fact that a profound controversy does exist regarding pathogen-induced IHD, there is evidence that can not be taken lightly, such as the presence of pathogen DNA in atherosclerotic lesions, the presence of antibodies against hsps in patients with IHD and the already described cross-reactivity of antibodies against microbes with vascular components, along with the activation of oxidative pathways or the induction of insulin resistance, in the presence of infection. Summing up, it must be stated that, however tempting, the notion of antimicrobial therapy use for lowering IHD hazard can not be fully supported by the existing evidence. Besides, further implications during their use exist, since according to the multiple hits theory, even the repeated ''strikes'' from the same previously treated and eradicated microorganisms are involved in the onset and progression of atheromas. Moreover, the conventional antimicrobial therapy for a certain microorganism ie *C. pneumoniae* with macrolides may also have an impact on *H. pylori* infection but is inadequate for its succesful eradication. Thus, it seems that other problems emerge such as that of antibiotic resistance, already a common condition in many countries around the globe, and have to be taken into account when weighing the ''pros'' and ''cons'' of such regimens. For all these reasons it becomes rather obvious that a more intensive and targeted approach, through carefully designed studies in large populations is mandatory, so that the role of infections as risk factors for IHD can be documented, a model probably combining different mechanisms is established, and only then through a consensus, the best treatment modality, preferably a combination of pharmaceutic agents of well-known value such as statins with free of hsps vaccines, and/or antibiotics (eradication of pathogens ± chemoprofylaxis with a broad range antibiotic) is chosen and then applied.

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Helicobacter pylori with or without its neutrophil-activating protein may be the common denominator associated with multiple sclerosis and neuromyelitis optica

In their paper, Li et al.[1] concluded that *Helicobacter pylori* (*Hp*) neutrophil-activating protein (*Hp*-NAP) may be associated with the anti-aquaporin-4 (AQP4) antibody-related neural damage in multiple sclerosis (MS)/neuromyelitis optica (NMO) patients. However, this conclusion might be incomplete, because, as mentioned by the authors, *Hp*-NAP did not bind to anti-AQP4 antibody, and thus molecular cross mimicry between *Hp*-NAP and AQP4 is unlikely to be a responsible underlying mechanism. On the other hand, the authors claimed that *Hp*-NAP, by promoting neutrophils and monocytes recruitment/activation and inducing mast cells to release proinflammatory molecules, that are able to activate these cells, could contribute to neural oxidative damage in MS/NMO patients. However, although the authors showed higher *Hp* seropositivity rates in anti-AQP4 antibody-positive MS/NMO patients, they disclosed a significantly lower *Hp* seropositivity rate in conventional MS patients than in healthy controls and a significant inverse association with mean Expanded Disability Status Scale score and fulfilment of the Barkhof criteria for brain MRI lesions;[2] they concluded that *Hp* infection (*Hp*-I) is a potential protective factor against conventional MS in Japanese,[2] which contradicts their latest conclusion that *Hp*-I 'seems to be one of the risk factors for the development of AQP4 + /MS'.[1] Finally, the authors mentioned that a similar low prevalence of *Hp*-I was also reported in Western populations,[3] that is again incomplete information.

By using histology, recognized as the practical gold standard for the diagnosis of current *Hp*-I, we showed a strong association between *Hp*-I and MS in a Greek cohort.[4] Although the serological test used by Li et al. establishes the presence of *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,[5] thereby contributing and possibly perpetuating neural tissue damage.[5–7] Moreover, eradicating *Hp*-I alters MS pathophysiology.

Importantly*, Hp* achieves its pathogenetic role by triggering an intense gastric mucosa leukocyte infiltration, and neutrophil activation provides a major source of reactive oxygen metabolites which can cause tissue damage mainly in the absence of antioxidants.[8] *Hp* virulence factors promote the release of various chemoattractants/inflammatory mediators including mainly the neutrophil attractant chemokine IL-8 and *Hp*-NAP, which, as a virulence factor, recruits leukocytes from the vascular lumen, activates neutrophils, monocytes, and mast cells;[8,9] *Hp*-NAP activates neutrophils through a Pertussis toxin-sensitive pathway, and extracellular regulated kinase and p38-mitogen-activated protein kinase activations are involved in many neutrophil functions stimulated by *Hp*-NAP.[9] In this respect, we reported that Chios mastic gum inhibits neutrophil activation in the presence of *Hp*-NAP, playing a crucial role in *Hp*-associated pathologies.[10] In addition, *Hp*-induced cytotoxin VacA exhibits chemotactic activities to the bone marrow-derived mast cells (BMDMCs) and induces BMDMCs to produce proinflammatory cytokines.[12] A series of factors have been implicated in inducing blood–brain barrier (BBB) disruption, including aforementioned inflammatory mediators (e.g. cytokines and chemokines induced by *Hp*-I) and oxidative stress.[11,12] BBB disruption permits access of AQP4-specific antibodies and T lymphocytes to the central nervous system, thereby playing a major role in MS/NMO pathogenesis.[12–14]

Therefore, apart from *Hp*-NAP, *Hp*-I itself, by inducing several mediators, appears to be a common denominator influencing the pathophysiology of MS/NMO (including relapsing type) that shares similarities both in Western and Japanese populations as mentioned by the authors.[1]

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CORRESPONDENCE

A New Second-Line Sequential Regimen for *Helicobacter pylori* Eradication Based on Levofloxacin: A Pilot Study

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To the Editor

H. pylori is a very common infection worldwide and has a substantial role in the development of gastritis, peptic ulcer, and gastric malignancies. Eradication rates of first-line regimens have been reduced in less than 80% during the last decade because of the increasing prevalence of antibiotic resistance. A 10-day sequential treatment containing a proton pump inhibitor (PPI) and amoxicillin for 5 days followed by a PPI, clarithromycin and tinidazole for an additional 5 days, has been proven to be very effective, achieving per-protocol (PP) cure rates around 90% [1]. Levofloxacin-based regimens have recently been shown to be encouraging second-line options. In a meta-analysis, levofloxacin triple therapy had higher eradication rates and was better tolerated compared to the quadruple therapy [2]. The aim of this pilot study was to evaluate the efficacy and tolerability of a 10-day sequential second-line levofloxacin-based regimen and to compare it to the usually prescribed 10-day concomitant one in patients after one *H. pylori* eradication failure. Fifty-six patients (F: 30, M: 26; mean age: 52, range: 18–75 years) were randomized to receive either a 10-day treatment with omeprazole 20 mg b.i.d., amoxicillin 1 g b.i.d. and levofloxacin 500 mg b.i.d. (group A) or a sequential 10-day treatment as follows: (a) from day 1 to 5: omeprazole 20 mg b.i.d. and amoxicillin 1 g b.i.d. (b) from day 6 to 10: omeprazole 20 mg b.i.d. and levofloxacin 500 mg b.i.d. (group B). *H. pylori* eradication was confirmed with a negative breath test performed at least 4 weeks after treatment end. Success rates were 72.4% (21/29) (95% CI: 52.8–87.2%) and 77.8% (21/27) (95% CI: 57.7–91.4%) in groups A and B, respectively, in the intention-to-treat analysis and 80.8% (21/26) (95% CI: 60.7–93.5%) and 84% (21/25) (95% CI: 63.9–95.5%), respectively, in the per-protocol analysis ($p > 0.05$, NS). Three patients failed to complete the treatment because of severe allergic reaction and another seven complained of minor side-effects. However, no significant differences were observed between the two groups concerning adverse effects.

The idea of a sequential regimen was launched in Mediterranean countries in an attempt to provide a more effective first-line and relatively simple therapy against *H. pylori*. In a meta-analysis, Essa et al. proved that a 10-day concomitant regimen with three antibiotics (amoxicillin, clarithromycin, and tinidazole) was superior over triple therapy and concluded that it was less complex than the sequential one [3]. To the best of our knowledge, our study is the first one to evaluate a second-line sequential, levofloxacin-based treatment, including patients where there was a failure to eradicate *H. pylori* after classical triple therapy. Aydin et al. treated both naive and previous treatment failures with a modified 14-day sequential regimen using rabeprazole and amoxicillin for 7 days, and rabeprazole, levofloxacin, and metronidazole for another 7 days and found acceptable eradication rates only in naive patients [4]. Graham et al. used a 12-day sequential therapy with high dose of esomeprazole and amoxicillin followed by gatifloxacin and showed that this was effective. However, in contrast to our study, amoxicillin was given during the whole period and patients were both naive and previous failures [5]. Minor adverse effects of levofloxacin can occur in up to 22% of the patients [6]. Although we did not observe any differences between the two groups, other authors found significantly less

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side-effects by reducing the number of the antibiotic pills given [7]. It is of note that the cost of the sequential regimen we used was 40% less compared to the concomitant one, since patients received 50% less tablets of amoxicillin and levofloxacin (80.5 vs. 134.2 €).

In conclusion, a novel sequential regimen containing omeprazole, amoxicillin, and levofloxacin is effective and safe in *H. pylori* eradication as a second-line treatment. Moreover, it is more simple and less expensive compared to the usually prescribed concomitant regimen.

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Helicobacter

LETTER TO THE EDITOR

Impact of TLR-4 Polymorphisms on Circulating Levels of Antibodies Against *Helicobacter pylori*

Dear Editor,

Over the years, *Helicobacter pylori* infection has been associated with a variety of intestinal, as well as extra-intestinal disorders [1], thus underlining the need for the development and application of successful diagnostic and therapeutic strategies [2]. Among the procedures used for the diagnosis of infection from this Gram-negative bacterium, serology antibodies against *H. pylori* is one of the most commonly applied in research and clinical practice [2]. On the other hand, various factors capable of modifying immune responses against pathogens are currently known, including polymorphisms of toll-like receptors (TLR) [3,4]. These molecules and their polymorphic alleles have been implicated in the recognition of several bacterial components i.e. lipopolysaccharides of Gram-negative bacteria from TLR-4 [3–5]. To investigate whether the presence of polymorphic alleles could influence the production of antibodies against *H. pylori*, antibody titers were compared between *H. pylori*-infected individuals, with either wild-type or mutant TLR-4 genotypes.

The TLR-4 Asp299Gly and Thr399Ile polymorphic alleles were genotyped using a polymerase chain reaction (PCR) and direct sequencing, in 246 individuals with documented, via both campylobacter-like organism (CLO) test and histology, *H. pylori* infection. For the purposes of the PCR amplification, a forward, 5′-TCTAGAGGGCCTGTGCAATT-3′, and a reverse primer, 5′-TGAAACTCACTCATTTGTTTCAA-3′, were used. Using an enzyme-linked immunosorbent assay (ELISA, Enzygnost; Dade Behring Marburg GmbH, Marburg, Germany), IgG and IgA antibodies against *H. pylori* were determined. All study participants lacked any disorder that could alter the immune response and were not under immuno-modulatory or -suppressive medication.

A total of 40 individuals were tested positive for both mutant alleles, heterozygotes, while the rest 206 were homozygotes for the wild-type allele. The simultaneous presence –cosegregation- of TLR-4 Asp299Gly and Thr399Ile alleles, in our study was 100%. The mean ± SEM of anti-*H. pylori* IgG titers were 51.90 ± 4.927 U/mL, in individuals with the wild-type genotype, and 48.94 ± 9.878 U/mL, in the group carrying

the mutant alleles ($p > .05$). On the contrary, a statistically significant difference was recorded when anti-*H. pylori* IgA titers were compared between carriers of wild-type (11.61 ± 1.330 U/mL) and mutant polymorphic alleles (5.887 ± 0.6506 U/mL) ($p < .001$).

Evidently, lower IgA levels were found in the group of TLR-4 Asp299Gly and Thr399Ile gene carriers. This observation does not come entirely as a surprise, because interactions between TLR-4 and Gram-negative bacteria have already been described [3–5]. The impact of the TLR-4 Asp299Gly and Thr399Ile polymorphic alleles, however, on immune responses and especially those triggered by pathogens, has been debated [3]. One of the major reasons for the lack of unanimity regarding this matter was that the simultaneous presence of the two alleles did not seem to increase susceptibility or alter the cytokine response to microorganisms [3]. The results from this study seem to contradict the notion that the cosegregated mutant alleles ''behave'' pretty much like the wild-type allele. As for the rather selective downregulation of IgA response against *H. pylori*, it could be hypothesized that it reflects a defect or a dysregulation in the mucosal defense against *H. pylori*. Besides, the production of IgAs, circulating and mucosal, which represent the most common immunoglobulin subclass in the mucosa, can be triggered by TLR-facilitated recognition of microorganisms, in the intestinal epithelium [4,5]. In addition, a similar blunt antibody production has been reported for anti-chitobioside (ACCA) and anti-outer membrane porin (Omp) IgA, in patients with inflammatory bowel disease carrying the TLR-4 Asp299Gly allele [6]. This, however, is the first attempt to record a potential interaction between widely studied polymorphisms, tightly linked to the host's immune responses toward Gram-negative bacteria, and *H. pylori*. In view of the notion that the presence of these specific TLR-4 polymorphic alleles could reduce the diagnostic utility of *H. pylori* serology, our findings are not consistent with this remark, because the IgG response against *H. pylori* remained intact. All in all, it seems that the idea that the host's genetic background can ''carve'' the reaction toward environmental challenges is once more verified this time via the suppression of anti-*H. pylori*

IgA production by the presence of mutant TLR-4 alleles.

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