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Evaluation of a Four-drug, Three-antibiotic, Nonbismuth–containing ''Concomitant'' Therapy as First-line *Helicobacter pylori* Eradication Regimen in Greece

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Keywords
Helicobacter pylori, eradication therapy, amoxicillin, clarithromycin, metronidazole, Greece.

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Abstract

Background: The eradication rates of *Helicobacter pylori* (*H. pylori*) with standard treatments are decreasing worldwide as in Greece. Studies with new antibiotic combinations are needed to find better methods of eradication. Therefore, the aim of this study was to evaluate efficacy and tolerability of a 10-day, four-drug, three-antibiotic, nonbismuth–containing concomitant regimen.

Materials and Methods: This is a prospective, open-label, multicenter study that included 131 patients infected with *H. pylori*. All patients were diagnosed with peptic ulcer disease or nonulcer dyspepsia by endoscopy. *H. pylori* infection was established by at least two positive tests among rapid urease test, gastric histology, and ^{13}C-urea breath test. For 10 days, all patients received esomeprazole 40 mg, amoxycillin 1000 mg, clarithromycin 500 mg, and metronidazole 500 mg, all b.d. eradication was assessed with ^{13}C urea breath test 8 weeks after the start of treatment. Intention-to-treat and per-protocol eradication rates were determined.

Results: One hundred and twenty-seven of the 131 patients completed the study. At intention-to-treat analysis, the eradication rate was 91.6% (95% confidence interval (CI), 85.5–95.7%). For the per-protocol analysis, the eradication rate was 94.5% (95% CI, 89–97.8%). Adverse events were noted in 42 of 131 (32.1%); drug compliance was excellent with 96.9% of the patients taking more than 90% of the prescribed medication.

Conclusion: A 10-day concomitant regimen appears to be an effective, safe, and well-tolerated treatment option for first-line *H. pylori* eradication in Greece.

Helicobacter pylori (*H. pylori*) is a global human pathogen playing a cardinal role in the development of prevalent diseases including peptic ulcer disease and gastric malignancy [1]. Triple therapies consisting of a proton-pump inhibitor (PPI), plus amoxicillin and clarithromycin remained the standard first-line treatment options for *H. pylori* eradication since their first acceptance by the international guidelines in 1996 [2–4]. However, in the last decade, we are witnessing a progressive decline in cure rates below the acceptability threshold of 80% in an intention-to-treat (ITT) basis [5,6]. In fact, success rates with standard triple therapies in Greece before the year 2000 clearly exceeded 85% [7], whereas by the year 2002, they were barely reaching 78–80% [8,9] and since then have decreased even further (close to 70%) according to more recent data [10]. Increasing antimicrobial resistance represents the main risk factor for treatment failure [11]. After this disappointing data, new treatment strategies have been proposed. According to numerous studies, 10-day sequential administration of four drugs (dual therapy combination of amoxicillin and a PPI twice a day for 5 days, followed

by another 5 days of the PPI plus clarithromycin and tinidazole/metronidazole; ''sequential'' therapy) has been repeatedly shown to be superior to conventional triple therapy [12–14]. However, the complexity of requiring the patient to switch from a dual to a triple therapy at midpoint has led to a decline in enthusiasm for these regimens. Investigators from Germany and Japan have proposed that these same four drugs (a PPI, clarithromycin, metronidazole, and amoxicillin) be given concomitantly as a nonsequential four-drug, three-antibiotic, nonbismuth–containing quadruple therapy (i.e., ''concomitant'' therapy) [15,16]. This approach appears to be as effective as sequential therapy, while being inherently less complex [17]. The aim of this study was to evaluate the efficacy and tolerability of a 10-day, four-drug, three-antibiotic, nonbismuth–containing ''concomitant'' treatment for the eradication of *H. pylori* in the Greek population.

Methods

This is a prospective, open-label, multicenter study. From January 2009 to December 2010, 131 consecutive patients with *H. pylori* infection (79 men, 52 women; mean age 47.9 years; range 18–80) were prospectively assigned to receive a four-drug concomitant regimen comprising of esomeprazole 40 mg, metronidazole 500 mg, clarithromycin 500 mg, and amoxicillin 1000 mg, all taken twice a day for 10 days. The clinical and demographic data of the treated population are presented in Table 1. All patients included in the study were diagnosed with peptic ulcer disease or nonulcer dyspepsia (dyspepsia and gastritis and/or duodenitis) by endoscopy. *H. pylori* infection was established by at least two positive tests among rapid urease test, gastric histology, and ^{13}C-urea breath test. Patients with previous *H. pylori* eradication therapy, liver cirrhosis, renal failure, other serious and debilitating diseases, patients who had been treated in the preceding 1 month with antibiotics, bismuth preparations, non steroidal anti-inflammatory drugs and in the preceding 2 weeks with PPIs, patients with known allergy to the medications used, patients with previous gastric surgery, and pregnant and lactating women were excluded. The local ethics committee of the participating hospitals approved the study protocol and a fully informed written consent was obtained from each of the patients included in the study. The efficacy of treatment was evaluated by means of the ^{13}C-urea breath test performed following the standard European protocol at 8 week following the initiation of therapy. For patients requiring a follow-up endoscopy because of gastric ulcer (n = 3, 2.3%), histologic examination of four samples taken from the body and the antrum was the diagnostic test. Side effects of treatment were investigated by means of a structured clinical interview immediately after completing therapy. During the interview, patients were asked to grade the severity of every side effect experienced as ''mild'' (transient and well tolerated by the patient), ''moderate'' (causing discomfort to the patient and partially interfering with common everyday activities), or ''severe'' (causing considerable interference with the patient's activities, which may be incapacitating or life-threatening). Drug compliance was assessed by counting unused medication. For this purpose, any tablet that was not consumed was brought back to the clinic for pill count. Compliance was considered to be poor if <90% of the total medication was taken.

Table 1 Clinical and demographic characteristics of the treated population

	n = 131
Age (mean, range)	47.9, 18–80
Sex (M/F)	79/52
Smokers n (%)	54 (41.2%)
Alcohol >40 g/day n (%)	19 (14.5%)
DUD n (%)	43 (32.8%)
GUD n (%)	3 (2.3%)
NUD n (%)	85 (64.9%)
Previous PPI use n (%)	35 (26.7%)

M, males; F, females; DUD, duodenal ulcer disease; GUD, gastric ulcer disease; NUD, non-ulcer dyspepsia; PPI, proton-pump inhibitors.

Statistical Analysis

Noncategorical values are given as median and range. Primary outcome was eradication of *H. pylori* infection. Evaluations were done using ITT and per-protocol (PP) analyses. Secondary outcomes were compliance and side effects. Calculations were performed using the SPSS version 19 for Windows statistical package.

Results

One hundred and twenty-seven of 131 patients (96.9%) completed the study protocol. The eradication rates according to ITT analysis were 91.6% (95% confidence interval (CI): 85.5–95.7%). Per-protocol cure rates were 94.5% (95% CI: 89–97.8%). Four of 131 patients (3.1%) failed to complete the treatment because of severe adverse events. One patient discontinued clarithromycin on day 2 of treatment because of abdominal discomfort (epigastric pain and distension). Two patients reported nausea as the reason for

Table 2 Adverse events resulting from antibiotic therapy in the study population

	Mild (n)	Moderate (n)	Severe (n)	Total (n, %)
Side effects	29	9	4	42 (32.1)
Abdominal discomfort	8	2	1	11 (8.4)
Nausea	6	1	2	9 (6.9)
Taste disturbance	6	2		8 (6.1)
Vomiting	3	1		4 (3)
Diarrhea	2	2		4 (3)
Headache	1	1		2 (1.5)
Skin rush			1	1 (0.1)
Other	3			3 (2.3)

discontinuing treatment on days 5 and 6 of treatment, and another reported an allergic skin rash as the reason for discontinuing on day 5 of therapy. In spite of this, *H. pylori* was successfully eradicated in all four cases, as indicated by the negative breath test on final follow-up. Adverse events were observed in 42 of 131 patients (32.1%) (Table 2). The most frequent symptoms were abdominal discomfort (11 patients; 8.4%) and nausea (nine patients; 6.9%). Less-frequent symptoms were taste disturbance (eight patients; 6.1%), vomiting (four patients; 3%), and diarrhea (four patients; 3%). Overall, 96.9% of the patients took more than 90% of the total medication prescribed. Compliance with therapy (i.e., the percentage of tablets taken) was 97.9% (95% CI: 96.2–99.6%).

Discussion

In the initial studies from Germany and Japan, a PPI and three antibiotics (amoxicillin, clarithromycin, and metronidazole) were prescribed for 5–7 days and high eradication rates were obtained [15,16]. In a recent meta-analysis of the results of nine published studies (10 treatment arms, 771 patients) between 1998 and 2002, the pooled eradication rate of concomitant therapy was of the order of 89.7% on ITT and 92.9% on PP analysis [18]. However, all of these studies were conducted before 2002, and duration of most of the trials was inherently short (5–7 days). In the present study, 10-day concomitant administration of esomeprazole plus the same three antibiotics achieved acceptable eradication rates (91.6% on ITT and 94.5% on PP analysis) and therefore seems to be a promising alternative for the treatment of *H. pylori* infection. To our knowledge, there is no previously published data or experience regarding therapeutic outcomes of the concomitant regimen in Greece. In this country, the incidence of clarithromycin resistance has been increasing from 6% to over 20% in different studies, while that to metronidazole has been reported to be among the highest in Europe (about 49%) [19–22]. This increased incidence of clarithromycin resistance has led to a significant decline in cure rates obtained with traditional triple regimens [23]. Historically, in a study of 2002, we report an 18.2% failure rate among 566 patients treated with a 10-day standard triple therapy between 1996 and 2001 [8]. In our experience, performance of this same regimen has continued to decline over the last 10 years, falling to levels below the threshold of 80% on ITT basis. Indeed, according to unpublished data from the medical records database of our departments (150 *H. pylori*-infected patients; recruiting phase 2004–2007), therapeutic outcomes of a 10-day standard regimen were already suboptimal during the period of 4–7 years ago, achieving disappointing eradications rates of 78.7% on ITT and 79.7% on PP analysis. On the contrary, concomitant therapy of the same duration with the same four drugs seems to be far more effective (ITT difference >12%), thus representing a valid treatment alternative for first-line *H. pylori* eradication. If one considers the continuing increase in antibiotic resistance from 2004 to 2007 up to the present time, the difference today in ITT rates between the two schemes could be even greater, resulting in a clear superiority of the concomitant approach. The superiority of sequential over standard triple therapy has been reported to be related to the higher eradication of clarithromycin-resistant *H. pylori* strains [12], and this is also likely to be the reason for the advantage of concomitant over triple therapy. In a recent clinical trial conducted in Taiwan [17], concomitant therapy has proved to be as effective as sequential regimens while being inherently less complex. In addition to this simplicity, concomitant therapy has shown to be more suitable than sequential therapy for areas with an increased prevalence of dual antibiotic resistances. In Greece, the endemic prevalence of resistance to metronidazole is high and the scenario of a high dual antibiotic resistance seems very probable. In fact, in a previous study published in 2002 [8], a combined clarithromycin–metronidazole resistance of 13.7% was found and may be even greater at present. However, a caveat for which our study maybe criticized is that pretreatment susceptibility testing for clarithromycin and metronidazole was not carried out, and consequently, the influence of antibiotic resistance in the eradication could not be evaluated.

Overall, side effects were mild and did not interfere with compliance in our study. A total of 32.1% of patients reported adverse events. This rate is comparable with the results reported by previous data in

patients treated with the concomitant quadruple regimen [18]. Only four patients receiving the concomitant regimen discontinued treatment, two reporting nausea, one abdominal discomfort and one because of allergic skin rush. Anaphylaxis to medication as the reason for discontinuing treatment has been also reported in three patients from three previous studies [16,24,25]. Although, in our experience, taste alterations, such as ''bitter mouth'', are extremely common among patients receiving the concomitant scheme, only eight patients (6.1%) reported significant taste disturbance as a side effect. However, none of these patients discontinued treatment.

The present study has several limitations. As mentioned previously, the main drawback is the lack of antibiotic pretreatment susceptibility testing. Whether the increased performance of the concomitant regimen is the result of an improved efficacy against antibiotic-resistant *H. pylori* strains could not be evaluated. However, according to our results and given that Greece may have a high prevalence of antibiotic resistances, it seems probable that this regimen is fairly effective in eradicating drug-resistant strains of *H. pylori*. Nonetheless, the high adherence rate observed in our study may not be reproducible in clinical practice especially if the treating physician does not emphasize the importance of completing the regimen as prescribed.

In conclusion, 10-day concomitant therapy based on esomeprazole plus three antibiotics (amoxicillin, clarithromycin, and metronidazole) proved to be an effective, safe, and well-tolerated treatment option for *H. pylori* eradication in Greece. Additional studies comparing treatment dose and duration, while evaluating pretreatment susceptibility, are needed to further evaluate the concomitant regimen as a new eradication protocol in our population.

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Competing interests: the authors have no competing interests.

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The Association Between *Helicobacter pylori* Infection and Insulin Resistance: A Systematic Review

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Abstract

Background: *Helicobacter pylori* infection has been associated with diverse extradigestive morbidity, including insulin resistance (IR) syndrome. The aim of this systematic review was to summarize the epidemiologic evidence concerning the association between *H. pylori* infection and IR quantitative indexes.

Materials and Methods: A computerized literature search in PubMed electronic databases and Cochrane Central Register of Controlled Trials was performed.

Results: Nine studies reporting data on 2120 participants were finally eligible for this systematic review. Seven of them were cross-sectional studies and two were nonrandomized, open-label, controlled trials investigating the effect of *H. pylori* eradication on IR. Homeostatic model of assessment insulin resistance (HOMA-IR) was used in all studies to quantify IR. There seems to be a trend toward a positive association between *H. pylori* infection and HOMA-IR, strengthened by regression analysis in one study. However, there was significant heterogeneity between studies regarding the method(s) of *H. pylori* infection diagnosis based on and the study populations. The studies for the effect of *H. pylori* eradication on HOMA-IR revealed conflicting results.

Conclusions: Although data seem to indicate a potential association between *H. pylori* infection and IR, further studies are needed to strengthen this association and to clarify whether there is a causative link between them. If a causal link is confirmed in the future, this may have a major impact on the pathophysiology and management of IR syndrome, including type 2 diabetes mellitus and nonalcoholic fatty liver disease.

The metabolic or insulin resistance (IR) syndrome refers to the clustering of cardiovascular risk factors including type 2 diabetes mellitus (T2DM), central obesity, dyslipidemia, hypertension, nonalcoholic fatty liver disease (NAFLD) and endothelial dysfunction [1]. The IR syndrome is a concept that identifies the centrally obese patient with increased risk for T2DM and cardiovascular disease [2]. IR and subsequent hyperinsulinemia are the key pathogenetic factors in metabolic syndrome. Despite its various definitions and criticism regarding its clinical usefulness [3], the concept of the IR syndrome improves our understanding of the pathophysiology of IR and its metabolic and vascular consequences [1].

The prevalence of IR syndrome and its components is rapidly increasing worldwide, largely as a consequence of the ongoing obesity epidemic and significantly increases with age; old age appears to be an independent risk factor for the development of metabolic abnormalities [4–6]. In this regard, although relative degenerative conditions, including IR syndrome, have an increasingly high impact on aged population, their association with *Helicobacter pylori* infection, which affects more than half of the world's population, has only recently been addressed [7,8].

A potential relationship between IR syndrome and *H. pylori* infection appears to exist based on the following comparable data: (1) both diseases affect mainly old

people in the world [4,6]; (2) *H. pylori* infection has been implicated in a variety of extradigestive vascular conditions including functional vascular disorders caused by the release of vasoactive and proinflammatory substances, arteriosclerosis-induced increased platelet activation/aggregation, ischemic heart disease and/or ischemic cerebrovascular disorders and stroke, also detected in IR syndrome and other degenerative diseases contributing to their clinical manifestations [9], but until now a definite conclusion has not been obtained.

The aim of this systematic review was to summarize the epidemiologic evidence concerning the association between *H. pylori* infection and IR quantitative indexes; studies with nonquantitative or semi-quantitative assessment of IR were not included, because currently there is no consensus on the definition of IR syndrome and different definitions may bias the conclusive results [10–14]. A positive link between *H. pylori* infection and IR could have certain therapeutic prospects, because eradication of *H. pylori* might contribute to insulin sensitization and minimization of the IR syndrome consequences.

Methods

Search Strategy

A computerized literature search in PubMed electronic databases and Cochrane Central Register of Controlled Trials (CENTRAL) was performed. Search limits, including publication time and language, were not used, because it has been shown that they may inadvertently eliminate an important number of articles [15]. MeSH database was used as a terminology search filter. From the combination of terminology (MeSH terms) and methodological search filters, relevant journal articles were retrieved [16]. More specifically, after a preliminary search of terms, we formatted the following query (last update 1 October 2010): ''((Metabolic[All Fields] AND (''syndrome''[MeSH Terms] OR ''syndrome''[All Fields])) OR (''metabolic syndrome x''[MeSH Terms] OR ''metabolic syndrome x''[All Fields] OR (''insulin''[All Fields] AND ''resistance''[All Fields] AND ''syndrome''[All Fields]) OR ''insulin resistance syndrome''[All Fields] OR ''insulin resistance''[MeSH Terms] OR (''insulin''[All Fields] AND ''resistance''[All Fields]) OR ''insulin resistance''[All Fields] OR (''insulin''[All Fields] AND ''resistance''[All Fields] AND ''syndrome''[All Fields]))) AND ((''*helicobacter pylori*''[MeSH Terms] OR (''*helicobacter*''[All Fields] AND ''*pylori*''[All Fields]) OR ''*helicobacter pylori*''[All Fields]) OR (''*helicobacter pylori*''[MeSH Terms] OR (''*helicobacter*''[All Fields] AND ''*pylori*''[All Fields]) OR ''*helicobacter pylori*''[All Fields] OR (''*campylobacter*''[All Fields] AND ''*pylori*''[All Fields]) OR ''*campylobacter pylori*''[All Fields]))''.

The literature search was extended to the ''Related citations'' links next to each selected article in PubMed and its references. Finally, automatic alerts (up to the submission of the study) were activated in PubMed (''My NCBI'') to add relevant articles published after the initial search.

Two reviewers (S.A.P. and J.K.) independently assessed the extracted data (titles, abstracts, references, and full-text articles). Any discrepancy was solved by consensus.

Eligibility of Relevant Studies

Studies of any design reporting both quantitative assessment of IR by any method (hyperinsulinemic-euglycemic clamp technique, quantitative insulin sensitivity check index (QUICKI), homeostatic model assessment-insulin resistance (HOMA-IR) or any other index) and *H. pylori* assessment by methods requiring gastric biopsies performed during endoscopy (rapid urease test, histology, culture) or by noninvasive methods (anti-*H. pylori* specific immunoglobulin G [IgG] antibody, urea breath and stool antigen tests) were eligible for this study.

Studies retrieved were excluded from the systematic review, if: (1) they were reviews, editorials, case reports, letters to the editor, hypotheses, studies on animals or cell lines, abstracts from conferences or unpublished studies; (2) IR syndrome assessment was performed by nonquantitative or semi-quantitative criteria, including those of World Health Organization [10], European Group for the study of IR [12], American Association of Clinical Endocrinologists [13], National Cholesterol Education Program-Adult Treatment Panel III [14], International Diabetes Foundation criteria [11]; (3) enrolled patients had previously received *H. pylori* eradication therapy or gastric surgery; (4) enrolled patients had received H2-receptor antagonists or proton-pump inhibitors (PPIs) within 4 weeks before enrollment; (5) there was evidence for patients' overlap and (6) there were serious methodological flaws and the corresponding authors did not provide additional data.

Apart from *H. pylori* infection and IR quantitative indexes, other variables were evaluated as selected on the basis of the criteria used for the definition of IR syndrome by different organizations [10–14]. These variables are presented in Tables 1 and 2. In all included studies body mass index (BMI) was calculated as: weight (kg)/[height (m)]^2 and HOMA-IR as:

Table 1 Main characteristics of studies including in the systematic review

First author, year, origin*	Study design	Participants	Upper gastrointestinal endoscopy/ biopsy	Patients with peptic ulcer/ gastritis inclusion	Diabetic patients inclusion	Hyperlipidemic patients inclusion	Hypertensive patients inclusion	Patients with CAD inclusion	Additional information
Aslan, 2006, Turkey [18]	Cross-sectional	Dyspeptic patients	Yes[a,b]	No	No	No	No	No	*Hp* negative if both tests were negative
Aydemir, 2005, Turkey [19]	Cross-sectional	NA	Yes[a]	No	No	NA	No	No	
Eshraghian, 2009, Iran [20]	Cross-sectional	Apparently healthy adults	No[c]	NA[†]	No	NA	No	No	
Gao, 2009, China [21]	Cross-sectional	Apparently healthy adults	Yes[b,c]	No	No	NA	NA	NA	(1) *Hp* negative if both tests were negative; (2) The main aim was the association of *Hp* and serum ghrelin and obestatin; (3) 100 of 257 participants were selected
Gen, 2010, Turkey [7]	Nonrando-mized, longitudinal, open-label, controlled[‡]	Dyspeptic patients	Yes[a,b]	Yes	No	No	No	No	*Hp* positive if both tests were positive
Gunji, 2009, Japan [22]	Cross-sectional	Apparently healthy adults	No[c]	NA[†]	No	No	No	No	IR was defined as HOMA-IR score ≥2.5
Ozdem, 2007, Turkey [23]	Cross-sectional	Dyspeptic children and adolescents	Yes[a,b]	Yes	No	Yes	NA	No	*Hp* positive if histologic analysis was positive
Park, 2005, Korea [24]	Nonrandomized, longitudinal, open-label, controlled	Patients with peptic ulcer and Hp infection	Yes[a,b]	Yes	No	No	No	No	Hp positive if either test was positive
So, 2009, China [25]	Cross-sectional	Workers subjected to check up	No[d]	NA[†]	Yes	Yes	Yes	NA	(1) Hypoadiponectinemia was defined as plasma adiponectin level <4 mg/mL; (2) The main aim was the association of endemic chronic infections and adiponectin

CAD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostatic model assessment-insulin resistance; *Hp*, *Helicobacter pylori*; IR, insulin resistance; NA, not available.

*References are presented in first author order.

†The authors stated that the patients with history of peptic ulcer were excluded; however no biopsy was performed at the time of study.

‡Cross-sectional data between *Hp* positive and *Hp* negative patients at baseline are also provided.

Method of *Hp* detection:

[a]Gastric mucosa histologic examination for *Hp* presence.

[b]Gastric mucosa rapid urease test (CLO test).

[c]Serum *Hp*-specific immunoglobulin G antibody concentration (ELISA).

[d]Serum *Hp*-specific immunoglobulin G antibody concentration (chemiluminescence).

Table 2 Main anthropometric and biochemical characteristics per group of included studies providing cross-sectional data at baseline

First author, year[a]	Group	N (male)	Age (years)	BMI (kg/m^2)	WC (cm)	FBG (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	SBP (mmHg)	DBP (mmHg)	HOMA-IR	*Hp* positive (N per cent)
Aslan, 2006 [18]	*Hp*−	48 (20)	35 ± 15	22.5 ± 3.1	NA	98 ± 15	NA	NA	NA	NA	0.89 ± 0.47	0
	Hp+	55 (26)	37 ± 12	23.5 ± 1.6	NA	98 ± 14	NA	NA	NA	NA	1.67 ± 0.99*	100
Aydemir, 2005 [19]	*Hp*−	27 (13)	48.5 ± 10.7	24.4 ± 3.0	NA	94 ± 8	NA	NA	NA	NA	1.73 ± 1.10	0
	Hp+	36 (15)	46.1 ± 10.1	23.5 ± 3.0	NA	92 ± 7	NA	NA	NA	NA	2.56 ± 1.54*	100
Eshraghian, 2009, [20]	*Hp*−	28 (13)	33.0 ± 8.1	24.0 ± 3.2	NA	77 ± 8	NA	NA	NA	NA	2.46 ± 1.90	0
	Hp+	43 (21)	32.2 ± 14.2	23.1 ± 4.5	NA	78 ± 11	NA	NA	NA	NA	3.54 ± 2.20*	100
Gao, 2009, [21]	*Hp*−	50 (25)	43.3 ± 2.9	21.5 ± 1.2	NA	90 ± 1.8	97 ± 18	50 ± 8	NA	NA	1.7 ± 0.3	0
	Hp+	50 (26)	45.9 ± 2.4	21.2 ± 0.7	NA	92 ± 1.8	106 ± 18	46 ± 8	NA	NA	1.6 ± 0.2	100
Gen, 2010 [7]	*Hp*−	71 (36)	33.2 ± 8.3	24.4 ± 5.0	NA	92 ± 11	147 ± 45	48 ± 8	NA	NA	2.21 ± 1.27	0
	Hp+	88 (45)	32.5 ± 7.6	24.2 ± 3.0	NA	94 ± 7	168 ± 37*	40 ± 13*	NA	NA	3.89 ± 1.18*	100
Gunji, 2009 [22]	IR−	1008 (892)	49.6 ± 8.6	23.0 ± 2.6	83.6 ± 7.7	97 ± 9.	118 ± 118	60 ± 14	125 ± 16	80 ± 10	1.16 ± 0.47	28.7
	IR+	99 (96)*	48.6 ± 8.7	27.1 ± 3.5*	94.6 ± 9.2*	104 ± 11*	171 ± 88*	50 ± 12*	135 ± 14*	86 ± 9*	3.51 ± 1.13*	39.4*
Ozdem, 2007 [23]	*Hp*−	29 (11)	13 (5–16)[b]	18.7 (15.1–25.7)[b]	NA	82 (66–101)[b]	69 (21–155)[b]	58 (20–83)[b]	NA	NA	0.91 (0.13–2.43)[b]	0
	Hp+	31 (10)	12 (6–17)[b]	18.7 (14.7–29.7)[b]	NA	87 (74–122)[b]	72 (39–206)[b]	53 (22–78)[b]	NA	NA	1.41 (0.60–4.87)[b,*]	100
So, 2009 [25]	Low adiponectin	107 (107)	40.9 ± 7.5	26.3 ± 3.6	88.5 ± 8.9	101 ± 30	129 (101–204)[c]	47 ± 10	128 ± 18	82 ± 12	1.39 ± 0.86	NA
	Normal adiponectin	181 (181)	40.7 ± 9.3	24.7 ± 3.9*	84.6 ± 10.0*	95 ± 23.9*	115 (73–160)[c,*]	52 ± 13*	122 ± 17*	77 ± 11*	1.22 ± 1.12	NA

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment-insulin resistance; *Hp*, *Helicobacter pylori*; IR, insulin resistance; N, number; NA, not available; −, negative; +, positive; SBP, Systolic blood pressure; WC, waist circumference.
Data are presented as numbers or mean ± standard deviation (indicated if they are presented differently).
[a]References are presented in first author order.
[b]Data presented as median (range).
[c]Data presented as median (interquartile range).
*$p < .05$ between groups.

[fasting insulin (mU/L) × fasting glucose (mmol/L)]/22.5 [17].

Results

We initially identified 45 potentially relevant articles in the database search. We included nine more articles found through the ''Related citations'' links and the references of initially identified articles (54 articles totally). A flow chart summarizing search results and identification of eligible studies is provided in Fig. 1. Nine of them were selected for final analysis [7,18–25].

The selected studies, which reported data on 2120 participants, were published between 2005 and 2010. The main characteristics of these studies are summarized in Table 1. Notably, all the nine studies were held in Asia. Seven were cross-sectional studies and two were nonrandomized, open-label, controlled trials [7,24], investigating the effect of *H. pylori* eradication therapy on IR. One of them [7], also provided cross-sectional data between *H. pylori* positive and *H. pylori* negative patients at baseline, whereas the other did not [24]. Subsequently, there were cross-sectional data from eight studies [7,18–23,25] (Table 2) and data for the effect of *H. pylori* eradication therapy from two studies [7,24] (Table 3). HOMA-IR was used in all studies to quantify IR; no study was identified in which hyperinsulinemic-euglycemic clamp technique, QUICKI index or other index for IR was used. There was significant heterogeneity between studies regarding the method(s) which the diagnosis of *H. pylori* infection was based on and the study populations (Table 1). Histologic analysis for documentation of *H. pylori* infection was performed in six of nine studies. Rapid urease test was also used in five studies. Serum *H. pylori*-specific IgG antibody concentration was measured by enzyme-linked immunosorbent assay (ELISA) in three studies and by chemiluminescence in one study. Urea breath and stool antigen tests were not used in the baseline assessment in the selected studies. When two of the diagnostic tests were performed, the participants were considered to be *H. pylori* positive if either or both tests were positive, thereby increasing the heterogeneity (Table 1). The participants were adults in all but one study [23]. The participants mainly consisted of apparently healthy individuals or dyspeptic patients; patients with peptic ulcer or gastritis were included in three studies [7,23,24]. Studied groups were divided according to: (1) *H. pylori* diagnosis (*H. pylori* negative or positive) in six studies [7,18–21,23]; (2) IR in one study [22]; (3) *H. pylori* eradication therapy introduced in one study [24] and (4) serum adiponectin levels in one study [25] (Table 2). Table 2 shows the main baseline anthropometric and biochemical data per group and Table 4 concisely presents whether there was an association or not in the studies where cross-sectional data were available at baseline [7,18–23,25].

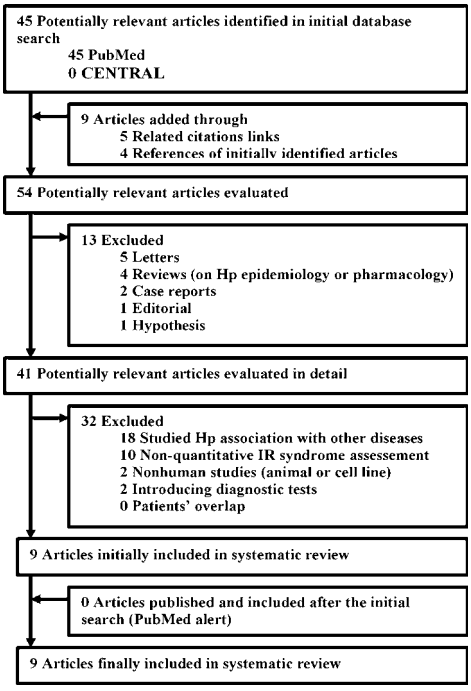

Figure 1 Identification of eligible studies.

Some additional highlight points were the following: (1) when compared with *H. pylori* negative patients, there was a higher serum oxidative stress (as quantified by total oxidant status and oxidative stress index) and lower serum total antioxidant capacity in *H. pylori* positive patients [18]; (2) in the Gunji et al. study, *H. pylori* serology was found to be an independent predictor for HOMA-IR after adjustment for confounders, including gender, age, BMI, waist circumference, visceral, and subcutaneous adipose tissue, smoking status, alcohol consumption, dietary habits, and physical activity [22]; (3) no difference in serum *H. pylori*-specific IgG antibodies was found between patients with low or normal serum adiponectin levels in Chinese men [25] and (4) serum ghrelin was significantly

Table 3 Main anthropometric and biochemical characteristics per group of studies providing data for the effect of *Hp* eradication therapy on IR

First author, year[a]	Group	Time between pre- and post-treatment measurements	N (male)	Age (years)	BMI (kg/m^2) pre-treatment	FBG (mg/dL) pre-treatment	Triglyceride (mg/dL) pre-treatment	HDL-C (mg/dL) pre-treatment	HOMA-IR pre-treatment	HOMA-IR post-treatment
Gen, 2010 [7]	Unsuccessful eradication treatment	6 weeks	39 (21)	32.1 ± 9.2	24.4 ± 3.0	93 ± 6	153 ± 59	39 ± 14	3.98 ± 1.20	3.87 ± 1.50
	Successful eradication treatment	6 weeks	47 (23)	32.8 ± 6.5	23.8 ± 1.8	95 ± 6	159 ± 63	37 ± 10	3.79 ± 1.28	2.21 ± 1.7*
Park, 2005 [24]	No eradication treatment	1 year	82 (63)	46.0 ± 8.0	24.1 ± 2.5	93 ± 16	176 ± 111	54 ± 16	1.80 ± 0.80	1.82 ± 1.54[b]
	Eradication treatment	1 year	87 (76)	44.7 ± 7.9	24.2 ± 3.1	90 ± 13	177 ± 193	53 ± 14	1.80 ± 0.90	1.82 ± 1.60[b]

BMI, body mass index; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment-insulin resistance; IR, insulin resistance; *Hp*, *Helicobacter pylori*; N, number.
Data are presented as numbers or mean ± standard deviation.
[a]References are presented in first author order.
[b]Estimated by calculation.
*$p < .05$ between groups.

Table 4. Summary of the main outcome of included studies with cross-sectional arm at baseline

First author, year[a]	Association between IR and *Hp* infection	*p*-value
Aslan, 2006 [18]	Yes	<.001
Aydemir, 2005 [19]	Yes	.021
Eshraghian, 2009, [20]	Yes	<.05
Gao, 2009, [21]	No	NA
Gen, 2010 [7]	Yes	<.05
Gunji, 2009 [22]	Yes	<.001
Ozdem, 2007 [23]	Yes	<.05
So, 2009 [25]	No	.157

Hp, *Helicobacter pylori*; IR, insulin resistance.
[a]References are presented in first author order.

lower in *H. pylori* positive compared with *H. pylori* negative patients [21].

Regarding the effect of *H. pylori* eradication regimen on HOMA-IR, two studies were retrieved with conflicting results [7,24]; no effect was initially reported [24], whereas improvement in HOMA-IR was recently shown [7]. In the last study, HOMA-IR decreased from 3.79 ± 1.28 to 2.21 ± 1.70 after successful *H. pylori* eradication, whereas it remained unchanged (3.98 ± 1.20 to 3.87 ± 1.5, respectively), in patients for whom *H. pylori* eradication had failed.

We initially intended to perform a meta-analysis within the results of this systematic review. In this meta-analysis we could have included the six studies in which groups were divided into *H. pylori* positive or *H. pylori* negative [7,18–21,23]. However, the high heterogeneity between studies and the low percentage of patients included in the meta-analysis (556 of 2120, 26%) could have seriously biased any result of the meta-analysis. On the other hand, subgroup analysis in order to investigate the source of heterogeneity could be hardly performed due to the small number of studies.

Discussion

This systematic review summarized the evidence for the association between *H. pylori* infection and quantitative indexes of IR. There seems to be a trend toward a positive association between *H. pylori* infection and HOMA-IR. The association between *H. pylori* infection and IR is independent of several confounders, including visceral and subcutaneous adipose tissues, at least as they are both quantified by computerized tomography [22]. More specifically, when studied groups were divided according to *H. pylori* diagnosis (*H. pylori*

negative or positive), higher HOMA-IR was found in all [7,18–20,23] but one study [21]. However, in the last study, the primary endpoint was not the association of *H. pylori* infection with HOMA-IR, but with ghrelin and obestatin, and a selection bias incurred, given that 100 of 257 participants were finally selected for the study [21]. There is one more study, where no difference in HOMA-IR was reported, but this study was also not focused on *H. pylori* infection – IR interaction and its studied groups were divided according to adiponectin levels [25] (Tables 2 and 4).

In this systematic review only studies that had quantitatively assessed IR were included. We initially intended to include studies having assessed IR not only by HOMA-IR but also by other quantitative indexes, but all related studies retrieved up to the submission of the manuscript had assessed IR by HOMA-IR. HOMA-IR is the most common method for assessment of IR in clinical practice and epidemiologic studies [26,27]. HOMA-IR is a minimally invasive index needing only fasting serum sampling and is well-correlated to hyperinsulinemic-euglycemic clamp technique, considered the gold standard in the assessment of IR. There is no currently widely accepted normal range for HOMA-IR; however, the upper cut-off value, which defines IR, has been proposed between 2.0 and 3.0 in different populations [17,22,28]. We did not include studies having assessed IR syndrome by nonquantitative or semi-quantitative criteria, as defined in the ''Methods'' section [22]. Some relative studies reported a significant link between *H. pylori* infection and metabolic syndrome (as defined by semi-quantitative definitions) or its parts (i.e., T2DM, dyslipidemia, hypertension, polycystic ovary syndrome) or cardiovascular disease [8,29–31]. However, the results of other studies failed to confirm this association [32–35]. The use of different nonquantitative or semi-quantitative criteria makes the inter-study comparison difficult and might have biased the conclusions of this systematic review. Moreover, the existence of metabolic or IR syndrome is debatable [36,37], whereas IR itself is measurable and not debatable. The debate concerning the metabolic syndrome lies on whether the cardiovascular risk accompanying it is higher compared with the sum of risks of its parts, i.e. T2DM, hypertension dyslipidemia etc.

It would be important to note that the evaluation of the *H. pylori* infection was not determined in all studies by histologic detection of organisms in mucosal biopsy specimens, considered the actual diagnostic gold standard. Although the diagnostic utility of serum anti-*H. pylori* specific IgG antibodies is well established [38], it is inevitable that some false-positive or false-negative subjects be included in these studies. This matter is further attenuated by the fact that anti-*H. pylori* specific IgG remain positive even after eradication of *H. pylori*, given that it is unknown whether *H. pylori* is only a trigger instigating IR or chronic active *H. pylori* infection is required to promote IR [39]. However, serologic tests are widely available, noninvasive, and cheap and thus suitable for screening and large epidemiologic studies.

There are limited and conflicting data regarding the effect of *H. pylori* eradication on HOMA-IR (Table 3). Gen et al., reported a HOMA-IR improvement [7], whereas Park et al., reported no effect [24]. However, there are significant methodological differences between these two studies. First, in the former study [7], all *H. pylori* positive patients received eradication therapy and comparison was made between those successfully and not successfully treated; in the latter study [24], *H. pylori* positive patients who consented were subjected to therapy, whereas patients who refused to receive eradication therapy served as controls (selection bias). Second, there were different eradication regimens and duration: sequential therapy regimen (pantoprazole and amoxicillin for 7 days followed by pantoprazole, metronidazole, and tetracycline for the remaining 7 days [7]), or standard PPIs-based triple therapy (omeprazole, amoxicillin, and clarithromycin for 7 days) [24], respectively. Third, post-treatment measurements were performed 6 weeks after treatment in the former study, whereas 1 year after treatment in the latter. Finally, the effect of eradication was estimated by ^{14}C-urea breath test at 6 weeks for all participants in the former study [7], whereas there was methodological dissociation in the latter study [24]: patients were subjected to repeat biopsy 6 weeks after the last dose of treatment and controls 1 year after the previous biopsy.

In line, there is a case report of an 84-year-old Japanese man with type II IR syndrome (a rare cause of diabetes) and immune thrombocytopenic purpura (ITP), both developed simultaneously. Both conditions improved after *H. pylori* eradication, and treatment for diabetes was no longer necessary, although diabetes was not effectively managed before *H. pylori* eradication [40]. Apart from ITP [41], *H. pylori* eradication has been reported to ameliorate other immunologic disorders, including anti-phospholipid antibody syndrome [42], rheumatoid arthritis [43] or autoimmune neutropenia [44].

Interestingly, the presence of 16S recombinant RNA gene of *H. pylori* spp. on liver samples of a patient with NASH has been reported [45]. This finding was validated by another study, in which the 16S recombinant RNA gene was found in five of 11 patients with NAFLD [46]. However, it remains unclear whether *H. pylori* in

these specimens was an incidental finding or it has the potential to affect the natural course of NAFLD. Given that NAFLD is the hepatic manifestation of IR syndrome, studies evaluating *H. pylori* infection in NAFLD patients would be of importance.

Chronic inflammation and alterations in counter-regulatory hormones are deemed responsible for IR pathogenesis and, although the pathogenetic link between *H. pylori* infection and IR remains elusive, this infection may influence the pathophysiology of IR and IR syndrome by the following mechanisms:

1 Releasing large amounts of proinflammatory and vasoactive substances, such as cytokines [interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12, TNF-α, interferon-γ], eicosanoids (leukotrienes, prostaglandins), and acute phase proteins (fibrinogen, C-reactive protein) [47,48], also involved in the pathogenesis of IR syndrome [1].
2 Promoting platelet activation and platelet–leukocyte aggregation [49]. Enhanced platelet aggregation and activation markers are found in IR syndrome accompanied by low adiponectin concentrations [50].
3 Inducing chronic atrophic gastritis with concomitant decrease in vitamin B12 and folate concentrations, thereby increasing the homocysteine [51], an independent risk factor for IR-related disorders. IR syndrome and total plasma homocysteine levels are significantly associated with the extent of coronary atherosclerosis [52].
4 Producing reactive oxygen species [18] also involved in the pathophysiology of IR syndrome [1].
5 Inducing impaired ghrelin production [53]. Low ghrelin levels are associated with elevated fasting insulin levels and IR [54]. *H. pylori* infection has been also associated with lower leptin levels [55], which are associated with impaired energy homeostasis, lipid metabolism, and insulin sensitivity [56].
6 Influencing the apoptotic process [47,48], which also plays an important pathophysiologic role in many IR-related disorders [1].

There is further evidence for association between other infections and IR. It is well established for chronic hepatitis C [57,58], also associated with a high prevalence of *H. pylori* co-infection [59], but not for chronic hepatitis B infection [58,60]. Human immunodeficiency virus (HIV)-infected patients also experience a dramatic increase in IR, but antiretroviral therapy plays an important role in the specific population [61]. Notably, apparently healthy individuals with exposure to common pathogens, such as herpes virus types I and II, enteroviruses, Chlamydia pneumonia, and Cytomegalovirus, usually manifesting chronic infections, have increased fatty mass and IR [62–64]. Generally, the greater the pathogen burden, the higher the fatty mass and IR [63,64]. These associations become of paramount importance given the high prevalence of the aforementioned infections, similar to *H. pylori* infection.

An important issue that should be highlighted is that the results of this systematic review do not necessarily imply a causative role of *H. pylori* for developing IR. Most included studies were cross-sectional, and the two prospective studies evaluating the effect of eradication therapy were nonrandomized and provided conflicting results [7,24]. Therefore, randomized-controlled trials are required to clarify the *H. pylori* infection–IR interaction. However, given that *H. pylori* infection is usually acquired early in life, it has been proposed that *H. pylori* infection might precede *H. pylori* infection–IR interaction [65], but further studies are needed to clarify which is the cause and the effect, if such a condition exists.

In conclusion, although data seem to indicate a potential association between *H. pylori* infection and IR, further studies are needed to strengthen this association and to clarify whether there is a causative link between them. If a causal link between *H. pylori* infection and IR is confirmed in the future, this may have a major impact on the pathophysiology and management of IR syndrome, including T2DM and NAFLD.

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RESEARCH REVIEW

Upper Gastrointestinal Carcinogenesis: *H. pylori* and Stem Cell Cross-Talk

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Chronic inflammation of the gastric epithelium has been associated with the pathogenesis of gastric cancer, as it was postulated by Corea's model of gastric carcinogenesis. *Helicobacter pylori* (*Hp*) regulates this inflammatory process and promotes gastric carcinogenesis through induction of gene mutations and protein modulation. Recent data raise the cancer stem cell hypothesis, which implies a central role of multipotent cancer cells in oncogenesis of various solid tumors. This review provides a synopsis of gastric cancer initiation and promotion through *Hp* and stem cell signaling pathways. The expanding research field of *Hp*-related cancer stem cell biology may offer novel implications for future treatment of upper gastrointestinal cancer.

Key Words: **gastric carcinogenesis; oncogenic signaling pathways; bone marrow-derived stem cells; epithelial cancer.**

INTRODUCTION

Gastrointestinal cancer accounts for 20% of all cancer causes worldwide. Infection and inflammation are associated with 15% to 20% of the world's malignancies and are predisposing risk factors for gastrointestinal cancers [1, 2]; it is becoming increasingly apparent that many cancers arise at the sites of chronic inflammatory process and infection [3, 4]. Although this association has long been documented, the underlying mechanisms remain unclear (Table 1). Within the gastrointestinal tract (GIT), there are several examples of inflammatory conditions associated with malignancy, such as gastroesophageal reflux disease and Barrett's esophageal adenocarcinoma, *Helicobacter pylori* (*Hp*) infection, and gastric malignancies, inflammatory bowel disease and colorectal cancer, and viral hepatitis resulting in hepatocellular carcinoma development [2, 5]. There are various mechanisms by which chronic inflammation has been postulated to result in cancer development, which includes gene mutations, inhibition of apoptosis, increased angiogenesis, and cell proliferation in an endless effort to heal injury, and the presence of a persistent inflammatory microenvironment creating a pro-carcinogenic environment; chronic inflammation induces epigenetic alterations associated with oncogenesis [1, 5]. In particular, the inflammatory microenvironment, as an altered stem cell niche, typically affects cancer promotion and can also affect cancer initiation and progression; tissue stem cells reside within a "niche" or a group of cells, and extracellular substrates that provides an optimal microenvironment for normal differentiation and alterations in the stem cell niche might be responsible for the transformation of stem or progenitor cells to tumor stem cells. The origin of the tumor cells is frequently attributed to tumor stem cells, a unique subpopulation within tumors that possesses the ability to initiate tumor growth and sustain self-renewal, and are mainly responsible for their metastatic potential [6]. In this respect, there is also a role for engraftment of circulating bone marrow-derived stem cells (BMDSCs), which may contribute to tumor formation in animal models with *Hp*-induced chronic gastric inflammatory process and in human beings [5].

Specifically, gastric cancer is the fourth most common cancer worldwide, making the search for its molecular and cellular basis an imperative priority [7, 8]. The

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TABLE 1

Risk Factors Involved in the Pathogenesis of Gastric Cancer

1. *H. pylori* bacterial factors (*cag* pathogenicity island)
2. Th1 immune response to *H. pylori*
3. Germline mutations of E-cadherin gene
4. Genetic polymorphism of cytokine genes (increased IL-1β, TNF-α, low IL-10)
5. Genetic polymorphism of genes encoding components of the stem cell signaling network (BMP6, GDF15, RUNX3)
6. Diet high in salt and nitrates, low in fresh fruits and vegetables
7. Smoking and metabolic syndrome
8. Increased serum gastrin levels
9. Achlorhydria and bacterial overgrowth

IL-1β = interleukin 1β; Th1 = T helper 1; TNFα = tumor necrosis factor α; IL-10 = interleukin 10; BMP6 = bone morphogenetic protein 6; GDF15 = growth differentiation factor 15; RUNX3 = runt-related transcription factor 3.

association of this lethal disease with *Hp* infection has provided new aspects in gastric carcinogenesis; recent advances in gastric stem cell biology and lessons from hematologic malignancies triggered the investigation of stem cell's implication in this solid tumor development [8, 9]. Two types of gastric stem cells may be involved in gastric oncogenesis. Resident adult or tissue stem cells might, in a chronically inflamed environment, slowly acquire a series of genetic and epigenetic changes that result in their emergence as "cancer stem cells." Otherwise, the chronic inflammatory process and gastric mucosal damage might result in loss of the indigenous gastric stem cells from their niches, and BMDSCs may then be recruited to and be engrafted into the gastric mucosa, thereby contributing to the development of tumor mass and the stromal components of the tumor [5, 8].

This review focuses on the link between *Hp*-induced inflammation and upper GIT carcinogenesis and the relationship between stem cells and cancer.

The Stem Cell Concept

Over the past two decades, the advances in our understanding of stem cell biology and the role of stem cells in pathologic conditions, such as gastrointestinal cancers, have been remarkable. In particular, discoveries related to the control of stem cell proliferation and how dysregulation of proliferation results in carcinogenesis have been in the forefront. In this regard, since tumors originally develop from normal cells that gain the ability to proliferate aberrantly and ultimately turn malignant, a crucial question in tumor biology is which cells can be transformed to form tumors. Relative recent data elucidated the existence of cancer stem cells that have the exclusive capacity to produce tumors [10]. The cancer stem cell hypothesis holds that tumors are driven by a cellular subcomponent that has stem cell properties, including self-renewal, tumorigenicity, and multilineage differentiation capacities [11]. Specifically, carcinogenesis is driven by the accumulation of various genetic alterations, which, in turn, provide the cancer stem cells with six distinct capabilities: self-sufficiency in growth signals; insensitivity to antigrowth signals; evading apoptosis; sustained angiogenesis; tissue invasion and metastasis, and limitless replicative potential [12–14].

Importantly, cancer stem cells are only a small subset of cancer cells constituting a reservoir of self-sustaining cells with the mentioned exclusive ability to self-renew and maintain the tumor; it was found that only a minority of cancer cells are capable of forming tumors, if transplanted into mice. These cells have the ability of independent growth, maintaining the tumor mass through proliferation and differentiation patterns similar to tissue stem cells [15]. Evidence suggests that solid tumors harbor cancer stem cells that are capable of initiating tumor growth in immunodeficient animals with as few as 10 cells [16]. These cells are identified by specific stem cell markers: antigens, molecules, and signaling pathways. Transcription factors and molecules associated with oncogenesis, including nuclear factor (NF)-κB, Wnt (wingless) β-catenin, polycomb gene BMI-1 (B-cell-specific Moloney murine leukemia virus integration site 1), Notch, sonic hedgehog (SHH), and their biochemical pathways, active only in a minority of cancer stem cells, may play important roles in determining the biology and the overall long-term behavior of cancer [17].

Regarding the GIT, the Wnt family of growth factors, and events such as the regulation of the nuclear localization of β-catenin, seem to be vital to normal homeostasis, and mutations in the components of these pathways appear to result in cancer development [18]. Signaling pathways such as BMI1 and Wnt have similar effects in normal and cancer stem cell self-renewal, thereby suggesting that common molecular pathways regulate both populations [10]. Cancer stem cells share many characteristics with normal stem cells. These are the capacity for self-renewal, the low proliferation rate, which renders them resistant to conventional chemotherapy, the ability to differentiate into all cell lines that compose the cancer, and the utilization of the same signal transduction pathways, as normal stem cells but different than those utilized by the differentiated cancer cells. Studies of normal and cancer stem cells from the same tissue have shed light on the cancers ontogeny. With the increasing evidence that cancer stem cells exist in a wide range of tumors, it is becoming increasingly imperative to understand the molecular mechanisms that regulate self-renewal and differentiation since corruption of genes involved in these pathways participates in cancer growth [10].

Markedly, apart from tumor development, the mentioned BMDSCs, recruited to and engrafted into the gastric mucosa, may also contribute to the tumor stroma growth; these stem cells comprise a subset of cells within the tumor stroma and within seemingly uninvolved epithelium and subepithelial spaces adjacent to cancers [5]. In this regard, components of the tumor microenvironment important in the final common pathway resulting in cancer include the tumor stroma, tumor-associated macrophages, cytokines and chemokines, and reactive oxygen and nitrogen species [19].

The definition of a cancer stem cell, however, does not necessarily imply its origin from a stem, progenitor, or differentiated cell. Therefore, the term tumor- or cancer-initiating cell is frequently used instead to avoid any implications. A relative proposed model suggests that tumor cells progressively acquire stem cell properties as a consequence of oncogene-induced plasticity [20].

In view of the aforementioned data, it appears that cancer stem cell hypothesis has great implications in understanding carcinogenesis and detection, prevention, and/or treatment of cancer; given the specific stem cell features, novel therapeutic pathways can be targeted.

Hp, Stem Cells, and Upper Gastrointestinal Carcinogenesis

Although *Hp* infection is the leading cause of gastric cancer worldwide, little is known about the early development and detection of this tumor. Advances have been made with respect to identifying the gastric epithelial stem cells and their immediate descendents, which act as progenitors giving rise to mucus-, acid-, pepsinogen-, and hormone-secreting cell lineages. Analyses of some genetically manipulated animal models in which the proliferation and differentiation program of the gastric stem cells was altered by different approaches have provided some clues to the cellular origin of gastric malignancy [21].

In the stomach, epithelial cell migration is bidirectional, with cells originating in a putative stem cell compartment in the gastric gland neck/isthmus region and migrating both upwards towards the lumen, and down into lower gland regions of the gland. Albeit fundamentally based upon circumstantial evidence, it is generally accepted that the gastric stem or progenitor cells are located and maintained within a mesenchymal niche, situated towards the center/isthmus of the corpus gastric glands giving rise to differentiated cells *via* bidirectional migration patterns. In the gastric antrum, they are located at the bottom of the glands, and descendents migrate toward the surface unidirectionally. Although the study of gastric epithelial homeostasis and cancer has been hampered by the lack of stem cell markers and *in vitro* culture methods, homeostasis of the stem cells seems to be maintained by a complex signaling network, which is composed of Wnt, Notch, fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and Hedgehog (Hh) signaling pathways. Homeostasis of stem cell signaling pathways is dysregulated during carcinogenesis; dysregulation of the stem cell signaling network due to the accumulation of germline mutation, single nucleotide polymorphism (SNP), *Hp* infection, epigenetic change, and genetic alteration gives rise to gastric malignancy (Table 2) [22].

Specifically, Wnt, Notch, and FGF signaling pathways are the core components of the stem cell signaling network. Canonical Wnt signals induce Notch and FGF signaling in progenitor cells. Although feedback loops from FGF and Notch signaling pathways to the Wnt signaling pathway remain to be elucidated, dickkopf (DKK) protein-2, a Wnt antagonist, is found to be a Notch signaling target in intestinal stem cells; the zonal expression of DKK protein-3 in gastric tissue, and DKK-2, DKK-3, and Wnt inhibitory factor-1 (Wif1) in colon tissue, with increased expression in the deep gastric glands/colonic crypt bases, where gastrointestinal stem cells reside, suggests that soluble Wnt antagonists, including soluble frizzled related proteins (SFRP), DKK proteins, and Wif1 might be crucial Wnt signaling regulators in these tissues, and might contribute to the maintenance of the stem cell pool [23]. The canonical Wnt and sonic Hh pathways have been independently linked to cell proliferation in a diversity of tissues and systems. However, interaction of these signals is also involved in the control of cell cycle progression [24].

BMP signaling is regulated by the balance between BMP family ligands and BMP inhibitors. Hh signals from epithelial cells induce BMP4 up-regulation in mesenchymal cells. Then, BMP signals (regulated by

TABLE 2

Proposed Mechanisms of *H. pylori* and Stem Cell Interaction

1. Up-regulation of Wnt signaling pathway
2. CagA-induced SHP2 dysfunction affecting the FGF signal transduction pathway
3. Activation of BMP/TGFβ signaling pathway
4. Down-regulation of SHH signaling through CpG hypermethylation of HHIP1 promoter region
5. Recruitment of mesenchymal stem cells through the production of IL-6 and IL-8 and/or BMDSCs cells through chronic inflammation and cagA-related CXCR-4 expression

SHP2 = Src-homology 2 phosphatase; FGF = fibroblast growth factor; BMP = bone morphogenetic protein; TGFβ = transforming growth factor β; SHH = sonic hedgehog; HHIP1 = hedgehog-interacting protein 1; BMDCs = bone marrow derived cells; CXCR-4 = CXC chemokine receptor-4.

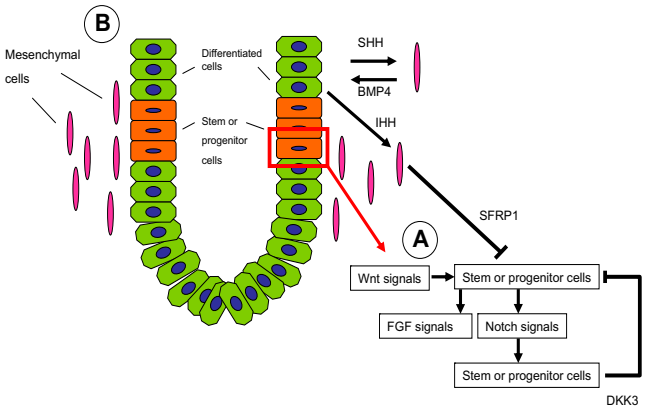

FIG. 1. Stem cell signaling network. (A) Wnt signals induce FGF and Notch signaling in progenitor cells. DKK3, a Wnt antagonist, found to be a Notch signaling target, in gastric stem cells. (B) SHH signals from epithelial cells up-regulate BMP4 in mesenchymal cells. BMP signals induce IHH up-regulation in the differentiated pit cells. IHH signals induce secretion of the Wnt antagonist SFRP1 from the mesenchymal cells. (Color version of figure is available online.)

the balance between BMP family ligands and BMP inhibitors), through the cooperation between SMAD complex and Runt-related transcription factor (RUNX) family transcription factors, induce Indian Hedgehog (IHH) up-regulation of Wnt antagonist SFRP1 in mesenchymal cells to inhibit the canonical Wnt signaling pathway. Hh-dependent SFRP1 induction keeps differentiated epithelial cells away from the effects of canonical Wnt signaling; BMP and Hh signaling pathways are the peripheral components of the stem cells signaling network (Fig. 1).

Hp - and Stem Cell-Related Wnt Oncogenic Signaling Pathway

Wnt signaling pathway is implicated in carcinogenesis and embryogenesis. Wnt signaling pathway includes secreted-type glycoproteins, which transduce signal through frizzled family receptors. Canonical Wnt signaling releases β-catenin from the AXIN/GSK3β degradation complex. Accumulation of β-catenin results in the transcriptional activation of target genes maintaining the stem/progenitor cells and inducing cell proliferation. Noncanonical signaling activates various intracellular cascades (RhoA, JNK, PKC, NFAT, and NLK) for the control of tissue polarity, cell adhesion, and cell movement [22, 25]. Specifically, distinct Wnt intracellular pathways including both canonical β-catenin/ T-cell factor (Tcf) signaling and noncanonical cascades mediated by JNK, PKC, Ca(2+), or Rho are implicated in the regulation of multipotential mesenchymal stem cells proliferation and differentiation [26]. Wnt signaling molecules, especially Wnt8A and Wnt8B, may play crucial roles in embryonal tumors and embryonic stem cells through synergistic activation of the β-catenin-T cell factor (Tcf) signaling pathway [27]. Wnt2 and Wnt5A are frequently up-regulated in gastric cancer [28]. Up-regulation of Wnt10B in human gastric mucosa may result in gastric oncogenesis through activation of the mentioned β-catenin-Tcf signaling pathway [29]. In this respect, up-regulation of Wnt10A induced by tumor necrosis factor (TNF)-α and *Hp* may play central roles in human gastric cancer through activation of Wnt- β-catenin-Tcf signaling pathway [30]. Of note, *Hp* infection induces the expression of interleukin (IL)-6 and TNF-α in gastric mucosa, which in turn up-regulate Wnt5A and Wnt10B, respectively, and finally triggers canonical and noncanonical Wnt signaling [29]. Collectively, the aforementioned data suggest that both *Hp* infection and stem cell-related Wnt- β-catenin-Tcf signaling pathway may be involved in gastric oncogenesis.

Hp - and Stem Cell-Related Notch Oncogenic Signaling Pathway

Notch family receptors and their transmembrane-type ligands compose the Notch signaling pathway. Upon ligand-binding Notch intracellular domain is released for the interaction with CSL or NF-κB transcription factors. This pathway enables cell–cell communication, acting to regulate cell fate decisions and thereby thus influences cell proliferation, differentiation, and apoptosis. Notch signaling is up-regulated in gastric cancer, and maintains stem/progenitor cells through the inhibition of epithelial differentiation [31]. In particular, Notch is activated during chronic *Hp* [22]. In addition, Notch homolog 1 (Notch1) belongs to the Notch family of transmembrane receptors and

plays a significant role in cell differentiation; Notch1 plays an important role in oncogenesis and can be both oncogenic and tumor suppressive. The dysregulation of Notch1 expression might correlate to the occurrence and development of gastric cancer and may be a novel prognostic marker of gastric malignancy [32]. Viewing the aforementioned data, it appears that Notch signaling related with *Hp* and stem/progenitor cells may play an important role in gastric cancer development with prognostic significance.

Hp - and Stem Cell-Related FGF/BMP Oncogenic Signaling Pathway

Apart from Wnt and Notch signaling pathways, FGFs constitute the stem cell signaling network, which plays a crucial role in a variety of processes including carcinogenesis [33]; FGFs and their signaling receptors have evoked interest as candidate oncogenes with the potential to initiate and/or promote tumorigenesis [34]. FGF signaling pathway is activated upon binding of FGF ligands to FGF-receptor (FGFR) and subsequent receptor homodimerization and autophosphorylation. FGF signals are transduced to PKC, NFAT, RAS-ERK, and PI3K-Akt signaling cascades [35]. Importantly, FGF/FGFR alterations have been involved in gastrointestinal neoplasms [36]; gene amplification or missense mutation of FGFR2 occurs in gastric cancer [37]. FGFR and BMP4 are particularly overexpressed in signet ring gastric carcinoma. This activation of FGF and BMP signaling pathway may contribute in down-regulation of SHH signaling in diffuse type gastric cancer [38]. Noticeably, *Hp* cytotoxin CagA-induced SHP2 dysfunction affects the FGF signal transduction through the FRS2/FRS3-SHP2-GRB2 protein complex to RAS-ERK and PI3K-Akt signaling cascade [22, 39]. Since FGF signaling pathway is also activated during chronic *Hp* infection [22], this pathway being related with the stem cell signaling network and *Hp* might play an additional role in gastric oncogenesis.

BMPs are included in the TGFβ superfamily and play a crucial role in stem and progenitor cell biology as regulators of the balance between expansion and differentiation. BMPs promote differentiation of stem cells, thus promoting exit from the stem cell compartment. BMP/TGFβ signaling pathway is also activated during chronic *Hp* infection [22] and plays a key role in carcinogenesis [33]. Of note, the BMP signaling utilizes R-SMAD proteins as transducers for the transcriptional activation of target genes [40]. *Hp* infection results in an influx of inflammatory cells that disturb the expression of morphogens in gastric epithelia; it was found that inflammatory cells secrete BMP2 and BMP4 to activate the BMP-IHH signaling loop in the epithelial cells. Interestingly, a shift in activity of the BMP pathway was observed towards the precursor cell compartment (isthmus) of the gastric units [41]. BMP2 and BMP4 activity have also been related to the diffuse-type gastric cancer.

Hp - and Stem Cell-Related Hh/ SHH Oncogenic Signaling Pathway

Hh is a secreted protein named for the bristle phenotype observed in *Drosophila* embryos that lack the corresponding gene. Three homologs have been characterized in vertebrates, all of which have crucial roles in the development of multiple organ systems; the Hh signaling pathway is critical to normal mammalian gastrointestinal development [42]. In addition, these proteins regulate stem cell production and appear to drive proliferation in a diversity of types of cancers, including gastric malignancy [42, 43]; Hh signaling is aberrantly activated in esophageal cancer, gastric cancer, pancreatic cancer, and other malignancies [44]. The role of Hh signaling in the evolution of cancer in areas of chronic inflammation could be the establishment of an up-regulated steady state of cell proliferation as well as an increased resistance of cells to apoptosis [33]. Comparable with aforementioned signalingpathways, Hh also constitutes the stem cell signaling network [33]; it is activated during chronic *Hp* infection [22], thereby being involved in gastric oncogenesis related to possible *Hp* and stem cell interactions.

Specifically, SHH is a morphogen involved in the homeostasis of the gastric fundic glands [45]; maturation and differentiation of parietal cells is regulated by the SHH signaling. During chronic infection with *Hp*, SHH is implicated in the repair process. On the other hand, in atrophic gastritis, SHH signaling is decreased due to the depletion of the parietal cell lineage [46]. Aberrant SHH signaling, in response to mucosal damage, could help repair the damaged epithelium. Moreover, SHH, IHH, patched family receptor 1 (PTCH1), and GLI1 expression, as well as down-regulation or CpG hypermethylation of Hedgehog-interacting protein 1 (HHIP1) promoter are frequently observed in esophageal and gastric malignancies [47–50]. Loss of SHH expression and aberrant expression of Caudal homeobox (CDX) 2 gene correlates with the type of intestinal metaplasia and may play a role in gastric carcinogenesis [51]; loss of SHH is an early change that occurs in the mucosa prior to neoplastic transformation [52]. Importantly, the observed suppressed SHH expression in the gastric mucosa by *Hp* infection is significantly restored following eradication of the infection [53, 54]. Therefore, SHH signaling seems to play a role in upper GIT oncogenesis possibly involving *Hp* and cancer and adult stem cells interactions.

OVERVIEW OF *HP*-STEM CELL INTERACTION

Strains of *Hp* can be divided into two major subpopulations based on their ability to produce a 120–145 kDa immunodominant protein called cytotoxin-associated gene A antigen (CagA). CagA protein, encoded by cagA gene, is delivered into gastric epithelial cells *via* the bacterial type IV secretion system, where undergoes tyrosine phosphorylation by Src family kinases (SFKs) and Abl kinases. Tyrosine-phosphorylated CagA then acquires the ability to interact with and deregulate Src-homology 2 (SH2) phosphatase, a *bona-fide* oncoprotein, deregulation involved in a diversity of human malignancies, including gastric cancer [55]; Src kinases are associated with malignancies, Src inhibition may have a cytostatic effect on malignant stem cells [56], and SHP-2 phosphatase is involved in Bcr-Abl tyrosine kinase hematopoietic stem cell transformation and oncogenesis [57]. In particular, gastric epithelial cells expressing CagA undergo a unique morphologic change termed the "hummingbird" phenotype, characterized by the formation of needle-like cell protrusions; the phosphorylated CagA binds and activates SHP-2 phosphatase, thereby inducing the growth factor-like morphologic change "hummingbird phenotype", characterized by long, thin cellular extensions. In addition, CagA disrupts the apical junctional complex that regulates the cell–cell contact and maintains the integrity of the epithelial structure, and CagA-induced SHP2 dysfunction is implicated in FGF signal transduction pathway activation. These CagA activities may collectively cause cellular dysfunctions, thereby promoting accumulation of multiple genetic changes involved in malignant transformation.

Moreover, CagA-SHP2 complex activates the ras-raf-MAP kinase-ERK kinase (MEK)-extracellular regulated MAP kinase (ERK) signaling cascade involved in the regulation of cell growth, differentiation and stem cell oncogenic transformation [58]; expression of CagA in gastric epithelial cells results in prolonged activation of ERK mitogen-activated protein (MAP) kinase activity.

Apart from *Hp* CagA-related interactions with stem cell, *Hp* infection itself interacts with all the aforementioned main components of the stem cell signaling network (i.e., Wnt, Notch, FGF/BMP, and Hh/ SHH oncogenic signaling pathways). It dysregulates normal stem cell signaling and more interestingly *Hp* may adapt to and influence stem cell biology, thus contributing to gastric tumorigenesis [59]; dysregulation of the mentioned stem cell signaling network due to *Hp* infection, accumulation of germline mutation, SNP, epigenetic change, and genetic alteration gives rise to gastric malignancy [22] (Fig. 2).

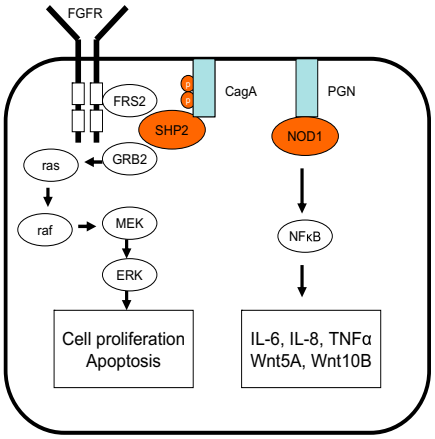

FIG. 2. CagA-induced SHP2 dysfunction is implicated in FGF signal transduction pathway activation, activating the ras-raf-MEK-ERK signaling cascade involved in the regulation of cell growth, differentiation, and stem cell oncogenic transformation. Peptidoglycan (PGN) derived from *H. pylori* is recognized by the cytoplasmic pathogen-recognition molecule NOD1 to activate the NFκB signaling cascade for the transcriptional activation of cytokines in epithelial cells (specifically IL-6 and TNFα) and, subsequently, the wnt signaling pathway. (Color version of figure is available online.)

Hp, Epithelial Stem Cells, BMDSCs, and Upper Gastrointestinal Carcinogenesis

Experimental studies directly demonstrate the existence of a common stem cell population in the gastric epithelium; long-lived committed progenitors/multipotential stem cells play a role in the gastric epithelium development [60].

In this respect, *Hp* infection evokes directly gastric inflammation and indirectly propels the supply of gastric epithelial stem cells and their differentiation to the mature cells. Gastric epithelial stem cells may be the product of an altered extracellular microenvironment in the neck portion of the gastric mucosa under *Hp* infection, thereby possibly suggesting the *in situ* occurrence of gastric malignancy under *Hp* infection [61]. Relative human studies show that gastric cancer contains gastric tumor-initiating stem-like cells with heterogeneity and distinct hierarchy in malignancy [62]. By using the stem cell marker CD44, the CD44(+) gastric cancer stem cells show the stem cell properties of self-renewal, the ability to form differentiated progeny and, moreover, increased resistance for chemotherapy- or radiation-induced cell death [63]; *Hp* either directly or through a local inflammatory response, is responsible for increased expression of CD44 and its variant CD44 v9 [64, 65], thereby suggesting a possible *Hp* induction of CD44(+) gastric cancer stem cells involved in gastric cancer production and progression.

On the other hand, studies of the cellular origins of malignancy in the *Hp*-infected mouse model has result in the surprising insight that gastric cancer may originate from circulating BMDSCs and not from resident tissue stem cells as previously believed [66]. Indeed, Houghton *et al.* [5, 67] showed that *Hp*-induced inflammation in mice caused migration of stem cells originating from bone marrow to the stomach, where they subsequently developed gastric tumors. In this regard, we recently conducted a pilot study using tissue sections of biopsies of human gastric cancer in which *Hp* was detected by Cresyl violet staining [9]. Moreover, stem cells and neovessels were detected by immunohistochemical method using a monoclonal antibody, anti-CD34; CD34 is a surface glycoprotein expressed on hematopoietic stem cells and is used as an important marker of these cells and neovessels. In addition, cyclin D1 involved in the regulation of cell proliferation was also detected by immunohistochemical method. Therefore, it would be reasonable to speculate that chronic infection of C57BL/6 mice and humans with *Hp* induces repopulation of the stomach with BMDSCs that may facilitate gastric cancer progression as well as colon cancer progression [68]. These findings present a new way of thinking about the pathogenesis of upper gastrointestinal malignancy. The observation that BMDSCs are the origin of *Hp*-induced gastric cancer can be also combined with supporting observations of BMDCs in other tumors such as Barrett's esophageal adenocarcinoma, Kaposi sarcoma, cancer-associated fibroblasts, or benign and malignant tumors of skin [5, 69].

Focusing on animal models, a "premetastatic" niche has been described, where BMDSCs migrate to specific sites before metastasis formation [70]. Why and how do BMDSCs metastasize into the stomach? *Hp* is unique in its ability to colonize the gastric mucosa, to induce chronic inflammation and, finally, atrophy of the acid producing mucosa. This cascade is dependent on the host's T helper (Th)1 immune response, which is partly predefined by host's factors, and specifically by proinflammatory cytokine gene SNP. The first cytokine studied is IL-1β; IL-1β SNP is associated with gastric cancer and its genotyping could help to identify high-risk individuals [71, 72]. Specifically, IL-1 cluster polymorphisms have been identified as a risk factor for the development of atrophic gastritis and gastric cancer in *Hp*-infected patients but not in uninfected patients [9]. Moreover, IL-1 gene cluster polymorphisms are associated with an increased risk of both hypochlorhydria induced by *Hp* and gastric cancer [9]. IL-1-β polymorphisms are associated with increased risk of gastric cancer not only in whites, but also in patients from the Far East (Japan) [9]. Interestingly, a combination of IL-1β, TNF-α, and IL-10 SNPs, which potentially results in a phenotype of elevated IL-1β and TNF-α and decreased IL-10, confers a 50-fold increased risk of gastric cancer [73]. This immune response is associated with elevations in chemokines and cytokines, which induce monocyte/leukocyte migration but also BMDSCs migration. Indeed, similar to white blood cells, many types of BMDSCs are mobilized into the circulation in response to inflammatory mediators [74]. IL-6 and IL-8 production, during inflammation, initiate neutrophil infiltration and recruitment of mesenchymal stem cells (MSC) [75]. Other chemoattractants for MSC are vascular endothelial growth factor (VEGF) and macrophage inflammatory protein 1α (MIP-1$^{\alpha}$). *Hp* modulates VEGF expression through IL-6 induction and the NF-κB and cyclooxygenase (COX)-2 pathways [9, 76]. Activated myofibroblasts, originated from MSC or gastric stromal cells, seem to form the "premetastatic" niche and produce chemokine IL-12 (CXCL12) for further recruitment of BMDSCs [77].

CXCL12, also known as stromal-derived factor 1 (SDF1), secreted by the marrow stromal cells, is essential in hemopoietic stem cell (HSC) homing within the microenvironment in the bone marrow. HSCs, exclusively, respond to CXCL12 chemotactic signals. It signals through CXC receptor-4 (CXCR4); CXCL12-CXCR4 axis functions in mobilization of HSCs as well as in tissue repair and regeneration [78]. During ischemic injury, hypoxia-inducible factor-1 (HIF1) induces CXCL12 expression on endothelial cells, which in turn attracts the circulating progenitor and stem cells forming a niche for tissue repair [79]. Additionally, HIF1 induces CXCR4 expression in malignant cells and, thus, it may play a role in metastatic spread of these cells to selectively CXCL12-expressing organs [80]. Indeed, it has been demonstrated that CXCR4 gene is included in the "bone metastatic signature" of various tumors [81]. Furthermore, Hansson *et al.* demonstrated that *Hp* induces CXCR4 expression on dendritic cells, resulting in their maturation and chronic inflammation of gastric mucosa [82]. This expression was related, specifically, to Cag pathogenicity island positive strains, also implicated in gastric carcinogenesis.

Although *Hp* initiates gastric carcinogenesis, establishing an inflammatory environment, in which further responses are related to *Hp* pathogenicity potential and host's factors, and, moreover, *Hp*-induced Th1 response results in BMDSC recruitment into the gastric mucosa, however, more work is needed to show that the BMDSCs move into areas of *Hp* chronic injury or inflammation with long-term malignant consequences. The importance of BMDSCs engraftment during human gastric neoplasia is an area requiring urgent investigation.

Viewing the aforementioned data, *Hp*-related gastric cancer seems to originate by both BMDSCs and a subset

of gastric epithelial stem cells. Important roles for signaling between epithelial and mesenchymal cells, particularly myofibroblasts, are also emerging. However, despite the challenges and the similarity between gastric epithelial progenitors and their differentiation program in mice and humans, it remains to be determined whether observations made in genetically engineered mice are also applicable to humans.

Closing Remarks

Gastrointestinal stem cells have the capacity for long-term self-replication and the ability to give rise to all other epithelial cell lineages. These properties make them essential since they maintain tissue homeostasis by regulating cell turnover, depending on the current demand; tissue-specific stem cells are responsible for the maintenance of the epithelium throughout the GIT. However, they are also crucial players in the gastrointestinal carcinogenesis, as they form a target for mutations to accumulate and result in the development of the malignant phenotype; the accumulation of mutations in these stem cells is the likely cause of most gastrointestinal cancers. Clonal analysis of these stem cell populations has revealed how normal homeostatic processes work and how neoplastic growth occurs. Carcinogenesis is characterized by initiation, promotion, and progression phases. Several *Hp* and gastric stem cell-related oncogenic signaling pathways have been recently proposed to involve in these phases of upper gastrointestinal cancer. Moreover, the setting of *Hp*-related chronic inflammatory gastric injury may result in the loss of the indigenous gastric stem cells from their niches; BMDSCs may then be recruited to and engraft into the gastric epithelium. Such recruited cells have the potential to contribute to the tumor mass. The aforementioned expanding research field of *Hp*-related cancer stem-cell biology may offer novel clinical implements in the diagnosis, treatment and prevention of upper gastrointestinal cancer.

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Gastrointestinal Immune System and Brain Dialogue Implicated in Neuroinflammatory and Neurodegenerative Diseases

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Abstract: A common characteristic of the central nervous system (CNS) neurodegenerative disorders is neuroinflammation, marked by augmented numbers of activated and primed microglia, increased inflammatory cytokines and decreased anti-inflammatory molecules. CNS neuroinflammation is a critical component in the progression of several neurodegenerative diseases which sensitize the brain to produce an exaggerated response to immune stimuli in the periphery. Neuroinflammation might initiate from the periphery and peripheral conditions through disrupted blood-brain barrier powerfully influence various brain pathologies. Gastrointestinal tract (GIT) represents a vulnerable area through which pathogens influence the brain and induce CNS neuroinflammation. The pathogens may access the CNS through blood, the nasal olfactory pathways and the GIT. Potential GI pathogens, such as *Helicobacter pylori*, induce humoral and cellular immune responses that, owing to the sharing of homologous epitopes (molecular mimicry), cross-react with CNS components thereby contributing and possibly perpetuating neural tissue damage. GIT is strictly connected to the CNS and a bi-directional communication exists between them. The brain is involved in regulating the immune and gut system. Conversely, limited attention has been paid on the GIT role in the development and regulation of the CNS autoimmune diseases. The GIT is the primary immune organ with specialized immunoregulatory and anti-inflammatory functions, represented by the gastrointestinal immune system (GIS). This review focuses on the potential GIS and brain dialogue implicated in neurodegenerative diseases. Gaining a better understanding of the relationship between GIS and CNS could provide an insight on the pathogenesis and therapeutic strategies of these disorders.

Keywords: Gastrointestinal immune system, central nervous system, dysbiosis, neuroinflammation, neurodegeneration, *Helicobacter pylori.*

INTRODUCTION

The central nervous system (CNS) is an isolated, relatively immune-privileged organ, wherein a well-instated barrier system, the blood-brain barrier (BBB), precludes the entry of blood cells into the brain with the exception of regular immune surveillance cells. Despite this tight security, immune cells are successful in entering the CNS where they lead to neuroinflammation, tissue damage and cell death [1]. Activated immune effectors (CD4+, CD8+ T cells, B cells, and macrophages), through the disrupted BBB, powerfully influence the neuroinflammatory processes in the brain. In the CNS, an immunological cascade triggers the activation of the innate immune system [microglia, dendritic cells (DCs), astrocytes and B cells], which leads to chronic inflammation. Neuroinflammation is a common feature of the neurodegenerative disorders of the CNS, characterized by augmented numbers of activated and primed microglia, increased steady-state levels of inflammatory cytokines and decreased levels of anti-inflammatory molecules. Notably, the 'cross-talk' between mast cells, lymphocytes, neurons and glia constitutes a neuroimmune axis implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component [2]. Both the innate and the ill-fated adaptive immune responses can potentially contribute to neuroinflammation. In the recent years, the involvement of innate immune cells in mediating neuroinflammatory pathogenesis is being evaluated; anti-inflammatory or pro-inflammatory function of DCs, microglial cells, natural killer (NK) cells, NK-T cells and gamma-delta (γδ) T cells, along with their interaction among themselves and with CNS components, contribute to neuroinflammatory and neurodegenerative pathophysiology. Moreover, the discovery of T helper (h)17 cells but also interleukin (IL)-17-secreting γδ T cells and their association with neuroinflammation has broken the dogma that interferon (IFN)-γ-producing Th1 cells have the exclusive capacity to invade and destroy the CNS tissue [2-4]. Activated microglia, by releasing cytotoxic factors, also participate in the induction of neuroinflammation. As part of the inflammatory process, toxic macrophage products, such as proteases, free radicals, nitric acid, and perforins, cause myelin and oligodendrocytes' damage; reactive oxygen species leading to oxidative stress, generated

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in excess primarily by macrophages, have been implicated as mediators of neuronal and axonal damage [5].

Although neurodegenerative disorders are multifactorial and multigenic disorders and the early events underlying them remain uncertain, neuroinflammation is currently recognized to be a feature of nearly all neuroinflammatory disorders and a critical component in the initial phase and progression of several neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis (MS), amyotrophic lateral sclerosis and, more recently, epilepsy [6-11]. These conditions sensitize the brain to produce an exaggerated response to the presence of an immune stimulus in the periphery or following exposure to a stressor; inappropriate (in time, place and extent) inflammation is progressively incriminated in diverse disease conditions, including, most relevant here, CNS pathologies [12, 13].

Neuroinflammation might initiate from the periphery and relative data suggest that peripheral conditions through the disrupted BBB powerfully influence various pathologic processes in the brain. For instance, potential pathogens have been implicated directly in the induction and development of MS, thus infections are emerging as the most consistent predictors of MS risk [14]; infections by pathogens such as Epstein-Barr virus, human herpes virus (HSV) 6, *Chlamydia pneumoniae* (*C. pneumoniae*), and *Helicobacter pylori* (*Hp*) might act synergistically with genes to induce or trigger the disease [10, 15-18]. Incidentally the consideration that microorganisms can cause AD has recently been addressed for HSV1 and *C. pneumoniae* [19, 20]. Likewise, *Hp* has been frequently implicated in the pathogenesis of AD [7, 8] and idiopathic parkinsonism [21, 22] and *Hp* eradication might benefit the course of these neurodegenerative diseases [22-24].

The pathogens may access the CNS through the blood, the nasal olfactory pathways and the gastrointestinal tract (GIT). Specifically, GIT represents a vulnerable area through which pathogens influence the brain and induce CNS neuroinflammation by the induction of pro-inflammatory cytokines in microglia, classically regarded as macrophage-like cells [25, 26]. GIT is strictly connected to the CNS, and a bi-directional communication exists between them. The brain is involved in regulating the immune and gut system [25]; functional and inflammatory gut diseases are regulated by the brain. Conversely, limited attention has been paid on the role of GIT in the development and regulation of the CNS autoimmune diseases.

The GIT is the primary immune organ with specialized immunoregulatory and anti-inflammatory functions, represented by the gastrointestinal immune system (GIS). The present study emphasizes the effects of GIS on the CNS and particularly the implications for neuroinflammatory-neurodegenerative diseases. Gaining a better understanding of the relationship between GIS and CNS could provide insight into the pathogenesis and treatment of these diseases.

I. Pivotal Role of GIS in Innate and Adaptive Immune System Function (Table 1)

The GIT is an essential immune organ consisting of a complex cellular network, secreted peptides and proteins and other host defenses. It is the primary immune organ of the body with specialized immunoregulatory and anti-inflammatory functions, represented by the GIS through innate defense mechanisms, survival of unique types of lymphocytes and their products, transport of polymeric immunoglobulins (Igs) and existence of immunoactivating components (e.g. lipopolysaccharide (LPS), heat shock proteins (Hsp), peptidoglycans, superantigens, bacterial DNA) [26]. GIS induces and maintains peripheral immune tolerance. Specifically, DCs are involved in the complex interactions between the gut microbiota and the innate and adaptive immune system, resulting in tolerance and immunity [27]; GIT mucosal DCs initiate and regulate local immune responses [28]. Innate immunity plays a crucial role in GIT immune defense against invading pathogens and also serves as a bridge to the activation of the adaptive immune system. Pattern recognition molecules of microorganisms are an important component for identifying invading pathogens. In this regard, toll-like receptors (TLRs), CARD15/NOD2 and scavenger receptors serve as the pattern recognition receptors in the innate immune defense system. Bactericidal peptides or defensins secreted by the intestinal epithelia represent another central element of innate mucosal immune defense. Likewise, autophagy is a conserved homeostatic process by which cells degrade and recycle cytoplasmic contents or organelles and an innate defense mechanism acting as a cell-autonomous system for elimination of intracellular pathogens [29]; it also plays a role in the development and education of the adaptive immune system [30]. In contrast, mutations in pattern recognition receptors and dysfunction of autophagy and secretory bactericidal peptides may impair host immune defenses resulting in an invasion of pathogens leading to chronic GIT inflammation [31, 32]; uncontrolled local immune responses and a disturbed balance of beneficial and pathogenic enteric microbiota, known as dysbiosis, may be critical factors in numerous immune-mediated diseases such as rheumatoid arthritis [33], inflammatory bowel disease [34], celiac disease [35], human allergy and asthma [36] and probably metabolic and degenerative disorders [37]. In this respect, a recent body of evidence suggests that GIS may be involved in neural development and function, both peripherally in the enteric nervous system (ENS) and centrally involving in the pathogenesis of neuroinflammatory diseases in the CNS; its dysregulation and the loss of immunosurveillance lead to diseases. For instance, mood and feelings are potentially influenced by gut microbiota and chronic

Table 1. The Role of Gastrointestinal Immune System in Innate and Adaptive Immune System Function: Immunological Phenomena Implicated in Neurodegenerative Diseases

➢ **Development and maintenance of the mucosal and systemic immune system**
➢ **Maturation of immunoregulatory pathways** (mucosal dendritic cells initiate and regulate local immune responses through induction of regulatory T cells and secretion of Immunoglobulin A)
➢ **Innate defense mechanisms**
▪ Defensin's production
▪ Scavenger receptors
▪ Toll-like receptors
▪ Homeostatic process for elimination of intracellular pathogens (autophagy)
➢ **Immune endocrine mediators** (serotonin, chromogranins)
➢ **Production of immunoactivating components**
▪ Lipopolysaccharide
▪ Heat shock proteins
▪ Peptidoglycans
▪ Superantigens
➢ **Production of pathogen-specific immunoglobulins**
➢ **Expansion of cytotoxic and T helper lymphocytes**
➢ **Dysbiosis:** a shift in the composition of enteric microbiota to a nonphysiologic composition
▪ Inflammatory cytokine stimulation
▪ Compliment pathway activation
▪ Increased vascular permeability
▪ Nitric oxide synthesis
▪ Proteoglycan synthesis
▪ Interleukin (IL)-17 production
▪ IL-22-producing NKp46+ cells

inflammation of GIT alters CNS biochemistry in mice and induces anxiety-like behavior [26, 38-40]. The implication of GIS and, specifically, the role of gut flora in neuroinflammatory disorders were demonstrated in the development of experimental allergic encephalomyelitis (EAE), the experimental model of MS in a way that is dependent on NK T cells [41]; gut flora might influence the development of EAE in a way that is dependent on Valpha14 invariant NK T cells, which has important implications for the treatment and prevention of autoimmune diseases [42]. Moreover, GIS greatly impacts the balance between pro- and anti-inflammatory immune responses during EAE and influences the Th/T regulatory cells (Tregs) axis implicated in the pathogenesis, prevention and treatment of neuroinflammatory diseases [43-45].

II. Bi-Directional Communication between GIT and CNS (Fig. 1)

Bi-directional communication between GIT and CNS occurs both in health and disease. This communication involves neural pathways as well as immune and endocrine mechanisms.

From Brain to GIT

GIT constitutes one of the largest organs whose motor, transport, secretory, storage, and sensory functions are controlled by the nervous system. GIT disorders such as irritable bowel syndrome and functional and inflammatory bowel diseases are also greatly regulated by the brain [26, 46] and the brain–gut axis plays an important role in the modulation of GIS and mucosal inflammation; in this sense, mucosal mast cells - at cellular level - and corticotropin releasing factor (CRF) - at molecular level - appear to play a critical role [47]. Specifically, chronic psychological stress results in reduced host defense and initiates intestinal inflammation *via* mast cell-dependent mechanisms, thereby signifying the activation of peripheral CRF receptors and mast cells as significant mechanisms involved in stress-related alterations of gut physiology [48]. CRF or other neuroendocrine factors might act directly or indirectly on the epithelium through mast cells, enteroglial cells or other immune cells. Cholinergic and adrenergic nerves seem to play a role in these pathways. Stress increases gut permeability, ion secretion by a mechanism involving neural stimulation or mast cells, mucin release and depletes goblet cells; these innate immune cells derive mucins and trefoil factors. It also results in increased bacterial adherence and decreased luminal lactobacilli (probiotic). As a consequence of all these changes luminal antigens may gain access to the epithelium, causing and perpetuating inflammation; intestinal

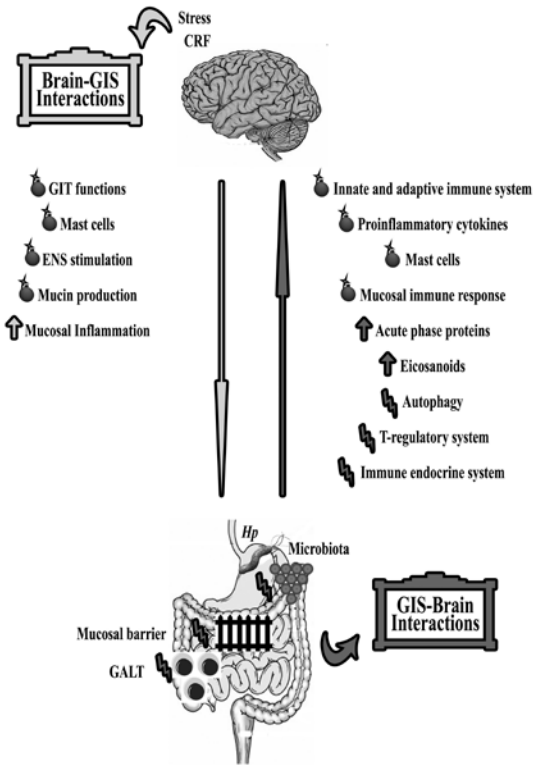

Fig. (1). Bi-directional interaction between brain and gastrointestinal immune system (GIS). The brain–gut axis plays an important role in the modulation of GIS and mucosal inflammation; GIS mechanisms influence central nervous system pathology through systemic inflammatory mediators and immunoregulatory pathway signals to the brain, contributing and possibly perpetuating neural tissue damage in neuroinflammatory disorders. Gut microbiota and *Helicobacter pylori* (*Hp*) represent major modulators of immune regulation and homeostasis. (CRF, corticotropin releasing factor; GIT, gastrointestinal tract; ENS, enteric nervous system; GALT, gut-associated lymphoid tissue; , activation; , defective; , increased).

permeability to large antigenic molecules leading to mast cell activation, degranulation, colonic mucin depletion and inflammation. The sequel of this inflammation may feedback centrally to further aggravate stress and stimulate further CRF-induced events [49]. Signals from various sources (e.g., pain, sound, sight, smell, somatic, and visceral sensations) access the brain. These inputs are modified by memory, cognition and affective mechanisms and are integrated within the neural circuits of the CNS, spinal cord, autonomic and ENS and have physiological effects including changes in motility, secretion, immune function, and blood flow to the GIT [50].

GIT Neurotransmitters and Immune Activation

It is surprising that 95% of the fibers located in the vagus nerve of the brain-gut axis are afferent fibers rising from the GIT to the brain. With regard to the GIT pathophysiology, an interface between immune and endocrine systems exists, particularly in the context of two major products of enteric endocrine systems, serotonin and chromogranins (Cgs), in relation to immune activation and induction of inflammation; gut hormones serotonin and Cgs may play an important role in the immune activation and induction of inflammation. Specifically, 95% of the serotonin (5-hydroxytryptamine, 5-HT) is located in the GIT. Serotonin is a major gastrointestinal paracrine hormone and pro-inflammatory enteric neurotransmitter; it activates the immune cells to secrete proinflammatory mediators and by manipulating the 5-HT system it is possible to modulate gut inflammation. The scenario of Cgs is more complicated, as these hormones exert

both pro-inflammatory and anti-inflammatory functions. It is also conceivable that interaction between 5-HT and Cgs might play a role in the modulation of immune and inflammatory responses [51]. Abnormalities of serotonergic function contribute to the pathogenesis of functional bowel disorders, inflammatory and infectious diseases of the gut. Therapies acting as agonists or antagonists of 5-HT receptors or that modulate 5-HT reuptake play prominent roles in managing these conditions [52]. Antidepressants such as selective serotonin uptake inhibitors may exert their action mainly on the gut and not in the brain and the antidepressant treatments have therapeutic efficacy in functional GIT disorders.

From GIT Inflammation to Brain Neuroinflammation

Inflammation in the brain might initiate from the periphery and relative data suggest that peripheral conditions powerfully influence various pathologic processes in the brain. Indeed, systemic infections influence CNS function, and microbial invasion and traversal of the BBB is a prerequisite for CNS infections. Current hypotheses suggest that microbes in some way break the self-tolerance against CNS antigens and chronically driven T-B cell collaboration, elicited by infection, can cause neuroinflammation in mice [53, 54]. Systemic infections or inflammation give rise to signals that communicate with the brain and result in changes in metabolism and behavior collectively known as sickness behavior. Symptoms including fever, malaise, lethargy and appetite loss, frequently referred to as sickness behavior, are a consequence of systemically produced pro-inflammatory mediators; sickness behavior process is triggered by pro-inflammatory cytokines [i.e., IL-1β, -6, -8, and tumor necrosis factor (TNF)] produced by peripheral phagocytic cells in contact with invading microorganisms [12, 13, 55]. These inflammatory mediators signal to the brain, leading to activation of microglial cells, which, in turn, signal to neurons to induce adaptive metabolic and behavioral changes. Systemic inflammation has an impact on primed microglia and switches them from a relatively benign to an aggressive phenotype with the enhanced synthesis of pro-inflammatory mediators [56]. Specifically, systemic inflammation leads to inflammatory responses in the brain, an exaggeration of clinical symptoms and augmented neuronal death both in animals and patients with MS, chronic neurodegenerative disease, stroke and even during normal aging [57]; it is involved in the exacerbation of acute symptoms of chronic neurodegenerative disease and might accelerate disease progression. Prompt systemic inflammation treatment or blockade of signaling pathways from the periphery to the brain might slow neurodegeneration and improve the quality of life of patients with chronic neurodegenerative disease [56].

Microbial translocation of the BBB is a key step for the acquisition of CNS infections and pathogens can induce BBB dysfunction, including increased permeability, pleocytosis, and brain pathologies [58, 59]; pathogens cross the BBB transcellularly, paracellularly, and/or in infected phagocytes (the so-called Trojan-horse mechanism).

The microorganism itself can initiate an immune response in GIT. Post-infection autoimmunity can be induced by multiple mechanisms, such as molecular mimicry, epitope spreading, bystander activation, persistence and polyclonal activation. For instance, molecular mimicry has been proposed as an explanation for autoimmune side effects/disorders of gut microorganisms implicated in MS [60]. Despite the fact that no single causal agent or event has yet been identified, a long-favored hypothesis in MS pathology is largely attributed to autoreactive effector T cells generated in the periphery that penetrate the BBB and become activated within the CNS [61]. The activated encephalitogenic immune effectors (CD4+, CD8+ T cells, B cells, and macrophages) express surface molecules that allow them to penetrate the BBB and enter the CNS [61, 62]. As a result, autoreactive CD4+ and CD8+ T cells have been found to invade and clonally expand in inflammatory CNS plaques in MS.

GIT represents a vulnerable area through which pathogens influence the brain and induce CNS neuroinflammation, through a slower humoral pathway and a faster neural pathway transported by the afferent neurons connecting the GIT to the brain leading to the expression of pro-inflammatory cytokines in microglia in the brain [25]. However, the explanation of the initial process of breakdown of normally tight BBB and the association of cellular infiltration into the CNS remains unanswered.

Hp Infection

A series of factors have been implicated in inducing BBB disruption, including inflammatory mediators (e.g., cytokines, chemokines induced by *Hp* infection) and oxidative stress [63]. *Hp* could indirectly affect the brain and other target organs, e.g., the heart, through the release of numerous cytokines such as TNF-alpha (α) acting at a distance [63]; TNF-α is involved in BBB disruption through a mechanism involving matrix metalloproteinases upregulation. TNF-α and IL-6 (TNF-α is the main trigger for the production of IL-6 by a variety of cells) play important roles in the regulation of the synthesis of other acute phase proteins which are established risk factors for atherosclerosis, such as fibrinogen and factor VIII. These cytokines also have profound effects on lipid metabolism directly at the site of the atherosclerotic lesion, but could influence the atheroma process through blood circulating levels, distant production of cytokines, or through stimulating circulating white blood cells to produce them, thereby contributing to BBB disruption and pathogenesis of heart and brain pathologies [56, 57, 63-65]; *Hp* may be involved in the pathophysiology mechanisms of BBB disruption including the release of large amounts of pro-inflammatory and vasoactive substances, such as IL-6, -8, and TNF-α, eicosanoids (leukotrienes, prostaglandins catalyzed by cyclo-oxygenase enzymes), and acute phase proteins (fibrinogen, C-

reactive protein) involved in a number of disorders [66] including MS [10, 19], AD [7, 8, 23, 24, 67, 68] and idiopathic parkinsonism [21, 22, 69] which can lead to long-term neurologic deficits. In addition, *Hp*-induced vacuolating A (VacA) cytotoxin exhibits chemotactic activities to the bone marrow-derived mast cells (BMD-MCs) and induces BMD-MCs to produce pro-inflammatory cytokines including TNF-α [70]; BMD-MCs reside adjacent to blood and lymphatic channels, mainly under epithelial surfaces including the BBB and GIT [71]. *Hp* stimulates MCs directly or *via* gastrin induction and MCs are actively involved in the pathogenesis of *Hp*-associated pathologies [66]. Apart from activated MCs, vascular endothelial growth factor (VEGF), IL-8, chymase or tryptase (a serine endoprotease released by mast cells) and mast cell growth factor linked to *Hp* infection [71, 72], MCs themselves can be stimulated by corticotropin-releasing hormone, secreted under stress, to release mediators including histamine, IL-8, tryptase and VEGF, which disrupt the BBB [72].

New findings provide an important suggestion as to why *Hp* causes a broad spectrum of diseases [7-10, 18, 21-24, 57, 66-69]. *Hp*-induced VacA cytotoxin promotes intracellular survival of the bacterium, modulates host immune responses and induces autophagy [73, 74]. Consequently, *Hp* as intracellular microorganism invades and replicates in the cells. The autophagy induction by *Hp* is not only found in macrophages, but also in DCs and gastric epithelial cells. The bacterium's residence inside infected cells will increase its resistance to antimicrobial treatment, avoid neutralization by anti-*Hp* antibodies, impair antigen presentation, and alter the cellular immune response [75]. In turn, the potential influx of activated monocytes infected with *Hp* through the disrupted BBB in the brain might lead to microglia activation and the development of brain pathologies. In this regard, an infection-based animal model initially demonstrates that, following intranasal inoculation of BALB/c mice with *C. pneumoniae*, amyloid plaques/deposits consistent with those observed in the AD brain develop, thereby implicating this infection in the etiology of AD [19, 20]; an alternative route of entry for *C. pneumoniae* into the CNS is through the olfactory pathways. Because *C. pneumoniae* readily infects epithelial cells and has direct access to the olfactory neuroepithelium of the nasal olfactory system, this route of infection would be likely given that *C. pneumoniae* is a respiratory pathogen. Moreover, the influx of activated monocytes infected with *C. pneumoniae* through the BBB could have disastrous consequences in the brain; as perivascular macrophages, pericytes, microglia, and astroglia have been shown to be infected with *C. pneumoniae* in the AD brain, this infection could account for a significant proportion of neuroinflammation and underlying pathology in the brain [20]. Therefore, as in the case of *C. pneumoniae*, the influx of the mentioned activated monocytes infected with *Hp,* due to defective autophagy resulting in *Hp* replication in autophagic vesicles [75], through the disrupted BBB in the brain might lead to microglia activation and other cells including astrocytes and DCs, thereby resulting in the development of degenerative diseases. Furthermore, as in the case of the olfactory route of entry for *C. pneumoniae* into the CNS, recent studies showed that from the oral cavity, a permanent reservoir of *Hp* [76], *Hp* may reach, through the nasal cavity, the anterior surface of the eye, causing blepharitis or worsening it, at least on the basis of cytological criteria [77]. Therefore, this bacterium through oral-nasal-olfactory pathway, might also access brain, thereby leading to the development of degenerative diseases. However, further studies are needed to elucidate these fields (Fig. **2**).

Infectious stimuli may also participate in the development of autoimmunity by inducing an increased expression of hsps, chaperones, and transplantation antigens, which results in abnormal processing and presentation of self antigens. Specifically, superantigens appear to be one of the most effective bacterial components to induce inflammatory reactions and to take part in the development and course of autoimmune mechanisms; defective immune system is associated with a higher risk of a development of autoimmune disease, including MS, associated with *Hp* infection [10, 18, 78]. Remarkably, current infections such as *Hp* infection induce humoral and cellular immune responses that, owing to the sharing of homologous epitopes (molecular mimicry), cross-react with components of CNS thereby contributing and possibly perpetuating neural tissue damage in neurodegenerative disorders [10, 18, 78, 79]. Interestingly, molecular mimicry of host structures by the saccharide portion of LPS of the GIT pathogens *Campylobacter jejuni* (*C. jejuni*) and *Hp* are thought to be connected with the development of autoimmune sequelae observed in inflammatory autoimmune neuropathies such as Guillain-Barré syndrome [80-82]. Moreover the implications of infections have been noticed in other degenerative neuropathies, such as glaucoma (defined as 'ocular AD'), supported by observations indicating that the titer of anti-*Hp* IgG antibodies in the aqueous humor of patients with glaucoma may reflect the severity of glaucomatous damage [63, 83, 84]. Specific antibodies are found in increased levels in glaucoma patients' sera, and when these antibodies access the brain due to blood-ocular barrier disruption, they are capable of killing retinal cells, thereby contributing to glaucoma pathologies [84].

Gut Microbial Ecosystem

Hippocrates (460-377 B.C.), father of Medicine, very wisely remarked: "all the diseases begin in the gut" and "death sits in the bowel" creating the hypothesis that the GIT contributed not only in the human nutrition but it is also responsible for many other biological and immunological activities. As early as the beginning of the 20th century, Metchnikoff proposed that bacteria contribute to various disease processes [85] and that

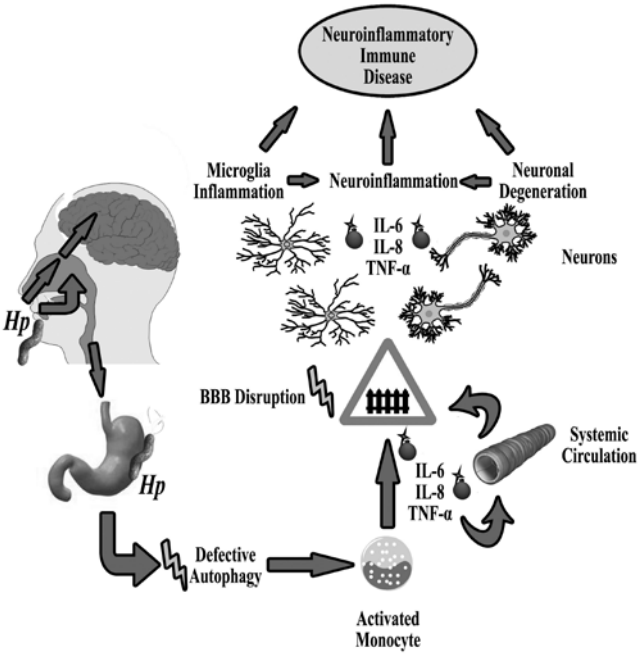

Fig. (2). Proposed mechanisms of neuroinflammation from gastrointestinal tract (GIT) pathogens including *Helicobacter pylori* (*Hp*) current infection. The pathogens may access the central nervous system (CNS) through the blood, the nasal olfactory pathways and the GIT. Neuroinflammation might initiate from the periphery, and systemic conditions, through disrupted blood-brain barrier (BBB) powerfully influence various brain pathologies. The potential influx of activated monocytes infected with *Hp,* due to defective autophagy, through the disrupted BBB in the brain might lead to activation of microglia, which, in turn, triggers neurons to induce neuroinflammation. (, activation/induction; , defective; , increased; IL, interleukin; TNF, tumor necrosis factor).

modification of microbiota composition through consumption of viable microbes might help to improve health and longevity [26, 34, 85].

Except for the appreciated fundamental concept that GIT plays an important role in motility, secretion and nutrition [86], it is the most complex part of the immune system of the human body being continually in a status of controlling inflammation. It is essential and necessary for the proper development of the mucosal and systemic immune system and maturation of immunoregulatory pathways [26-32].

GIS is represented by the commensal flora, the mucosal barrier (epithelial layer), and the gut-associated lymphoid tissues (GALT). Commensal gut microbiota represent a major modulator of immune homeostasis; they can either have an immuno-regulatory effect both in the local and systemic immune responses creating an immune status that is refractory to inflammation, or conversely, act as an adjuvant. Colonization of the GIT, begins at birth, continues in early development and remains throughout life; it is influenced by genetics and postnatal environmental exposure [87, 88]. The GALT is the largest pool of immunocompetent cells in the human body and needs to be hyporesponsive to commensal and dietary antigens while possessing the capacity to detect and attack pathogens. Relative data suggest that DCs play integral roles in managing this paradoxical condition and maintaining the gut complex homeostasis, which includes the induction of IgA synthesis [89]. During infection by enteric pathogens, the GALT plays a critical role in controlling infection by stimulating the production of pathogen-specific Igs or the expansion of cytotoxic and helper T lymphocytes [90]. In this regard, intraluminal bacterial antigens elicit specific responses in the GALT through the binding capacity of these antigens to epithelial cells, which allows antigen entry through enterocytes and aids in evading the tolerance function in Peyer's patches (PP). Such tonic immune responses in the GALT might allow control of the metabolic activity and balance of the gut microbial species [91].

The intestinal mucosa is a cellular barrier and the main location of interaction with foreign substances and exogenous microorganisms; it participates in the defense of the host through mucosal blood flow, mucosal secretions, epithelial cell functions, surface hydrophobicity, and defensin's production [92]. Specifically, the epithelial cells are equipped to phagocytose bacteria, sequester and neutralize toxins, detect prokaryocytic-associated molecular patterns, and respond by bolstering secreted defense, initiating wound repair and activating underlying innate and adaptive immunity. Epithelial crosstalk with underlying immunity is crucial in the regulation of the nature of the response to microbial and toxic stimuli [93]; interactions between the intestinal epithelium and the mucosa-associated immune system are critical for immunological functions [92, 94]. Remarkably, the GIT mucosa surface is the largest and primary and most complex immune organ in the body [95]; it consists of more than 10^6 lymphocytes/ g tissue and of 70-80% of all Ig-producing cells; and it produces more secretory IgA than the total production of Ig in the body.

Fed antigen passes from the intestinal lumen either *via* the villus epithelium and M (microfold) cells in the PP or the mucosal lamina propria to the PP and mesenteric lymph nodes or directly to peripheral lymphoid organs. Each of these sites contains distinctive populations of antigen-presenting cells (APCs) and has unique local microenvironments that may influence the immune response in different ways; Fed antigen can also exert systemic effects [96]. It has been proposed that antigens in high doses may induce clonal anergy, deletion, or altered differentiation because they gains direct access to resting APCs in the T cell areas of both the GALT and peripheral lymphoid organs, with presentation occurring in the absence of productive costimulation. Paneth cells residing at the base of the crypts fulfill a crucial role in innate immunity since they produce high quantities of defensins and several other antibiotic peptides and proteins; most defensins are potent antibiotics, and some have chemotactic and toxin-neutralizing activities [97, 98]. Populated with seemingly immature lymphoid cells and DCs, it has been suggested that cryptopatches maturate intraepithelial lymphocytes, Th17 cells, IL-22-producing NKp46+ cells, and lymphoid tissues in response to the gut microbiota [99-101]. Furthermore lamina propria has been identified as a site constitutively inhabited by Th17 cells which play a cardinal role in many CNS disorders characterized by neuroinflammation [102].

Gut Microbiota in Developmental and Maintenance Immune System Disorders

The fact that GIT microbiota influence the systemic immune system is strongly supported by Gustafsson's experiments comparing specific germ-free and normal mice and rats showing the influence of intestinal flora on the maturation and development of local and systemic immunity. Germ-free mice with no commensal gut microbiota exhibit an undeveloped immune system [103]. Additionally, recent studies revealed that these animals are characterized by having low densities of lymphoid cells in the gut mucosa, a reduced size of specialized follicle structures, and low concentrations of Igs in the peripheral blood [104-106]. Microbiota can alter the behavior causing stress-induced memory dysfunction in mice [107]; the existence or absence of conventional intestinal microbiota influences the behavior development, and is associated with brain neurochemical changes [38-40, 108]; behavior of germ-free mice differs from that of colonized mice; and adult germ-free mice show an exaggerated stress response. Germ-free mice showed no difference in basal stress hormones but showed increased plasma corticosterone and adrenocorticotrophic hormone levels in response to restraint stress [109].

Discrimination between beneficial commensal bacteria, harmless antigens, and pathogenic microorganisms is a fundamental issue in the role that GIT immune cells play in maintaining the balance between immune response and tolerance. Under the conditions of disturbed microflora homeostasis, impaired mucosal permeability, and immunocom-promisation, microbial translocation is pathologically increased, thereby eliciting systemic inflammatory responses which play an important role in the eventual outcome towards multiple-organ involvement including the brain [110]. The microorganism itself can initiate an immune response in GIT and molecular mimicry has been proposed as an explanation for autoimmune side effects/disorders of microorganism infections including MS [60].

Gut Microbiota Implication in the Immuno-pathogenesis, Immunoregulation and Prevention of EAE (Experimental Data)

Regarding the implication of gut in MS etiology, an autoimmune mechanism is also suspected based on significant analogies with EAE. To induce EAE in animals, both an immunogen and an adjuvant must be injected at the same time. By using immunogens, it is suggested that a microorganism responsible for autoimmune activity in MS could be a normally occurring gut bacterium. Moreover, adjuvant molecules are normally absent in human body fluids or tissue, except the gut [60]. Apparently, the two groups of substances, potential immunogens (mimics) and adjuvant molecules, known to be required for an autoimmune response are normally found in the human gut. The cell wall material of the microorganism responsible for a secondary infection is a source of the adjuvant. Although the adjuvant itself cannot cross the gut, the adjuvant–immunogen complex can probably cross the gut barrier, resulting in EAE development. Besides, many potential encephalitogens reside within bacteria and viral cells; the most probable source of these mimics is the normal gut. In addition, bacteria and viruses are powerful inflammatory cytokine stimulators and activators of the complement pathway [110, 111]. They affect vascular permeability; generate nitric oxide (contributing to the apoptotic neuronal cell

death in degenerative disorders); and induce proteoglycan synthesis and events leading to the pathological and biological hallmarks of neuroinflammation. Incoming bacterial signals include secreted chemoattractants, flagellin, bacterial nucleic acids, and cellular constituents such as LPS and peptidoglycans. Specifically, the mentioned bacterial inflammatory surface molecule LPS, a bacterial endotoxin, is a powerful inflammatory factor of Gram-negative bacteria. LPS and peptidoglycan are highly resistant to degradation by mammalian enzymes and thus may provide a persisting inflammatory stimulus [112]. In this regard, substantial demyelination results from the focal inflammatory lesion caused by the LPS injection directly into the rat dorsal funiculus, signifying that LPS-induced demyelination may serve as experimental model available for the study of MS [113]. At molecular level, both LPS-induced NF-kappaB activation and endogenous production of IFN-β that consequently induces STAT-1alpha activation play crucial roles in the transcriptional activation of the CD40 gene, expressed on macrophages and microglia, by LPS; aberrant CD40 expression is associated with autoimmune inflammatory diseases including MS [114].

Microbial of symbiotic or infectious colonization affects autoimmunity and impacts the balance between pro- and anti-inflammatory immune responses during EAE; filamentous bacteria promote IL-17 production in the gut and induce IL-17A-producing CD4+ T cells (Th17) in the CNS [43]. Furthermore, gut flora influences the development of EAE in a way that is dependent on NK T cells, which may have significant implications for the prevention and treatment of autoimmune diseases [41, 42].

The harmless microorganisms of GIT lead to immunoregulation through the maturation of DCs and Tregs, resulting in a constant background of bystander suppression. The prominent human commensal, *Bacteroides fragilis* (*B. fragilis*), directs the development of Foxp3+ Tregs with a unique "inducible" genetic signature; *B. fragilis* co-opts the Treg lineage differentiation pathway in the gut to actively induce mucosal tolerance [115]. This effect requires the innate immune system and suggests that in an environment which primes Treg activity less actively, immunoregulatory disorders will occur first in those individuals whose innate immune systems are less efficient at driving Tregs [116, 117]. Furthermore intestinal CD103+ DCs are important regulators of the immune response driving the induction of the chemokine receptor CCR9 and alpha(4)beta(7) integrin, both known as gut-homing receptors, and contribute to control inflammatory responses and intestinal homeostasis by promoting the modification of naïve T cells into induced Foxp3+ Tregs a mechanism that relies on transforming growth factor-beta and retinoic acid signaling [118]. Importantly, as autoreactive T cells are present in the blood of MS patients, other regulatory mechanisms exist to prevent autoreactive T cells from causing immune disorders. In this respect, the function of Tregs which play a key role in the control of self-antigen-reactive T cells and the induction of peripheral tolerance may be diminished in MS and may be correlated with impaired inhibitory activity [119].

The increased tendency of immune-mediated disorders in western countries may indicate that environmental factors do not prime Treg activity efficiently. This might precipitate immunoregulatory disorders more frequently in those individuals whose innate immune systems have genetic polymorphisms that further reduce the efficiency of Tregs induction. The mentioned *B. fragilis* of gut microflora producing a bacterial capsular polysaccharide antigen can affect a population of Foxp3+ Tregs that regulate demyelination and protect against EAE; experimental data suggest a significant role for commensal bacterial antigens, in particular *B. fragilis* expressing polysaccharide A, in protecting against CNS demyelination in EAE and possibly human MS [120]. Furthermore, antibiotic modification of gut commensal bacteria can modulate peripheral immune tolerance that can protect against EAE, thereby representing a new approach in the treatment of MS and possibly other autoimmune conditions [44]. Specifically, the commensal microflora reduction prevents the development of EAE in mice. When animals are recolonized with commensal bacteria, EAE clinical manifestations are reportable, whereas long-term control of bacterial populations with oral antibiotic treatment confers complete protection against EAE. These data do not reflect a direct neuroprotective effect of antibiotics. Protection might be caused by a nonspecific bystander effect of Foxp3+ Treg cells and a reduction in the global levels of proinflammatory responses due to the reduced presence of bacterial populations and/or their products [121]. Finally, probiotics exert their therapeutic effects mediated by IL-10 producing Tregs effect on EAE and perhaps on MS [44, 120, 121]. It seems, therefore, that alteration on gut flora could trigger or prevent the development of autoimmune conditions.

Summarizing all the aforementioned data, the repertoire of the CNS immune system is greatly regulated by GIS, enhancing our understanding of the pathogenesis of neuroinflammatory autoimmune diseases, and thus it could have a major impact on future therapeutic strategies.

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Letter to the Editor

Impact of *Helicobacter pylori* on chronic hepatitis C-related cognitive dysfunction

We read with considerable interest the paper by Hilsabeck et al. (2010), who concluded that "patients with chronic hepatitis C (CHC) with immune responses characterized by elevated interferon (IFN)-α may be at risk for cognitive difficulties; in patients with detectable IFN-α, higher levels of interleukin (IL)-6 and tumor necrosis factor (TNF)-α were related to poorer cognitive functioning". Discussing their results, the authors claimed that their "findings provide preliminary support for the hypothesis that indirect rather than direct effects of IFN-α may be associated with cognitive difficulties in CHC patients not undergoing antiviral therapy".

The association between *Helicobacter pylori* infection (*Hp*-I) and CHC and/or liver cirrhosis has been recognized in different parts of the world (Ponzetto et al., 2003; Rocha et al., 2005; Queiroz et al., 2006; El-Masry et al., 2010). The high seroprevalence of antibodies to *Hp* in CHC patients (Ponzetto et al., 2003) and the detection of *Hp* and *H. pullorum* DNA in the liver tissue of patients with CHC and hepatocellular carcinoma (HCC) suggest that these bacteria could be implicated in the progression of CHC to cirrhosis and HCC (Ponzetto et al., 2000; Dore et al., 2002); *Hp* species appear to be a co-risk factor in hepatitis C virus (HCV) chronic liver diseases (Rocha et al., 2005). Noticeably, HCV is only capable of limited inflammation, whereas *Hp* species are strong inducers of the inflammatory cascade (Crabtree 1995) with various proinflammatory cytokines involved, leading to the accumulation of an extraordinary number of lymphocytes and polymorphonuclear cells in the infected tissue (El-Omar et al., 2000). Several *Hp* species could also secrete a liver-specific toxin that causes hepatocyte necrosis in cell culture and might therefore be involved in damaging liver parenchyma *in vivo* (Meyer-ter-Vehn, et al., 2000).

Moreover, *Hp*-I has been implicated in extradigestive vascular conditions caused by vascular dysregulation, frequently detected neurodegenerative diseases including mild cognitive impairment (MCI), Alzheimer's disease (AD) and glaucoma (Kountouras et al., 2006; Kountouras et al., 2007; Kountouras, 2009; Kountouras et al., 2009a); in the latter studies the diagnosis of frequently involved *Hp*-I was based on histology, recognized as the practical gold standard for the diagnosis of current *Hp*-I. Although the serological test 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 (Deretzi et al., 2009), thereby contributing and possibly perpetuating neural tissue damage. Moreover, eradication of *Hp*-I might delay AD progression, particularly at early disease stages, including MCI. In this respect, we found that *Hp* eradication may positively influence AD manifestations at two and five-year clinical endpoints, 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 (Kountouras et al., 2009b; Kountouras et al. 2009c; Kountouras et al., 2010).

Taking together the aforementioned data, *Hp* may be involved in the pathophysiology of CHC-related cognitive dysfunction by several mechanisms including the release of large amounts of proinflammatory and vasoactive substances, such as IL -6, -8, and TNF-α, mentioned by the authors, or eicosanoids (leukotrienes, prostaglandins catalyzed by cyclo-oxygenase enzymes), and acute phase proteins (fibrinogen, C-reactive protein) (Kountouras et al., 2001; Kountouras et al., 2003a) involved in a number of vascular disorders including AD which can lead to long-term neurologic deficits. Specifically, a series of factors have been implicated in inducing blood brain barrier (BBB) disruption, mentioned by the authors, including inflammatory mediators (e.g., cytokines and chemokines induced by *Hp*-I) and oxidative stress (Kountouras et al., 2008; Keep et al., 2008). *Hp* could indirectly affect the brain and other target organs, e.g. the heart, through the release of numerous cytokines such as TNF-α acting at a distance; TNF-α is involved in BBB disruption through a mechanism involving matrix metalloproteinases upregulation (Candelario-Jalil et al., 2007). TNF-α and IL-6 (TNF-α is the main trigger for the production of IL-6 by a variety of cells) play important roles in the regulation of the synthesis of other acute phase proteins which are established risk factors for atherosclerosis, such as fibrinogen and factor VIII. These cytokines also have profound effects on lipid metabolism directly at the site of the atherosclerotic lesion, but could influence the atheromatous process through blood circulating levels, distant production of cytokines, or through stimulating circulating white blood cells to produce them, thereby contributing to BBB disruption and to the pathogenesis of heart and brain neurodegenerative diseases (Guerreiro et al., 2007; Tan et al., 2007; Kountouras et al., 2009a). In addition, *Hp*-induced cytotoxin VacA exhibits chemotactic activities to the bone marrow-derived mast cells (BMDMCs) and induces BMDMCs to produce proinflammatory cytokines including TNF-α (Supajatura et al., 2002); BMDMCs reside adjacent to blood and lymphatic channels, mainly under epithelial surfaces including the BBB and gastrointestinal tract (Kountouras et al., 2009d). Apart from activated mast cells, vascular endothelial growth factor (VEGF), IL-8, chymase or tryptase (a serine endoprotease released by mast cells) and mast cell growth factor linked to *Hp*-I (Kountouras et al., 2009d; Kountouras et al., 2004), mast cells themselves can be stimulated by corticotropin-releasing hormone, secreted under stress, to release mediators including histamine, IL-8, tryptase and vascular endothelial growth factor (VEGF), which disrupt the BBB (Theoharides et al., 2008). BBB disruption, in turn, could play an important role in promoting entry of immune cell infiltration and pathogens into the brain resulting in the development of brain pathologies (Itzhaki et al., 2004).

Apart from cytokine network, *Hp* may also be involved in the pathophysiology of CHC-related cognitive decline by: 1. Promoting platelet and platelet-leukocyte aggregation (Kountouras et al., 2007; Kountouras et al., 2009b). Platelet activation and aggregation have also been proposed to play pathophysiologic roles in the development and deterioration of AD (Sevush et al., 1998; Casoli et al., 2008).

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Patients with CHC also have increased platelet activation/aggregation, and liver fibrosis may play a role in the activation of platelets in CHC (Fusegawa et al., 2002). 2. Inducing chronic atrophic gastritis with concomitant decrease in vitamin B12 and folate concentrations, thereby increasing homocysteine (Hcy); the elevated Hcy, in turn, could trigger endothelial damage and result in atherothrombotic disorders and AD. In CHC, a high Hcy level is also associated with a high degree of steatosis and fibrosis (Borgia et al., 2009). 3. Stimulating mononuclear cells to produce a tissue factor-like procoagulant that converts fibrinogen into fibrin (Kountouras et al., 2002); fibrin is a mediator of inflammation and may accelerate neurovascular damage in AD (Paul et al., 2007). CHC patients with thrombosis also have high values of fragment F1 + 2, a marker of thrombin, and moreover fibrin granulomas have been observed in CHC (Violi et al., 1995; Glazer et al., 2007). 4. Producing reactive oxygen metabolites (ROMs) and circulating lipid peroxides (Kountouras et al., 2007; Kountouras et al., 2009b), that have also been involved in the pathophysiology and prognosis of AD (Pulido et al., 2005; Mangialasche et al., 2009); oxidative/nitrosative stress plays a potential role in the prognosis of the disease (Mangialasche et al., 2009). The increased generation of ROMs with the decreased antioxidant defense, also promote the development and progression of hepatic and extrahepatic complications of CHC (Choi and Ou, 2006). 5. Influencing the apoptotic process that may also be an important form of cell death in many neurodegenerative diseases, including AD (Kountouras et al., 2007; Kountouras et al., 2009a); apoptotic process contributes to the damage of brain cells and progression of the disease (Hoffmann et al., 2009). Apoptosis of liver cells may also play a significant role in the pathogenesis of CHC (Kountouras et al., 2003b).

It is therefore important to know if the authors have considered the association of *Hp*-I with CHC and if eradication of this infection might benefit cognitive function in their CHC patients.

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LETTER TO THE EDITOR

Association between *Helicobacter pylori* infection and fibromyalgia syndrome

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Dear Editor,

In the paper by Akkaya N, et al., *Helicobacter pylori* (*H. pylori*) infection was evaluated in 65 patients with fibromyalgia syndrome (FM) and in 41 healthy controls, using two different serological methods [1]. In particular, the authors used anti-*H. pylori* IgA antibodies, a serological test with unacceptably low sensitivity (63.4%) and specificity (67.6%), yielding a poor overall accuracy of 67.2% in all ages [2]. Furthermore, it was demonstrated that this test possesses an even lower overall accuracy rate in the ages over 18 years (51.8%) [2], thereby rendering it practically useless and a waste of time and resources for the patients evaluated in the study by Akkaya N, et al. [1]. Therefore, this comes to no surprise why the authors found no significant difference in the prevalence of *H. pylori* IgA antibodies between their FM patients and controls (p=0.169) [1]. It would be interesting to know why the authors chose this particular test for their study in spite of its poor performance shown before.

On another aspect, the authors reported that they found no statistically significant differences regarding mainly the clinical features (sleep disturbance, fatigue, stiffness, headache, subjective soft-tissue swelling, paresthesia, dysmenorrhea, female urethral syndrome, irritable bowel syndrome, and anxiety), anxiety and depression subscale of Fibromyalgia Impact Questionnaire, history of depression, and previous treatment for depression between the two study groups (FM patients and controls) [1]. We believe that this is a paradox, since one would expect that FM patients with a higher prevalence of *H. pylori* infection (according to the *H. pylori* IgG serology) should present with more profound clinical features compared with a group of healthy controls. This paradox, however, can be explained by the fact that although this serological test establishes the presence of previous *H. pylori* infection, it does not discriminate between current and old infections. Such a distinction is crucial because only current *H. pylori* infection induces both humoral and cellular immune responses that cross-react with host components, owing to the sharing of homologous epitopes (molecular mimicry), thereby contributing and/or perpetuating tissue damage [3], and possibly, the development of the clinical features of FM; immunological (cellular/humoral) responses are increasingly recognized as being essential in the initiation and progression of FM syndrome [4]. Moreover, eradicating *H. pylori* infection might delay the progression of this disease, particularly at earlier stages. Thus, it would be interesting to know why the authors chose a serological IgG test, instead of histology, the practical gold standard for the diagnosis of current *H. pylori* infection, or at least the ^{13}C-urease breath test, the noninvasive test of choice for *H. pylori* infection. Although the latter test 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 [5], this is considered a reliable alternative to the histologic method for the detection of *H. pylori* infection. By using these two methods signifying active *H. pylori* infection, the authors

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might have found significant differences regarding the clinical features of FM between their two study groups.

Disclosures None.

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Letters to the Editor

Helicobacter pylori may play an important role in both axonal type Guillain–Barré syndrome and acute inflammatory demyelinating polyradiculoneuropathy

Dear Editor,

In their paper, Ghabaee et al. [1] reported a relationship between *Helicobacter pylori* infection (*Hp*-I) and axonal damage rather than demyelination, which is to some extent against our study that proposed a possible association between *Hp*-I and acute inflammatory demyelinating polyradiculoneuropathy (AIDP) [2]. However, serum and cerebrospinal fluid anti-*Hp* IgG titers were significantly higher in their patients, consisting mainly of AIDP [10/15(67%)] patients and only 2 (13%) patients with acute motor axonal neuropathy (AMAN), than in controls. Moreover, the authors claimed that anti-*Hp* IgG may have an important role in Guillain–Barré syndrome; damage and leaky blood–nerve barrier (BNB) allows entry of these antibodies into endoneural space, and, through molecular mimicry, immune reactions occur between these antibodies and myelin or axon epitopes, thereby resulting not only in axonal damage, as the authors commented, but also in demyelination.

BNB damage has been increasingly implicated in inflammatory demyelinating neuropathies (IDNs) [3], and a variety of proinflammatory cytokines are instrumental in the course of IDNs. They increase vascular permeability and BNB disruption (tumor necrosis factor (TNF)-alpha, vascular endothelial growth factor, vascular permeability factor); induce transmigration of leukocytes into the nerve, activation and proliferation of macrophages (interferon-gamma) and T-cells (interleukins-1 and -2); and exert direct myelinotoxic activities (TNF-alpha and -beta) [4]. Besides, mast cells are located in close proximity to neurons in the peripheral and central nervous systems, signifying their role in normal and aberrant neurodegenerative conditions [5].

Hp-I, by releasing several of the aforementioned inflammatory mediators, could induce BNB/BBB breakdown, thereby possibly being involved in the pathogenesis of neuropathies including both AMAN and AIDP [6–9]. For instance, *Hp* could indirectly affect the peripheral and central nervous systems, through the release of numerous cytokines such as TNF-alpha acting at distance; TNF-alpha is involved in BBB/BNB disruption through matrix metalloproteinases upregulation [6,7]. Moreover, *Hp*-induced vacuolating cytotoxin A exhibits chemotactic activities to the bone marrow-derived mast cells (BMD-MCs) and induces BMD-MCs to produce proinflammatory cytokines involved in the BBB/BNB disruption [6,7]. Therefore, *Hp*-induced BBB/BNB breakdown might promote entry of immune cell infiltrations (autoreactive effector CD4+ and CD8+ T-cells) and *Hp* circulating antibodies into the peripheral and central neurons resulting in the development of neuronal pathologies [6,7,10–12] including AMAN and AIDP.

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LETTERS TO THE EDITOR

Readers are encouraged to write letters to the editor concerning articles that have been published in CLINICAL GASTROENTEROLOGY AND HEPATOLOGY. Short, general comments are also considered, but use of the Letters to the Editor section for publication of original data in preliminary form is not encouraged. Letters should be typewritten and submitted electronically to http://www.editorialmanager.com/cgh.

Helicobacter pylori Might Contribute to Persistent Cognitive Impairment After Resolution of Overt Hepatic Encephalopathy

Dear Editor:

We have read with interest the report by Riggio et al[1] who concluded that hepatic encephalopathy (HE) is not a fully reversible condition, and the mechanism behind the lack of reversibility of the neurocognitive status despite the resolution of mental status changes is unclear. In this regard, recent data suggest that chronic hepatitis C patients, including cirrhotic patients with detectable interferon-α, and higher levels of interleukin-6 and tumor necrosis factor–α, might be at risk for poorer cognitive function.[2]

Hepatitis B and hepatitis C infections are among the most common causes of liver cirrhosis worldwide, and *Helicobacter pylori* infection is strongly associated with hepatitis B- and hepatitis C-related cirrhosis in Italy; *H. pylori* infection is more common in cirrhotic patients with HE than in those without. Moreover, *H. pylori* infection has been frequently detected in neurodegenerative diseases, including mild cognitive impairment and Alzheimer's disease (AD).[3] In this respect, we found that *H. pylori* eradication may positively influence AD manifestations at 2- and 5-year clinical end points,[3] thereby supporting a role for this common infection in the pathobiology of the disease.

Summarizing the aforementioned data, *H. pylori* may be involved in the pathophysiology of the post-HE persistent cognitive impairment by several mechanisms,[3] such as the release of proinflammatory and vasoactive substances, ie, interleukin-6, -8, and tumor necrosis factor–α, involved, through blood-brain barrier disruption, in a number of vascular disorders including AD, which can lead to long-term neurologic deficits.[3] It is therefore important to know if the authors have considered the association between *H. pylori* infection and post-HE cognitive impairment in their cirrhotic patients.

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Conflicts of interest

The authors disclose no conflicts.

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Reply. We did not assess the presence of *Helicobacter pylori* infection in the patients included in the study because, at least from an epidemiological point of view, the role of *H. pylori* as an independent factor for hepatic encephalopathy (HE) is still controversial. In fact, HE prevalence did not significantly differ between cirrhotics with and without infection.[1] Moreover, some prospective studies have documented that mental status in HE cirrhotics was not significantly affected by *H. pylori* eradication.[2,3] Finally, the prevalence of *H. pylori* has been reported to be considerably high, even in cirrhotic patients without HE.[1] Until now, ammonia is supposed to play the major role in the relationship between *H. pylori* and HE, but other mechanisms may be possible. On the other hand, the mechanism behind the lack of reversibility of the neurocognitive impairment, despite the resolution of mental status changes in cirrhotic patients with previous episodes of HE, is unclear. It is also interesting to note that, despite therapy with ammonia-lowering strategies, these deficits can persist.[4] This evidence highlights the possibility that mechanisms different from ammonia may be implicated, and indeed, the role of inflammatory cytokines has been recently proposed among the possible factors involved in the pathogenesis of HE.[5,6] Therefore, the hypothesis proposed by Kountouras et al, that *H. pylori* may be involved in the pathophysiology of the post-HE persistent cognitive impairment through the release of proinflammatory and vasoactive substances (IL-6, IL-8, and TNF-α), is reasonable. Given the similar prevalence of *H. pylori* infection between patients with or without HE a similar alteration in inflammatory cytokines and learning ability should be expected in patients with and without previous HE. This was not the case in our study. Nevertheless, we do not have any way to possibly test this interesting hypothesis in our patients, and studies specifically directed at establishing the relationship between *H. pylori*, inflammatory cytokines, and HE are needed.

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Conflicts of interest

The authors disclose no conflicts.

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Correction

Berman K, Tandra S, Forssell K, et al. Incidence and predictors of 30-day readmission among patients hospitalized for advanced liver disease. Clin Gastroenterol Hepatol 2011;9:254–259.

The name of the fourth author, Raj Vuppalanchi, was incorrectly spelled as Raj Vuppalanch. This error has been corrected on the *Clinical Gastroenterology and Hepatology* website.

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Correspondence

Helicobacter pylori may be a common denominator associated with systemic and multiple sclerosis

Keywords:
Helicobacter pylori
Multiple sclerosis
Systemic sclerosis

Dear Editor,

In their paper, Radić et al. [1] concluded that *Helicobacter pylori* infection (*Hp*-I) might play a critical role in the pathogenesis of systemic sclerosis (SSc) whereas it may protect against the development of other autoimmune disorders including multiple sclerosis (MS). However, the latter conclusion might be incomplete, because, for instance, some authors initially concluded that *Hp*-I is a potentially protective factor against conventional MS in Japanese population, which contradicts their latest conclusion that *Hp*-I seems to be one of the risk factors for the development of anti-aquaporin-4(+) MS [2].

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 [3]. Moreover, by using the same diagnostic method, we also detected *Hp*-I in 87% of patients with primary or secondary Raynaud's phenomenon [4]. In addition, *Hp* eradication was associated with a significant improvement in both clinical and laboratory indices in 75% of patients who completed the study [4]. Comparable clinical benefit was also noticed in the studied reported by Radić et al. [1]. Although the serological test reported in the relative studies by Radić et al. and employed 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 only current *Hp*-I induces humoral and cellular immune responses that, owing to the sharing of homologous epitopes (molecular mimicry) mentioned by the authors, cross-react with host components, thereby contributing and possibly perpetuating neural and/or connective tissue damage [1,5,6]. Moreover, eradicating *Hp*-I may alter SSc and MS pathophysiology. Importantly, the coexistence of SSc and MS even in the same patient has been described [7], thereby suggesting the existence of a possible common denominator predisposing to the development of both diseases.

Although the above-mentioned data do not establish causality, because, apart from the other Koch and Hill's causation criteria, this requires showing that eradication of *Hp*-I alters the course of SSc and MS and the other relative autoimmune diseases, however, *Hp*-I may influence the pathophysiology of SSc and MS by:

- promoting platelet aggregation and activation also proposed to play pathophysiologic roles in SSc mentioned by the authors and MS [1,8];
- releasing proinflammatory and vasoactive substances such as cytokines (interleukin [IL]-1, -6, -8, -10, -12, tumor necrosis factor-α, interferon-γ), endothelin-1 or eicosanoids (leukotrienes, prostaglandins catalyzed by cyclo-oxygenase enzymes), involved in a number of vascular disorders including SSc and MS [8,9]; the Th2 cytokine response leads to tissue fibrosis, whereas Th1 and Th17 cytokines promote inflammation in SSc patients [9];
- stimulating mononuclear cells to produce a tissue factor-like procoagulant that converts fibrinogen into fibrin [8];
- causing the development of cross mimicry between endothelial and *H. pylori* antigens [1,8];
- producing reactive oxygen metabolites and circulating lipid peroxides also involved in the pathophysiology of SSc and MS [8,10];
- influencing the apoptotic process, an important form of cell death in many autoimmune diseases, including SSc and MS [8,10].

Conflict of interest statement

None of the authors has any conflicts of interest to declare.

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Reply to comment by Kountouras et al. about article untitled "*Helicobacter pylori* infection and systemic sclerosis – is there a link? by Radić et al."

Keywords:
Systemic sclerosis
Multiple sclerosis
Helicobacter pylori
Pathogenesis

Sir,

In their letter, Kountouras et al. raise some queries about possible role of *Helicobacter pylori* (*H. pylori*) infection in some autoimmune disorders such as multiple sclerosis (MS) reported in our review [1]. Recent evidence suggests that *H. pylori* infections play a role in the pathogenesis of a variety of autoimmune diseases. A big controversy is whether the *H. pylori* infection is a protective factor against conventional multiple sclerosis or not [2,3]. We agree that the serological test does not discriminate current from old infections and only current *H. pylori* infection induces humoral and cellular immune responses [1,4,5]. However, we performed a study to evaluate the possible association between *H. pylori* infection with systemic sclerosis (SSc) activity, biochemical and serological data [6]. Our preliminary results suggest that *H. pylori* infection is implicated in activity of SSc, especially in skin involvement of this disease. This study may indicate *H. pylori* infection as a possible cofactor in the development of SSc. Systematic studies examining the relationship between autoimmune entities and infection with *H. pylori* and documentation of the effect of *H. pylori* eradication are needed to complete our understanding on this topic. Gavalas et al. showed a strong association between *H. pylori* infection and MS in a Greek cohort [2]. Moreover, eradication of *H. pylori* infection may alter SSc and MS pathophysiology. Numerous infectious agents have been proposed as possible triggering factors in SSc and MS but very few infections are as rare as SSc and MS [6,7]. Therefore, development of SSc and MS is unlikely to depend exclusively on an infectious agent. Instead, it likely occurs as a result of interactions between the infectious agent and a cascade of host-specific factors and events. This is not surprising because immune response to infection is highly individual. It is controlled by multiple genes, age, and the route of infection. It may even be different in the same individual from one day to the next, owing to a number of factors, including co-infections, stress, and pregnancy. In addition, polymorphisms in genes unrelated to immunity may cause an infectious agent to induce disease through molecular mimicry in one person and not another. Therefore, in a disease as varied, complex, and rare as SSc and MS, infection prevalence alone should not be expected to provide sufficient evidence for or against a pathological role in the disease. Despite intensive studies, there is no definitive evidence to conclude that SSc and MS has a viral or bacterial origin. In SSc and MS, some viral or bacterial products could synergise with other factors in the microenvironment predisposing to SSc and MS development.

Conflicts of interest statement

None of the authors has any conflicts of interest to declare.

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individuals. Therefore, there is hope that biomarkers will help to predict AIS development.

Cartilage oligomeric matrix protein (COMP) is essential for the normal development of cartilage and for its conversion to bone during growth. It is expressed at high levels during skeletal development and long bone growth. Mutations in COMP produce clinical phenotypes of pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) [2,3]. These disorders are characterized by disproportionate short stature, brachydactyly, joint hypermobility, early-onset osteoarthritis, and scoliosis [4]. Interestingly, the phenotypes in PSACH/MED cartilage disorders is not caused by the reduced amount of COMP but rather due to dysfunctional mutated COMP. Abnormal COMP protein cannot be transported out of the cell but rather builds up inside the chondrocyte and it ultimately leads to early chondrocytes death preventing normal bone growth [5]. Recently, in a microarray approach evaluating primary human osteoblasts, we found that COMP is one of the most differentially regulated genes in AIS compared to unaffected individuals [6]. COMP was found to be significantly down-regulated by 4-fold in AIS [6]. Consistent with the microarray analysis, relatively low levels of COMP mRNA transcripts in AIS were detected by RT-qPCR analysis. Altogether, these data suggest that low expression of COMP is associated with AIS. This is the first down-regulated gene described in AIS. Thus, we hypothesized COMP down-regulation would result to a low COMP serum level in AIS patients and this could be an important and novel biomarker in predicting scoliosis development.

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Helicobacter pylori might be a potential therapeutic target in epilepsy

Dear Editor,

We have read with interest the concept by Yin et al. [1] that matrix metalloproteinase (MMP)-9, which could lead to epileptogenesis by causing neuroinflammation and neuronal death, may be a novel therapeutic target for epilepsy. Specifically, increased MMP-9 in seizures could degrade the extracellular matrix molecules to break down the blood–brain barrier (BBB), thereby leading to seizures induction and epilepsy progression.

Recent data suggest a probable association between *Helicobacter pylori* infection (*Hp*-I) and epilepsy, especially with poor prognosis [2]. Specifically, a series of factors have been implicated in inducing BBB disruption, including inflammatory mediators (e.g., cytokines and chemokines induced by *Hp*-I) and oxidative stress [3]. *Hp* could indirectly affect the brain through the release of numerous cytokines such as interleukin (IL)-1, -6, or tumour necrosis factor (TNF)-α acting at a distance; TNF-α is involved in BBB disruption through a mechanism involving matrix metalloproteinases' upregulation, mentioned by the authors. TNF-α and IL-6 may contribute to BBB disruption and pathogenesis of neuronal inflammatory damage in neurodegenerative diseases and epilepsy; cytokines, including IL-1β, IL-6 and TNF-α, influence the pathogenesis and course of epilepsy, the primary BBB lesion is involved in epileptogenesis [4], and dementia is an aetiologic factor for status epilecticus. Moreover, *Hp*-induced cytotoxin VacA exhibits chemotactic activities to the bone marrow-derived mast cells (BMDMCs) and induces BMDMCs to produce proinflammatory cytokines including TNF-α which disrupt the BBB, thereby promoting entry of immune cells and pathogens into the brain resulting in neurodegeneration. Likewise, activated monocytes (possibly infected with *Hp* due to defective autophagy resulting in *Hp* replication in autophagic vesicles) might also enter the brain due to BBB disruption, thereby possibly contributing to epilepsy development and progression; Hp VacA promotes intracellular survival of the bacterium and modulates host immune responses.

Viewing the aforementioned data we can consider that *Hp*-I, by inducing proinflammatory cytokine production and BBB disruption, might influence the pathophysiology and therefore the management of epilepsy.

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None.

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Letter to the Editor

Neuroprotection in glaucoma: Is there a future role of *Helicobacter pylori* eradication?

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Dear Editor,

We read with considerable interest the paper by Baltmr A et al. (Baltmr et al., 2010) summarizing the mechanisms in initiating the apoptotic glaucomatous damage and reviewing "current potential neuroprotective strategies targeting RGCs from the laboratory to the clinic". However, the authors did not mention the role of environmental agents involved in the pathophysiology of glaucomatous neuropathy and the potential neuroprotective strategies against these agents. In this regard, although degenerative diseases, including glaucoma, have an increasingly high impact on aged population, their association with *Helicobacter pylori* (*H. pylori*) infection has only recently been addressed (Kountouras et al., 2001, 2003; Kountouras et al., 2004a; Kountouras, 2009).

A relationship between glaucoma and *H. pylori* infection appears to exist based on the following comparable data: (a) both diseases affect mainly old people; (b) *H. pylori* infection has been implicated in a variety of extradigestive vascular conditions including functional vascular disorders caused by the release of diverse vasoactive and proinflammatory substances, hypertension, arteriosclerosis-induced increased platelet activation/aggregation, ischemic heart disease, and ischemic cerebrovascular disorders, also detected in glaucoma and other neurodegenerative diseases contributing to their clinical manifestations (Kountouras et al., 2001; Kountouras et al. 2003; Kountouras et al., 2004a; Kountouras, 2009); and (c) in the nervous system, *H. pylori* infection is thought to be associated with the development of autoimmune sequelae observed in peripheral neuropathies, Alzheimer's disease (AD) and glaucomatous optic neuropathy (defined as "ocular" AD) (Kountouras et al., 2001, 2003, 2004a, 2007; Kountouras, 2009).

Based on the aforementioned data, we documented a high prevalence of *H. pylori* infection in Greek patients with glaucoma, establishing a significant relationship between *H. pylori* infection and glaucoma (Kountouras et al., 2001). It is important to note that *H. pylori* infection was confirmed by histology, the gold standard for the diagnosis of *H. pylori* infection. Reports from other ethnic populations also showed a relationship between glaucoma and *H. pylori* infection (Kountouras, 2009; Kountouras et al., 2009a), although this has not been confirmed by all the relevant studies published so far (Table 1), and thus this association may only apply to a limited sub-population of glaucoma patients. In a subsequent study we documented a beneficial effect of *H. pylori* eradication on glaucoma progression (Kountouras et al., 2002), suggesting a possible causal link between *H. pylori* and glaucoma. Moreover, we reported an increased *H. pylori*-specific IgG antibody level in the aqueous humor of glaucoma patients, the titer of which correlated with the degree of vertical cupping, possibly reflecting the glaucomatous damage severity (Kountouras et al., 2003).

It would thus be interesting to know if the authors have considered these data that appear to be important, casting light in *H. pylori* infection because it may currently be claimed that this infection might influence the pathophysiology (Izzotti et al., 2009) and therefore the management of glaucoma by: promoting platelet and platelet-leukocyte aggregation, also involved in the pathophysiology of glaucoma (Kountouras et al., 2004a; Kountouras, 2009); releasing proinflammatory and vasoactive substances, including cytokines [interleukin (IL) −1, −6, −8, −10, −12, tumor necrosis factor (TNF)-α, interferon-γ], eicosanoids (leukotrienes, prostaglandins) and acute phase proteins (fibrinogen, C-reactive protein), involved in the mentioned vascular disorders and glaucoma (Kountouras et al., 2003, 2004a; Kountouras, 2009); stimulating mononuclear cells to induce a tissue factor-like procoagulant activity that converts fibrinogen into fibrin; causing the development of cross mimicry between endothelial and *H. pylori* antigens; producing oxidative stress and circulating lipid peroxides; and influencing mainly the apoptotic process mentioned extensively by the authors, parameters that may also exert their own effects in the induction and/or glaucoma progression and other neurodegenerative disorders (Guillain-Barré syndrome, AD, Parkinson's disease), associated with both *H. pylori* infection and glaucoma (Kountouras et al., 2004a, 2008a).

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Table 1
Studies from different ethnic glaucoma populations investigating a potential relationship between *Helicobacter pylori* infection and open-angle glaucoma. Negative indicates lack of association.

Year published	Authors	Country	Outcome
2000	Kountouras et al.	Greece	Positive
2000	Öztürk et al.	Turkey	Positive
2001	Kountouras et al.	Greece	Positive
2002	Kountouras et al.	Greece	Positive
2003	Kountouras et al.	Greece	Positive
2003	Galloway et al.	Canada	Negative
2003	Jahadi et al.	Iran	Negative
2004	Roozitalab et al.	Iran	Positive
2005	Quaranta et al.	Italy	Negative
2006	Razeghinejad et al.	Iran	Positive
2007	Abrishami et al.	Iran	Positive
2007	Hong et al.	China	Positive
2008	Kurtz et al.	Israel	Negative
2008	Deshpande et al.	India	Positive
2009	Öztürk et al.	Turkey	Positive
2011	Kim et al.	Korea	Positive

Importantly, *H. pylori* infection and glaucoma share the Fas/FasL and the mitochondria-mediated apoptotic pathways, thereby proposing an apoptotic link in the pathophysiology of both diseases (Kountouras et al., 2004b). In particular, increased endothelin-1 (a potent constrictor of arterioles and venules), nitric oxide (NO) and inducible NO synthase (iNOS) levels are associated with *H. pylori* infection (Kountouras et al., 2000). Endothelin-1-induced anterior optic nerve vessels vasoconstriction and NO vascular tone modulation in the ophthalmic artery may produce glaucomatous damage. Moreover, NO, a rapidly diffusing gas, is a potent neurotoxin that may facilitate the apoptotic retinal ganglion cells death in glaucomatous optic neuropathy (Kountouras et al., 2004a). Support for the consideration of NO neurotoxicity in glaucoma is provided by experimental evidence demonstrating that retinal ganglion cell apoptosis is attenuated by neutralizing antibodies against TNF-α or by selective inhibitors of iNOS, thereby suggesting that the inhibitors of TNF-α or of the inducible isoform NOS2 may provide novel therapeutic targets for neuroprotection in the treatment of glaucomatous optic neuropathy (Kountouras et al., 2004a). In addition, systemic *H. pylori*-induced oxidative damage may be the mechanism which links oxidative stress, *H. pylori* infection and the apoptotic damage to the trabecular meshwork and optical nerve head that results in glaucoma (Izzotti et al., 2009). In this regard, oxidative stress is an essential underlying cause of neuroinflammatory and neurodegenerative diseases, including glaucoma, and blood–brain barrier (BBB) damage is connected to them; oxidative stress activates protein tyrosine kinase and matrix metalloproteinases resulting in BBB dysfunction (Haorah et al., 2007; Grieshaber and Flammer, 2007).

Specifically, a series of factors have been implicated in inducing BBB disruption, including inflammatory mediators (e.g., cytokines and chemokines induced by *H. pylori* infection and the mentioned oxidative stress (Kountouras, 2009; Kountouras et al., 2008b). *H. pylori* could indirectly affect the brain including the eyes and other target organs, e.g. the heart, through the release of numerous cytokines such as TNF-α acting at a distance; TNF-α is involved in BBB disruption through a mechanism involving matrix metalloproteinases upregulation (Kountouras, 2009). TNF-α and IL-6 (TNF-α is the main trigger for the production of IL-6 by a variety of cells) play important roles in the regulation of the synthesis of other acute phase proteins which are established risk factors for atherosclerosis, such as fibrinogen and factor VIII. These cytokines also have profound effects on lipid metabolism directly at the site of the atherosclerotic lesion, but could influence the atheroma process through blood circulating levels, distant production of cytokines, or through stimulating circulating white blood cells to produce them, thereby contributing to BBB disruption and pathogenesis of heart and brain neurodegenerative diseases (Kountouras, 2009). In addition, *H. pylori*-induced cytotoxin VacA exhibits chemotactic activities to the bone marrow-derived mast cells (BMDMCs) and induces BMDMCs to produce proinflammatory cytokines including TNF-α (Kountouras, 2009); BMDMCs reside adjacent to blood and lymphatic channels, mainly under epithelial surfaces, including the BBB and gastrointestinal tract (Kountouras et al., 2009b). *H. pylori* stimulates mast cells directly or via gastrin induction and mast cells are actively involved in the pathogenesis of *H. pylori*-associated pathologies (Kountouras et al., 2009b). Apart from activated mast cells, vascular endothelial growth factor (VEGF), IL-8, chymase or tryptase (a serine endoprotease released by mast cells) and mast cell growth factor linked to *H. pylori* infection, mast cells themselves can be stimulated by corticotropin-releasing hormone, secreted under stress, to release mediators including histamine, IL-8, tryptase and VEGF, which disrupt the BBB (Kountouras, 2009). BBB disruption, in turn, could play an important role in promoting entry of immune cell infiltration and pathogens into the brain resulting in the development of brain pathologies (Itzhaki et al., 2004). Apart from pathogens' intranasal inoculation, the influx of activated monocytes infected with *Chlamydia pneumoniae* through the BBB could have dire consequences in the brain leading to the development of degenerative diseases, including AD (Itzhaki et al., 2004) and possibly glaucoma; infectants including herpes simplex virus 1, *Chlamydia pneumoniae*, and even *Borrelia* species have been found in brain regions demonstrating significant AD pathology. In this regard, as in the case of influx of activated monocytes infected with *Chlamydia pneumoniae* through the BBB, we can speculate that activated monocytes (possibly infected with *H. pylori* due to defective autophagy resulting in *H. pylori* replication in autophagic vesicles) (Wang et al., 2009) might also enter the aqueous circulation due to BBB/blood-ocular barrier (BOB) disruption possibly contributing to glaucoma development and progression; the *H. pylori* VacA promotes intracellular survival of the bacterium and modulates host immune responses (Raju and Jones, 2010). Besides, autoimmune injury to the optic nerve may occur directly by autoantibodies or indirectly via a "mimicked" autoimmune response to a sensitizing antigen, which, in turn, damages retinal ganglion cells. Specific antibodies are found in increased levels in glaucoma patients' sera, and when these antibodies access the brain due to BBB disruption, they are capable of killing retinal cells, thereby contributing to glaucoma pathologies (Kountouras et al., 2003). Comparable data could also be considered in the presence of BOB dysfunction. In this respect, we can also speculate that *H. pylori* antibodies circulating in the bloodstream may enter the aqueous circulation due to BOB disruption, possibly contributing to glaucoma development and progression (Kountouras et al., 2003).

In view of the aforementioned data, eradication of *H. pylori* infection may benefit the glaucomatous optic neuropathy (which accounts for approximately 90.8 million affected individuals) by ameliorating mainly the apoptotic loss of retinal ganglion cells and their axons, and the progressive loss of visual field sensitivity, thereby suggesting its future role in glaucoma neuroprotection.

Competing interests

None declared.

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blood pressure in AlP-poisoned patients, because myocardial suppression and resistant hypotension, as mentioned above, are the main features of AlP poisoning. Digoxin may have a role in combating cardiovascular shock in AlP poisoning. This hypothesis has not clinically been tested yet.

Conflict of interest

None declare.

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Modern industrialisation may increase primary open-angle glaucoma prevalence through easier transmission of *Helicobacter pylori* infection

Dear Editor,

We have read with interest the paper by Wang et al. who concluded that the prevalence of primary open-angle glaucoma (POAG) in Chinese is related to modern industrialisation and will increase in the future in the urban regions [1]. To support their theory, the authors underscored the previously established relationship between POAG and myopia and claimed that industrialisation increases the prevalence of myopia since it brings more chances for higher education and an intense near-work environment.

Although myopia can be considered as an independent risk factor for developing POAG, several other factors should be also considered before we conclude that industrialisation and POAG are related. For example, the population living in urban regions has better access to medical facilities and, furthermore, as the technology advances rapidly, it is very likely that more and more sophisticated methods for the early detection of POAG will be implemented in the future, thereby increasing the POAG prevalence. Moreover, as the population grows older, one could predict that POAG may also increase in the future.

Based on the latter epidemiological data, we first reported that *Helicobacter pylori* infection (*Hp*) and glaucoma might be associated causatively, since both diseases affect older adults in the developed world [2]. The existing published data pertaining to the prevalence of *Hp* infection in patients with different glaucoma types present geographic and ethnic similarities or diversities reflecting the geographic and ethnic variations in the prevalence of *Hp* infection and the different glaucoma types. More specifically, the Greek data show a high prevalence of *Hp* infection in Greek patients with POAG [2], *Hp* eradication was shown to benefit glaucoma progression [3], thereby suggesting a possible causal link between the bacterium and glaucoma, and *Hp*-specific IgG antibody concentration was increased in the aqueous humour of POAG patients and correlated with the degree of vertical cupping, possibly indicating the severity of glaucomatous damage [4]. Likewise, in a Chinese population, Hong et al. recently confirmed that *Hp* infection was significantly higher in POAG patients than in control participants ($p = 0.017$) [5].

Taking into consideration that the prevalence of *Hp* infection is higher in crowded household conditions, one would expect that in the urban areas of the low-income and booming industry countries such as China, where apartments are expensive, small and crowded accommodating the millions of workers and their families, *Hp* infection and, consequently, also POAG prevalence is likely to increase in the future, compared to the less crowded rural areas.

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Letter to the Editor

Helicobacter pylori infection may trigger Guillain–Barré syndrome, Fisher syndrome and Bickerstaff brainstem encephalitis

Dear Editor:

We read with considerable interest the Editorial by Yuki and Shahrizaila [1] who commented a case of Bickerstaff's brainstem encephalitis (BBE) complicating *Salmonella* Paratyphi A infection [2]. The authors considered mainly the antecedent infection with *Campylobacter jejuni* (*C. jejuni*) triggering Guillain–Barré syndrome (GBS), Fisher syndrome (FS) and BBE. On the other hand, they concluded that "*Helicobacter pylori* has also been suggested as a possible cause of GBS, but it is unlikely that such a chronic infection would induce an acute and monophasic, immune-mediated condition".

The latter consideration, however, appears to be rather incomplete. It is known that current *Helicobacter pylori* (*H. pylori*) infection may induce irregular humoral and cellular immune responses that, owing to the sharing of homologous epitopes (i.e., molecular mimicry), cross-react with components of nerves, thereby constantly triggering and possibly perpetuating neural tissue damage observed in neurodegenerative diseases, including acute and monophasic, immune-mediated conditions such as GBS [3,4]. In this respect, we have reported that *H. pylori* infection, documented by histology, is more frequent in patients with GBS than in controls [3]. We found that high serum concentrations of anti-*H. pylori* IgG antibodies closely correlated with a more advanced clinical stage of GBS. Furthermore, increased serum antibody concentrations were associated with involvement of the proximal parts of peripheral nerves in the disease [3]. These results are in accordance with previous data that showed positive antibodies against vacuolating cytotoxin (VacA) of *H. pylori* in the cerebrospinal fluid of patients with GBS, and delayed F-wave latencies in patients with GBS and positive antibodies to VacA of *H. pylori* in serum [5]. The target molecules of the specific antibody against VacA in the cerebrospinal fluid of patients with GBS are probably associated with some components of the peripheral nerve myelin, which suggests a potential role in the immune responses of patients with the demyelinating form of GBS [5]. Patients who have GBS with anti-GM1 ganglioside antibodies alone or associated with anti-*H. pylori* antibodies had a significantly longer hospitalization time to reach a low clinical score at discharge than patients who did not have anti-GM1 antibodies [4]. Moreover, previous data also showed positive antibodies against VacA of *H. pylori* in the cerebrospinal fluid of patients with FS; there was a sequence homology between VacA and some membrane ion transport proteins, raising the possibility that the specific antibody against VacA involves the ion channels in the node of Ranvier in some patients with FS [6].

The association between *H. pylori* and autoimmunity is now well established, and GBS, an acute inflammatory polyradiculoneuropathy, and FS are thought to be caused by autoimmune processes, triggered by preceding bacterial infections, as mentioned in the Editorial [1]. Autoantibodies to specific neural targets have been found to impair neural function and are specific to subtypes of GBS, including acute inflammatory demyelinating polyradiculoneuropathy (AIDP) as shown in our study [3] and as suggested by others [7]. In AIDP, the common epitopes of GM3, GD3, or GT3 can be shared with certain antigens localized in the peripheral nervous system that function as conduction-related molecules at the neuromuscular junction. Although studies have shown that GBS is frequently preceded by acute infections, the exact cause of GBS is not known and a specific immunological explanation has not been found. *H. pylori*, through the mentioned molecular mimicry, cross-reacts with ganglioside surface components of peripheral nerves. Immune reactions against target epitopes in the surface membranes of Schwann cells or myelin result in GBS [3]. Molecular mimicry of host structures by the saccharide part of lipopolysaccharides of the gastrointestinal pathogens *C. jejuni* mentioned by the authors and *H. pylori* are thought to be associated with the development of autoimmune sequelae seen in GBS [8].

Apart from molecular mimicry and cross-reactivity, *H. pylori* infection might influence the pathophysiology of GBS and other neurodegenerative diseases through several other mechanisms, including the release of proinflammatory and vasoactive substances (e.g., cytokines or eikosanoids), induction of oxidative stress, or apoptotic processes [4]. Specifically, blood-nerve barrier (BNB) damage has been increasingly implicated in inflammatory demyelinating neuropathies (IDNs) [9,10] and a variety of proinflammatory cytokines are instrumental in the course of IDNs. They increase vascular permeability and BNB disruption (tumor necrosis factor (TNF)-alpha, vascular endothelial growth factor (VEGF), vascular permeability factor), induce transmigration of leukocytes into the nerve, activation and proliferation of macrophages (interferon (IFN)-gamma) and T cells (interleukins 1 and 2), and exert direct myelinotoxic activities (TNF-alpha and TNF-beta). Moreover, downregulation of the immunosuppressive cytokine transforming growth factor (TGF)-beta 1 may provoke nerve inflammatory reactions [10]. In this respect, Schwann cell induces reactivation of CD4+ T cells, which, by producing TNF-alpha and IFN-gamma, could exacerbate BNB disruption thereby playing a role in IDNs [9]; intraneural activated T cells cause focal BNB disruption, and, moreover, increased circulating TNF-alpha might play a role in the BNB disruption and the pathogenesis of demyelination in chronic inflammatory demyelinating polyneuropathy [10]. Besides, mast cells are located in close proximity to neurons in the peripheral and central nervous systems, signifying their role in normal and aberrant neurodegenerative conditions [10]. Mast cell degranulation is able to secrete a range of potent mediators which could orchestrate neuroinflammation and affect the blood-brain barrier (BBB)/BNB integrity; mast cell degranulation induces profound changes in BNB permeability, thereby playing a role in neuropathies [11–13]. The 'cross-talk' between mast cells, lymphocytes, neurons and glia constitutes a neuroimmune axis implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component [11].

H. pylori infection, by releasing several mediators, could induce BNB/BBB breakdown, thereby possibly being involved in the pathogenesis of neuropathies including FS, acute motor axonal neuropaty (AMAN) and BBE, mentioned by the authors, and AIDP [10]. Indeed, a

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series of factors has been implicated in inducing BNB/BBB disruptions, including inflammatory mediators (e.g., cytokines and chemokines induced by *H. pylori* infection) and oxidative stress [10]. For, instance, *H. pylori* could indirectly affect the peripheral and central nervous systems, through the release of numerous cytokines such as TNF-α acting at distance; TNF-α is involved in BBB/BNB disruption through a mechanism involving matrix metalloproteinases upregulation [12,13]. Likewise, oxidative stress is an essential underlying cause of neuroinflammatory and neurodegenerative diseases and BBB/BNB damage is associated with them [13,14]. In addition, *H. pylori*-induced VacA exhibits chemotactic activities to the bone marrow-derived mast cells (BMD-MCs) and induces BMD-MCs to produce proinflammatory cytokines including TNF-alpha; BMD-MCs reside adjacent to blood and lymphatic channels, mainly under epithelial surfaces including the BBB/BNB and gastrointestinal tract [13]. Apart from activated mast cells, VEGF, interleukin-8, chymase or tryptase (a serine endoprotease released by mast cells) and mast cell growth factor linked to *H. pylori* infection, mast cells themselves can be stimulated by corticotropin-releasing hormone, secreted under stress, to release mediators including histamine, interleukin-8, tryptase and VEGF, which disrupt the BBB/BNB [13]. In turn, BBB/BNB breakdown could promote entry of immune cells (autoreactive effector CD4+ and CD8+ T cells) infiltrations, activated monocytes infected with *H. pylori*, due to defective autophagy resulting in *H. pylori* replication in autophagic vesicles [15] and/or *H. pylori* circulating antibodies into the peripheral and central neurons resulting in the development of neuronal pathologies [13] including AMAN and AIDP [10].

GBS, including the FS subtype of GBS, and BBE are immune-mediated disorders that have overlapping clinical features and the Chinese are associated with a high prevalence of *H. pylori* infection [16]. In view of the aforementioned data related to *H. pylori* infection, it is interesting to know if the authors have considered the possible role of *H. pylori* infection as a trigger of GBS, FS and BBE.

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Response to Letter to the Editor

Helicobacter pylori and the Guillain-Barré syndrome: The evidence is lacking☆

In our article we suggested four criteria to verify that a given microorganism is causative in the development of GBS and its related conditions [1]. Here we reiterate these criteria and for clarity, have expanded them as follows: (i) Epidemiological association is established between the microbial infection and the disease; (ii) The microorganism is isolated from patients at the acute progressive phase of the illness, and not at the recovery phase; (iii) Anti-neural antibodies are detected at the acute phase, and the titres decrease at the recovery phase; (iv) Molecular mimicry is identified between the microbial and neural antigens; (v) The anti-neural antibodies are induced by sensitization with the microbe itself, or its component in animals; and (vi) Animal models are reproduced by inoculation with the microbe itself, or its component, as well as with the neural antigen. At the very least, criteria (ii) to (v) should be fulfilled before concluding that certain microbes are probable causes of GBS or its related conditions. *Campylobacter jejuni* has satisfied all six definite criteria [2–6], confirming the pathogenic properties of this bacterium in the development of GBS. To our knowledge, no other microbes (*H pylori* included) can make a similar claim. We encourage researchers to satisfy the aforementioned definite or probable criteria to allow for more meaningful hypotheses to be formed in understanding the pathogenesis.

We note that the authors have raised a similar question in a previous letter [7]. We agree with van Doorn and colleagues in their reply. There has not been sufficient evidence to explain how *H pylori*, which can cause a chronic infection if untreated, can trigger GBS, a typically acute, monophasic condition. Eradication of *H pylori* infection can increase the platelet count in patients with immune thrombocytopenic purpura, suggesting that the bacterium may have a role in the pathogenesis of that condition [8]. However, there is no clear evidence that *H pylori* induces the development of other autoimmune diseases including GBS.

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☆ We thank Kountouras and colleagues for their response to our commentary. The authors argued that a more complete consideration was not given to the possibility of *Helicobacter pylori* infection triggering Guillain-Barré syndrome (GBS).

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LETTER TO THE EDITORS

Association between *Helicobacter pylori* infection and Alzheimer's disease in Japan

Jannis Kountouras · Christos Zavos · Marina Boziki · Emmanuel Gavalas · Panagiota Kyriakou · Georgia Deretzi

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Dear Sirs,

We read with interest the paper by Shiota et al. [1], who reported that older age and male sex were significantly associated with Alzheimer's disease. Moreover, the authors reported a lack of association between *Helicobacter pylori* infection (*Hp*-I) status and Alzheimer's disease in their Japanese cohort, suggesting that their findings might be explained by the much higher prevalence of *Hp*-I in the general Japanese than in the European population.

We herein wish to emphasize two essential concerns regarding the methodological limitations which may render the results of this study highly debatable. The first is that both age and sex, found to be associated with Alzheimer's disease, were not matched in the two study groups ($p < 0.001$ and $p = 0.01$, respectively), and thus comparisons between the two study groups (i.e., Alzheimer's disease patients and controls) cannot be expected to establish any firm conclusions.

The second concern is that the very high *Hp*-I prevalence in the general Japanese population around 70 years old, reported by the authors, and, deductively, in the control group of the study, renders the study underpowered, meaning it requires probably thousands of participants in order to prove whether an association between Alzheimer's disease and *Hp*-I can be established or excluded; it requires a very large population to be screened to prove or not a statistical difference in *Hp*-I among patients with Alzheimer's disease and the general Japanese population.

In view of the aforementioned methodological limitations, we deduce that this study can neither confirm the lack of association between the *Hp*-I status and Alzheimer's disease in the Japanese population, nor is it comparable with the European studies indicating such an association [2–4].

Conflict of interest None.

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LETTER TO THE EDITOR

Helicobacter pylori may be involved in stroke pathophysiology by altering tumor necrosis factor-α and matrix metalloproteinases

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Keywords: blood–brain barrier, *Helicobacter pylori*, matrix metalloproteinases, tumor necrosis factor-α

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Dear Editor,

We read with considerable interest the paper by Munshi A *et al*. [1] concluding that tumor necrosis factor (TNF)-α + 488 G/A variant but not matrix metalloproteinase (MMP)-3 1612 5A/6A polymorphism is an important risk factor for ischemic stroke in South Indians. However, the authors did not mention the role of environmental agents involved in the TNF-α/MMPs-induced pathophysiology of stroke. In this regard, systemic inflammatory events, such as infections, increase the risk of stroke, are associated with worse outcome, and the mediators of this clinically important effect may include cytokines such as TNF-α and MMP involved in the blood–brain barrier (BBB) disruption; BBB dysfunction is a hallmark of many central nervous system pathologies including ischemic stroke [2] and can lead to an increase in vascular permeability and brain edema, exacerbating the ischemic injury.

Specifically, *Helicobacter pylori* infection (*Hp*-I), a well-recognized cause of upper gastrointestinal pathologies affecting ~85% of South Indian population [3], is a risk factor for ischemic stroke [4]; *Hp* is present in carotid atherosclerotic lesions associated with features of inflammatory cell response, CagA-positive strains of *Hp* are significantly associated with atherosclerotic stroke and its recurrence, and *Hp* strains induce platelet aggregation, leading to thrombosis events.

Hp-I, by releasing several of the aforementioned inflammatory mediators, could induce BBB breakdown, thereby being involved in the pathogenesis of neuropathies including stroke [4,5]. For instance, *Hp* could indirectly affect the peripheral and central nervous systems through the release of numerous cytokines such as TNF-α acting at distance; TNF-α is involved in BBB disruption through MMP upregulation [5]. Moreover, *Hp*-induced vacuolating cytotoxin A exhibits chemotactic activities to the bone marrow-derived mast cells (BMD-MCs) and induces BMD-MCs to produce pro-inflammatory cytokines involved in the BBB disruption [5]. It would thus be interesting to know if the authors have considered the aforementioned data regarding the consideration that *Hp*-induced TNF-α and MMP might be involved in stroke development, through an impaired BBB, in their South Indian participants, expected to exhibit a possible high prevalence of *Hp*-I. If a role of *Hp*-I in the stroke pathophysiology is demonstrated, this might have a major impact on the management of this disease.

Conflict of interest

None declared.

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LETTER TO THE EDITOR

The potentially dual-faceted nature of fetuin-A in *Helicobacter pylori* infection and insulin resistance

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We read with interest the study by Kebapcilar et al.[1] on the effect of *Helicobacter pylori* (*HP*) eradication on serum fetuin-A concentrations. Specifically, baseline serum fetuin-A was lower in *HP*-positive participants than in *HP*-negative matched participants. Furthermore, fetuin-A levels significantly increased after successful *HP* eradication treatment.[1] To our knowledge, this is the first report on the effect of *HP* eradication on serum fetuin-A.

Recently, Manolakis et al. proposed serum fetuin-A as a mediator linking *HP* infection and insulin resistance (IR).[2] This is also an interesting concept with therapeutic potential because it suggests that *HP* eradication might decrease IR and IR-related morbidity, including type II diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD),[3] although the effect on CVD is highly debatable.[4] We have recently performed a systematic review on the association between *HP* infection and IR.[5] Existing data indicate a potential association between *HP* infection and IR, as assessed by homeostatic model assessment IR (HOMA-IR). However, although histology is considered the gold standard diagnostic test for *HP*, some studies used other methods to determine *HP* status. The serum anti-*HP*-specific IgG antibody level may be of diagnostic value, but it does not discriminate between past and current infections. Furthermore, given that serum remains positive for anti-*HP*-specific IgG even after eradication of *HP*, it is unclear whether *HP* infection is the inducer or the promoter of IR.[5]

However, in the study by Manolakis et al., baseline fetuin-A was higher in *HP*-positive participants than in *HP*-negative participants.[2] The reason for this discrepancy between the two studies[1,2] is largely unknown. Both studies recruited non-diabetic patients with dyspeptic symptoms, and their exclusion criteria were similar. However, the mean age and BMI of the *HP*-positive group were higher in the Manolakis et al. study[2] than in the Kebapcilar et al. study[1] (57.3 ± 16.1 versus 29.1 ± 7.5 years and 25.8 ± 12.7 versus 22.9 ± 4.5 kg/m^2, respectively, after converting SEM to SD in the first study), and these two differences could have affected IR. There were also ethnic differences, which might have affected IR and possibly serum fetuin-A. Finally, in the two studies, serum fetuin-A was measured by different commercial ELISA kits.

Fetuin-A is an acute-phase glycoprotein synthesized and secreted almost exclusively by the liver that plays a role in bone mineralization and insulin-signaling regulation.[6] Fetuin-A binds a form of insoluble calcium phosphate, inhibiting pathological extraosseous calcification and thereby playing a protective role in the evolution of arterial calcification. However, when dysregulated, fetuin-A can lead to ectopic calcification of soft tissues in the vasculature, thereby contributing to atherosclerosis. Likewise, by binding the extracellular portion of the β-subunit of the insulin receptor, fetuin-A inhibits insulin-receptor tyrosine kinase, thereby attenuating, under normal conditions, excessive insulin signaling. However, when dysregulated, fetuin-A results in excessive inhibition of insulin signaling in the liver and in skeletal muscle, thereby triggering IR. Subsequently, increased serum fetuin-A has been associated with IR-related morbidity, including T2DM, visceral obesity, NAFLD, CVD and ischemic stroke.[6,7] Fetuin-A also seems to play a role in inflammation by downregulating the pro-inflammatory cytokines produced by macrophages.[1] Fetuin-A is regarded as an anti-inflammatory mediator that contributes to macrophage deactivation, and moreover, it possesses anti-fibrotic activity and inhibits apoptosis in vascular smooth muscle cells. Nonetheless, fetuin-A downregulates adiponectin, an anti-inflammatory and insulin-sensitizing adipokine. Finally, although fetuin-A induces lipid accumulation in the liver, it may attenuate hepatic fibrosis by modifying the effects of transforming growth factor-β signaling in hepatocytes.[7] Summarizing all the above-mentioned data, fetuin-A may possess a dual-faceted nature in the metabolic and inflammatory milieu. It may be protective by inhibiting extraosseous calcification or excessive insulin signaling in lean, metabolically healthy individuals, but, in other situations, it may also be harmful, especially if its action is persistently elevated and prolonged. This dual-faceted nature may also apply to most cytokines and adipocytokines.[8]

In the Kebapcilar et al. study,[1] serum fetuin-A was low in young, *HP*-positive individuals with a negative history of diabetes, obesity and known cardiovascular risk factors. Furthermore, successful *HP* eradication was associated with a fetuin-A increase and a simultaneous decrease in both C-reactive protein and macrophage migration inhibitory factor. This result seems rational for this specific population, in whom the counteracting mechanisms, including fetuin-A, are beneficial. In the Manolakis et al. study,[2] serum fetuin-A was increased in non-diabetic, *HP*-positive individuals with established IR, who generally were older and had a higher BMI than the *HP*-positive individuals of the Kebapcilar et al. study.[1] This finding also

seems rational for this specific population, in whom the counteracting mechanisms may no longer be beneficial, and thus fetuin-A may contribute, at least in part, to IR. In conclusion, serum fetuin-A may represent a promising index for assessing the *HP*-related contributions to inflammation, IR and IR-related conditions, including T2DM, NAFLD and CVD; however, further clinical trials are necessary to confirm its diagnostic efficacy.

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LETTER TO THE EDITOR

Multiple sclerosis and seizures: possible role of *Helicobacter pylori*

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Keywords: *Helicobacter pylori*, multiple sclerosis, seizures

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We have read with interest the proposition by Anderson and Rodriguez [1] that an increase in interleukin (IL)-18, and its associated induction of indoleamine 2, 3-dioxygenase (IDO) and quinolinic acid, mediates seizure activity in multiple sclerosis (MS) at least partly via an increase in interferon-gamma (IFN-γ), accompanied by blood–brain barrier (BBB) permeability.

Recent studies showed an association between *Helicobacter pylori* infection (*Hp*-I) and epilepsy, especially with poor prognosis [2]. Moreover, using histology, the practical gold standard for current *Hp*-I diagnosis, we showed a strong association between *Hp*-I and MS in a Greek cohort [3]. In this respect, we proposed that *Hp*-I, by inducing pro-inflammatory cytokine production and BBB disruption [4], may lead to neuroinflammation and neuronal damage in epilepsy, thereby triggering seizures' induction and epilepsy progression [5].

Specifically, a series of factors have been implicated in inducing BBB disruption, including inflammatory mediators (e.g. cytokines and chemokines induced by *Hp*-I) and oxidative stress [5,6]. *Hp* could indirectly affect the brain through the release of numerous pro-inflammatory cytokines, such as IL-1b, IL-18, IFN-γ [7] mentioned by the authors, or tumor necrosis factor (TNF)-α acting at a distance. TNF-α may contribute to BBB disruption and pathogenesis of neuronal inflammatory damage in neurodegenerative diseases and epilepsy; IL-1b, IL-6, and TNF-α influence the pathogenesis of seizures and course of epilepsy, and the primary BBB lesion is involved in seizures' induction/epileptogenesis [8]. Likewise, an influx of *Hp*-infected monocytes, owing to defective autophagy resulting in *Hp* replication in autophagic vesicles, through the disrupted BBB might lead to brain degeneration and epilepsy development partially by potential activation of natural killer cells and IFN-γ production, detrimental for MS; *Hp* VacA cytotoxin promotes intracellular survival of the bacterium and modulates host immune responses. Of note, *Hp* is known to be associated with the increase in IDO-dependent mechanisms.

Viewing the aforementioned data and because half of the world's population is infected with *Hp*, it would be interesting to know whether the authors have considered *Hp*-I as a potential confounder involved in the pathophysiology of MS-associated increased seizure activity and therefore its management.

Disclosure of conflict of interest

The authors declare no financial or other conflict of interests.

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thalmics, Costa Mesa, California), allowing adequate fit of various-sized contact lenses and eliminating friction between the contact lens edge and the tube or plate; (3) decreasing the risk of conjunctival erosion or tube exposure and extrusion. We have observed cases of extrusion in pre-existing shunts placed with the conventional technique, but with the above technique, we have not observed any extrusion in 4 years of follow-up. However, longer follow-up is required, and we recommend close monitoring and early repair of any shunt exposure to avoid sight-threatening complications.

In summary, we believe that an interdisciplinary approach optimizing surgical techniques for glaucoma drainage devices and for the Boston type I keratoprosthesis can avoid shunt-related complications, and therefore can achieve the best visual rehabilitation in these patients.

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CONFLICT OF INTEREST DISCLOSURES: THE AUTHORS HAVE completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.

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REPLY

WE READ WITH APPRECIATION THE COMMENTS FROM CORtina and associates in response to our recent article on long-term complications associated with a glaucoma drainage device (GDD) in patients with Boston keratoprosthesis.[1] We were pleased by their interest in our publication, and we acknowledge their contributions to one of the most challenging aspects of keratoprosthesis surgery.

Their technique for pars plana Ahmed valve (FP7; New World Medical, Inc, Rancho Cucamonga, California, USA) placement with a partial thickness corneoscleral patch graft is described nicely in their case series.[2] Our series of 9 patients with GDD erosions did include 4 erosions that occurred in GDDs placed in the pars plana. The main difference in our series, however, was the use of Tutoplast allograft pericardium (Tutoplast; IOP, Inc, Costa Mesa, California, USA) to cover the tube. The suggestion that a partial thickness corneoscleral patch graft may provide a smoother surface than Tutoplast, and thus may decrease the mechanical effects that may contribute to GDD erosions in these patients, is worth exploring further. We also find that an advantage of a corneoscleral patch graft is that in the event of a recurrent erosion, the corneal material is more likely to re-epithelialize than Tutoplast. This simple modification of GDD technique easily could be adopted by glaucoma surgeons who are assisting in the management of these challenging patients.

Ultimately, this highlights once again the importance of an atmosphere of collaboration, as different centers and different ophthalmic disciplines continue to work together to restore and maintain vision for these patients.

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CONFLICT OF INTEREST DISCLOSURES: SEE THE ORIGINAL article[1] for any disclosures of the authors.

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Helicobacter pylori Infection as a Risk Factor for Both Primary Open-Angle Glaucoma and Pseudoexfoliative Glaucoma in Thessaloniki Eye Study

EDITOR:

TOPOUZIS AND ASSOCIATES' OBSERVATION THAT INcreased intraocular pressure (IOP) was the only risk factor associated with both primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (XFG) in the Thessaloniki Eye Study is interesting because recent evidence indicates that the prevalence of *Helicobacter pylori (H. pylori)* infection in glaucoma patients of this area is equally high, thereby

representing a common denominator underlying both POAG and XFG in Thessaloniki.[1–4]

It is known that current *H. pylori* infection may induce irregular humoral and cellular immune responses that, because of the sharing of homologous epitopes (ie, molecular mimicry), cross-react with components of nerves, thereby triggering and possibly perpetuating neural tissue damage observed in neurodegenerative diseases, including glaucoma.

In 2001, by using histologic analysis, representing the practical gold standard for *H. pylori* infection diagnosis, we documented for the first time a high prevalence of *H. pylori* infection in Thessaloniki patients with POAG and XFG, establishing a relationship between *H. pylori* infection and glaucoma.[2] These results may indicate either that a common factor that causes susceptibilities to both glaucoma and *H. pylori* infection or that *H. pylori* may be a causal factor for developing glaucoma. In a subsequent study,[3] we reported a beneficial effect of *H. pylori* eradication on glaucoma progression, including reduction in intraocular pressure mentioned by the authors, suggesting a possible causal link between the bacterium and glaucoma.[1] Moreover, we reported an increased *H. pylori*-specific immunoglobulin G antibody concentration in the aqueous humor of patients with POAG and XFG; the concentration of this antibody correlated with the degree of vertical cupping, possibly indicating the severity of glaucomatous damage.[4] Recently, we also showed for the first time the detection of *H. pylori* bacteria in the trabecula and iris of POAG patients, indicating that the bacterium is present locally and possibly is directly implicated in glaucomatous damage.[5]

Reports from other ethnic populations also showed a relationship between glaucoma and *H. pylori* infection, although this has not been confirmed by all the relevant studies published so far, and thus this association may apply only to a limited subpopulation of glaucoma patients; similar observations have been made in Korea, China, India, Turkey, and Iran.[4,6]

H. pylori infection, by releasing several inflammatory mediators,[4,5] could induce blood-brain barrier (BBB) and blood-ocular barrier breakdown, thereby being involved in the pathogenesis of neuropathies, including glaucoma.[4,5] For instance, *H. pylori* indirectly may affect the brain through the release tumor necrosis factor α acting at a distance; tumor necrosis factor α is involved in BBB disruption through the upregulation of matrix metalloproteinases. Furthermore, *H. pylori* circulating antibodies may also enter the aqueous circulation as a result of BBB and blood-ocular barrier disruption, possibly contributing to glaucoma progression; when serum-specific antibodies access the brain, they are capable of killing retinal cells.[4] Likewise, an influx of *H. pylori*-infected monocytes, owing to defective autophagy resulting in *H. pylori* replication in autophagic vesicles, through the disrupted BBB and blood-ocular barrier may lead to glaucoma neuropathy; *H. pylori* vacuolating toxin A (VacA) promotes intracellular survival of the bacterium and modulates host immune responses.[5] Finally, because the oral cavity may act as a permanent reservoir for *H. pylori*, this bacterium may reach the eye through the nasal cavity, causing ophthalmic pathologic changes and possibly including glaucoma.[5]

It thus would be interesting to know if the authors have considered *H. pylori* infection as a risk factor involved in POAG and XFG progression in their Thessaloniki Eye Study participants, who are expected to exhibit a high prevalence of *H. pylori* infection.

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CONFLICT OF INTEREST DISCLOSURES: THE AUTHORS HAVE completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.

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REPLY

WE THANK KOUNTOURAS AND ASSOCIATES FOR THEIR interest in our article.[1] We acknowledge their research on the association of *Helicobacter pylori* infection with neurodegenerative and other diseases. However, the association between *H. pylori* infection and glaucoma remains controversial. Specifically, the above association, initially found by Kountouras and associates in a case-control study of 41 glaucoma patients and 30 anemic controls,[2] was not confirmed in an independent study from Canada involving 97 patients and 94 controls and in a more recent study from Israel involving 51 glaucoma patients and 36 controls.[3,4] Also, in another study from India of 100 glaucoma

patients and 50 controls, the mean level of anti-*H. pylori* immunoglobulin G positivity in the sera was not statistically significantly different between subjects with primary open-angle glaucoma and the control group, whereas it was higher in controls compared with the pseudoexfoliative glaucoma group.[5] In the same study, the level of anti-*H. pylori* immunoglobulin G titers did not differ between the pseudoexfoliative glaucoma and the control groups. All of the above studies have the inherent limitations of the case-control design in investigating risk factors for a disease. Further, because glaucoma prevalence increases with age and *H. pylori* infection is very common in the older age groups, it is very difficult to determine whether there is an association between these 2 conditions or whether the reported associations have resulted from chance alone. For all of the above reasons, *H. pylori* infection has not been established as a potential risk factor for glaucoma, and therefore has not been included in recent major reviews on risk factors for glaucoma.[6,7]

Based on the current state of evidence, we did not investigate the potential role of *H. pylori* infection in glaucoma in the Thessaloniki Eye Study. Moreover, at the time that the Thessaloniki Eye Study was designed, the potential association between *H. pylori* infection and glaucoma had not been reported. Given that evidence for *H. pylori* infection being a risk factor for glaucoma is still conflicting and that population-based studies provide the best design to investigate risk factors for a disease, in retrospect it would have been desirable for us to have looked into this assocation, so as to determine more definitely whether *H. pylori* infection is associated with glaucoma.

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CONFLICT OF INTEREST DISCLOSURES: SEE THE ORIGINAL article[1] for any disclosures of the authors.

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Graft Rejection and Graft Failure After Anterior Lamellar Versus Penetrating Keratoplasty

EDITOR:

WE READ THE ARTICLE BY BORDERIE AND ASSOCIATES[1] with great interest. We commend the authors' effort and find their results consistent with available evidence.[2,3] While it is interesting to know that eyes undergoing anterior lamellar keratoplasty (ALK) had a lower incidence of rejection episodes and irreversible rejection compared to the penetrating keratoplasty (PK) group, could the authors kindly throw some light on the following questions that came up in our discussion?

In Figure 1, the authors highlight that only 77 patients in the ALK group completed follow-up at 36 months. In Figure 2, however, it is stated that the cumulative incidence of reversible and irreversible rejection episodes of 149 ALK patients with a 36-months-postoperative time period was 0% and 10%, respectively. It is unclear, however, how these figures were calculated given that only 77 patients were followed up to 36 months.

In addition, in terms of graft survival, it would be helpful if the authors would provide information on the remaining 72 patients of the ALK group, for whom no follow-up data are provided. That is, how many patients were lost to follow-up and what were the reasons for this? If even 1 or 2 more patients in the ALK group had graft failure, this would have a significant effect on the overall graft survival, particularly in comparison to the PK group. In addition, similar data for the 28 patients who had PK would be informative.

Essentially, graft failure and/or rejection episodes in the patients who were lost to follow-up could have a

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Key words: dysmorphophobia, delusional disorder somatic type, antipsychotics, antidepressants

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Low aqueous humor ghrelin levels in open-angle glaucoma patients may correlate with Helicobacter pylori-associated apoptotic mechanisms

Dear Editor,

Recent evidence indicates that the aqueous humor ghrelin levels are significantly lower in primary open-angle (POAG) and pseudoexfoliation glaucoma (PXG) patients compared with cataract controls[1]. This finding has been attributed to: 1) glaucomatous neuropathy per se; 2) glaucoma medications; or 3) a higher intraocular pressure level[1].

Concerning the first possible explanation, ghrelin, playing an essential role in the gastric mucosal defense mechanism, has been closely associated with gastritis and Helicobacter pylori infection (Hp-I), mainly by controlling the apoptotic processes induced by Hp lipopolysaccharide (LPS)[2]; apoptosis is involved in several important ocular and gastrointestinal diseases, including glaucoma, Hp-induced gastrointestinal and/or extraintestinal diseases, comprising autoimmune and neurodegenerative ones, also associated with glaucomatous apoptotic neuropathy. Recently, we showed a high prevalence of Hp-I in POAG and PXG patients, and Hp eradication may positively influence glaucoma parameters[3]. Hp-I may influence the pathophysiology of glaucoma by releasing various proinflammatory and vasoactive substances or by being involved in the apoptotic process[3].

Furthermore, it has been assumed that circulating ghrelin can affect aqueous humor ghrelin levels by crossing the blood-ocular barrier (BOB), based on previous studies showing that ghrelin can pass the blood-brain barrier (BBB) in a complex and highly regulated process[1]. In this respect, a series of factors has been implicated in inducing BBB disruption, including inflammatory mediators (e.g., cytokines and chemokines induced by Hp-I) and oxidative stress[3]. In addition, Hp-induced cytotoxin VacA exhibits chemotactic activities to the bone marrow-derived mast cells (BMDMCs) and induces BMDMCs to produce proinflammatory cytokines including tumor necrosis factor (TNF)-α acting at a distance; TNF-α is involved in BBB disruption through a mechanism involving matrix metalloproteinases upregulation. Apart from activated mast cells, vascular endothelial growth factor, interleukin (IL)-8, chymase or tryptase and mast cell growth factor linked to Hp-I, mast cells themselves can be stimulated by corticotropin-releasing hormone, secreted under stress, to release the aforementioned mediators, which disrupt the BBB; its disruption could play an important role in promoting immune cell infiltration and pathogens' entry to the brain[4]. Besides, specific antibodies are found in increased levels in glaucoma patients' sera, and when these antibodies access the brain, due to BBB disruption, they are capable of killing retinal cells, thereby contributing to glaucoma[3].

Given that Hp-I and its related inflammation scores are significantly associated with lower ghrelin levels and that ghrelin protects gastric mucosal cells against Hp LPS-induced apoptosis[4], it would be interesting to elucidate in the future the concept that the low aqueous humor ghrelin levels found in glaucoma patients possibly correlate with a responsible apoptotic mechanism induced or not by Hp-I.

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Key words: Helicobacter pylori; ghrelin; open-angle glaucoma; apoptosis

Conflict of interest: None

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Early enteral nutrition positively influences endocrine function in traumatic brain injury patients

Dear Editor,

Recent data provide evidence supporting the provision of early enteral nutrition (EEN) (within 24-48h) after admission to intensive care unit (ICU)[1]. Moreover, sufficient data demonstrate the influence of EEN on the deregulated endocrine system (ES) of traumatic brain injury (TBI) patients. The ES is directly or indirectly affected by TBI[2], while it participates in the metabolic and immunologic response following trauma. A recently published open-labeled randomized study investigated the effect of EEN on the ES of TBI patients[3].The effect of the onset of nutrition on the pituitary (thyroid-stimulating hormone; TSH), thyroid (free-triiodothyronine/f-T_3, free-thyroxine/f-T_4), gonadal (testosterone-males), and adrenal (cortisol) hormones were investigated on day 6 and day 12 after admission to the ICU. The mortality rate was similar in both groups (p=0.693). However, the duration of ICU stay tended to be longer in control patients with delayed enteral feeding (DEF) (p=0.06). The levels of TSH, f-T_3, f-T_4, and testosterone of DEF patients declined and cortisol concentration increased in comparison to the levels of the day of admission. Notably, the changes of hormonal values were less pronounced in the EEN group.

Overall, deficiency in hormones released via the hypothalamus-pituitary-thyroid-axis results in typical symptoms of hypothyroidism[4]. In traumatic patients the so-called "low T_3 syndrome" is supposed to counteract the catabolic processes appearing in severe illness. These data suggest that the provision of EEN to TBI patients can lead to a diminished drop of thyroid hormones; the latter may decrease the need to consume endogenous substrate storages, and therefore, intense down-regulation of TSH and thyroid hormones may no longer be required. Moreover, EEN clearly reduced increase in cortisol levels, which is very important, since injured patients with high cortisol levels show the highest risk of mortality[5]. TBI leads to hypogonadotropic hypogonadism and lowered testosterone levels in critically ill male polytrauma patients[6]. Of note, by decreasing the decline of testosterone, EEN contributes to the counteracting response against catabolic processes induced by TBI.

It is uncertain if the hormonal changes initiate the metabolic changes or they are the effect of it (thus, being a "marker" of increased catabolism or decreased anabolism). Taken together, an early onset of feeding (EEN) may exert beneficial effects on the hormonal profile of TBI patients (and also to other parameters, such as early-onset ventilator associated pneumonia reduction), possibly contributing to a better clinical outcome. Further studies are warranted to elucidate the mechanisms by which feeding is affecting the hormonal system in TBI patients.

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Letters to the Editor

Helicobacter pylori as a risk factor for cardiovascular disease: Is it or is it not?

We read with great interest the letter by Dr Kountouras et al. [1] about *Helicobacter pylori infection and the risk for cardiovascular disease*, commenting our previous article on the same subject [2]. We consider their input correct and indeed, a considerable body of past and more recent literature has linked *H. pylori* infection to cardiovascular disease (CVD). CagA-positive *H. pylori* strains have been related with a more intense immune response against the bacterium and subsequently some authors found a significantly higher prevalence of *H. pylori* infection and Cag-A seropositivity in patients with acute myocardial infarction. The authors of the letter have revealed a potential association between *H. pylori* and insulin resistance, the key pathway of the metabolic syndrome [3]. They also present the mechanisms through which *H. pylori* could influence the development of CVD.

Our study examined unselected *H. pylori* positive or negative patients for evidence of CVD and failed to show any clear association of *H. pylori* with CVD, but it revealed strong associations of CVD with well-established risk factors, such as age, hypertension, diabetes mellitus, abdominal periphery obesity and hyperlipidemia. In addition, a number of epidemiological and molecular studies have investigated the possible association between *H. pylori* and CVD. Some studies were in favor of this association, while others were against it. A population based case-control study showed a subsequent attenuation of the relationship between *H. pylori* and CVD after adjustment for age, sex, number of siblings, smoking and socioeconomic status [4], while a prospective study of 2512 cases reported that neither CVD incidence, nor heart disease mortality was related to the presence or absence of CagA strains [5].

Since eradication of proven *H. pylori* infection is usually warranted, prospective studies to investigate the impact of treatment on the incidence of CVD are beyond realistic consideration. In our opinion, the use of *H. pylori* testing to assess the risk of CVD is not warranted, since other risk factors are much more important and consistent, and eradication treatment should be reserved for patients with an appropriate indication.

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Response to Savarino *et al.*

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This letter underwent AJG editorial review.

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To the Editor: We appreciate the comments by Savarino *et al.* They suggested that *Helicobacter pylori* infection could be a cause of immune thrombocytopenia in cirrhotic patients and that rifaximin might have improved platelet counts in our study (1) by eradicating this infection (2). Indeed, the response to *H. pylori* infection may generate antibodies that crossreact with platelet antigens (3) and eradication therapy has been shown to increase platelet counts in patients with idiopathic thrombocytopenic purpura (4). However, the possibility that rifaximin treatment could have affected our results through its effects on *H. pylori* infection seems rather unlikely due to several reasons.

First, the prevalence of seropositivity to *H. pylori* infection in cirrhotic patients does not differ from that in the general population (5–7). Considering that only a minority of non-cirrhotic subjects will develop immune-mediated thrombocytopenia due to *H. pylori* infection, the contribution of *H. pylori* infection to cirrhosis-associated thrombocytopenia is reasonably expected to be limited. By contrast, rifaximin increased platelet counts in 9 of 10 thrombocytopenic cirrhotic patients in our study (1). This suggests that rifaximin improved thrombocytopenia by suppressing other more common mechanisms capable of decreasing platelet counts in the setting of cirrhosis rather than immune mechanisms triggered by *H. pylori* infection. In this regard, the administration of rifaximin in cirrhotic patients has been shown to reduce significantly endotoxemia (8), which, according to our recently reported hypothesis, could negatively affect platelet counts in cirrhosis (9).

Second, thrombocytopenia worsens with progression of cirrhosis (10) whereas the seroprevalence of *H. pylori* infection has been reported to be unrelated to liver disease severity (3) or higher in early than in advanced cirrhosis (6). By contrast, endotoxin concentrations increase proportionally to the severity of cirrhosis (11).

Finally, rifaximin alone has been proved to be effective for *H. pylori* infection, but even the highest dose (1,200 mg/daily) give a cure rate of only 30%. Eradication rates obtained with dual and triple rifaximin-based regimens are still below the standard set by current guidelines (2). Another major drawback of rifaximin could be its inability to reach sufficiently high concentrations in the gastric mucus layer under and within which *H. pylori* is commonly located. This would likely affect eradication rate and particularly in patients with portal hypertension and congestion gastropathy (2).

We conclude therefore that eradication of *H. pylori* infection by rifaximin could not be a major determinant of the beneficial effects of rifaximin on platelet counts in cirrhotic patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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High Prevalence of Significant Endoscopic Findings in Patients With Uninvestigated Typical Reflux Symptoms

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This letter underwent external review.

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To the Editor: We read with interest the article on prompt upper gastrointestinal endoscopy as an appropriate initial man-

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Letter to the Editor

Helicobacter pylori infection and the risk for cardiovascular disease

Keywords:
Cardiovascular disease
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Dear Editor:

We read with considerable interest the paper by Christodoulou DK et al. [1] concluded that there is no association of *Helicobacter pylori* (*H. pylori*) infection with documented cardiovascular disease (CVD) and eradication of *H. pylori* to prevent CVD is not warranted. Although this conclusion might be at least partially correct, it probably reflects only one side of the coin. A large body of past and more recent evidence has linked *H. pylori* infection to CVD and despite the debatable literature data, its relative role is recognizable, involving in the early events of the coronary vascular syndrome; regarding ischemic heart disease, there are new interesting data playing in favor of the association [2–7]. For instance, recent studies support the association between CagA-positive *H. pylori* infection and coronary atherosclerotic burden; the anti-CagA antibody titer is significantly higher in the patients with CVD. Moreover, a recent meta-analysis regarding 4241 cases revealed that in a subset of patients with unstable angina, an intense immune response against CagA-positive *H. pylori* strains might be critical to precipitate coronary instability mediated by antigen mimicry between CagA antigen and a protein contained in coronary atherosclerotic plaques [6]. The chronic CagA seropositive virulent strains have a trend of increasing the risk of ischemic strokes and coronary heart disease. Furthermore, patients with acute myocardial infarction (AMI) have a significantly higher prevalence of *H. pylori* infection and CagA seropositivity; this infection may influence AMI, through an inflammatory process [7].

Specifically, our recent review [8] revealed a potential association between *H. pylori* infection and insulin resistance (IR), the pathogenetic key of metabolic syndrome. It is well recognized that IR observed in patients with type 2 diabetes and obesity essentially increases the risk for CVD and the metabolic syndrome is associated with a 2-fold increase in cardiovascular outcomes; patients with metabolic syndrome show increased rates of all-cause mortality, ischemic heart disease and CVD. Importantly, the metabolic syndrome with IR and their consequences are essential factors in the pathogenesis of atherosclerosis. In this regard, the metabolic syndrome has a significant association with prior infections with *Chlamydia pneumoniae*, cytomegalovirus, herpes simplex virus type 1 and *H. pylori*; chronic infections with herpes simplex virus type 1, cytomegalovirus, *C. pneumoniae*, also mentioned by the authors, and *H. pylori* are linked with the development of atherosclerosis and coronary heart disease. Moreover, it has been reported that *H. pylori* infection appears to be one of the independent risk factors for the development of nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of IR or metabolic syndrome; NAFLD can be considered as an early mediator of the atherosclerotic process with which it shares some pathogenetic mechanisms (IR, oxidative stress, endothelial dysfunction, inflammatory activation).

Likewise, our series [2,9] demonstrated that increased fibrinogen levels (an independent risk factor for CVD) are associated with *H. pylori* infection and can be significantly reduced by *H. pylori* eradication. Other recent relative studies also reported that *H. pylori* eradication is associated with modification of certain clinical and biochemical parameters related to ischemic heart disease during a follow-up of five years; high density lipoprotein-cholesterol increases whereas C-reactive protein and fibrinogen levels diminish significantly [10]. Because this bacterium and, in particular, infection with Cag-A positive *H. pylori* strain may play a major role as a risk factor in development of ischemic heart disease through provocation of high inflammatory response or through other relative mechanisms [2], eradication of this infection might be of important as it is also much less expensive than long term treatment of the other risk factors.

Apart from its effect on fibrinogen levels, *H. pylori* could influence the development of CVD through the following possible mechanisms [2,3]: 1. *H. pylori* can coagulate blood by stimulating mononuclear cells. Under bacterial stimulation mononuclear leukocytes produce a tissue factor-like procoagulant activity, which, through the extrinsic pathway of blood coagulation, converts fibrinogen into fibrin. Thus, *H. pylori* has another activity (blood clotting) potentially contributing to CVD pathogenesis; 2. Von Willebrand factor antigen is strongly associated with *H. pylori* infection, providing solid evidence that *H. pylori*-positive patients have increased risk of CVD. In this respect, it has been demonstrated that some strains of *H. pylori* bind von Willebrand factor and interact with glycoprotein Ib to induce platelet aggregation in humans and *H. pylori* leads to diverse thrombotic and lipid protein changes; 3. *H. pylori* infection promotes formation of L- and P-selectin dependent platelet–leukocyte aggregates in murine gastric microvessels, and human *H.* pylori infection also induces platelet activation and aggregation as well as an increase of plasma levels of triglycerides and various proatherogenic factors including homocysteine. These phenomena may contribute to the proposed relationship between *H. pylori* and CVD; 4. *H. pylori* infection is associated with increased serum levels of tumor necrosis factor (TNF)-alpha, a circulating cytokine able to exert its effects at distance. TNF-alpha and interleukin (IL)-6 (TNF-alpha is the main trigger for the production of IL-6 by a variety of cells) play important roles in the regulation of the synthesis of other acute phase proteins which are established risk factors for atherosclerosis, such as the mentioned fibrinogen and factor VIII. These cytokines also have profound effects on lipid metabolism directly at the site of the atherosclerotic lesion, but could influence the atheroma process through blood circulating levels, distant production of cytokines, or through stimulating circulating white blood cells to produce them, thereby contributing to pathogenesis of heart and brain diseases via blood–brain barrier disruption. These findings shed light on the pathogenesis of some "extra-gastric" diseases (including CVD), which are significantly associated with *H. pylori* infection; 5. Anticardiolipin antibodies, linked to CVD, have also been detected in *H. pylori*-positive

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patients with CVD; and 6. Circulating concentrations of lipid peroxides, also associated with cardiovascular risk, are raised in patients with *H. pylori* infection. Regarding the latter mechanism, the combination of an antioxidant with classic triple *H. pylori* eradication therapy might improve the rate of *H. pylori* eradication.

Conflict of interest

All authors declared no conflict of interest related to this manuscript.

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2 May 2011

Helicobacter Pylori and Insulin Resistance Association: Not Just a Myth, Not Yet a Fact

Sir,

We read with interest the study of Albaker,[1] summarizing the evidence for the association between *Helicobacter pylori* (*Hp*) infection and metabolic syndrome (MetS)-related morbidity, including cardiovascular disease, the endpoint of MetS. Insulin resistance (IR) is a key pathogenetic factor in MetS. Despite its various definitions and the criticism regarding its clinical usefulness, the concept of MetS improves our understanding of the pathophysiology of IR and its metabolic and vascular consequences.[2] The association of *Hp* infection and MetS seems to be appealing, given that: a) almost half of the world's population is affected by *Hp* infection; b) the prevalence of MetS is increasing worldwide and, c) if an association is proved, *Hp* eradication might have a beneficial effect on MetS-related morbidity. However, as reported by Albaker, the existing evidence is controversial.[1] This may be partly attributed to the fact that different studies have adopted different criteria for MetS and also different criteria for the diagnosis of *Hp* infection.[3] MetS has been defined by different semi-quantitative or non-quantitative criteria, such as those of the World Health Organization, the European Group for the Study of IR, the American Association of Clinical Endocrinologists, the National Cholesterol Education Program-Adult Treatment Panel III,

and the International Diabetes Foundation. Furthermore, the diagnosis of *Hp* infection was set by serum anti-*Hp* specific IgG antibodies, rapid urease test, urea breath test, or stool antigen test, and not always by histologic detection of organisms in mucosal biopsy specimens, which is considered the practical diagnostic gold standard.[3]

We have previously published a systematic review summarizing the epidemiologic evidence concerning the association between *Hp* infection and IR quantitative-only indices.[3] The homeostatic model of assessment of IR (HOMA-IR) was used in all studies to quantify IR. There appeared to be a trend towards a positive association between *Hp* infection and HOMA-IR, which was strengthened by regression analysis in one study.[4] More specifically, when the study groups were divided according to *Hp* status (negative or positive), higher HOMA-IR was found in all but one study.[3] However, there was significant heterogeneity between studies with regard to the method(s) used for diagnosis of *Hp* infection and to the population differences among different studies. The studies on the effect of *Hp* eradication on HOMA-IR also revealed conflicting results, but there were methodological differences between them.

In conclusion, existing data indicate that the association between *Hp* infection and IR is more than a myth; however, further studies are needed to elucidate whether any causative link exists that may have potential therapeutic benefits.

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