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The Year in Helicobacter 2007

Guest Editors: Peter Malfertheiner

Francis Mégraud

Pierre Michetti

Pentti Sipponen

on behalf of the European Helicobacter Study Group

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CONTENTS

ORIGINAL ARTICLES

- 1 Epidemiology of *Helicobacter pylori* Infection
Philippe Lehours and Ozlem Yilmaz
- 4 Diagnosis of *Helicobacter pylori*
Meltem Yalinay Cirak, Yakut Akyön and Francis Mégraud
- 10 Pathogenesis of *Helicobacter pylori* Infection
Shin Maeda and Andreas F. Mentis
- 15 *Helicobacter pylori* Inflammation, Immunity, and Vaccines
Mario Milco D'Elios and Leif P. Andersen
- 20 *Helicobacter pylori* and Non-malignant Diseases
Theodore Rokkas, Ilkay Simsek and Spiros Ladas
- 23 *Helicobacter* and Gastric Malignancies
Steven F. Moss and Peter Malfertheiner
- 31 Treatment of *Helicobacter pylori*
B. J. Egan, M. Katicic, H. J. O'Connor, C. A. O'Morain
- 38 *Helicobacter pylori* Infection in Pediatrics
Gabor Veres and Ender Pehlivanoglu
- 45 Extragastric Manifestations of *Helicobacter pylori* Infection – Other *Helicobacters*
Ulrich R. M. Bohr, Bruno Annibale, Francesco Franceschi, Davide Roccarina and Antonio Gasbarrini

Epidemiology of *Helicobacter pylori* Infection

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Abstract

Helicobacter pylori infection is typically acquired in early childhood in both low- and high-income regions of the world and, once established, commonly persists lifelong unless treated. Social and economic development decreases the prevalence both within and between countries. The epidemiology of *H. pylori* infection highlights the geographic, ethnic, and racial differences throughout the world.

Helicobacter pylori is one of the most common bacterial pathogens in humans. *H. pylori* infection is now recognized as a worldwide problem. It causes chronic gastritis, peptic ulcer disease, and lymphoproliferative disorders and is a major risk factor for gastric cancer. Several reviews have focused on the epidemiology of *H. pylori* infection this year [1–6]. The present paper reviews epidemiologic studies published on *H. pylori* occurrence and transmission between April 2006 and March 2007.

Prevalence of Infection

The increase in *H. pylori* prevalence with age is largely due to a birth cohort effect rather than a late acquisition of infection. In Sao Paulo, Brazil, Zaterka et al. tested 993 blood donors with no dyspeptic symptoms for *H. pylori* infection and found a prevalence of 66.5% in men and 63.2% in women [7]. As usual, the prevalence increased with age and was higher in non-white populations; both phenomena were independent of gender. The common risk factors, i.e. crowding, type of drinking water, lack of toilet facilities during childhood, lower family income, lower educational level and previous gastrointestinal endoscopy, were observed.

There are considerable differences in *H. pylori* prevalence between high- and low-income countries and, concerning children, the prevalence ranges from less than 10% to more than 80%, respectively [8]. Goldman et al. studied 395 children with upper gastrointestinal symptoms in Buenos Aires, Argentina. *H. pylori* prevalence was 40%. They found that children with gastrointestinal symptoms were slightly older than asymptomatic children (9.9 ± 3.1 vs. 7.9 ± 4.6 years, respectively) [2].

It has been suggested that treating infected children reduces the transmission of infection and ultimately

prevents or reduces the incidence of gastric cancer in adults, however, re-infection can occur [9]. The aim of a study by Halitim et al. was to determine the rate of *H. pylori* re-infection after successful eradication in children and adolescents, and to identify risk factors associated with re-infection. Forty-five children (median age at the time of eradication: 10.9 years) were reviewed 1–9 years later; 18 had been reinfected, i.e. 24% of the children older than 10 years at the time of diagnosis [9]. The authors concluded that children are re-infected more frequently than adults and that close contact between young children, especially among siblings and children younger than 5 years, may be a more important risk factor than the age of the patient at the time of eradication treatment. Furthermore, *H. pylori* infection is also acquired in adulthood. In high-income countries, the seroconversion rate is approximately 0.5–1% per annum with a slightly higher seroreversion rate. In low-income countries, the rate of seroconversion tends to be higher and annual reinfection rates after *H. pylori* eradication in some low-income communities have been reported to be as high as 13–24%, thus being comparable to the incidence in childhood [8].

As noted by Muhsen et al., socioeconomic and living conditions are major risk factors for *H. pylori* infection and intrafamilial transmission in early childhood plays an important role [10]. Celinski et al. addressed this question by conducting a seroprevalence study in the Lublin region of Poland. The global prevalence was 78.5%. Eighty-seven percent of those born in rural areas were infected compared to 78.4% of those born in small towns and 64% for those born in big towns. A high prevalence was correlated with a lack of knowledge concerning personal hygiene. Indeed, the percentage of *H. pylori*-positive subjects neglecting basic hygiene rules sometimes exceeded 90% [11].

This year several papers focused on the influence of gender on *H. pylori* prevalence. In a large meta-analysis including 18 adult populations and 10 pediatric populations, de Martel and Parsonnet observed a male predominance in adults as a global and homogeneous phenomenon [12]. However, a similar predominance was not found in children. The authors hypothesized that differences in antibiotic exposure or differences in protective immunity between genders may explain the different results observed in the two populations. A male predominance of *H. pylori* infection was also found in a study performed in Hyderabad, South India, by Ahmed et al. [13]. Concerning the influence of immunity, Nguyen et al. showed that breastfeeding for longer than 6 months was negatively and independently associated with *H. pylori* seropositivity in 824 children aged 6 months to 15 years in Vietnam [14].

Transmission Routes

Human are the only known host of *H. pylori*. Its transmission route is not yet clearly understood. The human stomach is considered as the reservoir of this pathogen, and accepted routes of transmission are 1, the fecal–oral route in developing countries and 2, the gastro-oral route in developed countries, in which water could be a vehicle [1]. However, only molecular methods and not culture allow the detection of *H. pylori* in water. Queralt and Araujo studied the survival of *H. pylori* in a water model using culture, morphology and molecular methods. They showed that *H. pylori* survives in water but rapidly loses its cultivability and bacillar morphology, although it remains viable for long periods and its DNA is still detectable much later. They concluded that *H. pylori* could be considered as a waterborne pathogen and therefore its accidental presence in drinking water could be a risk factor for *H. pylori* transmission [15].

The importance of the type of water consumption was also noted in a study performed on the Hyderabad population in India [13]. Indeed, 71.6% male patients and 73.5% female patients who consumed municipal water were *H. pylori* positive compared to 12.6% of those consuming boiled or filtered water.

H. pylori has been cultured from vomitus, diarrheal stools, and saliva, demonstrating that the bacterium is potentially transmissible by these routes [6,8,11]. Exposure to an *H. pylori*-infected person with gastroenteritis and vomiting, presents an increased risk for infection. Indeed, in a study including household members in northern California, Perry et al. found that exposure to an infected household member with gastroenteritis was associated with a 4.8-fold increased risk, vomiting being a greater risk factor than diarrhea alone [16].

Transmission of such a ‘close-contact infection’ depends

on the degree of mixing and age distribution between susceptible and infected individuals [1,8]. De Schryver et al. studied the bacteria’s transmission from institutionalized persons with mental disabilities and a high seroprevalence of infection to 621 health-care workers. Using a multiple logistic analysis, they showed an association with fecal oral transmission but did not rule out the possibility of other forms of transmission [17].

A Turkish study was designed to evaluate the transmission routes of *H. pylori* and hepatitis A virus (HAV) by comparing the seroprevalence of these two pathogens in children. They found no significant correlation between seroprevalence of *H. pylori* and HAV. This study confirmed the existence of various transmission routes in addition to the fecal–oral route [18].

However, *H. pylori* transmission is believed to be mainly intrafamilial. Indeed, in a study conducted on family units of Japanese Brazilians living in Sao Paulo, the prevalence of *H. pylori* infection (39.2 and 9.3% for parents and children, respectively) supports the hypothesis of predominant role of mother–child transmission mainly via contact with regurgitated gastric juice from the mother’s mouth. A mother with nausea symptoms was considered to be a risk factor and a child with his or her own room had a significantly reduced risk of infection [19]. Moreover, in a commentary, Marshall indicates the case of a patient who married into an ulcer family and then developed duodenal ulcer. Spousal transfer of *H. pylori* to a *Helicobacter*-free partner might be another way of late transmission of *H. pylori* [20].

On the contrary, Delpont et al. reported that transmission has a strong nonfamilial component after performing a high-resolution analysis of nucleotide sequences of three genes. A population of 105 healthy individuals from a rural, South African, black community, for whom extensive past history information was available, were followed as part of a long-term surveillance program on *H. pylori* epidemiology. Their results contradict the hypothesis of a strict vertical transmission presented as an explanation for the strong correlation between human population history and *H. pylori* diversity and suggest the potential of recombination events in light of a horizontal transmission [21].

Interestingly, using sequencing from a large data set of *H. pylori* strains, Linz et al. showed that genetic diversity decreases with geographic distances from East Africa, the place where the modern humans are supposed to have emerged. Indeed, it appears that *H. pylori* would have spread from East Africa around 58,000 years ago [22].

Conclusion

While substantial progress has been made in understanding the role of genetic and environmental factors in the etiology

of *H. pylori*-associated disease, there is still much to be learned about the epidemiology of this infection. The association between *H. pylori* infection and low socioeconomic status and the identification of early childhood and the household as the common time and place of acquisition are important elements of the epidemiologic picture. The finding that transmission appears to occur primarily between mothers and offspring and among siblings fits into a scheme where close contact is important for transmission.

Conflicts of interest

The authors have declared no conflicts of interest.

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Diagnosis of *Helicobacter pylori*

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Abstract

Although there are attempts to perform *Helicobacter pylori* diagnosis directly in vivo using magnification endoscopy, most articles on diagnosis this year concerned non-invasive tests and molecular methods. For urea breath tests, there are attempts to have a quicker and cheaper test and to evaluate its role in cases of premalignant lesions. For stool antigens tests, evaluation of kits using monoclonal antibodies was carried out. Molecular tests have been applied for typing and detection of resistant mutants.

Endoscopy

Among the new methods of magnifying endoscopy, a prototype of endocytoscopy developed by Olympus was used for ex vivo visualization of *Helicobacter pylori* on experimentally infected gastric biopsies. Moving bacteria were observed at 1100× magnification, giving hope for a possible direct detection during endoscopy [1]. Kim et al. also used magnifying endoscopy on 103 patients to classify the gastric surface according to four patterns: flat, irregular, papillary or nonstructured, which were then compared to the updated Sydney System for histologic gastritis. Histologic gastritis was found in 91% of the biopsy sections with a nonflat type, and among them, 96% were confirmed to harbor *H. pylori* infection [2]. In another study, the magnified endoscopic findings in the gastric body were classified into four patterns and then correlated with histology results. Type 1 pattern corresponded to normal gastric mucosa, types 2 and 3 to *H. pylori*-infected mucosa and type 4 to atrophy. The sensitivity and specificity for these endoscopic findings were 92.7% and 100% for type 1, and 100% and 92.7% for types 2 and 3 together, respectively [3].

Histology

Few studies concerned histology and led to the following conclusions: nodular gastritis increases with gastritis score [4], examination of antral biopsies is sufficient to screen for lymphoid follicles [5], mast cells do not appear to be related to other inflammatory parameters [6], and methylene blue staining can be substituted for Giemsa stain to visualize *H. pylori* [7].

The presence of *H. pylori* in neoplastic tissue is a matter of controversy. Using transmission electron microscopy, Necchi et al. were able to see cytochemically proven *H. pylori* in six of eight intestinal metaplasia samples and nine of 20 cancer samples [8].

Complementary techniques to histology include immunohistochemistry and fluorescence in situ hybridization. The former technique was used to detect East Asian type *cagA* present both in the cell nucleus and in the cytoplasm, and was in agreement with *cagA* gene sequence [9]. The latter was used to detect the presence of *H. pylori* [10] and its clarithromycin resistance [11].

Rapid Urease Test

A urease test based on an immunological detection of urease was proposed for the first time in Japan. Its sensitivity was 96% but its specificity only 90% [12]. Two new rapid urease tests (RUT) based on pH change were also tested. Unfortunately, the dry RUT (GUT test) was not reliable at a 15-minute reading time [13]. The motility indole urease test, in contrast, had a high sensitivity [14].

Urea Breath Test

The ¹³C-urea breath test (¹³C-UBT) has been recognized as an excellent test because of its accuracy as well as of its robustness: the specimens can be transported without special conditions, and the result is independent of human interpretation.

Mauro et al. compared the DOB values of ¹³C-UBT samples collected every 5 minutes up to 30 minutes from 67 patients. The value after 10 minutes showed 98.6%

sensitivity and specificity compared to the test performed at 30 minutes [15].

Attempts were made to render the test cheaper by decreasing the dose of ^{13}C -urea. Yong et al. compared various additives to ^{13}C -urea in a capsule, and found that polyethylene glycol increased the initial dissolution rate of urea leading to an increased DOB and improved sensitivity of the test in volunteers [16].

The best cut-off for a positive test has been discussed at length. Based on 2232 patients explored for *H. pylori* infections with ^{13}C -UBT in a Canadian community and a cluster analysis, a cut-off point of 3.8‰ was validated. There was a slight difference between the results of those submitted to a first diagnosis (3.09) and those tested post-treatment (2.88) [17]. However, the cut-off for children younger than 5 years is higher. A study carried out in a single center on 30 *H. pylori*-positive children during 7.5 years confirmed that the best specificity was obtained with a cut-off of 8.8‰ [18].

Interestingly, Shmueli et al. compared DOB values from a large series of tests (7373) and noted an age-adjusted difference of 7 (95% CI 6.4–7.9) between genders, with a higher DOB value for females. This result may indicate a higher bacterial load in women [19]. However, other studies indicate that ^{13}C -UBT is not a quantitative test. No correlation was found between DOB values and bacterial count at any site or histologic grading of *H. pylori* in 19 subjects according to Tummala et al. [20]. A similar result was obtained in children [21].

A potential reason for false negative UBT is the presence of atrophy. However, studies have shown that in this particular case, UBT can be helpful in addition to serology to diagnose *H. pylori* [22]. Capurso et al. tested the hypothesis that UBT results are affected by gastritis phenotype. They compared UBT to intragastric pH in 66 patients in a multivariate analysis and found that the only risk factor for a false negative UBT was corpus predominant gastritis [23].

Among the variations of ^{13}C -UBT, ^{14}C -UBT using a microdose of ^{14}C has been proven to be accurate and economical [24,25]. A ^{13}C -urea blood test was also found to be reliable and well tolerated in children [26].

Stool Antigen Test

The stool antigen test is considered as a valuable noninvasive alternative to diagnose *H. pylori* when UBT is not available. A second generation of kits, based on monoclonal antibodies, has already been used for several years.

Gisbert et al. carried out a systematic review and meta-analysis on the accuracy of these tests for diagnosis and for treatment follow up [27]. They analyzed 22 studies, including a total of 2499 patients where the tests were performed prior to eradication. Pooled sensitivity and

specificity were 94% (95% CI: 93–95) and 97% (95% CI: 96–98), respectively. In 13 studies where polyclonal antibody-based stool antigen tests were compared to monoclonal antibody-based tests, a higher sensitivity was shown for the latter (95% vs. 83%). Twelve studies including a total of 957 patients assessed monoclonal antibody-based tests post-eradication, with pooled sensitivity and specificity of 93% (95% CI: 89–96) and 96% (95% CI: 94–97), respectively; again they showed a better sensitivity than polyclonal antibody-based tests (91% vs. 76%) in eight studies when both were performed.

In studies published this year, however, the results are not generally good. HpSTAR (Dako, Glostrup, Denmark) provided good results in some studies [28–30] but not in others. The pretreatment specificity was low in a study by Dominguez et al. [31] as was the post-treatment sensitivity as reported by Quesada et al. [32]: 70.7% and 73%, respectively.

Immunocard HpSA (Meridian, Diagnostic, Cincinnati, OH, USA) is a point-of-care test also using monoclonal antibodies. It had a good accuracy (96%) in a pediatric study [28] but a low sensitivity in adults according to Hooton et al. (79%) [29] and a low specificity according to Lu et al. (82.8%) [33]. Both sensitivity and specificity were satisfactory in another study (91% and 97%, respectively).

Antibody Detection

The detection of multiple antibodies in serum by protein array has been used for *H. pylori* diagnosis. This array is comprised of three recombinant *H. pylori* antigens: UreB, VacA and CagA immobilized on nitrocellulose membranes. Bound IgG are detected using staphylococcus protein A labeled with colloidal gold. Sensitivity and specificity were above 90% compared to ELISA [34]. This rapid and reproducible test may be a future competitor to immunoblot.

Indeed, attempts to correlate a specific disease with antibodies directed toward specific *H. pylori* antigens are still being made. It has been known for many years that antibodies against CagA are associated with peptic ulcer disease [35] but they are not specific enough to screen these patients among dyspeptic patients [36]. A specific immunoblot pattern indicating infection with a more virulent strain was associated with active inflammation as well as atrophy and intestinal metaplasia in the antrum [37]. A significant association (OR:19.5) was found between the presence of gastric cancer and the presence of IgG against three *H. pylori* antigens of 19.5, 33 and 136 kDa (CagA) [38]. CagA and VacA antibodies were valid markers of past *H. pylori* infection when standard *H. pylori* serology was negative following atrophic body gastritis [39]. Yang et al. also confirmed that immunoblot can detect *H. pylori* antibodies in gastric cancer patients when other tests are

negative [40]. However, the presence of antibodies to a VacAm region-specific antigen was not able to predict the risk of gastric cancer development [41].

In the past, ELISA performed with antigens obtained from local strains led to better results than when kits were used. In a study carried out in Vietnam, Pyloriset EIA-GIII (Orion Diagnostics, Espoo, Finland) as well as Helicoblot 2-1 (GeneLabs, Singapore) performed equally well in this population [42]. However, in Thailand Pyloriset EIA-GIII had a low specificity (75.3%) [43].

The problem of distinguishing false positive tests from acute or transient infection when a single test is positive was addressed by measuring pepsinogen levels, and the conclusion was that most transient infections are indeed false positives [44].

Molecular Methods

Molecular methods are widely used for the diagnosis of *H. pylori* infection as well as analyses of diversity, virulence, persistence and resistance patterns of these bacteria. Minami et al. proposed a novel and quick identification system for *H. pylori* which is a combination of the endoscopic brushing technique and the loop-mediated isothermal amplification method (LAMP). Among the samples from 200 patients, 123 brushing samples were *H. pylori* positive using LAMP primers constructed for the *glmM* gene within a 90-minute detection time with 100% sensitivity and specificity, whereas 100 patients were positive when only biopsy samples were tested [45].

Typing

Genetic diversity of *H. pylori* in the same patient is a challenging dilemma considering the accuracy of diagnosis. For differentiation of mixed infections with *H. pylori* strains, enterobacterial repetitive intergenic consensus-polymerase chain reaction (PCR) has a high discriminatory power and is time-efficient compared to random amplified polymorphic DNA (RAPD) fingerprinting. Finger et al. detected the presence of more than one *H. pylori* strain in more than half of the 63 patients studied [46]. In another study where multiple single *H. pylori* colonies from different regions of the stomach of eight adult and four pediatric patients were analyzed, the presence of two distinct genomic profiles of *H. pylori* strains was demonstrated in a single adult patient, differing at 113 gene loci including the *cag* PAI virulence genes, by using RAPD, amplified fragment length polymorphism and comparative genomic hybridization microarray [47]. A study on 250 Jordan patients using PCR showed this genetic diversity with a predominance of *iceA2* (73.6%), a high frequency of the *vacAs2* allele, and a low proportion of *cagA* genotype [48].

With regard to the importance of diagnosis in children, Oleastro et al. identified new candidate markers for childhood peptic ulcer disease by suppressive subtractive hybridization analysis [49]. Two *H. pylori* virulence genes, *jhp0870* and *jhp0562*, related to outer membrane protein and lipopolysaccharide biosynthesis, respectively, were shown to play a conspicuous role in the pathogenesis of peptic ulcer in children. The positivity rate for *jhp0870* was 80.0% in 15 ulcers versus 36.7% in the control group of 30 gastritis specimens and for *jhp0562* 80.0% versus 33.3%. A Brazilian study on *cagA* using both histology and PCR showed that 57 of 121 (47%) children were positive for *H. pylori*, of which *cagA* strains were found in 20 of 29 (69%) children with chronic gastritis and in 18 of 28 (64%) with normal mucosa, suggesting an initial infection with the bacteria [50].

The relationship between host gene polymorphisms and *H. pylori* genotypes has been emphasized in a certain number of studies as well. Among 302 *H. pylori*-infected cases in China, carriers of the proinflammatory *IL-1B-511* T allele and *H. pylori vacA* m1 genotype had an approximately fourfold higher risk of developing intestinal metaplasia [51]. Another study using oligonucleotide allele-specific PCR on samples from 233 patients detected a significant difference in the frequency of the IL-8-251 A/T polymorphism and *H. pylori vacA* gene polymorphisms among gastritis, peptic ulcer, and gastric cancer patients [52].

Multiplex PCR is also used for genotyping *H. pylori*. Bolek et al. found a correlation between *cagA*-positivity, *vacA-s1m1* genotype, and peptic ulcers [53]. In another genotyping study, a significant association between *H. pylori vacA* s1a, *cagA*, and *cagE* genotypes and duodenal ulcer and gastric cancer as well as between *iceA1* and *babA2* and gastric cancer, was demonstrated in Turkish patients with dyspepsia [54].

Genetic diversity of the 3' variable regions of the *cagA* gene and determination of the related EPIYA phosphorylation motifs were explored by one-step PCR in a Greek study of 75 adults and 60 children in order to identify closely related *H. pylori* subclones within the same patient; the results suggested that a prediction of the number of EPIYA repeats sheds light on the prognosis of infection [55]. In a Korean study on the diversity of the 3' end of the *cagA* gene and the relationship between EPIYA motifs and clinical outcome among 79 patients suffering from gastritis, peptic ulcer and gastric cancer, 76 (96.2%) harbored the East Asian type without any significant difference [56].

Antimicrobial Resistance

Owing to the difficulties of culturing these bacteria, molecular methods are of great interest in the detection of

antimicrobial resistance. Nishizawa et al. developed an allele-specific PCR for the detection of *gyrA* mutations in fluoroquinolone-resistant *H. pylori* strains [57]. The rate of *H. pylori* resistance to furazolidone was reported to be 8.7% in China, and six mutations in *porD* and *oorD* genes were identified in these resistant isolates [58].

Gerrits et al. found that multiple mutational changes in the *pbp1A* gene led to amoxicillin resistance in *H. pylori*, which renders the development of a molecular test difficult in contrast to cases of clarithromycin and tetracycline resistance [59]. At the same time, Kim et al. confirmed the association of *pbp1A* gene mutations and amoxicillin resistance using sequence analysis [60].

A study employing TaqMan technology showed no association between clarithromycin resistance and *cagA* and *vacA* status in paraffin-embedded biopsy specimens by real-time PCR [61]. The same group from Italy also showed a twofold increase from 10.2 to 21.3% in primary clarithromycin resistance rate in *H. pylori* strains over 15 years and determined the most prevalent point mutation as A2143G [62].

Among the PCR-based methods, PCR-RFLP is an appropriate technique for detecting point mutations. Raymond et al. detected mutations in the 23S rRNA genes of *H. pylori*, the most prevalent being A2143G. Furthermore, two different mutations were identified in the same biopsy specimen and the rate of resistance increased from 18.6% during the period 1993–96 to 41.6% during 2001–04 [63].

A novel biprobe, the ClariRes real-time PCR assay, used for the detection of *H. pylori* infection and simultaneous clarithromycin susceptibility testing in stool samples was evaluated. It was less effective than expected with 63% sensitivity for an accurate diagnosis in children [64].

Further research concerning DNA biosensors was carried out for single-base polymorphism detection. A label-free electrochemical DNA hybridization detection method using peptide nucleic acid probes was developed for the evaluation of A2143G in the 23S rRNA gene of *H. pylori* [65].

A novel diagnostic microarray for the identification of a group of pathogenic bacteria including *H. pylori* using competitive oligonucleotide probes with a high detection sensitivity range of 0.1% was developed by Kostic et al. as a new approach [66].

DNA microarray analysis is currently used as well for the comparison of *H. pylori* genomes. In a Chinese study, 1636 genes including 522 strain-specific genes were tested for the identification of pathogenic strains. Results of this kind concerning genome evaluation highlight the virulence and pave the way for candidate vaccines for *H. pylori* [67].

In summary, there were no great breakthroughs this year in the diagnosis of *H. pylori* infection. However, serology, previously considered not specific enough for diagnosis, was recommended in the Maastricht III

Conference Report because this method is not influenced by the consumption of proton-pump inhibitors which is currently a common treatment among patients seeking a specialized consultation [68].

Conflicts of interest

The authors have declared no conflicts of interest.

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Pathogenesis of *Helicobacter pylori* Infection

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Abstract

The clinical outcome of *Helicobacter pylori* infection is determined by a complex interaction between the bacterium and the host. The main bacterial factors associated with pathogenicity comprise outer membrane proteins, including BabA, SabA, OipA, AlpA, and AlpB, the vacuolating cytotoxin VacA and the products of *cag*PAI. The multitude of putative virulence factors makes it extremely difficult to test the contribution of each individual factor. Much effort has been put into identifying the mechanism associated with *H. pylori*-associated carcinogenesis. Interaction between bacterial factors such as CagA and host signal transduction pathways seems to be critical for mediating cell transformation, cell proliferation, invasion, apoptosis/anti-apoptosis, and angiogenesis. An animal model using the Mongolian gerbil is a useful model for showing gastric pathology due to *H. pylori* infection which is similar to that in humans and can be used to evaluate virulence factors including CagA, host responses, and environmental factors such as salt intake.

Colonization and Adherence

The majority of *Helicobacter pylori* cells are found in the gastric mucus layer overlying the epithelium; however, the organism has been reported to interact with gastric epithelial cells and even invade them. *H. pylori* biofilm formation has recently been reported to be involved in the colonization of the human stomach. In biopsies from human gastric mucosa, mature biofilms covering almost the entire mucosal surface were observed in urease-positive patients, while coverage was less than 2% in urease-negative patients [1]. *H. pylori* adherence to gastric epithelial cells facilitating access to nutrients and delivery of effector molecules has been considered essential for development of the disease. Several of the *H. pylori* Hop proteins (outer membrane proteins) have been identified as adherence factors, including BabA, SabA, OipA, AlpA, and AlpB; however, their exact role in *H. pylori* pathogenicity remains unclear. Unlike previous reports, *H. pylori* strains expressing low levels of BabA contributed to more severe mucosal injury and were more frequently associated with duodenal ulcer (DU) and gastric cancer (GC) than strains with a high-level expression of BabA or those lacking the *babA* gene. Moreover, Le(b)-binding activity or the presence of *H. pylori* strains with triple-positive status (*cagA*+/*vacAs1*/*babA*-H) did not accurately reflect the

severity of mucosal damage or relate to the clinical outcome [2]. Colbeck et al. found extensive genotypic diversity in *babA* and *babB* among different strains, as well as in a strain colonizing an individual patient, which may reflect selective pressures for adherence [3].

Dossumbekova et al. [4] reported that *hopH* (*oipA*) mutagenesis resulted in lower adherence to gastric epithelia in vitro, but did not alter the epithelial interleukin (IL)-8 secretion and confirmed previous observations that in-frame ("on" genotype) *hopH* alleles are associated with the presence of *vacAs1*, *vacAm1*, *bab2*, and *cagA* genotypes. OipA-positive expression status was significantly associated with the presence of DUs and GC, high *H. pylori* density, and severe neutrophil infiltration, while SabA positive status was associated with GC, intestinal metaplasia as well as corpus atrophy, and negatively associated with DU and neutrophil infiltration [5]. Another major point was that SabA expression frequently switched "on" or "off", suggesting a response to changing conditions in the stomach. Lu et al. [6] confirmed that AlpAB may be involved in cellular adherence as whole *alpA*/*alpB*-deleted mutants were poor colonizers of the stomachs of C57BL/6 mice, and were associated with lower mucosal levels of proinflammatory effectors KC and IL-6. Interestingly, deletion of *alpAB* reduced IL-8 induction by AGS cells following infection with East Asian but not with Western strains.

cagPAI, VacA

H. pylori strains with more EPIYA motifs induce higher levels of CagA phosphorylation and more cytoskeletal rearrangements, which are associated with atrophic gastritis and GC. By sequential immunoprecipitation and immunoblot, it was shown that: (1) CagA proteins carrying multiple EPIYA-C or EPIYA-D sites bound to and deregulated SHP-2 more strongly than those with a single EPIYA-C or EPIYA-D, and that (2) the ability of CagA to bind to Csk was correlated with the number of EPIYA-A and EPIYA-B sites [7]. Ren et al. [8] reported that CagA multimerization within the EPIYA-C segment as well as in sequences located immediately downstream of the EPIYA-C or EPIYA-D segments is a prerequisite for CagA-SHP-2 interaction and subsequent deregulation of SHP-2. However, from a clinical perspective, there was no significant correlation between the number of EPIYA motifs or CagA subtypes and various gastroduodenal diseases [9]. Isogenic *H. pylori* strains, from the same patient, expressing CagA with different numbers of EPIYA-C repeats have been reported to exist in approximately 10% of the population and should be taken into consideration during routine EPIYA screening of *H. pylori* isolates [10].

The *H. pylori vacA* gene encodes a secreted protein (VacA) that has been reported to exhibit a pleiotropic activity on gastric epithelial cells as well as on T lymphocytes. Shirasaka [11] proposed that VacA binding to its receptor PTP zeta/beta results in gastric epithelial detachment and eventually gastric ulceration through abnormal signaling. Based on their data, Terebiznik et al. [12] proposed that VacA-dependent *H. pylori*-containing vacuoles protect the bacterium from the bactericidal components of the lysosomal pathway, promoting bacterial survival and contributing to the persistence of infection.

Other Potential Virulence Factors

Potential new markers associated with disease-related strains include the gene jph0870 [13] and outer membrane proteins Omp26, Omp30, and Omp6 [14], the gene jph0562 for LPS biosynthesis [13], and the hp0015 homolog of *H. pylori* strain 26695 [15]. Lin et al. [16], using two-dimensional immunoblots, identified elongation factor EF-G (FusA), catalase (KatA), and urease alpha subunit (UreA) as DU-related antigens, showing a higher seropositivity in DU samples than in GC. A novel lipolytic enzyme (EstV) encoded by ORF HP0739 of *H. pylori* 26695, isolated, cloned, and purified by Ruiz et al. [17], was proposed to be involved in mucus degradation and the release of proinflammatory and cytotoxic compounds. Finally, Baldwin et al. [18], by monitoring the colonization ability of transposon mutants of two *H. pylori* strains in a mouse

model, identified 10 previously uncharacterized colonization gene loci candidates.

H. pylori Infection and Signal Transduction Pathway

Activation of signal transduction pathways caused by *H. pylori* infection has been studied extensively in recent years. Nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) activation are the critical regulators of innate immune responses and inflammation. Pathak et al. found that HP0175, which is a peptidyl prolyl *cis*-, *trans*-isomerase, was capable of interacting directly with the extracellular domain of Toll-like receptor 4 (TLR4). HP0175-induced IL-6 gene expression was critically dependent on TLR4-dependent NF- κ B and MAPK activation in monocytic cells [19]. Obonyo et al. also showed that TLR2 or TLR4 is required for *H. pylori*-induced cytokine expression such as IL-1 β and IL-6 in macrophages [20]. Moreover, Zhao et al. noted that NF- κ B and MAPK signaling linked to the TLR2 might be necessary for *H. pylori*-HSP60-induced IL-8 secretion [21]. These observations suggest that TLR-mediated signal transduction pathways such as NF- κ B and MAPK are critical for the pathogenesis of *H. pylori* infection. Interestingly, several reports revealed that polymorphisms in genes related to bacterial lipopolysaccharide/peptidoglycan signaling such as TLR or CD14 may be implicated in the development of gastric premalignant lesions and GC [22–24]. Cho et al. analyzed the crosstalk between MAPK and NF- κ B activation by *H. pylori* and found that extracellular signal-regulated kinase (ERK) induced phosphorylation of I κ B α in *H. pylori*-infected AGS cells [25].

Dysregulation of apoptotic and anti-apoptotic pathways has been recognized as an important factor in *H. pylori*-mediated pathogenesis [26]. *H. pylori*-infected antrum showed greater surface epithelial apoptosis that decreased after eradication therapy [27]. In vitro, *H. pylori*-induced apoptosis of T cells is mediated by the mitochondrial pathway and might necessitate a local environment that facilitates life-long infection [28]. In addition to the previous reports, virulence factors such as VacA, gamma-glutamyltranspeptidase, external membrane vesicles, have been shown to be inducers for apoptosis [29–31]. An anti-apoptotic function of *H. pylori* has also been reported. Low multiplicity of *H. pylori* infection suppresses apoptosis of B lymphocytes and suggests that the low levels of infection that occur in the human stomach are associated with cell survival, proliferation, and development of mucosa-associated lymphoid tissue lymphoma [32].

CagA/cagPAI and Host Response

As previously described, the CagA protein, an *H. pylori* virulence factor, induces morphologic changes in host cells

and may be associated with the development of peptic ulcer and GC. The mechanism concerning how CagA contributes to the pathogenesis of *H. pylori* infection is still obscure. Murata-Kamiya et al. reported that CagA can interact with E-cadherin independently of CagA tyrosine phosphorylation. This interaction impairs the complex formation between E-cadherin and β -catenin and causes nuclear accumulation of β -catenin. Dysregulated β -catenin transactivates genes such as *cdx1*, which encodes an intestinal specific CDX1 transcription factor, and may be implicated in the development of intestinal metaplasia [33]. They also reported that FAK, via SHP-2, plays a crucial role in the morphogenetic activity of CagA, and impaired cell adhesion and increased motility may be involved in the development of gastric pathology due to *H. pylori* infection [34]. Poppe et al. identified the nonreceptor tyrosine kinase c-Abl as a crucial mediator of *H. pylori*-induced migration and a novel CagA kinase in epithelial cells. C-Abl interacts directly with CagA and is localized in focal adhesion complexes and membrane ruffles, which are dynamic cytoskeletal structures necessary for cell motility [35].

Chang et al. explored the effect of CagA on the expression of cyclin D1, an important cell cycle regulator. *H. pylori*-induced cyclin D1 expression was attenuated in a *cagA* mutant, and AP1 and cAMP response elements (CRE) were involved in the induced cyclin D1 expression [36].

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine implicated in carcinogenesis. Beswick et al. revealed that *H. pylori* induced MIFs which were dependent on CagA. MIF can bind to CD74, which is highly expressed on the surface of gastric epithelial cells. They also investigated the role of the *H. pylori*-induced MIF on epithelial proliferation and procarcinogenic events and found that recombinant MIF and *H. pylori* increased cell proliferation. Proliferation was decreased when *cagA*-negative strains were used and when CD74 was blocked by mAbs. Furthermore, MIF binding to CD74 was also shown to decrease p53 phosphorylation and up-regulate Bcl2 expression [37].

A relationship between CagA and pro- and anti-apoptosis factors has been reported. Infection with *cagA*-positive strains is associated with an over-expression of pro-apoptotic proteins in the gastric mucosa, mainly at the antral lesser curvature, which may play a role in atrophy development [38]. Zhu et al. investigated whether CagA modulates the activation of MAPKs and their downstream apoptosis regulators in B lymphocytes. Transfection of lymphocytes with *CagA* transiently increased Erk1/2 phosphorylation, which was negatively regulated by MAPK phosphatases, MKP-1 and MKP-6. Activation of MAPK led to phosphorylation of Bad at Ser-112 which plays a role in anti-apoptosis [39].

The role of *cagPAI* in *H. pylori* cellular invasion remains controversial. Oliveira et al. [40] reported, in agreement with previous observations, that the invasion of AGS cells was a bacterial T4SS-dependent event involving the activation of c-Met receptors and the up-regulation of matrix metalloproteinase (MMP)-2 (gelatinase-2) and MMP-9 (gelatinase-9) activity. However, Kundu et al. [41] reported that a strain lacking the *cagPAI* and the strain SS1 with a non-functional CagPAI was equipotent in increasing pro-MMP-9 induction, but affected MMP-2 activity only moderately. They also provided data showing that up-regulation of MMP-9 may be mediated via proinflammatory cytokines through different pathways other than *cagPAI*.

Pathogenesis of *H. pylori* in an Animal Model

The Mongolian gerbil is a useful model because the gastric pathology caused by *H. pylori* infection in gerbils is similar to that in humans. Shibata et al. [42] reported that although the stomachs of Mongolian gerbils were colonized at similar densities by wild-type *H. pylori* strains and isogenic mutants with disrupted *cagA*, or *cagE* genes, the $\Delta cagA$ mutant induced milder gastritis. Kudo et al. analyzed the role of transcription factors in the gastric mucosa of *H. pylori*-infected gerbils and observed that the gastric mucosal transcription factors induced by *H. pylori* infection differed according to the phase and outcome of infection. AP-1 and CREB levels were early detectors related to inflammation and ulceration, whereas NF- κ B and ISRE were late detectors related to atrophy [43]. Cao et al. demonstrated that the term and severity of *H. pylori* infection might play important roles in gastric carcinogenesis, with an essential involvement in chronic inflammation [44]. Kato et al. studied dose-dependent enhancing effects of salt in chemical carcinogenesis in *H. pylori*-infected gerbils and found that a reduced salt intake could be one of the most important chemopreventive methods for human gastric carcinogenesis [45]. Mongolian gerbils seem to be a very valuable model for *H. pylori* infection; however, Otaka et al. reported that Mongolian gerbils might be special animals in which HSP70-induction is absent and the mucosal protective ability in HSP-dependent cytoprotection in the gastric mucosa is extremely poor. They suggest that the Mongolian gerbil model is not adequate to evaluate the effect of *H. pylori*-associated gastric inflammation followed by development of GC [46]. This assumption warrants a more thorough discussion.

H. pylori Infection and Carcinogenesis

Recently, epigenetic silencing of gene expression by CpG island methylation was recognized as an important

mechanism in inactivating tumor suppressor genes [47]. Leung et al. and Chan et al. reported that promoter methylation in E-cadherin was frequently detected in the stomach of *H. pylori*-infected patients. Eradication of *H. pylori* possibly reduced the methylation density in the E-cadherin gene and the chance of subsequent neoplastic transformation [48,49]. Another important factor for carcinogenesis might be gene mutations. Yao et al. reported that *H. pylori* might lead to an accumulation of genomic mutations, independently of inflammation. This is associated with a reduced DNA mismatch repair, and is in part associated with CpG methylation of the hMLH1 promoter [50]. In clinical samples, Watari et al. reported that K-ras mutations occurred at an early stage, and are influenced by *H. pylori* infection, and that some mutations may also be selected by eradication [51].

Gastric cancers express enhanced levels of MMPs and their tissue inhibitors (TIMPs). MMP-7 is produced in epithelia and increases with *H. pylori* infection. McCaig et al. studied the role of MMP-7 in signaling between epithelial cells and a stromal cell type, myofibroblasts, and found that MMP-7 from *H. pylori*-infected epithelial cell medium stimulated proliferation and migration of gastric myofibroblasts. Proliferation of gastric epithelial cells was also stimulated by MMP-7-treated myofibroblasts [52]. These results suggest that MMP-7 is a critical regulator for redefining the gastric microenvironment and leads to hyperproliferation in response to *H. pylori* infection. An elevated expression of tissue matrix metalloproteinase 1 (MMP-1) is also associated with GC. Wu et al. investigated the regulation of MMP-1 expression during *H. pylori* infection and found increased MMP-1 mRNA levels in the gastric mucosa and epithelial cells [53].

A basic and important concept concerning the pathogenesis of *H. pylori* was reported by Necchi et al. It is not clear how *H. pylori*, an apparently extracellular pathogen, colonizes the luminal side of the gastric epithelium. They showed that *H. pylori* penetrates normal, metaplastic, and neoplastic gastric epithelium in vivo, intracellularly or interstitially, causing a strong immune-inflammatory response and promoting gastric carcinogenesis [54].

Conflicts of interest

The authors have declared no conflicts of interest.

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Helicobacter pylori Inflammation, Immunity, and Vaccines

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Abstract

Helicobacter pylori infects almost 50% of the world population and is the major cause of gastroduodenal diseases. *H. pylori* colonizes the gastric mucosa, activates Toll-like and Nod-like receptors, and usually elicits a T helper 1 (Th1) type of immune response, fully polarized in peptic ulcer patients. Among several bacterial factors, the neutrophil-activating protein represents a key factor driving Th1 inflammation. A complex and fascinating balance between *H. pylori* and host factors takes part in the gastric niche and allows the majority of infected individuals to be without any symptom during their entire life. Novel insights into the innate and adaptive responses against *H. pylori*, dealing with regulatory T cells and cytokines, CTLA-4 molecule, cholesterol glucosylation, and immune evasion have been elucidated during the past year and are discussed for the development of an effective vaccine.

Helicobacter pylori chronically infects the stomach of more than half of the human population and represents the major cause of gastroduodenal pathologies. However, only 10–20% of *H. pylori*-infected patients develop severe diseases, such as peptic ulcer, gastric cancer, and lymphoma, during their lifetime. This fact suggests that the type of innate and acquired immune response to *H. pylori* may represent an important factor able to influence the outcome of the infection towards protection, evasion, or pathology. Here we present an overview of the major findings on the host response to *H. pylori* published over the past year.

Epithelial and Innate Immune Responses

H. pylori activates a wide spectrum of events resulting in modulation of host epithelial and innate defense. Natural immune responses strongly depend on host recognition of invariant structures, namely pathogen-associated molecular patterns, by different innate sensors, such as Toll-like receptors (TLR). The infiltration of inflammatory cells within the gastric mucosa is a common finding in *H. pylori* infection, and the degree of mucosal damage correlates with neutrophil infiltration. Different components present in *H. pylori* extracts directly attract and activate neutrophils and other inflammatory cells. The *H. pylori* heat-shock protein 60 is able to induce interleukin (IL)-8 production

in human monocytes via TLR2, by activation of either extracellular signal-regulated kinase (ERK) or mitogen-activated protein kinase pathways (MAPK) [1]. *H. pylori* neutrophil-activating protein (HP-NAP) is a 150-kDa oligomeric protein isolated from *H. pylori* that was found to promote neutrophil adhesion to endothelial cells, to induce production of reactive oxygen radicals by neutrophils, and to increase the synthesis of tissue factor (TF) and plasminogen activator inhibitor-2 in mononuclear cells. HP-NAP efficiently crosses the endothelia, promotes rapid neutrophil adhesion in vitro and in vivo, and stimulates neutrophils to synthesize and release several chemokines, including CXCL8 (IL-8), CCL3 (macrophage inflammatory protein-1 α), and CCL4 (macrophage inflammatory protein-1 β), that contribute to further recruitment of neutrophils, monocytes, dendritic cells, and lymphocytes [2]. HP-NAP induces also a progressive and consistent maturation of monocytes into mature dendritic cells showing high expression of surface class II major histocompatibility complex molecules, CD80, and CD86 [3].

H. pylori factors are able to elicit IL-6 production by macrophages in the gastric environment [4]. The secreted peptidyl prolyl *cis*-, *trans*-isomerase, HP0175 induces IL-6 gene expression and IL-6 release from macrophages, via direct interaction with the extracellular domain of TLR4. The HP0175-induced IL-6 gene expression was critically

dependent on nuclear factor- κ B (NF- κ B) and MAPK activation. TLR4-dependent ERK1/2 and p38 MAPK signaling converged upon activation of mitogen- and stress-activated protein kinase 1 and subsequent IL-6 gene transcription through chromatin modification at the IL-6 promoter [5].

Experimental evidence has suggested that epithelial cells can respond to conserved bacterial products via receptors other than TLRs. A new family of intracytoplasmic pathogen-recognition molecules, the Nod-like receptor, with homology to host plant resistance protein has been described. Nucleotide-binding oligomerization domain (Nod) 1 is an important sensor for *H. pylori* peptidoglycan, strongly dependent on the bacterial type IV 'syringe', encoded by the *cag* pathogenicity island (PAI). The relevance of Nod1 in antimicrobial immunity is demonstrated by higher susceptibility to *H. pylori* infection in Nod1-deficient mice. Moreover, innate immune sensing of peptidoglycan by Nod1 is a key factor for the onset of antigen-specific acquired immunity, priming either T-helper cell or antibody responses [6]. *H. pylori*-dependent NOD1 activation triggers human β -defensin (h β D)2, a critical component of host mucosal defense and is strictly related to the presence of the bacterial *cag*PAI, whether h β D3 expression is epidermal growth factor receptor and ERK pathway dependent [7]. Epithelial cells could contribute to the host response via activation or inhibition of suppressors of cytokine signaling-3 pathway or macrophage migration inhibitory factor [8,9]. *H. pylori* colonization induces local inflammation and oxidative stress in the stomach that can be modulated by a complex network of bacterial and host factors [10–13]. NF- κ B and apurinic/apurimidinic endonuclease-1/redox factor-1 have been recently shown to represent important regulators of host gene expression, during *H. pylori* infection [14].

Cytokine Network and *Helicobacter*

Interleukin-12 plays a key role in natural host defense by inducing natural killer cell interferon (IFN)- γ production and by favoring the differentiation of IFN- γ -secreting T helper (Th)1 cells. IL-12 is also involved in chronic inflammatory disorders characterized by excessive Th1 responses. Recently, a new member of the IL-12 cytokine family, IL-23, able to drive Th1 responses, was identified. IL-23, mainly secreted by activated dendritic cells and macrophages, consists of two subunits: one, p40, shared between IL-23 and IL-12, and the other, p19, specific for IL-23. *H. pylori*, and particularly HP-NAP, has shown a strong ability in inducing IL-23 and IL-12 production in human monocytes, dendritic cells, and neutrophils [3].

Using mouse models of *H. hepaticus*-induced T-cell-dependent colitis, it was demonstrated that IL-23 and not IL-12 is essential for the development of a severe disease.

Although IL-23 has been implicated in the genesis of other inflammatory disorders via the activation of IL-17-secreting Th cells, in this *H. hepaticus* model of colitis, IL-17 in the absence of IFN- γ did not appear sufficient to induce disease, the maximum intestinal inflammation depending on both IFN- γ and IL-17 [15]. In another model, colonization with *H. bilis* of LPS-nonresponder C3H/HeN mice, resulted in production of IL-12, TNF- α , IL-6, IFN- γ , and development of immune-mediated intestinal bowel disease [16,17]. *C. jejuni* infection, as well, triggered an IL-12 production and a polarized Th1 response [18].

Using different strains of *H. pylori*, it has been shown that CagE stimulates a high production of IL-12 by murine dendritic cells, whereas a higher multiplicity of infection or chronic exposure to *H. pylori* down-regulates IL-12 via IL-10. CD40 ligation indeed increases IL-12 release induced by *H. pylori* [19–22]. On the other hand, low multiplicity of infection suppressed apoptosis of B lymphocytes, and promoted B-cell survival and proliferation, potentially leading to mucosa-associated lymphoid tissue lymphoma [23,24].

Th1 Driving Factors in *H. pylori* Infection

T-helper cells orchestrate host defense against pathogens via different types of cytokine secretion and effector functions. In *H. pylori* infection, a predominant activation of Th1 cells with production of IFN- γ , IL-12, IL-18, IL-17, and TNF- α , occurs in vivo in the antrum. New data shed light on important mechanisms responsible for this mucosal Th1 polarization. Stimulation of neutrophils, monocytes, and dendritic cells with HP-NAP resulted in prompt and remarkable up-regulation of IL-12 and IL-23 mRNA expression and protein secretion, via TLR2 activation. In the gastric mucosa of *H. pylori*-infected patients, a remarkable proportion of Th cells show a significant proliferation to different *H. pylori* antigens, including HP-NAP. HP-NAP drives production of high levels of IFN- γ and TNF- α by antigen-specific gastric Th cells and elicits a powerful cytotoxic activity, thus promoting a polarized Th1 response [3]. *H. pylori* factors other than HP-NAP may contribute to the generation of the Th1-type milieu found in the gastric mucosa of *H. pylori*-infected subjects. Outer membrane preparations of *H. pylori*, but not urease, are very effective in eliciting IL-12, IFN- γ , and TNF- α secretion in vitro [25]. Accordingly, the products of the *cag*PAI island, as well as those of the plasticity region, the outer membrane protein 18, and the cysteine-rich protein A might be relevant in inducing IL-12 expression and Th1 polarized response in vivo. *H. pylori* lipopolysaccharide (LPS) was able to promote a Th1 type immune response in immunized mice [26]. T-bet is an important T-cell transcription factor, which is required for differentiation of IFN- γ -secreting Th1 cells. Using congenic and T-bet-knock-out mice, *H. pylori*

was able to induce gastric inflammation and disease either via T-bet-dependent and T-bet-independent mechanisms, suggesting that T-bet and other pathways of T-cell activation are involved in *H. pylori* inflammation [27].

***H. pylori* Persistence and Immune Evasion**

H. pylori has colonized the human stomach since the earliest times of evolution and is able to persist in the gastric niche of its host lifelong. Despite the severe inflammatory response induced by the bacterium, the immune system is not able to clear the infection. Different *H. pylori* factors, such as VacA and arginase, have defined immune-suppressive activity. In particular, VacA exerts immune suppression of specific responses by acting either on antigen-presenting cells or on T cells. VacA acts on antigen-presenting cells by inhibiting antigen-processing and presentation, and on T cells by disrupting actin rearrangement and inhibiting calcium mobilization, that ultimately results in defective activation of NF-AT transcription factor. Recent studies demonstrated that VacA inhibited T-cell activation and human immunodeficiency virus infection, via mitochondrial depolarization and ATP depletion [28,29].

CD25⁺/Foxp3⁺ regulatory T cells (Treg) are part of an integrated system physiologically devoted to regulate the effector immune responses in the different districts of the organism and play a crucial role for the maintenance of self-tolerance. Treg are able to suppress antigen-specific lymphocyte and antibody responses, and their dysfunction leads to severe autoimmune diseases. An abnormal Treg activation by microbial antigens may represent a mechanism of *H. pylori* evasion from host defense. Treg have been shown, both in mice and in humans, to be activated by *H. pylori*, and while limiting the extent of the immunopathology, they contribute to an increase in bacterial colonization [30]. Depletion of Treg in *H. pylori*-infected mice, while increasing the frequency of autoreactive T cells, has not always resulted in autoimmune gastritis [31]. Treg are able to suppress T-cell responses, either via cell contact or by soluble factors, such as IL-10 and transforming growth factor (TGF)- β . Moreover, TGF- β , which is highly expressed in *H. pylori*-infected gastric mucosa, is a survival factor for Treg and may play an important role in modulating the magnitude of gastritis, via fine-tuning of Smad signaling [32]. TGF- β 1 polymorphisms were relevant to host susceptibility to *H. pylori*-related diseases in a large cohort of Spanish patients [33]. Using a transgenic mouse model of infection, IL-10 secreting T-regulatory cells were shown to be important in the control of gastric and duodenal inflammation induced by *H. pylori* [34]. IL-10 regulates *H. pylori*-induced inflammation via the p50/p105 subunit of NF- κ B [35]. Pratt et al. showed that the cytolethal distending

toxin of *Helicobacter hepaticus* plays a key role in bacterial persistence, resulting in development of colitis in IL-10-deficient mice [36]. Accordingly, a rapid onset of ulcerative typhlocolitis was found in IL-10-knockout rats infected with *Helicobacter trogontum* [37].

Anergy, induced via cytotoxic T-lymphocyte antigen 4 (CTLA-4) T-cell surface molecule, is a further mechanism involved in *H. pylori* immune subversion. Specific T cells derived from infected mice, but not those of immunized mice, were hyporesponsive to *H. pylori* antigens, due to the CTLA-4 engagement. The anergic state could be reversed by addition of IL-2 together with the antigen or abrogated by blocking the CTLA-4 molecule [38]. The activity of another mediator of T-cell suppression, the indoleamine-pyrrole 2,3-dioxygenase, was found to be elicited by *H. pylori* and tightly regulated by CTLA-4 and TGF- β [39].

An intriguing new mechanism of immune evasion by *H. pylori* relates to cholesterol glucosylation and is based on the fact that *H. pylori* is markedly auxotrophic for cholesterol. *H. pylori* senses cholesterol in the milieu and moves along increasing concentrations of the nutrient. Excessive cholesterol promoted phagocytosis and antigen-specific T-cell activation, whereas cholesteryl- α -glucosides promote escape of *H. pylori* from phagocytosis and T-cell responses. By using knockout experiments, it was demonstrated that the HP0421 gene is critical for *H. pylori* evasion [40].

Towards an Effective Vaccine

Significant progress has been made in treating *H. pylori* infection with antibiotics and proton pump inhibitors. Nevertheless, rapid emergence of antibiotic resistance, possible recurrence of infection, high cost, side-effects and poor compliance of pharmacological therapy are making the need for an effective vaccine against *H. pylori* more urgent day by day. Over the last year several lines of research investigated the potential benefits of a DNA vaccine, an oral vaccine, and different routes of administration for the design of a successful vaccine.

A DNA vaccine has great potential in achieving protection against infections by eliciting specific immune responses. Potential advantages of a DNA vaccine are its simplicity in preparation and manipulation, temperature stability, and stimulation of cytotoxic T-cell responses, which may be important for infection cure and prevention. Nonetheless, the mechanisms involved in the processing of DNA vaccines are not fully understood and there are theoretical safety concerns, which have never been materialized, such as DNA integration into host cells or appearance of anti-DNA autoimmunity. Recombinant urease, while very effective for mucosal vaccination trials in different animal models, is not as effective in humans. A DNA vaccine based on urease subunit B gene (UreB) has been used

to immunize mice. The protection achieved was modest, and was slightly better in animals immunized by intramuscular and intranasal routes than by intragastric or intrarectal routes [41,42].

Another possible option is to deliver a DNA vaccine via food, using an oral vaccine. *H. pylori ureB* has been cloned and introduced into a rice genome by *Agrobacterium*-mediated transformation. The population of potential transgenic plants was selected for the presence of *ureB* in the nuclear genome of rice plants by polymerase chain reaction, and then verified by Western blot analysis. Gu et al. provided evidence that recombinant UreB protein accumulates in transgenic rice and proposed the potential utilization of transgenic rice for delivery of oral vaccines against *H. pylori* [43]. Considering that bacterial flagella play an important role in bacterial pathogenesis, a study was performed to see whether a specific immune response could be mounted against *H. pylori* flagellin. *H. pylori* flagellin itself was very weakly immunogenic and the specific antibody response was strictly T-cell dependent [44].

HP-NAP is able to induce neutrophil infiltration and is highly immunogenic both for T-cell and for B-cell responses, thus represents a good vaccine candidate. Oral immunization of mice with live attenuated *Salmonella typhimurium* expressing the HP-NAP gene achieved induction of specific immunoglobulins A and G both in sera and at the gastrointestinal level, suggesting that an oral DNA vaccine with HP-NAP may be part of a new effective vaccination program against *H. pylori* infection [45]. The use of an *H. pylori* DNA vaccine containing built-in adjuvants was able to significantly decrease *H. pylori* colonization in immunized mice [46].

Although it is not yet clear which type of immune response gives protection, several vaccination trials are expected to take place in the near future and will probably help to identify the best immunogens, adjuvants, and route of immunization to be used for developing a successful *H. pylori* vaccine.

Conclusion

H. pylori induces an inflammatory response in the gastric mucosa characterized by polymorphonuclear and mononuclear cell infiltration. Both TLR and NOD molecules play a frontline role in host innate defense, but also contribute to the generation of adaptive immunity. A polarized Th1 response occurs in the stomach of infected individuals and is associated with severe diseases. Different factors, related to genetics, age, sex, diet, environment, other concomitant or previous infections, influence the type of host gastric immune responses. HP-NAP represents a crucial bacterial factor able to promote Th1 gastric inflammation. The host usually fails to clear the infection, although an apparently

vigorous innate and adaptive immune response is mounted. The *H. pylori*-induced immune evasion has been shown to be related to different mechanisms, involving regulatory T cells, CTLA-4 molecule, IL-10, and TGF- β . Collectively, these findings not only contribute to the understanding of host-pathogen interactions but also to the design of vaccines.

Conflicts of interest

The authors have declared no conflicts of interest.

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Helicobacter pylori and Non-malignant Diseases

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Keywords

GERD, NSAIDs, peptic ulcer disease, non-ulcer dyspepsia.

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Abstract

In recent years, the focus of *Helicobacter pylori* clinical research has been mainly on gastric malignancy. However, the role of *H. pylori* in non-malignant diseases, such as peptic ulcer, gastroesophageal reflux disease (GERD) and non-ulcer dyspepsia, as well as non-steroidal anti-inflammatory drug consumption, is still of great interest. A 1- to 2-week course of *H. pylori* eradication therapy is an effective treatment for *H. pylori*-positive peptic ulcer disease and a positive *CagA* status is a predictor for successful eradication of *H. pylori*. Antral prostaglandin-E2-basal levels appear to be critical for the development of aspirin-induced gastric damage in subjects without *H. pylori* infection. In clinical practice, among patients treated with proton-pump inhibitors, *H. pylori* status has no effect on the speed or degree of GERD symptom relief. For the management of dyspepsia in primary care, antisecretory therapy confers a small insignificant benefit compared to strategies based on *H. pylori* testing while these latter strategies may be cost-effective. *H. pylori* eradication therapy has a small but statistically significant effect on *H. pylori*-positive non-ulcer dyspepsia. An economic model suggests that this modest benefit may still be cost-effective but more research is needed.

Peptic Ulcer Disease

Over the last year a number of papers concerning various aspects of the association of *Helicobacter pylori* with peptic ulcer disease (PUD) have been published. The majority examined the effectiveness of various *H. pylori* treatments and will be reviewed in the Treatment section.

Murakami et al. [1] examined the possible relationship between peptic ulcer recurrence and the presence or absence of maintenance therapy with an H₂-receptor antagonist administered until evaluation of *H. pylori* eradication. The results of this study suggested that maintenance therapy with an H₂-receptor antagonist post-eradication therapy is likely to greatly reduce the ulcer recurrence rate without affecting the evaluation of *H. pylori* eradication.

Eradication of *H. pylori* reduces the relapse rate of PUD. Ford et al. examined the magnitude of this effect in their systematic review and meta-analysis [2], which was an update of a previous systematic review [Cochrane Database Syst Rev. 2004;(4):CD003840]. The primary outcomes

were an increase in the proportion of peptic ulcers healed initially and an increase in the proportion of patients free from relapse following successful healing. Eradication therapy was compared to placebo or pharmacologic therapies in *H. pylori*-positive patients. Secondary aims included symptom relief and adverse events. Sixty-three trials were eligible and 56 trials were finally included. For duodenal ulcer healing, eradication therapy was superior to ulcer-healing drugs (34 trials, 3910 patients, relative risk [RR] of ulcer persistence = 0.66; 95% confidence interval [CI] 0.58–0.76) and no treatment (two trials, 207 patients, RR 0.37; 95% CI 0.26–0.53). For gastric ulcer healing, no significant difference was detected between eradication therapy and ulcer-healing drugs (14 trials, 1572 patients, RR 1.25; 95% CI 0.88–1.76). In preventing duodenal ulcer recurrence, no significant differences were detected between eradication therapy and maintenance therapy with ulcer-healing drugs (four trials, 319 patients, RR of ulcer recurrence = 0.73; 95% CI 0.42–1.25), but eradication therapy was superior to no treatment (27 trials, 2509 patients, RR 0.20; 95% CI 0.15–0.26). In preventing

gastric ulcer recurrence, eradication therapy was superior to no treatment (11 trials, 1104 patients, RR 0.29; 95% CI 0.20–0.42). The authors concluded that a 1- to 2-week course of *H. pylori* eradication therapy is an effective treatment for *H. pylori*-positive PUD. The role of the *cagA* status of *H. pylori* strains as a predictive factor for the outcome of eradication therapy is controversial. Suzuki et al. in their systematic review and meta-analysis [3] confirmed the importance of the presence of *cagA* as a predictor for successful eradication of *H. pylori*.

Non-Steroidal Anti-inflammatory Drug Consumption

Last year relatively few studies examined the association of *H. pylori* with non-steroidal anti-inflammatory drugs (NSAIDs). The mechanisms by which *H. pylori* and low-dose aspirin induce gastric damage are not completely elucidated. Thus, Venerito et al. [4] evaluated the effects of low-dose aspirin on gastric damage, mucosal prostaglandin-E2 levels, and cyclooxygenase-enzyme expression in relation to *H. pylori* status. They concluded that in healthy subjects, low-dose aspirin given for 1 week does not affect cyclooxygenase expression or mucosal prostaglandin-E2 levels. Antral prostaglandin-E2 basal levels appear to be critical for development of aspirin-induced gastric damage in subjects without *H. pylori* infection.

Gastroesophageal Reflux Disease

As with the association of *H. pylori* infection and NSAIDs, last year few studies were devoted to the association of *H. pylori* and gastroesophageal reflux disease (GERD). Several studies suggested that proton-pump inhibitors suppress gastric acid more effectively in *H. pylori*-infected than in non-infected patients, but no evaluation of the short-term clinical response was performed. De Boer et al. [5] studied whether *H. pylori* infection influences the response rate or speed of symptom control in patients with GERD treated with rabeprazole. They did not find an effect on either of these parameters according to *H. pylori* status. Infected patients and non-infected patients can therefore be treated with a similar dose of rabeprazole. When treating heartburn with rabeprazole, physicians do not need to consider the patients' *H. pylori* status and most patients (> 80%) have adequate symptom relief after just a few days of treatment. Rabeprazole (10 mg b.i.d.) is often administered as an eradication therapy for *H. pylori* and has also been proposed as a therapy for refractory GERD. However, there has not been a comprehensive assessment of its acid-suppressive effects. Shimatani et al. [6] compared the acid-suppressive effects of rabeprazole (10 mg b.i.d. or 20 mg b.i.d.). They found that the effects of the two

rabeprazole doses were the same in *H. pylori*-positive patients, whereas in *H. pylori*-negative subjects, 20 mg b.i.d. was superior for prevention of nocturnal acid breakthrough.

The effect of *H. pylori* eradication on the development of GERD is controversial. Vakil et al. [7] determined the incidence of symptoms of reflux disease and erosive esophagitis and also their relationship to changes in histologic gastritis in patients with non-ulcer dyspepsia (NUD) over 12 months. Gastric biopsies were scored using the modified Sydney classification. The results showed that antrum-predominant gastritis is the most common pattern of gastritis seen in NUD in Western populations. Heartburn and regurgitation improve after eradication therapy or placebo in patients with NUD and the development of esophagitis is uncommon.

The impact of long-term acid suppression on the gastric mucosa remains controversial. Lundell et al. [8] reported on further observations concerning an established cohort of patients with GERD, after a 7-year follow up. Among the original cohort randomized for either omeprazole treatment or anti-reflux surgery, 117 and 98 patients remained in the medical and surgical arms, respectively. Gastric biopsies were taken at baseline and throughout the study. Results showed that long-term omeprazole therapy does not alter the exocrine oxyntic mucosal morphology in *H. pylori*-negative patients, but mucosal endocrine cells appear to be under proliferative stimulation: changes in mucosal inflammation and atrophy were observed in *H. pylori*-positive patients.

Dyspepsia and Non-ulcer Dyspepsia

Hu et al. [9] compared empirical prokinetics, the *H. pylori* test-and-treat strategy and empirical endoscopy in a 1-year study on primary-care patients presenting with dyspepsia. They found the three strategies equally effective. Empirical prokinetic treatment was the least expensive but peptic ulcers were sometimes missed, whereas the *H. pylori* test-and-treat strategy was indeed the most cost-effective option. An economic evaluation of empirical antisecretory therapy versus *H. pylori* test-and-treat strategy in the management of dyspepsia patients presenting in primary care was performed by Jarbol et al. [10]. Thus, a randomized trial in 106 general practices in the County of Funen, Denmark, was designed in order to obtain clinical outcome measures and resource utilization data prospectively. Seven hundred and twenty-two dyspeptic patients presenting with more than 2 weeks of epigastric pain or discomfort were randomized in one of three initial management strategies: 1, empirical antisecretory therapy, 2, testing for *H. pylori*, or 3, empirical antisecretory therapy, followed by *H. pylori* testing if symptoms improved.

Cost-effectiveness and incremental cost-effectiveness ratios of the strategies were determined. They concluded that empirical antisecretory therapy confers a small but not significant benefit and costs more than test-and-treat strategies for *H. pylori*, therefore it is probably not a cost-effective strategy for the management of dyspepsia in primary care. Undoubtedly, *H. pylori* is the main cause of PUD but its role in NUD is less clear. Moayyedi et al. examined this question in their recent systematic review and meta-analysis [11] which was an update of a previous systematic review [Cochrane Database Syst Rev. 2005;(1):CD002096]. They determined the effect of *H. pylori* eradication on dyspepsia symptoms in patients with NUD. They included all parallel group randomized controlled trials (RCTs) comparing drugs to eradicate *H. pylori* with placebo or other drugs known not to eradicate *H. pylori* for patients with NUD and 21 RCTs met the inclusion criteria. Eighteen trials compared antisecretory dual or triple therapy with placebo antibiotics with or without antisecretory therapy, and evaluated dyspepsia at 3–12 months. Seventeen of these trials gave results as dichotomous outcomes evaluating 3566 patients and there was no significant heterogeneity between the studies. There was a 10% relative risk reduction in the *H. pylori* eradication group (95% CI 6–14) compared to the placebo. The number needed to treat in order to cure one dyspeptic patient was 14 (95% CI 10–25). Three further trials compared bismuth-based *H. pylori* eradication with an alternative pharmacologic agent. These trials were smaller and had a shorter follow up but suggested that *H. pylori* eradication was more effective than either H₂-receptor antagonists or sucralfate in treating NUD. *H. pylori* eradication therapy has a small but statistically significant effect in *H. pylori*-positive NUD patients. An economic model suggests that this modest benefit is cost-effective but requires confirmation.

Conflicts of interest

The authors have declared no conflicts of interest.

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Helicobacter and Gastric Malignancies

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Keywords

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Abstract

Over the past year *Helicobacter pylori* has been confirmed as the most important risk factor for non-cardia gastric adenocarcinomas and gastric mucosa-associated lymphoid tissue (MALT) lymphomas. Eradication therapy has been proven to be beneficial when given prior to the development of intestinal metaplasia, but is less efficacious when administered later. However, the best data from clinical trials indicate that *H. pylori* eradication alone will have only a moderate effect on gastric cancer incidence worldwide. The mechanisms responsible for *H. pylori*-associated gastric carcinogenesis continue to be dissected. Accumulating evidence suggests that some *H. pylori* may be able to invade through the gastric epithelial barrier, though pro-carcinogenic effects may also be related to the complex and evolving pathways of altering signal transduction pathways within gastric epithelial cells that are stimulated by adherence and translocation of *H. pylori* products through its type IV secretory system. Determinants of the host response to *H. pylori* infection continue to focus on polymorphisms in genes related to the innate and acquired immune responses, including NOD2, COX-2, and TLR-4. *H. pylori* eradication is indicated for low-grade gastric B-cell MALT lymphoma and may even provide “cure” in some apparently *H. pylori*-negative cases. How and why does *H. pylori* promote lymphomagenesis? Some evidence from human and murine models points to specific chromosomal translocations and host genetic polymorphisms as relating to the outcome of infection. Finally, *Helicobacter hepaticus* infection has been linked to both intestinal and breast tumorigenesis in susceptible strains of female mice – a provocative and novel finding warranting further investigation.

Epidemiology

The extent to which chronic *Helicobacter pylori* infection is responsible for the global burden of gastric cancer and non-Hodgkin's lymphoma was reassessed based on new cancers registered in 2002 [1]. In that year almost 20% of cancers were considered to be attributable to infectious diseases, with *H. pylori* being the leading cause (5.5% of all cancers) followed by human papilloma viruses, hepatitis B and C viruses, Epstein–Barr virus, HIV, and human herpesvirus 8. *H. pylori* was estimated to be responsible for about 75% of noncardia gastric cancers and gastric non-Hodgkin's lymphomas, and 65% of all stomach cancers worldwide.

Reviewing data from the European Prospective Investigation into Cancer and Nutrition trial, a multicenter case control study that started in 1992, Palli et al. [2] reported that anti-CagA antibodies were associated with a threefold increased risk of gastric cancer development. In contrast, no such association was observed for noncardia cases after

adjustment for multiple demographic, nutritional, and other confounding factors. The contribution of *H. pylori* to noncardia gastric cancers was also calculated from a nested case-control study of *H. pylori* infection within the Finnish chemopreventive cancer prevention trial conducted from 1985 to 1999 [3]. This report confirmed the results of most other studies from Western populations, i.e. *H. pylori*-infected subjects had a statistically significantly decreased risk of gastric cardia cancer [adjusted odds ratio (OR) 0.3], but an increased risk of noncardia gastric cancer (adjusted OR 8). In contrast, in China *H. pylori* infection was associated with increased risk of both noncardia and cardia cancers [4] – in line with previous reports from East Asia.

It has long been debated why the incidence of gastric cancer is so high in Japan. Diet, genetic susceptibility, and *H. pylori* infection have all been proposed as causes. Differences in interpretation of the gastric histology by Eastern- and Western-trained pathologists may further render the conclusion difficult. In an interesting comparative

study, Naylor et al. [5] compared the gastric histology of dyspeptic patients without ulcers, cancer, or endoscopic esophagitis attending endoscopy centers in Japan and the UK. After review by highly experienced Japanese and English 'blinded' pathologists, the Japanese stomachs clearly had much more extensive and severe gastritis, with greater numbers of inflammatory cells and a greater risk of progression to atrophy and intestinal metaplasia. About 90% of the gastritis cases from both countries were related to *H. pylori* infection; CagA-positive strains were more common in Japan than in the UK. This prospective observational study supports the assertion that the increased gastric cancer risk in Japan relates to the severity and extent of the underlying chronic gastritis.

Childhood living conditions are well established to be important determinants of *H. pylori* acquisition. Re-analyzing data from a long-term cohort of Japanese-American men followed for almost three decades, the risk of gastric cancer in men infected by Cag-positive *H. pylori* strains was especially high in those who had many siblings (OR 2, when comparing > 6 sibs with one to three sibs in a single family) [6]. Thus, having many brothers and sisters increases one's risk of gastric cancer associated with Cag-positive *H. pylori* infection, conceivably through earlier acquisition of *H. pylori*, infection by more virulent strains, or due to subtle changes in later socioeconomic status or lifestyle.

***H. pylori* Eradication and the Prevention of Gastric Cancer**

Despite the overwhelming evidence that *H. pylori* infection is a risk factor for noncardia gastric cancer, accumulating evidence indicates that although *H. pylori* eradication is relatively simple to achieve, impacting the global burden of gastric cancer will be a much more difficult challenge. One of the earlier studies providing some optimism that *H. pylori* eradication would decrease gastric cancer risk was the observation in a large cohort study that the gastric cancer risk after hip replacement (accompanied by antibiotic use) was lower than expected [7]. However, the hypothesis that antibiotics given at the time of surgery provided incidental eradication of *H. pylori* has not been supported by a much larger recent study from the same group [8]. Over half a million Swedish inpatients treated for infectious diseases between 1970 and 2003 had no evidence of a subsequent decrease in gastric cancer risk at all, demonstrating that 'incidental eradication', if it occurs, has no long-term benefits on gastric cancer incidence.

Well-conducted large, prospective, controlled interventional studies to examine the effects of *H. pylori* eradication on gastric cancer have been few and far between. This may be partly due to recruitment problems related to the

ethical dilemma of having a placebo arm, as *H. pylori* is designated as a definite carcinogen. You et al. [9] reported the largest study so far of *H. pylori* eradication in subjects at risk for gastric cancer. Following baseline endoscopy, patients in a high cancer incidence area in China who were *H. pylori*-positive were randomized in a factorial design to receive either *H. pylori* eradication therapy: amoxicillin and omeprazole; and/or garlic extracts; and/or a 'vitamin supplementation' of vitamin C, E, and selenium. Those who were not infected by *H. pylori* entered either the 'vitamin' and/or the 'garlic' arm with appropriate placebo *H. pylori* therapy. After > 7 years follow up (including an impressive 93% retainment of the initial recruits) those subjects who had received *H. pylori* eradication therapy had a reduced combined prevalence of gastric cancer and preneoplastic changes (the primary endpoint). The number of cases of gastric cancer was low, but *H. pylori* eradication provided some benefit with an overall reduction in incidence from 2.4% to 1.7%. However this was not statistically significant despite the investigators having entered > 3000 subjects in the trial. In subgroup analysis, those subjects who had already progressed to intestinal metaplasia at the time of entry into study had the least benefit from *H. pylori* eradication therapy. No beneficial effects accrued from either garlic extracts or the combination of vitamin C, E, and selenium. These sobering results from a large prospective interventional study contrast with many of the observational studies that appeared to indicate a much greater benefit of *H. pylori* eradication. One example is the study by Takenaka et al. [10], who observed gastric cancer in six of 1519 (0.4%) subjects who were treated with *H. pylori* eradication therapy after having an endoscopy in Japan versus five of 288 (1.7%) subjects who failed *H. pylori* eradication therapy. Fuccio et al. [11] attempted to systematically review the more rigorously designed observational and randomized studies of *H. pylori* eradication. Though one could debate the methodology used to select published studies for their pooled analyses, the authors concluded that in nonrandomized studies the risk of gastric cancer development was significantly reduced by *H. pylori* eradication (OR 0.23), whereas in the large and best conducted randomized studies of *H. pylori* eradication the ORs were much less impressive (0.67) and not statistically significant, owing to wide confidence intervals. The authors concluded very reasonably that '*H. pylori* eradication is a plausible intervention for gastric cancer prevention; however, it seems to be relevant in only a subset of subjects'. This was also the feeling of the authors of the recently published *Maastricht III Consensus Report* [12], who stated at the consensus meeting held in 2005 that 'the optimum time to eradicate *H. pylori* is before preneoplastic lesions (atrophy, intestinal metaplasia) are present'. Another important conclusion from the Maastricht III

consensus was that 'the potential for gastric cancer prevention on a global scale is restricted by currently available therapies'.

Micronutrients and other chemopreventive approaches have generally been even less impressive than *H. pylori* eradication. Is there a role for pharmacologic intervention? Two reports published this year provide a mixed picture of the effects of selective COX-2 inhibitors. Yang et al. [13] took multiple gastric biopsies from Taiwanese dyspeptic patients, approximately half of whom were chronic users of celecoxib. From the initial 366 patients, 103 were found to have *H. pylori* infection with intestinal metaplasia and received *H. pylori* eradication therapy. At the initial endoscopy, the prevalence of intestinal metaplasia was similar between the chronic celecoxib users and the controls, but after a year the chronic celecoxib users appeared to have less intestinal metaplasia than those who did not take this drug. The authors speculated that celecoxib may be beneficial along with *H. pylori* eradication in the prevention of gastric cancer. Leung et al. [14] addressed this issue with rofecoxib 25 mg daily in a randomized prospective placebo-controlled trial in 213 subjects with intestinal metaplasia. After 2 years absolutely no differences in the amount or severity of intestinal metaplasia, apoptotic, or proliferative scores were found between rofecoxib and placebo. This latter study provides no optimism for COX-2 inhibitor chemoprevention in the prevention of gastric cancer, especially in view of the large cloud that hangs over all selective COX-2 inhibitors related to increased cardiovascular side-effects [15].

Mechanisms of Gastric Carcinogenesis

Several very interesting articles were published on the pathogenesis of *H. pylori* infection and its relationship to gastric carcinogenesis. Detailed microscopic studies of gastric biopsies from patients with dyspepsia and gastric cancer have resuscitated the idea that *H. pylori* may be invasive. While intracellular *H. pylori* has been noted repeatedly in coculture, the dogma has been that *H. pylori* does not invade gastric epithelial cells in vivo. However, recent findings that *H. pylori* may modulate the expression and functions of junctional proteins encouraged Necchi et al. to re-evaluate this dogma [16]. Using transmission electron microscopy and immunogold labeling with a variety of antibodies against *H. pylori*, lysates, and purified proteins (including VacA and CagA), *H. pylori* bacteria were identified inside the cytoplasm of epithelial cells, between epithelial cells and in the underlying lamina propria, often close to immune cells, and occasionally inside blood vessels. These findings were evident in normal mucosa, intestinal metaplasia, and in nine of 20 gastric cancers. This study of a small number of patients will undoubtedly

reignite the long-simmering controversy regarding whether *H. pylori* should be regarded as invasive, especially as *H. pylori* may invade murine gastric progenitor cells [17].

Equally controversial has been the suggestion that *H. pylori* is directly mutagenic, over and above the potential mutagenicity of the accompanying inflammatory response. While chronic coculture of the AGS gastric cancer cell line with *H. pylori* led to increased mutations in the gastric epithelial cell line, in association with reduced expression of two proteins, hMLH1 and hMSH2 that are critical for DNA repair and DNA fidelity [18], it is feasible that these results could be explained by the selection pressure of *H. pylori* leading to the emergence of a previously under-represented cell population. However, in support of a directly mutagenic action in coculture, *H. pylori* was reported to induce aberrant expression of activation-induced cytidine deaminase (AID), a protein acting as a DNA and RNA editing enzyme, with the consequent accumulation of mutations in the p53 tumor suppressor gene [19]. This appears to be mediated through I κ B kinase-dependent NF- κ B activation and the Cag pathogenicity island, and was relatively specific for p53, with mutations occurring much less frequently in *beta catenin* and *c-myc*. Increased AID expression was also noted in *H. pylori*-infected human gastric biopsies and was decreased following *H. pylori* eradication. AID levels were elevated in gastric cancer, and similar up-regulation was seen in a mouse model of *H. pylori* infection although this did not lead to the accumulation of p53 mutations, even after 40 weeks infection. Nevertheless, owing to the frequency of p53 mutations in human gastric cancer this study provides an important potential mechanism by which chronic *H. pylori* leads to the aberrant expression of a protein involved in nucleic acid editing and somehow specifically to p53 mutation.

Genomic and proteomic technologies continue to be applied to explore *H. pylori* pathogenesis in relation to human gastric carcinogenesis. Following their important work defining the genomic changes induced by *H. pylori* in transgenic mouse models [20], Gordon's laboratory has now turned its attention to humans. In a prior publication [17], this group reported an *H. pylori* isolate from a patient with chronic atrophic gastritis that appeared to bind and persist within gastric stem cells. Customized Affymetrix gene chips representing the genome of this strain were then synthesized and used for genotyping other *H. pylori* isolates from chronic gastritis patients. By combining genomic expression data with extensive clinical follow up, this impressive publication describes the genetic signature of *H. pylori* that may determine progression to cancer in these patients, genes regulated by acid during in vitro growth, and document gene defines clusters expressed during the adaptation of *H. pylori* to growth in the achlorhydric stomach. Ellmark et al. [21] constructed

large-scale antibody microarrays with 127 antibodies against antigens thought to be important in immunoregulation, to assess simultaneously the expression of multiple antigens in *H. pylori*-positive and -negative gastric cancers compared with their 'normal' resection margins (the *H. pylori*-negative cases were obtained from pancreatic cancer patients). Both tumor-associated signatures and *H. pylori* infection-associated signatures were obtained after mathematical correction and clustering. In a summary Venn diagram, specific proteins in each of these categories were evident and only one protein (the complement subunit C1s) was exclusively expressed in *H. pylori*-infected gastric tissues but not in tumors. Lin et al. [22] also used a proteomic approach to identify serum antibodies recognizing specific *H. pylori* antigens present in strains from patients with gastric cancer but not present in patients infected by duodenal ulcer-associated strains. The most promising differentially recognized antigen of the gastric cancer-associated strains was the chaperonin GroES (recognized by 64% of gastric cancer serum samples, compared with 31% of the gastritis samples and 35% of the samples from duodenal ulcer patients). Recombinant GroES was synthesized and in vitro strongly stimulated interleukins (IL) 8 and 6, granulocyte macrophage colony-stimulating factor, IL-1 β , tumor necrosis factor α (TNF- α), cyclooxygenase 2, and prostaglandin E2 expression from peripheral blood monocytes. Moreover in gastric epithelial cells, GroES-up-regulated IL-8, *c-jun*, *c-fos* and cyclin D1 increased cell proliferation and decreased p27 expression. Many of these effects had been observed with whole *H. pylori* and are thought to be important in the pathogenesis of gastric cancer, as they are similarly regulated in vivo. It is noteworthy that this study using very large numbers of clinical samples, careful proteomic analysis, and in vitro verification to support the biologic significance of their findings clearly illustrates the power of proteomic technology to identify potentially important *H. pylori* proteins. Identifying candidate virulence factors expressed by gastric cancer-causing strains but not duodenal ulcer strains has been a major challenge previously.

Following the adherence of *H. pylori* to gastric epithelial cell lines, extensive arrays of downstream signaling pathways resulting in potentially pro-carcinogenic events have been described, many of which are dependent on an intact type IV secretion system. To these effects can now be added increased cellular invasion, a hallmark of increased malignancy of cells in culture [23]. This effect was not related to VacA but required both direct contact and activation of the *c-met* receptor, accompanied by increased activity of the matrix metalloproteinases MMP2 and MMP9. Whether similar events occur in vivo remains to be determined. In vivo *H. pylori* stimulates the production of macrophage migration inhibitory factor (MIF) from several

cell types. Beswick et al. showed that the production of MIF by gastric epithelial cell lines was dependent on CagA, that MIF binds to CD74 (the class II MHC-associated invariant chain) to promote epithelial proliferation and to decrease apoptosis, and that this was associated with decreased p53 phosphorylation and increased Bcl-2 expression [24].

Moving from studies in cell lines to the evaluation of molecular changes in human gastric cancer tissues, Griffiths et al. reported increased expression of hypoxia-inducible factor (HIF) 1 α with progression through the normal-*H. pylori* gastritis-intestinal metaplasia-dysplasia-carcinoma sequence [25]. Furthermore, increased HIF 1 α expression was associated with the worst prognosis in a large series of gastric and esophageal cancer cases, though this was true only in univariate analysis. These results are consistent with reports of increased HIF 1 α expression in some other malignancies. RUNX3 is a transcription factor that was proposed to act as a gastric cancer tumor suppressor in a high profile publication by Li et al. in 2002 [26]. However, some other groups could not confirm an abnormal gastric phenotype in RUNX3 knockout mice, nor did the mice develop gastric cancer. To further investigate the importance of gastric epithelial expression of RUNX3, Friedrich et al. [27] examined RUNX3 expression by immunohistochemistry, laser capture microdissection, and quantitative PCR and reported that the level of RUNX3 in gastric epithelium was very low and not influenced by *H. pylori* or the development of gastric cancer. Furthermore, RUNX3 was mainly expressed in the gastric mucosa by infiltrating inflammatory cells rather than epithelial cells, adding further uncertainty over RUNX3 as a gastric cancer tumor suppressor.

The association of *H. pylori* infection with gene methylation has provided another possible mechanistic link between chronic *H. pylori* colonization and gastric carcinogenesis. Of multiple genes targeted by *H. pylori*, E-cadherin is of great interest because it is frequently down-regulated in sporadic gastric cancer, germline mutations in E-cadherin are associated with the hereditary diffuse cancer syndrome, and the effects of E-cadherin down-regulation includes loss of tight junction function and dysregulation of cell proliferation. Of 28 *H. pylori*-infected dyspeptic patients studied in Hong Kong [28], over half had E-cadherin methylation. This was significantly decreased 1 year after *H. pylori* eradication, suggesting that E-cadherin methylation may be reversed with eradication of the organism and resolution of gastric inflammation. However, methylation status may be unrelated to *H. pylori* since even in individuals without any evidence of *H. pylori* infection, gastric cancer cases had increased methylation of promoters in multiple genes, especially in patients with multiple gastric cancers [29].

Host Genetic Polymorphisms and Cancer Susceptibility

The landmark publication of El-Omar et al. linking polymorphisms in genes regulating the gastric inflammatory responses to gastric cancer risk from *H. pylori* [30] has spurred many groups worldwide to investigate other susceptibility loci governed by polymorphic alleles, particularly those of the innate immune response. The pathogen-associated intracellular recognition molecules NOD1 and NOD2 have recently emerged as potentially important regulators of chronic inflammatory conditions. NOD2 mutations segregate with particular phenotypes of Crohn's disease and NOD1 appears to be involved in the activation of a key transcription factor, NF- κ B, by the Cag pathogenicity island [31]. Rosenstiel et al. reported that NOD1 and NOD2 were up-regulated in the gastric epithelial cells of patients with chronic *H. pylori* infection and that the Crohn's disease-associated NOD2 variant R702W was significantly more prevalent in patients with gastric lymphoma than in *H. pylori*-infected individuals with gastritis or gastric ulcers [32]. No similar correlation between genetic variants of NOD1 was found and gastric cancer was not addressed in this study. Cyclooxygenase2 (COX-2) has long been known to be over-expressed in gastric cancers and in *H. pylori* infection. In a large series of gastric cancer cases and controls with preneoplastic lesions from China, Liu et al. reported an association between specific COX-2 genotypes associated with high level COX-2 expression and gastric cancer risk [33]. However, they did not state whether this association holds true after adjusting for other known gastric cancer-associated polymorphisms such as IL-1 β and TNF- α .

Screening the genotype distribution and allele frequencies of single nucleotide polymorphisms of four matrix metalloproteinases and two tissue inhibitors of matrix metalloproteinases, Kubben et al. reported that a single allele of MMP-7 was associated with *H. pylori* status and prognosis in Dutch gastric cancer patients, while a polymorphism of TIMP-2 correlates with tumor-related survival [34]. Again, previously described polymorphisms were not adjusted for, and the data require verification in other populations. Toll-like receptor 4 (TLR-4) is a receptor for lipopolysaccharide and may be important for *H. pylori* signaling to macrophages, monocytes, and perhaps also gastric epithelial cells. TLR-4 polymorphisms and mutations have been associated with a variety of inflammatory conditions, where defective signaling through TLR-4 is thought to be responsible for activating an exaggerated and inappropriate inflammatory response. Hold et al. addressed this issue with respect to *H. pylori* infection in gastric carcinogenesis [35]. A large series of patients previously investigated for cytokine polymorphisms and

susceptibility to gastric cancer from Poland, gastric cancer cases and their achlorhydric family member controls from Scotland, and cases of gastric and esophageal cancer from a US cancer registry were all tested for TLR-4 polymorphisms. An association between a polymorphism in TLR-4 that renders cells hyporesponsive to LPS and an increased risk of noncardia gastric cancer and its precursor lesions including achlorhydria was subsequently identified. This association was specific for noncardia gastric cancer (it was not observed in esophageal or gastric cardia cases) and remained even after correcting for the polymorphic variations in IL-1 β and the IL-1 receptor previously documented by this group, although whether it remains after correction for all other cytokine polymorphisms was not stated in the article.

MALT Lymphoma

That *H. pylori* eradication is a definitive cure of low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma has found worldwide recognition and appreciation. The most recent study from Korea reports a median time of 3 months for reaching complete remission in 84 of 99 patients following successful *H. pylori* eradication and a long-term complete remission in 94% of those [36]. Tumors located in the distal stomach had a more favorable response than those in the proximal stomach [36].

In another study, a group of 90 *H. pylori*-infected patients with low-grade MALT lymphoma underwent eradication therapy for 14 days and 85 (94.4%) reached complete remission. Median follow-up period after complete remission was 45 months (range 15–109). Only eight (10.4%) patients were found with recurrence of MALT lymphoma. Cumulative recurrence rates were 2.7, 11.5, and 12.2% at 1, 2, and 3 years respectively. Persistence of *H. pylori* was identified as the most important risk factor involved in recurrence, and therefore an adequate eradication regimen and accurate regular evaluations for *H. pylori* status are needed during follow up of primary gastric low-grade MALT lymphoma [37].

Long-term follow up of patients with MALT lymphoma is recommended as it is also possible that a metachromous gastric cancer can be detected [38]. It is also not unusual for *H. pylori* to be associated with extragastric locations such as reported for primary orbital lymphoma [39].

Some peculiar aspects in the therapy of MALT lymphomas are that some patients respond to therapy even in the absence of detectable *H. pylori*, as well as others with *H. pylori*-associated extragastric MALT lymphomas [40,41].

Carrier of the rare allele T have more than doubled risk of developing lymphoma than controls. *H. pylori*-induced up-regulation of NOD1 and NOD2 in vivo may play a critical

role in the recognition of this common pathogen. A missense mutation in the leucine-rich region of NOD2 is associated with increased risk of gastric lymphoma [32,42].

Low levels of *H. pylori* infection as they occur in vivo are associated with B-cell survival and proliferation, consistent with their potential to evolve into MALT lymphoma [43].

CagA, like interleukin-3, can enhance lymphocytes ability to evade apoptosis through phosphorylation of Bad. This may account, at least in part, for the direct ability of CagA to promote lymphomagenesis [44].

Two insightful reviews dealt with the management of gastric MALT lymphoma in great detail [45,46], including the basic mechanisms involved in the pathogenesis of MALT lymphoma. A genetic link of CTLA4 gene polymorphisms with development of gastric MALT lymphoma has been identified which further supports the fundamental role of host-activated T cells in MALT lymphomagenesis [47]. Almost 100% of C57BL/6 mice infected with *H. heilmannii* developed gastric MALT lymphoma after a 6-month period and lymphatic neoplasia was associated with destruction of parietal cells [48]. Genetic variations as predisposing factors of primary gastric B-cell lymphoma development have been excluded in a study from Germany [49]. Different clonality is a common reason for the differential response of coexisting low-grade and high-grade MALT lymphoma to *H. pylori* eradication therapy. The immunohistochemical examination of BCL10 expression may help to identify the coexistence of these components [50].

T(11;18)/API2-MALT1 translocation is frequent, while IGH-involved translocation is rare in gastric MALT lymphoma in Japan. This may have important impact on the clinical outcome [51].

Interestingly, γ glutamyl transpeptidase has been reported as a novel immunosuppressive factor produced by *H. pylori* that inhibits regulatory T-cell proliferation by induction of a cell cycle arrest in the G(1) phase [52].

Gastric MALT lymphoma is an interesting human and experimental model that will continue to give us important insights into the specific and general principles neoplasia development.

H. pylori and Breast Cancer?

Advances in animal models of *Helicobacter* infection relevant to gastric carcinogenesis were few in the past year. However, during investigations of the intestinal bacterial flora in relationship to colon cancer in the APC/Min mouse model, Rao et al. found that orogastric administration of the murine intestinal species *Helicobacter hepaticus* increased not only the number of intestinal tumors but also breast cancers in susceptible strains of female mice [53]. Further experiments demonstrated that this was dependent on

TNF- α and the absence of a specific subset of regulatory T cells. These intriguing experiments led the authors to postulate that gastrointestinal microbial infection by *Helicobacter* species and related organisms may dysregulate systemic immune responses, resulting in cancers in anatomically unrelated sites such as the breast. If true in humans, then perhaps breast cancer could one day be screened for through examining the stool rather than imaging the breasts!

Conflicts of interest

The authors have declared no conflicts of interest.

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Treatment of *Helicobacter pylori*

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Abstract

Since the discovery of *Helicobacter pylori* in the early 1980s many treatment regimes have been developed to effectively treat this infection. International guidelines have allowed consensus on the best management and improved eradication rates. In recent years, increasing antimicrobial resistance has resulted in falling eradication rates with standard therapies. In this article, we review the most recent studies and guidelines in the treatment of *H. pylori*. Currently, the first-line treatment remains clarithromycin, amoxicillin or metronidazole and proton pump inhibitor twice daily, but a number of recent studies have shown low eradication rates with this treatment. Increased duration of therapy has been recommended to overcome the falling eradication rates. However, conflicting findings have been reported on the benefits of extending the length of traditional therapy. Sequential therapy may be an effective alternative to standard triple therapy in regions of increased antimicrobial resistance. Probiotics reduce side-effects from traditional regimens and may improve eradication rates. A quinolone-based second-line triple therapy appears to be effective and well tolerated. Bismuth-based quadruple therapy is also an effective alternative if available. In the future, regional antimicrobial resistance and eradication rates will determine the best treatment for *H. pylori*.

The treatment of *Helicobacter pylori* remains a challenging clinical problem despite extensive research over the last 25 years. Increasing antimicrobial resistance and falling eradication rates are the result of the widespread use of antibiotics. The third Maastricht Consensus Report agreed that effective treatment for *H. pylori* should achieve an intention-to-treat (ITT) eradication rate of over 80% [1]. However, in clinical practice eradication rates are lower than 80% for many of the standard treatment regimes. A number of factors such as duration of treatment, choice of antibiotics, new drug combination, improved patient compliance, and novel agents may help to improve eradication rates.

Antimicrobial Resistance

Antibiotic resistance is a major cause of treatment failure [2]. The prevalence of antimicrobial resistance in *H. pylori* shows regional variation both within and between countries. Alternative antibiotics based on local resistance rates may improve eradication rates. Clarithromycin resistance has a greater effect on treatment efficacy than nitroimidazole resistance [3]. The widespread and

sometimes indiscriminate use of antibiotics in developing countries has resulted in a higher prevalence of resistance than in industrialized countries [4]. Clarithromycin resistance rates in the USA have a prevalence of 10–12.5% [5,6]. In Canada, clarithromycin resistance is estimated to be less than 4% [7]. In Europe, there is a significant difference between clarithromycin resistance rates in Northern, Eastern, and Southern Europe with resistance rates of 4.2%, 9.3%, and 18%, respectively [8]. The prevalence of secondary clarithromycin resistance, i.e. after failure of a treatment including this drug, is extremely high, up to 60% [7]. Resistance to metronidazole is much more common than resistance to macrolides. In developed countries about 35% of *H. pylori* strains are resistant to nitroimidazoles, whereas in developing countries the resistance rates are even higher [4]. However, metronidazole resistance in vitro does not always predict treatment failure. There is poor correlation between different methods of metronidazole resistance detection and this may explain differing resistance rates between institutions. As with macrolides, the essential risk factor for resistance is the previous consumption of the drug. Metronidazole is more commonly used in developing countries for the treatment

Regimen	Country	Eradication rate 7 days	Eradication rate 14 days	Reference
PAC	Italy	57% (n = 117)	70% (n = 126)	13
PAM	Italy	52% (n = 122)	56% (n = 121)	
PAC	Croatia	74% (n = 122)	93% (n = 58)	14
PAM	Croatia	75% (n = 122)	95% (n = 58)	
PAC	Italy	80% (n = 301)	82% (n = 301)	15
PAC	Korea	71% (n = 337)	76% (n = 261)	16

P, proton pump inhibitor; A, amoxicillin; C, clarithromycin; M, metronidazole.

Table 1 *Helicobacter pylori* eradication rate according to length of treatment in proton pump inhibitor-amoxicillin-based triple therapies

of parasitic infections. In developed countries, it is mainly used for dental and gynecological infections, and in some studies resistance is more commonly found in females [7]. The prevalence of amoxicillin resistance is low (< 1%). In areas where penicillin is available without prescription, it may be higher. Tetracycline resistance is estimated to be less than 1%. Fluoroquinolones are being increasingly prescribed in recent years and thus has led to increasing resistance rates.

A recent Italian study showed very high primary resistance to antibiotics. *H. pylori* resistance rate was 16.9% for clarithromycin, 29.4% for metronidazole, and 19.1% for levofloxacin. Clarithromycin resistance was significantly higher in patients with non-ulcer dyspepsia than in patients with peptic ulcer (19.1% vs. 0%, $p = .02$). Metronidazole resistance was higher in foreign than in Italian patients (50% vs. 22.9%, $p = .0004$), and levofloxacin resistance was higher in older than in younger patients (28.4% vs. 14.4%, $p = .048$). Levofloxacin resistance was also more frequent in strains with either clarithromycin or metronidazole resistance [9]. Another study suggested that *H. pylori* resistance to fluoroquinolones is already high in Belgium (17%) [10]. In comparison a Dutch study highlights the European regional differences in antibiotic resistance rates. In this study, mean rates of primary resistance to metronidazole and clarithromycin were 14.4% and 1.0%, respectively. Primary metronidazole resistance was stable over the 6-year period (1997–2002), and primary clarithromycin resistance showed a decreasing trend. Patients of foreign descent and from secondary care had a higher chance of harboring primary metronidazole-resistant *H. pylori*. Patients with failed *H. pylori* eradication had a higher chance of harboring multi-resistant *H. pylori* than untreated patients [11]. In the Netherlands, sales of antibiotics are lower than in any other European Union country and four times lower than in some Mediterranean countries. A meta-analysis proved that drug resistance is a strong predictor of efficacy across triple therapies for the eradication of *H. pylori* in adults [3]. These findings suggest that regional specific treatment regimes based on local

antibiotic resistance may improve eradication rates. The third Maastricht guidelines recently recommended local reference centres to measure antibiotic resistance rates within countries to improve eradication [1].

First-Line Treatment

Proton pump inhibitor (PPI)-based triple therapy has been used as first-line treatment of choice for over a decade [12]. A combination of PPI, clarithromycin 500 mg, and amoxicillin 1 g or metronidazole 400 or 500 mg, all given twice a day, is still recommended by the European *Helicobacter* Study Group [1]. There is some controversy over the most effective length of treatment for this regime. The European guidelines acknowledged that a 14-day treatment course may be more effective than a 7-day course. A number of recent studies continue to provide conflicting evidence in regard to the length of treatment course (Table 1).

A Croatian study comparing 7-, 10-, and 14-day treatment courses of PPI, amoxicillin, and clarithromycin (PAC) or metronidazole (PAM) demonstrated that an eradication rate (ITT analysis) exceeding 80% was achieved only by a 14- and 10-day course of PAC and only by a 14-day course of PAM. This study failed to achieve an adequate eradication rate with a 7-day treatment course [13]. A northern and central Italian study also showed that a 14-day PAC therapy achieved a significantly higher eradication rate than 7-day or 14-day regimes with metronidazole (70% vs. 52%, $p < .01$) and the same therapy for 7 days (70% vs. 57%, $p = .05$). At per-protocol (PP) analysis, a 14-day therapy with omeprazole, amoxicillin, and clarithromycin showed a significantly higher eradication rate than a 7-day therapy with amoxicillin and metronidazole (77% vs. 62%; $p = .03$) but no difference with 1-week of the same regime (66%) [14]. This study concluded that 2-week therapies, independently of antibiotic combination, lead to a significant increase in *H. pylori* eradication rate compared to 1-week therapies. The compliance and tolerability were similar for 1-week and 2-week treatment groups.

Table 2 Efficacy of different first-line treatment regimens to eradicate *Helicobacter pylori*

Country	Regimen (twice daily)	Duration (days)	Patient no.	Eradication rate		Reference
				ITT	PP	
Italy	CAE	7	100	75%	79%	18
	CME	7	100	72%	77%	
	CLE	7	100	87%	91%	
Italy	CRE	7	24	58%		20
	LRE	7	24	42%		
Germany	CAE	7	31	84%	84%	19
	LEA	7	30	87%	93%	
Spain	BAL	10	64	84%	89%	21
Italy	EBMT	10	95	91%	95%	22
Spain	CAO	7	171	86%	77%	23
	OBMT (t.d.s.)	7	168	89%	83%	

C, clarithromycin; A, amoxicillin; E, esomeprazole; L, levofloxacin; R, rifaximin; B, ranitidine bismuth citrate; M, metronidazole; T, tetracycline; O, omeprazole. ITT, intention to treat; PP, per protocol.

Eradication rates for both 1 and 2 weeks were, however, inadequate and this study questions the efficacy of this therapy in this region in Italy.

However, another Italian study comparing 1 and 2 week regimes led to similar results in terms of efficacy, safety and patient compliance with ITT eradication rates of 79.9% and 81.7%, respectively [15]. A Korean study again failed to show a benefit in the longer duration of treatment [16]. Again neither the 7-day or the 14-day course of PAC achieved acceptable eradication rates on an ITT analysis.

These conflicting findings may reflect varying resistance rates within the populations studied and confirm the need for more local reference centres to determine the best treatment regimes. In these studies it is also noticeable that the eradication rate for standard triple therapy is not adequate and is often less than 80%.

Eradication rates have decreased in recent years and increased duration of therapy may not overcome increasing bacterial resistance. A Turkish study that determined eradication rates over a 10-year period showed a marked decrease in eradication after year 2000. Pooled eradication rates each year from 1996 through 2005 were 79.4%, 83.7%, 81.8%, 81.8%, 75.1%, 61.3%, 65.6%, 65.1%, 55.3%, and 61.1%, respectively. In this study, eradication rates were not affected by the duration of treatment, choice of PPI, or indication for treatment [17]. In this era of increasing clarithromycin use, the effectiveness of standard triple-therapy regimen for *H. pylori* eradication needs reassessment. The most promising alternatives include sequential therapy and quinolone-based therapy.

Levofloxacin has proven very effective in the treatment of *H. pylori* infection in a number of studies. In a comparative study in Italy, the eradication rate achieved with levofloxacin-based triple therapy as a first-line treatment was significantly higher than that with standard therapies in either ITT or PP analysis (Table 2). The incidence of side-effects was similar with both standard and levofloxacin treatment [18]. In a study of patients with known antimicrobial sensitivity in a German population, a 7-day levofloxacin treatment had an eradication rate of 92.2% and 86.7% on a PP and ITT analysis, respectively [19]. In contrast, rifaximin-based triple therapy was not effective as first-line treatment in Italy [20]. Quadruple therapies comprising PPI, metronidazole, tetracycline, and bismuth are effective alternative first-line treatments which may be advocated in areas of high antibiotic resistance [22,23]. A meta-analysis confirmed this regimen's effectiveness as a first-line treatment [3].

Sequential Therapy

In Italy, a substantial decline in *H. pylori* cure rates with standard triple therapy has led to the use of sequential therapy. Sequential therapy in which PPI plus amoxicillin are given for 5 days followed by PPI plus clarithromycin and tinidazole also for 5 days has eradication rates close to or greater than 90%. This sequential therapy has proved superior to standard triple therapy in a number of Italian studies (Table 3). The incidence in side-effects was similar with both regimes in these trials. This treatment regimen appears to overcome clarithromycin resistance. Further international study is required for this promising approach.

Table 3 Comparative studies of sequential and standard therapy for first-line treatment of *Helicobacter pylori*

Patient no.	Year	Eradication rate % (ITT)		Reference
		Sequential therapy	Triple therapy	
300	2007	89%	77%	24
213	2006	94%	76% (7 days) 82% (10 days)	25
179	2005	94%	80%	26
342	2004	94%	71% (7 days) 80%	27

ITT, intention to treat.

Treatment after One or More Eradication Failures

Following first-line treatment failure, a number of options are available for second-line treatment that may overcome bacterial resistance. Triple therapy, quadruple therapy and more recently levofloxacin-based therapy have been studied as second-line therapies.

A large French study using a combination of PPI, amoxicillin, and metronidazole for 14 days achieved an eradication rate of 81% and 59% for metronidazole-susceptible and metronidazole-resistant strains, respectively. The overall eradication rate was 69% [28]. Clarithromycin should be avoided in second-line treatment in most areas unless resistance tests confirm the *H. pylori* strain to be susceptible. In Japan, the PPI-amoxicillin-metronidazole regime used as second-line treatment has been more successful with a number of trials showing eradication rates of over 90%.

Bismuth-based quadruple therapy is the second-line treatment of choice in many countries. Bismuth salt reduces the bacterial load of *H. pylori* in the stomach. Several studies have obtained good results with the regimen of PPI, ranitidine bismuth citrate, metronidazole, and tetracycline [31]. However, this regime requires the administration of four drugs with a complex scheme and is associated with a high incidence of side-effects [32]. Also bismuth salts are not universally available due to toxicity. This bismuth-based regime still fails to eradicate *H. pylori* in up to 30% of patients. In a Taiwan study of quadruple therapies PPI, bismuth, metronidazole, and tetracycline had an eradication rate of 77% similar to a quadruple therapy where clarithromycin replaced bismuth [33]. Quadruple therapy with PPI, metronidazole, clarithromycin, and amoxicillin was effective in first-line treatment according to a meta-analysis [3].

Levofloxacin-based triple therapies are now the second-line treatment of choice in some European countries. A meta-analysis showed a mean eradication rate of 80%

with levofloxacin-based rescue regimes. *H. pylori* cure rates were higher with a 10-day than a 7-day regime. This suggests that a 10-day regime of levofloxacin 250 mg b.d., amoxicillin 1 g b.d., and PPI twice daily should be chosen as the second-line treatment. Levofloxacin is generally well tolerated and most adverse events associated with its use are mild to moderate and transient. The most frequent adverse effects affect the gastrointestinal system. In this meta-analysis, levofloxacin-based treatments had a lower incidence of adverse events than quadruple therapy (19% and 44%, respectively) [32].

A study comparing levofloxacin, amoxicillin, and omeprazole versus ranitidine bismuth citrate, tetracycline, and metronidazole after treatment failure found comparable eradication rates. The incidence of side-effects with these two regimes was also comparable [34].

Patients who are not cured following two consecutive treatments including clarithromycin and metronidazole are most likely to have a strain resistant to one if not both antibiotics. Eradication therapy after two failures should be based on susceptibility testing [1]. Empirical treatment regimes following two treatment failures depend on initial treatment administered. Levofloxacin therapy had an eradication rate of 60% in patients with two failed treatments with standard triple therapy and bismuth-based quadruple therapy, respectively. Rifabutin has been used with some degree of success in the treatment of *H. pylori*; however, in a one study it had poor eradication results [35]. Levofloxacin-based therapy is more effective than rifabutin-based therapy in third-line treatment [36]. Widespread use of rifabutin could induce resistance in mycobacteria and so it should be used with caution.

Adjuvant Therapy

Bacterial resistance and poor patient compliance are believed to be the primary factors in *H. pylori* treatment failure. The occurrence of side-effects can reduce the compliance of patients with treatment regimens and lead to the development of bacterial resistance [37]. This has led to the development of alternative treatment options in *H. pylori*. Adjuvant therapy with probiotics, bovine lactoferrin, and curcumin have been studied in recent years.

A probiotic is defined as a living microbial species that, on administration, may have a positive effect on bowel microecology and improve health conditions [38]. The most studied probiotics are lactic acid-producing bacteria, particularly *Lactobacillus* species [39]. Probiotics play a role in the stabilization of the gastric barrier function and decrease of mucosal inflammation [40]. Some probiotic species such as lactobacilli and bifidobacteria release bacteriocins that may inhibit *H. pylori* growth and its adherence to gastric epithelial cells [39].

Table 4 Studies of adjuvant therapy

Regimen	Adjuvant	Eradication rate (ITT)%		Significant reduction in side-effects	Compliance	Reference
		Placebo	Adjuvant			
CAE	bLF and Pbs	73%	89%	Diarrhea $p = .001$ Nausea $p = .005$	No difference	37
EBATi	Lactoferrin	89%	94%	Overall $p = .05$	No difference	41
CTiE	Lactoferrin	77%	90%	Not significant	No difference	42
CTiR	Lactobacillus	80%	76%	Taste $p = .0027$	No difference	43
	<i>Saccharomyces boulardii</i>	80%	81%	Diarrhea $p = .018$		
CAR	<i>Lactobacillus</i> and bifidobacteria	80%	86%			
	<i>Bacillus clausii</i>	71%	72%	Overall $p < .05$	No difference	44

C, clarithromycin; E, esomeprazole; A, amoxicillin; Ti, tinidazole; R, rabeprazole.

Bovine lactoferrin is an iron-binding glycoprotein that is found in body fluids and secretions of humans and bovines. It appears to play a role in the hosts defense against bacteria and has a bacteriostatic and bactericidal effect [42]. It inhibits adherence and iron uptake by *H. pylori*. A number of studies have compared triple therapy with and without adjuvant therapy (Table 4). Eradication rates may or may not improve with adjuvant therapy but the incidence of side-effects especially diarrhea, nausea, and taste disturbance is reduced significantly. A large scale meta-analysis in *H. pylori* treatment has shown a significant reduction in side-effects with adjuvant therapy. Tong et al. showed that eradication rates of combining probiotics with standard triple therapy were slightly higher in both ITT and PP analysis [45]. De Bartoli et al. showed a significant increase in eradication rates with a combination of standard triple therapy, probiotics, and bovine lactoferrin. There was also a significant reduction in side-effects such as diarrhea, nausea, and taste disturbance [37]. The addition of bovine lactoferrin 200 mg (b.i.d.) and a probiotic may improve eradication rates and reduce side-effects.

Factors Related to Eradication Failure

A number of other factors have been studied in *H. pylori* eradication. Smoking is an independent risk factor for *H. pylori* treatment failure [46,47]. In a Finnish study, smoking and coffee drinking reduced the efficacy of therapy [48]. In contrast, alcohol consumption may facilitate elimination of *H. pylori* infection among adults [49]. A number of studies have shown a positive effect of alcohol consumption on the success of eradication therapy [50]. In a Polish study nonsmokers who drink alcohol had the highest eradication rate of 92% with standard triple therapy. There is some controversy on the role of the CYP2C19 phenotype on eradication therapy. A number of

studies have failed to show any significance of this genetic polymorphism in eradication therapy [47].

Conclusion

Increasing evidence suggests that standard triple therapy may no longer be the most effective first-line treatment in certain regions. Two-week therapy may be more effective than 1 week but may not overcome bacterial resistance. Sequential therapy appears to be an effective alternative. Adjuvant therapy with probiotics and bovine lactoferrin can reduce side-effects and may improve eradication rates. Local reference centres are required to monitor antibiotic resistance and eradication rates and determine the best treatment regimes.

Conflicts of interest

The authors have declared no conflicts of interest.

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Helicobacter pylori Infection in Pediatrics

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Abstract

During the last year, epidemiologic studies have shown that spontaneous clearance of *Helicobacter pylori* infection has a less significant role in countries with high prevalence and, in contrast to adults, there is no male predominance of *H. pylori* infection in children. Early acquisition of *H. pylori* may play a role in the development of recurrent abdominal pain in children less than 5 years of age. In this very young age group, the adequate performance of stool antigen test and ¹³C urea-breath test demonstrated satisfactory sensitivity and specificity as non-invasive methods to diagnose *H. pylori* infection. In the current paper, the most relevant pediatric studies on *H. pylori* infection published between April 2006 and March 2007 are reviewed.

Despite the fact that *Helicobacter pylori* was discovered 25 years ago and that the Nobel Prize in Medicine or Physiology was awarded to Marshall and Warren 2 years ago, *H. pylori* infection is still a challenging subject for many researchers and physicians [1,2].

In the present paper, the most relevant studies on *H. pylori* infection in children published last year (between April 2006 and March 2007) are reviewed.

Basic Research

The *cagA* gene, at least in adults, is considered to be a marker for severe disease inducing a high production of proinflammatory cytokines in the gastric mucosa. However, Sokucu et al. found no association between *cagA* positivity and the development of mucosal lesions/ulcers in Turkish children infected with *H. pylori* [3]. Antral lymphonodular hyperplasia was strongly associated with *H. pylori* positivity but *cagA* positivity and esophageal lesions were significantly less common in *cagA*-positive children (20% vs. 76.7%). In another study from Portugal, a country with one of the highest gastric cancer incidences and mortality rates among European Union countries [4], Costa Lopes et al. also found that there was no association between the most prevalent genotypes of infected children (*cagA*-, *vacAs2m2*, and *iceA2*) and patients' demographic and gastroduodenal disease phenotype, suggesting a potential role of host and environmental factors in the development of distinct clinical disease at a later age [5].

Oleastro et al. used suppressive subtractive hybridization for comparative genomics between *H. pylori* strains

isolated from patients with ulcer and from patients with gastritis only [6]. Two of the examined genes, *jhp0562* (involved in lipopolysaccharide biosynthesis) and *jhp0870* (an outer membrane protein), were highly associated with peptic ulcer disease (PUD) in children, suggesting novel virulence factors of *H. pylori*.

Prevalence

It is well known that childhood is an important period for acquisition of *H. pylori* infection but regarding the change in epidemiology of infection, only a few cohort studies involving children have been published [7–11]. In 2006, Özen et al. published a prospective study to determine the acquisition and loss rates of *H. pylori* infection in 327 asymptomatic Turkish preschool and school children (3–12 years) by using ¹³C-urea breath test (UBT) [12]. The incidence of *H. pylori* infection among previously uninfected children was 14%, and the loss rate of infection among previously infected children was 5.5% during a 6-year follow up. This 2.5-fold higher rate of acquisition compared to loss of infection suggests that, at least in a country with a high prevalence rate, spontaneous clearance of *H. pylori* infection has no significant role. Low household density and antibiotic administration over the last 6 months were found to be protective factors against acquisition of the infection.

Frenck et al. conducted a well-designed study enrolling 108 Egyptian children between 2 and 17 years of age [13]. One of the strengths of this study was the large number of children included under 6 years of age (n = 52). Prevalence

of *H. pylori* was 33% in children younger than 6 years and 60% in children older than 6 years. Both invasive (histology, culture, and rapid urease testing) and non-invasive methods (UBT, stool, and serum ELISA) were used for *H. pylori* diagnosis.

The prevalence of *H. pylori* infection is declining in developed countries; however, the rate of infection is influenced by several factors such as ethnic origin [14]. In a recent study from the Netherlands, Mourad-Baars et al. also found a low seroprevalence (1.2%) of *H. pylori* infection in 1258 young children (2–4 years), with a significant difference between children with parents who were both Dutch (0.5%) and children with at least one non-Dutch parent (2.6%) [15].

New data on seroprevalence of *H. pylori* in Vietnam have been published [16,17]. Using an ELISA test on 824 Vietnamese children without gastrointestinal symptoms, aged 6 months to 15 years, seroprevalence was 34.0%. Age groups ranging from 3 to 6 years and 6 years and older, and number of offspring were positively and independently associated with seropositivity [adjusted odds ratio (OR) 2.9 (95% confidence interval (CI) 1.5–5.5), OR 1.9 (95% CI 1.1–3.1) and OR 1.8 (95% CI 1.1–2.6), respectively]. Only breastfeeding more than 6 months was negatively associated with *H. pylori* seropositivity [OR 0.5 (95% CI 0.3–0.9)].

Halitim et al. evaluated the *H. pylori* re-infection rate in 45 children (median 10.9 years) after successful eradication [18]. One to nine years after *H. pylori* eradication, 18% of the children were re-infected (5.4% to 6% per patient-year) indicating a higher re-infection rate in children than in adults.

In contrast to what was found in adults, a male predominance of infection was not found in the separate meta-analysis of 10 pediatric studies where the summary OR was close to 1 [summary OR 1.03 (95% CI 0.91–1.17)] [19]. Differential protective immunity (women have higher plasma IgM levels than men [20] and estrogen stimulates immune response [21]) or differential antibiotic exposure between genders may explain the differences observed in pediatric and adult studies.

Transmission

The role of infected mothers in transmission of *H. pylori* has been described in the past [22,23]. In 2006, several reports re-established this relationship.

Ito et al. conducted a community-based familial study (265 families: 507 children and 530 adults) on *H. pylori* infection among Japanese Brazilians living in Sao Paulo to identify risk factors associated with intrafamilial transmission [24]. *H. pylori* seropositivity was found in 9.3% of children and 39.2% of the parents. The prevalence

of *H. pylori* infection was 3.5% for children with uninfected parents, 9.9% for those with a seropositive father and a seronegative mother, 14.9% for those with a seropositive mother and a seronegative father, and 16.0% for those with both seropositive parents. Moreover, the mother's symptoms of nausea and vomiting and the use of pacifier were significantly associated with the risk of *H. pylori* infection in children. These data suggest that infected mothers are the main source of *H. pylori* infection of their children, mainly through contact with contaminated and regurgitated gastric juice from the mother's mouth.

Another study from Germany found a similar association using more reliable methods to detect *H. pylori* infection (UBT and monoclonal antigen stool test) [25]. In this community-based birth cohort, 2.4% of children (20 of 834) were infected. The odds ratio for infection of the child was 12.9 (95% CI, 3.2–52.5) if the mother was infected and 1.4 (95% CI, 0.4–4.6) if the father was infected.

Vertical transmission during pregnancy was investigated in an experimental study using Mongolian gerbils [26]. After experimental infection of pregnant animals, there was no detectable *H. pylori* infection, using bacterial culture, polymerase chain reaction and rapid urease test, in any of the fetuses during pregnancy nor in the litters at parturition.

Campell et al. analyzed the presence of *cagA*- and *vacA*-specific IgA monthly in breast milk of 48 mothers in Gambia [27]. Thirty-seven out of 48 infants (77%) had *H. pylori* infection by UBT. Low breast milk content of anti-*vacA* IgA antibody was correlated with weight loss in *H. pylori*-infected infants.

Stray-Pedersen et al. investigated the prevalence of *H. pylori* antigen in the stools (HpSA) of Norwegian neonates and very young children [28]. Interestingly, there was an unexpectedly high rate of positives in the neonates (52%, 36 of 69), suggesting either a lack of specificity of the test or a transient colonization during the neonatal period. Moreover, *H. pylori* antigen detection in the neonates was significantly associated with the mode of delivery (vaginal births, 59%, Cesarean section, 10%).

Clinical Manifestations

H. pylori is considered to be the major cause of chronic gastritis and duodenal ulcer in childhood. However, the association between *H. pylori* and non-ulcer dyspepsia, recurrent abdominal pain (RAP), gastric outlet obstruction, and extraintestinal manifestations is still controversial.

Intestinal Symptoms

Recurrent abdominal pain occurs in 10–15% of school-age children with a controversial association with *H. pylori* infection. Malaty et al. found no association between a

positive UBT and RAP among 243 symptomatic children (11% UBT+) versus 330 asymptomatic children (17% UBT+) [29]. A previously validated, multidimensional measure for RAP (MM-RAP) consisting of four scales previously described (pain intensity scale, symptoms scale, disability scale, and satisfaction scale) was applied to each child/parent described earlier in detail [30]. Unexpectedly, in symptomatic children the prevalence of *H. pylori* fell with age from 20% at age < 5 years to 7% for children > 10 years (OR 2.7, 95% CI 0.7–11.2). In contrast, the prevalence of *H. pylori* in children without symptoms increased with age from 11% for children < 5 years to 40% for children > 10 years (OR 5.4, 95% CI 2.0–13.8), as expected. In this second group, the mother's educational level was inversely correlated with *H. pylori* infection. The authors suggested that early acquisition of *H. pylori* may cause chronic abdominal symptoms.

In a study in Taiwan, 135 patients with RAP were investigated for *H. pylori* by rapid urease test, histology, and UBT [31]. The prevalence of *H. pylori* infection was 23.7%. After the 12-month follow up, *H. pylori* status did not significantly influence the disease course: persistence of RAP was noted among 59 of 84 (70.2%) non-infected patients, and in 13 of the 15 (86.7%) *H. pylori*-infected patients with failed eradication.

Yen et al. followed-up 11 children with gastric outlet obstruction [32]. In six cases, there was an anatomic abnormality and in five cases PUD. Two of the five patients had an *H. pylori* infection detected by rapid urease test. The authors concluded that PUD plays an important role in children with gastric outlet obstruction, however, compared to adult patients *H. pylori* is a less important etiologic factor. An interesting case report concerning this topic was recently published involving a rare case of gastric outlet obstruction in an 11-year-old girl with an ectopic pancreatic tissue, which was probably induced by *H. pylori*-associated PUD [33].

Extraintestinal Manifestations

H. pylori infection has been associated with extradigestive manifestations, such as anemia, short stature, immune thrombocytopenic purpura, and migraine, some of these lacking sufficient evidence. A study in Poland found no association between *H. pylori* infection and autoimmune hepatitis in children [34].

Yilmaz et al. cultured *H. pylori* from aspirated middle ear fluid and biopsies taken from the promontorium of children with otitis media with effusion [35]. There was a significantly increased colonization by *H. pylori* in patients with otitis media (n = 22) when compared with controls (n = 20).

The association between *H. pylori* infection and growth is still controversial [36]. Two studies addressed this issue.

Fialho et al. compared height and weight of 197 children with *H. pylori* infection to 156 children without infection [37]. In a low-income community in north-east Brazil, *H. pylori* infection was associated with short stature only in older children (8–14 years). Chimonas et al. investigated 650 children (7–11 years) in Alaska [38]. At baseline, 87% of children were infected with *H. pylori* (UBT). During the post-treatment follow up (2, 8, and 14 months), there was no positive correlation between *H. pylori* eradication status and improvement in any of the measured growth parameters (height, weight, and body mass index).

The negative correlation between plasma levels of ghrelin and *H. pylori* infection that may contribute to the alterations of the appetite and dyspeptic symptoms observed in infected patients was confirmed [39,40].

Yahav et al. assessed the prevalence of *H. pylori* and toxigenic *Clostridium difficile* infections in 30 consecutive patients with cystic fibrosis versus controls. Prevalence of *H. pylori* infection was lower (17%) than in similarly aged controls (30%) while about half of the patients with cystic fibrosis were asymptomatic carriers of *C. difficile* producing mostly toxin B [41].

Another study in Turkey showed a negative effect of *H. pylori* infection on serum ferritin and vitamin B₁₂ levels in children [42]. There was no significant effect of *H. pylori* status on serum folate or zinc levels.

It is of interest that in the previously mentioned Turkish cohort study, children with *H. pylori* infection were complaining more often of headaches than abdominal pain or dyspepsia. All three children with migraines were also *H. pylori* positive [12].

Ohno et al. from Japan reported long-lasting remission of primary gastric lymphoma of the mucosa-associated lymphoid tissue type in two immunocompromised pediatric patients (14- and 6-year-old boys) with *H. pylori* infection [43]. Treatment of *H. pylori* alone completely resolved the patients' lymphoma in the follow up of 10 years and 3 years, respectively.

Diagnostic Modalities

The accuracy of diagnostic tests remains uncertain in children less than 5–6 years of age. Even in the large, multicentre and multinational study carried out in Europe, only 13 *H. pylori*-positive children < 6 years were enrolled [44]. Such a study was performed on Egyptian children [13]. Histology, culture, and rapid urease testing were performed on all patients providing a strong gold standard for validation of the non-invasive tests. Moreover, 52 children < 6 years of age were enrolled in this study and the 33% global prevalence of *H. pylori* infection allowed a distribution of results by age. UBT and HpStar stool ELISA kit had the highest sensitivity and specificity (UBT, 98%

and 89%; HpStar, 94% and 81%, respectively). However, the serology kit (HM-CAP) had a very low sensitivity (50%). Sensitivity of the UBT was not influenced by the age of the patient but specificity was lower, although not statistically different, in children < 6 years of age (86%) versus 95%, for children > 6 years. The specificity of HpStar stool kit, although high, was slightly but significantly lower in younger children. Receiver operating curves found optimal cut-off points of UBT at 6.2 delta over baseline (DOB) which is higher than the level of 2.5–4 DOB that is usually accepted as a cut-off level for *H. pylori* infection in adults [45].

Dondi et al. also observed satisfactory sensitivity and specificity for UBT, 93.3% and 95.5%, respectively, and for HpSA, 93.3% and 98.7%, respectively, in 30 children under 5 years of age with *H. pylori* infection [46]. Increasing the cut-off from a DOB of 5–8 improved UBT specificity from 95.5 to 98.1. Polyclonal HpSA tests were performed without transportation to the hospital laboratory, within a few days after collection, providing good results. Authors suggested that transportation or storage of the stools may negatively influence the accuracy.

No correlation between the UBT results and histologic grades for mononuclear infiltrate, neutrophilic infiltrate, and bacterial density was found in Brazilian children [47], indicating that UBT in children should be interpreted qualitatively.

A study in Taiwan suggested that a lower dose of urea for UBT (1 mg/kg of bodyweight, maximum 25 mg) is also effective to detect *H. pylori* infection in children [48].

The sensitivity and specificity of the ¹³C-urea blood test were 83% and 91%, respectively, in comparison to histology and rapid urease test in 40 children [49].

Pelerito et al. investigated the accuracy of Assure *H. pylori* Rapid Test (Genelabs Diagnostics, Singapore) which is intended for the rapid detection of antibodies to *H. pylori* in human serum, plasma, or whole blood [50]. A total of 130 Portuguese children were enrolled, of whom, according to the gold standard (culture/histology and rapid urease test), 70 were *H. pylori* positive. The sensitivity, specificity, and positive and negative predictive values of the test on serum were 75.7%, 95.0%, 94.6%, and 77.0%, respectively. Interestingly, when a longer reading time of 45 minutes was introduced, the rapid test showed better performances (sensitivity 98.6% and specificity 95%). However, this rapid test was not evaluated with finger-pricked whole blood which is more realistic in the practitioner's clinic setting.

The monoclonal antibody-based ELISA HpStar used by Kolho et al. in a study on 102 stool samples [51] showed a sensitivity and specificity of 95% and 90%, respectively. Half of the specimens were also tested with two other antibody stool tests with excellent performance. The accuracy

rates of the tests were 98% for the HpSA and 96% for the ImmunoCardSTAT.

GastroPanel (Biohit, Helsinki, Finland), a serum test kit that measures *H. pylori* antibodies (HPABs) and pepsinogens I and II and gastrin 17, was tested on 30 children with *H. pylori*-positive gastritis [52]. The test had a sensitivity too low (47–73%) to be recommended for *H. pylori* screening; even the assays of pepsinogens and gastrin did not improve sensitivity.

Muhsen et al. tested the HpELISA kit (URINELISA, Otsuka Pharmaceuticals Co, Ltd, Tokyo, Japan) for detection of *H. pylori* IgG antibodies in urine of 159 healthy Israeli Arab children aged 3–5 years. This test was not sensitive (34–66%), indicating limited diagnostic value [53].

***H. pylori* Treatment**

Triple treatment including a proton-pump inhibitor (PPI) and clarithromycin, combined with either amoxicillin or metronidazole, has been recommended to treat children with *H. pylori* infection [54]. Nevertheless, a growing body of evidence showed that antibiotic resistance is increasing, thus new drugs and therapeutic regimens are clearly needed [55].

A prospective randomized study comparing a triple therapy (ranitidine bismuth citrate, amoxicillin, and clarithromycin) given for 4 days versus 7 days was conducted by Tam et al. on 206 children with *H. pylori* infection [56]. The 7-day regimen was superior to the 4-day regimen with eradication rates of 89% and 78%, respectively ($p = .02$).

A register was established by European pediatricians (Pediatric European Register for Treatment of *Helicobacter pylori*, PERTH) and is available on the European Society for Pediatric Gastroenterology Hepatology and Nutrition website to collect data on treatment performed. The results show a significantly higher eradication rate in children with ulcer than in those without, 79.7% and 63.9%, respectively. Bismuth-containing triple therapies were more efficacious than PPI-based regimens (77% vs. 64%, OR 1.88, 95% CI 1.1–3.3). Moreover, a 2-week therapy was not superior to a 1-week therapy. The authors concluded that treatment recommended for adults may be not suitable for children [57].

In contrast, when Bahremand et al. compared a triple therapy (omeprazole, clarithromycin, and amoxicillin for 10 days) to a quadruple therapy (omeprazole, amoxicillin, metronidazole, and bismuth citrate for 10 days) in Iranian children with *H. pylori* infection, the eradication rates were higher in triple versus bismuth quadruple therapy (92–75.5% vs. 84–68.8%, respectively) [58].

A 1-week esomeprazole-based triple therapy was highly effective for eradication of *H. pylori* in 58 children (92–93%) [59]. Resistance rates to clarithromycin and metronidazole were 9% and 16%, respectively.

Cadranel et al. showed that standard triple therapy (omeprazole, amoxicillin, and clarithromycin) offered a better eradication rate (69%) than dual therapy (amoxicillin and clarithromycin) (15%) in 46 children with *H. pylori* gastritis [60].

Kawakami et al. conducted a prospective open trial in 38 children with *H. pylori* infection administering a 7-day course of omeprazole, clarithromycin, and furazolidone (100 mg < 30 kg, 200 mg > 30 kg) twice daily [61]. Intention-to-treat and per-protocol analysis showed moderate efficacy in *H. pylori* treatment (73.7% and 84.8%, respectively).

In 2006, two randomized placebo controlled trials evaluated the effect of probiotic food as an adjuvant to the standard triple therapy for eradication of *H. pylori* infection in children. One of them showed a beneficial effect of *Lactobacillus reuteri* administration to reduce antibiotic-associated side-effects [62], while the other using *Bifidobacterium animalis* and *Lactobacillus casei* did not [63].

The first meta-analysis assessing the efficacy of *H. pylori* treatment in children concluded that additional well-designed randomized placebo-controlled pediatric trials are needed, especially in developing countries [64].

Bacterial resistance and low compliance for drug intake are the main factors for the treatment failure in *H. pylori* infection [65]. Koletzko et al. conducted a prospective multicentre study on antibiotic resistance of *H. pylori* strains obtained from 1233 children from 14 European countries [66]. Resistance to clarithromycin was observed in 20% before therapy, with significantly higher percentage in boys and children under 6 years of age. Similarly, primary resistance to metronidazole was 23% with almost no resistance to amoxicillin (0.6%). The authors suggested that administration of antibiotics for other indications may be the major risk factor for development of primary resistance.

Another study was performed in Iran [67]. The primary resistance was 54.2% to metronidazole, 8.3% to amoxicillin, and 4.2% to clarithromycin, with no resistance to tetracycline and furazolidone.

Conflicts of interest

The authors have declared no conflicts of interest.

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Extragastric Manifestations of *Helicobacter pylori* Infection – Other *Helicobacters*

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Abstract

Today there is evidence that *Helicobacter pylori* has a critical role in different extragastric diseases. The discovery of a number of other novel *Helicobacter* species has stimulated the research in different extragastric diseases, in which an infectious hypothesis is plausible. Enterohepatic *Helicobacter* species have been hypothesized to play a role in different disorders, including hepatocellular carcinoma, gallstones formation and cholangiocellular carcinoma, as well as enteric diseases and inflammatory bowel diseases. Concerning the extragastric manifestations of *H. pylori* infection, idiopathic thrombocytopenic purpura, and sideropenic anemia represent, based on the current data, the diseases in which the pathogenic link appears to be strongest. There is also an increasing evidence for a possible association of *H. pylori* with cardiovascular disease.

The discovery of *Helicobacter pylori* has opened a new world in the field of gastroduodenal diseases. Culture of the slow-growing bacterium *H. pylori* from the gastric mucosa has abolished the dogma that the stomach is sterile and enables us to cure peptic ulcer disease that has formerly been classified as a chronic recurrent disorder with severe complications. Here we review recent results concerning the extragastric manifestations of *H. pylori* infection as well as different diseases that may be associated with the other novel *Helicobacter* species.

Helicobacter pylori Infection

Cardiovascular and Cerebrovascular Diseases

This area records the higher number of studies published during the last year. In particular, Lenzi et al. determined the overall prevalence of *H. pylori* and CagA-positive *H. pylori* infection and the prevalence of other bacterial and viral causes of chronic infection in patients with coronary artery disease (CAD), and the potential role of anti-heat-shock protein 60 (Hsp60) antibodies in increasing the risk of cardiovascular disease (CVD) development. Eighty patients with CAD and 160 controls were enrolled. *H. pylori* infection and CagA status were determined serologically. The results of this study demonstrate that infection with CagA-positive *H. pylori* strains may concur to the development of CAD, as high levels of anti-Hsp60

antibodies have been shown to constitute a marker and/or a concomitant pathogenic factor of the disease [1].

Di Bonaventura et al. investigated, for the first time, circulating and gastric mucosal levels of IL-1alpha, IL-6, IL-8, and TNF-alpha in patients with ischemic heart disease (IHD) and in matched controls, according to the presence or absence of active *H. pylori* infection. Furthermore, the lipidic profile was also evaluated. There was no correlation between mucosal and circulating cytokine levels. Active *H. pylori* infection was not associated with a modified lipid profile in either controls or IHD patients, although ApoAI levels were significantly higher in *H. pylori*-positive controls compared to *H. pylori* negative. Taken together, the results of the present study provide evidence that active *H. pylori* infection may play a role as a trigger factor in the pathophysiology of IHD by inducing an inflammatory cascade concentrated on gastric mucosa [2].

The relationship between the serologic status for both *Chlamydia pneumoniae* and *H. pylori*, and the presence of CAD remains a controversial issue in the general literature [3]. While Vijayvergiya et al. seemed to support this concept [4], another study by Sotiropoulos et al. added evidence against this association. In the latter study, in particular, the seropositivity to *C. pneumoniae* was 91% in patients with CAD and 86% in controls ($p > .05$) and *H. pylori* seroprevalence was 77% and 68%, respectively ($p > .05$). Multivariate analysis, adjusted for age, gender, educational level, diabetes, hypertension, obesity, smoking,

family history of CVD, and lipids, confirmed the results of the univariate analysis [5].

Based on the findings obtained by Pellicano and Rizzetto, the role of *H. pylori* seems to be more important in unstable angina than in stable angina, in which the prevalence of *H. pylori* resulted to be lower [6].

Eskandarian et al. studied the association between *H. pylori* infection and cardiac syndrome X (CSX). *H. pylori* infection was detected by urea breath test (UBT) in patients with CSX, and in sex- and age-matched controls. In particular, 95% of patients with CSX were infected by *H. pylori* compared to only 47.5% of controls ($p < .001$). Based on these findings, the authors proposed a possible causative effect of *H. pylori* infection in the pathogenesis of CSX [7].

Concerning functional CVD, Lugon et al. studied patients with mild or moderate chronic gastritis and *H. pylori* infection and subjects with the same diagnosis but *H. pylori* negative. Noninvasive cardiovascular tests were applied before and after feeding. Interestingly, the results demonstrated a blunted sympathetic reactivity and exacerbated vagal response to feeding in *H. pylori*-positive patients [8].

Altintas et al. attempted to determine carotid intima-media thickness in patients with or without *H. pylori*-induced atrophic gastritis. Esophagogastroduodenoscopy was performed on 123 patients. No significant differences in carotid intima-media thickness were reported between the two groups; therefore carotid intima-media thickness seemed not to be associated with *H. pylori*-induced atrophic gastritis [9].

Arias et al. utilizing a seminested polymerase chain reaction (PCR), detected *H. pylori* DNA in 83% of the atherosclerotic carotid tissue samples, 64% of which were *cagA* positive [10], while Weiss et al. did not find evidence for a direct role of *H. pylori* nor *Mycoplasma pneumoniae* in carotid artery atherosclerosis [11].

Finally, a meta-analysis by Pasceri et al. who analyzed the results of 10 retrospective case-control studies (with 1527 patients and 1661 control subjects) and three prospective cohort studies (with 701 patients and 1439 control subjects) on *CagA* status and IHD and four retrospective case-control studies (with 513 patients and 590 control subjects) on *CagA* status and cerebral ischemia, reported a small but significant association between vascular diseases and *CagA*-positive strains of *H. pylori* [12].

Lung Diseases

Gencer et al. studied the seroprevalence of *H. pylori* in patients with chronic obstructive pulmonary disease (COPD) and its relation to pulmonary function tests. Forty-nine patients with COPD and 50 age- and sex-matched controls were included in this study. *H. pylori*-specific IgG was measured with a commercially available kit from blood

samples. Serum levels of *H. pylori*-specific IgG were significantly higher in patients with COPD than in control subjects. In addition, when patients with COPD were grouped according to *H. pylori* IgG seropositivity, forced expiratory volume values were lower in the seropositive patients compared to seronegative [13].

Gulhan et al. using a PCR in bronchoalveolar lavage fluid and bronchiectatic lung tissue studied the role of *H. pylori* in bronchiectasis; their findings provided evidence that there is no direct association between *H. pylori* and bronchiectasis, even though an indirect role of soluble products of *H. pylori* could not be excluded [14].

A study by Angrill et al. investigated the presence of *H. pylori* in bronchial biopsies of patients with bronchiectasis, by histochemical and immunochemical staining. The presence of *H. pylori* in bronchial specimens from patients with bronchiectasis could not be demonstrated [15].

Yahav et al. investigated the prevalence of *H. pylori* and toxigenic *Clostridium difficile* infection and its relationship with gastrointestinal symptoms and pancreatic sufficiency or insufficiency in cystic fibrosis patients. The findings of this study supported that cystic fibrosis patients with pancreatic sufficiency or a history of distal intestinal obstruction syndrome and those carrying mutations associated with a severe phenotype are protected against *H. pylori* infection [16].

Hematologic Diseases

The link between *H. pylori* and iron deficiency anemia (IDA) has been investigated in the last years, contributing to clarify its pathophysiologic role. The data regarding adults and children are not overlapping. In fact, considering studies on adult, Hershko et al. prospectively investigated 44 IDA male patients and documented that in those with unexplained IDA, *H. pylori* was highly prevalent (86.2% vs. 33.3%, $p < .0001$) and that, after eradication (mean 38 months \pm 15) all patients achieved normalization of hemoglobin levels [17].

Interestingly, a study evaluating the influence of *H. pylori* infection on iron accumulation in hepatitis C (HCV) patients has shown that HCV patients with coexistent *H. pylori* infection had significantly lesser serum ferritin levels than HCV patients without *H. pylori* infection (99 vs. 150 ng/mL) and also a reduced grade of hepatic iron deposit ($p < .001$), suggesting that *H. pylori* infection may affect hepatic iron accumulation in HCV-related liver disease [18].

Considering studies in children or adolescent, where the balance between iron intake and its utilization is more complex for the physiologic need and loss (growth and menses), data are less robust. In a short report that investigated a cohort of 124 female adolescents with a median age of 16 years (14–18 years), Choi et al. showed that

serum-soluble transferrin receptor (sTfR) was significantly higher in IDA *H. pylori*-positive patients than in negatives, although serum iron and ferritin levels did not differ between groups. The authors concluded that serum sTfR reflects more accurately body iron status than serum iron or ferritin in *H. pylori*-positive adolescents [19].

One large epidemiologic study performed in Alaska, involving school-aged children (n = 688) of an aboriginal community, documented that active *H. pylori* infection is independently associated with iron deficiency and IDA [20]. However, the same group reported that *H. pylori* treatment did not improve iron deficiency in an open label study involving 181 children with a median follow up of 14 months [21]. Nevertheless, the existence of some limitation in the design and the analysis of the data (inclusion of only iron-deficient children, high failure rate in the eradication treatment, and ferrous sulfate treatment only in controls) may have affected the results of this study, as admitted by the authors [22].

The role of *H. pylori* in the pathogenesis of idiopathic thrombocytopenic purpura (ITP), which has already been well described in the past, has been confirmed by other studies. Kodama et al. demonstrated the improvement in platelet counts after eradication of *H. pylori* in patients with ITP. They also reported that the anti-CagA antibody titer before eradication did not differ between responders and nonresponders; however, reduction in the anti-CagA antibody titer after eradication therapy was significantly greater in responders than in nonresponders, thus confirming a possible role of the CagA antigen of *H. pylori* in the pathogenesis of ITP [23].

Matsukawa et al. observed, in their study, that the eradication of *H. pylori* reduced peripheral platelet count in patients with gastritis and gastric ulcer but without ITP [24].

Suvajdzic et al. investigated the prevalence of *H. pylori* infection in a cohort of 54 adult Serbian patients with ITP, and examined the effects of its eradication on their platelet count. A significant mean platelet recovery was seen 6 months after successful *H. pylori* eradication. No platelet recovery was registered in either *H. pylori*-negative, untreated *H. pylori*-positive patients, or *H. pylori*-positive patients who failed eradication. The results of this small prospective study supported the current view that *H. pylori* eradication may be an effective non-immunosuppressive treatment for chronic ITP [25].

The findings of Asahi et al. supported the fact that, after *H. pylori* eradication therapy, platelet recovery results from the disappearance of *H. pylori* itself, and is mediated, in part, through suppression of anti-platelet autoantibody production [26].

The prospective cohort study of Jaing et al. did not find statistically significant relationship between *H. pylori* infection and acute ITP in children [27]. On the other

hand, Uchiyama et al. reported a case of ITP associated with either *H. pylori* infection or hepatitis B in a patient with liver cirrhosis [28].

Finally, Takahashi et al. administered metronidazole, amoxicillin, and a proton-pump inhibitor as a second-line treatment in patients with ITP. *H. pylori* was successfully eradicated in both patients, with a rapid recovery of platelet count [29].

Hepato-Biliary Diseases

Wang et al., in a prospective study, evaluated the relationship among *H. pylori* infection, blood ammonia concentrations, and hepatic encephalopathy status, and investigated the effect of *H. pylori* eradication on blood ammonia levels and hepatic encephalopathy status in cirrhotic patients. The results of this interesting study showed that *H. pylori* infection is an important source of ammonia, concurring in the development of hepatic encephalopathy in cirrhotic patients and therefore *H. pylori* eradication may help to prevent hepatic encephalopathy in those patients [30].

Krasinkas et al., in another study, showed a possible role of *H. pylori* in the pathogenesis of primary sclerosing cholangitis (PSC). They enrolled 25 patients with end-stage PSC and 31 healthy controls: *H. pylori* DNA was more frequently detected in microdissected hilar biliary epithelium of PSC patients than in controls, supporting the hypothesis that bile reflux from the duodenum into the biliary tract might carry *H. pylori* organisms into the proximal biliary system, possibly contributing to PSC development and/or progression, at least in some of the patients [31].

Finally, *H. pylori* was also found by Li et al. in the liver of patients with hepatocellular carcinoma (HCC). In particular, they studied liver samples resected from 34 patients with HCC and from 20 patients without primary liver carcinoma. In particular, 65% (22 of 34) of HCC samples tested positive for *Helicobacter*-specific 16S rRNA gene by in situ hybridization compared to none of the controls [32].

Intestinal Diseases

Villanacci et al. investigated whether *H. pylori*-infected celiac disease (CD) patients may have different clinicopathologic features from non-infected subjects, and whether the histopathologic responses to a gluten-free diet could be different in *H. pylori*-positive compared to *H. pylori*-negative patients. The results of this study showed that the clinical features of CD patients are unrelated to *H. pylori* gastritis, and a gluten-free diet is equally effective in infected and non-infected patients [33].

An interaction between *H. pylori* infection and untreated CD on gastric histologic pattern was reported by Santarelli

et al. They observed that in patients with *H. pylori* infection, untreated CD could represent a risk factor for follicular gastritis and may be associated with a lower prevalence of atrophic gastritis. The complex interaction between *H. pylori* and untreated CD on Th-1/Th-2 balance in the gastric mucosa could explain those results [34].

Zumkeller et al., in a population-based case-control study, investigated the association between *H. pylori* seroprevalence and colorectal adenocarcinoma (CRC). Interestingly, a higher prevalence of *H. pylori* infection was found in patients with CRC than in controls; moreover, a positive association between *H. pylori* seroprevalence and CRC risk was found, which also persisted after adjustment for known potential confounders, including socioeconomic status. Presence of specific *H. pylori* CagA antibodies did not significantly affect the observed risk [35].

Neurologic Diseases

Interesting results were reported by Kountouras et al. on the association between *H. pylori* and Alzheimer's disease; *H. pylori* infection appeared to induce irregular humoral and cellular immune responses that, due to molecular mimicry, cross-react with components of nerves, thereby contributing and possibly perpetuating the apoptotic neural tissue damage observed in this neurodegenerative disease [36].

The same authors also suggested that *H. pylori* can be a possible common underlying risk factor in normal-tension glaucoma and Alzheimer's disease [37].

There are studies supporting the opinion that *H. pylori* infection might be a significant risk factor for migraine. During the infection, superoxide radicals and nitric oxide are produced, and prolonged oxidative injury caused by the persistent infection might be involved in regional cerebral flow changes during migraine. However, results of a study by Tunca et al. do not support the role of oxidative stress in patients suffering from *H. pylori* infection and migraine [38].

Attallah et al. have investigated the presence of a *H. pylori* antigen in serum and cerebrospinal fluid (CSF) samples from 173 individuals with meningitis. The influence of *H. pylori* infection on CSF levels of Th1/Th2 cytokines was also evaluated. *H. pylori* antigen was detected in the CSF samples of 75% of meningitis patients, also showing *H. pylori* antigen in their sera. A significant correlation was found between serum and CSF levels of a 58-kDa *H. pylori* antigen. Only the levels of Th1 cytokine (IFN- γ) were significantly higher in CSF of meningitis patients positive for the above-mentioned *H. pylori* antigen, thus allowing to conclude that the 58-kDa *H. pylori* antigen, which may cross the blood-brain barrier and enter the CSF of patients with meningitis, may play a role in that disease [39].

Fiddian-Green has tested whether *H. pylori* eradication could improve the pharmacokinetic and clinical response to L-dopa in patients with Parkinson disease and motor fluctuations. Their data demonstrated a reversible *H. pylori*-induced interference with L-dopa clinical response related to the impaired drug absorption, probably due to active gastroduodenitis. Therefore, the author suggests that *H. pylori* eradication may improve the clinical status of infected patients with Parkinson disease and motor fluctuations by modifying L-dopa pharmacokinetics [40].

Other Diseases

A study by Afsar et al. reported that *H. pylori* infection may increase renal resistive index [41]. Furthermore, Pietroiusti et al. showed that CagA-positive strains of *H. pylori* may represent a risk factor for the development of microalbuminuria in type 2 diabetes. They hypothesized that the causative effect could be due to the fact that these strains have antigenic sequences common to endothelial cells, which may be responsible for an antigenic mimicry [42].

More studies reported the association between diabetes mellitus and *H. pylori*: diabetic-infected patients have been found to require higher doses of insulin and to have higher levels of HbA1c than those uninfected; while the mechanisms are unknown the authors have proposed that the infection with *H. pylori* may induce gastric inflammation with production of several cytokines, which may stimulate the secretion of insulin counter-regulatory hormones, affecting the carbohydrate metabolism. Nevertheless, Moghimi et al. suggested that *H. pylori* treatment in patients with type 2 diabetes mellitus has no role in short-term control of the disease [43]. Ojetti et al. found a significantly higher incidence of *H. pylori* re-infection, after 5 years of follow up, in type 1 diabetic patients compared with controls [44].

A study by Longo-Mbenza et al. has reported the association between certain components of the metabolic syndrome and gender, cardiovascular diseases, and *H. pylori*: in particular they demonstrated a positive response of these factors after *H. pylori* eradication [45]. Similarly, Nabipour et al. reported an association of metabolic syndrome and *C. pneumoniae*, *H. pylori*, cytomegalovirus, and herpes simplex virus type 1 [46].

Sterzl et al. examined the prevalence of anti-*H. pylori* antibodies in patients with autoimmune thyroiditis, with and without different polyglandular involvement, and in healthy controls. A higher prevalence was found in patients with isolated autoimmune thyroiditis compared to patients with autoimmune thyroiditis coupled with a polyglandular syndrome [47].

An association between *H. pylori* seropositivity and pre-eclampsia was investigated by Ponzetto et al.; the results showed that *H. pylori* infection is higher in mothers with

pre-eclampsia (51.1%) compared with mothers with uneventful pregnancy; the difference was even greater when stratified for CagA seropositivity [48].

Aytac et al. tested the hypothesis that *H. pylori* infection may cause hyperemesis gravidarum (HG). There was no significant difference between pregnant women diagnosed as HG and controls [49].

Karatas et al. showed that *H. pylori* could be an occult risk factor for chronic prostatitis [50] while Kanbay et al. demonstrated a relationship between *H. pylori* infection and proteinuria [51].

A study by Zavos et al. has reported that mitogen-activated protein kinase intracellular signalling in the aqueous humor activated by *H. pylori* may play a role in glaucoma [52]. *H. pylori* infection has also been linked to other ophthalmic disorders, including central serous chorioretinopathy, uveitis and blepharitis, through different pathogenic mechanisms. In particular *H. pylori* infection may produce some noxious compounds, e.g. ammonia, hydrogen nitrate, and hydrogen cyanide, in exhaled breath of infected individuals. On this view, Hosseini et al. have hypothesized that chronic exposure of ocular surface to these compounds may explain these manifestations [53].

An association of CagA-positive *H. pylori* strains with adenotonsillar hypertrophy has been demonstrated by Bulut et al. [54], while Agirdir et al. have investigated the presence of *H. pylori* in the middle ear effusion by *Campylobacter*-like organisation test and whether this bacterium may have a role in the pathogenesis of chronic otitis media with effusion (OME); the results showed the presence of *H. pylori* in the middle ear effusion, possibly playing a role [55]. Furthermore, an association between *H. pylori* and OME was also reported by Yilmaz et al. [56].

Several studies, including those published by Tezer et al. have described that *H. pylori* eradication may increase the incidence of gastroesophageal reflux disease and laryngopharyngeal reflux disease [57], while the results of Kountouras et al. did not support a protective role of *H. pylori* infection in these diseases [58].

Kim et al. have investigated the prevalence of *H. pylori* in the nasal cavity of patients with chronic rhinosinusitis. Interestingly, intranasal *H. pylori* was more prevalent in patients with chronic rhinosinusitis than in healthy controls. However, there was no significant correlation between the severity of sinusitis and intranasal *H. pylori* colonization [59]. A preliminary study by Aladag et al. has demonstrated a positive association between high prevalence of *H. pylori* infection and chronic nonspecific pharyngitis [60].

Golan et al. did not find an association between *H. pylori* infection and seasickness susceptibility [61]. Cuevas Acuna et al. found an association between *H. pylori* infection and chronic urticaria [62].

Finally, Ozel et al. investigated the effect of *H. pylori* infection and eradication therapy on interleukin-6 levels in patients with familial Mediterranean fever (FMF): their results showed that the correlation between *H. pylori* infection and FMF seems to be unlikely, but further studies will be needed to better clarify this issue [63].

Other Helicobacters

The genus *Helicobacter* has been continuously growing in the recent years. Twenty-three formally named *Helicobacter* species and two *Candidatus* species have been described in the Bergey's *Manual of Determinative Bacteriology* [64]. Moreover, there is a number of further isolates that were not formally named, but which are likely to represent further *Helicobacter* species. The most recently published *Helicobacter* species are *H. cynogastricus* [65], *H. callitrichis* [66], *H. equorum* [67], *H. anseri* [68], and *H. brantae* [68].

Due to specific abilities, *Helicobacter* species are able to colonize various ecological niches in the gastrointestinal tract. With respect to their preferential site of colonization, *Helicobacter* species are divided into two subgroups: the better known gastric *Helicobacter* species, which preferably colonize the host's stomach, represent only one-third of the known species of *Helicobacteraceae*. The remaining two-thirds of *Helicobacter* species are referred to as enterohepatic because they predominantly colonize the intestine and the hepatobiliary system.

Helicobacter species are highly specialized bacteria. They are well adapted to various niches in their host's body and are able to colonize sites which are hostile to most other bacteria. With its acidic milieu, the gastric mucosa represents such a hostile environment. The stomach was formerly believed to be sterile until the gastric *Helicobacter* species were successfully colonized from gastric mucosa. There is a complex system of genes and mechanisms explaining how gastric *Helicobacter* species are adapted to the acidic environment; certainly urease is of capital importance [69]. Recently, the importance of urease has also been underlined by the discovery that *H. felis* has even got a second urease system: UreA2B2 [70]. Similar to gastric juice, also bile and pancreatic juice are highly bactericidal. Nevertheless, a number of enterohepatic *Helicobacter* species use the bile ducts as a preferential habitat. *H. cholecystus* is currently the only species that is known to colonize the pancreas. *Helicobacter* species were also identified within the crypts of Lieberkühn in the intestinal mucosa which contain high concentrations of antimicrobial factors such as defensins, lysozymes, IgA, and mucins. There is evidence that some enterohepatic *Helicobacter* species are able to inhibit the innate immune responses [71], but taken together the mechanisms how enterohepatic *Helicobacter* species defy the innate immune response

or survive in the presence of bile or pancreatic juice are largely unknown and need further investigation.

Helicobacteraceae may infect a wide variety of vertebrates. Host species comprise humans, as well as various non-human primates, carnivores, ruminants, horses, rodents, cetaceans, and birds. The bacteria have been evolving with their hosts since thousands of years and therefore are well adapted to their specific hosts. For example, comparative analysis of the genomes of *H. acinonychis* and the closely related *H. pylori* revealed that a host jump occurred approximately 50,000–400,000 years ago. On the basis of unique features within the genome of *H. acinonychis* the direction of the host jump was deduced, namely from early humans to cats. Hence, it can be assumed that large felines were infected with *Helicobacter* by eating early humans together with their gastric inhabitant *H. pylori* [72]. *H. acinonychis* possesses an unusually large number of highly fragmented genes. Many of these encode outer membrane proteins, which may have been destroyed in order to bypass deleterious responses from the feline host immune system and therefore are believed to be molecular events that allowed *H. acinonychis* to adapt to novel animal hosts [72].

Although *Helicobacteraceae* are well adapted to their specific host organisms, transfer from one host to another may occur. There is evidence that a number of gastric *Helicobacter* spp. can be transferred from dogs [73], as it was shown from cats [74], to their owners. In larger series *Candidatus H. suis*, *H. felis*, *H. bizzozeronii*, *H. salomonis*, *Candidatus H. bovis*, as well as formally unnamed *Helicobacteraceae* were detected in human samples. *Helicobacter* spp. other than *H. pylori* are found in 0.2–0.6% of human gastric biopsies. There are no studies on specific pathologies or therapies yet. It is suggestive that birds serve as a zoonotic source of enterohepatic *Helicobacter* species. Recently, two further novel *Helicobacter* spp., *H. anseris* and *H. brantae*, were isolated from geese living in public waterways, parks, and golf courses, which were contaminated with bird feces [68]. However, human transmission with these novel bird helicobacters have not yet been described.

Helicobacter Infection in Pets, Livestock, Zoo, and Laboratory Animals

Helicobacter infections may cause serious health problems in captive animals. Especially animals kept in mass stocks are at risk of getting infected. Once the infection is introduced into a facility, it may easily spread due to the close contact among the animals. The best example for *Helicobacter*-infected animals kept in mass stocks is broiler chickens and commercial laying hens which are frequently infected with *H. pullorum* [75–77]. Usually, animals kept in animal farms or breeding facilities are infected with enterohepatic *Helicobacter* spp., which result in permanent

infections of the animal's intestines and spread of infection by fecal–oral transmission [78,79]. However, close contact of animals does not only increase the risk of infection in small animals. Recently, it has been demonstrated that prevalence of *H. equorum*, a novel *Helicobacter* species that was isolated from the feces of horses, is rare in feces of privately owned horses, but prevalence increases from 0.8% to 3.1% in riding-school horses and 7.9% in hospitalized horses [80]. Also laboratory animals are frequently infected with enterohepatic *Helicobacter* species [79]. While in earlier studies *H. bilis* and *H. hepaticus* were identified as the most common *Helicobacter* species in laboratory mice, the most frequent *Helicobacter* species found in a recent study were *H. ganmani* and *H. typhlonicus* as well as several *Helicobacter* species that had not been formally named so far [79]. Because *Helicobacter* infection may significantly influence the results of animal experiments, even if there is no apparent pathology, *Helicobacter* testing is especially important and should be required in animal models.

Helicobacter-Related Pathologies

Helicobacter species other than *H. pylori* infecting different animal species are used as models for *H. pylori* infection in humans. This year a new mouse model using C57/BL6 mice and *H. heilmannii* allowed to study the apoptosis and angiogenesis of low grade gastric mucosa-associated lymphoid tissue lymphoma [81].

Helicobacter species other than *H. pylori* may also play a role in chronic liver diseases, gallstone formation and hyatobiliary neoplasia. A recent study on gallbladder carcinoma carried out in Germany failed to detect significant numbers of patients infected with *Helicobacter* species [82]. This study confirms a trend that in regions with a high incidence of gallbladder carcinoma, infections with enterhepatic *Helicobacter* species are more frequent than in regions with a low incidence of gallbladder carcinoma.

Enterohepatic helicobacters have been implicated in inflammatory bowel disease in some animals, and also in humans [83]. A positive study carried out in children was also published this year [84].

Conclusions

Several studies performed during the past year supported a possible role for *H. pylori* infection in the pathogenesis of several extragastric diseases. Among them, ITP and IDA represent the diseases in which the pathogenic link appears to be well defined. Interest in a role for *H. pylori* in IHD continues. For other extragastric diseases, the level of evidence is still weak. There is an increasing number of gastric *Helicobacter* spp. which colonize the

stomach of various vertebrates and may be transmitted to humans as a zoonosis. Enterohepatic *Helicobacter* species are important emerging pathogens. Their pathogenic potential on different hepatobiliary and intestinal diseases has been proven in various animal models and there is increasing evidence that enterohepatic *Helicobacter* spp. also play a crucial role in human diseases.

Conflicts of interest

The authors have declared no conflicts of interest.

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