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DERLEME / *REVİEW*

Helicobacter Pylori: Patofizyoloji, Sıklık, Risk Faktörleri, Tanı ve Tedavi

Helicobacter Pylori: Pathophysiology, Prevalence, Risk Factors, Diagnosis and Treatment

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ÖZET

Helicobacter pylori (H pylori) populasyonun %50'sinden fazla görülen ve gastrik mukozaya yerleşen spiral şekilli, flajelli, mikroaerofilik, gram (-) bir basildir. Dünya genelinde en yükseği gelişmekte olan ülkelerde bildirilen değişken bir görülme sıklığına sahiptir. Risk faktörleri ile ilgili çalışmalar özellikle sosyoekonomik faktörler üzerinde durmaktadır. İnsanlarda gastrit ve ülser ile ilişkisi net olarak kanıtlanmıştır. Enfeksiyon çocukluk çağında sıklıkla oral yolla bulaşmaktadır. Üre nefes testi, dışkı antijen testi, antikor tayini, endoskopi, histolojik inceleme, üreaz testi ve kültür tanıda kullanılan yöntemlerdendir. Antibiyoterapi ve antiasitler tek başına yeterli olmadığından birlikte kullanımları tercih edilmektedir. N-asetilsistein gibi mukolitik bir ajan ile *H pylori* tabakasının ortadan kaldırılması da tedavi öncesinde etkili olabilmektedir. *Lactobacillus, Saccharomyces, Bifidobacterium* ve *Bifidobacterium clausii* gibi probiyotik suşların eklenmesi de diğer bir tedavi yaklaşımıdır. İlk tercih tedaviler yetersiz kaldığında farklı antibiyotikleri içeren ikinci adım tedavilere gerek duyulabilmektedir.

Anahtar Kelimeler: Helicobacter pylori, gastrit, antibiyotik, tanı, tedavi

ABSTRACT

Helicobacter pylori (H pylori) is a spiral-shaped, flagellated, micro- aerophilic gram-negative bacillus that colonizes the gastric mucosa of more than 50% of the human population. There are different findings for the prevalence of *H pylori* across the world with the highest prevalence in developing countries. Most of the reports on risk factors focused on socioeconomic indicators. Its relationship with gastritis and peptic ulcer in humans was proven. The infection is transmitted within the family in childhood, likely by oral transmission. Urea breath test, stool antigen test, antibody detection, endoscopy, histology, urease test, and culture are used for the diagnosis. Antibiotics and antiacidics are not sufficient alone, therefore combination treatment is preferred. Pretreatment with N-acetylcysteine as a mucolytic agent to destroy the biofilm of *H pylori* is effective. The addition of probiotics such as *Lactobacillus spp*., *Saccharomyces spp*., *Bifidobacterium spp*., and *Bifidobacterium clausii* as an adjunctive agent is another approach. If the first-line therapy fails, the second-line options should include different antibiotics.

Keywords: Helicobacter pylori, gastritis, antibiotics, diagnosis, treatment

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Introduction

Helicobacter pylori (*H pylori*) was discovered at the beginning of the 1980s and its relationship with gastritis and peptic ulcer in humans was proven[1, 2].

H pylori is a spiral-shaped, flagellated, micro-aerophilic gram-negative bacillus that colonizes the gastric mucosa of more than 50% of the human population, with the highest prevalence in developing countries.

The infection is transmitted within the family in childhood, likely by fecal– oral transmission. There is some evidence of *H pylori* presence in the oral cavity that a recent meta-analysis related to gastric colonization and possible reinfection. Presence of *H pylori* in tonsils is controversial; if confirmed, it could help further understanding of *H pylori* transmission and reinfection[3]. There is also evidence that *H pylori* infection is a risk factor for gastric mucosa-associated lymphomas (MALT lymphomas)[4]. Gastric adenocarcinoma is one of the few malignant neoplasms for which infectious agents have been recognized as having an important etiologic role[5]. In 1994, based mostly upon epidemiologic evidence, the International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO), recognized infection by *H pylori* as a primary cause of gastric adenocarcinoma[6]. If left untreated, *H pylori* infection leads to life-long chronic active gastritis, which is a risk factor for both intestinal and diffuse gastric adenocarcinomas[7]. However, *H pylori*-associated preneoplastic lesions are a feature of intestinal-type gastric cancer and not the diffuse-type. The diffuse type is more likely to have a primary genetic etiology, and the involvement of *H pylori* is probably limited to a subset of sporadic cases[8].

Prevalence

There are different findings for the prevalence of *H pylori* across the world. The reported prevalence ranged from 4% in Japanese children to 82% in African refugee children in Australia. A prevalence of 15% or lower was reported for Australian lab patients, Malaysian blood donors, and Chinese and Japanese school children. A prevalence of 24–25% was reported for Israeli children attending daycare units and unspecified individuals from Turkey[10, 11]. Among the Italian villagers (mean age; 59 years), the prevalence was 58%, considerably higher than the 34% observed in an earlier similar study of adults from northern Swedish communities (mean age; 52 years). A prevalence of 60% or more was reported for groups in Albania, Egypt, Iran, Turkey, and China[9-13].

Risk Factors

Most of the reports on risk factors focused on socioeconomic indicators. Most of the studies examined cross-sectional associations between exposures of interest and being infected at the time of screening, which cannot differentiate determinants of acquisition from determinants of persistent infection. Relationship did not appear to be independent from other factors in multivariable analyses[12,13].

Two studies examined occupational exposures that increase the risk of infection. In a Belgian-Swiss study, using seroconversion as an

endpoint for survival analysis, no clear effect of exposure to sewage was observed, when controlling for education level, nationality, country of childhood, smoking, and alcohol intake[14,15].

Among African refugee in Australia, ethnicity, country of transit and premigration antimalarial treatment history were associated with *H pylori* infection, but in a multivariable logistic regression model, only premigration antimalarial treatment appeared to retain an independent association, in the direction of reduced odds of infection[13].

Diagnosis

Urea breath test (UBT)

13C-UBT has been shown numerous times to be the most accurate *H pylori* diagnostic test. The effect of the test meal was explored further. Indeed, it was already known that citric acid was the best test meal to be used in 13C-UBT, the hypothesized mechanism being a delay in gastric emptying. In some studies, it was clearly showed that the increased intragastric urease activity could not be attributed only to gastric emptying. It was suggested that citric acid could have a direct effect on UreI, a proton gated urea channel, making urea more accessible to the intrabacterial urease. The use of citric acid also led to a higher accuracy of the 14C-UBT, allowing to a decrease in the dose of radioactivity (1 μCi instead of 2.5) and the measurement time (10 minutes instead of 20)[16].

The possibility of false-positive results due to urease-positive bacteria from the oral cavity in patients with atrophic gastritis was highlighted by Osaki et al. indicating that the histologic status of the stomach, i.e. presence or absence of atrophy, must be considered in interpretation of the results[17]. To avoid false-positive results, the capsule UBT can be used[18].

Stool antigen test

Stool antigen detection kits for the diagnosis of *H pylori* infection have been widely used because of their full noninvasive nature. Blanco et al. evaluated the results of 6 tests which are under use and found that sensitivity and specificity were 52.5-95% and 55.5-94.4%, respectively[19]. These results are very promising and deserve to be confirmed because this test could possibly turn out to be the best noninvasive test.

Antibody detection

This is a cheap and easy ELİSA test in the detection of antibodies against to *H pylori*. But such serologic tests can not be used in the evaluation of *H pylori* eradication since antibody titres decrease within 6-12 months despite an efficient eradication[20].

CagA (cytotoxic associated protein) antibodies persist longer than *H pylori* antibodies detected in a global test, and can help in linking gastric carcinoma to *H pylori* infection. In a study, the serological status assessed by a CagA commercial immunoblot had no predictive value for the severity of disease while the CagA status of the isolate had[16].

Endoscopy

To obtain biopsies, upper gastrointestinal tract endoscopy must be performed. Cho et al. proposed a new method of standard endoscopic diagnosis of *H pylori*: the phenol red mucosal

pH test. A 0.1% phenol red solution was sprayed on the gastric mucosa. The extent of staining, expressed as a staining score, was positively correlated with the urea breath test values and with *H pylori* density as measured by histology. The pH measured by this technique with an antimony electrode was significantly higher in *H pylori* infected mucosa. Therefore, endoscopic phenol red staining may be an alternative method for the diagnosis of *H pylori* infection[21].

Advantage of this endoscopic method is easy use in both pre- and post-treatment evaluations. Both sensitivity and specificity are in high levels. *H pylori*-associated pathologies can be easily detected during endoscopy, and it is suitable to take cultures for the antibiotic sensitivity[22].

Histology

Biopsy specimens are stained by hematoxilen-eosin, warthin-starry gumus, gram, akridin orange, and modified giemsa. Histogical examination detects chronic active inflammation, lymphoid aggregates, athropy, intestinal metaplasia, and malignancy besides *H pylori*.

An article referred to the new staging system for atrophy (OLGA) and its application in diagnostic practice. It was also used to assess atrophic gastritis in 63 *H pylori* positive patients with various gastric diseases. They found the OLGA staging system useful for the assessment of the severity of atrophic gastritis and simple to use. In another study concerning different risks of gastric cancer in populations, the OLGA staging mirrored the gastric cancer incidence[22-25].

Urease test

Quantitative analysis of urease activity of *H pylori* present in gastric mucosa is possible by this test. As a solution to the low sensitivity of the rapid urease test (RUT), some authors proposed to increase the number of biopsies up to four. Comparing one biopsy to four, the positive results increased from 52 to 96%, respectively[26].

Culture

Although is the most specific method for the diagnosis, failure in providing optimum conditions decreases sensitivity of this test. Sainsus et al. tried to develop a liquid culture medium for the rapid isolation, identification, and subsequent antibiotic susceptibility testing of *H pylori* from biopsy specimens. They selected Ham's F12 medium with 5% horse serum with antibiotics which provided the most rapid and reliable growth. The CIM medium seems a promising solution for some of the current problems concerning *H pylori* culture in solid media[27].

Treatment

Antibiotics and/or antiacidics are not sufficient alone. So combination treatment is preferred. Efficieny of combination treatment is well known[22]. The efficacy of the standard first-line triple therapy is declining, most likely from increased antibiotic resistance. Several attempts have been made to overcome treatment failure and newer regimens with new combinations of antibiotics have been introduced including sequential, and concomitant quadruple therapies[28].

Pharmacological agents have been studied with the goal to make the bacteria more

susceptible to antibiotics. Pretreatment with N-acetylcysteine as a mucolytic agent with the intention to destroy the biofilm of *H pylori* and thus to overcome *H pylori* antibiotic resistance has been successfully tested in patients, but more studies are needed before its possible introduction to clinical practice.

The addition of probiotics as an adjunctive agent is another approach. Various *lactobacilli* or their metabolic products can inhibit or eradicate *H pylori* in vitro. A recent meta-analysis investigated the effects of *Saccharomyces boulardii* as a supplementation to the standard triple therapy. The adjunctive treatment with *Saccharomyces boulardii* had little effect on the eradication rate but reduced *H pylori* therapy-related adverse effects. A recent review of the literature, including all available randomized, double-blind, placebo-controlled trials, concluded that a variety of 'probiotic' bacteria and yeasts, including *Lactobacillus spp*., *Saccharomyces spp*., *Bifidobacterium spp*., and *Bifidobacterium clausii*, when added to standard *H pylori* eradication regimens, did not affect eradication rates but reduced adverse effects such as nausea, taste disturbance, diarrhea, and epigastric pain, thus increasing tolerability of *H pylori* eradication therapies[29-31].

If first-line therapy fails, the second-line options should include different antibiotics. If standard triple therapy was used, the second attempt should be performed with the bismuth containing quadruple therapy. If bismuth-based quadruple fails, second-line option should be levofloxacin-based triple therapy. As quinolone resistance (i.e.

Other causes include constipation, previous hemorrhoids, and excess weight. Rectal bleeding, itching around the anus, discomfort and mucosal changes can negatively affect sexual activity[43].

Disparoni: The incidence of sexual activity in the active phase is 46% [3,44]. In the study of Aslan et al. (2005), it was reported that especially in the third trimester, the disparonin increases in pregnancy[21]. Reamy and White dyspareunia in pregnancy stated in 1985 that many physical factors such as vaginal congestion and decreased lubrication, deep fetal headache, candidiasis, urinary tract infections, trichomonas vaginalis and also fatigue, body image change and anxiety cause of disparoni[45].

Erectile Dysfunction in Men During Pregnancy: A large number of males may experience erection problems once during the pregnancy period of their couples. This is not a sign of erectile dysfunction. This can usually be associated with fatigue, intense sadness, or getting too much alcohol. Sometimes men can not have an erection or continue their erection while their partners are pregnant. Sexual function can be blocked if the partner does not get attractive. In addition, fear of harm to the baby and the mother may affect sexual function[5,6].

The Situations Prohibiting Sexuality During Pregnancy

In the past, couples were recommended to avoid sexual intercourse in order to avoid abortions in the first three months and to prevent infection in recent weeks. It is thought that in today's healthy pregnancy, it is not

levofloxacin) rises, the efficacy of this regimen needs to be monitored and cautiously used in treatment of patients with chronic pulmonary infections who may have been received quinolones before[32].

Several clinical studies were published during 2011, aiming at assessing the efficacy of modifications of the current treatment regimens. A large study in Latin America indicated that the first-line 14-day standard triple therapy (lansoprazole, amoxicillin, clarithromycin) in this area remains more efficacious than a 5-day quadruple concomitant therapy with the addition of metronidazole, or sequential therapy of 5 days of lansoprazole and amoxicillin followed by 5 days of lansoprazole, clarithromycin, and metronidazole[32]. On the other hand, different sequential regimens produced good results in other studies of first-line or second-line treatment[33-39]. Also, some second-line therapies achieved good eradication rates in Japanese studies[37,38]. A large study performed in 39 European sites with a first-line quadruple therapy (bismuth subcitrate potassium, metronidazole, tetracycline hydrochloride, omeprazole) achieved excellent eradication rates in comparison with a standard triple therapy (amoxicillin, clarithromycin, omeprazole) (80 vs. 55%, intention to treat; 93 vs. 70%, per protocol)[39]. Moreover, this study included three-in-one capsules with the aim of increasing patient compliance by making easier administration. Also different quadruple therapies achieved good results in Turkey[40-41].

Helicobacter pylori and Non-malignant Diseases

Gastritis and H pylori Infection

It is well known that *H pylori* infection causes histologic gastritis. There are inter-individual differences in the severity or patterns of gastritis which are then associated with the further development of different kinds of disorders, such as duodenal ulcer, gastric ulcer, and gastric cancer. Genetic differences in host and bacterial factors have been considered to be one of the reasons for the inter-individual differences.

For the explanation of these inter-individual differences in response to *H pylori* infection, polymorphisms of cytokines, such as interleukins (ILs) and tumor necrosis factor-alpha (TNF-α), have been studied intensively since the year 2000. These cytokine polymorphisms are associated with different patterns of gastritis among different individuals. In 2008, several new polymorphisms associated with *H pylori*-induced gastritis were reported[42].

There have been several important reports on the polymorphism of bacterial factors. *H pylori* strains have been classified into two groups: strains with high virulence and low virulence. The differences between the two groups are partly explained by the status of *cagA* and *vacA*, which are well known to be polymorphic. For *vacA*, strains with an s1/m1 genotype have been thought to be more virulent than those with *s2/m2* [43]. Chomvarin et al. attempted to determine whether any correlation exists between genotypes of *vacA*, *cagA*, *cagE*, *iceA*, and *babA2* and clinical

manifestations in dyspeptic patients infected with *H pylori* and concluded that neither a single gene nor a combination of *vacA*, *cagA*, *cagE*, *iceA*, and *babA2* genes was significantly helpful in predicting the clinical outcome of *H pylori* infection in their country[44]. However, Basso et al. studied *cagA* and *vacA* polymorphisms as well as the number of type C Glu-Pro- Ile-Tyr-Ala motif (EPIYA) (EPIYA-C) segments, which increase phosphorylation-dependent *cagA* activity in *H pylori* positive Italian patients with different disorders and they confirmed the association of *cagA* and *vacA s1 / m1* polymorphisms with peptic ulcer diseases and cancers and noted that the most important factors in western countries were the number of *cagA* EPIYA-C segment for cancer risk and the intermediate region type of *vacA* for peptic ulcer diseases risk[45]. Because the EPIYA-C segment is the *Src* homology 2 domain-containing protein tyrosine phosphatase (SHP-2) binding site of *cagA* is clearly associated with *RAS / MAP kinase*, EPIYA-C will be the key factor for elucidating the bacterial types and their corresponding clinical outcomes, including gastric cancer.

As stated before, a variety of polymorphisms from both bacterial and host sides were reported to be associated with the severity and / or the type of gastritis. In contrast, Kim et al. evaluated risk factors of atrophic gastritis and intestinal metaplasia with respect to *H pylori* virulence factors (i.e., *cagA*, *vacA m1*, and *oipA*), and environmental factors (i.e., smoking and alcohol) and host polymorphisms (i.e., IL-1b-511, IL-1RN, TNF-A-308, IL-10-592, IL-10-819, IL-10-1082, IL-8-251, IL-6-572, GSTP1, p53 codon 72, and ALDH2) and found that the bacterial factors were important risk factors for atrophic gastritis but that environmental and host factors were more important for intestinal metaplasia.

Conclusion of the article is that; to understand the inter-individual differences in response to *H pylori* infection among different subjects, not only genetics of hosts and bacteria, but also environmental factors have to be studied. The useful marker that predicts the individual response to *H pylori* infection remains to be elucidated in relation to environmental factors[42,46].

Gastroduodenal Ulcer and H pylori Infection

It is a common knowledge that *H pylori* infection is, along with nonsteroidal anti-inflammatory drugs (NSAIDs) / aspirin, a major factor of peptic ulcer. Peptic ulcer diseases remains a common condition despite a decrease in incidence and prevalence owing to a decrease in *H pylori* infection. Wu et al. reported a dramatic decrease in the incidence of admissions for complicated or uncomplicated peptic ulcer diseases correlated with a significant increase in eradication therapy and use of proton-pump inhibitors from 1997 to 2006. Eradication of *H pylori* infection is known to be effective in the prevention of bleeding ulcers. Van Leerdam et al. evaluated the epidemiological surveys on gastrointestinal bleeding and observed that *H pylori* infection was found in about 50% of bleeding peptic ulcer patients. They concluded that, all ulcer patients should be examined for

H pylori infection and treatment for eradication should be given to those who are positive[47,48].

Gastroesophageal Reflux Disease and H pylori Infection

Studies have shown that the prevalence of *H pylori* infection is lower in patients with gastroesophageal reflux disease than in patients with non-gastroesophageal reflux disease. *H pylori* infection has been considered to be possibly protective against the development of gastroesophageal reflux disease. The fact that the eradication of *H pylori* favors gastroesophageal reflux disease and / or exacerbates symptoms in patients with gastroesophageal reflux disease remains controversial. Different conclusions have been reported on this subject in several studies[47-50].

Several studies were performed to clarify the relationship between *H pylori* status, gastric atrophy, and gastroesophageal reflux disease. Anderson et al. performed a case-control study including a large number of patients with esophageal adenocarcinoma, Barrett's esophagus, reflux esophagitis, and healthy controls. They found an inverse association of *H pylori* seropositivity and also atrophy determined by the pepsinogen I / II ratio with esophageal adenocarcinoma, Barrrett's esophagus, and reflux esophagitis. However, although gastric atrophy was involved, it might not fully explain the inverse association with *H pylori* infection. Similarly, Kwon et al., who compared a group of 45 patients having erosive esophagitis with a group of 66 control patients, found that the rate of infection of *H pylori* was lower in the esophagitis group and the pepsinogen I / II ratio was higher than that in the control group, suggesting an inverse association between gastroesophageal reflux disease and *H pylori*-related gastric atrophy. In contrast, Monkemuller et al. did not find any correlation between serum gastrin and pepsinogen I and II with the severity of gastroesophageal reflux disease[50-52].

Gastric Polyps and H. pylori Infection

Since some gastric polyps may disappear after eradication of *H pylor*i, the pathophysiological role of *H pylori* infection in the development of gastric hyperplastic polyps has been suggested. Ohnishi et al. studied the pathophysiologic role of *cagA* using *cagA* transgenic mice and found that wild-type *cagA* transgenic mice developed gastric epithelial hyperplasia and some of the mice developed gastric polyps and adenocarcinomas of the stomach and small intestine, suggesting that *cagA* is an oncogenic protein[54]. Interestingly, such pathologic abnormalities were not observed in transgenic mice expressing phosphorylation-resistant *cagA*, indicating the importance of *cagA* tyrosine phosphorylation in the development of *H pylori*-associated neoplasms.

NSAIDs/Aspirin-Induced Gastric Injury and H. pylori Infection

For antiplatelet therapy, the recommendation is to examine *H pylori* infection in patients with a history of peptic ulcer diseases and to eradicate *H pylori* infection when present. The PPIs are recommended to prevent recurrence of complications[59].

H pylori infection is associated with many nonmalignant disorders as described before. Genetics of hosts and bacteria as well as environmental factors are responsible for the inter-individual differences in response to *H pylori* infection in different individuals. Unfortunately, the impact of newly discovered polymorphisms is still unclear. Therefore, comparative studies are needed to clarify the important single-nucleotide polymorphisms associated with a response to *H pylori* infection. Although the pathophysiologic role of *H pylori* in nonmalignant diseases has not been fully elucidated, eradication of the bacteria is sometimes effective for the treatment of these disorders. Eradication of *H pylori* infection has also been recommended for patients treated with NSAID ⁄ aspirin and ⁄ or antiplatelet agents. Indeed, there are no disorders for which eradication of *H pylori* infection is contraindicated; therefore, the ‘‘test and treat strategy’’ appears to be useful in *H pylori*-positive patients with certain symptoms, such as dyspepsia. However, further studies are needed to clarify more precisely the association of *H pylori* infection with these nonmalignant disorders, which will contribute to higher quality of clinical practice in the treatment of digestive diseases.

Helicobacter pylori and gastric cancer

H pylori infection is the strongest known risk factor for gastric cancer, and epidemiologic studies have estimated that, in the absence of *H pylori* infection, 75% of gastric cancers would not exist. *H pylori* is considered to be the most common causative agent of infection-related cancers, and is estimated to be responsible for 5.5% of all cancers world-wide. Although it is clear that *H pylori* is the strongest causative agent for gastric cancer, the precise mechanisms for gastric cancer development in response to *H pylori* infection are less well defined, and a complex interplay of strain-specific bacterial constituents, inflammatory responses governed by host genetic diversity, and/or environmental influences are involved in determining the fate of the host that is persistently colonized by *H pylori.* This review focuses on the specific mechanisms used by *H pylori* to drive gastric carcinogenesis[60-63].

The *cag* pathogenicity island (cag PAI) is a well-characterized and intensively studied *H pylori* virulence determinant, and strains that harbor the *cag* PAI increase the risk for distal gastric cancer compared with strains that lack the *cag* island[64]. Genes within the *cag* island encode proteins that form a bacterial type IV secretion system (T4SS) that translocates proteins across the bacterial membrane into host gastric epithelial cells[65-67]. The terminal gene product of the *cag* island is *CagA*, and this is one of the substrates that is translocated into host cells by the T4SS[68]. *CagA* translocation occurs through the interaction of the *H pylori* protein *CagL*, which is located on the distal tip of the T4SS pilus, with integrin a5b1 on host epithelial cells[69]. *CagI* and *CagY* have also been shown to interact with b1 integrin and mediate *CagA* translocation, and *CagL* physically associates with *CagI* and *CagH*[70,71]. In addition, *CagA* facilitates its own translocation through specific binding to b1 integrin. *CagA* is also reported to be delivered

into host epithelial cells by T4SS-induced externalization of phosphatidylserine from the inner leaflet of the cell membrane. The N-terminus of *CagA* then interacts with phosphatidylserine to gain entry into host epithelial cells. Once inside host cells, *CagA* is tyrosine phosphorylated by Src and Abl kinases at glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs located within the carboxyl-terminus of *CagA*[60].

Once phosphorylated by members of the Abl and Src family kinases, phospho-CagA targets and interacts with numerous intracellular effectors to lower the threshold for carcinogenesis. Phospho-CagA activates a eukaryotic tyrosine phosphatase (SHP-2), leading to sustained activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2), Crk adaptor, and C-terminal Src kinase, and induces morphologic transformations similar to the changes induced by growth factor stimulation. Interaction of phospho-CagA with C-terminal Src kinase rapidly activates a negative feedback loop to downregulate Src signaling and subsequently the generation of phospho-CagA[60].

CagA is not the only bacterial product delivered through the T4SS; components of *H pylori* peptidoglycan are also delivered into host cells and trigger signaling pathways that lower the threshold for carcinogenesis. Peptidoglycan interacts with the host intracellular pattern recognition molecule Nod1, which leads to activation of NF-kB-dependent proinflammatory responses such as secretion of IL-835 or b-defensin-2, as well as production of type I interferon (IFN). Translocated peptidoglycan can also activate phosphatidylinositol 3-kinase (PI3K)/Akt signaling, leading to decreased apoptosis, increased proliferation, and increased cell migration[72-75].

H pylori-associated gastric cancer is a major worldwide health care burden. Although the incidence is declining in developed countries, over the coming decades the incidence of gastric cancer in developing countries will actually increase, largely because of aging of the population. Thus, it is in developing countries that early detection is most needed. Since resources are limited, biomarker tests must be non-invasive, simple, and cheap, which makes the task of biomarker discovery and development even more difficult. To be most efficient and economical, biomarkers will also have to be utilized in the right context. For example, it will be important to validate single nucleotide polymorphisms or other markers in different ethnic groups, and to use markers of unregulated inflammatory response, such as altered mRNA, DNA methylation, or altered glycomics and proteomics, only in older adults (probably > 40 y) where precancerous lesions are more likely. Gastric cancer is a multifactorial disease, and a proper combination of biomarkers, together with age, gender, family history, and perhaps even blood group, may improve their utility to identify patients at risk. Finally, since the neoplastic response to *H. pylori* infection is delayed in germ free mice, other members of the gastric microbial community might also be informative. Early detection with a combination of biomarkers, together with more

intensive screening of high-risk individuals, offers the most realistic hope to bend the gastric cancer curve[76].

Helicobacters and Extragastric Diseases

Atherosclerotic Disease

Two aspects of *H pylori*, *H pylori* involvement in atherosclerotic disease were investigated: epidemiology and pathogenesis. Regarding IHD, Aiello et al. evaluated the socioeconomic and psychosocial gradients of pathogen burden of four infectious agents (*cytomegalovirus, herpes simplex virus-1, H. pylori* and *Chlamydia pneumoniae*). The authors showed that low education and a higher level of chronic psychosocial stress were significant independent predictors of higher pathogen burden after adjustment for covariates[77]. In a study from Turkey, the authors focused on the seroprevalence of antibodies to *H pylori* in patients with acute coronary syndrome. They showed a significantly higher rate of positivity in patients than in controls. However, no adjustment for socioeconomic factors was made[78]. Similar results were reported in India, where the seroprevalence of IgA and IgG to *H pylori* was significantly higher in patients with an incident or prevalent IHD with respect to age and sex-matched controls. The level of CRP was higher in subjects positive for IgA, but not for IgG to *H pylori*. On the basis of these findings, the authors proposed that the association of CRP with IgA to *H pylori* be used as marker to target the population at high risk for IHD[79].

The study by Nikolopoulou et al. supported the association between seropositivity for *anti-H pylori* IgG and coronary atherosclerosis, but not in its acute phase. Furthermore, a potential causal role involving the overexpression of TNF-α and vascular cell adhesion molecule-1 is not supported by data[80]. To clarify if more virulent *H pylori* strains (expressing the CagA antigen) were involved in coronary instability, Franceschi et al. performed a clinico-pathological study and a meta-analysis on 4241 cases. In their study, the authors showed that the anti-CagA antibody titer was significantly higher in patients with unstable angina compared to those with stable angina, normal coronary arteries or healthy controls. Moreover, anti-CagA antibodies recognized antigens localized inside coronary atherosclerotic plaque in all specimens from both stable and unstable patients. In the meta-analysis, seropositivity to CagA was significantly associated with the occurrence of acute coronary events[81]. These findings support the potential role of more virulent *H pylori* strains in the acute phase of IHD, a pathogenic model postulated on the basis of previous observations[82], and are not mutually exclusive with the association of the infection with increased circulating low-density lipoprotein cholesterol and triglyceride levels[83].

Arrhythmias

Besides ischemic heart disease, the possible association between *H pylori* infection and atrial fibrillation has been previously published. Platonov et al. reported, in a case–control study, that permanent atrial fibrillation is associated with elevated CRP levels, but the latter is not the result of earlier infection with *H pylori* or *C. pneumoniae*[84]. This is in

agreement with the conclusion of an editorial that, in light of the existing results, the responsibility of *H pylori* infection has been excluded in the development of atrial fibrillation[85].

Idiopathic Thrombocytopenic Purpura (ITP)

After the pioneer report by Gasbarrini et al. (86), the association between *H pylori* and ITP obtained a formal recognition in the Maastricht III Consensus report which recommended that *H pylori* infection should be sought after and treated in patients with ITP[87]. It was found that patients infected with *H pylori* have low thrombocyte count[88].

During the last year, a Canadian prospective study showed that in subjects with ITP, 48 months after *H pylori* eradication, 75% achieved a complete or a partial response and 50% had a long-term ongoing response[89]. Unfortunately, the small sample size (four *H pylori*-positive patients) limits the value of the long-term follow-up. In a 7-year follow-up prospective study conducted in Japan, *H pylori* eradication had a short-term efficacy in about half of the *H pylori* positive ITP patients[90]. In Korea, in patients who did not respond to steroid and ⁄ or danazol therapy for ITP, a combination therapy consisting of *H pylori* eradication plus immunosuppressive therapy induced, after 6 months, a statistically higher response than *H pylori* eradication alone. Furthermore, the median response duration was also longer in the former than in the latter group[91]. In contrast, in Australia, four of nine ITP patients receiving eradication treatment showed no response and underwent splenectomy, and one relapsed after 3 months[92]. In a systematic review, original articles reporting 15 or more total patients were included. The authors found 25 studies including 1555 patients, of whom 696 were evaluable for the effect of *H pylori* eradication on platelet count. The complete response and overall response (at least doubling of the basal count) were 42.7% and 50.3%, respectively. The response rate tended to be higher in countries with a high background prevalence of *H pylori* infection (e.g. Japan) and in patients with a milder degree of ITP[93].

Iron-deficiency Anemia (IDA)

Several seroepidemiologic studies have suggested a link between *H pylori* infection and IDA both in adults and in children[94]. Moreover, pregnant women with IDA had a significantly high prevalence of active *H pylori* infection[95].

Some investigators observed that cure of the bacterial infection is followed by improvement and normalization of mean cell volume, ferritin, and iron, with disappearance of anemia[96]. During a follow-up of 40 months of children in rural Alaska, *H pylori* eradication modestly reduced the prevalence of iron deficiency and substantially reduced that of IDA[97]. Different results have been achieved in Iran, where the frequency of *H pylori* infection in children with and without anemia was similar[98]. Similar findings have been reported in Northwest Turkey where authors hypothesized that IDA might be explained by inadequate dietary intake[99]. In Bangladeshi children, the authors observed a significantly higher effect of iron alone therapy compared to *anti-H pylori* therapy in improving iron status.

Even *anti-H pylori* treatment compared with placebo was not effective in improving iron status at day 90. No additional impact of combined *anti-H pylori* plus iron therapy over iron therapy alone was observed[100]. Muhsen and Cohen performed a systematic review and a meta-analysis on *H pylori* infection and iron stores. Although very few studies controlled for multiple potential confounders, most investigations reported a positive association between *H pylori* and decreased body iron stores in symptomatic and asymptomatic infected subjects. *H pylori* may be considered a risk factor for reduction of body iron stores, iron deficiency and IDA, especially in high-risk groups. The meta-analysis showed an increased risk of IDA as well as iron deficiency[101].

Conclusion

Since the discovery of *H pylori* and its relationship with severe gastroduodenal disease, including gastric cancer, incessant research has been performed, attempting to find a definitive weapon against this pathogen. In the absence of a licensed efficacious vaccine, continuous efforts have been made to improve the efficacy of the treatment, with the aim of overcoming the antibiotic resistance and the frequent lack of patient compliance. Indeed, some recent attempts to modify the treatment and/or the regimen were successful. On the other hand, the results of the studies on *H pylori* infection and pathogenesis, also exploiting data obtained in animal models, revealed aspects that could be exploited in the near future to develop new treatments and/or to better understand how to induce protective immunity.

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