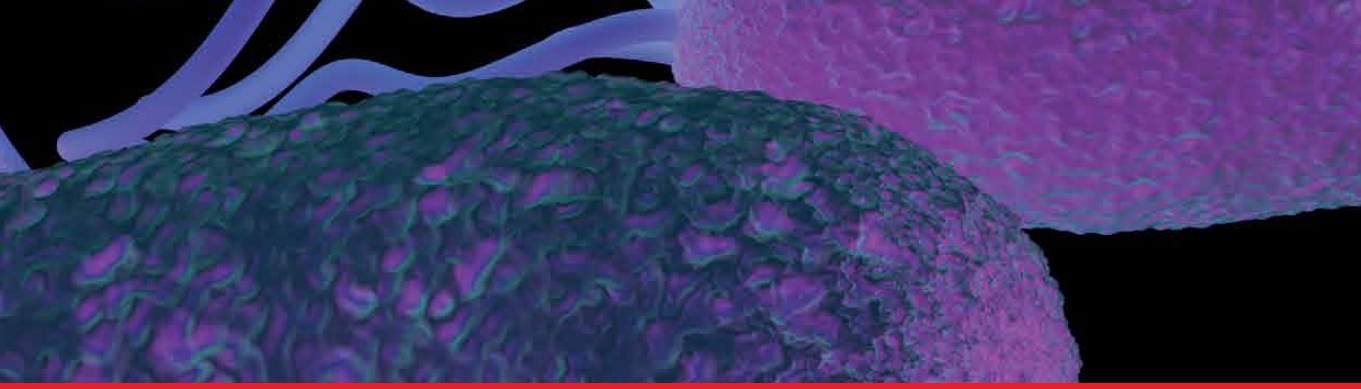

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Helicobacter pylori

From First Isolation to 2021

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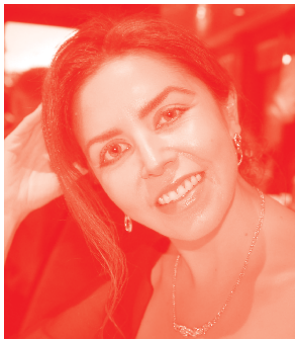

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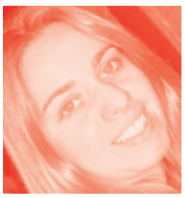

Dr. Bruna Maria Roesler is a pharmacist biochemist and holds a master´s degree in Pharmacology and a doctoral degree in Basic Sciences–Internal Medicine from the State University of Campinas (Campinas, SP, Brazil) where she has identified the principal genotypes of *Helicobacter pylori* strains in patients with chronic gastritis, peptic ulcer disease, and gastric cancer (early and advanced stages) through molecular biology techniques. She has published her work in several peer-reviewed journals and given oral and poster presentations at various congresses. Her research also includes the etiology, epidemiology, and physiopathology of gastrointestinal diseases. She has also participated in some studies that reported the possible relationship between *H. pylori* and idiopathic thrombocytopenic purpura, as well as between *H. pylori* and liver diseases.

Contents

Preface

Despite novel human pathogens emerging worldwide, such as COVID-19, which is responsible for the severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) development, *Helicobacter pylori* remains one of the principal human infections. *H. pylori* is a condition with a relative risk of developing various clinical disorders of the upper gastrointestinal tract, such as chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT lymphoma), and gastric adenocarcinoma.

The infection has also been associated with the development of some extradigestive diseases, such as hepatobiliary, cardiovascular and pancreatic diseases, and iron deficiency anemia, among others.

All individuals with *H. pylori* infection have histological gastritis, which corresponds to classical chronic gastritis and is characterized by the infiltration of neutrophils and other inflammatory cells. However, most patients are asymptomatic for life, while only some will come to develop a digestive disease. Studies have been demonstrating that different disease developments are probably due to the bacterial pathogenicity and due to the host susceptibility factors that include the role of bacterial genetic diversity in host colonization and persistence, outer membrane proteins, and modulation of adhesin expression and virulence factors (VacA, CagA, and BabA, among others).

The routes of transmission of *H. pylori* are not completely clarified, but infection is usually acquired during childhood and is characterized as being chronic, with greater prevalence in developing countries in all ages. Guides for the management of *H. pylori* have been developed in various countries in order to identify which cases are indicated to eradicate the bacterium. Therefore, various guidelines for the management of *H. pylori* infection are available. Nevertheless, there are discussions concerning the eradication treatment in asymptomatic individuals that receive a positive diagnosis for *H. pylori* in routine examinations.

Various aspects of this important microorganism are explored in this book, which is divided into two sections: the first one "*Helicobacter pylori* - A Very Old Human Microorganism" with two chapters and the second one, with chapters concerning the diseases associated with *H. pylori* infection and their new approaches.

Dr. Maldonado describes the bacterium in important aspects, considering that the interaction of the strain's characteristics, the host's characteristics, and the environment is responsible for several diseases that can be developed due to this infection, which affects more than half of the world population.

Prof. Cheng An-Lii attempts to provide evidence that early-stage gastric diffuse large B-cell lymphoma with (DLBCL[MALT]) and without ("pure" DLBCL) histological features of MALT origin is closely related to *H. pylori* infection.

Dr. Zhenguo reports and discusses the relationship between *H. pylori* infection and extradigestive diseases, especially cardiovascular diseases, like hypertension, and

atherosclerosis. He also reports the possible interactions between this infection and endothelial dysfunction and the role of exosomes in mediating the effect of the microorganism's presence on the endothelial function.

Dr. Rendón-Huerta discusses the principal mechanisms by which *H. pylori* is able to disrupt the tight junctions and invade the gastric epithelial mucosa.

Dr. Vasilievna attempts to study and analyze the MALT gastric lymphomas and the chapter is divided into two principal sections: the morphological aspects of the diagnosis of MALT gastric lymphomas and the clinical aspects of MALT gastric lymphomas.

Dr. Mascellino reports the principal virulence factors that have been studied in this important microorganism, which has accompanied human physiology in its complex migration history. Besides, the genotypic resistance related to phenotypes, antibiotics, and updated treatment strategies are also described and discussed.

Dr. Oti attempts to evaluate and study the antibody *H. pylori* seromarkers in students from Nasarawa State University, Nasarawa State, Nigeria.

Finally, the editor expresses her sincere thanks for the excellent work of the contributing authors. It was really a real challenge to prepare our book in the midst of a pandemic situation that hit humanity as a whole! The editor thanks Ms. Sara Debeuc, Author Service Manager at IntechOpen, for her excellent work and help in all aspects. In addition, the editor is especially thankful for the essential support given by Ms. Lucija Tomicic-Dromgool during the book's development, as well as the entire IntechOpen publishing team. It was a great pleasure working with you again!

Dr. Bruna Maria Roesler
State University of Campinas,
Brazil

Section 1

Helicobacter pylori - A Very Old Human Microorganism

Chapter 1

Helicobacter pylori; a Way to Gastric Cancer?

Norma Sánchez-Zauco, Erandi Pérez-Figueroa and Carmen Maldonado-Bernal

Abstract

Gastric cancer is one of the types of cancer that is associated with *Helicobacter pylori* infection. The infection starts in childhood, and 50–90% of the population in the world is infected. The clinical symptoms can be stomach pain, gastritis, atrophy gastric, and only 2–3% of the infected population developed gastric cancer. The majority of gastric cancers are adenocarcinomas. From Lauren's histological classification, gastric cancer is divided into two large groups: intestinal and diffuse. The cells that gives rise to them are different and the epidemiologic features and diagnosis are different according to gender and age; however; the survival rate is approximately of 5-years. Surgery is the only radical treatment, but the adjuvant treatment is chemotherapy and radiotherapy which unfortunately lead to only a modest survival benefit. On this review, we describe the major risk factors associated with the bacteria: cagPAI, CagA, VacA, HOPs, as well as host immune and inflammatory responses: immune cells, Toll-like receptors, cytokines, immune signal pathway, genetic predisposition, such as single nucleotide polymorphisms (SNP's) and environmental factors: age, high salt intake, diets low in fruit and vegetables, alcohol intake, and tobacco use. Finally, we included the interaction of all factors for the development of gastric cancer. Knowing and understanding the role of all factors in the development of gastric cancer will allow the implementation of better therapies and improve patient prognosis.

Keywords: *Helicobacter pylori*, inflammatory response, dysplasia, metaplasia, gastric cancer

1. Introduction

Helicobacter pylori is a Gram-negative bacterial pathogen that infects more than half of the human population worldwide. It is usually acquired during childhood and it is able to establish a lifelong chronic infection [1]. *H. pylori* is characterized by an exceptionally high genetic diversity and variability. Some pathogenic mechanisms include relatively inefficient DNA repair mechanisms as well as natural competence for transformation and the ability to integrate small fragments of homologous DNA into the chromosome. The species of Helicobacter are divided into large phylogeographic populations with distinct geographical distributions. In 22 countries in Central and South America and Asia, a prevalence of approximately 70% or higher around age 60 years was reported in the late 1990s and early 2000s, with a decreasing tendency in different time periods in most countries where data were available [1]. This text is a historical review of the data which exist on host-helicobacter interaction.

2. Virulent factors from *H. pylori* and its interaction with the host

2.1 Virulence factors and their association with gastric cancer

2.1.1 Cag Pathogenicity Island and Cag A

The major protein that was identified as a product of the multigene *cag* Pathogenicity Island (*cag*PAI) is CagA. The *CagPAI Island* has 27–31 putative genes and 20 genes encode to the type IV secretion system.

CagA is a protein considered an oncoprotein; it is translocated into gastric epithelial cells by the type IV secretory system of the pathogen, inducing multiple signaling cascades [1]. There are two distinct types of *H. pylori:* cagA-positive and cagA-negative strains. Only the CagA-positive strains induce the onco-transformations in animal models and contribute in the development of gastric cancer. The gene of *cagA* has different repeated sequences in the 3'region; each repeat region contains EPIYA motifs; its term describes specific sequence of amino acid (Glu-Pro-Ile- Tyr-Ala). If the first repeat region has two EPIYA motifs, they are called EPIYA A and EPIYA B; however, if there are two in the second repeat region, they are call EPIYA-C and EPIYA-D [2].

The CagA is injected into the host cell through the type IV secretion system (T4SS). In the cytoplasm, CagA is phosphorylated at its EPIYA motifs; CagA alters the host cell signaling in both manners, phosphorylation- dependent and phosphorylation-independent. After its translocation into the host epithelial cells, the EPIYA-motifs of CagA undergo tyrosine (Y)-phosphorylation via cellular kinases, such as Csk, c-Src, and c-Abl. The phosphorylated tyrosine interacts with the Src homology 2 phosphatase (SHP2) or with the adapter protein Grb2 and hinders cell–cell adhesion, cellular proliferation, IL-8 expression, and cellular elongation via the activation of cell signaling pathways, such as Ras–ERK MAP kinases (Rap1 → B-Raf → Erk) and Wnt-β-signaling [3].

Li et al., 2018 demonstrated that CagA stimulates YAP signaling pathway activation leading to gastric tumorigenesis in AGS cells. This in vitro result was also supported by the finding that *H. pylori* infection could enhance YAP expression activation together with the E-cadherin suppression in chronic gastritis tissues infected with *H. pylori* compared to *H. pylori* negative patients [4]. Infection with CagA-positive strains is thus associated with an increased risk of developing atrophic corpus gastritis compared to CagA-negative *H. pylori*-positive subjects [1]. The association with atrophy in the stomach is more in developed countries, but in developing countries, there is a balance of the immune response for there are co-infections with helminthes, with increased T regulatory cells and polarize inflammation to Th2 responses, which reduces gastric atrophy in *H. pylori*-infected subjects [5] (**Figure 1**). CagA has another target in the CDX1 cells, which is a home box transcription factor that plays an important role in the development of the human intestine. In an abnormal environment, CDX1 produces cell proliferation, invasion, and migration, induces the change from gastric characteristic to intestinal characteristics, and induces the stream cell-like phenotype. These cells have a Lrig-1 marker in their surface; a high number of steam-like cells is associated to premalignant lesions, such as atrophic gastritis and intestinal metaplasia, which develop resistance to chemotherapies [6, 7]. Some gastric cancer case–control studies have demonstrated that *H. pylori* and CagA positive increased risk in both intestinal-type gastric cancer and diffuse-type gastric cancer when compared with non-cancer control [8, 9].

The interaction of *H. pylori* with the environment of the host can be an important element in the infection. There are many environmental factors that may

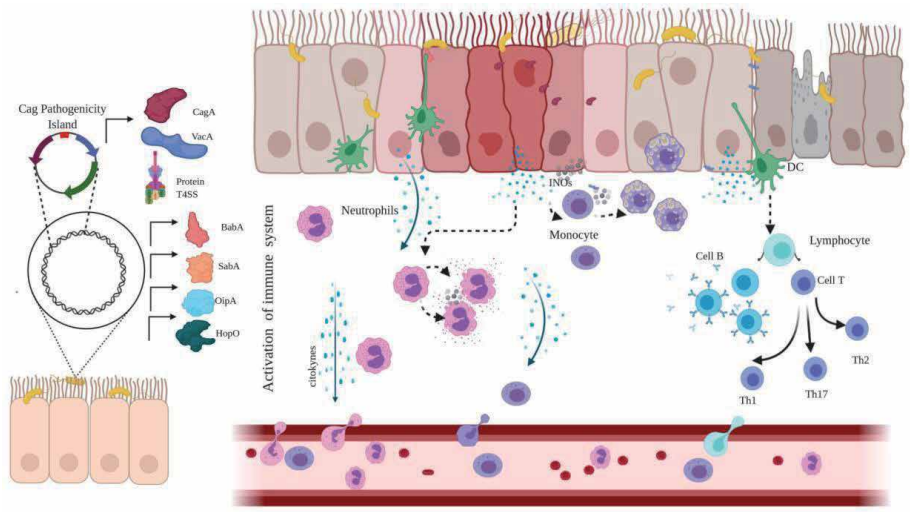

Figure 1.
Infection of Helicobacter pylori *activates immunity system.* Helicobacter pylori *(*H. pylori*) has different virulence factors which contribute to the activation of the immune system. CagA and VacA are proteins coded by the pathogenic island to induce the secretion of inflammatory cytokines in the gastric epithelium, which attracts neutrophils and monocytes to the infection site. The activation of these cells causes inflammatory conditions by triggering their effector mechanisms such as degranulation and ROS production. Subsequently, DC processes and present the antigen which induces a specific response through the lymphocyte.*

change the expression of CagA; for example, pH 6.0 induces higher expression of CagA when compared with pH 7.0, even at a pH 4.0, it increased the expression of CagA but decreased the survival of *H. pylori,* especially strains with a high number of EPIYA repeat regions [1].

A high-salt diet modifies the expression of the CagA protein and transcriptome level in *H. pylori* [10]. In a gerbils infection model, a high-salt diet induced higher *cagA* expression compared with *H. pylori*-infected gerbils with a regular diet; however, the first group had higher levels of inflammatory cytokines (IL-1, IL-6, IL-17, and gamma interferon [IFN-γ]), anti-inflammatory cytokines (IL-10), chemokines (KC, CCL12), and inducible nitric oxide synthase (iNOS); in consequence, there were higher inflammation scores as well as a higher frequency of gastric carcinoma [11].

Using an animal model, Noto *et al*., 2013, demonstrated that gerbil iron-depleted diets and infected with CagA positive strains enhanced virulence and induced robust proinflammatory responses. All of these things induce premalignant and malignant lesions; lower levels of iron are a risk factor for gastric cancer [12].

2.1.2 VacA

VacA is a pore-forming cytotoxin that plays a role interacting with gastric cells, induces vacuole formation, and apoptosis in mammalian cells; it is a pro-toxin of 140 kDa that is secreted through the auto-transporter pathway. The mature protein 88 kDa secreted toxin undergoes proteolysis in two fragments: p33 and p55 [3]. The *vacA* gene is not part of cagPAI; it has three polymorphic regions: the signal (s) intermediate (i) and middle (m) regions [13]. The s-region of *vacA* is divided into s1 (s1a, s1b, and s1c) and s2 genotypes. The m-region, which is composed of about 300 amino acids, is classified into m1 (m1a and m1b) and m2 genotypes; there is a segment located in the middle region of the 148 amino acid that exploits the cell binding specificity of VacA and plays a in a better survival for *H. pylori* inside the

gastric epithelial cells [3]. The i-region is located between the s- and m-regions of *vacA* and it is composed of different combinations of 3 clusters (A, B, and C). The i-region is classified into the i1 and i2 genotypes, according to the combination of clusters that are present [3].

The studies have revealed that the combination of different sequences in the three regions can determine the capability of vacuolation. *s*1-type VacA has been suggested to be associated with peptic ulcers, *m*1-type VacA induces vacuolation in HeLa cells, and strains with the *i*1 genotype are strongly associated with gastric cancer and vacuolating cytotoxin activity [13]. There are western strains that have a deletion (*d*) region located between the *i* and the m regions, the *d* region can be *d*1 or *d*2.

VacA is a risk factor for gastric mucosal atrophy. All s1/i1/d1 strains are called East Asia *Helicobacter* type [2]. Do Carmo *et al*., 2011, demonstrated that only the presence of *vacA* s1 and the absence of *cagA* have a major role in the development of gastric cancer [14].

The secretion inside the VacA cell induces the production of antibodies against VacA; however, there is a meta-analysis in which an association of VacA antibody with peptic ulcer disease and gastric cancer risk is observed; furthermore, the higher level of antibody response to VacA is associated with a risk of extra-gastric disease, such as colorectal cancer in African Americans [3].

Another target of VacA inside the cells is the endoplasmic reticulum; it produces stress and activates autophagy and increased cellular death. VacA is very important to survival efficacy through a transient receptor potential membrane channel mucolopin 1 (TRPML1) activity that inhibits the lysosomal and autophagy killing of bacterial cells to promote the establishment of an intracellular niche that allows bacterial survival, in addition to an altered host immune response, mainly through the inhibition of T cell activation and proliferation (**Figure 1**) [2].

2.1.3 Outer membrane proteins and gastric cancer

The outer membrane is the outer barrier of Gram-negative bacteria that contains outer membrane proteins (OMPs) resistant to the external environment. The OMPs have a variety of biological functions, such as maintaining the outer membrane structure and guaranteeing the material transportation especially, the OMPs participate in the adherence of *H. pylori* to the gastric mucosa, they play important roles in the initial colonization and long-term persistence on the gastric mucosa, as well as in the intensity of the resulting inflammatory response [14, 15]. OMPs in *H. pylori* mainly include lipoproteins, porins, iron-regulated proteins, efflux pump proteins, and adhesins. 4% of the whole-genoma encode OMPs; the expression of OMPs is variable and depends on geographical differences, being the five best characterized: BabA (HopS), SabA (HopP), OipA (HopH), HopQ and HopZ [15].

2.1.3.1 BabA (HopS)

Blood group antigen-binding adhesin (babA) is a 78-kDa outer membrane protein encoded by the babA2 gene, which binds the fucosylated Lewis b antigen (Le^b) on the surfaces of gastric epithelial cells. The specific manner by hydrogen bonds network structure formed between four residues of Lewis b and eight amino acids of BabA. *H. pylori* has blood group binding preferences; some strains combine only with O antigen residues. Persons with type O blood are more likely to suffer peptic ulcer than those with other blood types for it overexpresses Lewis b [16]. A special study demonstrated that the expression of BabA is acid-sensitive and has nothing to do with the binding affinity of Lewis b [17]. However, the expression loss of BabA is

not related to adaptive immunity or the Toll-like receptors (TLRs) [18]. The protein encoded for three genomic loci has been found in *bab*, namely, *babA*, *babB,* and *babC*; from these, only the babA2 is functional for binding activity. Almost 18,000 strains have two babA alleles, which are babA1 (silence) and babA2 (expression) [15]. In patients from Saudi Arabia, *H. pylori* was isolated and PCR were performed; the *babA2* gene strains were associated with a high risk of gastric cancer and a strong relation with VacAs1 and the prevalence of these genotype were higher. The strains of *H. pylori* carrying *babA2*, *cagA*, and *vacAs1* genotypes were associated with the risk of intestinal cancer [19].

2.1.3.2 Sialic acid-binding adhesin (SabA)

SabA binds to gangliosides with fucose substitutions of the N-acetyllactosamine like the dimeric sialyl-Lex antigen [3]. SabA is the second most commonly reported adhesin in *H. pylori,* also known as HopP or OMP17. The *sab* gene has two alleles, sabA and sabB [15]; however, *H. pylori* selectively expresses SabA during the colonization. This is caused by slipped-strand mispairing in 5'dinucleotide repeat region, which affects the on–off states of *sabA* and *sabB* [15]. This state reflects the ability of *H. pylori* to adapt to the host. The acid-responsive signaling, such as the environment pH activates the signal transductions to *H. pylori* to expresse SabA. *H. pylori* alters the gastric mucosa glycosylation and up regulates the sialyl-Lex antigens, promoting the attachment of SabA to the gastric mucosa. The SabA binding produces inflammation and allows bacterial persistence and gastric pathogenicity establishment. Several studies have reported an association between SabA expression and an increased risk of chronic gastritis, intestinal metaplasia, corpus atrophy, and even gastric cancer. The construction of a profile with the combination of SabA with other virulence factors, such as OipA and BabA, can be used as a prognostic marker, for it could distinguish the patients with gastric cancer from duodenal cancer patients and healthy individuals [3]. However, the only presence of SabA related with gastric cancer risk was showed in a Japanese population [20]. Nevertheless, another study showed that SabA-positive strains were no related with gastric cancer [21].

2.1.3.3 OipA

The outer inflammatory protein A (OipA), also called HopH, *oip* gene, is approximately 100 kb from the cagPAI, and usually CagA positive strains have OipA expressed. OipA is a protein with a molecular weight of 34 kDa; it can increase the secretion of interleukin-8 (IL-8) to cause neutrophil infiltration that produces inflammatory environment, which helps *H. pylori* colonization. However, OipA can induce inflammation and affect actin dynamics through the phosphorylation of multiple signaling pathways that usually interact with cagPAI-related pathway. Therefore, OipA inhibits the apoptosis of gastric cells and has a role in the activation of focal adhesion kinase (FAK), which is a cytoplasmic non-receptor tyrosine kinase and can regulate the shape of the cells, cell movement, and this is an essential role in the occurrence and invasive growth of tumors [22]. The Asian and Western strains that have expressed OipA are correlated with peptic ulcer, gastric cancer, and MALT. The signaling pathway related to carcinogenesis is regulated by the activation of the phosphoinositide-3 kinase (PI3K)/Akt, and, at the same time, the same signaling pathway regulates IL-8 secretion through forehead transcription factors of class O (FoxO) 1/3a inactivation.

In an animal model, mice were inoculated with immunogenic OipA and *H. pylori* at the same time and showed that the colonization and inflammation were

reduced [23]. Maybe the OipA vaccine is a therapeutic target to the *H. pylori* infection and could prevent the development of gastric cancer. On the other hand, the inactivation of OipA produced a decreased level of nuclear β-catenin *in vitro* and a reduced incidence of cancer in gerbils; OipA is very important in the *H. pylori* infection [24].

2.1.3.4 HopQ

Another OMP that plays an important role in the initial colonization is the *hopQ* gene; it is present in 2 forms: type I and type II. The presence of type I *hopQ* alleles and another *H. pylori* virulence markers, including type s1 *vacA* alleles, *hopQ* are essential for CagA translocation and transformation of the hummingbird phenotype and cell scattering, for the targets are β-strands. G, F, and C in the N-terminal domain (C1ND) and the IgV-like domain of carcinoembryonic antigen-related cell adhesion molecule family (CEACAMs), mainly CEACAM1, CEACAM3, CEACAM5 and CEACAM6, and the interaction of HopQ-CEACAM interaction facilitates the transference of CagA to the host cells and induces signal transduction [15]. The suppression or deletion of *hopQ* reduces T4SS-dependent activation of NF-κB, induction of MAPK signaling and the secretion of IL-8 in the host cells, but it does not affect the attachment of the bacteria to the host cells. Patients with hopQ type I strains have more inflammatory cell infiltration and atrophy than those with hopQ type II strains [24].

So far, we already know which virulence factor helps Helicobacter to colonize and persistent in the stomach, but what happens with the immune response of the host and their genetic susceptibility?

2.2 Host characteristics

2.2.1 Genetic susceptibility

Host genetic susceptibility depends on polymorphisms of genes involved in *H. pylori*-related inflammation and the response of cytokines in gastric epithelial and immune cells. *H. pylori* strains differ in their ability to induce a deleterious inflammatory response. *H. pylori*-driven cytokines accelerate the inflammatory response and promote malignancy by DNA damage, the impairment of repair processes, and increase the rate of mutation [25].

The receptor which recognizes multiples virulence factor of *H. pylori* or of any microorganism are the Toll-like receptors (TLR's). These receptors are present in the surface and inside of the intracellular vesicle of any mammalian cell, but mainly in immunological cells, such as macrophages, neutrophils, T cells, and B cells.

Groups of receptors are simultaneously engaged in the recognition of *H. pylori* compounds and the development of gastric cancer; these are TLR2, TLR3, TLR4, TLR5, and TLR9 (**Figure 2**) [25].

It has been shown that *H. pylori* LPS, as the TLR2 ligand, induces the secretion of chemokines by gastric epithelial cells; however, TLR4 also recognizes LPS [25]. *Moran AP. 2001*, proposed that *H. pylori* LPS reduced immunogenicity by uncommon phosphorylation and acylation of *H. pylori* lipid A [26]. Another work showed *H. pylori* LPS has anti-phagocytic properties *in vitro* [27].

Chochi *et al*. 2008, showed that TLR4 increased the growth of gastric cancer [28]. There are studies that showed that single nucleotide polymorphisms (SNPs) of TLR2 and TLR4 receptors were associated with an increased risk of gastric carcinoma in some population [29].

Metanalysis of TLR2–196 to –174 showed in a Japanese population that this deletion decreased the induction of IL-8 and is associated with a risk of gastric cancer

compared with controls group [29], but this correlation failed in a Chinese population; this may indicate an ethnic consideration in the incidence of stomach cancer.

Single nucleotide polymorphisms (SNPs) of the TLR4 receptor were associated with an increased cell death against *H. pylori*, whose effectiveness affects the risk of gastric carcinoma, including TLR4 rs4986790 (Asp299Gly), TLR4 rs4986791 (Thr399Ile), TLR4 rs10116253, TLR4 rs10983755, TLR4 rs11536889 (C3725G/C), TLR4 rs1927911. TLR4 Asp299Gly and Thr399Ile polymorphisms generate less stability of the extracellular domain [30]. In an Iranian population, the TLR4 (Asp299Gly) G and DG *alleles* were associated with chronic active gastritis [31]; in a Western population, the G allele as well as the TLR4 rs11536889 C allele and the CC genotype increased the risk of gastric cancer [32]. Different associations were obtained with the polymorphisms, for it depends of the genetic background of the population; therefore, the risks of cancer or inflammatory gastric disease totally depend on ethnicity.

The second part of the activation of the pattern recognition receptors on the leukocyte and epithelial/endothelial cells induce the production of cytokines. All these elements are part of the inflammatory environment, they could regulate tumor growth and metastasis, cause discomfort symptoms, and potentially influence the tumor prognosis [33].

Another pathogenic factor of *H. pylori* is flagellin; its principal role is motility and colonization. The flagellin is made of two separate subunits, FlaA and FlaB and it is recognized by TLR5 [34], Andersen-Nissen *et al.,* 2005 showed that dimer TLR5-flagellin failed to induce the nuclear factor (NF)-kB activation, allowing the evasion of the immune response (**Figure 2**) [35].

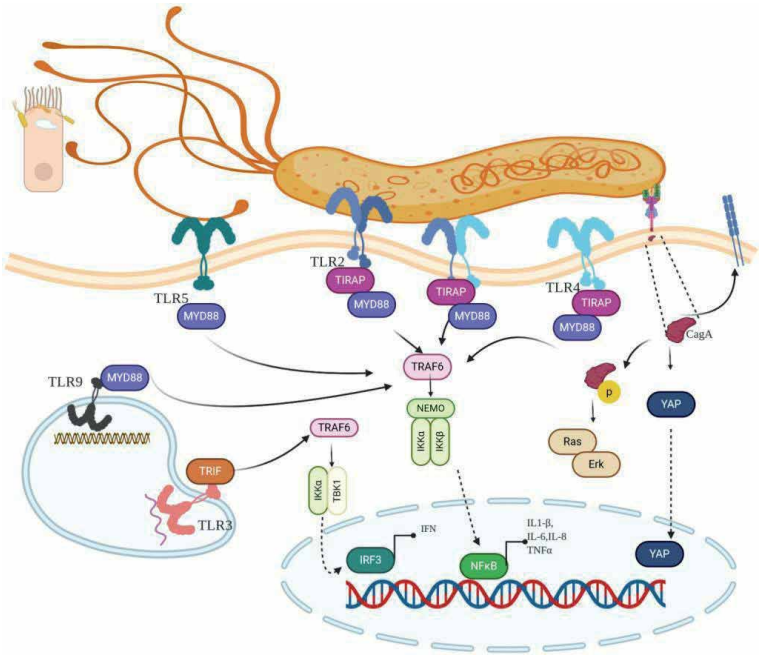

Figure 2.
Signaling of TLRs activate for Helicobacter pylori. *Toll-like receptors (TLRs) are pattern recognition receptors which distinguish conserved microbial products of* H. pylori. *TLR2, TLR4, TLR5 and TLR9 are expressed over the cell membrane. TLR3 and TLR9 are expressed over the endosome. All TLRs expected for the TLR3 activate MyD88-dependent pathway to induce NF-κB and p38/JNK to activate AP1, to induce the production of proinflammatory cytokines. However, TLR3 requires IRIF to activate the production of IFNα/β. additionally,* H. pylori *infection induces YAP and downstream effects over the gastric epithelial cell.*

After the activation of immune and epithelial cells, there is a production and secretion of pro-inflammatory and anti-inflammatory cytokines such as tumor necrosis factor (TNF-α), IL-1β, IL-8, and IL-10 [25], which leads to the recruitment of macrophages, neutrophils, and lymphocytes to the gastric tissue [36].

H. pylori has a different mechanism to evade the immune response, some examples are; VacA alters the antigen presentation by B cells, VacA affects endosomal traffic, preventing the development of the TH1 response. *H. pylori* affects the host's trafficking pathways, it produces modifications in GTPases in macrophages, and deletes the expression of *Rgs1/2, Fgd2* and *Dock8,* which are regulators of Rho, Pac, and Cdc42 GTPases, respectively. This disrupts the actin cytoskeleton and phagocyte function [36].

H. pylori can inhibit the killing by reactive oxygen species and nitric oxide for it disrupts NADPH oxidase. On the other hand, *H. pylori* activates the inducible iNOS in macrophages by urease and the arginase, which produces less amount of oxide nitric.

Once the infection starts, dendritic cells are the first cells to arrive to the gastric mucosa and produce IL-6, IL-1β, IL-12, and TNF-α, which causes inflammation and Th1 response. During atrophic gastritis, the macrophages are polarized to M1 subtype and induce proliferation of T cells; however, *H. pylori* can stimulate M2 macrophages. In consequence, there are less inflammatory cytokines and the immune response is balanced (**Figure 3**) [37, 38].

In contrast, *H. pylori* activates the ERk1/2 pathway and then the activation of the AP-1 complex. This complex generates an increased expression of ornithine decarboxylase that induces apoptosis in macrophages [36]. Another mechanism of *H. pylori* that induces apoptosis is through the Fas pathway using the HP986 protein [39]. The last mechanism to induce monocyte apoptosis is through the p52 fragment of VacA, which activates NF-κB pathways and induces proinflammatory cytokines such as TNF-α, IL-1β, NOS, and ROS, subsequently causing apoptosis.

In dendritic cells (DC), VacA causes a decreased expression of CD40, CD80, CD86, MHC class II, and decreases the secretion of IL-1β, IL12p70 and TNF-α; the major effect of the down expression is the inhibition of the T cell response [40].

Patients infected with *H. pylori* showed an increase of CD4+ CD25 T cells (Treg); these cells allow an increase of bacterial load and induce chronic infection by suppressing the immune response; additionally, the Treg induces the secretion of TGF-β and IL-10 [36]. *H. pylori* can up-regulate the expression of B7-H1 and, at the same time, down regulate the B7-H2 expression on epithelial gastric cells; this change can induce alterations of the T cell subpopulation, increasing Treg and decreasing Th17 [41].

There is some evidence that virulence factors such as VacA and CagA cause damage in gastric cells and result in peptic ulcer, even gastric cancer. Now we know that the severity of gastric diseases depends on the virulence factor together with the host factor.

2.2.2 Immune factors

Immune dysregulation plays a pathogenic role in the development of cancer.

Colonization of *H. pylori* induces acute inflammation; this response is highly reactive but it is unable to eradicate the infection. If with time the infection persists, the inflammation will be regulated and is considered a chronic inflammation.

Chronic inflammation of the gastric mucosa evolves in three forms: (1) antral-predominant, (2) corpus-predominant, and (3) diffuse [25]. Antral-predominant gastritis promotes duodenal ulcers whereas corpus-predominant gastritis promotes gastric ulcers [25].

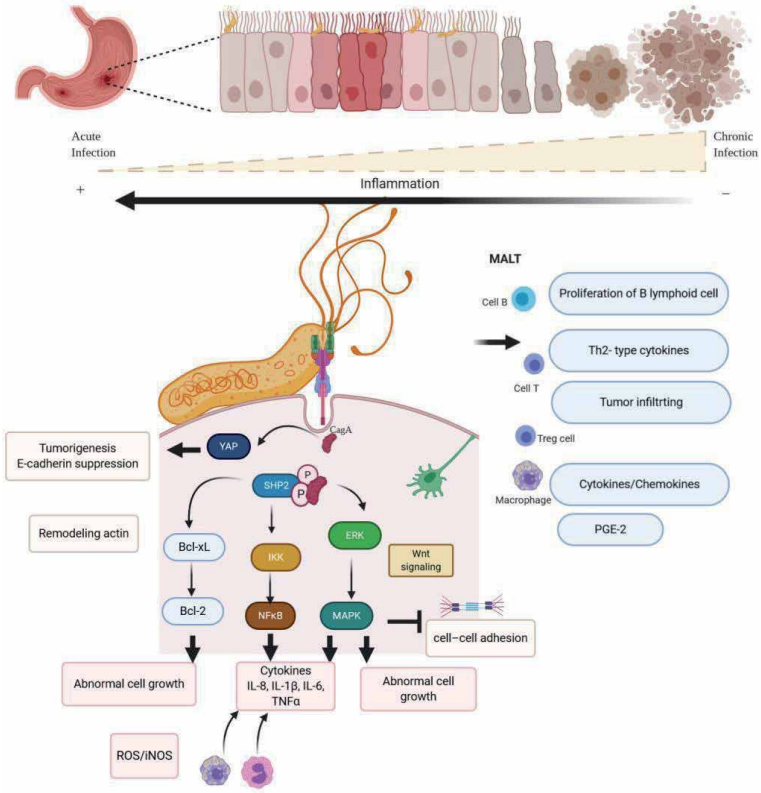

Figure 3.
Role of Helicobacter pylori *in peptic ulcer and gastric cancer.* H. pylori *Infection modulates a variety of host cell signal pathways and the crosstalk of MAPk, ERK, WNT, and YAP pathways.* Helicobacter pylori *colonizes the gastric mucosa in the human stomach and represents a major risk factor for peptic ulcer disease and gastric cancer.* H. pylori *Manipulates host signal networks, to trough, by the cag pathogenicity island (cagPAI)-encoded type IV secretion system (T4SS).* H. pylori *Infection includes the disruption of cell–cell junctions and cytoskeletal rearrangements, as well as proinflammatory, cell cycle-related, proliferative, antiapoptotic, and DNA damage responses and epithelial mesenchymal transition (EMT).*

In atrophic gastritis, the principal and parietal cells are replaced by globlet cells as the lesion progresses giving way to intestinal cancer; this transformation is called metaplasia. In general, there are two different types of metaplasia, intestinal metaplasia and spasmolytic polypeptide-expressing metaplasia (SPEM); both types of metaplasia are associated with the progression of intestinal-type gastric cancer.

In intestinal metaplasia, globet cells express intestinal markers such as Muc2 and Trefoil factor 3 (TFF3) [42].

In spasmolytic polypeptide-expressing metaplasia (SPEM), the cells have a morphological characteristic more typical of deep antral gland cells or Brunner's glands, expressing Muc6 and Trefoil factor 2 (TFF2).

The type II cytokines, including IL-4, IL-5, and IL-9, can activate type II innate lymphoid cells (ILC2). These lymphocytes respond to IL-33 producing IL-13 [43].

The development of metaplasia involves alarming cytokines, such as IL-33 and IL-13. IL-13 induces chief cell trans differentiation into SPEM, following the loss of parietal cells from the corpus of the stomach, and activated macrophages, which promotes the resolution of inflammation and wound repair [44]. Subsequently, it drives the progression of metaplasia to become more proliferative with increased intestinal characteristics; furthermore, SPEM is a phenotype in the atrophic gastritis and correlates with intestinal-type gastric cancer [43].

In general, gastric cancer shows an immunosuppressive character. There are tumor-infiltrating leukocytes, such as CD8+ T cells, CD68+ macrophages, and CD4+ T cells, that represent 15%, 13%, and 11% of all intratumorally cells, respectively. The role of the immune response has a strong selective pressure on the tumor and allows its growth; finally, it helps the cancer-immunoediting process, one of the mechanisms to demonstrate it is the down expression of PD1/PDL1. This response induces pro-tumoral effects such as angiogenesis and metastasis [45]. In our experience, the immune response can be a diagnostic marker to gastric cancer independently of the histological subtype (**Figure 3**) [33].

2.3 Environmental factors

2.3.1 Dietary factors

The dietary factors that have an important impact on gastric cancer are low intake of fresh fruits and vegetables, high-sodium diet, salt-preserved food, red and cured meat; all these are associated with gastric cancer risk.

2.3.2 Other

High alcohol intake, tobacco smoking, and high weight were associated with gastric cancer risk in a prospective study in a studied cohort, demonstrating that 62% of cardia gastric cancer could have been prevented if the population had followed a healthy lifestyle [46]. The primary prevention of gastric cancer includes healthy diet, anti-*H. pylori* therapies, and screening for early detection [47].

2.4 Development of gastric cancer

Gastric cancer is a carcinoma that occurs sporadically most of the times. It is associated to *H. pylori* infection and is commonly caused by coincidence with many risk factors. There is a geographical variation in cancer gastric variation, 70% the of cases occurs in developing countries and half of the total case occurs in Eastern Asia, especially China. This country has the highest mortality rates, and the highest mortality rates in Central and Eastern Europe, Central and South America, whereas the lowest rates occur in North America [48].

Most gastric cancers are diagnosed at an advanced stage; 25–50% of the cases will develop metastasis. The main treatment with curative-intent in gastric cancer patients is surgery, being associated with approximately a 5-years survival rate of 20–25%; therefore, additional treatments are chemotherapy and radiotherapy but unfortunately they lead only to a modest survival benefit [43].

In 1965, Lauren described two histologically different stomach adenocarcinomas, diffuse and intestinal. The diffuse type is considered an endemic cancer type.

Diffuse adenocarcinoma affects mostly women and younger populations. The intestinal type is related to preneoplastic changes, such as chronic atrophic gastritis and intestinal metaplasia of mucous membranes. This type causes tumors in the peripheral part of the stomach. Intestinal adenocarcinoma is an epidemic type of cancer for it occurs in regions with a high risk of gastric cancer morbidity. It affects mostly men and older populations [36, 45].

The ratio of intestinal and diffuse types varies among countries. For example, intestinal type is more common and occurs more often in the distal stomach, in high-risk area and it is often preceded by long-standing precancerous lesion in European countries.

Another classification was proposed by the Word Health Organization (WHO). There are four histological subtypes: papillary, tubular, mucinous and poorly cohesive. Both classifications are inadequate, for they stratify patients regarding tumor behavior, prognosis, and response to specific treatment.

There is also the molecular classification in which gastric cancer is divided into four genomic subtypes: Chromosomal instability, Microsatellite instability, Genomic stability, and Epstein Barr virus-associated [49].

Chromosomal instability is associated with loss or gain of tumor suppressor genes, such as TP53, and receptor tyrosine kinase mutation that affects the cell cycle gene and MET, RAS, BRAF, HER2, and EGFR; the tumors are located at the gastroesophageal junction [49].

Microsatellite instability is associated with abnormal absences of the protein expression and it is diagnosed by immunohistochemistry or polymerase chain reaction (PCR); the sensitivity of the test is between 83 and 89% and the specificity is 89–90% [50].

Genomic stability: in diffuse gastric cancer the main somatic genomic alterations are CDH1, ARIDIA and RHOA, and they are involved in cellular motility [48].

Epstein Barr virus-associated: 10% of gastric cancer patients have been detected with Epstein bar virus, especially in far East Asian patients and it is more frequent in younger persons [48].

Development of gastric cancer associated to *H. pylori* strains carrying the cagPAI, induces the NF-kB transcription factor, and CagA and VacA are dispensable for direct NF-kB activation. NF-kB-driven gene products include cytokines/chemokines, growth factors, anti-apoptotic factors, angiogenic regulators, and metalloproteinases. Many of the genes are transcribed by NF-kB promote gastric carcinogenesis. All of the pro-inflammatory mediators lead the accumulation of genetic and epigenetic changes in differentiated and steam cells. Chronic inflammation is the initial step towards atrophy, metaplasia, and dysplasia and a promoter of cancer development [51]. Furthermore, the recruitment and activation of inflammatory cells, genetic predisposition, the activation of the cells with the environmental factors, and more pathogenic strains induce the progression and metastasis of gastric cancer.

3. Conclusion

Cancer gastric is a multifactorial cancer and many factors can play a role in its incidence. In the present review, several *H. pylori* pathogenic factors, environmental factors, genetic susceptibility, and immune factor in the host were described in the contributing role in the development of cancer gastric.

Different histological types of gastric cancer and anatomic location might suggest different etiologies of gastric cancer; however, genetic predisposition and inflammatory response have a consequence in a process regulating proliferation, evasion of apoptotic development of synergistic complex for the development gastric cancer, and, eventually, metastasis.

It is necessary to know the different risk factors involved in the development of gastric cancer in order to implement better therapies and a better prognosis for patients.

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Conflict of interest

"The authors declare no conflict of interest."

Abbreviations

ARID1A	AT-richnteractive domain-containig protein 1 A
BabA	Blood group antigen-binding adhesin
BRAF	Serine–threonine kinase
CDH1	gene coding E-cadherine
C1ND	C in the N-terminal domain
cag PAI	cag Pathogenicity Island
CEACAMs	Carcinoembryonic antigen-related cell adhesion molecules
DC	Dendritic cells
EGRF	Epidermal growth factor receptor
FAK	Focal adhesion kinase
FoxO	transcription factors of class O
HER2	Human epidermal growth factor receptor
IFN-γ	Gamma interferon
IL	Interleukin
ILC	Innate lymphoid cells
iNOS	inducible nitric oxide synthase
Leb	Lewis b antigen
MET	Receptor tyrosine kinase
NF-κB	Nuclear factor kappa B
OipA	Outer inflammatory protein A
OMPs	Outer membrane proteins
PCR	Polymerase chain reaction
PD1/PD-L1	Programmed death 1/ligands *PD-L1*
PI3K	Phosphoinositide-3 kinase
RHOA	Ras homolog family member A
SHP2	Src homology 2 phosphatases
SNPs	Single nucleotide polymorphisms
SPEM	Spasmolytic polypeptide-expressing metaplasia
T4SS	Type IV secretion system
TFF2	Trefoil factor 2
TFF3	Trefoil factor 3
TLRs	Toll-like receptors
TRPML1	Transient receptor potential membrane channel mucolopin

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References

[1] Park JY, Forman D, Waskito LA, Yamaoka Y, Crabtree JE. Epidemiology of Helicobacter pylori and CagA-Positive Infections and Global Variations in Gastric Cancer. Toxins (Basel). 2018;10(4):163. doi: 10.3390/toxins10040163.

[2] Sokic-Milutinovic A, Alempijevic T, Milosavljevic T. Role of Helicobacter pylori infection in gastric carcinogenesis: Current knowledge and future directions. World J Gastroenterol. 2015;21(41):11654-11672. doi: 10.3748/wjg.v21.i41.11654.

[3] Ansari S, Yamaoka Y. *Helicobacter pylori* Virulence Factors Exploiting Gastric Colonization and its Pathogenicity. Toxins (Basel). 2019;11(11):677. doi: 10.3390/toxins11110677.

[4] Li N, Feng Y, Hu Y, He C, Xie C, Ouyang Y, Artim SC, Huang D, Zhu Y, Luo Z, Ge Z, Lu N. Helicobacter pylori CagA promotes epithelial mesenchymal transition in gastric carcinogenesis via triggering oncogenic YAP pathway. J Exp Clin Cancer Res. 2018;37(1):280. doi: 10.1186/s13046-018-0962-5.

[5] Du Y, Agnew A, Ye XP, Robinson PA, Forman D, Crabtree JE. Helicobacter pylori and Schistosoma japonicum co-infection in a Chinese population: helminth infection alters humoral responses to H. pylori and serum pepsinogen I/II ratio. Microbes Infect. 2006;8(1):52-60. doi: 10.1016/j.micinf.2005.05.017.

[6] Choi SI, Yoon C, Park MR, Lee D, Kook MC, Lin JX, Kang JH, Ashktorab H, Smoot DT, Yoon SS, Cho SJ. CDX1 Expression Induced by CagA-Expressing *Helicobacter pylori* Promotes Gastric Tumorigenesis. Mol Cancer Res. 2019;17(11):2169-2183. doi: 10.1158/1541-7786.MCR-19-0181.

[7] Wroblewski LE, Choi E, Petersen C, Delgado AG, Piazuelo MB, Romero-Gallo J, Lantz TL, Zavros Y, Coffey RJ, Goldenring JR, Zemper AE, Peek RM Jr. Targeted mobilization of Lrig1+ gastric epithelial stem cell populations by a carcinogenic Helicobacter pylori type IV secretion system. Proc Natl Acad Sci U S A. 2019;116(39):19652-19658. doi: 10.1073/pnas.1903798116.

[8] Nomura AM, Pérez-Pérez GI, Lee J, Stemmermann G, Blaser MJ. Relation between Helicobacter pylori cagA status and risk of peptic ulcer disease. Am J Epidemiol. 2002;155(11):1054-1059. doi: 10.1093/aje/155.11.1054.

[9] Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. Gut. 1997;40(3):297-301. doi: 10.1136/gut.40.3.297.

[10] Loh JT, Torres VJ, Cover TL. Regulation of Helicobacter pylori cagA expression in response to salt. Cancer Res. 2007;67(10):4709-4715. doi: 10.1158/0008-5472.CAN-06-4746.

[11] Gaddy JA, Radin JN, Loh JT, Zhang F, Washington MK, Peek RM Jr, Algood HM, Cover TL. High dietary salt intake exacerbates Helicobacter pylori-induced gastric carcinogenesis. Infect Immun. 2013;81(6):2258-2267. doi: 10.1128/IAI.01271-12.

[12] Noto JM, Gaddy JA, Lee JY, Piazuelo MB, Friedman DB, Colvin DC, Romero-Gallo J, Suarez G, Loh J, Slaughter JC, Tan S, Morgan DR, Wilson KT, Bravo LE, Correa P, Cover TL, Amieva MR, Peek RM Jr. Iron deficiency accelerates Helicobacter pylori-induced carcinogenesis in rodents and humans. J Clin Invest. 2013;123(1):479-492. doi: 10.1172/JCI64373.

[13] Inagaki T, Nishiumi S, Ito Y, Yamakawa A, Yamazaki Y, Yoshida M, Azuma T. Associations Between CagA, VacA, and the Clinical Outcomes of Helicobacter Pylori Infections in Okinawa, Japan. Kobe J Med Sci. 2017;63(2):E58-E67

[14] Do Carmo AP, Rabenhorst SH. Importance of vacAs1 gene in gastric cancer patients infected with cagA-negative Helicobacter pylori. APMIS. 2011;119(7):485-486. doi: 10.1111/j.1600-0463.2011.02739.x.

[15] Xu C, Soyfoo DM, Wu Y, Xu S. Virulence of Helicobacter pylori outer membrane proteins: an updated review. Eur J Clin Microbiol Infect Dis. 2020;39(10):1821-1830. doi: 10.1007/s10096-020-03948-y.

[16] Moonens K, Gideonsson P, Subedi S, Bugaytsova J, Romaõ E, Mendez M, Nordén J, Fallah M, Rakhimova L, Shevtsova A, Lahmann M, Castaldo G, Brännström K, Coppens F, Lo AW, Ny T, Solnick JV, Vandenbussche G, Oscarson S, Hammarström L, Arnqvist A, Berg DE, Muyldermans S, Borén T, Remaut H. Structural Insights into Polymorphic ABO Glycan Binding by Helicobacter pylori. Cell Host Microbe. 2016;19(1):55-66. doi: 10.1016/j.chom.2015.12.004.

[17] Kable ME, Hansen LM, Styer CM, Deck SL, Rakhimova O, Shevtsova A, Eaton KA, Martin ME, Gideonsson P, Borén T, Solnick JV. Host Determinants of Expression of the Helicobacter pylori BabA Adhesin. Sci Rep. 2017;7:46499. doi: 10.1038/srep46499.

[18] Bugaytsova JA, Björnham O, Chernov YA, Gideonsson P, Henriksson S, Mendez M, Sjöström R, Mahdavi J, Shevtsova A, Ilver D, Moonens K, Quintana-Hayashi MP, Moskalenko R, Aisenbrey C, Bylund G, Schmidt A, Åberg A, Brännström K, Königer V, Vikström S, Rakhimova L, Hofer A, Ögren J, Liu H, Goldman MD, Whitmire JM, Ådén J, Younson J, Kelly CG, Gilman RH, Chowdhury A, Mukhopadhyay AK, Nair GB, Papadakos KS, Martinez-Gonzalez B, Sgouras DN, Engstrand L, Unemo M, Danielsson D, Suerbaum S, Oscarson S, Morozova-Roche LA, Olofsson A, Gröbner G, Holgersson J, Esberg A, Strömberg N, Landström M, Eldridge AM, Chromy BA, Hansen LM, Solnick JV, Lindén SK, Haas R, Dubois A, Merrell DS, Schedin S, Remaut H, Arnqvist A, Berg DE, Borén T. Helicobacter pylori Adapts to Chronic Infection and Gastric Disease via pH-Responsive BabA-Mediated Adherence. Cell Host Microbe. 2017;21(3):376-389. doi: 10.1016/j.chom.2017.02.013.

[19] Bibi F, Alvi SA, Sawan SA, Yasir M, Sawan A, Jiman-Fatani AA, Azhar EI. Detection and Genotyping of Helicobacter pylori among Gastric ulcer and Cancer Patients from Saudi Arabia. Pak J Med Sci. 2017;33(2):320-324. doi: 10.12669/pjms.332.12024.

[20] Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, El-Zimaity HM, Reddy R, Arnqvist A, Graham DY. Helicobacter pylori outer membrane proteins and gastroduodenal disease. Gut. 2006;55(6):775-781. doi: 10.1136/gut.2005.083014.

[21] Yanai A, Maeda S, Hikiba Y, Shibata W, Ohmae T, Hirata Y, Ogura K, Yoshida H, Omata M. Clinical relevance of Helicobacter pylori sabA genotype in Japanese clinical isolates. J Gastroenterol Hepatol. 2007;22(12):2228-2232. doi: 10.1111/j.1440-1746.2007.04831.x.

[22] Tabassam FH, Graham DY, Yamaoka Y. OipA plays a role in Helicobacter pylori-induced focal adhesion kinase activation and cytoskeletal re-organization. Cell Microbiol. 2008;10(4):1008-1020. doi: 10.1111/j.1462-5822.2007.01104.x.

[23] Mahboubi M, Falsafi T, Sadeghizadeh M, Mahjoub F. The role of

outer inflammatory protein A (OipA) in vaccination of theC57BL/6 mouse model infected by Helicobacter pylori. Turk J Med Sci. 2017;47(1):326-333. doi: 10.3906/sag-1505-108.

[24] da Costa DM, Pereira Edos S, Rabenhorst SH. What exists beyond cagA and vacA? Helicobacter pylori genes in gastric diseases. World J Gastroenterol. 2015;21(37):10563-10572. doi:10.3748/wjg.v21.i37.10563.

[25] Chmiela M, Karwowska Z, Gonciarz W, Allushi B, Stączek P. Host pathogen interactions in Helicobacter pylori related gastric cancer. World J Gastroenterol. 2017;23(9):1521-1540. doi: 10.3748/wjg.v23.i9.1521. PMID: 28321154.

[26] Moran AP. Molecular Structure, Biosynthesis, and Pathogenic Roles of Lipopolysaccharides. In: Mobley HLT, Mendz GL, Hazell SL, editors. *Helicobacter pylori*: Physiology and Genetics. Washington (DC): ASM Press; 2001. Chapter 8. PMID: 21290749.

[27] Grebowska A, Moran AP, Matusiak A, Bak-Romaniszyn L, Czkwianianc E, Rechciński T, Walencka M, Płaneta-Małecka I, Rudnicka W, Chmiela M. Anti-phagocytic activity of Helicobacter pylori lipopolysaccharide (LPS)--possible modulation of the innate immune response to these bacteria. Pol J Microbiol. 2008;57(3):185-192. PMID: 19004238.

[28] Chochi K, Ichikura T, Kinoshita M, Majima T, Shinomiya N, Tsujimoto H, Kawabata T, Sugasawa H, Ono S, Seki S, Mochizuki H. Helicobacter pylori augments growth of gastric cancers via the lipopolysaccharide-toll-like receptor 4 pathway whereas its lipopolysaccharide attenuates antitumor activities of human mononuclear cells. Clin Cancer Res 2008; 14: 2909-2917.

[29] Castaño-Rodríguez N, Kaakoush NO, Goh KL, Fock KM, Mitchell HM. The role of TLR2, TLR4 and CD14 genetic polymorphisms in gastric carcinogenesis: a case-control study and meta-analysis. PLoS One. 2013;8(4):e60327. doi: 10.1371/journal.pone.0060327.

[30] Zhou Q, Wang C, Wang X, Wu X, Zhu Z, Liu B, Su L. Association between TLR4 (+896A/G and +1196C/T) polymorphisms and gastric cancer risk: an updated meta-analysis. PLoS One. 2014 Oct 7;9(10):e109605. doi: 10.1371/journal.pone.0109605.

[31] Bagheri N, Azadegan-Dehkordi F, Sanei H, Taghikhani A, Rahimian G, Salimzadeh L, Hashemzadeh-Chaleshtori M, Rafieian-kopaei M, Shirzad M, Shirzad H. Associations of a TLR4 single-nucleotide polymorphism with H. pylori associated gastric diseases in Iranian patients. Clin Res Hepatol Gastroenterol. 2014;38(3):366-371. doi: 10.1016/j.clinre.2013.12.004.

[32] Castaño-Rodríguez N, Kaakoush NO, Mitchell HM. Pattern-recognition receptors and gastric cancer. Front Immunol. 2014;5:336. doi: 10.3389/fimmu.2014.00336.

[33] Sánchez-Zauco N, Torres J, Gómez A, Camorlinga-Ponce M, Muñoz-Pérez L, Herrera-Goepfert R, Medrano-Guzmán R, Giono-Cerezo S, Maldonado-Bernal C. Circulating blood levels of IL-6, IFN-γ, and IL-10 as potential diagnostic biomarkers in gastric cancer: a controlled study. BMC Cancer. 2017;17(1):384. doi: 10.1186/s12885-017-3310-9

[34] Andersen-Nissen E, Smith KD, Strobe KL, Barrett SL, Cookson BT, Logan SM, Aderem A. Evasion of Toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci U S A. 2005;102(26):9247-9252. doi: 10.1073/pnas.0502040102.

[35] Pachathundikandi SK, Tegtmeyer N, Arnold IC, Lind J, Neddermann M,

Falkeis-Veits C, Chattopadhyay S, Brönstrup M, Tegge W, Hong M, Sticht H, Vieth M, Müller A, Backert S. T4SS-dependent TLR5 activation by Helicobacter pylori infection. Nat Commun. 2019 Dec 16;10(1):5717. doi: 10.1038/s41467-019-13506-6. PMID: 31844047; PMCID: PMC6915727.

[36] Lina TT, Alzahrani S, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE. Immune evasion strategies used by Helicobacter pylori. World J Gastroenterol. 2014;20(36):12753-12766. doi: 10.3748/wjg.v20.i36.12753.

[37] Quiding-Järbrink M, Raghavan S, Sundquist M. Enhanced M1 macrophage polarization in human helicobacter pylori associated atrophic gastritis and in vaccinated mice. PLoS One 2010; 5: e15018. doi: 10.1371/journal. pone.0015018]10.1371/journal. pone.0015018]

[38] Fehlings M, Drobbe L, Moos V, Renner Viveros P, Hagen J, Beigier-Bompadre M, Pang E, Belogolova E, Churin Y, Schneider T, Meyer TF, Aebischer T, Ignatius R. Comparative analysis of the interaction of Helicobacter pylori with human dendritic cells, macrophages, and monocytes. Infect Immun 2012; 80: 2724-2734. doi: 10.1128/IAI.00381-12.

[39] Alvi A, Ansari SA, Ehtesham NZ, Rizwan M, Devi S, Sechi LA, Qureshi IA, Hasnain SE, Ahmed N. Concurrent proin- flammatory and apoptotic activity of a Helicobacter pylori protein (HP986) points to its role in chronic persistence. PLoS One 2011; 6: e22530. DOI: 10.1371/ journal. pone.0022530.

[40] Kim JM, Kim JS, Yoo DY, Ko SH, Kim N,Kim H,Kim YJ. Stimulation of dendritic cells with Helicobacter pylori vacuolating cytotoxin negatively regulates their maturation via the restoration of E2F1. Clin Exp Immunol 2011; 166: 34-45. doi: 10.1111/j.1365-2249.2011.04447.x

[41] Lina TT, Pinchuk IV, House J, Yamaoka Y, Graham DY, Beswick EJ, Reyes VE. CagA-dependent downregulation of B7-H2 expression on gastric mucosa and inhibition of Th17 responses during Helicobacter pylori infection. J Immunol 2013; 191: 3838-3846. doi: 10.4049/jimmu- nol.1300524.

[42] Bizzaro N, Antico A, Villalta D. Autoimmunity and Gastric Cancer. Int J Mol Sci. 2018;19(2):377. doi: 10.3390/ ijms19020377.

[43] Meyer AR, Goldenring JR. Injury, repair, inflammation and metaplasia in the stomach. J Physiol. 2018;596(17): 3861-3867. doi: 10.1113/JP275512.

[44] Petersen CP, Meyer AR, De Salvo C, Choi E, Schlegel C, Petersen A, Engevik AC, Prasad N, Levy SE, Peebles RS, Pizarro TT, Goldenring JR. A signalling cascade of IL-33 to IL-13 regulates metaplasia in the mouse stomach. Gut. 2018;67(5):805-817. doi: 10.1136/gutjnl-2016-312779.

[45] Kim TS, da Silva E, Coit DG, Tang LH. Intratumoral Immune Response to Gastric Cancer Varies by Molecular and Histologic Subtype. Am J Surg Pathol. 2019;43(6):851-860. doi: 10.1097/PAS.0000000000001253.

[46] Buckland G, Travier N, Huerta JM, Bueno-de-Mesquita HB, Siersema PD, Skeie G, Weiderpass E, Engeset D, Ericson U, Ohlsson B, Agudo A, Romieu I, Ferrari P, Freisling H, Colorado-Yohar S, Li K, Kaaks R, Pala V, Cross AJ, Riboli E, Trichopoulou A, Lagiou P, Bamia C, Boutron-Ruault MC, Fagherazzi G, Dartois L, May AM, Peeters PH, Panico S, Johansson M, Wallner B, Palli D, Key TJ, Khaw KT, Ardanaz E, Overvad K, Tjønneland A, Dorronsoro M, Sánchez MJ, Quirós JR, Naccarati A, Tumino R, Boeing H, Gonzalez CA. Healthy lifestyle index and risk of gastric adenocarcinoma in the EPIC cohort study. Int J Cancer. 2015 1;137(3):598-606. doi: 10.1002/ijc.29411.

[47] Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res. 2018;10:239-248. doi:10.2147/CMAR.S149619

[48] Lazăr DC, Avram MF, Romoșan I, Cornianu M, Tăban S, Goldiș A. Prognostic significance of tumor immune microenvironment and immunotherapy: Novel insights and future perspectives in gastric cancer. World J Gastroenterol. 2018 Aug 28;24(32):3583-3616. doi: 10.3748/wjg.v24.i32.3583. PMID: 30166856; PMCID: PMC6113718.

[49] Lauwers GY, Carneiro F, Graham DY. Gastric carcinoma. In: Bowman FT, Carneiro F, Hruban RH, eds. Classification of Tumours of the Digestive System. Lyon: IARC, 2010

[50] Velho S, Fernandes MS, Leite M, Figueiredo C, Seruca R. Causes and consequences of microsatellite instability in gastric carcinogenesis. World J Gastroenterol 2014; 20: 16433-16442 [DOI: 10.3748/wjg.v20.i44.16433]

[51] Sokolova O, Naumann M. NF-κB Signaling in Gastric Cancer. Toxins (Basel). 2017;9(4):119. doi:10.3390/toxins9040119.

Chapter 2

Revisiting the Full Spectrum of *Helicobacter pylori*-Related Gastric Lymphoma

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Abstract

Early stage gastric diffuse large B-cell lymphomas (DLBCLs) with histological features of mucosa-associated lymphoid tissue (MALT) origin (DLBCL[MALT]) are also closely related to *Helicobacter pylori* (*Hp*) infection, apart from the classical gastric MALT lymphoma, and are cured by *Hp* eradication therapy (HPE). Whether some gastric "pure" DLBCLs (without histological features of MALT) are also *Hp*-related is clinically very important, since this subtype of gastric lymphoma is relatively common in the population and is still universally treated with intensive systemic chemotherapy. A large proportion of early stage gastric "pure" DLBCL can achieve long-term complete remission after HPE. However, the precise mechanisms of *Hp*-dependent (with complete regression of tumors after HPE) lymphomagenesis of gastric "pure" DLBCL, DLBCL(MALT), and MALT lymphoma remain uncertain. In the classical conception, gastric MALT lymphoma is indirectly caused by *Hp* through T-cell stimulation, with the aid of costimulatory molecules. To explore the direct interactions between *Hp* and lymphoma B-cells of *Hp*-dependent gastric MALT lymphoma, DLBCL(MALT), and "pure" DLBCLs, we assessed the participation of *Hp*-encoded cytotoxin-associated gene A (CagA) in the lymphomagenesis of these tumors. We discovered that CagA oncogenic protein and its regulated signaling molecules including phospho-Src homology-2 domain-containing phosphatase (p-SHP-2) and phospho-extracellular signal-regulated kinase (p-ERK) correlated significantly with *Hp*-dependence of gastric MALT lymphoma. This finding supports previous observations that the CagA protein of *Hp* can be translocated into B-cell lymphoma cells, thereby leading to survival signals. Furthermore, we demonstrated that *Hp*-positive and CagA-expressing gastric "pure" DLBCLs behave in a less biologically aggressive manner, and have better clinical outcomes; this is a distinguishing entity, and its cell origin may include germinal center B cells. In addition, we found that the expression of CagA, p-SHP-2, and p-ERK correlated significantly with the *Hp*-dependence of gastric DLBCL(MALT) and "pure" DLBCL. These findings indicate that the spectrum of *Hp*-related gastric lymphomas including MALT lymphoma, DLBCL(MALT), and "pure" DLBCL, is much wider than was previously thought. Further explorations of the spectrum, lymphomagenesis, and therapeutics of *Hp*-related gastric lymphoma are warranted.

Keywords: *Helicobacter pylori*, MALT, DLBCL, Stomach, CagA

1. Introduction

Gastric lymphoma, the most common non-Hodgkin lymphoma, has become an interesting research topic because of its unique clinicopathological features, wide spectrum of histological subtypes, and specific treatment strategies [1–4]. Histologically, gastric lymphomas are the most common B-cell neoplasms; mucosa-associated lymphoid tissue (MALT) lymphoma (renamed as marginal zone B-cell lymphoma with MALT type) and diffuse large B-cell lymphoma (DLBCL) with and without histological evidence of MALT origin are the most common subtypes according to the World Health Organization (WHO), in addition to rare mantle cell lymphoma, follicular lymphoma, and Burkitt lymphoma [3–6]. MALT lymphoma, which histologically consists primarily of diffuse small- and medium-sized lymphocytes, resembling centrocytes (centrocyte-like cells, CCLs) and lymphoepithelial lesions (LELs), was first described by Isaacson and Wright et al. in 1983 [1, 2, 7, 8]. At the same time, Marshall and Warren described the direct link between *Helicobacter pylori* (*Hp*) (a gram-negative, spiral rod-shaped bacterium) infection, and gastritis and peptic ulcer disease [9]. After one decade, Wotherspoon and Isaacson et al. found that 31% of patients with *Hp*-positive gastritis had lymphoid follicles, and 92% of patients with gastric MALT lymphoma had *Hp* infections, indicating a close association between *Hp* infection and the development of MALT and MALT lymphoma of the stomach [10]. Subsequently, they demonstrated that approximately 60% of patients with gastric MALT lymphoma achieved complete remission (CR) after being treated with antibiotics that eradicate *Hp* infection [11]. Subsequently, most investigators started administrating first-line *Hp* eradication therapy (HPE) by combining proton pump inhibitors (PPIs), amoxicillin, clarithromycin, bismuth, metronidazole, or tetracycline in the treatment of localized *Hp*-positive gastric MALT lymphoma [12–14]. By reviewing 32 clinical studies of first-line HPE for gastric MALT lymphoma patients (most prospective studies), Zullo et al. demonstrated that 1091 (77.5%) of 1408 patients achieved CR after successful first-line HPE; among these patients, patients with stage I disease had a higher CR rate than those with stage II disease (78.4% vs. 55.6%, P = 0.0003) [15]. Although some patients with gastric MALT lymphoma may take more than 12 months to achieve CR after completing HPE, most patients achieved CR within 12 months after completing HPE [15–17]. Therefore, eradication of *Hp* infection by antibiotics in addition to a PPI has been well conceded as the first-line treatment for early-stage *Hp*-positive gastric MALT lymphoma.

In contrast to gastric MALT lymphoma, high-grade transformed MALT lymphoma, relabeled as DLBCL with histological evidence of MALT origin DLBCL(MALT), is conventionally considered as *Hp*-independent (the lack of CR of lymphoma after HPE) according to the WHO [4–6]; as per WHO, patients with DLBCL(MALT) should be treated with systemic chemotherapy [18–20]. However, in the past decade, our group and other investigators have found that early-stage gastric DLBCL(MALT) is as responsive to first-line antibiotics as its low-grade counterpart, MALT lymphoma [21–25]. These observations have led to a drastic change in the standard therapy for patients with gastric DLBCL(MALT); many of these patents are now spared from experiencing the severe toxicity of intensive systemic chemotherapy.

Gastric "pure" DLBCL (DLBCL without histological evidence of MALT origin) is generally assumed as originating de novo instead of originating from high-grade transformed MALT lymphoma, and is thus regarded as having a rare association with *Hp* infection [3–6]. Considering that gastric "pure" DLBCLs comprise approximately half of gastric lymphomas, and this subgroup of patients are conventionally treated with systemic chemotherapy [4, 6, 8], it is worthwhile to explore whether some *Hp*-positive gastric "pure" DLBCL remain *Hp*-dependent. Our explorative study showed that antibiotics alone resulted in CR in 69% of patients with early

stage gastric "pure" DLBCL, and these *Hp*-dependent (the presence of CR of lymphoma after HPE) patients remained in CR after a 4-year rigorous endoscopic follow-up, whereas patients without CR after antibiotic treatment were still responsive to subsequent salvage chemotherapy [26]. Two other studies also demonstrated that some patients with *Hp*-positive early stage gastric "pure" DLBCL achieved CR through antibiotic eradication of *Hp*, and most *Hp*-dependent patients remained lymphoma-free after long-term follow up [27, 28]. In addition, among patients with gastric "pure" DLBCL receiving systemic chemotherapy, the *Hp*-positive group had less aggressive behaviors and better clinical outcomes than the *Hp*-negative group [29–31]. These findings suggest that for patients with *Hp*-positive localized gastric "pure" DLBCL, the administration of first-line antibiotic treatment, followed by careful monitoring of tumor response before and after antibiotic treatment using meticulous endoscopic examination, may allow certain patients to avoid the adverse effects of chemotherapy. Importantly, the explanation as to why some "pure" DLBCLs are still *Hp*-dependent can allow us to explore the precise molecular mechanisms of *Hp*-dependent lymphomagenesis of gastric DLBCL.

Regarding the lymphomagenesis of gastric MALT lymphoma, the classical concept is that *Hp* can only stimulate T cells, and then *Hp*-specific T cells transform the marginal-zone B cells into lymphoma [32–35]. Direct interaction between *Hp* and B cells was not considered to exist. However, several studies have observed that *Hp*-encoding cytotoxin-associated gene A (CagA) can be translocated into B cells, thereby activating survival signals of B-lymphoma cells, including tyrosine phosphorylation-dependent and -independent signaling [35–37]. Our group further observed that the CagA molecule and its triggering signaling molecules such as phospho–Src homology-2 domain-containing phosphatase (p-SHP-2), phospho–extracellular signal-regulated kinase (p-ERK), phospho-*p38* mitogen-activated protein kinases (p-p38 MAPK), B-cell lymphoma (Bcl)-2, and Bcl-xL are expressed in tumor cells of gastric MALT lymphoma patients [38–40]. Furthermore, CagA and its controlled signaling molecules significantly correlated with the *Hp*-dependence of these tumors [40]. In addition, our group showed that CagA, p-SHP-2, and p-ERK were closely associated with the *Hp*-dependence of gastric DLBCL(MALT) and "pure" DLBCL [41]. These observations pose a strong challenge to the classical concept of indirect *Hp*-specific T-cell stimulation, and suggest the possibility that a direct interaction between *Hp* and B cells exists in a wide spectrum of gastric lymphoma including MALT lymphoma, DLBCL(MALT), and "pure" DLBCL.

In this chapter, we will describe the association between *Hp* infection and MALT lymphoma, the novel use of first-line HPE in curing gastric DLBCL with and without histological evidence of MALT, and a wide spectrum of *Hp*-related gastric lymphomas; in addition, we present the possible molecular mechanisms and cellular origins of *Hp*-related gastric lymphoma.

2. The close link between *Hp* infection and gastric MALT lymphoma

Hp, a gram-negative and spiral rod-shaped bacterium that has evolved to grow in the environment of the stomach, infects approximately 50% of the population worldwide [42, 43]. Epidemiologic studies have shown that the prevalence of *Hp* infection documented by the histological detection of bacteria or positive serology was significantly higher in gastric MALT lymphoma patients than in healthy populations [44, 45]. Asenjo et al. reviewed studies exploring the association between *Hp* prevalence and gastric MALT lymphoma patients, and revealed that the incidence of *Hp* was approximately 79% in 1844 patients with gastric MALT lymphoma, and the differential prevalence may result from the number of assessments of *Hp*

infection. For example, a higher positive rate of *Hp* infection is observed with the use of more than two methods than with the use of a single method [46]. Moreover, the *Hp* infection rate was 74% for patients whose tumors were limited to the mucosa or submucosa, whereas the *Hp* infection rate was 44% for patients whose tumors invaded the muscularis or beyond [46].

3. Gastric MALT lymphoma is cured by first-line antibiotics eradicating *Hp* infection

Zullo et al. reviewed 1408 patients with gastric MALT lymphoma from 32 studies including 23 prospective and nine retrospective studies, to explore the treatment efficacies of first-line HPE and predictive markers of *Hp*-dependence [15]. In their studies, the determination of CR was mainly based on the tumors that regressed to achieve less than grade 2 (chronic active gastritis with florid lymphoid follicle formation) of Wotherspoon's scoring system [11]. Zullo et al. reported that tumors limited to the mucosa or submucosa were significantly closely associated with a higher CR rate (mucosa/submucosa vs. muscularis propria involvement and beyond: 82.2% vs. 54.5%; P = 0.0001) [15]. In addition, patients with tumors located at the distal lesions of the stomach (antrum and/or angulus) had a higher CR rate than those with tumors located at proximal lesions (gastric body and/or fundus) (91.8% vs. 75.7%; P = 0.0037) [15]. In a long-term follow-up of 994 patients, 72 patients (7.24%) experienced relapse of MALT lymphoma; among these, 12 patients had *Hp* infections [15].

In a retrospective multicenter study from Japan, Nakamura et al. showed that among 420 patients with successful HPE, 284 (67.6%) patients achieved pathological CR (pCR, absence of CLLs and without aggregation of small lymphocytes), and 39 (9%) patients had probable minimal residual disease (pMRD; presence of atypical lymphoid aggregates or nodules in at least two following assessments) according the Groupe d'Etude des Lymphomes de l'Adult (GELA) criteria, with an overall CR rate of 77% (323 patients) [47]. The histological scoring system proposed by GELA is currently recommended to assess whether tumors of gastric MALT lymphoma can achieve CR in a series of examinations after HPE using combined endoscopic findings and histological manifestations, in which CR was defined as the total vanishing of the gross tumor and a negative histological finding (pCR or pMRD) [48, 49]. Nakamura also showed that the median time to CR for these *Hp*-dependent tumors was four months (range from 1 to 94 months), and proximal or multiple locations, and non-superficial manifestations (such as hyperemia patches) were significantly associated with *Hp*-independence [47]. Tsai et al. and Kuo et al. also showed that ulcerative lesions, proximal locations of tumors, and tumors invading the muscularis propria or serosa correlated significantly with the *Hp*-independence of gastric MALT lymphoma [25, 39]. In another large cohort study of a Korean population, Gong et al. reported that *Hp* infection was detected in 317 (91.9%) of 345 patients with gastric MALT lymphoma using histology, a urea breath test, a rapid urease test, or a serologic test [50]. Gong showed that among *Hp*-positive patients, HPE resulted in a CR rate of 84.5% (n = 268), with a median time of 9.8 months (range 7.1 to 15.6 months) to CR, whereas 29 patients (10.7%) with CR developed relapses of MALT lymphoma [50].

Regarding the duration for achieving CR of tumors after completion of HPE, most studies showed that intervals are varied, ranging from quick (one to six months) to moderate (more than six months to 12 months) [15, 16, 39, 47, 51, 52]. In a prospective study, Hong et al. revealed that 85 (94.4%) of 90 patients with gastric MALT lymphoma achieved CR after HPE, with a median time to CR of three months (range, 1 to 24 months); 79 (92.9%) patients achieved CR at six months,

and another six (7.1%) patients achieved CR at 12 months [53]. Zullo et al. also showed that the median interval to CR was five months for patients with gastric MALT lymphoma after treating HPE, whereas few patients needed at least two years to achieve CR [15]. Fischbach et al. analyzed 108 gastric MALT lymphoma patients who underwent gross normalization but had histologically minimal residuals at 12 months after HPE, and found that 35 patients (32.4%) achieved subsequent CR after more than 24 months of follow-up post-HPE [54]. Terai et al. reported that among 74 patients with gastric MALT lymphoma, 56 (75.7%) patients had CR and 12 had gross tumor regression with histological residual tumor (hRD) at 12 months after successful HPE, while 11 patients with hRD achieved CR (ranging from 14 to 40 months after HPE) at a subsequent follow-up [55]. Tsai et al. further showed among patients with *Hp*-positive gastric MALT lymphoma who entered into a prospective study of the Taiwan Cooperative Oncology Group (TCOG) 3206 trial, the median time to CR was 4.0 months (range, 1 to 16 months) after HPE; among these patients, six (23%) patients achieved CR within 6 to 12 months, and four (15.4%) patients required 12 to 24 months to attain CR [25]. These results suggest that longer observation and refraining from the immediate administration of second-line therapy (including chemotherapy or radiotherapy) are recommended for gastric MALT lymphoma patients who had improved symptoms, partial remission (PR), or stable status of tumors even at 12 months or longer after HPE. In other words, a "watch and wait" treatment strategy may be advisable for patients with gastric MALT lymphoma who achieved probable minimal residual disease or tumor PR (according to GELA criteria) after successful HPE.

4. High-grade transformation does not confer *Hp*-independence of gastric lymphoma

It was previously believed that the transformation of MALT lymphoma into high-grade MALT lymphoma, is associated with the acquisition of *Hp*-independence (lack of CR of tumors after HPE); high-grade MALT lymphoma is thus considered as a *Hp*-independent tumor that is non-responsive to antibiotics (**Figure 1**) [18–20, 56]. Clinicians who administer first-line HPE to treat patients with high-grade gastric MALT lymphoma do so as they may regard MALT lymphoma as similar to low-grade MALT lymphoma that is highly responsive to HPE. Thus, to avoid such a confusion, Harris et al. in a 1999 WHO classification advised that high-grade MALT lymphoma should be renamed as DLBCL with histologic evidence of MALT (DLBCL[MALT]), and not as transformed high-grade MALT lymphoma [57]. In 2008, the WHO lymphoma classification advocated that histological manifestations of gastric lymphoma that display large-cell B-cell transformation in the MALT lymphoma background should be classified as DLBCLs rather than as high-grade transformed MALT lymphomas [5]. In this milieu, the presence of large B cells comprising LELs does not alter the pathological diagnosis of DLBCL [5, 6]. Regardless of this, the existence of complementary MALT lymphoma components in DLBCL should be appraised consistently [4, 5, 58].

However, two independent prospective studies and one retrospective study have revealed that a certain proportion of early-stage *Hp*-positive gastric DLBCL(MALT) patients were still responsive to first-line HPE, and thus achieved CR and subsequent long-term remission [21–24]. In the first prospective study, Chen et al. (Taiwan study group) demonstrated that 10 (62.5%) of 15 patients with early-stage gastric DLBCL(MALT) achieved CR after receiving first-line HPE, and remained lymphoma-free during a long-term follow-up [21]. In another prospective study assessing the association between clinicopathological features and tumor response

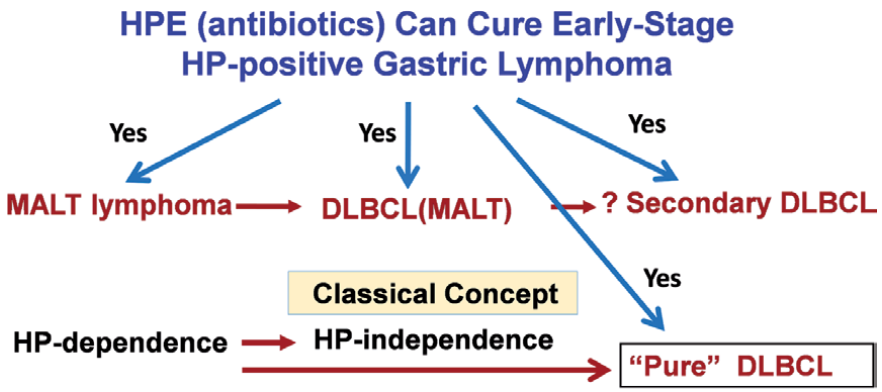

Figure 1.
Gastric diffuse large B-cell lymphoma as well as gastric mucosa-associated lymphoid tissue (MALT) lymphoma are Helicobacter pylori*-dependent, and are cured by first-line Hp eradication therapy (HPE). In contrast to the classic concept that gastric diffuse large B-cell lymphomas (DLBCLs) with and without histological evidence of MALT lymphoma are not responsive to HPE, several evidences demonstrated that* Hp*-related gastric lymphoma does not involve loss of* Hp*-dependence and is responsive to HPE, indicating that the spectrum of* Hp*-related gastric lymphoma is much wider than was originally thought.*

to HPE in gastric MALT lymphoma and DLBCL(MALT), Nakamura et al. reported that five (50%) of 10 gastric DLBCL(MLAT) patients had CR, whereas 4/6 with mucosa and submucosa involvement and 1/4 patients with tumor involvement in the muscularis propria were *Hp*-dependent [22]. Of these, five *Hp*-dependent patients were still free of lymphoma after a median follow-up of five years [22]. Mongnar et al. retrospectively analyzed eight patients with gastric DLBCL(MALT) who initially received HPE, of whom seven patients had CR [23]. Among these *Hp*-dependent patients, four patients did not receive further treatment, whereas one patient developed recurrence at six months after completing HPE, and another two patients underwent surgery later (one patient received chemotherapy because of residual MALT lymphoma in the surgical specimen) [23].

In 2005, Chen et al. reported the clinical outcome of a prospective study using first-line HPE for treating 24 patients with gastric DLBCL(MALT) and 36 patients with gastric MALT lymphoma, and demonstrated that 24 (80%) of 30 patients with MALT lymphoma and 14 (63.6%) of 22 patients with DLBCL(MALT) achieved CR after successful HPE [24]. The median time to CR after the completion of HPE for *Hp*-dependent patients was six months for DLBCL(MALT), and 10 months for MALT lymphoma [24]. Interestingly, after a median long-term follow-up (MALT lymphoma, 70 months; DLBCL[MALT], 56 months) for these *Hp*-dependent patients, the tumor did not recur in the DLBCL(MALT) case, but recurred in three cases of MALT lymphoma [24]. Regarding the depth of tumor infiltration associated with tumors responsive to HPE, the CR rate was 80% (8/10) for tumors limited to the mucosa or submucosa, and 29.4% (5/17) for tumors invading the muscularis propria or beyond (P = 0.018) [24].

In 2008, Cavanna et al. [59] reviewed the anecdotal cases series reporting the CR after antibiotic treatment for gastric DLBCL(MALT) and the results of HPE for patients with gastric DLBCL(MALT) obtained from Chen et al. [21, 24], Nakamura et al. [22], Morgan et al. [23], Hiyama et al. [60], and Alpen et al. [61]. Cavanna et al. showed that 42 (68.9%) of 61 cases of gastric DLBCL(MALT) responded completely to antibiotics eradicating *Hp*; most patients in this study presented with stage IE (30 cases), with tumor invasion to the mucosa or submucosa (21 of 33 cases were evaluable) [59]. However, age, sex, and tumor location (proximal or distal components) did not predict the response of tumors to HPE [59]. Although depth of invasion and stage of gastric DLBCL(MALT) were closely associated with

Hp-independence, there were a few cases of stage IIE1 (perigastric lymph node involvement) tumors that were dependent on *Hp* and achieved CR after HPE [59]. Zullo et al. analyzed the pooled data obtained from 1271 patients with gastric MALT lymphoma or DLBCL(MALT) through 34 studies exploring the treatment efficacy of first-line HPE, and revealed that a *Hp* eradication rate of 91% can be achieved using dual therapy for 14 days or triple therapy for seven to 14 days [62]. In their analyses, the CR rate was 78.5% for MALT lymphoma patients (n = 1215), and 62% for DLBCL(MALT) patients (n = 52) [62]. In the report of therapeutic efficacies of first-line HPE in gastric DLBCL(MALT) patients by Kuo et al. [26], the CR rate was 56.3% (18/32) with a median interval to CR of 5.0 months; the CR rate was significantly associated with the tumor extent (mucosa/submucosa vs. beyond: 80% [8/10] vs. 29.4% [5/17], P = 0.018) [26].

It should be noted that if patients with gastric DLBCL(MALT) do not respond well to antibiotic treatment, the tumor may rapidly progress and cause potential morbidities in these patients. However, for *Hp*-independent gastric DLBCL(MALT) patients, subsequent systemic chemotherapy could result in CR and let patients remain disease-free during long-term follow-up [21–25, 63]; this implies that a delay in the administration of systemic chemotherapy to 6–8 weeks after HPE with antibiotics is unlikely to influence the response of these tumors to conventional immunochemotherapy. These findings may support the contention that

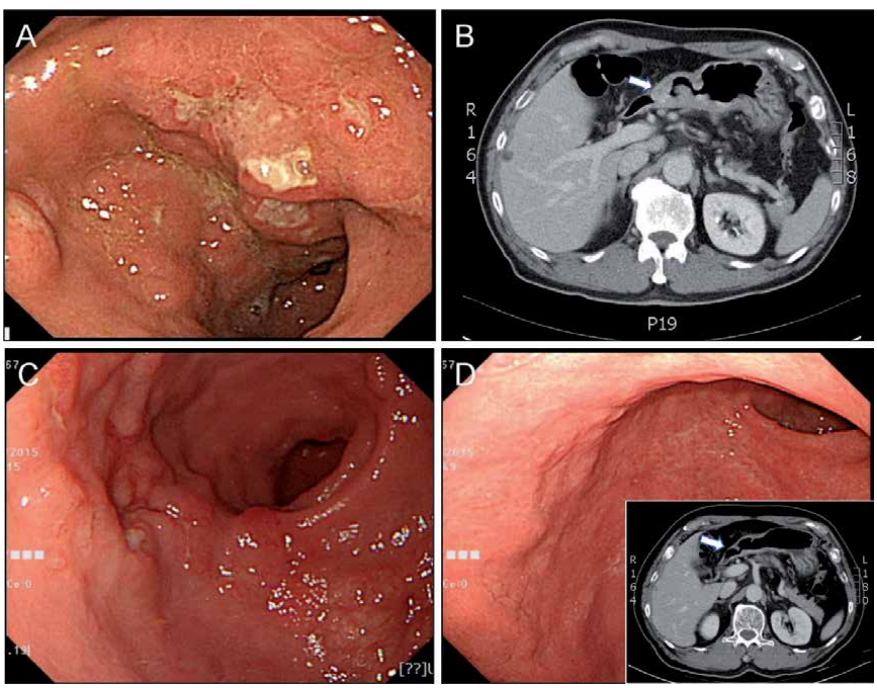

Figure 2.
Changes of endoscopic features in an example of stage IE Helicobacter pylori *(*Hp*)-dependent localized gastric diffuse large B-cell lymphoma (DLBCL) with mucosa-associated lymphoid tissue (MALT) (DLBCL[MALT]) before and after completion of Hp eradication therapy (HPE) (A) Endoscopy shows several ulcerative mass lesions with nodular and irregular margins at the antrum of a 74-year-old man before HPE (Histopathology disclosed DLBCL[MALT], DLBCL-predominant). (B) Computed tomography (CT) shows moderate wall thickening (white arrow) in the gastric antrum but no enlargement of perigastric lymph nodes in the same case A. (C) One month after the completion of HPE, endoscopy shows two partially regressed ulcerative masses at the gastric angle in the same case A (D) Four months after completion of HPE, complete remission was achieved in the same case A. Right bottom, CT shows no gastric wall thickening (white arrow).* Hp, Helicobacter pylori*; HPE, Hp eradication therapy; DLBCL, diffuse large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; CT, computed tomography; CR, complete remission.*

Authors (Ref.)	Study Group	Patients number (Men/ Women)	Age, years (median; range)	Histological subtype	Stage	First-line HPE regimen (for 2 weeks)	CR rate	Median time to CR (ms)	Follow-up period for *Hp*-dependent patients	Salvage treatment regimen for *Hp*-independent patients
Nakamura et al. [22]	Japan (2001)	10	NA	DLBCL(MALT)	IE	Proton pump inhibitor plus Clarithromycin 600 mg D Amoxicillin 2000 mg D Metronidazole 750 mg D	5/10 (50%)	NA	NA	NA
Morgner et al. [23]	Germany (2001)	4/4	26–85	DLBCL(MALT)	IE: 6 IIE1: 2	Dural regimen (2 weeks): Omeprazole 40 mg tid Amoxicillin 750 mg tid Triple regimen (1 week): Omeprazole 20 mg twice D Clarithromycin 250 mg twice D Metronidazole 400 mg twice D	7/8 (87.5%)	1 to 4 ms	6 to 66 ms	CHOP
Hiyama et al. [60]	Japan (2001)	4	NA	DLBCL(MALT)	IE	Proton pump inhibitor plus antibiotics for 1 to 2 weeks	2/4 (50%)	6 ms	18 ms	CHOP
Alpen et al. [61]	Germany (2001)	2	73–76	DLBCL(MALT)	IE	Omeprazole 40 mg D Clarithromycin 500 mg D Metronidazole 800 mg D	1/2 (50%)	1.3 ms	5.7 ms	CHOP
Chen et al. [21, 25] Kuo et al. [26]	Taiwan (2001, 2005, 2012)	12/22	55 (35–83)	DLBCL(MALT)	IE: 30 IIE1: 4	Amoxicillin 500 mg 4 times D Clarithromycin 500 mg twice D Omeprazole 20 mg twice D	18/32 (56.3%)	5.0 ms (95% CI, 2.8–7.5)	11.7 years (95% CI, 7.8–14.4)	CHOP, CEpiOP, R-CHOP, or R-CEpiOP
Tari et al. [27]	Japan (2009)	7/8	63–91 (mean: 72.9)	Pure DLBCL	IE	Rabeprazole 20 mg D Amoxicillin 750 mg twice D Metronidazole 250 mg twice D	4/15 (26.7%)	3 ms	7–100 ms (no recurrence)	R-CHOP followed by RT

Authors (Ref.)	Study Group	Patients number (Men/ Women)	Age, years (median; range)	Histological subtype	Stage	First-line HPE regimen (for 2 weeks)	CR rate	Median time to CR (ms)	Follow-up period for *Hp*-dependent patients	Salvage treatment regimen for *Hp*-independent patients
Kuo et al. [26]	Taiwan (2012)	6/10	63 (34–88)	Pure DLBCL	IE: 9 IIE1: 7	Amoxicillin 500 mg 4 times D Clarithromycin 500 mg twice D Omeprazole 20 mg twice D	11/16 (68.8%)	2.1 ms (95% CI, 0.7–6.3)	3.5 years (95% CI, 0.7 ~ 6.3)	R-CHOP or R-CEpiOP
Ferreri et al. [28]	Italy (2002)	16	NA	Pure: 11 DLBCL (MALT): 5	IE*	Clarithromycin 500 mg twice D Tinidazole or Metronidazole 500 mg twice D Omeprazole 20 mg twice D	8/16 (50%)	NA	14–114 ms (9/10 patients remain-free)	PR-rituximab SD or PD -chemo-RT
Tsai et al. [25]	Taiwan (2020)	6/4	71.5 (48–85)	DLBCL(MALT)	IE: 8 IIE1: 2	Amoxicillin 500 mg 4 times D Clarithromycin 500 mg twice D Omeprazole 20 mg twice D	8/10 (80%)	4.0 ms (range 1–10)	59 ms (95% CI, 23–95)	R-COP

Abbreviations: HPE, H. pylori *eradication therapy; DLBCL, diffuse large B-cell lymphoma; DLBCL(MALT),DLBCL with histological evidence of MALT lymphoma; "pure" DLBCL, DLBCL without histological evidence of MALT lymphoma; CR, complete remission in patients with successful HPE.* Hp*-dependent (CR after HPE);* Hp*-independent (non-CR after HPE); D, daily; ms, months; NA, non-analysis; R, rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone; RT, radiotherapy; CEpiOP, cyclophosphamide, epirubicin, vincristine, and prednisolone; PR, partial remission; SD, stable disease; PD, progressive disease. COP, cyclophosphamide, vincristine, and prednisolone.*IE: Perigastric lymph nodes of diameter < 1.5 cm.*

Table 1.
A summary of the efficacies of first-line Helicobacter pylori *eradication therapy for treating patients with early-stage* Helicobacter pylori*-positive gastric DLBCL(MALT) or "pure" DLBCL.*

first-line HPE should be administered to additional populations of stage IIE1 gastric DLBCL(MALT) patients. This concept is further supported by a prospective clinical trial (T3206) designed by the TCOG in evaluating the treatment efficacy of first-line HPE consisting of omeprazole, amoxicillin, and clarithromycin for 14 days in patients with *Hp*-positive stage IE or stage IIE1 MALT lymphoma, and DLBCL(MALT) of the stomach [25]. This trial revealed that 8 (80%) of 10 patients with DLBCL(MALT) and 26 (76.5%) of 36 patients with MALT lymphoma achieved CR (**Figure 2**); the CR rate was not different between stage IE (75%) and stage IIE1 (66.7%) [25]. After a median follow-up of 59 months, all eight *Hp*-dependent DLBCL(MALT) patients remained lymphoma-free, whereas three (7.7%) of the 26 *Hp*-dependent MALT lymphoma patients relapsed after a median follow-up of 82 months [25]. Notably, tumor invasions to the perigastric lymph nodes are not exclusive to *Hp*-dependent cases, suggesting that pivotal mechanisms exist in these tumors; this is because lymphoma cells of the perigastric lymph nodes communicate indirectly with the *Hp* bacteria.

Taken together, these findings show that the tumor remission rates after HPE are identical between MALT lymphoma and DLBCL(MALT) of the stomach (**Table 1**), which overthrows the classical concept that the transformation of MALT lymphoma into high-grade DLBCL(MALT) is associated with the acquisition of *Hp*-independence and thus DLBCL(MALT) is unlikely to respond to HPE. These clinical discoveries are in line with previous molecular studies showing the difference in clonalities between MALT lymphoma components and DLBCL components of the same stomach [64–66]. Kuo et al. compared the patterns of *IgH* rearrangement between DLBCL and MALT lymphoma components of the same gastric DLBCL(MALT) patients receiving HPE, and revealed that different clonal origins of the two co-existing components contributed to the differential response to HPE [67]. In the long-term follow-up of gastric MALT lymphoma without remission, Liu et al. showed that the frequency of development of DLBCL from MALT lymphoma was less than 2%, suggesting rare high-grade transformation in gastric MALT lymphoma [68]. These results indicate that some DLBCL components may evolve independently from their co-existing MALT lymphoma counterparts in gastric DLBCL(MALT). Overall, with reference to clinical impact, first-line HPE resulting in a durable CR rate of approximately 60% has revolutionized the treatment of gastric DLBCL(MALT), and has helped 60% of DLBCL(MALT) patients to avoid the risks of systemic chemotherapy (**Table 1**). With reference to molecular impact, the high-grade transformation of gastric MALT lymphoma does not confer *Hp*-independence to the tumor cells (**Figure 1**); this finding will eventually lead to the revision of the current lymphoma classification, such as the Revised European American Lymphoma Classification (REAL)/WHO. In the REAL/WHO, high-grade gastric MALT lymphoma is classified as DLBCL with histological evidence of MALT (DLBCL[MALT]), and is recommended to be treated as common DLBCL [3, 5, 57].

5. A proportion of gastric "pure" DLBCL patients can be cured by first-line HPE

Although the origin of gastric "pure" DLBCL is usually considered as de novo and not from high-grade transformed MALT lymphoma, there are several evidences demonstrating the epidemiological link between *Hp* infection and gastric "pure" DLBCL [44, 69–71]. In a case–control study in a Japanese population, Ishikura et al. disclosed a close association between *Hp* infection and risk of development of gastric lymphoma, in which the odds ratios for MALT lymphoma and DLBCL were 1.96 (95% confidence interval [CI], 1.00–3.86), and 1.92 (95% CI, 0.74–4.95), respectively [72].

Because the differentiation between "pure" DLBCL and DLBCL(MALT) is not clearly defined as per histopathological manifestations [57, 58], exploration of the therapeutic efficacy of first-line HPE in gastric "pure" DLBCL has rarely been studied as these patients should be treated with aggressive chemotherapy according to recommendations from the WHO advisory committee [5, 57]. However, a proportion of gastric "pure" DLBCL patients are elderly and have a relatively large number of comorbidities; such patients cannot tolerate the adverse effects of systemic chemotherapy. For these elderly gastric "pure" DLBCL patients with comorbidities, several anecdotal case reports showed that the administration of first-line HPE can cause complete regression of tumors [73–75], suggesting that a certain proportion of gastric "pure" DLBCLs are *Hp*-related, and the growth of these lymphoma cells is *Hp*-dependent.

Since 2001, our group and other investigators have shown that early stage gastric DLBCL(MALT) is *Hp*-dependent and is cured by first-line HPE [21–24]. Consequently, HPE has become the first-line treatment for patients with localized gastric DLBCL(MALT) at our institution. Kuo et al. further designed a pilot study to investigate the therapeutic efficacies of first-line HPE in stage IE/IIE1 gastric "pure" DLBCL patients who are monitored by the following approaches: (1) intensive endoscopic regular follow-up, and (2) immediate administration of systemic rituximab-based chemotherapy (immunochemotherapy) if tumors are stable or progressive [26]. This pilot study revealed that first-line HPE resulted in CR in 11 (68.8%) of 16 *Hp*-positive gastric "pure" DLBCL patients, with a median interval to CR of 2.1 months (**Figure 3**) [26]. Although there were a limited number of gastric "pure" DLBCL patients, the CR rate showed a trend of being higher in tumors involving the mucosa/submucosa than in tumors spread into the muscularis propria or beyond (100% [5/5] vs. 54.5% [6/11], P = 0.119) [26]. After a median follow-up of 3.9 years (95% CI, 3.7 to 4.1), 10 *Hp*-dependent patients were alive and free of lymphoma, but one patient died of lung cancer [26]. Considering that the tumors may rapidly progress and cause morbidity or mortality if tumors are unresponsive to antibiotic treatment, *Hp*-independent gastric "pure" DLBCL patients were immediately treated with immunochemotherapy, and the 5-year overall survival (OS) rate was compatible with patients receiving first-line conventional immunochemotherapy (88.9% vs. 78.3%, P = 0.551) [26].

In another retrospective study conducted in a Japanese population, Tari et al. revealed that among 15 patients with stage IE gastric "pure" DLBCL, four (26.7%) patients achieved CR after successful HPE with a HPE regimen consisting of rabeprazole, amoxicillin, and metronidazole [27]. In their studies, all four patients with CR presented with superficial endoscopic findings and remained lymphoma-free for 7–100 months, whereas 11 *Hp*-independent patients responded completely to rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) for three courses, followed by radiotherapy [27]. Endoscopic ultrasound staging also showed the CR rate for tumors limited to the mucosa and the shallow portion of the submucosa was 80% (4/5 cases), whereas that for tumors extending to the deep portion of the submucosa or beyond, it was 0% (0/10 cases) [27]. The HG-L1 trial was a multicenter phase II study which explored first-line HPE consisting of clarithromycin, tinidazole or metronidazole, and omeprazole in 16 patients with stage I *Hp*-positive gastric DLBCL ("pure" DLBCL, n = 11; DLBCL(MALT), n = 5) [28]. Reporting on this trial, Ferrei et al. revealed that eight (50%) patients achieved CR and three patients achieved PR at two months after HPE, and the remaining two patients with PR achieved CR after receiving single rituximab treatment [28]. In addition, the remaining patients with stable or progressive diseases were all converted to CR after receiving salvaged management with R-CHOP [28]. This prospective trial demonstrated that the therapeutic efficacies of HPE in Western populations with gastric DLBCL are the same as those in Asian populations.

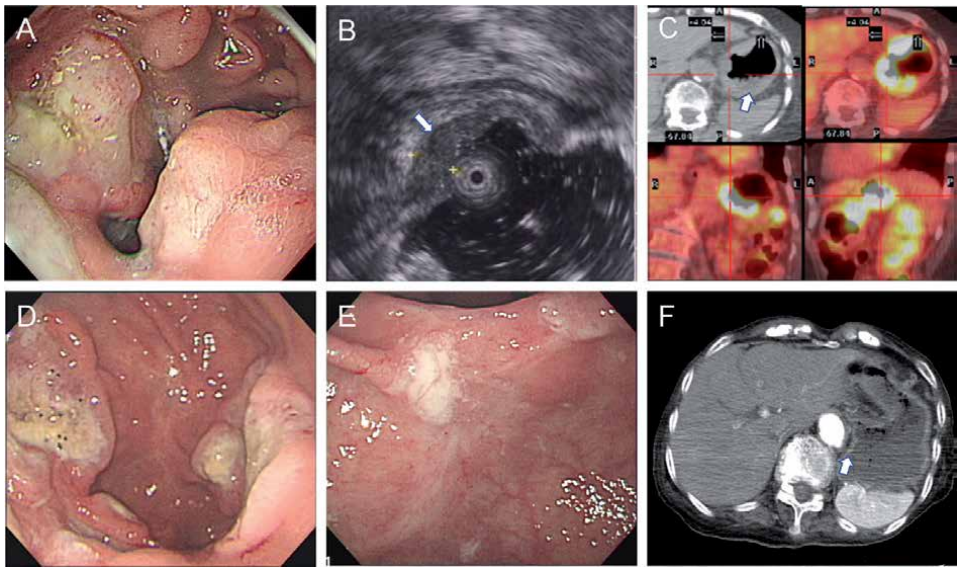

Figure 3.
Changes of endoscopic features and images in an example of Helicobacter pylori *(*Hp*)-dependent stage IIE1 gastric "pure" diffuse large B-cell lymphoma (DLBCL) before and after completion of Hp eradication therapy (HPE) (A) Endoscopy shows multiple ulcerative tumors at the anterior and posterior walls of the greater curvature of the gastric body in an 89-year-old woman before HPE (Histopathology disclosed "pure" DLBCL without histological evidence of mucosa-associated lymphoid tissue). (B) Endoscopic ultrasound examination shows increased thickness of 2nd/3rd layers (submucosal involvement) with maximal thickness (0.7 cm) (white arrow) and multiple perigastric lymphadenopathies in the body of the stomach. (C) Positron emission tomography and computed tomography (CT) reveal intense hot areas (white arrow) at the upper to middle gastric wall of the stomach (standard uptake value max early/delay = 21.5/37.6) (demonstrated at axial, coronal, and sagittal views). (D) One month after completion of HPE, endoscopy shows regressed ulcerative masses at the gastric body in the same case A. (E) Eight months after completion of HPE, complete remission was achieved except for ulcerative scarring (Histopathology disclosed chronic gastritis without intestinal metaplasia) in the same case A. (F) At the same time, CT shows no gastric wall thickening (white arrow) in the gastric body in the same case A.* Hp, Helicobacter pylori*; HPE, Hp eradication therapy; DLBCL, diffuse large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; CT, computer tomography; SUV, standard uptake value; CR, complete remission.*

Taken together, these findings indicate that approximately 50% of gastric "pure" DLBCL patients whose tumors are still *Hp*-dependent, are highly responsive to first-line HPE (**Table 1**). Importantly, HPE treatment may result in a quick response of tumors to CR and to long-lasting CR in these *Hp*-dependent gastric "pure" DLBCL patients. Even if the tumors invade the deep layer or the perigastric lymph nodes, some patients with gastric "pure" DLBCL are still cured by first-line HPE (**Figure 3**). Furthermore, gastric "pure" DLBCL patients who do not respond to first-line HPE are successfully salvaged with rituximab-based chemotherapy. Therefore, the use of first-line HPE as an exclusive treatment to avoid potential adverse effects of chemotherapy or radiotherapy for localized *Hp*-positive for gastric "pure" DLBCL patients is suggested, because this subgroup of patients is frequently older than 70 years.

6. *Hp*-positive gastric DLBCL may be part of *Hp*-related gastric malignancies

In addition to gastric DLBCL(MALT), gastric "pure" DLBCL is epidemiologically linked with *Hp* infection, and most gastric "pure" DLBCLs are diagnosed with limited-stage disease [42–44, 76]. Conventionally, patients with gastric "pure" DLBCLs are treated with immunochemotherapy, whereas immunochemotherapy

with or without subsequent radiotherapy is considered as the standard therapy for patients with localized disease [77, 78]. Considering that first-line surgical resection cannot improve the survival outcome for gastric "pure" DLBCL patients, a surgical approach is recommended for patients with severe bleeding or perforation [79–81]. Studies in Taiwanese, Japanese, and Western populations clearly demonstrated that certain patients with localized *Hp*-positive gastric "pure" DLBCL are highly responsive to first-line antibiotics eradicating *Hp*, and these patients have persistent CR for a long time after HPE [26–28]. These discoveries imply that gastric "pure" DLBCLs can be biologically classified into two types, *Hp*-related lymphoma, and *Hp*-unrelated lymphoma, each with different cellular origins, paths of tumorigenesis, and clinicopathological manifestations (**Figure 1**).

Hsu et al. retrospectively analyzed the clinicopathological features and clinical outcomes of patients with gastric DLBCL who received systemic chemotherapy, including DLBCL(MALT) (n = 17) and "pure" DLBCL (n = 26), and showed that patients with DLBCL had higher levels of serum lactate dehydrogenase (LDH 50% vs. 12%, P = 0.01) and a lower chemotherapy response rate (57.7% vs. 88.2%, P = 0.03) than those with DLBCL(MALT) [82]. Multivariate analysis identified that the presence of MALT lymphoma components, stage I and IIE1, and response to chemotherapy were independent and good prognostic factors [82]. This finding provides evidence that *Hp* infection may be associated with a better chemotherapy response and good prognosis; this is because gastric DLBCL(MALT) is generally transformed from its MALT lymphoma components in which gastric MALT lymphoma is always linked with *Hp* infection, although *Hp* examinations were not assessed in the study by Hsu et al. [82].

This hypothesis is further supported by a report from Kuo et al. who assessed the correlation between clinicopathological features and clinical outcomes, and *Hp* infection in patients (n = 95) with primary gastric "pure" DLBCLs who received conventional chemotherapy (anthracycline-based-regimens, such as CHOP or CEpirubicinOP) or immunochemotherapy (rituximab/anthracycline or rituximab-COP) as first-line therapy [29]. Kuo et al. found that the presence of *Hp* infection confirmed by histological examination, urease test, or bacterial culture, was associated with a lower International Prognostic Index score (0–1; *Hp*(+) [n = 46] vs. *Hp* (−) [n = 49]) = 65% vs. 43%, P = 0.029), a lower clinical stage (I-IIE1, 70% vs. 39%, P = 0.003), and a better CR rate (76% vs. 64%, P = 0.004) [29]. In addition, patients with *Hp* infection had a significantly better 5-year event-free survival (EFS) (71.7% vs. 31.8%, P < 0.001) and OS (76.1% vs. 39.8%, P < 0.001) than those without *Hp* infection [29]. Even among stage I-IIE1 patients, the *Hp*-positive group had a better trend for CR (88% vs. 68%, P = 0.097) and a better 5-year EFS (80.6% vs. 46.8%, P = 0.003) and OS (90.6% vs. 68.0%, P = 0.023) than the *Hp*-negative group [29]. Using multivariate analysis, Kuo et al. identified the absence of *Hp* infection as an independent predictor of worse EFS (hazard ratio [HR] = 2.509; P = 0.007) and OS (HR = 2.666; P = 0.009) [29].

The findings of Kuo et al. were endorsed by the Cheng et al. who conducted a retrospective analysis of 129 patients with primary gastric "pure" DLBCLs, in which the presence of *Hp* infection was based on a positive test for histological examination and urease breath tests [30]. In their study, the presence of *Hp* infection was significantly associated with limited stage (Stage I-II: *Hp*(+) vs. *Hp* (−), 78.6% vs. 60.3%, P = 0.027) and lower IPI score (0–1: *Hp*(+) vs. *Hp* (−), 92.2% vs. 78.2%, P = 0.022); all *Hp*-positive patients received HPE using PPI, bismuth compounds, and antibiotics, including clarithromycin, amoxicillin, tetracycline, or metronidazole, for eradicating *Hp* [30]. In addition, patients with *Hp* infections had a significantly better 5-year progression-free survival (PFS) rate (89.3% vs. 74.1%, P = 0.040) and 5-year OS (89.7% vs. 71.8%, P = 0.033) than those without *Hp* infection [30]. However,

58.9% of *Hp*-positive patients and 78.6% of *Hp*-negative patients underwent surgery as the initial treatment. Furthermore, multivariate analyses showed that in addition to ECOG performance status 0–1, the presence of *Hp* infection was a better independent predictor for PFS (HR = 0.379; P = 0.045) and OS (HR = 0.292; P = 0.021) [30].

Traditionally, MALT lymphoma is believed to be of marginal zone B-cell origin [3, 8]. Unlike MALT lymphoma, DLBCL is generally thought to originate de novo from germinal center B cells (GCB) or activated B cells [83, 84]. Previous studies showed that patients with GCB-subtype DLCBLs had better EFS and OS than those without GCB-subtype DLBCLs [85, 86]. However, in the reports of clinical outcomes of first-line chemotherapy for gastric "pure' DLBCL, Kuo et al. showed that the EFS and OS were not significantly different between GCB subtype and *non-GCB* subtype tumors according to Han's subclassification [29, 87]. Further analysis showed that the GCB subgroup lost its prognostic value in *Hp*-positive gastric DLBCL patients, but showed better survival in *Hp*-negative gastric DLBCL [29]. These results suggest that *Hp*-negative gastric DLBCL may originate de novo, and its biological significance and histologic subclassification may be more similar to that of nodal DLBCL. Similar to Kuo et al. [29], Chang et al. reported that the non-GCB subtype was not associated with the poor PFS and OS in gastric DLBCLs patients [30]. In addition, Kuo et al. showed that a proportion of *Hp*-dependent gastric DLBCLs, including DLBCL(MALT) and "pure" DLBCL, are classified as the GCB-subtype based on Han's subclassification (preliminary data) [88]. Ferreri et al. also showed that among patients with gastric "pure" DLBCL who received first-line HPE, the CR rate was comparable between GCB and non-GCB subgroups [89]. These results indicate that *Hp* may transform not only marginal zone B cells, but also GCB cells into high-grade large lymphoma cells in *Hp*-related gastric lymphoma. However,

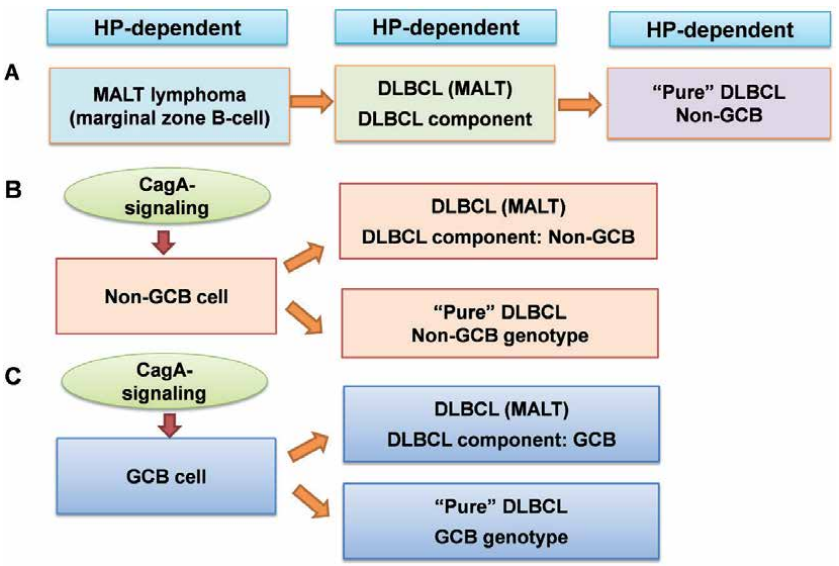

Figure 4.
Schema illustrating a hypothesis of heterogeneous cell origin of Helicobacter pylori *(*Hp*)-dependent gastric diffuse large B-cell lymphoma (DLBCL). (A) Large lymphoma B cells of* Hp*-dependent gastric DLBCL of non-GCB origin may be differentiated from* Hp*-dependent MALT lymphoma components, in which lymphoma cells often lack t(11;18)(q21;q21) [134, 135, 137]. (B) Large lymphoma B cells of* Hp*-dependent gastric DLBCL in which the non-GCBs may originate de novo. The* Hp *CagA and its CagA signaling molecules may directly affect non-GCB cells, which also contribute to the development of* Hp*-dependent large B-cell lymphoma components of gastric DLBCL. (C) Large lymphoma B cells of* Hp*-dependent gastric DLBCL in which the GCBs may originate de novo. The* Hp *CagA and its CagA signaling molecules may directly affect GCB cells, which also contribute to the development of* Hp*-dependent large B-cell lymphoma components of gastric DLBCL.*

further explorations of the cellular origins of these *Hp*-related gastric DLBCLs are warranted. Although the immunophenotypic phenomenon of the GCB subtype cannot explain the better prognosis of *Hp*-related gastric DLBCLs, the higher level of mi-R200 in *Hp*-positive tumors functionally hampering zinc-finger E-box-binding homeobox 1 (ZEB1) may thus contribute to the less aggressive behaviors of these tumors [31], since ZEB1 overexpression was reported to significantly correlate with lymph node metastases of DLCBL, and T-cell leukemia progression [90, 91].

These findings reveal that *Hp*-related gastric "pure" DLBCLs share similar clinicopathological features with gastric MALT lymphoma, such as less aggressive behavior, more limited stages, and better prognosis; this suggests an overlapping etiology between these two gastric lymphoma groups. However, the molecular mechanisms of *Hp* infection contributing to the less aggressive nature and better prognosis of gastric "pure" DLBCL remains unclear. The possible mechanisms are as follows: (1) *Hp* CagA may directly participate in the lymphomagenesis of certain portions of gastric "pure" DLBCL, (2) *Hp* may trigger antigen presentation and regulate the triggering immune communications responsible for gastric MALT lymphoma, which may also lead to *Hp*-related gastric "pure" DLBCL, and (3) *Hp* may transform not only the marginal zone B cells, but also GCB-DLBCL cells, and the mechanisms may include a direct CagA interaction with B cells (**Figure 4**). The following is a brief summary of the possible mechanisms of *Hp*-related gastric lymphoma including MALT lymphoma, DLBCL(MALT), and "pure" DLBCL.

7. *Hp*-specific CagA oncoprotein may play an important role in the molecular mechanism of *Hp*-dependent gastric lymphoma

Previous studies have demonstrated that one of the factors promoting the proliferation of *Hp*-related gastric MALT lymphoma is dependent on the communication between tumor-infiltrating T cells and tumor B cells [32–35]. In addition, CD40-mediated signaling, T helper-2-type cytokines, chemokines such as interleukin-22, the costimulatory molecule CD86, and regulatory T cells (Foxp3+) have been reported to help and promote the proliferation of MALT lymphoma cells [32–35, 92–94]. Through the eradication of *Hp*, malignant B cells are no longer subjected to antigen stimulation, *Hp*-related T-cell interaction, and immune-related regulations; this leads to their gradual regression and death [93, 94]. These findings may explain why MALT lymphomas are more likely to remain localized, and most of them are cured by HPE (**Figure 5**).

Previous studies have shown that the *Hp* strain associated with inflammatory and virulent processes carries the pathogenicity island (*cag*PAI) which includes the *cagA* gene which encodes the CagA protein [95–97]. The CagA protein contains three distinct domains (Domain I to III) at the N-terminal region, and a 5-amino acid-repetitive tandem motif (EPIYA, glutamic acid-proline-isoleucine-tyrosine-alanine) at the C-terminal region [98–100]. The EPIYA motif is characterized by the presence of a tyrosine phosphorylation site; the CagA is phosphorylated at this site by the Src-family kinase (SKFs) and c-Abl [100–102]. The *Hp* CagA-positive strain has been reported to be epidemiologically linked with the development of lymphoid follicles and neoplasms of the stomach [45, 103–105]. For example, Eck et al. reported that the positive rate of serum CagA immunoglobulin G antibodies was higher in *Hp*-positive gastric MALT lymphoma patients than in *Hp*-positive patients with chronic active gastritis (95.5% vs. 67.0%) [45]. In addition to epidemiological studies, other studies have found that CagA promotes the proliferation of B-lymphocytes through CagA-phosphorylation-dependent and -independent signaling pathways [36, 106, 107]. Ohnishi et al. reported that in CagA-transgenic

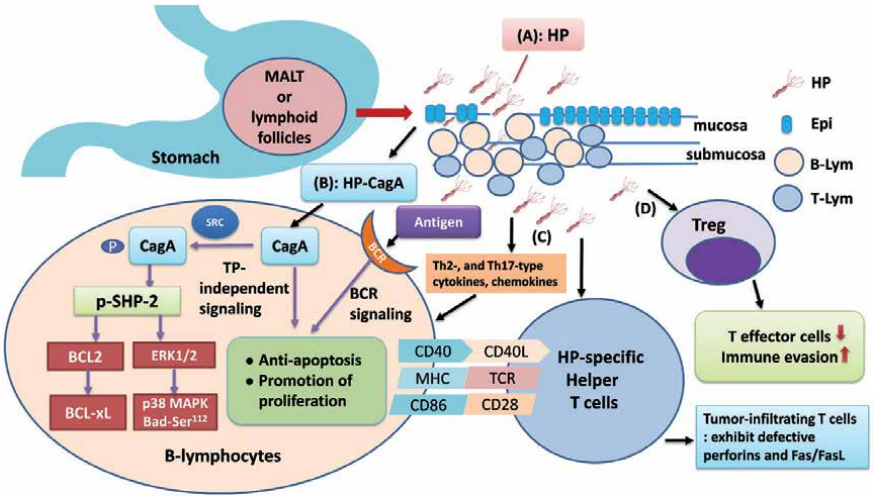

Figure 5.
Schema illustrating the direct and indirect lymphomagenesis of Helicobacter pylori *(*Hp*)-dependent gastric lymphomas, including mucosa-associated lymphoid tissue (MALT) lymphoma, diffuse large B-cell lymphoma (DLBCL) (MALT), and "pure" DLBCL. (A) After a long-term* Hp *infection,* Hp *can cause inflammation and destroy gastric epithelial cells, and stimulate immune B-lymphocytes to migrate into these lesions and progressively develop MALT or lymphoid follicle; and this phenomenon may allow the* Hp *to directly contact B-lymphocyte. Simultaneously, the* Hp *could stimulate the production of* Hp*-specific intratumoral T-helper cells. (B) When* Hp *directly communicates with B-lymphocyte, cytotoxin-associated gene A (CagA), an* Hp*-specific oncoprotein, can translocate into the subcellular area of B-lymphocyte and can undergo tyrosine phosphorylation (TP); phosphorylated CagA can interact with the cytoplasmic Src homology region 2 domain-containing phosphatase-2 and further trigger the activation of extracellular signal-regulated kinase, p38 mitogen activated protein kinase, and the production of anti-apoptosis proteins, Bcl-2, Bcl-xL, and Bad. CagA can also promote tumor growth and differentiation by activating TP-independent signaling. (C)* Hp *infection also indirectly produces antigens, which can communicate with B-cell receptors (BCRs) and elicit the BCR-related survival signal. Simultaneously,* Hp *can indirectly foster growth and differentiation of lymphoma B-cells with exhaustive help from* Hp*-stimulating helper T-cells, T helper (Th)2- or Th17-type cytokines-mediated signaling, the interaction with chemokines and their receptors, the co-communication of CD40/CD40 ligand, and stimulatory molecules such as CD86/CD28. (D)* Hp *infection can engender responses of* $CD4^+CD25^+$ *regulatory T-cells (Tregs) in the gastric microenvironment, and these Tregs could suppress immune-mediated pathogenesis and further cause immune evasion of these lymphoma B-cells. In addition,* Hp*-producing cytotoxic T-cells exhibit defective functionality of perforin and Fas/Fas-Ligand interaction and, thus, have less roles of cytotoxicity and apoptosis for lymphoma B-cells.* Hp, Helicobacter pylori*; Lym, lymphocyte; DLBCL, diffuse large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; CagA, cytotoxin-associated gene; TP, tyrosine phosphorylation; SHP-2, Src homology region 2 domain-containing phosphatase-2; ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinase; BCR, B-cell receptor; Th, T helper; Tregs:* $CD4^+CD25^+$ *regulatory T-cells.*

mice, CagA caused the occurrence of myeloid leukemia and malignant B-cell neoplasms (histologically similar to DLBCL) through SHP-2 phosphorylation-dependent signaling [37]. Other investigators showed that the deregulation of SHP-2 was associated with the proto-oncogenic functions associated with the creation of lymphoid and hematopoietic progenitor cells [108, 109].

Lin et al. revealed that CagA that is injected into B cells via type IV secretion systems (T4SS) can activate ERK, p38 MAPK, Bcl-2, and Bcl-xL molecules through SHP-2 phosphorylation-dependent signaling [38]. Kuo et al. found that in tumor samples of 64 gastric MALT lymphoma patients who received first-line HPE, the expression of CagA was significantly associated with *Hp*-dependence (*Hp*-dependence vs. *Hp*-independence: 68.4% [26/38] vs. 19.2% [5/26], $P < 0.001$) [39]. Among *Hp*-dependent patients, those with tumors expressing CagA responded more rapidly to HPE when compared to patients with tumors without CagA expression (median time to CR after completing HPE, 3.0 vs. 6.5 months, $P = 0.025$) [39]. In addition, in gastric MALT lymphoma patients with known tumor invasion depth, CagA expression was closely associated with less depth of tumor invasion (tumors

limited to mucosa/submucosa vs. tumor involved muscularis propria or beyond: 58.5% vs. 22.2%, P = 0.010) [39]. Furthermore, Kuo et al. found that CagA expression significantly correlated with p-SHP-2, p-ERK, p-38 MAPK, Bcl-2, and Bcl-xL expression, and the expression of the aforementioned molecules correlated with the *Hp* dependence of these tumors [40]. When compared with CagA expression alone, the co-expression of CagA, p-SHP-2, and p-ERK provided an augmented positive predictive value (93.3% vs. 81.8%) and better specificity (95.5% vs. 81.8%) for *Hp* dependence in gastric MALT lymphoma patients [40]. Based on the prospective T3206 trial, Tsai et al. reported that CagA expression correlated significantly with *Hp* dependence in gastric MALT lymphoma and DLBCL(MALT) (85.3% [29/34] vs. 33.3% [4/12], P = 0.001) [25]. Furthermore, downregulation or absence of CagA expression was documented in the residual tumor cells of *Hp*-dependent cases after HPE [25]. Ben Younes et al. also showed that CagA correlated with p-PAKT expression in the tumor cells of *Hp*-positive gastric MALT lymphoma and DLBCL patients, although this study did not show an association between CagA and *Hp* dependence [110]. In addition to CagA expression in tumor cells, Sumida et al. found that among patients with t(11;18)(q21;q21)-negative gastric MALT lymphoma, the serum titer of the CagA antibody was significantly higher in patients with *Hp*-dependent tumors than in those with *Hp*-independent tumors [111]. Taken together, these findings imply that the *Hp* CagA oncogenic protein may participate directly in the molecular mechanisms of *Hp*-dependent gastric MALT lymphoma (**Figure 5**).

In addition to gastric MALT lymphoma, the *Hp* CagA strain may be associated with the development of *Hp*-related gastric DLBCL [112, 113]. Peng first showed that the rate of detection of the CagA gene in gastric biopsy samples was significantly higher in gastric DLBCL(MALT) patients (76.7% [23/30]) than in gastric MALT lymphoma patients (37.8% [14/37]) or in patients with gastritis (30.3% [17/56]) [112]. Delchier et al. also revealed that the *Hp*-seropositive rate was greater in gastric DLBCL patients (100% [16/16]) than in those with gastric MALT lymphoma (78% [29/37]), whereas the CagA-seropositive rate was also higher in DLBCL patients than in MALT lymphoma patients (75% [12/16] vs. 44.8% [13/29], P < 0.05) [113]. Considering that a certain proportion of gastric DLBCLs, including DLBCL(MALT) and "pure" DLBCL, as well as gastric MALT lymphoma are responsive to antibiotics eradicating *Hp*, the clues from *Hp*-specific intratumor T cells and the interacting co-stimulatory molecules, and *Hp* CagA-triggering signaling in MALT lymphoma may also participate in the lymphomagenesis of gastric DLBCL [32–35, 92–94]. Regarding the interaction between *Hp*-specific T cells and co-stimulator molecule CD86 expressed in lymphoma B cells [94], Kuo et al. first showed that CD86 expression in tumor cells was significantly associated with *Hp* dependence in gastric DLBCL(MALT) (68.8% vs. 0%, P = 0.001) [114]. This finding is in line with the findings that *Hp*-dependent gastric MALT lymphoma exhibited a higher expression of CD86 than *Hp*-independent gastric MALT lymphoma [115]. Furthermore, Kuo et al. showed that among gastric "pure" DLBCL patients receiving first-line HPE, CD86 expression was more frequently found in *Hp*-dependent tumors than in *Hp*-independent tumors (61.5% [8/13] vs. 25% [2/8], P = 0.023) [41]. Lin et al. also revealed that in cocultures of *Hp* and B-lymphoma cells, *Hp* CagA upregulated CD86 expression in B cells [38], indicating that a proportion of gastric DLBCLs are still dependent on the triggering of T cells by *Hp* CagA for promoting proliferation (**Figure 5**).

When exploring the possible role of CagA in the lymphomagenesis of gastric DLBCLs, Kuo et al. revealed that CagA expression significantly correlated with p-SHP-2 expression and limited stages (I-IIE1, 82% vs. 47%, P = 0.017) in *Hp*-positive gastric "pure" DLBCL patients receiving systemic chemotherapy [29]. Kuo et al. also showed that CagA expression was associated with significantly better CR (CagA[+]

vs. CagA[−]: 89% vs. 59%, P = 0.030), 5-year EFS (CagA[+] vs. CagA[−]: 85.2% vs. 46.3%, P = 0.002), and OS (CagA[+] vs. CagA[−]: 88.9% vs. 52.9%, P = 0.003) [29]. These findings suggest that *Hp* CagA and its regulated signaling participate in the lymphomagenesis of *Hp*-related gastric "pure" DLBCL. Furthermore, Kuo et al. showed a close association between CagA expression and *Hp* dependence of patients with gastric DLBCL (including DLBCL(MALT) and "pure' DLBCL) who received first-line HPE (*Hp*-dependence vs. *Hp*-independence: 74.3% [26/ 35] vs. 25.0% [7/28], P < 0.001) [41]. Furthermore, CagA expression significantly correlated with the expression of p-SHP-2 and p-ERK, and the expression of these molecules was significantly associated with *Hp* dependence of gastric DLBCL [41]. Among *Hp*-dependent gastric DLBCL patients, the median time to CR after completing HPE was quicker in tumors with CagA expression than in tumors without CagA expression (4.0 months vs. 5.0 months, P = 0.050) [41]. Kuo et al. also observed that CagA and CagA signaling molecules were diminished or absent in a series of biopsy samples after HPE [41]. These results indicate that CagA and its regulated signaling molecules may be involved in the pathogenesis of *Hp*-dependent gastric DLBCL (**Figure 5**).

Epidemiological studies have reported that the incidence of gastric MALT lymphoma in East Asia (including Taiwan, Korea, and Japan) is higher than that in Western countries (Netherlands, Italy, and USA) [70, 72, 116–119]. In addition to the distinct prevalence of gastric lymphoma, the rate of CagA-positivity in *Hp* strains was higher in East Asian populations (at nearly 90%), when compared with that in Western populations (at approximately 60%) [120–125]. In contrast to CagA from Western *Hp* isolates containing EPIYA-A, EPIYA-B, and EPIYA-C segments, the EPIYA motifs in East Asian *Hp* strains (including those from Taiwan), mainly consist of EPIYA-A, EPIYA-B, and EPIYA-D segments [36, 97, 100, 126–128]. CagA activates ERK/MAPK signaling in gastric epithelial cells or lymphoma B-cells mainly by interacting with SHP-2; the CagA-SHP-2 complex is characterized by an interaction between the tyrosine-phosphorylated EPIYA-C or EPIYA-D segment of CagA with the SH2 domain of SHP-2 [97, 128, 129]. In addition, the *Hp* CagA strains bearing the EPIYA-D motif had a greater affinity for binding SHP-2, a capacity for phosphorylating tyrosine, and conferred a risk for developing gastric cancer [97, 126, 129, 130]. Chuang et al. assessed the intensity of tyrosine-phosphorylated CagA (p-CagA) in *Hp* strains isolated from Taiwanese patients with a distinct disease status, including gastric cancer, gastric ulcer, duodenal ulcer, and gastritis; the authors reported that the p-CagA intensity was higher in patients with gastric cancer or gastritis accompanied by intestinal metaplasia than in patients with gastritis but without intestinal metaplasia [131], indicating that a higher tyrosine phosphorylation activity of CagA may be associated with a risk of developing precancerous lesions and subsequent gastric cancer [131]. In *Hp* strains isolated from *Hp*-dependent cases of gastric lymphoma including five DLBCLs and six MALT lymphomas, Kuo et al. showed that all cases were CagA-positive strains [41]. In their studies, the positive CagA *Hp* strains were significantly associated with a rapid time to CR after completing HPE in *Hp*-dependent gastric lymphoma patients, including MALT lymphoma and DLBCLs [39, 41]. In addition, CagA expression correlated significantly with p-SHP-2 expression and the expression of tyrosine phosphorylation-dependent molecules such as ERK and p38 MAPK, in the lymphoma cells [39–41]. These findings further support the findings of a systematic review of HPE for treating gastric MALT lymphoma from Zullo et al. the authors investigated the reason for the significantly higher CR rate of tumors in Asian populations (84.1%) than that of tumors in Western populations (73.8%) (P < 0.0001) [62]. Although the association between tumors expressing CagA and the *Hp* dependence of gastric lymphoma may explain why most CagA-positive gastric MALT lymphomas (even for gastric DLBCLs) remain localized and show a quick response to HPE, approximately 30–50% of *Hp*-dependent gastric

lymphoma patients lack CagA expression in their tumors [39, 41]; this suggests that other underlying molecular mechanisms responsible for antibiotic responsiveness may exist, and need to be explored.

8. Discussion

We and other investigators have demonstrated that a large proportion of localized (stage IE to IIE1) gastric DLBCLs, including DLBCL(MALT) and "pure" DLBCL remain *Hp*-dependent and can possibly be treated by first-line HPE [21–28], indicating that DLBCL transformation is not associated with the loss of *Hp* dependence. We first discovered that most patients with *Hp*-dependent gastric DLBCL(MALT), in whom with DLBCL and MALT lymphoma showed the same clonality using laser capture microdissection and *IgH* CDR3 rearrangement analyses [67]. Starostik et al. compared the genetic aberrations of t(11;18)(q21;q21)-negative MALT lymphoma and DLBCL of the stomach [132]. They demonstrated that both lymphomas share allelic imbalances, such as 3q26.2–27 amplification [132]. Furthermore, Barth et al. found a high pathogenetic similarity between MALT lymphoma and small-cell components of DLBCL(MALT) and between DLBCL and large-cell components of DLBCL(MALT) of the stomach, through expression profile analysis [133]. These findings suggest that some of the large-cell components in patients with gastric DLBCL may be transformed from *Hp*–related MALT lymphoma [133]. Whether *Hp*-related gastric "pure" DLBCLs share biological, immunological, and molecular features of *Hp*-related gastric MALT lymphoma and DLBCL(MALT) is worth investigating in the future.

Although first-line antibiotic HPE has saved approximately 60% of early stage gastric DLBCL patients from the risks of systemic chemotherapy, approximately 40% of early stage and most patients with advanced, gastric DLBCL still receive immunochemotherapy as the primary treatment. To avoid delaying the administration of immunochemotherapy for these *Hp*-independent patients, the identification of molecular markers predicting antibiotic unresponsiveness has become an emergent issue. Previously, we discovered that canonical and noncanonical NF-κB signalings contribute to *Hp*-independent tumor growth of gastric lymphoma, including MALT lymphoma, DLBCL(MALT), and "pure" DLBCL of the stomach, and their respective determinant molecular markers, nuclear BCL10, nuclear NF-κB(p65), and B-cell-activating factor of TNF family (BAFF), closely correlated with *Hp* independence of these tumors [41, 134–136]. The incorporation of *Hp*-independent molecular markers with clinicopathological features into further personalized treatment for *Hp*-positive early stage gastric DLBCL patients is warranted. Our ongoing prospective trial (ClinicalTrials.gov, NCT02388581) is the first study to evaluate the efficacy of first-line antibiotic HPE, as determined by the CR rate and time to tumor progression, in patients with *Hp*-positive localized (stage IE and IIE1) gastric "pure" DLBCL. This trial will also validate the accuracy (sensitivity and specificity) of molecular markers, including CagA, BCL10, NF-κB(p65), and BAFF, in predicting antibiotic responsiveness. This ongoing phase II study hopes to answer the question of "whether not just MALT lymphoma," "pure" DLBCL of the stomach is still responsive to antibiotic treatment".

However, genetic abnormalities found so far in gastric MALT lymphoma, such as t(11;18)(q21;q21), are far from providing a complete understanding of the molecular mechanisms of *Hp*-related gastric DLBCL [93, 137]. *Hp* infection perturbs or changes the epigenetic status, including the methylation profiles, DNA methyltransferase, cytokines, and the inflammatory responses, and causes aberrant hypermethylation [138–140]; these *Hp*-regulated epigenetic and genetic changes are worth exploring as to their relationship with lymphomagenesis of *Hp*-related gastric lymphoma.

Although we and other investigators have demonstrated that CagA-signaling and tumor-infiltrating T-cells co-participate in the molecular mechanisms of *Hp*-related gastric lymphoma [94], *Hp* in the gastric microenvironment may alter immune responses [141–144]. In a murine model of *H. felis*-induced gastric MALT lymphoma, Craig et al. showed that the development of MALT lymphoma requires B-cell receptor signaling through the poly-reactivation of tumor immunoglobulin with certain antigens and tumor-infiltrating T-cells [145]. Most of the tumor-infiltrating CD4+ cells in gastric MALT lymphoma were shown to be $Foxp3^+CD4^+CD25^+$ regulatory T-cells (Tregs), and these Tregs were recruited by tumor cells through the chemokines, CCL17 and CCL22, secreted by $Foxp3^+$Tregs [145]. In addition, the systemic depletion of $Foxp3^+$Tregs *in vivo* efficiently resulted in the regression of MALT lymphoma [145]. Two recent *in vivo* studies examined the possible mechanisms of Tregs involvement in the immunomodulation of gastric MALT lymphoma and showed that the presence of $Foxp3^+$ expression was significantly higher in patients who achieved CR after HPE than in those without CR, suggesting that Tregs-mediated signaling contributes to *Hp*-dependent lymphomagenesis of gastric MALT lymphoma [146, 147]. Whether *Hp*-regulated chemokines, IL-22, CCL17, and CCL12, and Tregs and *Hp*-altering immune responses also contribute to the *Hp*-dependent lymphomagenesis of gastric DLBCL remains uncertain, and these clues from MALT lymphoma would push us to comprehensively explore the mechanisms by which *Hp*-related immune responses participate in the lymphomagenesis of *Hp*-related gastric DLBCL.

9. Conclusions

During the past two decades, we have discovered that the spectrum of *Hp*-related gastric lymphoma is much wider than was previously thought. In addition to the classical MALT lymphoma, we demonstrated that DLBCL with (DLBCL[MALT]) and without ("pure" DLBCL) histological evidence of MALT lymphoma are all closely related to *Hp*. Most importantly, they can all be cured by HPE, a clinical practice that saves thousands of lives and allows patients to avoid systemic immunochemotherapy-related adverse effects. Furthermore, we have identified the existence of *Hp* CagA in the entire spectrum of gastric lymphomas, including MALT lymphoma, DLBCL(MALT), and "pure" DLBCL; it is also important to note that CagA-regulated signaling molecules such as p-SHP-2 and p-ERK were also expressed in tumor cells of *Hp*-dependent gastric MALT lymphoma as well as of gastric DLBCLs. In addition, *Hp*-positive gastric "pure" DLBCLs, especially those exhibiting CagA expression, are not the same as *Hp*-unrelated gastric "pure" DLBCL in terms of clinicopathological manifestations and biological behavior. These findings indicate that CagA may lead to direct lymphomagenesis in these *Hp*-related gastric lymphomas via the regulation of pivotal tyrosine phosphorylation-related signal transduction. This is a big paradigm shift, since until very recently, the classical belief has been that MALT lymphoma is caused indirectly by the interaction of *Hp* with T cells. Since many DLBCLs are of GCB origin, the canonical concept that all *Hp*-related lymphomas are of a marginal-zone B cell origin, needs to be reappraised. However, we and other investigators have reported that a certain proportion of *Hp*-dependent gastric DLBCLs may be of GCB origin. These clues indicate that the spectrum of *Hp*-related gastric lymphomas should be revised to include not only MALT lymphoma, but also DLBCL(MALT) and "pure" DLBCL. Further investigations of the spectrum, lymphomagenesis, therapeutics, and cellular origins of *Hp*-related gastric lymphoma are warranted.

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References

[1] Isaacson PG. Primary Gastric Lymphoma. Pathol Oncol Res. 1996;2 (1-2):5-10. DOI: 10.1007/BF02893940.

[2] Isaacson PG. Update on MALT lymphomas. Best Pract Res Clin Haematol 2005;18(March (1)):57-68. DOI: 10.1016/j.beha.2004.08.003.

[3] Jaffe ES, Harris NL, Stein H, Vardiman JW, (Eds): World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001.

[4] Juárez-Salcedo LM, Sokol L, Chavez JC, Dalia S. Primary Gastric Lymphoma, Epidemiology, Clinical Diagnosis, and Treatment. Cancer Control. 2018 Jan-Mar;25(1): 1073274818778256. DOI: 10.1177/ 1073274818778256.

[5] Swerdlow S, Campo E, Harris NL, eds; International Agency for Research on Cancer. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue. Geneva, Switzerland: World Health Organization; 2008.

[6] Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, Jaffe ES. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016;127(May (20)):2375-2390. DOI: 10.1182/blood-2016-01-643569.

[7] Isaacson PG, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinct type of B-cell lymphoma. Cancer. 1983;52(8):1410-1416. DOI: 10.1002/1097-0142(19831015)52:8<1410::aid-cncr2820520813>3.0.co;2-3.

[8] Isaacson PG, Du MQ. Gastrointestinal lymphoma: where morphology meets molecular biology. J Pathol. 2005; 205(2):255-274. DOI: 10.1002/path.1703.

[9] Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1(8390):1311-1315. DOI: 10.1016/s0140-6736(84)91816-6.

[10] Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori associated gastritis and primary B-cell gastric lymphoma. Lancet. 1991;338(8776):1175 -1176. DOI: 10.1016/0140-6736(91)92035-z.

[11] Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet. 1993;342(8871):575-577. DOI: 10.1016/0140-6736(93)91409-f.

[12] Du MQ, Isaccson PG. Gastric MALT lymphoma: from aetiology to treatment. Lancet Oncol. 2002;3(2):97-104. DOI: 10.1016/s1470-2045(02)00651-4.

[13] Stolte M, Bayerdorffer E, Morgner A, Alpen B, Wündisch T, Thiede C, Neubauer A. Helicobacter and gastric MALT lymphoma. Gut 2002; 50(Suppl. 3): III19-24. DOI: 10.1136/gut.50.suppl_3.iii19.

[14] Gisbert JP, Martin-Asenjo L. Efficacy of Helicobacter pylori erradication treatment in gastric MALT lymphoma: a systematic review. Gastroenterology 2006; 130(Suppl. 2): T1907

[15] Zullo A, Hassan C, Cristofari F, Andriani A, De Francesco V, Ierardi E, Tomao S, Stolte M, Morini S, Vaira D. Effects of Helicobacter pylori eradication on early stage gastric mucosa-associated lymphoid tissue lymphoma. Clin Gastroenterol Hepatol. 2010;8(2):105-110. DOI: 10.1016/j.cgh.2009.07.017.

[16] Fischbach W. Gastric MALT lymphoma - update on diagnosis and

treatment. Best Pract Res Clin Gastroenterol. 2014;28(6):1069-1077. DOI: 10.1016/j.bpg.2014.09.006.

[17] Neumeister P, Troppan K, Raderer M. Management of gastric mucosa-associated lymphoid tissue lymphoma. Dig Dis. 2015;33(1):11-18. DOI: 10.1159/000366030.

[18] Hussell T, Isaacson PG, Crabtree JE, Spencer J. The response of cells from low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue to Helicobacter pylori. Lancet 1993; 342(8871):571-574. DOI: 10.1016/0140-6736(93)91408-e.

[19] Bayerdörffer E, Neubauer A, Rudolph B, Thiede C, Lehn N, Eidt S, Stolte M. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of Helicobacter pylori infection. Lancet 1995;345(8965):1591-1594. DOI: 10.1016/s0140-6736(95)90113-2.

[20] Neubauer A, Thiede C, Morgner A, Alpen B, Ritter M, Neubauer B, Wündisch T, Ehninger G, Stolte M, Bayerdörffer E. Cure of Helicobacter pylori infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. J Natl Cancer Inst. 1997;89(18):1350-1355. DOI: 10.1093/jnci/89.18.1350.

[21] Chen LT, Lin JT, Shyu RY, Jan CM, Chen CL, Chiang IP, Liu SM, Su IJ, Cheng AL. Prospective study of Helicobacter pylori eradication therapy in stage I(E) high-grade mucosa-associated lymphoid tissue lymphoma of the stomach. J Clin Oncol. 2001;19(22):4245-4251. DOI: 10.1200/JCO.2001.19.22.4245.

[22] Nakamura S, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M, Yao T, Tsuneyoshi M, Iida M, Fujishima M. Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphomas after eradication of Helicobacter pylori. *Gut. 2001;***48(4)**:454-460. DOI: 10.1136/gut.48.4.454.

[23] Morgner A, Miehlke S, Fischbach W, Schmitt W, Müller-Hermelink H, Greiner A, Thiede C, Schetelig J, Neubauer A, Stolte M, Ehninger G, Bayerdörffer E. Complete remission of primary high-grade B-cell gastric lymphoma after cure of Helicobacter pylori infection. *J Clin Oncol. 2001;***19(7)**: 2041-2048. DOI: 10.1200/JCO.2001.19.7.2041.

[24] Chen LT, Lin JT, Tai JJ, Chen GH, Yeh HZ, Yang SS, Wang HP, Kuo SH, Sheu BS, Jan CM, Wang WM, Wang TE, Wu CW, Chen CL, Su IJ, Whang-Peng J, Cheng AL. Long-term results of anti-Helicobacter pylori therapy in early-stage gastric high-grade transformed MALT lymphoma. J Natl Cancer Inst. 2005;97(18):1345-1353. DOI: 10.1093/jnci/dji277.

[25] Tsai HJ, Tai JJ, Chen LT, Wu MS, Yeh KH, Lin CW, Wang TE, Wang HP, Yu FJ, Liou JM, Hsiao CF, Cheng TY, Yeh HJ, Ko CW, Chen MJ, Lo GH, Hsu PI, Chang CS, Hwang WS, Chuang SS, Lee HW, Shun CT, Chiu CF, Wang WM, Hsieh CY, Liu TW, Lin JT, Kuo SH, Cheng AL; Taiwan Cooperative Oncology Group. A multicenter prospective study of first-line antibiotic therapy for early-stage gastric mucosa-associated lymphoid tissue lymphoma and diffuse large B-cell lymphoma with histological evidence of mucosa-associated lymphoid tissue. Haematologica. 2020;105(7):e349-e354. DOI: 10.3324/haematol.2019.228775.

[26] Kuo SH, Yeh KH, Wu MS, Lin CW, Hsu PN, Wang HP, Chen LT, Cheng AL. Helicobacter pylori eradication therapy is effective in the treatment of early-stage H pylori-positive gastric diffuse large B-cell lymphomas. Blood. 2012;119(21):4838-4844; quiz 5057. DOI: 10.1182/blood-2012-01-404194.

[27] Tari A, Asaoku H, Kashiwado K, Yoshino T, Kitadai Y, Tanaka S, Fujihara M. Predictive value of endoscopy and endoscopic ultrasonography for regression of gastric diffuse large B-cell lymphomas after Helicobacter pylori eradication. Dig Endosc. 2009;21(4):219-227. DOI: 10.1111/j.1443-1661.2009.00896.x.

[28] Ferreri AJ, Govi S, Raderer M, Mulè A, Andriani A, Caracciolo D, Devizzi L, Ilariucci F, Luminari S, Viale E, Müllauer L, Dell'Oro S, Arcidiacono PG, Ponzoni M, Patti C. Helicobacter pylori eradication as exclusive treatment for limited-stage gastric diffuse large B-cell lymphoma: results of a multicenter phase 2 trial. Blood. 2012;120(18):3858-3860. DOI: 10.1182/blood-2012-06-438424.

[29] Kuo SH, Yeh KH, Chen LT, Lin CW, Hsu PN, Hsu C, Wu MS, Tzeng YS, Tsai HJ, Wang HP, Cheng AL. Helicobacter pylori-related diffuse large B-cell lymphoma of the stomach: a distinct entity with lower aggressiveness and higher chemosensitivity. Blood Cancer J. 2014;4(6):e220. DOI: 10.1038/bcj.2014.40.

[30] Cheng Y, Xiao Y, Zhou R, Liao Y, Zhou J, Ma X. Prognostic significance of helicobacter pylori-infection in gastric diffuse large B-cell lymphoma. BMC Cancer. 2019;19(1):842. DOI: 10.1186/s12885-019-6067-5.

[31] Huang WT, Kuo SH, Cheng AL, Lin CW. Inhibition of ZEB1 by miR-200 characterizes Helicobacter pylori-positive gastric diffuse large B-cell lymphoma with a less aggressive behavior. Mod Pathol. 2014;27(8):1116-1125. DOI: 10.1038/modpathol.2013.229.

[32] Farinha P, Gascoyne RD. Helicobacter pylori and MALT lymphoma. Gastroenterology. 2005;128(6):1579-1605. DOI: 10.1053/j.gastro.2005.03.083.

[33] Bergman MP, D'Elios MM. Cytotoxic T cells in *H. pylori*-related gastric autoimmunity and gastric lymphoma. J Biomed Biotechnol. 2010;2010:104918. DOI: 10.1155/2010/104918

[34] Sagaert X, Van Cutsem E, De Hertogh G, Geboes K, Tousseyn T. Gastric MALT lymphoma: a model of chronic inflammation-induced tumor development. Nat Rev Gastroenterol Hepatol 2010;7(6):336-346. DOI: 10.1038/nrgastro.2010.58.

[35] Kuo SH, Cheng AL. Helicobacter pylori and mucosa-associated lymphoid tissue: what's new. Hematology Am Soc Hematol Educ Program. 2013;2013:109-117. DOI: 10.1182/asheducation-2013.1.109.

[36] Wang HP, Zhu YL, Shao W. Role of Helicobacter pylori virulence factor cytotoxin-associated gene A in gastric mucosa-associated lymphoid tissue lymphoma. World J Gastroenterol. 2013;19(45):8219-8226. DOI: 10.3748/wjg.v19.i45.8219.PMID: 24363512

[37] Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M, Matsui A, Higashi H, Musashi M, Iwabuchi K, Suzuki M, Yamada G, Azuma T, Hatakeyama M. Transgenic expression of Helicobacter pylori CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci U S A. 2008;105(3):1003-1008. DOI: 10.1073/pnas.0711183105.

[38] Lin WC, Tsai HF, Kuo SH, Wu MS, Lin CW, Hsu PI, Cheng AL, Hsu PN. Translocation of Helicobacter pylori CagA into Human B lymphocytes, the origin of mucosa-associated lymphoid tissue lymphoma. Cancer Res. 2010;70(14):5740-5748. DOI: 10.1158/0008-5472.CAN-09-4690.

[39] Kuo SH, Chen LT, Lin CW, Wu MS, Hsu PN, Tsai HJ, Chu CY, Tzeng YS, Wang HP, Yeh KH, Cheng AL. Detection of the Helicobacter pylori CagA protein in gastric mucosa-associated lymphoid tissue lymphoma cells: clinical and biological significance. Blood Cancer J.

2013;3(7):e125. DOI: 10.1038/bcj.2013.22.

[40] Kuo SH, Yeh KH, Chen LT, Lin CW, Hsu PN, Wu MS, Liou JM, Tsai HJ, Tzeng YS, Cheng AL. Helicobacter pylori CagA Translocation Is Closely Associated With the Expression of CagA-signaling Molecules in Low-grade Gastric Mucosa-associated Lymphoid Tissue Lymphoma. Am J Surg Pathol. 2015;39(6):761-766. DOI: 10.1097/PAS.0000000000000437.

[41] Kuo SH, Chen LT, Lin CW, Yeh KH, Shun CT, Tzeng YS, Liou JM, Wu MS, Hsu PN, Cheng AL. Expressions of the CagA protein and CagA-signaling molecules predict Helicobacter pylori dependence of early-stage gastric DLBCL. Blood. 2017;129(2):188-198. DOI: 10.1182/blood-2016-04-713719.

[42] de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol. 2012;13(6):607-615. DOI: 10.1016/S1470-2045(12)70137-7.

[43] Burkitt MD, Duckworth CA, Williams JM, Pritchard DM. Helicobacter pylori-induced gastric pathology: insights from in vivo and ex vivo models. Dis Model Mech. 2017 Feb 1;10(2):89-104. DOI: 10.1242/dmm.027649.

[44] Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. N Engl J Med. 1994 May 5;330(18):1267-1271. DOI: 10.1056/NEJM199405053301803.

[45] Eck M, Schmausser B, Haas R, Greiner A, Czub S, Müller-Hermelink HK. MALT-type lymphoma of the stomach is associated with Helicobacter pylori strains expressing the CagA protein. Gastroenterology. 1997;112(5):1482-1486. DOI: 10.1016/s0016-5085(97)70028-3.

[46] Asenjo L M, Gisbert JP. [Prevalence of Helicobacter pylori infection in gastric MALT lymphoma: a systematic review]. 2007;99(7):398-404. DOI: 10.4321/s1130-01082007000700006.

[47] Nakamura S, Sugiyama T, Matsumoto T, Iijima K, Ono S, Tajika M, Tari A, Kitadai Y, Matsumoto H, Nagaya T, Kamoshida T, Watanabe N, Chiba T, Origasa H, Asaka M; JAPAN GAST Study Group. Long-term clinical outcome of gastric MALT lymphoma after eradication of Helicobacter pylori: a multicentre cohort follow-up study of 420 patients in Japan. Gut. 2012;61(4):507-513. DOI: 10.1136/gutjnl-2011-300495.

[48] Ruskoné-Fourmestraux A, Fischbach W, Aleman BM, Boot H, Du MQ, Megraud F, Montalban C, Raderer M, Savio A, Wotherspoon A; EGILS group. EGILS consensus report. Gastric extranodal marginal zone B-cell lymphoma of MALT. Gut. 2011;60(6):747-758. DOI: 10.1136/gut.2010.224949.

[49] Copie-Bergman C, Wotherspoon AC, Capella C, Motta T, Pedrinis E, Pileri SA, Bertoni F, Conconi A, Zucca E, Ponzoni M, Ferreri AJ. Gela histological scoring system for post-treatment biopsies of patients with gastric MALT lymphoma is feasible and reliable in routine practice. Br J Haematol. 2013;160(1):47-52. DOI: 10.1111/bjh.12078.

[50] Gong EJ, Ahn JY, Jung HY, Park H, Ko YB, Na HK, Jung KW, Kim do H, Lee JH, Choi KD, Song HJ, Lee GH, Kim JH. Helicobacter pylori Eradication Therapy Is Effective as the Initial Treatment for Patients with *H. pylori*-Negative and Disseminated Gastric Mucosa-Associated Lymphoid Tissue Lymphoma. Gut Liver. 2016;10(5):706-713. DOI: 10.5009/gnl15510.

[51] Morgner A, Schmelz R, Thiede C, Stolte M, Miehlke S. Therapy of gastric mucosa associated lymphoid tissue lymphoma. World J Gastroenterol

2007;13(26): 3554-3566. DOI: 10.3748/wjg.v13.i26.3554.

[52] Suzuki H, Saito Y, Hibi T. Helicobacter pylori and gastric mucosa-associated lymphoid tissue (MALT) lymphoma: updated review of clinical outcomes and the molecular pathogenesis. Gut Liver. 2009;3(2):81-87. DOI: 10.5009/gnl.2009.3.2.81.

[53] Hong SS, Jung HY, Choi KD, Song HJ, Lee GH, Oh TH, Jo JY, Kim KJ, Byeon JS, Myung SJ, Yang SK, Hong WS, Kim JH, Min YI. A prospective analysis of low-grade gastric malt lymphoma after Helicobacter pylori eradication. Helicobacter 2006;11(6): 569-573. DOI: 10.1111/j.1523-5378.2006.00460.x.

[54] Fischbach W. Goebeler ME, Ruskone-Fourmestraux A, Wündisch T, Neubauer A, Raderer M, Savio A. Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of Helicobacter pylori can be managed safely by a watch and wait strategy: experience from a large international series. Gut. 2007;56(12):1685-1687. DOI: 10.1136/gut.2006.096420.

[55] Terai S, Iijima K, Kato K, Dairaku N, Suzuki T, Yoshida M, Koike T, Kitagawa Y, Imatani A, Sekine H, Ohara S, Shimosegawa T. Long-term outcomes of gastric mucosa-associated lymphoid tissue lymphomas after Helicobacter pylori eradication therapy. Tohoku J Exp Med. 2008;214(1):79-87. DOI: 10.1620/tjem.214.79.

[56] Andriani A, Zullo A, Di Raimondo F, Patti C, Tedeschi L, Recine U, Caruso L, Bonanno G, Chiarenza A, Lizzani G, Miedico A, Romanelli A, Costa A, Linea C, Marrone C, Mirto S, Mistretta A, Montalbano L, Restivo G, Vinci M, Bibas M, Hassan C, Stella F, Cottone M, Morini S. Clinical and endoscopic presentation of primary gastric lymphoma: a multicentre study. Aliment Pharmacol Ther. 2006;23(6): 721-726. DOI: 10.1111/j.1365-2036.2006.02826.x.

[57] Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol. 1999;17(12):3835-3849. DOI: 10.1200/JCO.1999.17.12.3835.

[58] Doglioni C, Ponzoni M, Ferreri AJ, Savio A; Gruppo Italiano Patologi Apparato Digerente (GIPAD); Societ`a Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP). Gastric lymphoma: the histology report. Dig Liver Dis. 2011;43(suppl 4):S310-S318. DOI: 10.1016/S1590-8658(11)60587-2.

[59] Cavanna L, Pagani R, Seghini P, Zangrandi A, Paties C. High grade B-cell gastric lymphoma with complete pathologic remission after eradication of Helicobacter pylori infection: report of a case and review of the literature. World J Surg Oncol. 2008;6:35. DOI: 10.1186/1477-7819-6-35.

[60] Hiyama T, Haruma K, Kitadai Y, Ito M, Masuda H, Miyamoto M, Tanaka S, Yoshihara M, Sumii K, Shimamoto F, Chayama K. Helicobacter pylori eradication therapy for high-grade mucosa associated lymphoid tissue lymphomas of the stomach with analysis of p53 and K-ras alteration and microsatellite instability. Int J Oncol. 2001;18(6):1207-1212. DOI: 10.3892/ijo.18.6.1207.

[61] Alpen B, Röbbecke J, Wündisch T, Stolte M, Neubauer A. Helicobacter pylori eradication therapy in gastric high grade non Hodgkin's lymphoma (NHL). Ann Hematol. 2001;80 Suppl 3:B106-107. DOI: 10.1007/pl00022770.

[62] Zullo A, Hassan C, Andriani A, Cristofari F, De Francesco V, Ierardi E, Tomao S, Morini S, Vaira D. Eradication therapy for Helicobacter pylori in patients with gastric MALT lymphoma: a pooled data analysis. Am J Gastroenterol. 2009;104(8):1932-1937. quiz 1938. DOI: 10.1038/ajg.2009.314.

[63] Ferreri AJ, Montalban C. Primary diffuse large B-cell lymphoma of the stomach. Crit Rev Oncol Hematol 2007;63(1):65-71. DOI: 10.1016/j.critrevonc.2007.01.003.

[64] Matolcsy A, Nagy M, Kisfaludy N, Kelenyi G. Distinct clonal origin of low-grade MALT-type and high-grade lesions of a multifocal lymphoma. Histopathology 1999;34(1):6-8. DOI: 10.1046/j.1365-2559.1999.00568.x.

[65] Cabras AD, Candidus S, Fend F, Kremer M, Schulz S, Bordi C, Weirich G, Höfler H, Werner M. Biclonality of gastric lymphomas. Lab Invest. 2001;81(7):961-967. DOI: 10.1038/labinvest.3780308.

[66] Cabras AD, Weirich G, Fend F, Nahrig J, Bordi C, Hofler H, Werner M. Oligoclonality of a "composite" gastric diffuse large B cell lymphoma with areas of marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue type. Virchows Arch. 2002;440(2):209-214. DOI: 10.1007/s004280100504.

[67] Kuo SH, Chen LT, Wu MS, Kuo KT, Yeh KH, Doong SL, Yeh PY, Hsu HC, Tzeng YS, Lin CW, Lin JT, Cheng AL. Differential response to *H. pylori* eradication therapy of coexisting diffuse large B-cell lymphoma and MALT lymphoma of stomach-significance of tumour cell clonality and BCL10 expression. J Pathol. 2007;211(3):296-304. DOI: 10.1002/path.2117.

[68] Liu TY, Dei PH, Kuo SH, Lin CW. Early low-grade gastric MALToma rarely transforms into diffuse large cell lymphoma or progresses beyond the stomach and regional lymph nodes. J Formos Med Assoc. 2010;109(6):463-471. DOI: 10.1016/S0929-6646(10)60078-2.

[69] Suzuki T, Matsuo K, Ito H, Hirose K, Wakai K, Saito T, Sato S, Morishima Y, Nakamura S, Ueda R, Tajima K. A past history of gastric ulcers and Helicobacter pylori infection increase the risk of gastric malignant lymphoma. Carcinogenesis. 2006;27(7):1391-1397. DOI: 10.1093/carcin/bgi334.

[70] Chang JS, Kuo SH, Chu PY, Shan YS, Tsai CR, Tsai HJ, Chen LT. The Epidemiology of Gastric Cancers in the Era of Helicobacter pylori Eradication: A Nationwide Cancer Registry-Based Study in Taiwan. Cancer Epidemiol Biomarkers Prev. 2019;28(10):1694-1703. DOI: 10.1158/1055-9965.EPI-19-0355.

[71] de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. Lancet Glob Health. 2020;8(2):e180-e190. DOI: 10.1016/S2214-109X(19)30488-7.

[72] Ishikura N, Usui Y, Ito H, Kasugai Y, Oze I, Kato S, Yatabe Y, Nakamura S, Matsuo K. Helicobacter pylori (HP) infection alone, but not HP-induced atrophic gastritis, increases the risk of gastric lymphoma: a case-control study in Japan. Ann Hematol. 2019;98(8):1981-1987. DOI: 10.1007/s00277-019-03721-y.

[73] Salam I, Durai D, Murphy JK, Sundaram B. Regression of primary high-grade gastric B-cell lymphoma following Helicobacter pylori eradication. Eur J Gastroenterol Hepatol. 2001;13(11):1375-1378. DOI: 10.1097/00042737-200111000-00018.

[74] Sugimoto M, Kajimura M, Sato Y, Hanai H, Kaneko E, Kobayashi H. Regression of primary gastric diffuse large B-cell lymphoma after eradication of Helicobacter pylori. Gastrointest

Endosc. 2001;54(5):643-645. DOI: 10.1067/mge.2001.118647.

[75] Alsolaiman MM, Bakis G, Nazeer T, Mac Dermott RP, Balint JA. Five years of complete remission of gastric diffuse large B cell lymphoma after eradication of Helicobacter pylori infection. Gut. 2003;52(4):507-509. DOI: 10.1136/gut.52.4.507.

[76] Gisbert JP, Calvet X. Review article: common misconceptions in the management of Helicobacter pylori-associated gastric MALT-lymphoma. Aliment Pharmacol Ther. 2011;34(9):1047-62. DOI: 10.1111/j.1365-2036.2011.04839.x.

[77] Haim N, Leviov M, Ben-Arieh Y, Epelbaum R, Freidin N, Reshef R, Ben-Shahar M. Intermediate and high-grade gastric non-Hodgkin's lymphoma: a prospective study of nonsurgical treatment with primary chemotherapy, with or without radiotherapy. Leuk Lymphoma 1995;17(3-4):321-326. DOI: 10.3109/10428199509056838.

[78] Binn M, Ruskone-Fourmestraux A, Lepage E, Haioun C, Delmer A, Aegerter P, Lavergne A, Guettier C, Delchier JC. Surgical resection plus chemotherapy versus chemotherapy alone: comparison of two strategies to treat diffuse large B-cell gastric lymphoma. Ann Oncol. 2003;14(12):1751-1757. DOI: 10.1093/annonc/mdg495.

[79] Aviles A, Nambo MJ, Neri N, Huerta-Guzmán J, Cuadra I, Alvarado I, Castañeda C, Fernández R, González M. The role of surgery in primary gastric lymphoma: results of a controlled clinical trial. Ann Surg. 2004;240(1):44-50. DOI: 10.1097/01.sla.0000129354.31318.f1.

[80] Raderer M, Chott A, Drach J, Montalban C, Dragosics B, Jäger U, Püspök A, Osterreicher C, Zielinski CC. Chemotherapy for management of localised high-grade gastric B-cell lymphoma: how much is necessary? Ann Oncol. 2002; 13(7):1094-1098. DOI: 10.1093/annonc/mdf178.

[81] Liu HT, Hsu C, Chen CL, Chiang IP, Chen LT, Chen YC, Cheng AL. Chemotherapy alone versus surgery followed by chemotherapy for stage I/IIE large-cell lymphoma of the stomach. Am J Hematol. 2000;64(3):175-179. DOI: 10.1002/1096-8652(200007)64:3<175::aid-ajh6>3.0.co;2-7.

[82] Hsu C, Chen CL, Chen LT, Liu HT, Chen YC, Jan CM, Liu CS, Cheng AL. Comparison of MALT and non-MALT primary large cell lymphoma of the stomach: does histologic evidence of MALT affect chemotherapy response? Cancer. 2001;91(1):49-56. DOI: 10.1002/1097-0142(20010101)91:1<49::aid-cncr7>3.0.co;2-y.

[83] Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. Blood 2008 Dec 1;112(12):4384-99. DOI: 10.1182/blood-2008-07-077982.

[84] Liu TY, Chen SU, Kuo SH, Cheng AL, Lin CW. E2A-positive gastric MALT lymphoma has weaker plasmacytoid infiltrates and stronger expression of the memory B-cell-associated miR-223: possible correlation with stage and treatment response. Mod Pathol. 2010;23(11):1507-1517. DOI: 10.1038/modpathol.2010.139.

[85] Miyazaki K. Treatment of Diffuse Large B-Cell Lymphoma. J Clin Exp Hematop. 2016;56(2):79-88. DOI: 10.3960/jslrt.56.79.

[86] Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. Am J Hematol. 2019;94(5):604-616. DOI: 10.1002/ajh.25460.

[87] Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J,

Ott G, Müller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO, Chan WC. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103(1):275-282. DOI: 10.1182/blood-2003-05-1545.

[88] Kuo SH, Chen LT, Lin CW, Yeh KH, Tsai HJ, Lee HW, Wu MS, Hsu PN, Cheng AL. Heterogeneous cell origin of *Helicobater pylori*-dependent high-grade gastric lymphomas (Vol 33, No 15_suppl (May 20 Supplement), 2015: e19520) 2015 ASCO Annual Meeting

[89] Ferreri AJ, Govi S, Ponzoni M. The role of Helicobacter pylori eradication in the treatment of diffuse large B-cell and marginal zone lymphomas of the stomach. Curr Opin Oncol. 2013;25(5): 470-479. DOI: 10.1097/01.cco.0000432 523.24358.15.

[90] Lemma S, Karihtala P, Haapasaari KM, Jantunen E, Soini Y, Bloigu R, Pasanen AK, Turpeenniemi-Hujanen T, Kuittinen O. Biological roles and prognostic values of the epithelial-mesenchymal transition-mediating transcription factors Twist, ZEB1 and Slug in diffuse large B-cell lymphoma. Histopathology. 2013;62(2): 326-333. DOI: 10.1111/his.12000.

[91] Nakahata S, Yamazaki S, Nakauchi H, Morishita K.. Downregulation of ZEB1 and overexpression of Smad7 contribute to resistance to TGF-beta1-mediated growth suppression in adult T-cell leukemia/lymphoma. Oncogene. 2010;29(29):4157-4169. DOI: 10.1038/ onc.2010.172.

[92] Chen JP, Wu MS, Kuo SH, Liao F. IL-22 negatively regulates Helicobacter pylori-induced CCL20 expression in gastric epithelial cells. PLoS One. 2014;9(5):e97350. DOI: 10.1371/journal. pone.0097350. eCollection 2014.

[93] Du MQ. MALT lymphoma: many roads lead to nuclear factor-κb activation. Histopathology. 2011;58(1):26-38. DOI: 10.1111/j.1365-2559.2010.03699.x.

[94] Kuo SH, Wu MS, Yeh KH, Lin CW, Hsu PN, Chen LT, Cheng AL. Novel Insights of Lymphomagenesis of Helicobacter pylori-Dependent Gastric Mucosa-Associated Lymphoid Tissue Lymphoma. Cancers (Basel). 2019; 11(4):547. DOI: 10.3390/cancers 11040547.PMID: 30999581

[95] Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, Bukanov NO, Drazek ES, Roe BA, Berg DE. Analyses of the cag pathogenicity island of Helicobacter pylori. Mol Microbiol. 1998;28(1):37-53. DOI: 10.1046/j.1365-2958.1998.00770.x.

[96] Azuma T, Yamakawa A, Yamazaki S, Fukuta K, Ohtani M, Ito Y, Dojo M, Yamazaki Y, Kuriyama M. Correlation between variation of the 3' region of the cagA gene in Helicobacter pylori and disease outcome in Japan. J Infect Dis. 2002 Dec 1;186(11):1621-1630. DOI: 10.1086/345374.

[97] Hatakeyama M. Oncogenic mechanisms of the Helicobacter pylori CagA protein. Nat Rev Cancer. 2004; 4(9):688-694. DOI: 10.1038/nrc1433.

[98] Murata-Kamiya N, Kikuchi K, Hayashi T, Higashi H, Hatakeyama M. Helicobacter pylori exploits host membrane phosphatidylserine for delivery, localization, and pathophysiological action of the CagA oncoprotein. Cell Host Microbe. 2010;7(5):399-411. DOI: 10.1016/j. chom.2010.04.005.

[99] Hayashi T, Senda M, Morohashi H, Higashi H, Horio M, Kashiba Y, Nagase L, Sasaya D, Shimizu T, Venugopalan N, Kumeta H, Noda NN, Inagaki F, Senda T, Hatakeyama M. Tertiary structure-function analysis reveals the pathogenic signaling

potentiation mechanism of Helicobacter pylori oncogenic effector CagA. Cell Host Microbe. 2012;12(1):20-33. DOI: 10.1016/j.chom.2012.05.010.

[100] Stein M, Ruggiero P, Rappuoli R, Bagnoli F. Helicobacter pylori CagA: from pathogenic mechanisms to its use as an anti-cancer vaccine. Front Immunol. 2013;4:328. DOI: 10.3389/fimmu.2013.00328.

[101] Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ, Covacci A. c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol. 2002;43(4):971-980. DOI: 10.1046/j.1365-2958.2002.02781.x.

[102] Tammer I, Brandt S, Hartig R, König W, Backert S. Activation of Abl by Helicobacter pylori: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology. 2007;132(4):1309-1319. DOI: 10.1053/j.gastro.2007.01.050.

[103] Ghoshal UC, Tiwari S, Dhingra S, Pandey R, Ghoshal U, Tripathi S, Singh H, Gupta VK, Nagpal AK, Naik S, Ayyagari A. Frequency of Helicobacter pylori and CagA antibody in patients with gastric neoplasms and controls: the Indian enigma. Dig Dis Sci. 2008;53(5):1215-1222. DOI: 10.1007/s10620-008-0229-7.

[104] Achyut BR, Moorchung N, Srivastava AN, Gupta NK, Mittal B. Risk of lymphoid follicle development in patients with chronic antral gastritis: role of endoscopic features, histopathological parameters, CagA status and interleukin-1 gene polymorphisms. Inflamm Res. 2008;57(2):51-56. DOI: 10.1007/s00011-007-7033-2.

[105] Floch P, Mégraud F, Lehours P. Helicobacter pylori Strains and Gastric MALT Lymphoma. Toxins (Basel). 2017;9(4):132. DOI: 10.3390/toxins9040132.

[106] Umehara S, Higashi H, Ohnishi N, Asaka M, Hatakeyama M. Effects of Helicobacter pylori CagA protein on the growth and survival of B lymphocytes, the origin of MALT lymphoma. Oncogene 2003;22(51):8337-8342. DOI: 10.1038/sj.onc.1207028.

[107] Zhu Y, Wang C, Huang J, Ge Z, Dong Q, Zhong X, Su Y, Zheng S.. The Helicobacter pylori virulence factor CagA promotes Erk1/2-mediated Bad phosphorylation in lymphocytes: a mechanism of CagA-inhibited lymphocyte apoptosis. Cell Microbiol 2007;9(4):952-961. DOI: 10.1111/j.1462-5822.2006.00843.x.

[108] Qu CK, Nguyen S, Chen J, Feng GS. Requirement of Shp-2 tyrosine phosphatase in lymphoid and hematopoietic cell development. Blood. 2001;97(4):911-914. DOI: 10.1182/blood.v97.4.911.

[109] Niogret C, Birchmeier W, Guarda G. SHP-2 in Lymphocytes' Cytokine and Inhibitory Receptor Signaling. Front Immunol. 2019;10:2468. DOI: 10.3389/fimmu.2019.02468. eCollection 2019.

[110] Ben Younes K, Doghri R, Mrad K, Bedhiafi W, Benammar-Elgaaied A, Sola B, Ben Aissa-Fennira F. PTEN Loss and Cyclin A2 Upregulation Define a PI3K/AKT Pathway Activation in Helicobacter pylori-induced MALT and DLBCL Gastric Lymphoma With Features of MALT. Appl Immunohistochem Mol Morphol. 2020; 29(1):56-61. DOI: 10.1097/PAI.0000000000000839.

[111] Sumida T, Kitadai Y, Hiyama T, Shinagawa K, Tanaka M, Kodama M, Masuda H, Ito M, Tanaka S, Yoshihara M, Chayama K. Antibodies to Helicobacter pylori and CagA protein are associated with the response to antibacterial therapy in patients with *H. pylori*-positive API2-MALT1-negative gastric MALT lymphoma. Cancer Sci. 2009;100(6):1075-1081. DOI: 10.1111/j.1349-7006.2009.01139.x.

[112] Peng H, Ranaldi R, Diss TC, Isaacson PG, Bearzi I, Pan L. High frequency of CagA+ Helicobacter pylori infection in high-grade gastric MALT B-cell lymphomas. J Pathol. 1998; 185(4):409-412. DOI: 10.1002/(SICI)1096-9896(199808)185:4<409::AID-PATH121>3.0.CO;2-T.

[113] Delchier JC, Lamarque D, Levy M, Tkoub EM, Copie-Bergman C, Deforges L, Chaumette MT, Haioun C. Helicobacter pylori and gastric lymphoma: high seroprevalence of CagA in diffuse large B-cell lymphoma but not in low-grade lymphoma of mucosa-associated lymphoid tissue type. Am J Gastroenterol. 2001;96(8):2324-2328. DOI: 10.1111/j.1572-0241.2001.04036.x

[114] Kuo SH, Chen LT, Chen CL, Doong SL, Yeh KH, Wu MS, Mao TL, Hsu HC, Wang HP, Lin JT, Cheng AL. Expression of CD86 and increased infiltration of NK cells are associated with Helicobacter pylori-dependent state of early stage high-grade gastric MALT lymphoma. World J Gastroenterol. 2005;11(28):4357-4362. DOI: 10.3748/wjg.v11.i28.4357.

[115] de Jong D, Vyth-Dreese F, Dellemijn T, Verra N, Ruskoné-Fourmestraux A, Lavergne-Slove A, Hart G, Boot H. Histological and immunological parameters to predict treatment outcome of Helicobacter pylori eradication in low-grade gastric MALT lymphoma. J Pathol. 2001;193(3):318-324. DOI: 10.1002/1096-9896(2000)9999:9999<::AID-PATH811>3.0.CO;2-Z.

[116] Capelle LG, de Vries AC, Looman CW, Casparie MK, Boot H, Meijer GA, Kuipers EJ. Gastric MALT lymphoma: epidemiology and high adenocarcinoma risk in a nation-wide study. Eur J Cancer. 2008;44(16):2470-2476. DOI: 10.1016/j.ejca.2008.07.005.

[117] Luminari S, Cesaretti M, Marcheselli L, Rashid I, Madrigali S, Maiorana A, Federico M. Decreasing incidence of gastric MALT lymphomas in the era of anti-Helicobacter pylori interventions: results from a population-based study on extranodal marginal zone lymphomas. Ann Oncol. 2010;21(4):855-859. DOI: 10.1093/annonc/mdp402.

[118] Khalil MO, Morton LM, Devesa SS, Check DP, Curtis RE, Weisenburger DD, Dores GM. Incidence of marginal zone lymphoma in the United States, 2001-2009 with a focus on primary anatomic site. Br J Haematol. 2014;165(1):67-77. DOI: 10.1111/bjh.12730. Epub 2014 Jan 12

[119] Huh J. Epidemiologic overview of malignant lymphoma. Korean J Hematol. 2012;47(2):92-104. DOI: 10.5045/kjh.2012.47.2.92.

[120] Yang JC, Wang TH, Wang HJ, Kuo CH, Wang JT, Wang WC. Genetic analysis of the cytotoxin-associated gene and the vacuolating toxin gene in Helicobacter pylori strains isolated from Taiwanese patients. Am J Gastroenterol. 1997;92(8):1316-1321. PMID: 9260797

[121] Chang WL, Yeh YC, Sheu BS. The impacts of *H. pylori* virulence factors on the development of gastroduodenal diseases. J Biomed Sci. 2018;25(1):68. DOI: 10.1186/s12929-018-0466-9.

[122] Rezaeifar A, Eskandari-Nasab E, Moghadampour M, Kharazi-Nejad E, Hasani SS, Asadi-Saghandi A, Hadadi-Fishani M, Sepanjnia A, Sadeghi-Kalani B.. The association of interleukin-18 promoter polymorphisms and serum levels with duodenal ulcer, and their correlations with bacterial CagA and VacA virulence factors. Scand J Infect Dis. 2013;45(8):584-592. DOI: 10.3109/00365548.2013.794301.

[123] Shiota S, Suzuki R, Yamaoka Y. The significance of virulence factors in Helicobacter pylori. J Dig Dis. 2013; 14(7):341-349. DOI: 10.1111/1751-2980.12054.

[124] Liou JM, Chang CY, Chen MJ, Chen CC, Fang YJ, Lee JY, Wu JY, Luo JC, Liou TC, Chang WH, Tseng CH, Wu CY, Yang TH, Chang CC, Wang HP, Sheu BS, Lin JT, Bair MJ, Wu MS; Taiwan Gastrointestinal Disease and Helicobacter Consortium. The primary resistance of Helicobacter pylori in Taiwan after the national policy to restrict antibiotic consumption and its relation to virulence factors-a nationwide study. PLoS One. 2015; 10(5):e0124199. DOI: 10.1371/journal.pone.0124199.

[125] den Hollander WJ, Holster IL, van Gilst B, van Vuuren AJ, Jaddoe VW, Hofman A, Perez-Perez GI, Kuipers EJ, Moll HA, Blaser MJ. Intergenerational reduction in Helicobacter pylori prevalence is similar between different ethnic groups living in a Western city. Gut. 2015;64(8):1200-1208. DOI: 10.1136/gutjnl-2014-307689.

[126] Hatakeyama M. Structure and function of Helicobacter pylori CagA, the first-identified bacterial protein involved in human cancer. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(4):196-219. DOI: 10.2183/pjab.93.013..

[127] Argent RH, Hale JL, El-Omar EM, Atherton JC. Differences in Helicobacter pylori CagA tyrosine phosphorylation motif patterns between western and East Asian strains, and influences on interleukin-8 secretion. J Med Microbiol. 2008;57(Pt 9):1062-1067. DOI: 10.1099/jmm.0.2008/001818-0.

[128] Lai YP, Yang JC, Lin TZ, Wang JT, Lin JT. CagA tyrosine phosphorylation in gastric epithelial cells caused by Helicobacter pylori in patients with gastric adenocarcinoma. Helicobacter. 2003;8(3):235-243. DOI: 10.1046/j.1523-5378.2003.00148.x.

[129] Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci U S A. 2002;99(22):14428-14433. DOI: 10.1073/pnas.222375399.

[130] Li Q, Liu J, Gong Y, Yuan Y. Association of CagA EPIYA-D or EPIYA-C phosphorylation sites with peptic ulcer and gastric cancer risks: a metaanalysis. Medicine (Baltimore). 2017;96(17):e6620. DOI: 10.1097/MD.0000000000006620.

[131] Chuang CH, Yang HB, Sheu SM, Hung KH, Wu JJ, Cheng HC, Chang WL, Sheu BS. Helicobacter pylori with stronger intensity of CagA phosphorylation lead to an increased risk of gastric intestinal metaplasia and cancer. BMC Microbiol. 2011;11:121. DOI: 10.1186/1471-2180-11-121.

[132] Starostik P, Patzner J, Greiner A, Schwarz S, Kalla J, Ott G, Müller-Hermelink HK. Gastric marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. Blood. 2002;99(1):3-9. DOI: 10.1182/blood.v99.1.3.

[133] Barth TF, Barth CA, Kestler HA, Michl P, Weniger MA, Buchholz M, Möller P, Gress T. Transcriptional profiling suggests that secondary and primary large B-cell lymphomas of the gastrointestinal (GI) tract are blastic variants of GI marginal zone lymphoma. J Pathol. 2007;211(3):305-313. DOI: 10.1002/path.2096.

[134] Yeh KH, Kuo SH, Chen LT, Mao TL, Doong SL, Wu MS, Hsu HC, Tzeng YS, Chen CL, Lin JT, Cheng AL. Nuclear expression of BCL10 or nuclear factor kappa B helps predict Helicobacter pylori-independent status of low-grade gastric mucosa-associated lymphoid tissue lymphomas with or without t(11;18)(q21;q21). Blood. 2005;106(3):1037-1041. DOI: 10.1182/blood-2005-01-0004.

[135] Kuo SH, Chen LT, Yeh KH, Wu MS, Hsu CH, Yeh PY, Mao TL, Chen CL, Doong SL, Lin JT, Cheng AL. Nuclear expression of BCL10 or nuclear factor kappa B predicts Helicobacter pylori-independent status of early-stage, high-grade gastric mucosa-associated lymphoid tissue lymphomas. J Clin Oncol. 2004;22(17):3491-3497. DOI: 10.1200/JCO.2004.10.087.

[136] Kuo SH, Yeh PY, Chen LT, Wu MS, Lin CW, Yeh KH, Tzeng YS, Chen JY, Hsu PN, Lin JT, Cheng AL. Over-expression of B cell-activating factor of TNF family (BAFF) is associated with Helicobacter pylori-independent growth of gastric diffuse large B-cell lymphoma with histologic evidence of MALT lymphoma. Blood. 2008;112(7):2927-2934. DOI: 10.1182/blood-2008-02-137513.

[137] Du MQ. MALT lymphoma: Genetic abnormalities, immunological stimulation and molecular mechanism. Best Pract Res Clin Haematol. 2017; 30(1-2):13-23. DOI: 10.1016/j.beha.2016.09.002.

[138] Simala-Grant JL, Lam E, Keelan M, Taylor DE. Characterization of the DNA adenine 5'-GATC-3' methylase HpyIIIM from Helicobacter pylori. Curr Microbiol. 2004.;49(1):47-54. DOI: 10.1007/s00284-004-4244-4.

[139] Min KO, Seo EJ, Kwon HJ, Lee EJ, Kim WI, Kang CS, Kim KM. Methylation of p16(INK4A) and p57(KIP2) are involved in the development and progression of gastric MALT lymphomas. Mod Pathol. 2006;19(1):141-148. DOI: 10.1038/modpathol.3800505.

[140] Kondo T, Oka T, Sato H, Shinnou Y, Washio K, Takano M, Morito T, Takata K, Ohara N, Ouchida M, Shimizu K, Yoshino T. Accumulation of aberrant CpG hypermethylation by Helicobacter pylori infection promotes development and progression of gastric MALT lymphoma. Int J Oncol. 2009;35(3):547-557. DOI: 10.3892/ijo_00000366.

[141] Rad R, Brenner L, Bauer S, Schwendy S, Layland L, da Costa CP, Reindl W, Dossumbekova A, Friedrich M, Saur D, Wagner H, Schmid RM, Prinz C. CD25+/Foxp3+ T cells regulate gastric inflammation and Helicobacter pylori colonization in vivo. Gastroenterology. 2006;131(2):525-537. DOI: 10.1053/j.gastro.2006.05.001.

[142] Yang ZZ, Novak AJ, Stenson MJ, Witzig TE, Ansell SM. Intratumoral CD4+CD25+ regulatory T-cell-mediated suppression of infiltrating CD4+ T cells in B-cell non-Hodgkin lymphoma. Blood. 2006;107(9):3639-3646. DOI: 10.1182/blood-2005-08-3376.

[143] Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. Nat Immunol. 2005;6(4):353-360. DOI: 10.1038/ni1181.

[144] Liao F, Hsu YC, Kuo SH, Yang YC, Chen JP, Hsu PN, Lin CW, Chen LT, Cheng AL, Fann CS, Lin JT, Wu MS. Genetic polymorphisms and tissue expression of interleukin-22 associated with risk and therapeutic response of gastric mucosa-associated lymphoid tissue lymphoma. Blood Cancer J. 2014;4(10):eXX. DOI: 10.1038/bcj.2014.70.

[145] Craig VJ, Cogliatti SB, Arnold I, Gerke C, Balandat JE, Wündisch T, Müller A. B-cell receptor signaling and CD40 ligand-independent T cell help cooperate in Helicobacter-induced MALT lymphomagenesis. Leukemia. 2010;24(6):1186-1196. DOI: 10.1038/leu.2010.76.

[146] García M, Bellosillo B, Sánchez-González B, García-Payarols F, Seoane A, Ferrer AM, Gimeno E, Barranco LE, Torner A, Solé F, Besses C, Serrano S, Salar A. Study of regulatory T-cells in patients with gastric malt lymphoma: influence on treatment

response and outcome. PLoS One. 2012;7(12):e51681. DOI: 10.1371/journal.pone.0051681.

[147] Iwaya Y, Kobayashi M, Momose M, Hiraoka N, Sakai Y, Akamatsu T, Tanaka E, Ohtani H, Fukuda M, Nakayama J. High levels of FOXP3+ regulatory T cells in gastric MALT lymphoma predict responsiveness to Helicobacter pylori eradication. Helicobacter. 2013 Oct;18(5):356-62. DOI: 10.1111/hel.12051.

Section 2

Diseases Associated with *H. pylori* Infection and Their New Approaches

Chapter 3

Helicobacter pylori Infection and Endothelial Dysfunction

Xiujuan Xia, Linfang Zhang, Canxia Xu, Hao Hong and Zhenguo Liu

Abstract

Endothelial cells play a critical role in maintaining the integrity of vascular structure and function. Endothelial dysfunction is closely associated with the development and progression of cardiovascular diseases (CVDs) like hypertension (HTN) and atherosclerosis. Gut microorganisms significantly contribute to atherosclerosis and related CVDs. *Helicobacter pylori* (*H. pylori*) colonizes in human gastric epithelium in a significant portion of general population in the world. Patients with *H. pylori* infection have significantly increased risk for CVDs including atherosclerosis, HTN, coronary heart disease, and cerebrovascular disease especially in younger patients (< 65 years old). *H. pylori* infection significantly impairs vascular endothelial function through multiple mechanisms including increased reactive oxygen species production and oxidative stress, inflammation, decreased nitric oxide formation, modification of the expression of cytokines and microRNAs, abnormalities of lipid and glucose metabolisms, and exosomes-mediated pathways. Endothelial dysfunction associated with *H. pylori* infection is reversible in both animal model and human subjects. Accumulating data suggests that *H. pylori* infection is an important risk factor for endothelial dysfunction and CVDs especially in young patients. Screening young male population for *H. pylori* infection and treating accordingly could be an effective approach for early prevention of CVDs especially premature atherosclerosis associated with *H. pylori* infection.

Keywords: *Helicobacter pylori*, atherosclerosis, endothelial dysfunction, cardiovascular disease, exosomes

1. Introduction

Atherosclerosis is among the principal contributors to cardiovascular diseases (CVDs) especially coronary artery diseases (CAD) and stroke [1]. Despite in-depth understanding of the traditional cardiovascular risk factors including diabetes mellitus (DM), hypertension (HTN), hyperlipidemia, smoking, and obesity, and effective control of these known risk factors, CVDs remain the leading cause of mortality and morbidity in developed countries including the US [2, 3]. It is worrisome that the decline of all cardiovascular mortality rate has been slowing down since 2011 [4]. It is very problematic that patients presenting with ST elevation myocardial infarction over the past 20 years are getting younger [5], and the total number of death from CAD and stroke is projected to increase by

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about 18% by 2030 [6]. Clearly, there are other risk factors that have not been defined, and yet contribute significantly to the development and progression of atherosclerosis and related CAD and stroke.

Gut microorganisms significantly contribute to the development of atherosclerosis and related CVDs [7–9]. The microaerophilic Gram-negative bacterium *Helicobacter pylori* (*H. pylori*) colonizes in the epithelium of human stomach in a significant portion of general population in the world with the infection rate ranging from 30% - 50% in developed countries up to 80% in developing countries especially in Asia [10, 11]. Most patients with *H. pylori* infection have no symptoms clinically [12]. However, *H. pylori* infection could cause progressive damage to gastric mucosa, and is closely associated with a number of important diseases including (but not limited to) chronic gastritis, peptic ulcers, and gastric cancer [13]. Recent data indicate that *H. pylori* infection could also contribute to some important extra gastrointestinal diseases such as hematological diseases (especially idiopathic thrombocytopenia), neurological abnormalities, dermatological pathologies, and autoimmune disorders like inflammatory bowel diseases, chronic liver disease, and DM [14–22]. Thus, *H. pylori* infection is a significant cause of morbidity and mortality in humans. In 2005, Dr. Barry Marshall and Dr. Robin Warren were awarded the Nobel Prize in Physiology for their pioneering work on *H. pylori*.

Growing evidences indicate that *H. pylori* infection could also contribute to CVDs. A recent meta-analysis with a large population showed that *H. pylori* infection increased the risk of adverse cardiovascular events by 51%, mostly due to myocardial infarction and cerebrovascular disease [23]. Data also suggests that *H. pylori* infection increases the risk of coronary heart diseases (CHD) and related events predominantly in a patient's early life [24], and is positively associated with HTN [25, 26]. In this chapter, efforts will be focused on: 1) brief overview on the association of *H. pylori* infection and CVDs; 2) relationship between *H. pylori* infection and atherosclerosis; 3) *H. pylori* infection and endothelial dysfunction; 4) role of exosomes in mediating the effect of *H. pylori* infection on endothelial function, and 5) significance and clinical implications.

2. Brief overview on *H. pylori* infection and cardiovascular diseases

The role of *H. pylori* infection in the development and progression of CVDs has been established for the past two decades. Early epidemiology studies have suggested an association between *H. pylori* infection and increased prevalence of atherosclerosis [27] An early study that included 96 patients with CAD and 96 patients without CAD has revealed the followings: 1) there is a significant link between CAD and infection with *H. pylori*, especially the one expressing the virulence factor cytotoxin-associated gene A (CagA) proteins, 2) patients infected with CagA-positive *H. pylori* show significantly greater coronary artery lumen loss and arterial re-stenosis after percutaneous transluminal coronary angioplasty (PTCA) with stent implantation, 3) *H. pylori* eradication significantly attenuates the reduction in coronary artery lumen in CAD patients after PTCA [28]. Diabetic subjects with *H. pylori* infection have more severe peripheral arterial stiffness compared with those without *H. pylori* infection, and a higher cardiovascular risk score and 10-year cardiovascular risk stratification [29, 30]. After adjusting for traditional CVD risk factors, *H. pylori* infection is found to be the only independent predictor of incident carotid plaque with the multivariate odds ratio (OR) of 2.3, and incident acute stroke (with multivariate OR of 3.6) [31]. *H. pylori* infection was positively associated with the prevalence of HTN among Chinese adults [25, 26].

Recently, a study using cardio-ankle vascular index reported that subjects with positive *H. pylori* serology were significantly associated with increased arterial stiffness [32].

A recent study, using a large database with a total of 208,196 patients diagnosed with peptic ulcer diseases, compared the cardiovascular outcome for subjects with and without *H. pylori* eradication. A total of 3,713 patients with *H. pylori* eradication treatment within 365 days of the index date were included in the study with randomly selected same number of patients using propensity scores as cohort of non-eradication patients for comparison. The study demonstrated that there was a significant decrease in composite end-points for CHD and death in the early eradication group. The cumulative CHD rate was significantly lower in younger patients (< 65 years old) with *H. pylori* eradication therapy started <1 year of the index date compared to those patients without eradication at all. Interestingly, the study also showed that eradication treatment did not appear to have a significant effect in older patients (≥ 65 years old). Multivariate analysis shows that HTN and renal diseases are risk factors for CHD in patients without eradication, while younger age (< 65 years old) was a protective factor for CHD for the patients with *H. pylori* therapy [33]. Thus, there is little doubt that *H. pylori* infection is indeed associated with significant CVDs including atherosclerosis, HTN, CHD, cerebrovascular disease, and peripheral arterial diseases, as well as their clinical outcomes especially in younger patients (< 65 years old).

3. *Helicobacter pylori* infection and carotid atherosclerosis

The relationship between *H. pylori* infection and atherosclerosis has been inconsistent and sometimes controversial with the findings from a strong positive association, and a mild association, to no association [27, 34–36]. Compared to those without *H. pylori* infection, patients with *H. pylori* infection, especially with CagA+ *H. pylori*, have much higher incidence of atherosclerosis (29% vs. 63%) [37], and acute ischemic stroke (45% vs. 77%) [17]. The prevalence of serologically confirmed *H. pylori* infection was significantly higher in patients with angiographically documented CAD, supporting a positive association between *H. pylori* infection and CAD [38–40]. However, a meta-analysis with inclusion of 18 epidemiological studies and over 10,000 patients showed no positive relationship between *H. pylori* infection and CAD [41]. In contrast, the data supporting a positive relationship between *H. pylori* infection and carotid atherosclerosis with increased carotid intima-media thickness (CIMT) were consistent in most of the studies with patients [17, 42–45]. The reason(s) for the significant difference in consistency on the relationship between *H. pylori* infection and CAD vs. carotid atherosclerosis is unclear. It could be very likely due to the different imaging modalities used for the detections of CAD (using coronary angiogram) and carotid atherosclerosis (using carotid ultrasound). Carotid ultrasound could easily detect early atherosclerotic lesions without significant loss of vascular lumen, while coronary angiogram could not. In a recent study, the investigators used cardiac multidetector computed tomography to identify subclinical coronary atherosclerotic lesions in healthy subjects without clinical CVD, and found that patients with current *H. pylori* infection was 3-fold more likely to have subclinical and yet significant coronary atherosclerosis than the patients without *H. pylori* infection [15]. One of the major features of atherosclerosis is thickening of the intima-media in the arteries that could not be detected with angiogram. Carotid artery is considered an early site of atherosclerosis, and superficially located. Thus, carotid ultrasound examination is an ideal and sensitive non-invasive image modality to diagnose and monitor the

progression of atherosclerosis [46], although it has not been widely used clinically for atherosclerosis screening at this point.

Recently, a large patient database of 17,613 adult patients with carotid ultrasound examination and a ^{13}C-urea breath *H. pylori* test was analyzed [47]. Based on the study designs, the patients were divided into two groups: a cross-sectional study for single measurement group, and a retrospective cohort study for the patients with follow up measurements up to 5 years. Patients were excluded from the study if any of the following conditions was present: 1) history of *H. pylori* eradication, 2) use of any antibiotics, proton pump inhibitors, or H_2-receptor blockers 3 months before the tests, 3) age < 20 or > 70 years, 4) connective tissue diseases or immunological diseases, 5) mental disorders, 6) asthma or COPD, 7) hematological disorders, 8) thyroid diseases, 9) malignancies, 10) recent (within 3 months) or chronic infection (over 3 months) except *H. pylori* infection, 11) congestive heart failure, and 12) abnormal liver or kidney function. Patients with CAD were not excluded from the study since carotid atherosclerosis and CAD share similar risk factors, and it was felt that exclusion of the subjects with CAD could remove the subgroup population who might be at increased risk for carotid atherosclerosis with *H. pylori* infection, leading to potential selection bias. Of note, the patients with CAD accounted only for about 3% of all participating subjects for this study, and there was no stroke in the patients in the database.

The data showed that, after adjusting for age, sex, body mass index, lipid profile, HTN, DM, and smoking, *H. pylori* infection was an independent risk factor for carotid atherosclerosis in male patients ≤50 years, but not in older males or females (OR of 1.229, p = 0.009). The data also demonstrated that *H. pylori* infection was associated with a significant increase in CIMT for males, not females. To further evaluate the relationship between *H. pylori* infection and carotid atherosclerosis, the investigators studied the 5 years follow up data on additional 2,042 subjects with and without *H. pylori* infection for progression on the prevalence of carotid atherosclerosis with annual carotid ultrasound examination and a ^{13}C-urea breath test. The data showed that for males with age of <50 years, there was a 22.5% increase in the prevalence of carotid atherosclerosis in the subjects with *H. pylori* infection compared with the ones without *H. pylori* infection. These data demonstrated that *H. pylori* infection selectively increased the risk for carotid atherosclerosis in young males under 50 years old [47]. However, how *H. pylori* infection could lead to atherosclerosis, and why only in young males is unknown.

4. *H. pylori* infection and endothelial dysfunction

4.1 *H. pylori* infection and endothelial dysfunction in patients

Endothelial cells play a critical role in maintaining the integrity of vascular structure and function. Endothelial dysfunction is an important contributing factor to the pathogenesis of CVDs including HTN and atherosclerosis [4]. Early studies with small patient samples suggested that there was no clear association between chronic infections, including infection with Chlamydia pneumoniae, cytomegalovirus, Epstein–Barr virus, and *H. pylori*, and decreased endothelial function [48]. A small study with a total of 53 pediatric patients using Doppler ultrasonography of the brachial artery showed that percent ratio of the change in systolic diameters during hyperemic phase to the basal diameter (endothelium-dependent) was not significantly different between *H. pylori*-negative and -positive groups in pediatric population [49].

However, accumulating data clearly supports the concept that *H. pylori* infection could lead to significant endothelial dysfunction in patients. Using high-frequency ultrasonographic imaging of the brachial artery, it was found that endothelium-dependent flow-mediated vasodilation (FMD) was significantly lower in the subjects with seropositive antibodies to *H. pylori* than in the ones with seronegative antibodies to *H. pylori*, while endothelium-independent nitroglycerin-induced vasodilation was similar in both groups [50]. Similarly, another study with patients with chronic gastritis associated with *H. pylori* infection demonstrated that the level of FMD in patients with positive *H. pylori* infection was significantly lower than those with negative *H. pylori* infection and the healthy control group [51]. Studies also showed that the levels of C-reactive protein and soluble intercellular adhesion molecule-1 (ICAM-1) were significantly higher in subjects with seropositive antibodies to *H. pylori* than in those with seronegative antibodies to *H. pylori* [50]. The levels of endothelial dysfunction biomarkers, including endothelin-1 (ET-1), E-selectin, and ICAM-1, were found to be significantly higher in *H. pylori* (+) patients than in *H. pylori* (−) subjects [52].

One of the important questions is whether endothelial dysfunction associated with *H. pylori* infection is reversible. In a study in 2011, vascular function measurements (ankle brachial index and flow-mediated diameter percent change) were made in patients with *H. pylori* infection at the time of study enrollment and 3 months afterwards with *H. pylori* eradication. Subjects with *H. pylori* infection were treated with standard triple antibiotics therapy. It was found that *H. pylori*-positive subjects had severe endothelial dysfunction that improved significantly after *H. pylori* eradication with triple antibiotics. Subjects without *H. pylori* infection also had endothelial dysfunction, however, that was not improved after treatment with triple antibiotics. These data suggests that endothelial dysfunction in patients with *H. pylori* infection appear to be reversible [53].

In a recent study, the investigators carefully selected 18 young patients (both male and female) with *H. pylori* infection without any known risk factors for endothelial dysfunction to evaluate endothelium-dependent FMD of the brachial artery with ultrasound. A group of 13 age- and sex-matched young healthy volunteers served as the controls. The diagnosis of *H. pylori* infection was confirmed with gastric endoscopic biopsy and ^{13}C urea breath test for each patient. No other confounding variables except the conditions listed in the exclusion criteria were considered for subject selection. Young patients were recruited to minimize the risk factors for endothelial dysfunction. Patients were excluded from the study if any of the following conditions was present: 1) history of *H. pylori* eradication, 2) use of any medications including antibiotics, proton pump inhibitors, or H_2-receptor blockers 3 months before the study, 3) age < 18 or > 35 years, 4) connective tissue diseases or immunological diseases, 5) mental disorders, 6) asthma or COPD, 7) hematological disorders, 8) thyroid diseases, 9) malignancies, 10) recent (within 3 months) or chronic infection except *H. pylori* infection, 11) congestive heart failure, 12) abnormal renal or liver function; 13) congenital heart diseases, 14) hypertension, 15) smoking, 16) diabetes mellitus, 17) lipid abnormalities, 18) stroke, 19) obesity, 20) sedentary life style, 21) alcohol use, 22) any use of energy drinks or coffee or tea within 48 hours, and 23) unresponsive to anti-*H. pylori* therapy. After fasting for 8 to 12 hours, brachial artery FMD was evaluated for patients and control subjects, and presented as percent change in post-ischemia diameter over baseline. The data showed that patients with *H. pylori* infection exhibited a significant reduction in endothelium-dependent vasodilatation compared with the controls (**Figure 1A**). When patients with *H. pylori* infection were treated with BIS-based quadruple oral anti-*H. pylori* therapy (100 mg furazolidone, 100 mg doxycycline, 5 mg ilaprazole, and 220 mg colloidal bismuth tartrate, twice a day for 2 weeks) [54], their endothelium-dependent FMD of the brachial artery was effectively restored (**Figure 1B**) [55]. The effectiveness of *H. pylori* eradication

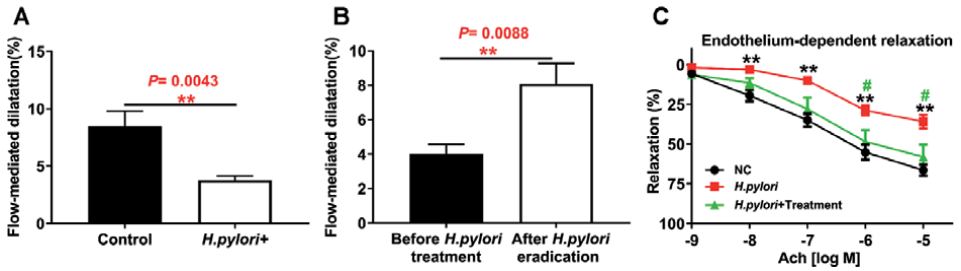

Figure 1.
H. pylori *infection significantly impairs endothelium-dependent flow-mediated dilatation (FMD) in human subjects and endothelium-dependent vascular relaxation in mice. Patients with* H. pylori *infection and healthy control subjects were evaluated for endothelium-dependent flow-mediated dilatation (***FMD***) of the brachial artery with ultrasound. The diagnosis of* H. pylori *infection was confirmed with gastric endoscopic biopsy and* ^{13}C *urea breath test for each patient. Patients with* H. pylori *infection (n = 18 patients) displayed a significant reduction in their endothelium-dependent FMD compared with the controls (n = 13 subjects) (A). Eradication of* H. pylori *infection with anti-*H. pylori *therapy effectively restored the endothelium-dependent FMD in patients with* H. pylori *infection (n = 10 patients with anti-*H. pylori *therapy) (B). Mice infected with CagA*$^+$ H. pylori *for 1 week significantly decreased acetylcholine (***Ach***)-induced endothelium-dependent relaxation of thoracic aorta without change in nitroglycerin (NTG)-induced endothelium-independent vasorelaxation (***data not shown***) after sub-maximal contraction with phenylephrine (***PE***)* $(10^{-6}$ *M). The impaired ach-induced endothelium-dependent vasorelaxation persisted for as long as the infection was present for at least 24 weeks (***C***) without change in NTG-induced endothelium-independent vasorelaxation (***data not shown***). Eradication of* H. pylori *infection with anti-*H. pylori *therapy effectively restored ach-induced endothelium-dependent vasorelaxation in mice with 12 weeks of chronic CagA*$^+$ H. pylori *infection,* $^{\#}p < 0.05$ *(compared to CagA*$^+$ H. pylori *+ treatment mice);* $^{**}p < 0.01$ *(compared to NC mice) (C), n = 8 mice for each group.* **NC***: Normal control;* **Ach***: Acetylcholine;* **NTG***: Nitroglycerin. Data were presented as mean ± SE. [adopted and modified from (55) with permission].*

with antimicrobial therapies was confirmed using ^{13}C urea breath test for the study patients. These data confirms that endothelial dysfunction in patients with *H. pylori* is indeed reversible (very likely within 1 year of infection).

4.2 *H. pylori* infection and endothelial dysfunction in animal models

In the same recent study, the investigators used specific-pathogen-free male C57BL/6 mice to establish a mouse *H. pylori* infection model to determine if impaired endothelial function in human subjects with *H. pylori* infection could be re-produced in animal model using the *H. pylori* bacteria isolated from patients. Since the vast majority (>90%) of *H. pylori* infection patients in East or Southeast Asian countries are infected with CagA$^+$ *H. pylori* [56, 57], and CagA is considered to be involved in the extragastric diseases associated with *H. pylori* infection [44, 58–60], CagA$^+$ *H. pylori* bacteria isolated from gastric ulcer patients were prepared, characterized, and used for the animal experiments with phosphate buffer solution (PBS) as control. After fasting overnight, mice were infected with *H. pylori* inoculum in PBS by intragastric gavage once per day for 3 days to achieve 100% infection rate. Successful infection with CagA$^+$ *H. pylori* in mice was confirmed with both positive Rapid Urease Test (RUT) and Giemsa staining as described [61]. A 100% infection rate was achieved in C57BL/6 mice with this method. Control mice received the same volume of PBS by intragastric gavage.

Thoracic aorta was collected to evaluate endothelium-dependent relaxation to acetylcholine (Ach) and endothelium-independent relaxation to nitroglycerin (NTG) at week 1, 8, 12, and 24 after *H. pylori* infection to determine if there was a significant difference in endothelial dysfunction after acute (1 week) and chronic (24 weeks) *H. pylori* infection. Indeed, Ach-induced endothelium-dependent relaxation was significantly reduced in mice 1 week after *H. pylori* infection without

change in NTG-induced endothelium-independent relaxation. The impaired Ach-induced endothelium-dependent relaxation persisted for as long as the infection was present for at least 24 weeks in the infected mice without change in vascular contraction to either phenylephrine or potassium chloride (**Figure 1C**), while NTG-induced endothelium-independent relaxation remained intact [55]. These data demonstrated that *H. pylori* infection selectively impairs endothelium-dependent relaxation, not endothelium-independent relaxation, of thoracic aorta in mice that are similar to the findings in human subjects with *H. pylori* infection.

Efforts were made to examine if eradication of *H. pylori* infection could improve endothelium-dependent vasodilation to confirm if *H. pylori* infection was indeed the reason for endothelial dysfunction. As expected, elimination of *H. pylori* infection in mice with anti-*H. pylori* therapy (123.3 mg/Kg bismuth potassium citrate, 102.75 mg/kg tinidazole, and 51.38 mg/kg clarithromycin once daily for 2 weeks via intragastric gavage) significantly improved Ach-induced endothelium-dependent vasorelaxation without change in NTG-induced endothelium-independent relaxation (**Figure 1C**). For the control group, *H. pylori* infected mice were given the same volume of normal saline. The effectiveness of *H. pylori* eradication with antimicrobial therapies in mice was confirmed using RUT and Giemsa staining [55, 61]. These findings confirm that impairment of endothelium-dependent vasodilation associated with *H. pylori* infection is reversible in mouse model, similar to the observations in human subjects.

4.3 Potential mechanisms for the effect of *H. pylori* infection on endothelial function

It is important to know how *H. pylori* infection leads to endothelial dysfunction. *In vitro* study using bovine aortic endothelial cells (BAECs) showed that treatment of BAECs with *H. pylori*-conditioned medium from *H. pylori* 60190 (vacuolating cytotoxin A) significantly decreased the proliferation, tube formation, and migration of the cells (by up to 44%, 65%, and 28%, respectively) through VacA-dependent reduction in the production of endothelial nitric oxide (NO) [62]. Culture of human umbilical vein endothelial cells (HUVECs) with *H. pylori* significantly inhibited the proliferation, migration, and tube formation of HUVECs, and increased the production of the inflammatory factor Chitinase 3 Like 1 (CHI3L1) and phosphorylated p38 in endothelial cells associated with an increased expression of GATA3. Increased levels of GATA3 and CHI3L1 were also found in the arteries of mice with *H. pylori* infection. Knockdown of GATA3 could prevent *H. pylori*-induced dysfunction of HUVECs. These findings suggest that *H. pylori* might impair endothelial function through increased expression of GATA3 and production of CHI3L1 [63].

H. pylori urease (HPU) is considered a key virulence factor that enables bacteria to colonize and survive in the stomach. It has been shown that HPU could trigger the production of reactive oxygen species (ROS) in endothelial cells. Increased intracellular ROS could lead to activation of nuclear factor kappa B (NF-κB) and upregulate expressions of cyclooxygenase-2, hemeoxygenase-1, interleukin (IL)-1β, and ICAM-1, thus increasing oxidative stress and endothelial dysfunction [64]. *H. pylori* infection of primary human endothelial cells is reported to stimulate secretion of important inflammatory cytokines, IL-6 and IL-8 (especially IL-8) in endothelial cells [65]. Treatment of HUVECs with different CagA positive and negative *H. pylori* derived products could enhance the expressions of microRNAs (miRNAs) including miR-21, miR-155, and miR-663 in the cells that are associated with inflammation, apoptosis and necrosis of the cell [66]. Recently, it was reported that *H. pylori* infection could impair endothelial function through exosomes-mediated mechanism [55]. This will be discussed in details below.

5. Role of exosomes in mediating the effect of *H. pylori* infection on endothelial function

H. pylori do not enter the blood circulation themselves because of the gastric tissue barrier and a unique survival and growth environment [67]. However, *H. pylori* virulence factor CagA and *H. pylori* DNA are present in human atherosclerotic lesions and human aorta, carotid and coronary arteries [44, 68–70]. Many cells are known to release extracellular vesicles with unique biophysical and biochemical properties [71, 72], that are referred as exosomes (with diameters from 30 to 200 nm) [73]. Exosomes are found in various body fluids including blood, urine, saliva, and breast milk, and play an important role in cell-to-cell communications through transport of a wide spectrum of bioactive constituents including proteins, lipids, and miRNAs [74, 75]. Recent studies have demonstrated that exosomes are critically involved in the transfer of proteins during infections like prion protein in neurodegenerative disease [76], human immunodeficiency virus-related proteins [77], and human T-cell leukemia virus type-1 proteins [78]. Indeed, it is shown that *H. pylori* infection increases the expression of miR-25 in gastric epithelial cells and is associated with increased levels of exosome-transmitted miR-25 in peripheral blood in human subjects. Further studies demonstrate that Kruppel-like factor 2 (KLF2) is a direct target of exosome-transmitted miR-25 in vascular endothelial cells. MiR-25/KLF2 axis is involved in the regulation of NF-κB signaling pathway, resulting in increased expression of IL-6, monocyte chemoattractant protein-1, vascular cell adhesion molecule-1, and ICAM-1 [79].

To determine how *H. pylori* infection impairs endothelial function, a recent study tested the hypothesis that *H. pylori* could interact with gastric epithelial cells (GES-1), leading to the release of CagA-containing exosomes into the circulation that in turn impair endothelial function [55]. Indeed, Western blotting analysis and immunofluorescence staining demonstrated that the unique *H. pylori* virulence factor CagA entered into human GES-1 after incubation with $CagA^+$ *H. pylori* (**Figure 2A** and **B**). Further studies showed that characteristic exosomes were present in the conditioned media of human GES-1cultured with $CagA^+$ *H. pylori* as defined by specific biomarkers (HSP70 and CD9) using Western blotting, by specific morphologic features using transmission electron microscopy, and by size distribution using a Zetasizer Nano ZS instrument [80]. Western blotting analysis demonstrated that the exosomes from the conditioned media of human GES-1 cultured with $CagA^+$ *H. pylori* contained the unique CagA protein, while exosomes from the control conditioned media of GES-1 (without culture with *H. pylori*) had no CagA protein (**Figure 2C-E**). When the labeled GES-1-derived exosomes with PKH67 were cultured with HUVECs, a detectable amount of PKH67-labeled exosomes was present in HUVECs using 3-D confocal microscopy after 12 hours of culture (**Figure 2F**), confirming the entry of exosomes into HUVECs. Treatment with human GES-1-derived CagA-containing exosomes significantly inhibited the function of HUVECs with decreased proliferation, migration, and tube formation as compared with the control exosomes (**Figure 2G-I**).

Further studies [55], using the serum exosomes from patients with $CagA^+$ *H. pylori* infection and from healthy age-and sex-matched volunteers, revealed that serum exosomes from both patients and healthy subjects exhibited the characteristics similar to the exosomes from human gastric epithelial cells GES-1 cultured with CagA+ *H. pylori* in their morphology using transmission electron microscopy, size distribution using a Zetasizer Nano ZS instrument, and unique biomarkers (HSP70 and CD9) using Western blotting. As expected, CagA protein was detected in the serum exosomes from patients with $CagA^+$ *H. pylori* infection, but not from control

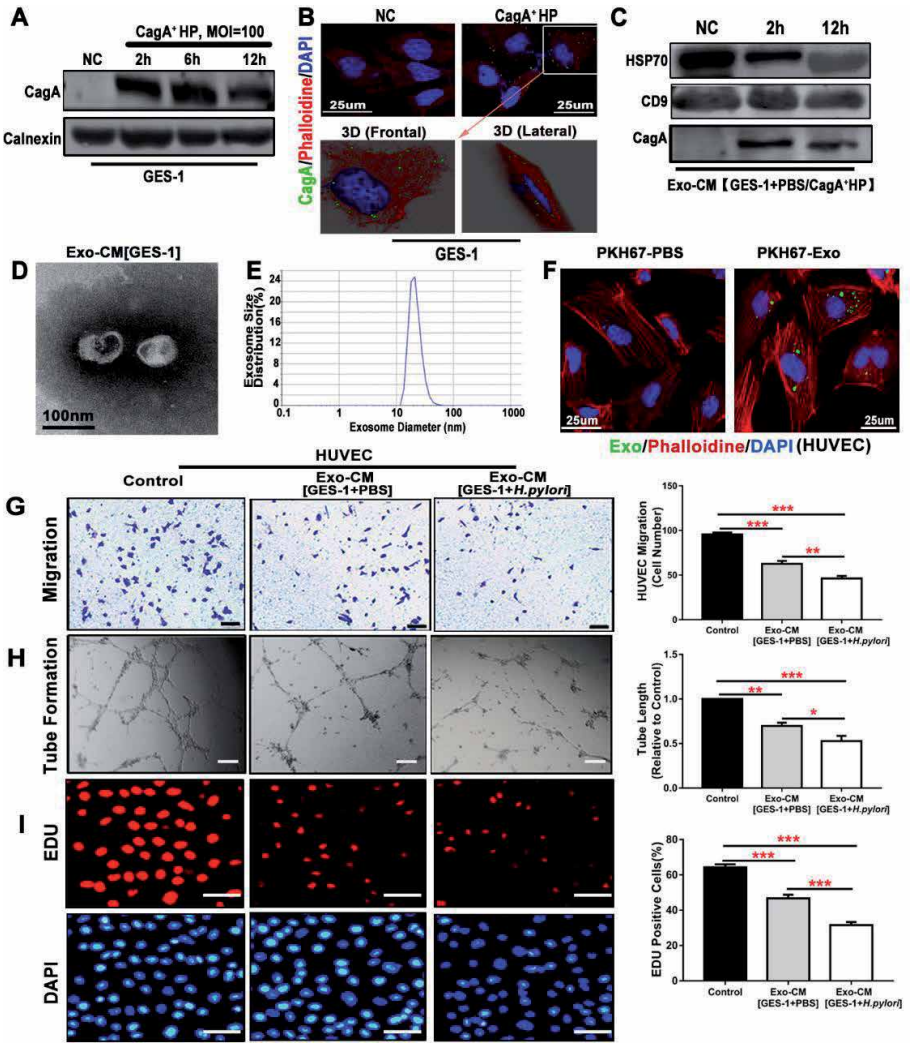

Figure 2.
Exosomes from human gastric epithelial cells GES-1 cultured with CagA⁺ H. pylori *significantly inhibited endothelial cell function in vitro. Western blotting analysis (A) and immunofluorescence staining (***B***) with 3-D confocal microscope demonstrated that the unique* H. pylori *virulence factor CagA entered into GES-1 after culture with CagA⁺* H. pylori*. Exosomes isolated from the conditioned media of GES-1 cultured with CagA⁺* H. pylori *displayed typical features for exosomes including characteristic biomarkers HSP70 and CD9 by western blotting (***C***), morphologies on transmission electron microscopy (***D***), and size using a Zetasizer Nano ZS instrument (***E***). Western blotting analysis confirmed the presence of CagA protein in the exosomes from the conditioned media of GES-1 cultured with CagA⁺* H. pylori*, but not in the ones from GES-1-conditioned media without CagA⁺* H. pylori *(***C***). PKH67-labeled GES-1-derived exosomes (***green***) were incubated with* **HUVECs** *(30 μg protein/5 × 10⁴ cells), and a significant amount of PKH67-labeled exosomes were detected inside the HUVECs as visualized using a 3-D confocal microscope (***F***), confirming that the exosomes entered into the cells. Treatment of HUVECs with CagA protein-containing exosomes (50ug/ml) from GES-1-conditioned media for 24 hours significantly inhibited the function of HUVECs with decreased migration (***G,** *scale bars = 200 μm), tube formation (***H,** *scale bars = 200 μm), and proliferation (***I,** *scale bars = 50 μm).* **NC:** *Normal control;* **CagA+ HP:** *CagA⁺* H. pylori*;* **GES-1***: Human gastric epithelial cells;* **HUVEC:** *Human umbilical vein endothelial cell;* **Exo-CM***: Exosomes derived from conditioned medium. *p < 0.05, **p < 0.01, ***p < 0.001. Data were presented as mean ± SE, n = 3 independent experiments (experiment was repeated 3 times for every measurement). [adopted from (55) with permission].*

subjects using Western blotting analysis. When labeled human serum exosomes with PKH67 were cultured with HUVECs, a significant amount of PKH67-labeled exosomes was present in HUVECs using 3-D confocal microscopy after 12 hours of culture, confirming the entry of serum exosomes into HUVECs. Treatment with

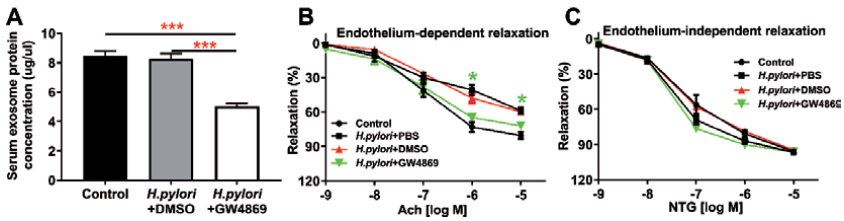

Figure 3.
Inhibition of exosome secretion by GW4869 significantly improved endothelium -dependent vascular relaxation in mice with CagA⁺ H. pylori *infection. Treatment with GW4869 significantly decreased the serum exosome level in the mice with CagA⁺* H. pylori *infection (A) as reflected by the significantly decreased total exosome protein levels (***p < 0.001 by one-way ANOVA with Bonferroni's test), and significantly improved acetylcholine (***ach***)-induced endothelium-dependent relaxation (B) of the aorta in the mice with CagA⁺* H. pylori *infection without change in nitroglycerin (***NTG***)-induced endothelium-independent relaxation (C). *p < 0.05 (when CagA⁺* H. pylori *+ GW4869 group compared with CagA⁺* H. pylori *+ DMSO group).* **Ach***: Acetylcholine;* **NTG***: Nitroglycerin;* **DMSO***: Dimethylsulfoxide (solubilizer of GW4869). Data shown were mean ± SE. n = 8 mice for control group and 10 mice for other groups. [adopted from (55) with permission].*

serum-derived CagA-containing exosomes from patients with CagA⁺ *H. pylori* infection significantly inhibited the function of HUVECs with decreased migration, proliferation, and tube formation. Of note, culture with serum exosomes from healthy control subjects also moderately and yet significantly inhibited endothelial function with decreased migration, tube formation, and proliferation, suggesting that some endogenous substances in the normal serum exosomes could also lead to endothelial dysfunction. However, the serum exosomes from patients with CagA⁺ *H. pylori* infection exhibited significantly greater inhibitory effects on endothelial functions than the ones from healthy subjects.

Studies were also performed to determine if blocking exosomes release with GW4869 *in vivo* could improve endothelial function in mice with CagA⁺ *H. pylori* infection [55]. Indeed, treatment with GW4869 significantly decreased the level of serum exosomes in the mice with *H. pylori* infection (**Figure 3A**), and effectively preserved Ach-induced endothelium-dependent relaxation of the aorta without change in NTG-induced endothelium-independent relaxation (**Figure 3B** and **C**). These findings suggest that *H. pylori* (especially CagA⁺ *H. pylori*) infection could lead to significant endothelial dysfunction in both patients and mice through exosomes-mediated mechanisms.

6. Effect of *H. pylori* infection on other cardiovascular risk factors

It is not surprising that *H. pylori* infection increases the risk for atherosclerosis and other CVDs including HTN and stroke. It has been reported that *H. pylori* infection promotes the release of IL-1, IL-6, TNF-α, and other cytokines, and activates local and systemic inflammatory response, thus leading to endothelial dysfunction and atherosclerosis [81–83]. *H. pylori* infection could also lead to malabsorption of vitamin B12, which could increase serum level of homocysteine, and promote the development and progression of atherosclerosis [84]. In addition, *H. pylori* could enhance the oxidation of low-density lipoproteins (LDL) and increase atherosclerotic plaque formation with decreased plaque stability [85, 86]. We also observed that the levels of LDL-cholesterol in patients with *H. pylori* infection were significantly higher than those without *H. pylori* infection, while the level of high-density lipoprotein cholesterol (HDL-C) were significantly decreased in the patients with *H. pylori* infection than those without *H. pylori* infection [47]. Patients with *H. pylori* seropositivity were shown to have increased brachial-ankle

pulse wave velocity (a marker of atherosclerosis), and impaired glucose metabolism [87]. It is believed that *H. pylori* could interact with gastric epithelial cells to up-regulate the expression of adhesion molecules, and secrete cytokines, which could activate leukocytes, damage the vascular endothelium, aggravate local and systematic inflammatory responses, and thus promote the development and progression of atherosclerosis and related CVDs.

7. Significance and clinical implications

It is very concerning that cardiovascular mortality has been increasing since 2010 especially for males for unknown reasons [6]. It is also reported that the patients with ST elevation myocardial infarction over the past 20 years are getting younger [5]. The reasons for this reverse trend in cardiovascular mortality and mobility have yet to be defined. *H. pylori* infection selectively increased the risk for carotid atherosclerosis in young male patients (≤ 50 years), not in older males or female patients. A recent study [33] that analyzed a large database with a study population of 208,196 showed that the mortality rate was significantly lower in patients with early eradication of *H. pylori* infection. The cumulative CAD rate was significantly decreased in younger patients (<65 years old) with *H. pylori* eradication therapy within 1 year of infection compared to those patients without eradication at all. Interestingly, the treatment of *H. pylori* eradication did not have a benefit in older patients (>65 years old). These data strongly suggested that *H. pylori* infection could be a significant risk factor for endothelial dysfunction, atherosclerosis and CAD in young patients, and could provide a potential explanation for young patients who develop CAD without a clear etiology. It is unclear why *H. pylori* infection does not increase the risk for atherosclerosis for patients older than 50 years. It is possible that other significant risk factors like DM, HTN, and hyperlipidemia play a dominant role that could mask the contribution of *H. pylori* infection to the development and progression of atherosclerosis in this age group of patients. Further studies are needed to investigate the mechanism(s) on the selective effect of *H. pylori* infection on atherosclerosis in young population.

There are substantial sex differences in many CVDs including (but not limited to) myocardial infarctions, heart failure, hypertension, and cardiac hypertrophy [88]. It is well known that premenopausal women are relatively protected from CVDs when compared to men. Typically, women are almost 10 years older than men when they have their first myocardial infarction [89]. It was believed that the decreased cardiovascular morbidity and mortality in young females was due to possible cardio-protective effects of estrogen [90]. However, several large clinical studies, including the HERS trials and the Women's Health Initiative study [91, 92] showed that hormone replacement therapies (HRT) had no cardiovascular benefit in post-menopausal women. In contrast, there might have been an increased risk of CAD during the first year of HRT, and there was an increased risk of nonfatal ventricular arrhythmias among the women on HRT [91]. Thus, the mechanism(s) for decreased CVD risk in premenopausal women is still unclear. The prevalence of *H. pylori* infection was the same in males and females, and yet, *H. pylori* infection only increased the risk for carotid atherosclerosis in male patients≤50 years, not in older males or female patients. It is possible that the significant sex and age difference in the development of atherosclerosis associated with *H. pylori* infection may be one of the reasons for decreased risk for CAD in young females. Further studies are needed to confirm these findings with both patients and experimental animal models.

Currently available data strongly suggest that *H. pylori* infection is an important risk factor for endothelial dysfunction and CVDs especially in young male population. The available data also provide solid evidence to support screening young male population for *H. pylori* infection once a year and treating accordingly for early prevention of CVDs especially premature atherosclerosis associated with *H. pylori* infection.

8. Conclusions

H. pylori infection significantly increases the risk for CVDs including atherosclerosis, HTN, CHD, cerebrovascular disease, and peripheral arterial diseases especially in younger patients (< 65 years old). *H. pylori* infection significantly impairs vascular endothelial function through multiple mechanisms including increased ROS production and oxidative stress, inflammation, decreased NO formation, modification of the expression of cytokines and miRNAs, interruption of lipid and glucose metabolisms, and exosomes-mediated pathways as shown in **Figure 4**. Endothelial dysfunction associated with *H. pylori* infection is reversible in both animal model and human subjects if the infection could be eliminated in a timely fashion (within one year of infection for human subjects and 6 months for mice). Accumulating data suggests that *H. pylori* infection is an additional risk factor for endothelial dysfunction and CVDs. Screening young male population for *H. pylori* infection once a year and treating accordingly could be an effective approach for early prevention of CVDs especially premature atherosclerosis associated with *H. pylori* infection.

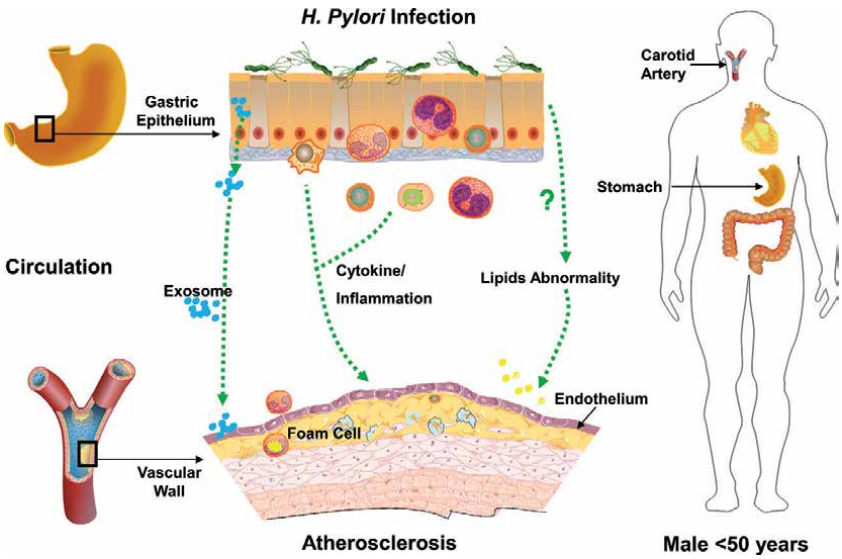

Figure 4.
Schematic illustration of the mechanism on endothelial dysfunction and atherosclerosis associated with H. pylori *infection. It is proposed that* H. pylori *infection could impair endothelial function through exosome-mediated mechanisms. CagA protein is only from CagA*$^{+}$ H. pylori*, and could serve as an ideal tracking molecule for exosome trafficking in vivo. CagA*$^{+}$ H. pylori *translocate CagA protein into gastric epithelial cells (GES-1). CagA-containing exosomes are released into circulation from GES-1, then enter into endothelial cells, leading to endothelial dysfunction.* H. pylori *Infection could also decrease endothelial function through increased production of reactive oxygen species, oxidative stress, and inflammation, decreased cellular nitric oxide formation, modification of the expression of cytokines and miRNAs, and interruption of lipid and glucose metabolisms. [adopted and modified from (47) with permission].*

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Conflict of interest

None.

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References

[1] Bhatt DL, Steg PG, Ohman EM. International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis. Jama. 2006;295(2):180-9. DOI:10.1001/jama.295.2.180.

[2] Jokinen E. Obesity and cardiovascular disease. Minerva pediatrica. 2015;67(1):25-32. DOI: 10.1161/CIRCRESAHA.115.306883

[3] Benjamin EJ, Blaha MJ, Chiuve SE. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. Circulation. 2017;135(10):e146-e603. DOI:10.1161/cir.0000000000000485.

[4] Gimbrone MA, Jr., García-Cardeña G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circulation research. 2016;118(4):620-36. DOI:10.1161/circresaha.115.306301.

[5] Mentias A, Hill E, Barakat AF. An alarming trend: Change in the risk profile of patients with ST elevation myocardial infarction over the last two decades. Int J Cardiol. 2017;248:69-72. DOI:10.1016/j.ijcard.2017.05.011.

[6] Pearson-Stuttard J, Guzman-Castillo M, Penalvo JL. Modeling Future Cardiovascular Disease Mortality in the United States: National Trends and Racial and Ethnic Disparities. Circulation. 2016;133(10):967-78. DOI:10.1161/CIRCULATIONAHA.115.019904.

[7] Chistiakov DA, Bobryshev YV, Kozarov E, Sobenin IA, Orekhov AN. Role of gut microbiota in the modulation of atherosclerosis-associated immune response. Frontiers in microbiology. 2015;6:671. DOI:10.3389/fmicb.2015.00671.

[8] Catry E, Bindels LB, Tailleux A. Targeting the gut microbiota with inulin-type fructans: preclinical demonstration of a novel approach in the management of endothelial dysfunction. Gut. 2018;67(2):271-83. DOI:10.1136/gutjnl-2016-313316.

[9] Brown JM, Hazen SL. Microbial modulation of cardiovascular disease. Nature reviews Microbiology. 2018;16(3):171-81. DOI:10.1038/nrmicro.2017.149.

[10] Mentis A, Lehours P, Mégraud F. Epidemiology and Diagnosis of Helicobacter pylori infection. Helicobacter. 2015;20 Suppl 1:1-7. DOI:10.1111/hel.12250.

[11] Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. Helicobacter. 2014;19 Suppl 1:1-5. DOI:10.1111/hel.12165.

[12] Chew CA, Lye TF, Ang D, Ang TL. The diagnosis and management of *H. pylori* infection in Singapore. Singapore medical journal. 2017;58(5):234-40. DOI:10.11622/smedj.2017037.

[13] Yamaoka Y, Graham DY. Helicobacter pylori virulence and cancer pathogenesis. Future oncology (London, England). 2014;10(8):1487-500. DOI:10.2217/fon.14.29.

[14] Xu Z, Li J, Wang H, Xu G. Helicobacter pylori infection and atherosclerosis: is there a causal relationship? Eur J Clin Microbiol Infect Dis. 2017;36(12):2293-301. DOI: 10.1007/s10096-017-3054-0

[15] Lee M, Baek H, Park JS. Current Helicobacter pylori infection is significantly associated with subclinical coronary atherosclerosis in healthy subjects: A cross-sectional study. PloS one. 2018;13(3):e0193646. DOI:10.1371/journal.pone.0193646.

[16] Kodama M, Kitadai Y, Ito M. Immune response to CagA protein is associated with improved platelet count after Helicobacter pylori eradication in patients with idiopathic thrombocytopenic purpura. Helicobacter. 2007;12(1):36-42. DOI: 10.1111/j.1523-5378.2007.00477.x

[17] Sawayama Y, Ariyama I, Hamada M. Association between chronic Helicobacter pylori infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). Atherosclerosis. 2005;178(2):303-9. DOI:10.1016/j.atherosclerosis.2004.08.025.

[18] Kronsteiner B, Bassaganya-Riera J, Philipson C. Systems-wide analyses of mucosal immune responses to Helicobacter pylori at the interface between pathogenicity and symbiosis. (1949-0984 (Electronic)). DOI: 10.1080/19490976.2015.1116673

[19] Izzotti A, Durando P, Ansaldi F, Gianiorio F, Pulliero A. Interaction between Helicobacter pylori, diet, and genetic polymorphisms as related to non-cancer diseases. Mutat Res. 2009;667(1-2):142-57. DOI: 10.1016/j.mrfmmm.2009.02.002

[20] Rocha M, Avenaud P, Menard A. Association of Helicobacter species with hepatitis C cirrhosis with or without hepatocellular carcinoma. Gut. 2005;54(3):396-401. DOI: 10.1136/gut.2004.042168

[21] Franceschi F, Gasbarrini A, Polyzos SA, Kountouras J. Extragastric Diseases and Helicobacter pylori. Helicobacter. 2015;20 Suppl 1:40-6. DOI: 10.1111/hel.12256

[22] Sonnenberg A, Genta RM. Helicobacter pylori is a risk factor for colonic neoplasms. The American journal of gastroenterology. 2013;108(2):208-15. DOI: 10.1038/ajg.2012.407

[23] Wang B, Yu M, Zhang R, Chen S, Xi Y, Duan G. A meta-analysis of the association between Helicobacter pylori infection and risk of atherosclerotic cardiovascular disease. Helicobacter. 2020;25(6):e12761. DOI:10.1111/hel.12761.

[24] Sun J, Rangan P, Bhat SS, Liu L. A Meta-Analysis of the Association between Helicobacter pylori Infection and Risk of Coronary Heart Disease from Published Prospective Studies. Helicobacter. 2016;21(1):11-23. DOI:10.1111/hel.12234.

[25] Xiong X, Chen J, He M, Wu T, Yang H. Helicobacter pylori infection and the prevalence of hypertension in Chinese adults: The Dongfeng-Tongji cohort. Journal of clinical hypertension (Greenwich, Conn). 2020;22(8):1389-95. DOI:10.1111/jch.13928.

[26] Wan Z, Hu L, Hu M, Lei X, Huang Y, Lv Y. Helicobacter pylori infection and prevalence of high blood pressure among Chinese adults. (1476-5527 (Electronic)). DOI: 10.1038/s41371-017-0028-8

[27] Chen BF, Xu X, Deng Y. Relationship between Helicobacter pylori infection and serum interleukin-18 in patients with carotid atherosclerosis. Helicobacter. 2013;18(2):124-8. DOI:10.1111/hel.12014.

[28] Kowalski M. Helicobacter pylori (*H. pylori*) infection in coronary artery disease: influence of *H. pylori* eradication on coronary artery lumen after percutaneous transluminal coronary angioplasty. The detection of *H. pylori* specific DNA in human coronary atherosclerotic plaque. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 2001;52(1 Suppl 1):3-31.

[29] Yang YF, Li Y, Liu JH. Relation of Helicobacter pylori infection to

peripheral arterial stiffness and 10-year cardiovascular risk in subjects with diabetes mellitus. Diabetes & vascular disease research. 2020;17(5): 1479164120953626. DOI:10.1177/1479164120953626.

[30] Ohnishi M, Fukui M, Ishikawa T. Helicobacter pylori infection and arterial stiffness in patients with type 2 diabetes mellitus. Metabolism: clinical and experimental. 2008;57(12):1760-4. DOI:10.1016/j.metabol.2008.08.001.

[31] Longo-Mbenza B, Nsenga JN, Mokondjimobe E. Helicobacter pylori infection is identified as a cardiovascular risk factor in Central Africans. Vascular health and risk management. 2012;6:455-61. DOI:10.2147/vhrm.s28680.

[32] Choi JM, Lim SH, Han YM. Association between Helicobacter pylori infection and arterial stiffness: Results from a large cross-sectional study. PloS one. 2019;14(8):e0221643. DOI:10.1371/journal.pone.0221643.

[33] Wang JW, Tseng KL, Hsu CN. Association between Helicobacter pylori eradication and the risk of coronary heart diseases. PloS one. 2018;13(1):e0190219. DOI:10.1371/journal.pone.0190219.

[34] Ameriso SF, Fridman Ea Fau - Leiguarda RC, Leiguarda Rc Fau - Sevlever GE, Sevlever GE. Detection of Helicobacter pylori in human carotid atherosclerotic plaques. (1524-4628 (Electronic)). DOI: 10.1161/01.str.32.2.385

[35] Mayr M, Kiechl S, Tsimikas S. Oxidized low-density lipoprotein autoantibodies, chronic infections, and carotid atherosclerosis in a population-based study. Journal of the American College of Cardiology. 2006;47(12): 2436-43. DOI:10.1016/j.jacc.2006.03.024.

[36] Hagiwara N, Toyoda K, Inoue T. Lack of association between infectious burden and carotid atherosclerosis in Japanese patients. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2007;16(4):145-52. DOI:10.1016/j.jstrokecerebrovasdis.2007.02.001.

[37] Shmuely H, Passaro DJ, Vaturi M. Association of CagA+ Helicobacter pylori infection with aortic atheroma. Atherosclerosis. 2005;179(1):127-32. DOI: 10.1016/j.atherosclerosis.2004.09.010

[38] Schottker B, Adamu MA, Weck MN, Muller H, Brenner H. Helicobacter pylori infection, chronic atrophic gastritis and major cardiovascular events: a population-based cohort study. Atherosclerosis. 2012;220(2):569-74. DOI:10.1016/j.atherosclerosis.2011.11.029.

[39] Yu XJ, Yang X, Feng L, Wang LL, Dong QJ. Association between Helicobacter pylori infection and angiographically demonstrated coronary artery disease: A meta-analysis. Exp Ther Med. 2017;13(2):787-93. DOI:10.3892/etm.2017.4028.

[40] Tabata N, Sueta D, Akasaka T. Helicobacter pylori Seropositivity in Patients with Interleukin-1 Polymorphisms Is Significantly Associated with ST-Segment Elevation Myocardial Infarction. PloS one. 2016;11(11):e0166240. DOI:10.1371/journal.pone.0166240.

[41] Danesh J, Peto R. Risk factors for coronary heart disease and infection with Helicobacter pylori: meta-analysis of 18 studies. BMJ (Clinical research ed). 1998;316(7138):1130-2. DOI: 10.1136/bmj.316.7138.1130

[42] Gabrielli M, Santoliquido A, Cremonini F. CagA-positive cytotoxic *H. pylori* strains as a link between plaque instability and atherosclerotic stroke.

European heart journal. 2004;25(1): 64-8. DOI: 10.1016/j.ehj.2003.10.004

[43] Markus HS, Risley P, Mendall MA, Steinmetz H, Sitzer M. Helicobacter pylori infection, the cytotoxin gene A strain, and carotid artery intima-media thickness. Journal of cardiovascular risk. 2002;9(1):1-6. DOI: 10.1177/174182670200900101

[44] Rozankovic PB, Huzjan AL, Cupic H, Bencic IJ, Basic S, Demarin V. Influence of CagA-positive Helicobacter pylori strains on atherosclerotic carotid disease. Journal of neurology. 2011;258(5):753-61. DOI:10.1007/s00415-010-5824-9.

[45] Shan J, Bai X, Han L, Yuan Y, Yang J, Sun X. Association between atherosclerosis and gastric biomarkers concerning Helicobacter pylori infection in a Chinese healthy population. Exp Gerontol. 2018;112:97-102. DOI:10.1016/j.exger.2018.09.009.

[46] Nagai Y, Matsumoto M, Metter EJ. The carotid artery as a noninvasive window for cardiovascular risk in apparently healthy individuals. Ultrasound in medicine & biology. 2002;28(10):1231-8. DOI: 10.1016/s0301-5629(02)00578-1

[47] Zhang L, Chen Z, Xia X. Helicobacter pylori infection selectively increases the risk for carotid atherosclerosis in young males. Atherosclerosis. 2019;291:71-7. DOI:10.1016/j.atherosclerosis.2019.10.005.

[48] Khairy P, Rinfret S, Tardif JC. Absence of association between infectious agents and endothelial function in healthy young men. Circulation. 2003;107(15):1966-71. DOI:10.1161/01.Cir.0000064895.89033.97.

[49] Coskun S, Kasirga E, Yilmaz O. Is Helicobacter pylori related to endothelial dysfunction during childhood? Pediatrics international : official journal of the Japan Pediatric Society. 2008;50(2):150-3. DOI:10.1111/j.1442-200X.2008.02542.x.

[50] Oshima T, Ozono R, Yano Y. Association of Helicobacter pylori infection with systemic inflammation and endothelial dysfunction in healthy male subjects. Journal of the American College of Cardiology. 2005;45(8):1219-22. DOI:10.1016/j.jacc.2005.01.019.

[51] Judaki A, Norozi S, Ahmadi MRH, Ghavam SM, Asadollahi K, Rahmani A. Flow mediated dilation and carotid intima media thickness in patients with chronic gastritis associated with Helicobacter pylori infection. Arquivos de gastroenterologia. 2017;54(4): 300-4. DOI:10.1590/s0004-2803.201700000-39.

[52] Rasmi Y, Rouhrazi H, Khayati-Shal E, Shirpoor A, Saboory E. Association of endothelial dysfunction and cytotoxin-associated gene A-positive Helicobacter pylori in patients with cardiac syndrome X. Biomedical journal. 2016;39(5):339-45. DOI:10.1016/j.bj.2016.01.010.

[53] Blum A, Tamir S, Mualem K, Ben-Shushan RS, Keinan-Boker L, Paritsky M. Endothelial dysfunction is reversible in Helicobacter pylori-positive subjects. The American journal of medicine. 2011;124(12):1171-4. DOI:10.1016/j.amjmed.2011.08.015.

[54] Malfertheiner P, Megraud F, O'Morain CA. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut. 2012;61(5):646-64. DOI:10.1136/gutjnl-2012-302084.

[55] Xia X, Zhang L, Chi J. Helicobacter pylori Infection Impairs Endothelial Function Through an Exosome-Mediated Mechanism. Journal of the American Heart Association.

2020;9(6):e014120. DOI:10.1161/jaha.119.014120.

[56] Sahara S, Sugimoto M, Vilaichone RK. Role of Helicobacter pylori cagA EPIYA motif and vacA genotypes for the development of gastrointestinal diseases in Southeast Asian countries: a meta-analysis. BMC Infect Dis. 2012;12:223. DOI: 10.1186/1471-2334-12-223

[57] Yamaoka Y, Orito E, Mizokami M. Helicobacter pylori in North and South America before Columbus. FEBS Lett. 2002;517(1-3):180-4. DOI: 10.1016/s0014-5793(02)02617-0

[58] Rasmi Y, Raeisi S Fau - Seyyed Mohammadzad MH, Seyyed Mohammadzad MH. Association of inflammation and cytotoxin-associated gene a positive strains of helicobacter pylori in cardiac syndrome x. (1523-5378 (Electronic)). DOI: 10.1111/j.1523-5378.2011.00923.x

[59] Suzuki H, Franceschi F Fau - Nishizawa T, Nishizawa T Fau - Gasbarrini A, Gasbarrini A. Extragastric manifestations of Helicobacter pylori infection. (1523-5378 (Electronic)). DOI: 10.1111/j.1523-5378.2011.00883.x

[60] Suzuki N, Murata-Kamiya N, Yanagiya K. Mutual reinforcement of inflammation and carcinogenesis by the Helicobacter pylori CagA oncoprotein. (2045-2322 (Electronic)). DOI: 10.1038/srep10024

[61] Naumov I, Fenjvesi A. [Correlation between rapid urease test and pathohistological gastrobiopsy finding with positive immunological test in detecting Helicobacter pylori infection]. Med Pregl. 2011;64(7-8):413-7. DOI: 10.2298/mpns1108413n

[62] Tobin NP, Henehan GT, Murphy RP. Helicobacter pylori-induced inhibition of vascular endothelial cell functions: a role for VacA-dependent nitric oxide reduction. American journal of physiology Heart and circulatory physiology. 2008;295(4):H1403-13. DOI:10.1152/ajpheart.00240.2008.

[63] Chi J, Xia X, Zhang L. Helicobacter Pylori Induces GATA3-Dependent Chitinase 3 Like 1 (CHI3L1) Upregulation and Contributes to Vascular Endothelial Injuries. Medical science monitor : international medical journal of experimental and clinical research. 2019;25:4837-48. DOI:10.12659/msm.916311.

[64] de Jesus Souza M, de Moraes JA, Da Silva VN. Helicobacter pylori urease induces pro-inflammatory effects and differentiation of human endothelial cells: Cellular and molecular mechanism. Helicobacter. 2019;24(3):e12573. DOI:10.1111/hel.12573.

[65] Tafreshi M, Guan J, Gorrell RJ. Helicobacter pylori Type IV Secretion System and Its Adhesin Subunit, CagL, Mediate Potent Inflammatory Responses in Primary Human Endothelial Cells. Frontiers in cellular and infection microbiology. 2018;8:22. DOI:10.3389/fcimb.2018.00022.

[66] Kalani M, Hodjati H, GhamarTalepoor A, Samsami Dehaghani A, Doroudchi M. CagA-positive and CagA-negative Helicobacter pylori strains differentially affect the expression of micro RNAs 21, 92a, 155 and 663 in human umbilical vein endothelial cells. Cellular and molecular biology (Noisy-le-Grand, France). 2017;63(1):34-40. DOI:10.14715/cmb/2017.63.1.7.

[67] Buzas GM. Metabolic consequences of Helicobacter pylori infection and eradication. World journal of gastroenterology. 2014;20(18):5226-34. DOI: 10.3748/wjg.v20.i18.5226

[68] Iriz E, Cirak MY, Engin ED. Detection of Helicobacter pylori DNA in

aortic and left internal mammary artery biopsies. Tex Heart Inst J. 2008; 35(2):130-5.

[69] Kilic A, Onguru O Fau - Tugcu H, Tugcu H Fau - Kilic S. Detection of cytomegalovirus and Helicobacter pylori DNA in arterial walls with grade III atherosclerosis by PCR. Pol J Microbiol. 2006;55(4):333-7.

[70] Kaplan M, Yavuz Ss Fau - Cinar B, Cinar B Fau - Koksal V. Detection of Chlamydia pneumoniae and Helicobacter pylori in atherosclerotic plaques of carotid artery by polymerase chain reaction. (1201-9712 (Print)). DOI: 10.1016/j.ijid.2004.10.008

[71] Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2(8):569-79. DOI: 10.1038/nri855

[72] Borges FT, Reis LA, Schor N. Extracellular vesicles: structure, function, and potential clinical uses in renal diseases. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 2013;46(10):824-30. DOI: 10.1590/1414-431X20132964

[73] Pegtel DM, Gould SJ. Exosomes. (1545-4509 (Electronic)). Annu Rev Biochem. 2019 Jun 20;88:487-514. DOI: 10.1146/annurev-biochem-013118-111902

[74] Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654-9. DOI: 10.1038/ncb1596

[75] Mittelbrunn M, Gutierrez-Vazquez C, Villarroya-Beltri C. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nature communications. 2011;2:282. DOI: 10.1038/ncomms1285

[76] Bellingham SA, Guo Bb Fau - Coleman BM, Coleman Bm Fau - Hill AF, Hill AF. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? (1664-042X (Electronic)). DOI: 10.3389/fphys.2012.00124

[77] Campbell TD, Khan M, Huang MB, Bond VC, Powell MD. HIV-1 Nef protein is secreted into vesicles that can fuse with target cells and virions. Ethn Dis. 2008;18(2 Suppl 2):S2-14-9.

[78] Jaworski E, Narayanan A, Van Duyne R. Human T-lymphotropic virus type 1-infected cells secrete exosomes that contain Tax protein. J Biol Chem. 2014;289(32):22284-305. DOI: 10.1074/jbc.M114.549659

[79] Li N, Liu SF, Dong K. Exosome-Transmitted miR-25 Induced by *H. pylori* Promotes Vascular Endothelial Cell Injury by Targeting KLF2. (2235-2988 (Electronic)). DOI: 10.3389/fcimb.2019.00366

[80] Shimoda A, Ueda K, Nishiumi S. Exosomes as nanocarriers for systemic delivery of the Helicobacter pylori virulence factor CagA. Scientific reports. 2016;6:18346. DOI: 10.1038/srep18346

[81] Russo F, Jirillo E, Clemente C. Circulating cytokines and gastrin levels in asymptomatic subjects infected by Helicobacter pylori (*H. pylori*). Immunopharmacology and immunotoxicology. 2001;23(1):13-24. DOI:10.1081/iph-100102563.

[82] Consolazio A, Borgia MC, Ferro D. Increased thrombin generation and circulating levels of tumour necrosis factor-alpha in patients with chronic Helicobacter pylori-positive gastritis. Alimentary pharmacology & therapeutics. 2004;20(3):289-94. DOI:10.1111/j.1365-2036.2004.02074.x.

[83] Maciorkowska E, Kaczmarski M, Panasiuk A, Kondej-Muszynska K, Kemonai A. Soluble adhesion molecules ICAM-1, VCAM-1, P-selectin in children with Helicobacter pylori infection. World journal of gastroenterology. 2005;11(43):6745-50. DOI: 10.3748/wjg.v11.i43.6745

[84] Sipponen P, Laxen F, Huotari K, Harkonen M. Prevalence of low vitamin B12 and high homocysteine in serum in an elderly male population: association with atrophic gastritis and Helicobacter pylori infection. Scandinavian journal of gastroenterology. 2003;38(12):1209-16. DOI: 10.1080/00365520310007224

[85] Hoffmeister A, Rothenbacher D, Bode G. Current infection with Helicobacter pylori, but not seropositivity to Chlamydia pneumoniae or cytomegalovirus, is associated with an atherogenic, modified lipid profile. Arteriosclerosis, thrombosis, and vascular biology. 2001;21(3):427-32. DOI: 10.1161/01.atv.21.3.427

[86] Laurila A, Bloigu A, Nayha S, Hassi J, Leinonen M, Saikku P. Association of Helicobacter pylori infection with elevated serum lipids. Atherosclerosis. 1999;142(1):207-10. DOI: 10.1016/s0021-9150(98)00194-4

[87] Yoshikawa H, Aida K, Mori A, Muto S, Fukuda T. Involvement of Helicobacter pylori infection and impaired glucose metabolism in the increase of brachial-ankle pulse wave velocity. Helicobacter. 2007;12(5):559-66. DOI:10.1111/j.1523-5378.2007.00523.x.

[88] Czubryt MP, Espira L, Lamoureux L, Abrenica B. The role of sex in cardiac function and disease. Can J Physiol Pharmacol. 2006;84(1):93-109. DOI:10.1139/y05-151.

[89] Radovanovic D, Nallamothu BK, Seifert B. Temporal trends in treatment of ST-elevation myocardial infarction among men and women in Switzerland between 1997 and 2011. Eur Heart J Acute Cardiovasc Care. 2012;1(3):183-91. DOI:10.1177/2048872612454021.

[90] Bell JR, Bernasochi GB, Varma U, Raaijmakers AJ, Delbridge LM. Sex and sex hormones in cardiac stress--mechanistic insights. J Steroid Biochem Mol Biol. 2013;137:124-35. DOI:10.1016/j.jsbmb.2013.05.015.

[91] Grady D, Herrington D, Bittner V. Cardiovascular disease outcomes during 6.8 years of hormone therapy: Heart and Estrogen/progestin Replacement Study follow-up (HERS II). JAMA. 2002;288(1):49-57. DOI: 10.1001/jama.288.1.49

[92] Rossouw JE, Anderson GL, Prentice RL. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-33. DOI: 10.1001/jama.288.3.321

Chapter 4

Effect of *Helicobacter pylori* on Tight Junctions in Gastric Epithelia

Erika Patricia Rendón-Huerta,
Carlos Abraham García-García
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Abstract

Molecular complexes grouped under the names of tight, adherent or gap junction regulate the flow of water, ions and macromolecules through epithelium paracellular spaces. The main constituents of tight junctions are claudins, a family of 26 different proteins whose expression and distribution are tissue specific but varies in tumors. A change in claudin 1, 3, 4, 5, 6, 7, 9 and 18 expression, that contributes to lose epithelial cohesion, has been associated to enhanced cell proliferation, migration, and invasiveness in gastric neoplastic tissue. Chronic inflammation process induced by *H. pylori* infection, a major risk factor for gastric cancer development, disrupts tight junctions via CagA gene, Cag pathogenicity island, and VacA, but the effect upon the epithelial barrier of *H. pylori* lipopolysaccharides or *H. pylori*-induced up-regulation of mTOR and ERK signaling pathways by microRNA-100 establishes new concepts of proof.

Keywords: gastric epithelia, *H. pylori*, tight junctions, claudins

1. Introduction

Disruption of the epithelium apical-junctional complex is an initial step of the process which allows many bacteria and/or its toxins to permeate across an otherwise tight mucosa. Normally, the most likely target are claudins, a family of 27 different molecules [1], essential for the maintenance of intercellular tight junctions, that viruses and bacteria such as Hepatitis C virus or *Clostridum perfringens* enterotoxin, bind to mediate their entry in hepatocytes or in human ileum epithelial cells [2, 3]. The aim of this review is to recognize the mechanisms that *Helicobacter pylori* uses to disrupt the tight junctions and invade the gastric epithelial mucosa.

2. *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) is a 3 micrometer long gram-negative spiral bacteria that colonizes the human gastric epithelium's luminal surface of approximately 50% of humans worldwide. Once acquired, it establishes a chronic persistent infection that

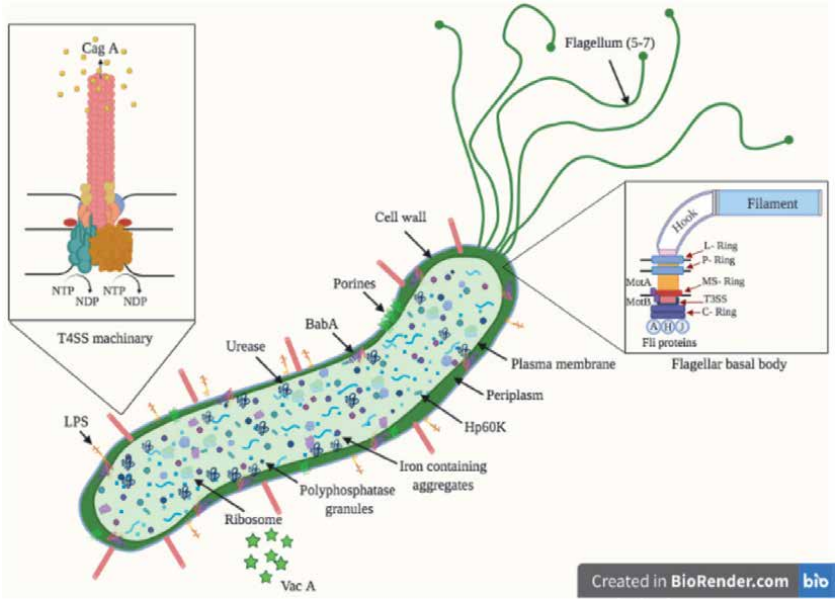

Figure 1.
Helicobacter pylori *components.*

leads to ulcer, cancer or MALT lymphoma [4]. *H. pylori* is conformed by BabA, SabA, OipA and HopQ bacterial colonization factors, and the effector proteins CagA, VacA, urease, catalase, flagellin, mucinase, lipase, neutrophil activating protein, lipopolysaccharides, Cholesterol-Glucosyltransferase and HtrA considered as virulence/pathogenicity factors, and the outer membrane vesicles [5–9]. **Figure 1** shows the complete structure and components of *H. pylori*. Although it has been clearly established that *H. pylori* disrupts gastric epithelial barrier function [10, 11] the precise mechanism(s) remain elusive. A major structure of *H. pylori* is the syringe-like Type IV secretion system which is found in many species of bacteria [12, 13]; this system plays an essential role in the translocation of CagA into host cells [14].

3. Epithelial barrier

The epithelial barrier is a fence composed by intercellular structures termed tight junctions, located at the apical border between gastric epithelial cells, formed by four different transmembrane proteins [occludin, claudins, junction-adhesion-molecules, and CAR –Coxsackievirus and Adenovirus Receptor- proteins] anchored to actin filaments and myosin light chains (MLC) by the actin cytoskeleton and linker proteins zonula occludens ZO-1, ZO-2 and ZO-3 which are members of the membrane-associated guanylate kinase cytoplasmic adaptors. Other highly important members of the barrier are the Adherens Junctions, the Desmosome, the Gap junctions and the Hemidesmosomes. Occludin and claudins interact with adjacent cells through their extracellular loops, whereas JAMs and CAR contain extracellular IgG-like domains [15, 16]. Different proteins form the regulatory complex (Rac, Cdc42, Par3, Par6, PKC). **Figure 2** shows the structural conformation of tight junctions[1]. Claudins, a family of 27 different proteins, are essential to establish and maintain the barrier function as they regulate paracellular permeability [18] whereas occludin is important for epithelial differentiation but not for establishing

[1] A profound review of the gastric epithelal barrier can be found at Tegtmeyer and Backert [17].

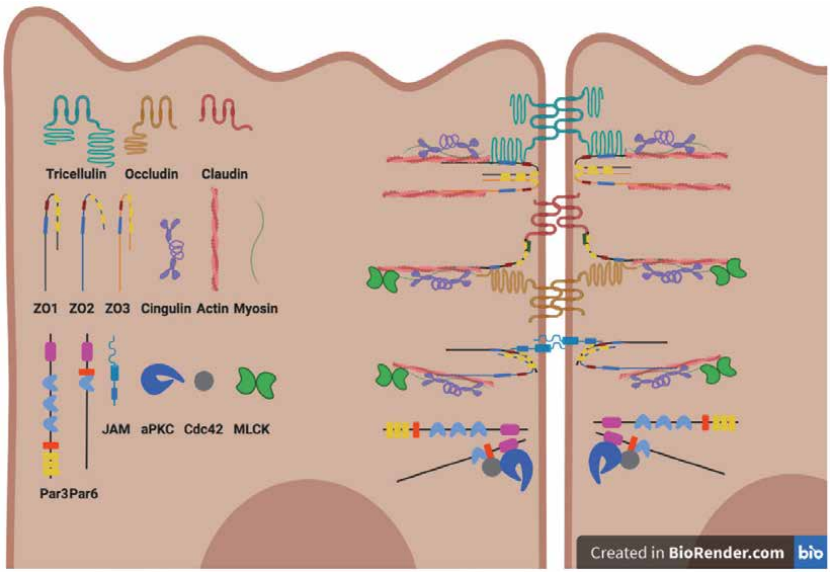

Figure 2.
Gastric epithelia tight junction structure.

the barrier [19]. Paracellular transport across the tight junctions is achieved through the leak pathway which is size-dependent and/or the pore pathway which is size and charge-dependent; size-dependance enables transportation of proteins and lipopolysaccharides and it is controlled by MLC kinase and occludin [20] whereas the pore pathway, controlled by claudins, enables the permeability of cations and anions across different epithelia and exclude molecules larger than 4A [21].

Claudins are responsible for watertight stability and transit of cations and anions. Claudins expression and regulation is tissue specific and their physiological and regulatory function varies according to the organ where they are being expressed [22, 23]. As an example, claudin-4 in ovarian cancer has a pro-angiogenic function whereas in pancreatic cancer it suppresses invasion [24, 25]. The expression of claudins is dysregulated in various cancers, and in gastric tissue the expression of claudin-1, −4, −6 and − 17 is modified when cancer develops but many other claudins such as −3, −5, −7 and − 18 have also been implicated; the loss or gain of claudins is linked to inflammation and inflammatory cytokines such as IFNy, IL-1, IL-6, IL-10, IL-17, IL-22, EGF, TGFb and TNF [26], as well as to several malignancies, drugs, antibiotics, toxins, pesticides, chemicals, microbiota imbalance and stress [27]. The integrity or modifications in tight junctions that affect claudin distribution is via the MAPK/ERK1/2 pathway [28–30]. It has been postulated that in *G. lamblia* infection the loss of epithelial barrier function could be caspase-3 dependent [31] but it does not seem the case in *H. pylori* infection.

The effect of the secretory molecules released by of *H. pylori* known to affect gastric mucosa tight junctions is discussed.

4. VacA

Amongst the major toxins that *H. pylori* possesses, the vacuolating cytotoxin A (VacA) contributes to host-pathogen interactions. After the 140 kDa VacA protein is translated, an active toxin of 88 kDa emerges after cleavage [32]. The toxin is conformed by two domains and three distinct segments: the signal region with

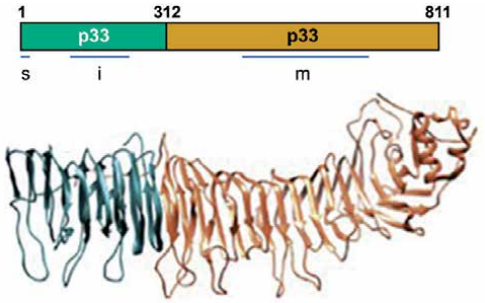

Figure 3.
Organization of VacA p88 protein. From Su et al. [33] and Foegeding et al. [34].

two allelic variations (s1, s2), the intermediate region, and the mid-region with two alleles (m1, m2) (**Figure 3**) [35, 36]; mosaicism has been reported for all the alleles (s1a, s1b, m1a, m1b) [34]. The relevance of these toxin components lays in the fact that s1 causes vacuolation of mammalian cells whereas s2 do not [37]; the discrepancy may be attributed to differences in channel-forming properties [38]. The combination of different VacA alleles is associated with more virulent strains and severe gastric disease: s1a/m2 strains are found in 87.5% of patients with peptic ulcer and in 93% of patients with gastric carcinoma [39]; other highly pathogenic associations include s1a/m1b, s1b/m1b, and s2/m2 [33]. VacA is involved in bacterial colonization of epithelial cells of the gastric mucosa via formation of low conductance membrane pores that are selective for anions over cations [40], and the induction of vacuole formation [41]. These vacuoles, once inside the epithelial cells, alter the transepithelial resistance but do not alter the localization or abundance of ZO-1 and occludin [42]. VacA exert other effects, mainly: endosomal, mitochondrial and epithelial barrier alterations, autophagy, atypical cell signaling and induction of apoptosis in epithelial cells [34]. AGS cells treated with *H. pylori* culture supernatants show rearrangement and disruption of the actin cytoskeleton due to a lack of actin stress fibers; these changes were not VacA dependent [43].

5. CagA

Of major relevance for this review is the effector protein CagA, one of the most important virulence factors [44, 45]. The cytotoxin-associated gene pathogenicity island (cagPAI) comprises 30 genes [46]. The cytotoxin-associated gene A is a 125-140 kDa protein encoded by the cag pathogenicity island [47], a chromosomal region that simultaneously encodes a type IV secretion system specialized in transferring peptidoglycan and CagA to the cytosol of the target cell in an ATP-dependent manner [45, 48]; once translocated, it interacts with numerous proteins in a phosphorylation dependent and independent manner within the epithelial cells, stimulating inflammatory responses, perturbing intracellular actin trafficking, and disrupting cellular tight junctions probably via the ERK1/2 signaling pathway [49–51]. Phosphorylated CagA interacts with Shp2, a host protein that binds to CagA, this complex dephosphorylates the focal adhesion kinase and in turn activates a signal pathway that involves ERK proteins [52, 53]. The transferred peptidoglycan promotes the activation of the pattern-recognition molecule Nod1 within the cytosol of the host cell [54] and subsequently induces the expression of IL-6 and IL-8 as well as MAPK phosphorylation [55–57]. The phosphorylation independent activity of CagA disrupts E-cadherin and ZO-1 and consequently cell-to-cell junctions in polarized epithelial cells [10, 49, 58, 59]. CagA modifies the

polarity of the infected cells by interacting with Par1b/MARK-2 [60, 61]. CagA also stimulates the expression of NfkB, which subsequently activates the IL-8 promoter and stimulates the release of the chemokine IL-8 into the gastric lumen [62], which disrupts epithelial tight junctions organization [63].

CagA is known to affect intercellular junctions and disrupt junction-mediated functions [64] as it causes an ectopic assembly of tight-junction components by recruiting ZO-1 and JAM to sites of bacterial attachment (Amieva 2003), and disrupts the epithelial barrier function [10]. CagA colocalizes with ZO-1 and JAM proteins, binds Par1b and, by inhibiting atypical PKC-mediated phosphorylation of Par1b, disrupts cell polarity and consequently tight junctions. CagA also targets Cdx2 and therefore claudin-2 expression thus suggesting a novel mechanism for gastric epithelial cells dedifferentiation [65]. Another pathophysiological mechanism by which *H. pylori* affect the epithelial barrier is by Rho kinase dependent manner that induces IL-1R type 1 phosphorylation and claudin-4 expression [66].

6. HtrA

One recently recognized mechanism by which CagA disrupts the barrier is mediated by a HtrA (high-temperature requirement A) serine protease [67]. This enzyme is part of a four proteases specific family identified in *E. coli*, *C. jejuni*, *C. coli* and *H. pylori,* all of which enhance adhesion, cellular invasion, and bacterial transmigration via the paracellular route [68]. The HtrA family of proteases contain a chymotrypsin-like protease domain and at least one C-terminal PDZ domain [69].

HtrA are bacterial proteins that provide tolerance to oxidative and heat stress; they undergo oligomerization when denatured proteins are encountered (**Figure 4**) [70]. HtrA can be expressed at the bacterial cell surface, or transported into the extracellular space, or shed in outer membrane vesicles. It favors bacterial paracellular transmigration by cleaving cell-to-cell junction factors such as components of tight junctions that leads to disruption of the epithelial barrier [71]. It has been shown that HtrA1 expression in gastric cancers correlates with better response to cisplatin-based chemotherapy [72].

Although *H. pylori*-infection and –related gastric diseases are clearly associated with downregulation of E-cadherin [73, 74], the mechanism remained elusive. The

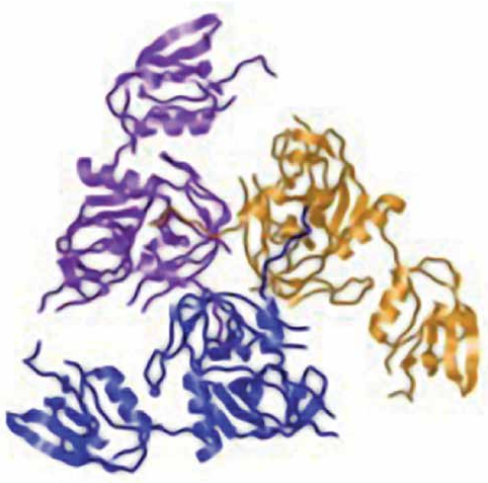

Figure 4.
Tridimensional modeling of H. pylori *trimeric HtrA. From Albrecht et al. [70].*

bacteria disrupts E-cadherin by upregulating the expression of several metalloprotease-1, –3, –7, –9, –10 and ADAM-10 and -15 all of which cleave E-cadherin on the cell surface [6, 68, 75–78]. It has recently been established that HtrA allows access of *H. pylori* to the basolateral side of the gastric epithelium through cleavage of the N-terminal fragment domain of E-cadherin [79] apparently affecting occludin expression on the epithelial cell membrane leading to destruction of adherence junctions and downregulation of the barrier function thus facilitating CagA delivery [80–82]. Phosphorylation of MLC by the specific MLC kinase regulates paracellular permeability [83]. It has been shown that certain strains of *H. pylori* induce the rearrangement of claudin-4 and claudin-5 in a MLC Kinase dependent but in a CagA- and VacA-independent manner [84]; the exact mechanism was not determined although ammonium produced by *H. pylori* urease has been implicated [85, 86].

7. Lipopolysaccharide

Gut bacterial lipopolysaccharides (LPS) are known to affect intracellular signaling as well as tight junctions of the blood brain barrier [87] and the intestinal barrier [88]. LPS, an important structural component of bacterial walls' outer membrane, is recognized by the membrane toll-like receptor 4, and alterations in permeability induced by LPS are via a TLR-4 dependent process associated to the adaptor protein focal adhesion kinase, which has been shown to co-localize with claudin-1 [89], and the activation of the MyD88-dependent pathway [90]. *H. pylori* LPS has an agonist function upon TLR-2 and not TLR-4 [91, 92]. We have shown that *H. pylori* LPS induces the expression of TLR-2 and that the greater expression of the receptor was accompanied by an initial increase in claudin-4 followed by claudin-6, –7 and – 9; this initial process was STAT3-dependent whereas the expression of claudin-6, –7 and – 9 was ERK1/2-dependent (**Figure 5**) [93]. The same pathway has been reported in claudin-1 downregulation in keratinocytes [94].

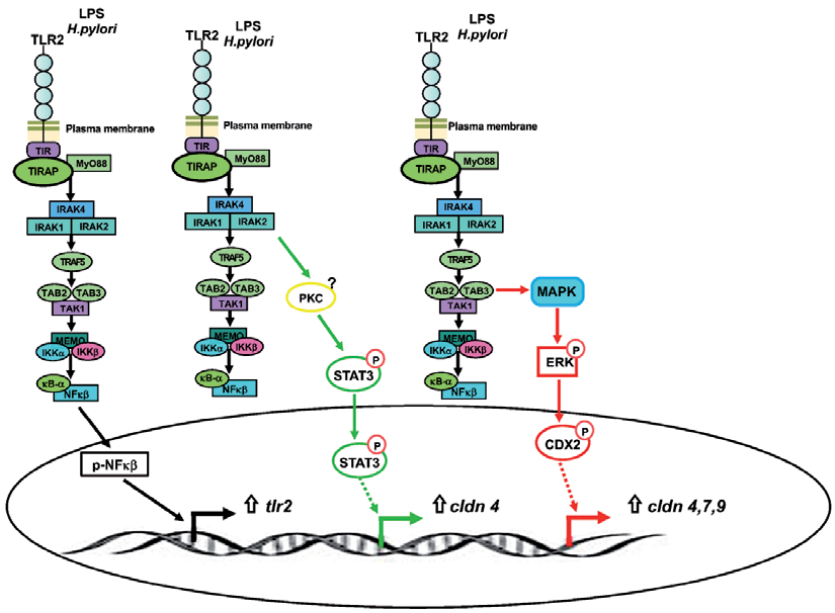

Figure 5.
Effect of H. pylori *LPS on TLR2 activation and claudin expression. From Chavarría-Velázquez et al. [93].*

8. Inflammation

Persistent *H. pylori* infection induces chronic inflammation, pro-inflammatory cytokines IL-1, Il-6, IL-8, TNF and micro RNAs, especially those of the let-7 family [95] that correlates significantly with one or various other pro-inflammatory cytokines [96]. Although it would be interesting to determine the role of pro-inflammatory cytokines in modulating tight junction dysfunction, it is clear that *H. pylori* infection does induce a local inflammatory process by activating nuclear transcription factors NFkB and the chemokine AP-1 [97] where IL-8 enhanced secretion plays an important role [98]. The phosphorylation of the IL-1 receptor after exposure to *H. pylori* reduces the expression of claudin-4 [66]. IL-8 exposure is known to disrupt the organization of epithelial tight junctions leading to "leaky" tight junctions due to a reduced expression of claudin-18 [63].

9. N-nitroso compounds

Exposure to N-nitroso compounds (NOCs) is clearly related to development and increased mortality of gastric cancer (**Figure 6**) [99, 100]. It has been established that nitrogenous constituents of gastric juice can be reduced and lead to the *in situ* formation of N-nitroso compounds [101] although the involvement of *H. pylori* in the development of NOCs and premalignant lesions was controversial until recently [102]. Gastric epithelial cells exposed to N-Nitroso compounds (NOCs) such as MNNG (N-methyl-N-nitro-N-nitrosoguanidine), N-nitrosodimetilamine, N-nitroso-N-ethylurea, or N-nitrosopiperidine through diet (bacon, smoked fish, sausages), high salt consumption, alcoholic beverages, and/or tobacco smoke[2], which also contains NOCs and favors the prevalence of *H. pylori* [103], induce

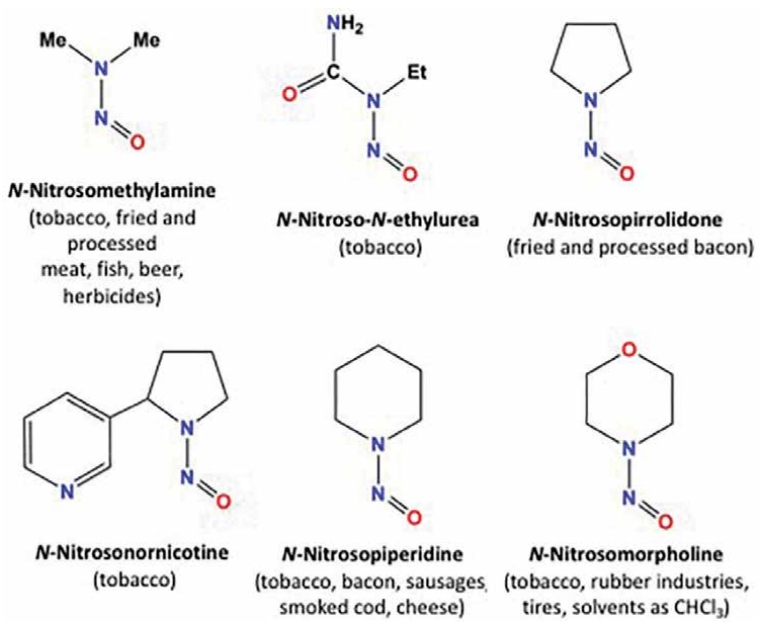

Figure 6.
Structure of relevant N-nitrosamine carcinogenic compounds. From NTP (National Toxicology Program), NIH, USA, 2014.

[2] For a complete list of NOCs compounds go to http://ntp. niehs.nih.gov/pubhealth/ roc/roc13

the expression of epithelial-mesenchymal transition markers in the presence of CagA positive *H. pylori* strains [104] which is mediated by Akt or ERK activation [105], both of which are involved in tight junction assembly [28]. N-etil-N-nitro-N-nitrosoguanidine, a compound that behaves similar to MNNG [106] and induces gastric carcinoma in nonhuman primates [107], synergizes with *H. pylori*, especially CagA+ strains [108] and induces gastric carcinogenesis [109]. Therefore, protagonism of these compounds in individuals with *H. pylori* infection cannot be belittled.

10. Conclusions

Modulation of polarized gastric epithelial cells tight junctions by *H. pylori* involves not only the direct action of some of the most recognized virulence factors of the bacteria that target individual TJ components by different pathways, but also the effect of some *H. pylori*-induced secondary or indirect mechanisms. It is clear that *H. pylori* has developed several mechanisms to endure in an organism and that invasion of the gastric mucosa is just the beginning of the bacteria survival and replicative process where suppression of the immune response is a key component that needs to be continuously explored. Nevertheless, the adhesion and invasion of the gastric mucosa epithelial cells through mechanism that favor the opening of the cell-to-cell tight junction is a bacterial strategy that allows persistent colonization and enhances its ability to cause damage to the host.

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References

[1] Lal-Nag, M. & P. J. Morin (2009) The claudins. Genome Biol, 10, 235.

[2] Veshnyakova, A., J. Protze, J. Rossa, I. E. Blasig, G. Krause & J. Piontek (2010) On the interaction of Clostridium perfringens enterotoxin with claudins. Toxins (Basel), 2, 1336-1356.

[3] Zheng, A., F. Yuan, Y. Li, F. Zhu, P. Hou, J. Li, X. Song, M. Ding & H. Deng (2007) Claudin-6 and claudin-9 function as additional coreceptors for hepatitis C virus. J Virol, 81, 12465-12471.

[4] Ernst, P. B. & B. D. Gold (2000) The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol, 54, 615-640.

[5] Ansari, S. & Y. Yamaoka (2019) Virulence Factors Exploiting Gastric Colonization and its Pathogenicity. Toxins (Basel), 11.

[6] Costa, A. M., R. M. Ferreira, I. Pinto-Ribeiro, I. S. Sougleri, M. J. Oliveira, L. Carreto, M. A. Santos, D. N. Sgouras, F. Carneiro, M. Leite & C. Figueiredo (2016) Helicobacter pylori Activates Matrix Metalloproteinase 10 in Gastric Epithelial Cells via EGFR and ERK-mediated Pathways. J Infect Dis, 213, 1767-1776.

[7] Mobley, H. L. T., G. L. Mendz & S. L. Hazell. 2001. : Physiology and Genetics.

[8] Odenbreit, S., J. Püls, B. Sedlmaier, E. Gerland, W. Fischer & R. Haas (2000) Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science, 287, 1497-1500.

[9] Sgouras, D., N. Tegtmeyer & S. Wessler (2019) Activity and Functional Importance of Helicobacter pylori Virulence Factors. Adv Exp Med Biol, 1149, 35-56.

[10] Amieva, M. R., R. Vogelmann, A. Covacci, L. S. Tompkins, W. J. Nelson & S. Falkow (2003) Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. Science, 300, 1430-1434.

[11] Terrés, A. M., J. M. Pajares, A. M. Hopkins, A. Murphy, A. Moran, A. W. Baird & D. Kelleher (1998a) Helicobacter pylori disrupts epithelial barrier function in a process inhibited by protein kinase C activators. Infect Immun, 66, 2943-2950.

[12] Chung, J. M., M. J. Sheedlo, A. M. Campbell, N. Sawhney, A. E. Frick-Cheng, D. B. Lacy, T. L. Cover & M. D. Ohi (2019) Structure of the. Elife, 8.

[13] Grohmann, E., P. J. Christie, G. Waksman & S. Backert (2018) Type IV secretion in Gram-negative and Gram-positive bacteria. Mol Microbiol, 107, 455-471.

[14] Backert, S., R. Haas, M. Gerhard & M. Naumann (2017) The Helicobacter pylori Type IV Secretion System Encoded by the cag Pathogenicity Island: Architecture, Function, and Signaling. Curr Top Microbiol Immunol, 413, 187-220.

[15] Guttman, J. A. & B. B. Finlay (2009) Tight junctions as targets of infectious agents. Biochim Biophys Acta, 1788, 832-841.

[16] Krause, G., L. Winkler, S. L. Mueller, R. F. Haseloff, J. Piontek & I. E. Blasig (2008) Structure and function of claudins. Biochim Biophys Acta, 1778, 631-645.

[17] N. Tegtmeyer and S. Backert (eds), *Molecular Pathogenesis and Signal Transduction by Helicobacter pylor*i, Current Topics in Microbiology and Immunology 400, Springer International Publishing AG, 2017. pp.: 195-226

[18] Krause, G., J. Protze & J. Piontek (2015) Assembly and function of claudins: Structure-function relationships based on homology models and crystal structures. Semin Cell Dev Biol, 42, 3-12.

[19] Schulzke, J. D., A. H. Gitter, J. Mankertz, S. Spiegel, U. Seidler, S. Amasheh, M. Saitou, S. Tsukita & M. Fromm (2005) Epithelial transport and barrier function in occludin-deficient mice. Biochim Biophys Acta, 1669, 34-42.

[20] Shen, L., C. R. Weber, D. R. Raleigh, D. Yu & J. R. Turner (2011) Tight junction pore and leak pathways: a dynamic duo. Annu Rev Physiol, 73, 283-309.

[21] Amasheh, S., N. Meiri, A. H. Gitter, T. Schöneberg, J. Mankertz, J. D. Schulzke & M. Fromm (2002) Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. J Cell Sci, 115, 4969-4976.

[22] Capaldo, C. T. & A. Nusrat (2015) Claudin switching: Physiological plasticity of the Tight Junction. Semin Cell Dev Biol, 42, 22-29.

[23] Van Itallie, C. M. & J. M. Anderson (2014) Architecture of tight junctions and principles of molecular composition. Semin Cell Dev Biol, 36, 157-165.

[24] Li, J., S. Chigurupati, R. Agarwal, M. R. Mughal, M. P. Mattson, K. G. Becker, W. H. Wood, Y. Zhang & P. J. Morin (2009) Possible angiogenic roles for claudin-4 in ovarian cancer. Cancer Biol Ther, 8, 1806-1814.

[25] Michl, P., C. Barth, M. Buchholz, M. M. Lerch, M. Rolke, K. H. Holzmann, A. Menke, H. Fensterer, K. Giehl, M. Löhr, G. Leder, T. Iwamura, G. Adler & T. M. Gress (2003) Claudin-4 expression decreases invasiveness and metastatic potential of pancreatic cancer. Cancer Res, 63, 6265-6271.

[26] Han, X., E. Zhang, Y. Shi, B. Song, H. Du & Z. Cao (2019) Biomaterial-tight junction interaction and potential impacts. J Mater Chem B, 7, 6310-6320.

[27] Bhat, A. A., S. Uppada, I. W. Achkar, S. Hashem, S. K. Yadav, M. Shanmugakonar, H. A. Al-Naemi, M. Haris & S. Uddin (2018) Tight Junction Proteins and Signaling Pathways in Cancer and Inflammation: A Functional Crosstalk. Front Physiol, 9, 1942.

[28] Aggarwal, S., T. Suzuki, W. L. Taylor, A. Bhargava & R. K. Rao (2011) Contrasting effects of ERK on tight junction integrity in differentiated and under-differentiated Caco-2 cell monolayers. Biochem J, 433, 51-63.

[29] Dörfel, M. J. & O. Huber (2012) Modulation of tight junction structure and function by kinases and phosphatases targeting occludin. J Biomed Biotechnol, 2012, 807356.

[30] Kim, B. & S. Breton (2016) The MAPK/ERK-Signaling Pathway Regulates the Expression and Distribution of Tight Junction Proteins in the Mouse Proximal Epididymis. Biol Reprod, 94, 22.

[31] Chin, A. C., D. A. Teoh, K. G. Scott, J. B. Meddings, W. K. Macnaughton & A. G. Buret (2002) Strain-dependent induction of enterocyte apoptosis by Giardia lamblia disrupts epithelial barrier function in a caspase-3-dependent manner. Infect Immun, 70, 3673-3680.

[32] Telford, J. L., P. Ghiara, M. Dell'Orco, M. Comanducci, D. Burroni, M. Bugnoli, M. F. Tecce, S. Censini, A. Covacci & Z. Xiang (1994) Gene structure of the Helicobacter pylori cytotoxin and evidence of its key role in gastric disease. J Exp Med, 179, 1653-1658.

[33] Su, M., A. L. Erwin, A. M. Campbell, T. M. Pyburn, L. E. Salay, J. L. Hanks, D. B. Lacy, D. L. Akey, T. L. Cover & M. D. Ohi (2019) Cryo-EM Analysis Reveals Structural Basis of Helicobacter pylori VacA Toxin Oligomerization. J Mol Biol, 431, 1956-1965.

[34] Foegeding, N. J., R. R. Caston, M. S. McClain, M. D. Ohi & T. L. Cover (2016) An Overview of Helicobacter pylori VacA Toxin Biology. Toxins (Basel), 8.

[35] Atherton, J. C., P. Cao, R. M. Peek, M. K. Tummuru, M. J. Blaser & T. L. Cover (1995) Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem*, 270, 17771-17777.

[36] Go, M. F., L. Cissell & D. Y. Graham (1998) Failure to confirm association of vac A gene mosaicism with duodenal ulcer disease. Scand J Gastroenterol, 33, 132-136.

[37] McClain, M. S., P. Cao, H. Iwamoto, A. D. Vinion-Dubiel, G. Szabo, Z. Shao & T. L. Cover (2001) A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin activity and alters membrane channel formation. J Bacteriol, 183, 6499-6508.

[38] Tombola, F., C. Pagliaccia, S. Campello, J. L. Telford, C. Montecucco, E. Papini & M. Zoratti (2001) How the loop and middle regions influence the properties of Helicobacter pylori VacA channels. Biophys J, 81, 3204-3215.

[39] Chen, X. J., J. Yan & Y. F. Shen (2005) Dominant cagA/vacA genotypes and coinfection frequency of H. pylori in peptic ulcer or chronic gastritis patients in Zhejiang Province and correlations among different genotypes, coinfection and severity of the diseases. Chin Med J (Engl), 118, 460-467.

[40] Iwamoto, H., D. M. Czajkowsky, T. L. Cover, G. Szabo & Z. Shao (1999) VacA from Helicobacter pylori: a hexameric chloride channel. FEBS Lett, 450, 101-104.

[41] Junaid, M., A. K. Linn, M. B. Javadi, S. Al-Gubare, N. Ali & G. Katzenmeier (2016) Vacuolating cytotoxin A (VacA) - A multi-talented pore-forming toxin from Helicobacter pylori. Toxicon, 118, 27-35.

[42] Papini, E., B. Satin, N. Norais, M. de Bernard, J. L. Telford, R. Rappuoli & C. Montecucco (1998) Selective increase of the permeability of polarized epithelial cell monolayers by Helicobacter pylori vacuolating toxin. J Clin Invest, 102, 813-820.

[43] Bebb, J. R., D. P. Letley, J. L. Rhead & J. C. Atherton (2003) Helicobacter pylori supernatants cause epithelial cytoskeletal disruption that is bacterial strain and epithelial cell line dependent but not toxin VacA dependent. Infect Immun, 71, 3623-3627.

[44] Backert, S., N. Tegtmeyer & M. Selbach (2010) The versatility of Helicobacter pylori CagA effector protein functions: The master key hypothesis. Helicobacter, 15, 163-176.

[45] Noto, J. M. & R. M. Peek (2012) The Helicobacter pylori cag Pathogenicity Island. Methods Mol Biol, 921, 41-50.

[46] Essawi, T., W. Hammoudeh, I. Sabri, W. Sweidan & M. A. Farraj (2013) Determination of Helicobacter pylori Virulence Genes in Gastric Biopsies by PCR. ISRN Gastroenterol, 2013, 606258.

[47] Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang & N. Figura (1993)

Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A, 90, 5791-5795.

[48] Backert, S. & N. Tegtmeyer (2012) Helicobacter pylori CagA tertiary structure reveals functional insights. Cell Host Microbe, 12, 3-5.

[49] Tohidpour, A. (2016) CagA-mediated pathogenesis of Helicobacter pylori. Microb Pathog, 93, 44-55.

[50] Wang, H. P., Y. L. Zhu & W. Shao (2013) Role of Helicobacter pylori virulence factor cytotoxin-associated gene A in gastric mucosa-associated lymphoid tissue lymphoma. World J Gastroenterol, 19, 8219-8226.

[51] Zhu, Y., C. Wang, J. Huang, Z. Ge, Q. Dong, X. Zhong, Y. Su & S. Zheng (2007) The Helicobacter pylori virulence factor CagA promotes Erk1/2-mediated Bad phosphorylation in lymphocytes: a mechanism of CagA-inhibited lymphocyte apoptosis. Cell Microbiol, 9, 952-961.

[52] Higashi, H., A. Nakaya, R. Tsutsumi, K. Yokoyama, Y. Fujii, S. Ishikawa, M. Higuchi, A. Takahashi, Y. Kurashima, Y. Teishikata, S. Tanaka, T. Azuma & M. Hatakeyama (2004) Helicobacter pylori CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. J Biol Chem, 279, 17205-17216.

[53] Higashi, H., R. Tsutsumi, S. Muto, T. Sugiyama, T. Azuma, M. Asaka & M. Hatakeyama (2002) SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. Science, 295, 683-6.

[54] Viala, J., C. Chaput, I. G. Boneca, A. Cardona, S. E. Girardin, A. P. Moran, R. Athman, S. Mémet, M. R. Huerre, A. J. Coyle, P. S. DiStefano, P. J. Sansonetti, A. Labigne, J. Bertin, D. J. Philpott & R. L. Ferrero (2004) Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. Nat Immunol, 5, 1166-1174.

[55] Allison, C. C., T. A. Kufer, E. Kremmer, M. Kaparakis & R. L. Ferrero (2009) Helicobacter pylori induces MAPK phosphorylation and AP-1 activation via a NOD1-dependent mechanism. J Immunol, 183, 8099-8109.

[56] Guo, M., F. Wu, Z. Zhang, G. Hao, R. Li, N. Li, Y. Shang, L. Wei & T. Chai (2017) Characterization of Rabbit Nucleotide-Binding Oligomerization Domain 1 (NOD1) and the Role of NOD1 Signaling Pathway during Bacterial Infection. Front Immunol, 8, 1278.

[57] Gunawardhana, N., S. Jang, Y. H. Choi, Y. A. Hong, Y. E. Jeon, A. Kim, H. Su, J. H. Kim, Y. J. Yoo, D. S. Merrell, J. Kim & J. H. Cha (2017) -Induced HB-EGF Upregulates Gastrin Expression via the EGF Receptor, C-Raf, Mek1, and Erk2 in the MAPK Pathway. Front Cell Infect Microbiol, 7, 541.

[58] Bagnoli, F., L. Buti, L. Tompkins, A. Covacci & M. R. Amieva (2005) Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells. Proc Natl Acad Sci U S A, 102, 16339-16344.

[59] Murata-Kamiya, N., Y. Kurashima, Y. Teishikata, Y. Yamahashi, Y. Saito, H. Higashi, H. Aburatani, T. Akiyama, R. M. Peek, T. Azuma & M. Hatakeyama (2007) Helicobacter pylori CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene, 26, 4617-4626.

[60] Lu, H. S., Y. Saito, M. Umeda, N. Murata-Kamiya, H. M. Zhang, H. Higashi & M. Hatakeyama (2008) Structural and functional diversity in

the PAR1b/MARK2-binding region of Helicobacter pylori CagA. Cancer Sci, 99, 2004-2011.

[61] Nesić, D., M. C. Miller, Z. T. Quinkert, M. Stein, B. T. Chait & C. E. Stebbins (2010) Helicobacter pylori CagA inhibits PAR1-MARK family kinases by mimicking host substrates. Nat Struct Mol Biol, 17, 130-132.

[62] Brandt, S., T. Kwok, R. Hartig, W. König & S. Backert (2005) NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein. Proc Natl Acad Sci U S A, 102, 9300-9305.

[63] Reynolds, C. J., K. Quigley, X. Cheng, A. Suresh, S. Tahir, F. Ahmed-Jushuf, K. Nawab, K. Choy, S. A. Walker, S. A. Mathie, M. Sim, J. Stowell, J. Manji, T. Pollard, D. M. Altmann & R. J. Boyton (2018) Lung Defense through IL-8 Carries a Cost of Chronic Lung Remodeling and Impaired Function. Am J Respir Cell Mol Biol, 59, 557-571.

[64] Hazell, S. L., A. Lee, L. Brady & W. Hennessy (1986) Campylobacter pyloridis and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. J Infect Dis, 153, 658-663.

[65] Song, X., H. X. Chen, X. Y. Wang, X. Y. Deng, Y. X. Xi, Q. He, T. L. Peng, J. Chen, W. Chen, B. C. Wong & M. H. Chen (2013) H. pylori-encoded CagA disrupts tight junctions and induces invasiveness of AGS gastric carcinoma cells via Cdx2-dependent targeting of Claudin-2. Cell Immunol, 286, 22-30.

[66] Lapointe, T. K., P. M. O'Connor, N. L. Jones, D. Menard & A. G. Buret (2010) Interleukin-1 receptor phosphorylation activates Rho kinase to disrupt human gastric tight junctional claudin-4 during Helicobacter pylori infection. Cell Microbiol, 12, 692-703.

[67] Boehm, M., D. Simson, U. Escher, A. M. Schmidt, S. Bereswill, N. Tegtmeyer, S. Backert & M. M. Heimesaat (2018) Function of Serine Protease HtrA in the Lifecycle of the Foodborne Pathogen. Eur J Microbiol Immunol (Bp), 8, 70-77.

[68] Hoy, B., T. Geppert, M. Boehm, F. Reisen, P. Plattner, G. Gadermaier, N. Sewald, F. Ferreira, P. Briza, G. Schneider, S. Backert & S. Wessler (2012) Distinct roles of secreted HtrA proteases from gram-negative pathogens in cleaving the junctional protein and tumor suppressor E-cadherin. J Biol Chem, 287, 10115-10120.

[69] Pallen, M. J. & B. W. Wren (1997) The HtrA family of serine proteases. Mol Microbiol, 26, 209-221.

[70] Albrecht, N., N. Tegtmeyer, H. Sticht, J. Skórko-Glonek & S. Backert (2018) Amino-Terminal Processing of. Front Microbiol, 9, 642.

[71] Hoy, B., M. Löwer, C. Weydig, G. Carra, N. Tegtmeyer, T. Geppert, P. Schröder, N. Sewald, S. Backert, G. Schneider & S. Wessler (2010) Helicobacter pylori HtrA is a new secreted virulence factor that cleaves E-cadherin to disrupt intercellular adhesion. EMBO Rep, 11, 798-804.

[72] Chien, J., G. Aletti, A. Baldi, V. Catalano, P. Muretto, G. L. Keeney, K. R. Kalli, J. Staub, M. Ehrmann, W. A. Cliby, Y. K. Lee, K. C. Bible, L. C. Hartmann, S. H. Kaufmann & V. Shridhar (2006) Serine protease HtrA1 modulates chemotherapy-induced cytotoxicity. J Clin Invest, 116, 1994-2004.

[73] Terrés, A. M., J. M. Pajares, D. O'Toole, S. Ahern & D. Kelleher (1998b) H pylori infection is associated with downregulation of E-cadherin, a molecule involved in epithelial cell adhesion and proliferation control. J Clin Pathol, 51, 410-412.

[74] Zhang, Y., D. Li, Y. Dai, R. Li, Y. Gao & L. Hu (2019) The Role of E-cadherin

in Helicobacter pylori-Related Gastric Diseases. Curr Drug Metab, 20, 23-28.

[75] Kundu, P., A. K. Mukhopadhyay, R. Patra, A. Banerjee, D. E. Berg & S. Swarnakar (2006) Cag pathogenicity island-independent up-regulation of matrix metalloproteinases-9 and -2 secretion and expression in mice by Helicobacter pylori infection. J Biol Chem, 281, 34651-34662.

[76] Pillinger, M. H., N. Marjanovic, S. Y. Kim, Y. C. Lee, J. U. Scher, J. Roper, A. M. Abeles, P. I. Izmirly, M. Axelrod, M. Y. Pillinger, S. Tolani, V. Dinsell, S. B. Abramson & M. J. Blaser (2007) Helicobacter pylori stimulates gastric epithelial cell MMP-1 secretion via CagA-dependent and -independent ERK activation. J Biol Chem, 282, 18722-18731.

[77] Sougleri, I. S., K. S. Papadakos, M. P. Zadik, M. Mavri-Vavagianni, A. F. Mentis & D. N. Sgouras (2016) Helicobacter pylori CagA protein induces factors involved in the epithelial to mesenchymal transition (EMT) in infected gastric epithelial cells in an EPIYA- phosphorylation-dependent manner. FEBS J, 283, 206-220.

[78] Yin, Y., A. M. Grabowska, P. A. Clarke, E. Whelband, K. Robinson, R. H. Argent, A. Tobias, R. Kumari, J. C. Atherton & S. A. Watson (2010) Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7. Gut, 59, 1037-1045.

[79] Schmidt, T. P., C. Goetz, M. Huemer, G. Schneider & S. Wessler (2016) Calcium binding protects E-cadherin from cleavage by Helicobacter pylori HtrA. Gut Pathog, 8, 29.

[80] Boehm, M., B. Hoy, M. Rohde, N. Tegtmeyer, K. T. Bæk, O. A. Oyarzabal, L. Brøndsted, S. Wessler & S. Backert (2012) Rapid paracellular transmigration of Campylobacter jejuni across polarized epithelial cells without affecting TER: role of proteolytic-active HtrA cleaving E-cadherin but not fibronectin. Gut Pathog, 4, 3.

[81] Tegtmeyer, N., Y. Moodley, Y. Yamaoka, S. R. Pernitzsch, V. Schmidt, F. R. Traverso, T. P. Schmidt, R. Rad, K. G. Yeoh, H. Bow, J. Torres, M. Gerhard, G. Schneider, S. Wessler & S. Backert (2016) Characterisation of worldwide Helicobacter pylori strains reveals genetic conservation and essentiality of serine protease HtrA. Mol Microbiol, 99, 925-944.

[82] Waskito, L. A., N. R. Salama & Y. Yamaoka (2018) Pathogenesis of Helicobacter pylori infection. *Helicobacter,* 23 Suppl 1, e12516.

[83] Turner, J. R., B. K. Rill, S. L. Carlson, D. Carnes, R. Kerner, R. J. Mrsny & J. L. Madara (1997) Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am J Physiol, 273, C1378-C1385.

[84] Fedwick, J. P., T. K. Lapointe, J. B. Meddings, P. M. Sherman & A. G. Buret (2005) Helicobacter pylori activates myosin light-chain kinase to disrupt claudin-4 and claudin-5 and increase epithelial permeability. Infect Immun, 73, 7844-7852.

[85] Lytton, S. D., W. Fischer, W. Nagel, R. Haas & F. X. Beck (2005) Production of ammonium by Helicobacter pylori mediates occludin processing and disruption of tight junctions in Caco-2 cells. Microbiology (Reading), 151, 3267-3276.

[86] Wroblewski, L. E., L. Shen, S. Ogden, J. Romero-Gallo, L. A. Lapierre, D. A. Israel, J. R. Turner & R. M. Peek (2009) Helicobacter pylori dysregulation of gastric epithelial tight junctions by urease-mediated myosin

II activation. Gastroenterology, 136, 236-246.

[87] Feng, S., L. Zou, H. Wang, R. He, K. Liu & H. Zhu (2018) RhoA/ROCK-2 Pathway Inhibition and Tight Junction Protein Upregulation by Catalpol Suppresses Lipopolysaccaride-Induced Disruption of Blood-Brain Barrier Permeability. Molecules, 23.

[88] Ghosh, S. S., H. He, J. Wang, T. W. Gehr & S. Ghosh (2018) Curcumin-mediated regulation of intestinal barrier function: The mechanism underlying its beneficial effects. Tissue Barriers, 6, e1425085.

[89] Ma, Y., S. Semba, R. I. Khan, H. Bochimoto, T. Watanabe, M. Fujiya, Y. Kohgo, Y. Liu & T. Taniguchi (2013) Focal adhesion kinase regulates intestinal epithelial barrier function via redistribution of tight junction. Biochim Biophys Acta, 1832, 151-159.

[90] Guo, S., M. Nighot, R. Al-Sadi, T. Alhmoud, P. Nighot & T. Y. Ma (2015) Lipopolysaccharide Regulation of Intestinal Tight Junction Permeability Is Mediated by TLR4 Signal Transduction Pathway Activation of FAK and MyD88. J Immunol, 195, 4999-5010.

[91] Nagashima, H., S. Iwatani, M. Cruz, J. A. Jiménez Abreu, T. Uchida, V. Mahachai, R. K. Vilaichone, D. Y. Graham & Y. Yamaoka (2015) Toll-like Receptor 10 in Helicobacter pylori Infection. J Infect Dis, 212, 1666-1676.

[92] Smith, M. F., A. Mitchell, G. Li, S. Ding, A. M. Fitzmaurice, K. Ryan, S. Crowe & J. B. Goldberg (2003) Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. J Biol Chem, 278, 32552-32560.

[93] Chavarría-Velázquez, C. O., A. C. Torres-Martínez, L. F. Montaño & E. P. Rendón-Huerta (2018) TLR2 activation induced by H. pylori LPS promotes the differential expression of claudin-4, −6, −7 and −9 via either STAT3 and ERK1/2 in AGS cells. Immunobiology, 223, 38-48.

[94] Ryu, W. I., H. Lee, H. C. Bae, J. Jeon, H. J. Ryu, J. Kim, J. H. Kim, J. W. Son, Y. Imai, K. Yamanishi, S. H. Jeong & S. W. Son (2018) IL-33 down-regulates CLDN1 expression through the ERK/STAT3 pathway in keratinocytes. J Dermatol Sci, 90, 313-322.

[95] Matsushima, K., H. Isomoto, N. Inoue, T. Nakayama, T. Hayashi, M. Nakayama, K. Nakao, T. Hirayama & S. Kohno (2011) MicroRNA signatures in Helicobacter pylori-infected gastric mucosa. Int J Cancer, 128, 361-370.

[96] Isomoto, H., K. Matsushima, N. Inoue, T. Hayashi, T. Nakayama, M. Kunizaki, S. Hidaka, M. Nakayama, J. Hisatsune, M. Nakashima, T. Nagayasu, K. Nakao & T. Hirayama (2012) Interweaving microRNAs and proinflammatory cytokines in gastric mucosa with reference to H. pylori infection. J Clin Immunol, 32, 290-299.

[97] Backert, S. & M. Naumann (2010) What a disorder: proinflammatory signaling pathways induced by Helicobacter pylori. Trends Microbiol, 18, 479-486.

[98] Fiorentino, M., H. Ding, T. G. Blanchard, S. J. Czinn, M. B. Sztein & A. Fasano (2013) Helicobacter pylori-induced disruption of monolayer permeability and proinflammatory cytokine secretion in polarized human gastric epithelial cells. Infect Immun, 81, 876-883.

[99] Hidajat, M., D. M. McElvenny, P. Ritchie, A. Darnton, W. Mueller, M. van Tongeren, R. M. Agius, J. W. Cherrie & F. de Vocht (2019) Lifetime exposure to rubber dusts, fumes and N-nitrosamines and cancer mortality in a cohort of British rubber workers with 49 years

follow-up. Occup Environ Med, 76, 250-258.

[100] Jakszyn, P., S. Bingham, G. Pera, A. Agudo, R. Luben, A. Welch, H. Boeing, G. Del Giudice, D. Palli, C. Saieva, V. Krogh, C. Sacerdote, R. Tumino, S. Panico, G. Berglund, H. Simán, G. Hallmans, M. J. Sanchez, N. Larrañaga, A. Barricarte, M. D. Chirlaque, J. R. Quirós, T. J. Key, N. Allen, E. Lund, F. Carneiro, J. Linseisen, G. Nagel, K. Overvad, A. Tjonneland, A. Olsen, H. B. Bueno-de-Mesquita, M. O. Ocké, P. H. Peeters, M. E. Numans, F. Clavel-Chapelon, A. Trichopoulou, C. Fenger, R. Stenling, P. Ferrari, M. Jenab, T. Norat, E. Riboli & C. A. Gonzalez (2006) Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. Carcinogenesis, 27, 1497-1501.

[101] Kyrtopoulos, S. A. (1989) N-nitroso compound formation in human gastric juice. Cancer Surv, 8, 423-442.

[102] Sobala, G. M., B. Pignatelli, C. J. Schorah, H. Bartsch, M. Sanderson, M. F. Dixon, S. Shires, R. F. King & A. T. Axon (1991) Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of the stomach. Carcinogenesis, 12, 193-198.

[103] Ferro, A., S. Morais, C. Pelucchi, N. Aragonés, M. Kogevinas, L. López-Carrillo, R. Malekzadeh, S. Tsugane, G. S. Hamada, A. Hidaka, R. U. Hernández-Ramírez, M. López-Cervantes, D. Zaridze, D. Maximovitch, F. Pourfarzi, Z. F. Zhang, G. P. Yu, M. Pakseresht, W. Ye, A. Plymoth, M. Leja, E. Gasenko, M. H. Derakhshan, E. Negri, C. La Vecchia, B. Peleteiro & N. Lunet (2019) Smoking and Helicobacter pylori infection: an individual participant pooled analysis (Stomach Cancer Pooling- StoP Project). Eur J Cancer Prev, 28, 390-396.

[104] Lin, L., H. Wei, J. Yi, B. Xie, J. Chen, C. Zhou, L. Wang & Y. Yang (2019) Chronic CagA-positive Helicobacter pylori infection with MNNG stimulation synergistically induces mesenchymal and cancer stem cell-like properties in gastric mucosal epithelial cells. J Cell Biochem, 120, 17635-17649.

[105] Yong, X., B. Tang, B. S. Li, R. Xie, C. J. Hu, G. Luo, Y. Qin, H. Dong & S. M. Yang (2015) Helicobacter pylori virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. Cell Commun Signal, 13, 30.

[106] Yoda, K., S. Sakiyama & S. Fujimura (1982) Interaction of N-ethyl-N'-nitro-n-nitrosoguanidine with nucleic acids and proteins in comparison with N-methyl-N'-nitro-N-nitrosoguanidine. Chem Biol Interact, 41, 49-59.

[107] Ohgaki, H., H. Hasegawa, K. Kusama, K. Morino, N. Matsukura, S. Sato, K. Maruyama & T. Sugimura (1986) Induction of gastric carcinomas in nonhuman primates by N-ethyl-N'-nitro-N-nitrosoguanidine. J Natl Cancer Inst, 77, 179-186.

[108] Gantuya, B., D. Bolor, K. Oyuntsetseg, Y. Erdene-Ochir, R. Sanduijav, D. Davaadorj, T. Tserentogtokh, D. Azzaya, T. Uchida, T. Matsuhisa & Y. Yamaoka (2018) New observations regarding Helicobacter pylori and gastric cancer in Mongolia. Helicobacter, 23, e12491.

[109] Liu, H., D. S. Merrell, C. Semino-Mora, M. Goldman, A. Rahman, S. Mog & A. Dubois (2009) Diet synergistically affects helicobacter pylori-induced gastric carcinogenesis in nonhuman primates. *Gastroenterology*, 137, 1367-79.e1-6.

Chapter 5

MALT Stomach Lymphomas: Aspects of Diagnosis and Treatment

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Abstract

Marginal zone lymphoma (LMZ) accounts for 5–15% of all NHL in Europe. This option includes splenic (0.7%), nodal (2.4%) and extranodal (MALT-Mucosa-Associated Limphoid-Tissue) LMZ –5%. Extranodal variants of MALT lymphomas can occur in any organ due to chronic antigenic stimulation. The most frequent localization associated with *Helicobacter pylori* (*Hp*) infection is the stomach - 30%. The gastrobiopsy material of 115 patients with lymphoid cell infiltrates in the gastric mucosa was studied, a complex of morphological diagnostic criteria for MALT gastric lymphoma for gastrobiopsy was developed based on a combination of histological and immunohistochemical characteristics of tumor cells, the nature of their growth. It is known that the mandatory initial therapy for local stages of *Hp*-positive MALT lymphoma of the stomach is the eradication of *Hp*. 68 patients with stages I – II of gastric MALT lymphomas were observed. Anti *Hp* therapy resulted in 87.8% of complete remissions, with a median duration of 51 months. The median time to the onset of *Hp*-eradication was 3 months, and the median time to the implementation of the antitumor process was 5.5 months. With a median follow-up of 58 months, the median overall and relapse-free survival was not achieved: 10-year OS - 100%, 10-year RFS - 92. 3%.

Keywords: MALT lymphoma, stomach, gastrobiopsy, morphology, immunohistochemistry, anti-*Helicobacter pylori* therapy

1. Introduction

Mucosa-associated lymphoid tissue (MALT) lymphoma is an indolent extranodal marginal zone B-cell lymphoma, originating in acquired MALT that is induced in mucosal barriers as part of a normal adaptive immune response to a chronic immunoinflammatory stimulus, most notably chronic infection by *Hp* [1].

MALT lymphomas can show a wide morphologic spectrum. MALT gastric lymphoma is characterized by mature cellular elements, the presence of lymphoid follicles and lymphoepithelial lesions (LELs) [2]. Histological diagnosis of MALT lymphoma of the stomach is rather difficult and is made basing on a combination

of morphological features because none of them is exclusively pathognomic for this lymphoma type [3, 4]. Immunohistochemical analysis is much importance in the diagnosis of MALT-lymphoma, especially in controversial cases [5–7].

In recent years, the principles of MALT lymphoma treatment have been defined. According to the recommendations of the European Society of Medical Oncologists (ESMO), at the first stage of treatment of patients with *Hp* + MALT gastric lymphoma, regardless of the stage, it is recommended to prescribe anti-*Hp* eradication therapy. However, there is an addition in the guidelines of the general cancer network. According to the addition Eradication Antibiotic Therapy is preferable for patients with stages I and II1 according to Lugano, in the absence of translocation t(11; 18), which is found in 15-40% of patients with gastric MALT lymphoma and is a predictor of refractoriness to antibiotic therapy. These patients with translocation t(11, 18) should in any case receive additional treatment. Alternatively, rituximab alone or radiation therapy can be used.

As an antibacterial anti-*Hp* therapy, a 3-component regimen is usually used: a proton pump inhibitor with clarithromycin, in combination with amoxicillin or metronidazole for 10-14 days. The effect should be confirmed morphologically 6 weeks after eradication therapy and at least 2 weeks after discontinuation of the proton pump inhibitor.

Patients with a widespread tumor process - more than stage IIe require a systematic approach in the presence of indications for therapy.

2. Part 1. Morphoimmunological aspects of the diagnosis of MALT gastric lymphomas

2.1 Materials and methods

The study was performed on gastrobiopsies from 115 patients with lymphoid cell infiltration of gastric mucosa including 71 patients with MALT lymphoma of the stomach, 23 patients with reactive (nontumor) lymphoid infiltrations, 21 patients with non MALT type peripheral small B-cell gastric lymphomas (cytological grade I or I–II FL, MCL), Burkitt's lymphoma (BL). There were 48 males and 67 females in the study group. The study was carried out using histological and immunohistochemical methods. Immunohistochemical study was performed on cryostatic and paraffin sections. The reaction was assessed using fluorescent microscopy. **Table 1** shows the antibodies that were used to stain the preparations.

Antibody	Application	Manufacturer
CD19, CD20, CD37	antibodies to linear antigens of B-lymphocytes	DAKO
CD3, CD4, CD5, CD7, CD8	antibodies to linear antigens of T-lymphocytes	DAKO
CD38	determination of plasma cells	DAKO
CD21 и CD23	determination of follicular dendritic cells	DAKO
HLADR, CD10	lymphoid progenitor cells, granulocytes	DAKO
immunoglobulin classes D, G, M	Ig expression	DAKO
light chains of immunoglobulins λ и κ.	determination of clonality	DAKO

Table 1.
List of antibodies used.

2.2 Morphoimmunological characteristics of MALT lymphoma

The study was performed in 71 patients aged 14 to 83 years, mean age 54.32 years. MALT lymphoma is always accompanied by active chronic inflammation which in half of cases is associated with H. p. infection; mucosal defects are found in two thirds of cases. MALT lymphoma has a very polymorphous morphological picture due to heterogeneous composition of the tumor component and a great interpatient variability of combinations. We assessed the following histological signs:

1. Cell composition with respect to nucleus shape;

2. The presence of monocytoid B-lymphocytes;

3. The presence of plasma cells;

4. The presence of tumor cell plasmocytoid differentiation;

5. LELs;

6. The presence of follicles (reactive, colonized);

7. The presence of blasts.

2.2.1 Cellular composition according to the shape of the nuclei of lymphoid cells

1. Cells with mainly round nuclei (morphologically closest to small lymphocytes: with clearcut nucleus outline, highly dispersed chromatin, non-visualized nucleoli, cytoplasm looking like a narrow, poorly outlined rim) — 5 cases (7.04%) (**Figure 1**).

2. Cells with centrocytoid morphology (small cells with cleaved or wedge-like nuclei, moderately dispersed or granulated chromatin, invisible nucleoli and a moderately wide cytoplasm rim) — 7 cases (9.86%).

3. Cells with mixed morphology (round and centrocytoid nuclei) — 53 cases (74.65%).

4. Cells with irregular nuclei — 6 cases (8.45%).

2.2.2 Tumor cell size

Tumor cell size ranged from small, similar to small lymphocytes to medium, similar to prolymphocyte and twofold greater than small lymphocytes. All MALT lymphomas were conventionally divided into 3 histological groups depending on the size of tumor cells (**Table 2**). The larger were the cells the greater were their degree of atypia and similarity to blasts.

2.2.3 Cell elements with a different morphology

Cell elements having a different morphology were present in the tumor infiltrations in parallel with the predominant type (**Table 3**).

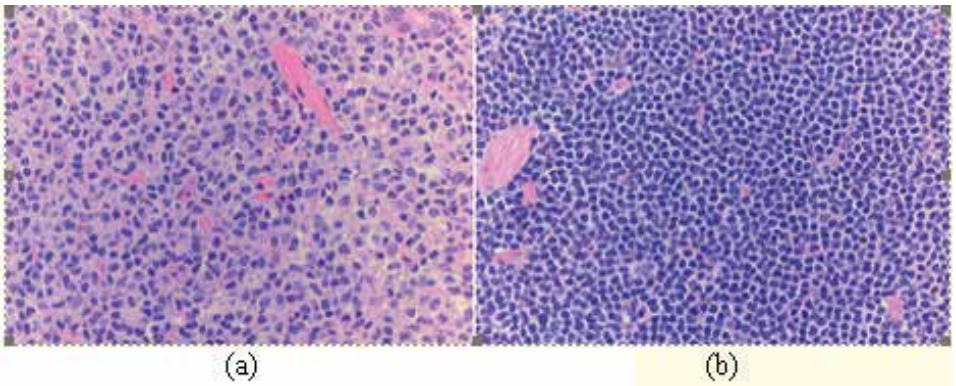


Figure 1.
Cytological features of MALT lymphoma can range from small lymphocytic (b) morphology to monocytoid (a, c) morphology (hematoxylin–eosin, original magnifications ×400 [2].

Cell nucleus shape	Small cells (n = 26)	Small and medium cells (n = 40)	Medium cells (n = 5)
round	4 (15,4%)	1 (2,5%)	0
round /centrocyt-like	17 (65,4%)	35 (87,5%)	1
centrocyt-like	5 (19,2%)	1 (2,5%)	1
irregular	0	3 (7,5%)	3

Table 2.
MAL lymphoma: Cell element morphology of lymphoid infiltration with respect to three groups.

Morphological sign	small cells (n = 26)	small and medium cells (n = 40)	medium cells (n = 5)
Monocytoid B-lymphocytes	n – 22 (84,6%) e –4 (15,4%)	n – 24 (60%) y – 16 (40%)	n – 1 y – 4
Plasma cells	n-1 (3,9%) s – 7 (26,9%) m – 18 (69,2%)	n-7 (17,5%) s – 16 (40%) m-17 (42,5%)	n-0 s – 4 m – 1
Cells with plasmocytoid differentiation	32%	18,2%	20%
Lymphoepithelial lesions	n – 12 (46,2%) y – 14 (53,8%)	n – 23 (57,5%) y - 17 (42,5%)	n - 1 y - 4
Reactive follicles-colonized	n-18 (69,2%) y – 7 (30,8%) c – 18 (37,5%)	n – 29 (72,5%) y – 11 (27,5%) c - 7 (63,6%)	n – 4 y - 1 c – 1
Blasts	n – 15 (57,7%) y – 11 (42,3%) s – 81,8%	n – 4 (10%) y – 36 (90%) s – 38,9% l – 36,1%	n – 0 y - 5

Note: n - no, y- yes, s- single, m-multiple, c-colonized, l- layers of blast more than 20.

Table 3.
MALT-lymphoma: Morphological characteristics of lymphoid cell infiltration with respect to three groups.

Monocytoid B-lymphocytes (35,2%). They were with a bean-shaped or irregular nuclei, wide and poorly stained cytoplasm.

Plasma cells (85,9%). In most cases plasma cells were located immediately under covering epithelium as a massive sheet (46.47%), or less frequently were dispersed in small portions in surface layers of gastric proper mucous plate among leukocytes

(39.43%). The plasma cells had typical appearance: nuclei, more frequently of a round or slightly irregular shape, wide cytoplasm in the form of a rim or a tongue of flame of intense pink color after Brachet staining.

Cells with plasmocytoid differentiation (21,13%). They resembled plasma cells, that was better observed after Brachet staining.

Lymphoepithelial lesions (LELs) as aggregations of 3 or more marginal zone cells destroying glandular epithelium were found in 50,7%. 6 cases (17.14%) presented with 'blast' LELs formed by large blasts (**Figure 2**).

Follicles or follicle-like structures with light-color proliferation centers consisting of centrocytes and centroblasts, a small number of mitotic figures and individual macrophages with cellular detrite in their cytoplasm were present in 14,09% cases. Proliferation centers could be surrounded by a partially preserved, thin mantle zone. Lymphoid follicle colonization when preexisting lymphoid follicle was replaced by small neoplastic cells was found practically in half the cases with follicles (15,49%).

Blasts. They were with either roundIoval/slightly irregular nuclei and 1 to 3 nucleoli, or with round bottle-shape nuclei and 1 compact, centrally located nucleolus. Amount of large blast cells varies from single cells to small group of cells (not more than 20 cells in one group).

2.3 Immunophenotyping of MALT- lymphoma

49 cases were analyzed. MALTI-ymphoma neoplastic elements were free from characteristic pathognomic immunological signs.

1. CD45 - common leukocyte antigen. There was a positive monomorphic reaction with diffuse growth of infiltrate. Cells, less frequently expressing this antigen, formed large clusters among the glands

2. B-cell markers (CD19, CD20). The infiltration cells expressed pan-B-cell markers as diffused, dense positive staining in most cases or, less frequently, as follicle-like areas on positive cell groups.

3. CD5 – antigen. Tumor cells were CD5-negative in all cases.

4. T-cell markers (CD8, CD4). T-cell component was present in most cases as single diffused cells, with CDI8 cytotoxic lymphocytes slightly predominating over CD4-helpers.

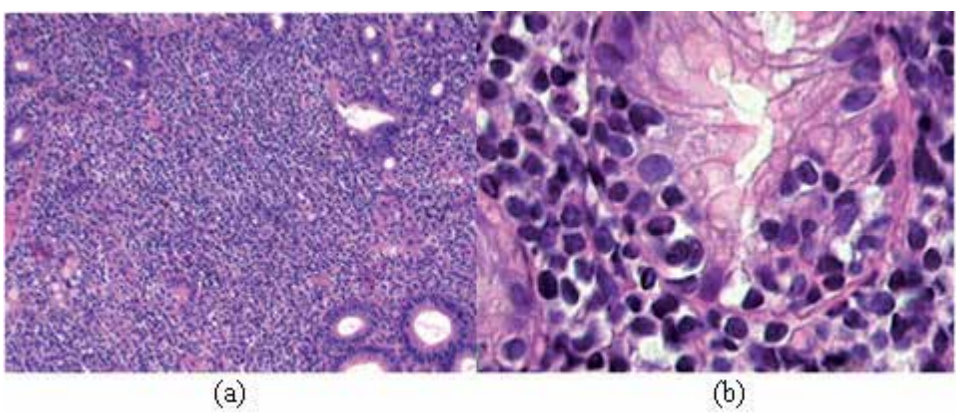


Figure 2.
Lymphoepithelial lesions (LELs) as aggregations of 3 or more marginal zone cells destroying glandular epithelium.

5. CD23 marker. Tumor cell reaction with CD23 was negative in more than 90% of cases.

6. CD21 and CD23 markers. FDCs were found in 19 cases. They formed a network among CD23-negative cells of tumor infiltration, though more frequently (17 cases, 89.47%) looked as loose clusters in CD21-positive infiltration (5 cases) or in CD21-negative infiltration (12 cases).

7. CD38 marker was assessed on both tumor cells and plasma cells. The tumor cell reactivity was negative; CD38- positive plasma cells were located in surface mucosa as foci or layers.

8. CD10 marker. All cases were CD10-negative.

Expression of CD20, CD3, IgD, IgG, IgM, and Ig λ and κ light chains was studied in 35 paraffin sections:

- clear-cut CD20 expression on tumor infiltration cells was found in all cases.
- CD3 was expressed on single diffuse cells among B-cells.
- Ig of all three classes were expressed on plasma cells.
- tumor cells demonstrated no Ig expression.
- Ig λ and κ light chains were expressed on plasma cells and very weakly on lymphoid neoplastic cells, though restriction of one of the chains was not found.

Therefore, all paraffin sections demonstrated plasma cell polyclonality, i.e. expression of different classes of Ig without restriction of one of the chains.

2.4 Differentiation criteria in the diagnosis of MALT-lymphoma of the stomach and morphologically similar lesions

2.4.1 Reactive lymphoid infiltrations of the stomach

The study was performed in gastrobiopsies from 23 cases aged 18 to 85 years, mean age was 53.47 years. More than half (56.52%) of the gastrobiopsies demonstrated mucosal defects of different severity: from surface erosion to ulcer. All 23 cases had signs of active chronic inflammation, mainly of moderate or severe degree (39.13 and 56.52% respectively). Twenty two (95.65%) cases presented with *Helicobacter pylori* in mucosa or in gland lumens on epithelial surface, the degree of contamination correlating with inflammation activity.

2.4.1.1 Tumor cell size

Lymphoid cells of the studied infiltrates were predominantly small (86.96%). There were no infiltrations consisting of medium-sized cells among the cases studied.

2.4.1.2 Cellular composition according to the shape of the nuclei of lymphoid cells

Morphological signs of reactive lymphoid cell infiltrates were as follows: cells with centrocytoid morphology of nuclei, without signs of cytological atypia, or with mild cytological atypia, predominated.

2.4.1.3 Cell elements with a different morphology

1. Monocytoid B-lymphocytes were not detected in any of the investigated infiltrates.

2. Plasma cells in all 23 cases were found in large numbers in the form of layers under the surface integumentary epithelium.

3. Cells with plasmacytoid differentiation were not found.

4. Lymphoepithelial lesions were present in about 1/5 of cases. LELs consisted of more than 3 small lymphoid cells, and the glands were distorted. Infiltration of the epithelium of the glands with segmented leukocytes or so called crypts of abscesses were mainly observed.

5. Follicles were found in 34,78% of samples as round or slightly irregular, light-color centers of proliferation with a typical centrocyte or centroblast composition and single macrophages with wide cytoplasm and phagocyted bodies. Germinal centers were surrounded by a narrow, denser and darker (after hematoxylin and eosin staining) mantle zone of small lymphoid cells with round nuclei. There were no cases with follicle colonization.

6. Two (8.7%) cases presented with single large (blast) cells. These cells looked like centroblasts or immunoblasts and were diffused in small cell infiltration.

2.4.1.4 Immunophenotyping of reactive lymphoid infiltrates

1. CD45 marker. A positive reaction was detected on all cells of the infiltrate, which were mainly in the form of small groups or loose diffuse clusters.

2. T, B-cell markers (CD19, CD20, CD8, CD4). There was a mixed cell infiltrate, where T-lymphocytes predominated over B-lymphocytes, or less often were in equal proportions, while CD8-positive T-lymphocytes predominated.

3. CD21, CD23, CD5, CD10. There was a negative reaction of infiltrate cells.

4. When reacting with CD21 and CD23, the presence of a positive, well-expressed, arranged network of FDC reactive follicles was noted.

5. CD38 - marker. Expression on plasma cells was noted.

To sum up, majority cases of reactive infiltrates have mild cytology atypia, severe atypia cases were not found at all, monocytoid B-cells were absent, and lymphoid cells did not undergo plasmocytoid differentiation.

Thereby, lymphoid infiltrate of MALT-lymphoma, in contrast to reactive infiltrate, has tendency to be more destructive and expansive. One third of all the MALT-lymphoma cases has mild cytology atypia. As cytological atypia grows, monocytoid cells appear more frequently, amount of plasma cells progressively decrease, moreover, the frequency of follicle appearance decrease, while the majority of cases have follicle colonization.

2.4.2 Peripheral small B-cell lymphoma of the stomach

The study was performed in 21 cases aged 14 to 83 years, mean age was 48 years. The cases were represented by follicular lymphoma of I, I – II, II cytological degree (n = 10), lymphoma from cells of the mantle zone (n = 6), Burkitt's lymphoma (n = 5). Defects of the mucous membrane from surface erosion to ulcer were noted in two-thirds of the material examined. Inflammation was mild or moderate in all variants of lymphomas. *Helicobacter pylori* infection was observed in 8 cases (38.1%). Infiltration growth was focal diffuse in about a third of cases with mantle cell lymphoma and follicular lymphoma, and diffuse in 2/3 of cases. In Burkitt's lymphoma the infiltration growth was diffuse in all 5 cases. Burkitt's lymphoma consists of cells of medium size, but it can be misinterpreted as small cell lymphoma due to artificial deformation after the tissue fixation. Morphological characteristics of follicular lymphoma, lymphoma from cells of the mantle zone, Burkitt's lymphoma are compared in **Table 4**.

Thereby, morphological features, typical for MALT-lymphoma (atypical lymphoid small cells, reactive follicles, LELs) can be seen in other peripheral B-cell lymphomas, that is why ICH-research is decisive.

Among peripheral small-cell B-cell lymphomas, 7 cases of follicular lymphoma and 4 cases of mantle cell lymphoma were studied by immunohistochemistry. All studied lymphomas (lymphoma from cells of the mantle zone, follicular lymphoma) were B-cells and contained single or small groups of diffuse T-cells. In this case, the diagnosis of follicular lymphoma was made on the basis of the totality of CD23, CD38, CD10 occurring in various combinations. It should be noted that all 3 cases of gastric follicular lymphoma were CD10-negative, while the cells of the tumor infiltrate were either CD38-positive or CD23-positive. For lymphoma from cells of the mantle zone the reaction with CD5 was diagnostically significant: all cases were positive. Morphological characteristics of lymphoid cell infiltrations in gastric mucosa for lymphomas and reactive infiltrations are compared in **Table 5**.

Morphological sign	**Follicular lymphoma (n = 10)**	**Lymphoma from cells of the mantle zone (n = 6)**	**Burkitt's lymphoma (n = 5)**
Tumor cell size	small cells	small and medium	medium cells
Monocytoid B-lymphocytes	1	1	0
Plasma cells	7	2	0
Cells with plasmocytoid differentiation	1	0	0
Lymphoepithelial lesions	4	1	2
Reactive follicles-colonized	2	0	0
Blasts	9	4	5

Table 4.
Peripheral small B-cell lymphomas, Burkitt's lymphoma: Morphological characteristics of lymphoid cell infiltration in gastric mucosa.

Morphological sign	MALT lymphoma	Reactive lymphoid infiltrates	Other lymphomas (FL, MCL)
Monocytoid B-lymphocytes	35,2%	Absent	FL –10%, MCL – 16,6%
Plasma cells	85,9%	100%	FL –70%, MCL – 33,3%
Cells with plasmocytoid differentiation	21,1%	Absent	FL –10%, MCL – 0%
Lymphoepithelial lesions	50,7%	Single in 20%	FL –40%, MCL – single in 16,6%
Reactive follicles-colonized	30% (15% - signs of col.)	30%, no signs of col.	FL - 20% with col. MCL – 0%
Blasts	73,2%	10%	FL - 90%MCL – 66,6%

Note: FL- follicular lymphoma, MCL- lymphoma from cells of the mantle zone, col- colonization.

Table 5.
Diagnostically meaningful morphological characteristics.

3. Part 2. Clinical aspects of MALT gastric lymphomas

3.1 Materials and methods

Blokhin National Medical Research Center of Oncology analyzed the data of 68 patients with early stages of gastric MALT lymphoma who received initial treatment from 1973 to 2004 [8]. The median follow-up was 61 months. The prevalence of female patients was noted - 64.71% versus 35.29%, almost half of the patients - 48.7% were over 60 years old. In accordance with the criteria for dividing gastric MALT lymphomas into categories depending on the number and nature of growth of blast-transformed cells, the patients were distributed as follows: small cell subvariant - 31 (45.6%); intermediate - 22 (32.4%) and mixed - 15 (22.0%). *H. pylori* infection was found in 54 patients - 85.7%.

A comprehensive assessment of the effectiveness of therapy consisted in summarizing clinical, morphoimmunological, radiological, ultrasound and endoscopic data. To assess the long-term results, the indicators of the duration of the delaying time to relapse, event-free and overall survival were used. To determine the reliability of the results obtained, the X-2 criterion and the Student's test were used to plot the survival curves - according to the Kaplan and Meier method, the comparison of the curves was performed using the Lograng test.

3.2 Analysis of clinical characteristics

A comparative analysis of the clinical characteristics of 3 groups of patients was carried out according to morphological variants, which made it possible to identify the clinical features of each of them (**Table 6**):

- For small-cell gastric MALT lymphomas, the most characteristic was the predominance of women, elderly age (63 years), the presence of a long gastroenterological history, scant clinical symptoms, an isolated lesion of the stomach body with a predominance of ulcerative and gastritis-like macroscopic growth, stage I of the disease and the presence of *Helicobacter pylori* infection

- For the intermediate subvariant of gastric MALT lymphoma, the prevalence of women is also characteristic, the median age is 61 years, the rapid development of the disease (within 1 year), early onset of intense pain syndrome, a more pronounced tendency to the spread of the tumor in the stomach (larger lesion

Characteristics	Morphological variant of MALT lymphoma		
	Small cell	**Intermediate**	**Mixed cell**
Number of patients - 68	31 (45.6%)	22 (32.4%)	15 (22%)
Median age	63	61	41
Women (%)	67.7%	68.2%	53.3%
Duration of disease development up to 12 months	61.3%	100%	80%
Hp-infection	96.7%	90%	53.8%
Pain syndrome	80.7%	84.6%	100%
Growth form: Infiltrative ulcerative Gastritis-like Mixed	48.5% 29% 6.4%	72.7% - 9.1%	60% - 13.3%
Stage: I Stage: II	90.3% 9.7%	68.3% 40%	40% 60%

Table 6.
Characteristics of patients with stage I-II gastric MALT lymphomas.

volume) and on adjacent organs and lymph nodes, the presence of *Helicobacter pylori* infection is detected in the form of a moderate degree of bacterial content.

- Mixed-cell gastric MALT lymphomas develop in young people (median - 41 years), with intense permanent pain syndrome, with a predominance of infiltrative-ulcerative and ulcerative forms of growth, with a tendency to early spread to regional lymph nodes - by the time of diagnosis in 2 / 3 patients, stage II is detected, and *Helicobacter pylori* infection is observed in only half of the patients.

3.3 Treatment results depending on morphological subvariant

3.3.1 The immediate effectiveness of various therapies

Various approaches have been used in treating patients, including anti-*Helicobacter pylori* therapy, surgery, and systemic chemotherapy. When using eradication antimicrobial therapy in the general population, the overwhelming majority of patients achieved a complete response (CR) - 87.5%, the median duration of CR - 51 months (range: 5 months - 81 months). The effect was realized quickly: the mediana of *Hp* eradication was 3 months, and the mediana of the CR observance was 5.5 months. Surgical treatment was performed for 28 patients (41.12%).

The efficacy of the therapy, depending on the morphological subvariant was evaluated (**Table 7**).

The efficacy of treatment in the group of patients with small cell subvariant of gastric MALT lymphoma was evaluated in all 31 patients. The median follow-up in this group was 64 months. Eradication anti-*Hp* therapy, which was used as the only method of treatment only in stage I (Lugano classification, 1993) of small-cell gastric MALT lymphomas, was performed for 16 patients (51.6%).

This approach allowed achieving CR in the vast majority of patients - 14 (87.5%), and the median duration of complete remissions was 51 months (5 - 81 months). Thus, anti-*Helicobacter pylori* therapy is competent and effective in primary small-cell gastric MALT-lymphomas of stage I with *H. pylori*, i.e. positive tumors with limited tumor lesion of the stomach (stage I) (**Figure 3a**, **b**).

Treatment type	CR (%)	CR mediana/ months	Stabilization (%)	Progression (%)	Patients total (abs - %)
Small cell variant of gastric MALT lymphoma - 26 patients					
anti-H.pyloi -therapy	87.5	51	12.5	—	16 – 51.6
Surgical	100	73	—	—	12 – 38.7
Chemotherapy	—	—	100	—	3 – 9.7
Total	**26 – 83.9**		**5 – 16.1**	**—**	**31 – 100**
Intermediate variant of gastric MALT lymphoma - 22 patients					
Chemotherapy + anti-H.pyloi	71.4	37.5	7.1	—	14 – 63.7
Surgical	100	54	—	—	8 – 46.3
Total	**18 – 81.8**		**3 –13.6**	**—**	**1 – 4.6**
Mixed subvariant of gastric MALT lymphoma - 15 patients					
Surgical treatment + PCT	11	57	—	1 (7%)	12-80
Chemotherapy	1 (7%)	39	1(7%)	1(7%)	3-20
Total	**12- 80**	**—**	**1 (7%)**	**2 (13%)**	**15 – 100**

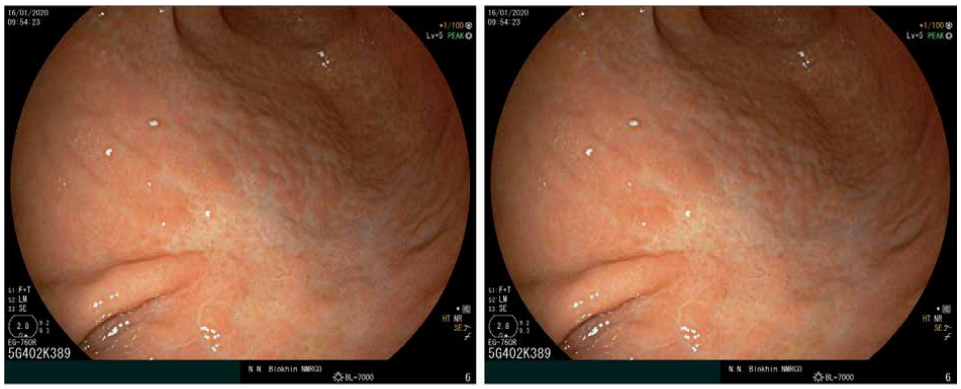
Table 7.
Direct efficacy of various types of therapy depending on the morphological subvariant of gastric MALT lymphomas.

In an earlier historical period (since 1973), 12 patients underwent surgical treatment of small-cell gastric MALT lymphomas: 11 patients underwent radical surgical treatment. The median duration of complete remissions in radically operated patients was 73 months (21 - 168 months), however, with a significant negative impact on the quality of life of patients.

The indication for chemotherapy (LVPP, COP, CHOP regimens) was the absence of *H. pylori* infection, while in all patients stabilization of the tumor process was only achieved. The efficacy of treatment in the group of intermediate subvariant of gastric MALT lymphoma was evaluated in 22 patients. The majority - 14 (63.7%) - received anthracycline-containing chemotherapy in combination with anti-*Helicobacter pylori* therapy. *H. pylori* eradication occurred in all patients within 1 to 8 months (median - 3 months), and complete remissions were achieved in 10 of them (71.4%). The median duration of CR was 37.5 months (1 - 53 months). Our experience of using induction standard chemotherapy in combination with anti-*Hp*-therapy for *H. pylori*, a positive MALT lymphoma of the intermediate subvariant, demonstrates a high frequency of achieving remissions and a stable long-term effect.

Radical surgical treatment was performed for 7 patients (31.8%): 2 - gastrectomy, 5 - distal subtotal gastrectomy were performed. The median duration of CR was 50 months (2–96 months).

The efficacy of treatment of the mixed subvariant MALT gastric lymphoma was assessed in all 15 patients, most of whom underwent surgical treatment at one of phase of disease (12/15) and only three received systemic chemotherapy alone. The immediate results of treatment of mixed gastric MALT lymphoma were better in 7 initially radically operated patients with subsequent adjuvant chemotherapy - the duration of CR was 57 months (mediana).



(a) (b)

Figure 3.
a) Endoscopic findings in subject with Hp + *MALT-lymphoma prior anti-*Hp*-therapy. b) -after anti-*Hp*-therapy (from the archive of Malikhova O.A.).*

The results of standard anthracycline-containing chemotherapy turned out to be less impressive: out of 3 patients, complete remission at stage I of the process was achieved and persists for 39 months in 1 patient.

3.3.2 Relapses

With *H. pylori* - positive MALT gastric lymphomas of a small cell structure, out of 14 patients treated with antibiotics, relapse occurred only in 1 patient (7.1%) - 7 months after the CR. In this group, among radically operated patients, relapses were detected in 3 cases (27.3%).

In the group of patients with an intermediate variant, who received fundamentally different therapy (surgical treatment and systemic PCT (palliative chemotherapy)), the course of the disease was comparable in terms of the frequency of relapse: 30.0% and 37.5%, respectively. The difference is noted only in the timing of the detection of relapse: with conservative treatment, all relapses occurred in the first 2 years, and after surgical treatment later - in terms of 2 to 5 years (after 2, 3 and 5 years).

Attention should be paid to the predominant development of local recurrence in the stomach, regardless of the type of treatment and the type of NHL.

With the mixed sub-variant MALT, no relapses were detected.

3.3.3 Long-term results

Survival rates were calculated for 5-year and 10-year periods, which is explained by the long period of observation of patients. The overall 5-year survival rate (OS) was 96.8% in the case of MALT lymphoma of the small-cell subvariant. The indicators of 5-year OS are significantly lower with the intermediate subvariant - 71.4% ($p = 0.008$). These differences become even more pronounced to 10 years follow-up (**Table 8**). A small number of cases of mixed-type MALT lymphoma does not allow us to speak of significant differences.

The analysis of the influence of other characteristics on survival allowed us to establish that gender and age are not prognostically significant. Information about the further life of patients, depending on the methods of therapy used, is strategically important: a sparing conservative approach - anti-*H. pylori* antibiotic therapy - and an aggressive surgical method provide a high 5-year OS in the small

Morphological subvariant	N	OS -Survival rate (%)			
		1 year	3 years	5 years	10 years
Overall survival					
Small cell	31	96.8 ± 3.2	96.8 ± 3.2 p = 0,00	96.8 ± 3.2	89.1 ± 0.6
Intermediate	22	85.7 ± 7.6	71.4 ± 9.9	71.4 ± 9.9	52.9 ± 13.6
Mixed	15	92.9 ± 6.9	85.7 ± 9.4	85.7 ± 9.4	85.7 ± 9.4
Disease-free survival (DFS) (%)					
Small cell	25	92 ± 5.4	88 ± 6.5	88 ± 6.5	78.7 ± 8.5
Intermediate	16	80.2 ± 10.3	66.7 ± 12.2	59.3 ± 12.9	50.8 ± 13.5
Mixed	15	100	100	100	100
Event-free survival (EFS) (%)					
Small cell	31	86.6 ± 6.2	79.7 ± 7.4	77.8 ± 8.2	68.7 ± 8.7
Intermediate	22	66.7 ± 10.3	52.4 ± 10.9	47.6 ± 10.9	28.2 ± 13.6
Mixed	15	86.7 ± 8.8	86.7 ± 8.8	80 ± 10.3	80 ± 10.3

Table 8.
Long-term results of MALT lymphoma therapy depending on the morphological subvariant.

cell subvariant of gastric MALT lymphoma, but with a significant deterioration in the quality of life in using a surgical approach.

Comparison of the conservative (chemotherapy + anti-Helicobacter antibiotic therapy) approach with the surgical approach in the intermediate variant demonstrates that OS indicators are comparable only in the first year of observation and amount to 83.8% and 80%, respectively; on the 3rd and 5th year of observation, the difference in these OS indicators increases by 25%: 81.8% and 55.8%, respectively. Disease-free survival is 15% higher with conservative therapy by the end of the first year (83.3% versus 67.5% with surgical treatment). This means that surgery for an intermediate variant of MALT lymphoma does not provide long-term control of the disease.

The analysis of survival rates in mixed gastric MALT lymphoma was not performed due to insufficient number of observations. Thus, the analysis of long-term results confirmed the clinical, morphological and prognostic heterogeneity of this tumor, a favorable prognosis of primary MALT gastric lymphoma in general, and significant differences in survival in different morphological subvariants.

4. Discussion

Currently, according to practical recommendations, as a second-line treatment for patients in whom antibiotic therapy did not lead to remission, rituximab is prescribed as a monotherapy: 4 weekly administrations at a standard dose of 375 mg/m2 [9, 10].

In a study by G. Martineli, E. Zucca et al. 27 patients were enrolled exclusively with NHL gastric MALT lymphoma who received weekly rituximab injections [10]. In this work, the clinical activity of rituximab was confirmed in patients with gastric MALT lymphoma, refractory to antibiotic therapy or without association with *Helicobacter pylori*: the overall response (OR) was 77%, CR - 46%. With a median follow-up of 33 months, only 2 relapses were detected - after 26 and 14 months.

The IELSG-19 study as of today is the largest randomized trial comparing rituximab + chlorambucil with chlorambucil alone, in which the combination has

shown the best results (OR) - 95% vs. 85%; CR - 79% vs. 63%). Based on data from this study (NCT 00210353) [11]. C. Thieblemont et al. the International Prognostic Index of Extranodal Lymphoma - MALT-IPI was developed [12]. Three factors were identified that influence event-free survival: age ≥ 70 years, Ann Arbor stage III or IV, and increased LDH levels. 5-year EFS in the low, intermediate and high risk groups was 70%, 56% and 29%, respectively.

The addition of rituximab to bendamustine provided significant advantages in a prospective study involving 60 patients with untreated extranodal MALT lymphoma: OR was 100, CR - 98%, 7-year EFS and PFS (progression-free survival) were 88% and 93%, respectively [13].

Fludarabine-containing regimens are excluded by most of the clinical recommendations from treatment standards and NHL due to the increased risk of developing second tumors [14].

In August 2020, a new treatment option for MZL relapses was registered in the Russian Federation - the lenalidomide + rituximab (R2) regimen. The high efficacy of this combination compared to rituximab + placebo was confirmed in the AUGMENT study for phase III, involving 358 patients with recurrent/refractory follicular lymphoma (295 patients) and marginal zone lymphoma (63 patients): median PFS was 39.4 months in the R2 group compared to 14.1 months in the placebo group [15].

The efficacy and safety of ibrutinib in relapses and refractory MZL was demonstrated in a st median udy of phase II involving 63 patients, of which 32 (51%) with extranodal MALT lymphomas [16]. With a median follow-up of 19.4 months median response time was not reached, and PFS - 14.2 months (mediana).

Blockade of the phosphatidylinositol 3-kinase (PI3K) pathway seems to be a very promising direction in the treatment of relapses or refractory MZL. However, drugs from this group are currently not registered in the Russian Federation.

5. Conclusion

MALT gastric lymphoma is a variant of MZL with an indolent prognosis and long survival, but a high tendency to develop relapses over time.

Diagnosis and differential diagnosis of lymphoma is difficult and includes morphological and immunohistochemical analysis. In this study, morphoimmunological criteria for the diagnosis of MALT gastric lymphoma and differential diagnosis with processes similar in histological picture (reactive infiltrates, peripheral small-cell B-cell lymphomas of the stomach) were developed.

Most informative morphological combinations were selected to differentiate MALT-lymphoma from neoplasias with a similar morphological pattern or reactive lymphoid infiltration. Tumor cell size was considered the most important morphological criterion in tumor tissue analysis: the larger the tumor cells, the greater the changes in their cytomorphological appearance involving nuclear shape, chromatin status and increased cytological atypia.

Potential value of frozen and paraffin section immunohistochemistry was assessed in the common and differential diagnoses of MALT-lymphoma. The immunological diagnosis must be made basing on changes in immunoarchitecture if other immunological criteria are absent.

The consistent application of different treatment methods is the key to the success in treating this category of patients. Conservative anti-*Helicobacter pylori* therapy in the early stages in primary patients gives good results and is a strategy necessary to maintain adequate organ function and prevent the toxic effects of chemotherapy, which, in turn, is used in the progressive refractory course of the disease. New drugs and targeted agents open up the possibility of treating people

with severe pre-treatment (inadequate therapy in the early stages of the disease), as well as in certain categories of elderly patients with severe comorbidity.

Conflict of interest

We hereby inform you that there is no conflict of interest.

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References

[1] Pereira MI, Medeiros JA. Role of Helicobacter pylori in gastric mucosa-associated lymphoid tissue lymphomas. World J Gastroenterol. 2014. 21;20(3): 684-98. doi: 10.3748/wjg.v20.i3.684. PMID: 24574742

[2] Skinnider BF. Lymphoproliferative Disorders of the Gastrointestinal Tract. Arch Pathol Lab Med. 2018;142(1):44-52. doi: 10.5858/arpa.2016-0610-RA. Epub 2017 Aug 22.PMID: 28829152

[3] Malikhova OA, Poddubnyĭ BK, Poddubnaia IV, Moskalenko OA, Kokosadze NV, Probatova NA, Kovrigina AM. Clinical and morphological aspects of MALT-gastric lymphoma. Eksp Klin Gastroenterol. 2010;(6):24-9.PMID: 20731161

[4] Tzankov A, Hittmair A, Müller-Hermelink HK, Rüdiger T, Dirnhofer S.Virchows Primary gastric follicular lymphoma with parafollicular monocytoid B-cells and lymphoepithelial lesions, mimicking extranodal marginal zone lymphoma of MALT. Arch. 2002;441(6):614-7. doi: 10.1007/s00428-002-0670-5. PMID: 12461620

[5] Hiyama T., Haruma K., Kitadai Y., et al. B cell monoclonality in Helicobacter pylori associated chronic atrophic gastritis. Virchows Arch.2001; 438: 232-237.

[6] Zett A., Meister S., Katzenberger T., et al. Immunohistochemical analysis of B cell lymphoma using tissue microarrays identifies particular phenotypic profiles of B cell lymphomas. Histopath. 2003;43:209-219.

[7] Burke JS. Lymphoproliferative disorders of the gastrointestinal tract: a review and pragmatic guide to diagnosis. Arch Pathol Lab Med. 2011;135(10):1283-97. doi: 10.5858/arpa.2011-0145-RA.PMID: 21970484

[8] Moskalenko O.A., Osmanov D.S., Probatova N.A., et al. Pervichnye MALT-limfomy zheludka u bol'nykh pozhilogo vozrasta // Journal of Modern Oncology. 2001; Vol. 3: N. 4: P. 146-151.

[9] NCCN clinical practice guidelines in oncology (NCCN guidelines) B-cell Lymphomas. Version 1. 2020. https://www.nccn.org/professionals/physician_gls/default.aspx/.

[10] Martineli G., Zucca E. et al.Clinical activity of rituximab in gastric marginal zone non-Hodgkin's lymphoma resistant to or not eligible for anti-Helicobacter pylori therapy. J Clin Oncol. 2005 Mar 20;23(9):1979-83. doi: 10.1200/JCO.2005.08.128

[11] Zucca E, Conconi A, Martinelli G, et al. Final Results of the IELSG-19 Randomized Trial of Mucosa-Associated Lymphoid Tissue Lymphoma: Improved Event-Free and Progression-Free Survival With Rituximab Plus Chlorambucil Versus Either Chlorambucil or Rituximab Monotherapy. J Clin Oncol. 2017;35(17): 1905-1912. doi: 10.1200/JCO.2016.70.6994.

[12] Thieblemont C.. Cascione L., Conconi A. et al. A MALT lymphoma prognostic index. Blood. 2017 Sep 21;130(12):1409-1417. doi: 10.1182/blood-2017-03-771915.

[13] Salar A, D. E, Panizo C, et al. First-line response-adapted treatment with the combination of bendamustine and rituximab in patients with mucosa-associated lymphoid tissue lymphoma (MALT2008-01): a multicentre, single-arm, phase 2 trial. Lancet Haematol. 2014;1(3):e104-e111. http://dx.doi.org/10.1016/S2352-3026(14)00021-0.

[14] Zinzani PL, Pellegrini C, Broccoli A, et al. Fludarabine-Mitoxantrone Rituximab regimen in untreated indolent non-follicular non-Hodgkin's

lymphoma: experience on 143 patients. Hematol Oncol. 2015;33(3): 141-146. doi: 10.1002/hon.2151.

[15] LeonardJ. P., Trneny M., IzutsuK. et al. AUGMENT: A Phase III Study of Lenalidomide Plus Rituximab Versus Placebo Plus Rituximab in Relapsed or Refractory Indolent Lymphoma. J Clin Oncol. 2019 Mar 21: doi: 10.1200 / JCO. 19.00010.

[16] Noy A., De Vos S., Thieblemont C. Targeting Bruton tyrosine kinase with ibrutinib in relapsed/refractory marginal zone lymphoma. Blood .2017/ 129 (16): 2224-2232. doi: 10.1182/blood-2016-10-747345.Subsections

Chapter 6

Virulence Markers, Genotypic versus Phenotypic Resistance and New Treatment Strategies in *Helicobacter pylori* Infection

Maria Teresa Mascellino, Massimiliano De Angelis, Dania Al Ismail and Alessandra Oliva

Abstract

This chapter aims at studying the microbial virulence determinants and markers of *Helicobacter pylori (Hp*), the molecular diagnostic of *Hp*, the growing antibiotic resistance with the related problem of heteroresistance, the genotypic resistance to antimicrobials compared with the phenotypic methods and the new treatment strategies for *Hp* eradication also evaluating new antimicrobial agents (furazolidone, vonoprazan). The virulence markers cover an important area in *Hp* pathology due to the correlation between these and the different diseases. The *Hp* molecuar diagnosis is fast, accurate and reliable over the traditional methods that are expensive and time- consuming. Therapy regimens used over the past decade are declining in efficacy being the *Hp* treatment bedevilled by drug-resistant strains. New treatment strategies are under study worldwide. The determination of the genetic resistance to antibiotics is very useful when used directly on gastric biopsies for prediction of antibiotics ineffectiveness or for addressing changes in previous treatments.

Keywords: *Helicobacter pylori* infection, virulence markers, molecular diagnostic, heteroresistance, antibiotic resistance, updated treatment strategies

1. Introduction

Helicobacter pylori (Hp) was first isolated in culture media by Warren and Marshall in 1983. Since then much progress has been made regarding all the characteristics, the pathology and the resistance to antibiotics of this microorganism, However the history of this bacterium goes back a long time ago. In 1892 Bizzozzero described the presence of helical microorganisms in gastric mucosa of dogs and cats. In 1896 Salomon demonstrated the transmission to rats. In 1899 Iaworski & Krientis evidenced helicoidal microorganisms in human gastric biopsies. In 1967 Luck, in 1975 Steer, in 1979 Fung and in 1982 Gregory showed the bacterial ultrastructural morphology. In 1983 Marshall & Warren identified for the first time *Hp* [1]. In 1984 Langenberg hypothesized a relationship between stomach urease and spiral germs [2].

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Hp is a Gram-negative, spiral-shaped bacterium, with positive findings for urease, oxidase and catalase. It colonizes the human gastric epithelium. The main pathologies related to *Hp* infection are the following: chronic active gastritis, peptic ulcer disease, gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Further, epidemiological and eradication studies have demonstrated a casual relationship between *Hp* infections and endothelial dysfunction, leading to vascular diseases [3, 4]. Generally, the colonization occurs primarily during childhood especially in the developing areas, usually in the same family for a cohort effect [5]. This colonization is widely asymptomatic even if a long-lasting infection can be established in some subjects. After colonization all patients with *H. pylori* infection develop histological gastritis, which corresponds to classical chronic gastritis and is characterized by the infiltration of neutrophils and other inflammatory cells. However, most patients are asymptomatic for life, while few of them will develop a digestive disease. Infection is virtually lifelong in the absence of treatment, implying that evasion of the host response is efficient.

Hp infection is widespread with about 50% of world population infected. In developing countries, especially in lower socioeconomic classes, the prevalence is higher (about 80%), whereas in the developed areas such as the USA, Canada, Japan, and Western Europe, the prevalence is much lower (about 25–30%) [6–9].

The infection outcome mainly depends on three factors: strain virulence, host response and environmental factors. The strain pathogenicity depends on the virulence markers present in the bacterium. Host response shows the peculiarity that it is not protective, indeed in some cases may worsen the patients situation. Environmental factor, such as cigarette smoking is a major risk factor for duodenal ulceration among *Hp*-infected persons. Other important factors include stress, childhood living conditions, diet, alcohol and NSAIDs (non- steroidal anti-inflammatory drugs) use [10, 11].

Aim of this chapter was to study the virulence markers involved in the pathogenicity of *Hp* such as Vacuolating cytotoxin (VacA) and vacA gene (vacuolating cytotoxin gene A), intermediate region (i) of vacA, CagA protein and the cag pathogenicity island. The molecular diagnostic of the microorganism, the genotypic resistance related to the phenotypic one, the antibiotic resistance and the updated treatment strategies including also non-antibiotics therapy are similarly studied.

2. Pathogenicity and virulence markers

2.1 Pathogenicity

The pathogenicity of *Helicobacter pylori* is shown in **Figure 1**.

Here it is reported the course of *Hp* infection in the patients beginning from the childhood to the advanced age considering what may happen at high level or at low level of acidity. The infection starts with the colonization of the microorganism in the normal gastric mucosa which can lead to an acute *Hp* infection at a low level of acid production. This in turn can result in a chronic *Hp* infection which may be asymptomatic for a lifetime or produce a non- atrophic pangastritis which can lead to MALT lymphoma. At high level of acidity the infection can result in an antral predominant gastritis with an evolution to duodenal ulcer. At a low level of acidity it can result in a corpus predominant atrophic gastritis which may evolve in gastric ulcer, intestinal metaplasia, dysplasia and gastric cancer in 2% of infected patients [12].

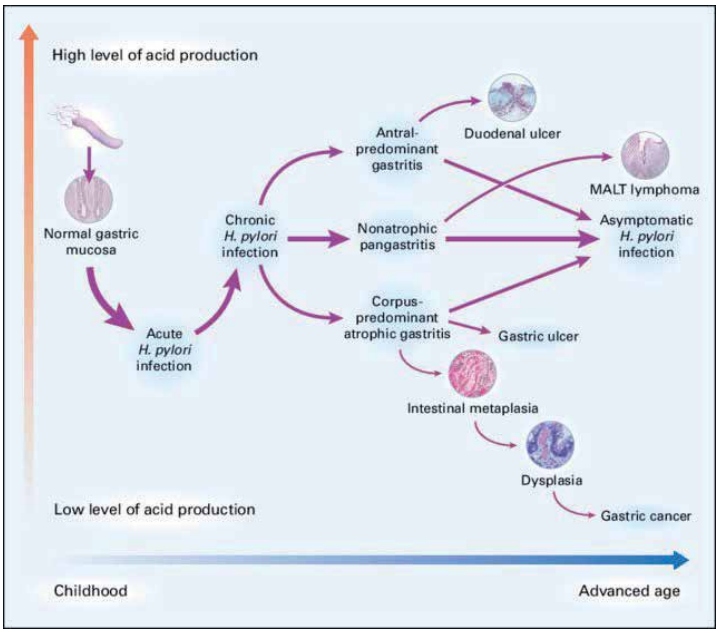

Figure 1.
Pathogenicity of Helicobacter pylori. *From Suerbaum et al. [11].*

2.2 Markers of virulence

Gastric colonization is a prerequisite for *Hp*-associated disease and this is mediated by both flagella and urease: mutant strains lacking these features cannot establish infection. The most important determinants of virulence are the following: (a) Vacuolating cytotoxin (VacA) and vacA gene (vacuolating cytotoxin gene A), (b) Intermediate region (i) of vacA, (c) CagA protein and the cag pathogenicity island, (d) Hps60 superficial protein, (e) BabA adhesion, (f) Urease virulence determinant.

2.2.1 Vacuolating cytotoxin (VacA) and vacA gene

This is a protein found in culture supernatant that induces vacuolation in a variety of cultured epithelial cell lines, The expression of *Hp* vacA gene leads to the production of a vacuolating cytotoxic protein VacA, (present only in about 40% of isolates), which is responsible for inducing the formation of acidic vacuoles. This secreted protein toxin is responsible for the gastric epithelial erosion observed in infected hosts [13].

In **Figure 2** the schematic structure of Vac A is reported [10].

In this figure the mosaicism of VacA is underlined [13]. In fact the vacA gene contains two variables regions: the s- (signal) region encoding part of the signal peptide with the N-terminus of the mature protein (hydrophilic part) and the m- (middle) region encoding C-terminal portion of the final processed polypeptide (hydrophobic part). These regions are both cleaved upon secretion to yield a mature toxin monomer of 87–95 kilodaltons [14]. The combination between the *s* and *m* regions causes the strains virulence and is correlated with the kind of disease. In fact s1-type strains are associated with vacuolating activity, s2-type is non-vacuolating, m-region causes the specificty of cell vacuolating: m1 alleles are more toxigenic than strains with m2 alleles, vacA s1m1 is the most toxigenic combination and is associated with duodenal and gastric ulceration.

The subtype s1a m1 is the most virulent strain involved in patients with ulcer.

A further region of the vacA gene (i- intermediate region) has been reported in literature to be associated with gastric cancer [15].

2.2.2 CagA protein and the cag pathogenicity island

Cag pathogenicity island is a chromosomal region with about 37,000 bp and 29 genes. Four genes are similar to the components of the type IV secretion system. Proteins encoded by the island are involved in two major processes: the induction of interleukin-8 production by gastric epithelial cells and the translocation of CagA

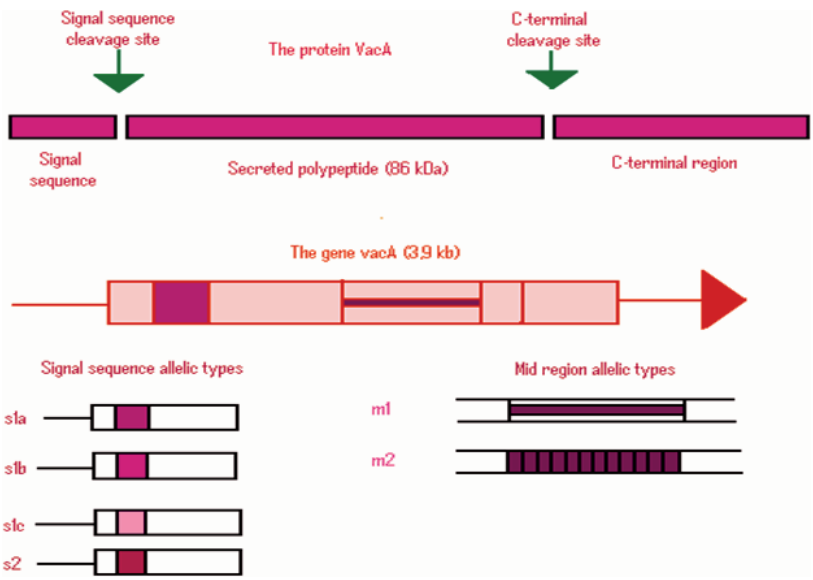

Figure 2.
The protein VacA. From Atherton et al [10].

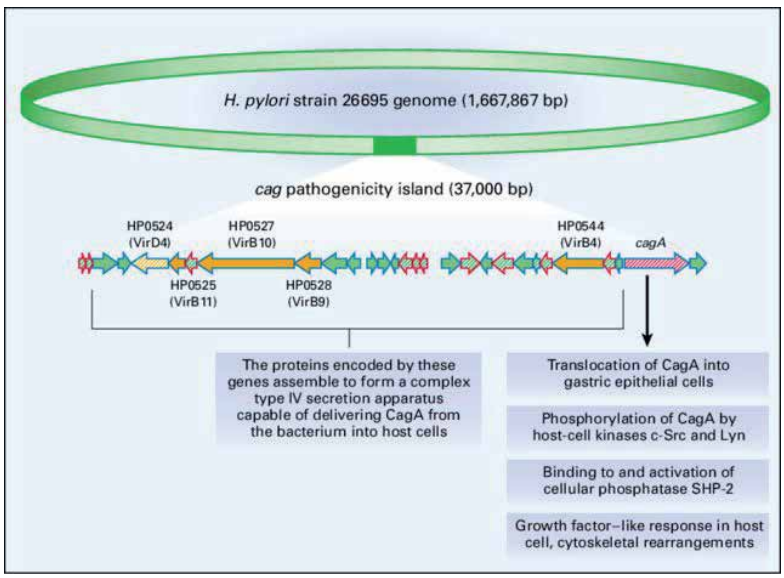

Figure 3.
The Cag pathogenicity Island. Modified by Suerbaum et al [11].

from the bacterium into the host cell by forming a syringe and a needle apparatus that delivers CagA protein (a protein of about 1200 amino-acids whose size varies between strains). Some genes are essential for the induction of interleukin-8; other genes are required for the translocation of CagA. The cagA-positive strain increases the risk of athrophic gastritis development and mucosal inflammation [16]. (see **Figure 3**).

2.2.3 Heat shock proteins (Hsps) or stress proteins

Hsps are families of highly conserved proteins serving as a strong antigenic target for the immune response linked with pathology. *H. pylori* produces 2 Hsps: a gro Es-like HspsA (size 13 kd) and a gro E1-like HspsB (size 54–60 kd). Hsp60 is shared by *H.pylori* and eukariotic cells (autoimmune response) [17, 18]. The role of heat-shock proteins in immune reactions is complex especially for the cellular effects of this proteins during the recognition processes by innate immunity. Heat-shock proteins (HSPs) are expressed at high levels by bacterial pathogens during adaptation to intracellular survival. The Hsps of both pathogens and hosts are involved in the activation of the receptors in innate immune response and in the presentation of antigens for the adaptive immune response [17, 18].

2.2.4 Bab A adhesions

The microorganism needs of adhesins for beginning its infective process. The presence of bacterial adhesins devoted to the attachment to human gastric epithelium, is essential [19]. *Helicobacter pylori* adherence to the human gastric mucosa involves specific bacterial adhesins and cognate host receptors. *babA* gene codes for the blood group antigen-binding adhesin BabA whereas the babB product is associated with a non-binding phenotype. BabA major adhesin is directed to the fucosylated Lewis b blood group antigen not present in all kinds of gastric cells [19].

2.2.5 Urease virulence determinants

The enzymatic activity of urease manages to break the urea molecule in bicarbonate ion and ammonia so that it is able to neutralize the gastric acid and is also correlated with the dual function of adhesivity and immunogenicity. The urease binds to the CD74 receptors of gastric epithelial cells [20]. It can be suggested that urease is specifically important for the attachment of *Hp* and for the pro-inflammatory immune response initiated by the bacterium. The binding of the subunit urease B to CD74 expressed on gastric cells may therefore contribute to increase the bacterial virulence during infection [20].

3. Molecular diagnostic of *Helicobacter pylori*

Helicobacter pylori is a fastidious bacterium difficult to grow in the common media culture. Essential conditions for *Hp* culture were the following: microaerophilic atmosphere, temperature of 37° (range 33°-40°), presence of 0.5% glycine. The appropriate culture medium such as Pylori Selective Agar (bio-Merieux, Marcy L'Etoile France) contains 5% sheep blood and antibiotics (amphotericin, vancomycin and trimethoprim). The incubation lasts 10 days under CO_2 atmosphere. This method is complicated, expensive and time-consuming so that when the isolation of the strain is unnecessary, the molecular method is very useful for its rapidity and appropriateness.

Helicobacter pylori presence in gastric specimens can be assessed by various methods including molecular PCR assay (GenoType HelicoDR kit) [21]. PCR is a more sensitive method (84.3% sensitivity, 75.0% specificity), with a higher *H. pylori* detection rate compared to culture [22].

Tissue obtained from gastroscopic biopsy was minced using a sterile scalpel, lysed by tissue lysis buffer and proteinase-K enzyme (Bioneer, Daejeon, Korea), and incubated for 10 min at 60°C. Ten total DNA was extracted with an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). This kit contains a glass filter in a column tube that can bind efficiently to DNA in the presence of salts. Additional washing steps were performed for proteins and salt removal. Aliquots of 50 µL were used for PCR amplification as reported elsewhere [23].

Molecular methods such as PCR offer marginal improvements when done on biopsy material, but have the advantage of being able to accurately identify *H. pylori* in areas outside the stomach where cultures usually fail. PCR can detect low numbers of organisms in gastric juice, bile, stool and oral secretions. Because of its high sensitivity it can also be used for epidemiologic investigations of environmental sources [24]. Molecular methods have been used in variable specimens other than gastric mucosa [25].

4. Antibiotic resistance and heteroresistance

4.1 Antibiotic resistance

The antibiotics once regarded as the first choice for *Hp* infection (such as metronidazole and clarithromycin), included in all therapeutic regimens, are now declining in efficacy because of their extensive use in many areas for unrelated infections. Metronidazole (MZ) mostly showed a very high resistance worldwide achieving a level up to 78.2% in China [26]. Clarithromycin (CLA) resistance rates have currently reached high levels, such as 30% in Italy and Japan, 40% in Turkey, and more than 70% in China, although rates in Sweden, Taiwan, and Germany are lower [27]. CLA was once considered the most powerful antibiotic for *Hp* infection. The local pattern of *Hp* resistance to CLA results as being crucial in each area, considering that in countries where CLA resistance is above 15–20%, this drug should not be used. CLA-resistant *Hp* has been extensively studied: its prevalence has become increasingly higher in many geographical areas. Fluoroquinolone (the most common fluoroquinolone is Levofloxacin LEV) resistance has been increasing worldwide in recent years achieving 20% in Italy, 13.3% in Germany, and 19.2% in China [26, 28]. These data are especially important in those regions planning a levofloxacin-based therapy because resistance to fluoroquinolones generally shows a major impact on the success of treatment [29].

In a study conducted in an Academic Hospital in Rome by our group on 80 *Hp* strains isolated from 80 biopsy samples in infected patients, the resistance percentage measured phenotypically through MIC values to MZ, CLA, LEV, TE (tetracycline) and AMX (amoxicillin) resulted as follows: 61.6% (50/80), 35% (28/80), 20% (16/80), 2.5% (2/80), and 1.25% (1/80), respectively. The MICs (Minimum Inhibitory Concentrations) resulted as being very high mainly to MZ showing three strains with MIC = 256 µg/mL and to CLA showing four strains with MIC ranging from 64 µg/mL to 128 µg/mL. As for LEV the MIC ranged from 0.25 µg/mL to 32 µg/mL (only one strain showed a MIC equal to 32 µg/mL) [30].

Multidrug resistance (the contemporaneous resistance to two or more antibiotics) is reported in **Figure 4**.

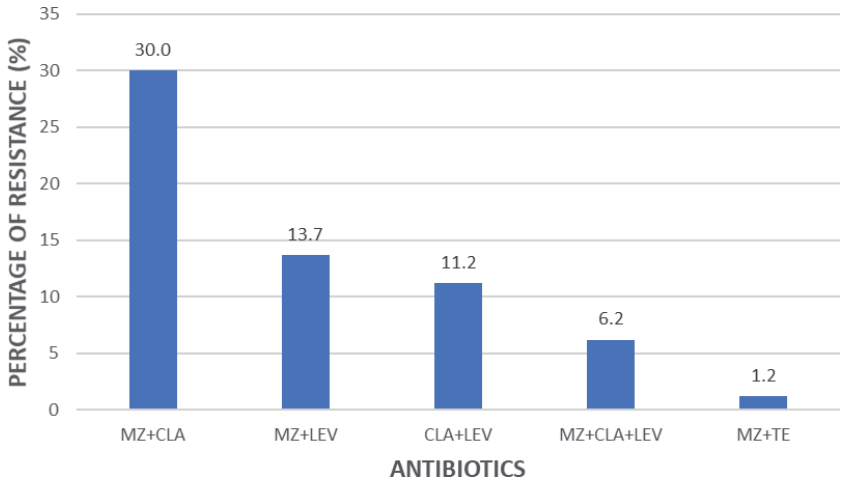

Figure 4.
Characteristics of phenotypic antibiotic resistance in Helicobacter pylori *strains. From Mascellino et al. [30]. Notes: Proportion of phenotypic resistant* Helicobacter pylori *isolates (%) to the respective antibiotics in an Academic Hospital in Rome (Italy). Abbreviations: MZ: metronidazole; CLA: clarithromycin; LEV: levofloxacin; TE: tetracycline. Resistance belonging to the association CLA + MZ was the highest one being detected in 30% of samples (24/80), resistance to the combinations MEZ + LEV and CLA + LEV ranged between 13.75% (11/80) and 11.25% (9/80 strains) respectively. Only 6.25% samples (5/80) harbored the triple resistance MZ + CLA + LEV. As far as the dual combination MZ + TE is concerned, resistance was found just in one strain (1.25%). In China too, a similar trend of multidrug resistance of* Hp *is found [26]. In general, the antibiotic combinations consisting of MET, CLA and LEV had higher combined resistance rate, whereas the antibiotic combinations recommended in bismuth-containing quadruple therapy (Bismuth, Omeoprazole, MZ, TE) had lower combined resistance rates.*

Antibiotics Patients	**MZ Strain genotype**	**CLA Strain genotype**	**AMX Strain genotype**	**LEV Strain genotype**	**TE Strain genotype**
1		S (A) R(C/F) cagA+s1m2	S (A) R(C/F) cagA+s1m2		
2	S (A) R(C/F*) cagA+s1m1	S (A) R(C/F*) cagA+s1m1			
3		S (A) R(C/F) cagA+s2m2	S (A) R(C/F) cagA+s2m2	S (A/C) R(F) cagA+s2m2	S (A) R(C/F) cagA+s2m2
4		S (A) R(C/F) cagA+s1m1		S (A) R(C/F) cagA+s1m1	
5	S(A) R(C/F) cagAs1m1	S (A) R(C/F) cagA+s1m1	S (A) R(C/F) cagA+s1m1	S (A) R(C/F) cagA+s1m1	

MZ-metronidazole, CLA-clarithromycin, AMX-amoxicillin, LEV-levofloxacin, TE-tetracycline S-susceptible, R-resistant, A-antrum, C-corpus, F-fundus.
** No growth.*

Table 1.
Heteroresistance of 5 strains (Mascellino, unpublished data).

4.2 Heteroresistance (HR)

Heteroresistance is defined as the concomitant presence of a different susceptibility pattern in the different districts of a single stomach in the same patient [31]. This is a common occurrence in *Hp* population and can be explained either as the result of multiple infections (unrelated isolates) or as the presence of susceptible

and resistant variants of the same strain (related isolates). In the latter case, HR has been described either as intra-district when susceptible and resistant isolates are present at the same time in the same site of gastric mucosa or as inter-district when multiple strains colonize different areas of the stomach [32, 33]. Heteroresistance of 5 strains out of 80 considered above is reported in **Table 1**.

For each of these patients, we found a different antimicrobial pattern in the strains isolated from antrum (susceptible) and corpus/fundus (resistant) towards CLA (five patients), MZ (two patients), AMX (three patients), LEV (three patients) and TE (one patient). The strain genotypes, identified on the basis of virulence genes (cagA and vacA), were the same for both loci in pairs of isolates (susceptible and resistant) obtained from different regions (A,C and F) of a single stomach (cag + s1m2 in one patient, cagA+s1m1 in three patients and cagA s2m2 in one patient).

5. Phenotypic resistance versus genotypic resistance

The phenotypic resistance was calculated on the basis of MIC (Minimum Inhibitory Concentration) performed on the biopsy samples. Interpretation of susceptibility test results was performed in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2020) recommendations [34]. In order to define strains resistance the following MIC breakpoints were used: $S \leq 0.125$ μg/ml and $R > 0.125$ μg/ml for AMX; $S \leq 0.25$ μg/ml and $R > 0.5$ μg/ml respectively for CLA; $S \leq 1$ μg/ml and $R > 1$ μg/ml for both TE and LEV; $S \leq 8$ μg/ml and $R > 8$ μg/ml for MZ.

The genotypic resistance is based on the point mutations present on the chromosome. Common resistance mechanisms to clarithromycin include point mutations in the bacterial domain V of 23S rRNA, which prevents antibiotic binding [35]. There are 3 point mutations in the 23S rRNA gene: A2143G, A2142G, and A2142C; these account for 90% of cases for primary resistance in Western countries [36]. Fluoroquinolone agents (mainly levofoxacin, which is a broad-spectrum quinolone) are an alternative therapy for infections caused by *H. pylori* and serve as second-line treatment [37]. Fluoroquinolone targets are the DNA gyrase, an enzyme responsible for negative supercoiling during the DNA replication process. This enzyme contains two A subunits and two B subunits, encoded by *gyrA* and *gyrB*, respectively [38]. Resistance to clarithromycin and fuoroquinolones, which is mostly acquired through point mutations, can be detected by molecular techniques. GenoType HelicoDR (Hain Life Science, Germany) test is a molecular diagnostic method for easy and simultaneous detection of frequent point mutations responsible for clarithromycin and fuoroquinolones resistance [21].

Genotypic susceptibility testing can be also performed by a real-time PCR followed by melting curve analyses using fluorochrome labeled hybridization probes for identifying the mutations on the 16S rRNA conferring resistance to TE [28].

5.1 Correlation between genotypic and phenotypic methods

The correlation between genotypic and phenotypic methods is reported in **Table 2**.

The PCR method results as being very useful in detecting the resistant strains in comparison with the E-test technique which primarily detects the susceptible ones. Mixed *Hp* infections were demonstrated only through the PCR in patients concomitantly yielding both wild type and resistant strains in a single gastric region. The resistance with both methods (phenotypic tests and PCR molecular tests) for CLA

Clarithromycin (CLA) Resistance 21 patients/60 (35%)		**Levofloxacin (LEV) Resistance 12 patients/60 (20%)**	
PCR	**E-test**	**PCR**	**E-test**
4 Wildtype + A2143G*	3 R	6 *gyr A* mutation codon 87	5 R
12 A2143G	8 R	4 *gyr A* mutation codon 91	MIC>1mcg/ml
	MIC>0.5 mcg/ml		
16/21 R (76%)	12/21 R (57%)	10/12 R (83%)	6/12 R (41%)

S = susceptible, R = Resistant, A2143G = point mutation on CLA23SrRNA gene conferring resistance to CLA, gyrA = gene conferring resistance to LEV.
**Refers to mixed iinfections (from Mascellino et al. [39]).*

Table 2.
Correlation between genotypic and phenotypic methods.

and TE demonstrated the superiority of PCR over the phenotypic test (data not shown). As for CLA, the resistance was detected in 76% (16/21) of patients by PCR and 57% (12/21) by E-test, whereas for LEV the same values corresponded to 83% (10/12) and 41% (6/12) respectively. In this last case the difference turned out to be statistically significant ($p < 0.05$).

The genotypic test performed directly on the biopsy samples shows some great advantages over the E-test. In fact the genotypic-resistance resulted very useful to identify mixed infections that represent a real problem possibly leading to a resistance underestimation or in the case of absence of live bacteria (coccoid forms) or contamination. The real-time PCR detects the resistant population at a lower concentration than the phenotypic tests which primarily show susceptible bacteria. Following our results [30], the E-test is unable to detect all resistant strains because when there are many susceptible bacteria compared with the resistant ones, these susceptible bacteria are identified first leading to a misclassification. On the contrary through a direct examination of gastric samples by genetic tests, a diversity of strains (susceptible, resistant or both) can be detected at the same time. Mixed *Hp* infections were demonstrated in patients yielding both wild type and resistant strains in a single gastric region at the same time. The use of genotypic tests directly on the clinical specimens could predict the antibiotic resistance addressing changes in previous treatments or for evaluating the primary resistance to antibiotics (ie CLA) in order to avoid administration of ineffective antimicrobials [23, 38].

6. Antibiotic therapy

Therapy regimens used over the past decade are declining in efficacy being the *Hp* treatment affected by drug-resistant strains. New treatment strategies are under study worldwide. The knowledge of the local susceptibility to the antibiotics in a single geographical area is crucial in order to establish a correct therapy. For instance as far as CLA susceptibility is concerned, it is stated that in those regions that show a resistance percentage > 15%, this antibiotic should not be used [30]. Indeed it would be possible to predict the efficacy of any treatment knowing the prevalence of antibiotic resistance for a regimen or even for a specific patient. As a matter of fact empiric therapy that takes into consideration the regional and mostly the local resistance patterns may be superior to the tailored therapy in predicting the efficacy of any regimen [40]. Hence, the regional resistance patterns and the eradication rates in the context of local environment are crucial for a correct establishment of *Hp* cure in real-world settings [41].

The old triple therapy (PPI=Proton Pump Inhibitors + clarithromycin and either amoxicillin or metronidazole) should be considered only in areas where the resistance to CLA is low (<15%) or where a high eradication success with these regimens (>85%) is well known. In general, in Western countries, clarithromycin-containing triple and sequential therapy should be considered obsolete as empiric therapies.

The resistance to CLA is reported to be increasing all over the world, and in some countries, it depends on the local seropositivity rate [42]. As far as LEV is concerned, there is a more limited number of studies evaluating susceptibility to LEV. In Italy LEV resistance has been reported to be 22–24% as well as in Portugal [43]. MZ shows a high rate of resistance (50–80%) in almost all the studied countries achieving a rate of 80% especially in developing areas [26]. Nevertheless in spite of its high resistance in vitro, it is included in the BQT (Bismuth Quadruple Therapy). This discrepancy between in vitro MZ resistance and treatment outcome may partially be explained by changes in oxygen pressure in the gastric environment, as MZ-resistant *Hp* isolates become MZ susceptible under low oxygen conditions in vivo [44]. The Bismuth Quadruple Therapy (PPI + Bismuth +MZ + TE] for 14 days has proven high efficacy in spite of MZ resistance in Europe bypassing also the quinolone resistance [29].

The new guidelines for the cure of *Hp* recommend to prolong the therapy from 10 to 14 days [45]. As first line therapy a concomitant non-bismuth quadruple therapy (PPI + AMX + MZ + CLA) may be used in those countries where the resistance to CLA is <15% otherwise the traditional bismuth quadruple therapy unaffected by CLA resistance, should be used. The BQT (PPI + Bismuth+MZ + TE, PBMT) results as being very useful in the countries where particular *Hp* high resistance is detected and when the AST (Antimicrobial Susceptibility Testing) is complicated to perform. In contrast if a bismuth-based quadruple therapy is used in these different situations, it is not recommended to perform AST because a risk of having a TE-resistant strain is extremely low and it was shown that MZ-resistance has no impact in the treatment of patients [29]. Recommended rescue therapy includes LEV as a second line of treatment ie PPI, AMX, LEV (PAL) [45] these therapeutic options are reported in **Table 3**.

Recommendations	Type of therapy	Drug
First line	• Old triple therapy*	PPI + CLA + either AMXorMZ
	• Concomitant non- bismuth quadruple therapy	PPI + AMX + MZ + CLA**
	• Bismuth quadruple therapy (BQT)	PPI + bismuth+MZ*TE ***
Second line	• Concomitant non bismuth quadruple therapy.	See above
	• BQT	See above
	• LEV- containing therapy	PPI + AMX + LEV
Rescue therapy	• Rifabutin containing therapy (undetermined)	PPI + AMX + RIF
	• LEV-containing therapy	See above

Notes: The therapy should be prolonged for 14 days.
PPI = proton pump inhibitor, CLA = clarithromycin, MZ = metronidazole; TE = tetracycline; AMX = amoxicillin, RIF = rifabutin.
**Obsolete therapy.*
***Used in the countries where CLA – resistance is <15%.*
****Unaffected by CLA - resistance.*

Table 3.
Antibiotic therapy.

7. New treatment strategies

Hp antibiotic resistance has been increasing all over the world in the last decade and this phenomenon constitutes an important challenge for the treatment of this fastidious bacterium. This has prompted the researchers to an obstinate search for new solutions such as the vaccine development and new treatments based on the use of natural resources such as plants, probiotics, and nutraceuticals [46, 47].

A new compound, a guanidine derivative bearing adamantane-1-carbonyl 2-bromo-4,6-difluouro-phenyl substituents (H-BDF), seemed to be promising against the strains tested [48]. Other substances were studied against *Hp* such as three known and five unknown N-substitute-2-oxo- H-1-benzopyran-3- carboxamides (coumarin-3-carboxamides). The compounds with a 4-acyl-phenyl group showed the best activity against *H. pylori* metronidazole- resistant strains [49].

Non-traditional therapies have been indicated as a means to target this important gastric pathogen. This approach also includes the use of antimicrobial peptides (core component of innate immune system of numerous eukaryotes) that interact with the anionic Gram-negative cell wall because of charge electrostatic attractions [50]. It also seems reasonable to investigate other options aimed at reinforcing the immune system of these patients, The potential role of N-acetil-cysteine which is capable of destroying bacterial biofilm, is an emerging treatment for recalcitrant infections [51]. Vonoprazan (potassium-competitive acid blocker P-CAB) is a new compound which could improve eradication rates by raising the intragastric pH and thus increasing bacterial antibiotic susceptibility [52]. Recent studies revealed that P-CAB-based triple therapy was more effective than PPI-based triple therapy (76.1% versus 40.2%) as a first-line *Hp* eradication method [53]. Furthermore, even in the presence of CLA-resistant strains, P-CAB-based triple therapy showed good eradication rates [52].

Furazolidone (FUR) is a new antibiotic studied mainly in China which has demonstrted a great activity either alone or in combination with other antibiotics against *H. pylori*. It is a monoamine oxidase inhibitor which can interact with the metabolism of tyramine causing side-effects such as vomiting, diarrhea and nervous system disorders. In China, the FUR is available for the treatment of patients. Therefore, quadruple therapy with proton pump inhibitors, bismuth and a combination of two antibiotics specifically FUR, TET or AMX would be more suitable for Chinese patients [26]. This treatment is also recommended in the Maastricht V/Florence Consensus Report [29] the new treatment strategies are shown in **Table 4**.

Compounds	**Activity**
Guanidine derivate	Promising activity against *Hp* strains
Coumarin-3-carboxamides	Activity against *Hp*-MZ--R
Antimicrobial peptides	Interaction with Gram- neg cell wall
N-acetyl-cysteine	Activity on the bacterial biofilm
Vonoprazan (potassium competitive acid blocker)	Raises the intragastric pH
New antibiotic	**Action**
Furazolidone * (monoamine oxidase inhibitor)	PPI + bismuth+FUR+ either TE or AMX

Fur = Furazolidone, MZ = Metronidazole, TE = tetracycline, AMX = amoxicillin, R = Resistant.
**Used alone or in combination with other antibiotics (in western countries).*

Table 4.
New treatment strategies and non-traditional therapies.

8. Conclusions

Helicobacter pylori is a complex microorganism that is difficult to cultivate, and treat also presenting particular characteristics of pathogenicity and virulence markers. Much progress has been made since 1983 when it was discovered first by Warren and Marshall [1] making it one of the most studied bacteria. *Hp* is well adapted to the human host as evidenced by its chronical persistence in the gastric niche and by the finding that the bacterial surface carries structures (antigens) which are identical to those found on human cells [54].

The markers of virulence such as CagA and VacA are seen to greatly influence the outcome of *Hp* infection. The combination of different virulence genotypes is the most important factor that strongly affects the bacterial virulence making some strains more pathogenic than others.

The heteroresistance is a big problem in the evaluation and management of *Hp* resistance in the infections. In fact the heteroresistance detected in five of our patients worsens the situation being an important issue as an isolate could be mistakenly considered susceptible if a single biopsy is used for antimicrobial tests possibly leading to a resistance underestimation [31].

The emergence of PCR method provides a quick, convenient way to guide tailored therapy in clinical practice of *H. pylori* [55]. In this situation without performing the susceptibility tests which are time-consuming and expensive, the gastroenterologists could establish a suitable treatment for *Hp* infected patients who in any case underwent a gastroscopy. The use of genotypic tests directly on the clinical specimens could predict the antibiotic resistance addressing changes in previous treatments. The real-time PCR detects the resistant population at a very low concentration not detectable by phenotypic tests which primarily show susceptible bacteria. The genotypic-resistance is useful in case of absence of live bacteria, contamination or for identifying mixed infections.

The CLA-resistance levels and mainly the local susceptibility turn out to be crucial to establish a correct therapy. The quadruple therapy (BQT) with proton pump inhibitor, bismuth and a combination of two antibiotics, specifically MZ and TE then unaffected by CLA and LEV resistance, turns out to be appropriate in the *Hp* infected- patients resulting in an effective eradication rate.

The lack of data on local susceptibility patterns and on eradication success rates was identified as a knowledge gap that has a major impact on the choice of therapy and hence best management. Periodic susceptibility testing should be considered by health authorities and clinicians should be encouraged to record their successes.

All in all we can say that this microorganism since its discovery in 1983 by Marshall and Warren, has been deeply studied and considered one of the most important gastric pathogen involved in a wide range of pathologies other than the classical ones, such as cancer, atherosclerosis and endothelial dysfunction leading to vascular diseases.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 1983 Jun 4;1(8336):1273-1275. PMID: 6134060

[2] Langenberg ML, Tytgat GNJ, Schipper MEI, Rietra PJGM, Zanen HC. Campylobacter-like organisms in the stomach of patients and healthy individuals. Campylobacter pylori, 1984: 151-153

[3] Ando T, Minami M, Ishiguro K, Maeda O, Watanabe T, MizunoT, et al.. Changes in biochemical parameters related to atherosclerosis after *Helicobacter pylori* eradication. Aliment Pharmacol Ther 2006:24:58-64. https://doi.org/10.1111/j.1365-2036.2006.00026.x

[4] Kanbay M, Gur G, Yucel M, Yilmaz U, Boyacioglu S. Does eradication of Helicobacter pylori infection help normalize serum lipid and CRP levels? Dig Dis Sci 2005:50:1228-1231. DOI: 10.1007/s10620-005-2764-9

[5] Goodman KJ, Correa P.. The transmission of *Helicobacter pylori:* a critical review of the evidence. Int J Epidemiol 1995:24: 875– 87. DOI: 10.1093/ije/24.5.875

[6] Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. Helicobacter. 2014;19 (Suppl 1):1-5. DOI: 10.1111/hel.12165

[7] Ford AC, Axon AT. Epidemiology of Helicobacter pylori and public health implication. Helicobacter. 2010;15(1):1-6. https://doi.org/10.1111/j.1523-5378.2010.00779.

[8] Goh KL, Chan WK, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori infection and public health implication. Helicobacter. 2011;16(1):1-9. DOI: 10.1111/j.1523-5378.2011.00874.x

[9] Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am. 2000:29(3):559-578. doi: 10.1016/s0889-8553(05)70130-8.

[10] Atherton JC.. H. pylori virulence factors. British Medical Bulletin. 1998:54:105-120. doi: 10.1093/oxfordjournals.bmb.a011662.

[11] Suerbaum S, Michetti P. Helicobacter pylori infection. New England J of Med 2002:347: 1175-1186. DOI: 10.1056/NEJMra020542

[12] Ansari S, Yamaoka Y. Helicobacter pylori Virulence Factors Exploiting Gastric Colonization and its Pathogenicity Toxins (Basel) 2019 Nov; 11(11): 677. Published online 2019 Nov 19. doi: 10.3390/toxins11110677 PMCID: PMC6891454].

[13] Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover T, et al. Mosaicism in vacuolating cytotoxin alleles of Helicobacter: association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem. 1995:270: 17771-7. doi: 10.1074/jbc.270.30.17771.

[14] Evans DG, Queiroz DM, Mendes EN, Doyle JE. Helicobacter pylori cagA status and s and m alleles of vacA in isolates from individuals with a variety of *H. pylori*-associated gastric diseases. J Clin Microbiol. 1998:36:3435-3437. PMC105353

[15] Rhead JL, Letley PD, Mohammad M, Hussein N, Mohagheghi MA, Hosseini ME, et al. A new Helicobacter pylori Vacuolating Cytotoxin Determinant, the Intermediate Region, Is Associated

With Gastric Cancer. Gastroenterology. 2007:133:926-936. doi: 10.1053/j.gastro.2007.06.056.

[16] Kaklikkaya N, Cubukcu K, Aydin F, Bakir T, Erkul S, Tosun I Y, et al.. Significance of cagA status and vacA subtype of Helicobacter pylori in determining gastric histopatology. J Gastroenterol Hepatol. 2006:21: 1042-1047. https://doi.org/10.1111/j.1440-1746.2006.04199.x

[17] Lindquist S, Craig EA.. The Heat shock proteins. Annu Rev Genet. 1988:22: 631-677.

[18] Wick G, Knoflach M, Henderson B, Bernhard D. Heat shock proteins and stress in atherosclerosis. Autoimm Rev 2004:3: 30-31. PMID: 15309783

[19] Ilver D, Arnqvist A, Ögren J, Kersulyte D, Incecik ET, Berg DE, et l.Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. 1998:279: 373-77. DOI: 10.1126/science.279.5349.373

[20] Uotani T, Graham DY. Diagnosis of Helicobacter pylori using the rapid urease test. Ann Transl Med. 2015:3: 9-15. doi: 10.3978/j.issn.2305-5839.2014.12.04

[21] Cambau E, Allerheiligen V, Coulon C, Céline Corbel, Christine Lascols, Lionel Deforges, et al. Evaluation of a new test, genotype HelicoDR, for molecular detection of antibiotic resistance in Helicobacter pylori. J Clin Microbiol. 2009:47:3600-7. doi: 10.1128/JCM.00744-09

[22] Lee JW, Kim N, Nam RH, Park JH, Choi YJ, Kim JM, et al. GenoType HelicoDR test in the determination of antimicrobial resistance of Helicobacter pylori in Korea. Scand J Gastroenterol. 2014;49:1058-67. DOI: 10.3109/00365521.2014.894117

[23] Binyamin D, Pastukh N, On A, Paritsky M, Peretz A. Phenotypic and genotypic correlationas expressed in Helicobcter pylori resistance to clarithromycin and fuoroquinolones. Gut Pathog. 2017:9:48-55 doi 10.1186/s13099-017-0198-5

[24] Westblom TU. Molecular diagnosis of Helicobacter pylori Immunol Invest. 1997:26(1-2):163-74. Doi 10.3109/08820139709048924

[25] Wang YK, Kuo FC , Liu CJ , Wu MC, Shih HY , Wang SW, et al Diagnosis of Helicobacter pylori infection: Current options and developments World J Gastroenterol. 2015 Oct 28;21(40):11221-35. doi: 10.3748/wjg.v21.i40.11221

[26] Liu DS, Wang YH, Zeng ZR, Zhang,Z.Y, Lu H, Xu JM, et al. Primary antibiotic resitance of Helicobacter pylori in Chinese patients: a multiregion prospective 7-year study Clin Microbiol Infect. 2018:, 24:780e5-780e8; doi: 10.1016/j.cmi.2017.11.010.

[27] Thung I.,Aramin H, Vavinskaya V, Gupta S., Park JY, Crowe SE, et al Review article: the global emergence of Helicobacter pylori antibiotic resistance. Aliment Pharmacol Ther. 2016; 43(4):514-533; doi: 10.1111/apt.13497

[28] Bluemel H, Goelz B, Goldma J, Gruger J, Hamel H, Loley K, et al. Antimicrobial resistance of Helicobacter pylori in Germany, 2015-2018 Clin Microbiol Infect. 2020, Feb:26(2):235-239; doi: 10.1016/j.cmi.2019.06.007.

[29] Malfertheiner P, Megraud F., O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. European Helicobacter and Microbiota Study Group and Consensus panel. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017: 66(1):6-30; doi:10.1136/gutjnl-2016-312288.

[30] Mascellino MT, Oliva A, Miele MC, De Angelis M, Bruno G, Severi C. Secondary Antibiotic Resistance, Correlation between Genotypic and Phenotypic Methods and Treatment in Helicobacter pylori Infected Patients: A Retrospective Study. Antibiotics. 2020:9(9):549-563. doi 10.3390/antibiotics9090549

[31] Norazah A, Rasinah WZ, Zaili Z, Aminuddin A, Ramelah M. Analysis of PCR-Rapid DNA and antibiotic susceptibility profiles of antrum and corpus of the same isolates of Helicobacter pylori from Malaysian patients. Malays J Pathol. 2009;31(1):29-34. PMID: 19694311

[32] de la Obra P, Alarcon T, Domingo D, Garcia J, Lopez-Brea M. Heteroresistance to metronidazole and genetic relationship of Helicobacter pylori clinical isolates. Gut. 2001:49(4):P.A4

[33] Marzio L, Cellini L, Amitrano M, Grande R, Serio M, Cappello G, et al. Helicobacter pylori isolates from proximal and distal stomach of patients never treated and already treated show genetic variability and discordant antibiotic resistance Eur J Gastroenterol Hepatol. 2011 Jun :23(6):467-472 doi:10.1097/MEG.0b013e328345d40f.

[34] https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf

[35] Papastergiou V, Georgopoulos SD, Karatapanis S. Treatment of Helicobacter pylori infection: meeting the challenge of antimicrobial resistance. World J Gastroenterol. 2014:20:9898-911. doi: 10.3748/wjg.v20.i29.9898

[36] Tanaka SK, Graham DY, Go MF. Mutations in 23S rRNA are associated with clarithromycin resistance in Helicobacter pylori. Antimicrob Agents Chemother. 1996:40(2):477-80. PMID: 8834903

[37] Romano M, Cuomo A, Gravina AG, Miranda A, Iovene MR, Tiso A,et al. Empirical levofoxacin-containing versus clarithromycin-containing sequential therapy for Helicobacter pylori eradication: a randomised trial. Gut. 2010:59:1465-70. doi: 10.1136/gut.2010.215350

[38] Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. J Antimicrob Chemother. 2003:51(5):1109-17. doi: 10.1093/jac/dkg222.

[39] Mascellino MT, Oliva A, De Angelis M. Pontone S, Porowska B. Helicobacter pylori infection: antibiotic resistance and eradication rate in patients with gastritis showing previous treatment failures. New Microbiol. 2018, Oct,41:(4):306-309. PMID: 30252924

[40] Wu JY, Liou JM, Graham DY. Evidence based recommendations for successful Helicobacter pylori treatment. Expert Rev Gastroenterol Hepatol. 2014:8(1):21-28. https://doi.org/10.1586/17474124.2014.859522

[41] Yi H, Yi Z, Lu NH. Recent progress of Helicobacter pylori treatment. Chin Med J (Engl). 2020 Feb 5:133(3): 335-343; doi: 10.1097/CM9.0000000000000618

[42] Gao W, Cheng H, Hu F, Li J, Wang L, Yang G, et al. The evolution of *Helicobacter pylori* antibiotics resistance over 10 years in Beijing, China. *Helicobacter*. 2010: 15(5):460-46. doi.org/10.1111/j.1523-5378.2010.00788

[43] Saracino JM., Zullo A, Holton J, Castelli V, Fiorini G, Zaccaro C, et al. High prevalence of primary antibiotic resistance in Helicobacter pylori isolates in Italy. J Gastrointestin Liver Dis. 2012:21(4):363-365. PMID: 23256118

[44] Gerrits MM, Van der Wouden EJ, Bax DA, van Zwet AA, van Vliet HM, de Jong AG, et al. Role of the rdxA and frxA genes in oxygen dependent metronidazole resistance of Helicobacter pylori. J Med Microbiol: 2004:53(11):1123-1128.doi: 10.1099/jmm.0.45701-0

[45] Fallone CA, Chiba N, van Zanten SV, Fischbach L, Gisbert JP, Hunt RH, et al. The Toronto consensus for treatment of Helicobacter pylori infection in adults. Gastroenterology. 2016:151(1):51-69. doi: 10.1053/j.gastro.2016.04.006.

[46] Ayala G, Escobedo-Hinojosa WI, de la Cruz-Herrera CF, Herrera C, Romer I. Exploring alternative treatment for Helicobacter pylori infection. World J Gastroenterol. 2014:20(6):1450-1469. doi: 10.3748/wjg.v20.i6.1450

[47] Ruggiero P. Use of probiotics in the fight against Helicobacter pylori. World J Gastrointest Pathophysiol. 2014:5(4):384-391. doi: 10.4291/wjgp.v5.i4.384

[48] SaeedA, Bosch A, Bettiol M, Nossa González D.L, Erben M.F, Lamberti Y. Novel Guanidine Compound against Multidrug-Resistant Cystic Fibrosis-Associated Bacterial Species., Molecules. 2018: 23(5):1158-76; doi.org/10.3390/molecules23051158

[49] Chimenti F, Bizzarri B, Bolasco, A, Secci D, Chimenti ,P, Carradori S, et al. Synthesis and in vitro selective anti-Helicobacter pylori activityofN-substituted-2-oxo-2H-1-benzopyran-3-carboxamides, Europ J Med Chem, 2006:41(2): 208-212. doi:10.1016/j.ejmech.2005.11.001

[50] Gopal R, Jeong E, Seo CH, Park Y. Role of antimicrobical peptides expressed by host cells upon infection by Helicobacter pylori. Protein Pept Lett. 2014:21(10):1057-1064. doi: 10.2174/0929866521666140708092032.

[51] Makipour K, Friedenberg FK. The potential role of N-acetylcysteine for the treatment of Helicobacter pylori. J Clin Gastroenterol . 2011: 45(10): 841-3;doi: 10.1097/MCG.0b013e31822be4d6

[52] Matsumoto H, Shiotani A, Katsumata R, Fujita M, Nakato R, Murao T. Helicobacter pylori eradication with proton pump inhibitors or potassium-competitive acid blockers: the effect of clarithromycin resistance. Dig Dis Sci. 2016: 61(11):3215-3220; doi: 10.1007/s10620-016-4305-0

[53] Noda H, Noguchi S, Yoshimine T, Goji S, Adachi K, Tamura Y et al. A novel potassium-competitive acid blocker improves the efficacy of clarithromycin-containing 7-day triple therapy against Helicobacter pylori. J Gastrointestin Liver Dis. 2016:25(3):283-288. doi: 10.15403/jgld.2014.1121.253.7hp.

[54] Monteiro MA, Chan KH, Rasko DA, Taylo TE, Zheng PY, Appelmelk BJ, et al. Simultaneous expressionof type 1 and type 2 Lewis blood group antigens by Helicobacter pylori lipopolisaccharides. Molecular mimicry between *H. pylori* lipopolisaccarides and human gastric epithelial cell surface glycoforms. J Biol Chem. 1998:273:11533-11543.

[55] Chen Q, Long X., Ji Y, Xiao,L, Dongping L, Hong G, et al. Randomized controlled trial: susceptibility-guided therapy versus empiric bismuth quadruple therapy for first-line Helicobacter pylori treatment. Aliment Pharmacol Ther. 2019:49(11):1385-1394. doi: 10.1111/apt.15273

Chapter 7

Helicobacter pylori Seromarkers in a University Students Population in Central Nigeria

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Abstract

Infection due to *Helicobacter pylori* is a public health challenge worldwide as over 3 billion persons are infected with the bacterium globally. There is a serious need to update the knowledge on the epidemiology of this bacterial pathogen and its probable risks factors to generate intervention programs that will reduce the morbidity and mortality of infected individuals. This chapter evaluated the seromarkers of *H. pylori* infection and its predisposing factors among students of Nasarawa State University, Keffi, Central Nigeria. This study was done between June through August 2019; blood and stool specimens were collected from 400 students of the institution. Before the commencement of the study, ethical clearance and informed consent were retrieved and a structured questionnaire was administered to each participant. Specimens were screened for *H. pylori* antigen and antibody using rapid test kits (CTK Biotech, Inc., San Diego, USA and Biotest Biotech, China). Information obtained were analyzed using SSP version 2.80. P values <0.05 were reflected statistically significant. Out of the 400 students tested, 166 (41.5%) and 128 (32.0%) showed positive for anti-*H. pylori* IgG and Ag markers respectively. The antibody seromarker was higher in female while the *H. pylori* antigen was higher in males. Those students aged 21–30 years old reported the highest prevalence of the seromarkers while those of more than 41 years old had the least prevalence. Location, type of toilet facility and place of residence were statistical associated between *H. pylori* antigen ($P < 0.05$). There was a statistically significant association between anti-*H. pylori* IgG and the sources of water of the students ($P < 0.05$). This is the first public report that has successfully reported the prevalence of these seromarkers among students of a tertiary institution in Nasarawa state. The overall outcomes of this study stressed the need for student-based intervention programs to stem the transmission of this infection in Nasarawa State, Nigeria.

Keywords: *Helicobacter pylori*, students, Keffi, seromarkers, prevalence

1. Introduction

Infections due to *Helicobacter pylori (H. pylori)* is a public health challenge globally as approximately 50% (over 3 billion) of the world population are known to be infected with this organism [1]. In 1983, Warren and Marshall identified *H. pylori*, which were formally called *Campylobacter pylori*, as a flagellated spiral, Gram negative

organism that have the capacity to synthesize urease in greater amounts [2]. Before then, laboratory analysis of upper gastrointestinal diseases that shows dyspepsia was with much complexity [3]. However, *H. pylori* has become a key etiological factor considered in cases of peptic ulcer disease (PUD), chronic gastritis, gastric cancer, and gastric mucosa associated lymphoid tissue (MALT) lymphoma in recent times [4–7].

Transmission of this organism could be person-to-person spread by either fecal-oral or oral-oral routes. Studies have shown that the organism can be isolated in feces, dental plaque, and saliva of few infected persons [8–9]. Most individuals infected with this organism are asymptomatic [10]. But in most situations, infected individuals are shows associated symptoms like mild to severe scorching intestinal ache that outspreads from the navel to the chest region. Nausea, loss of appetite, weight loss, vomiting, indigestion, and melena are other symptoms of this infection [5, 6, 9]. This bacterial pathogen is the primary cause of ulcers and its exact route of spread is still not known [4], there are other factors that has impact in ulcer generation including use of non-steroidal anti-inflammatory drugs (NSAIDs) like; ibuprofen, aspirin and piroxican [11]. Other fewer shared predictors may include smoking, cocaine, severe illness, Crohn's disease, alcoholism, autoimmune problems and radiation treatment among others [9, 12].

There are several diagnostic protocols for *H. pylori* infection [9, 13]. However, fecal antigen and urea breath tests shows utmost precision to check and approve the pathogen [13]. Nevertheless, serologic assays that detect the antibodies of *H. pylori* presence in human serum is commonly used especially in low resource countries like Nigeria [6, 14]. Practicing good hygiene and hand washing, especially with food preparation are keys for prevention of *H. pylori* infection while treatment is best by combination of antibiotics and proton pump inhibitors [4, 15].

In developing countries the bacterial infection is high when compared to developed nations, maybe because of lack of basic social amenities, poor sanitary conditions, low socio-economic status, and reduced use of antibiotics for dissimilar pathologies [10, 16–18]. The prevalence rate of the infection ranges from 30–40 percent in the United States and parts of Europe [19–20], 80–90 percent in South America and 70–90 percent in Africa continent [6, 15, 16]. In a hyper-endemic area of this bacterial infection like Nigeria [6, 7, 21, 22], there is a serious need to update the knowledge on the epidemiology of this bacterial infection and its related predictors to generate intervention programs that will reduce the morbidity and mortality of infected individuals. Therefore, this chapter principally determined the seromarkers for *H. pylori* infection among students of Nasarawa State University Keffi, Central Nigeria. It also identified possible predisposing factors such as gender, age, marital status, sources of water, toilet facilities among others from results obtained.

2. Material and methods

2.1 Study area

This study was done at Nasarawa State University, Keffi (NSUK), Nasarawa State, Nigeria. NSUK is a higher educational institution with a student population of approximately twenty thousand. The institution is situated in Keffi which is about 68 Km from Abuja, the Federal Capital Territory and 128 Km from Lafia, the Capital of Nasarawa State. The area lies in latitude eight 5'N of the equator and longitude seven 8′E, it is situated on height of 850 M up sea level [23]. The mean yearly shower is ±2,000 millimeters (79 in), and is heavy in the rainy months with its highest downpour during July to September [24]. The inhabitants mostly engage in trading, farming, schooling and petty jobs.

2.2 Study population

Four hundred (400) students of both sexes with a mean age of 24.8 years who are studying at the institution were recruited to be part of this cross-sectional study between June through August 2019. After knowledgeable agreement was retrieved from each student who was between the ages of 16 and above and eligible for the study was identified during the study period and involved in the study. Data concerning the participants socio-demographic and risk factors were retrieved by a self-structured questionnaire.

2.3 Sample size determination

Determination of sample size used for this study was done with the formula by Naing, [25] for calculation at 0.05 level of precision;

$$n = \frac{Z^2 pq}{d^2}$$

Where:

n = required sample size.

Z = standard normal deviation at the necessary confidence interval (1.96) which agrees to 95% confidence interval.

p = prevalence of *H. pylori* from previous study (56.3%) (0.2) [26].

q = 1 – p = 0.9.

d = degree of precision expected (0.05)

$$n = (1.96)^2 (0.2)\ (0.9)/(0.05)^2 = 3.8416 \times 0.18/0.0025 \quad (1)$$

$$3.8416 \times 0.18/0.0025 = 0.6915/0.0025 \quad (2)$$

$$0.6915/0.0025 = 276.6 \quad (3)$$

$$n = 277 \quad (4)$$

To minimize error, this was however rounded up to 400 samples.

2.4 Ethical approval and administration clearance

Ethical clearance for this chapter was collected from the Health Research Ethics Committee (HREC) of the Federal Medical Centre, Keffi, Nigeria (FMC/KEF/HREC/212/19). Official permission and administrative clearance was also received from the management of South Atlantic Petroleum Medical Center Keffi, Nasarawa State where specimens were obtained. In addition, each participant included in this study willingly completed and signed an informed consent form. Individual anonymity was treated with privacy and for the aim of the study.

2.5 Blood sample collection

Approximately 3 ml of blood specimen was drawn from each study participant by venipuncture into a plain tube and was labeled. Samples were left to clot at a minimal room atmosphere and spun at 3, 000 rpm for 5 minutes. The subsequent sera were collected into labeled cryovials and kept at -20 °C till set for analysis.

2.6 Stool sample collection

Each participant was given a labeled, germ-free leak proof universal bottle and instructed on how to obtain the stool sample aseptically [27]. The specimens were stored at 4 °C in the refrigerator until ready for the test.

2.7 Laboratory analysis

2.7.1 Detection of H. pylori *serum antibody*

All sera were tested for *H. pylori* antibody (IgG) presence using the one-step *H. pylori* antibody quick diagnostic screening kit (CTK Biotech, Inc., San Diego, USA). The tests protocol and results readings were done based on the instructions of the manufacturer.

2.7.1.1 Test procedure

The test kit and other reagents were taken to room temperature. The test device was placed on a flat dehydrated surface and about 2 droplets of the separated serum were added to the specimen well. It was allowed for 10 mins before reading was taken.

2.7.2 Detection of H. pylori *stool antigen*

H. pylori antigen was identified from stool samples using a one-step *H. pylori* antigen rapid test device (Biotest Biotech, China). The tests protocol and results readings were done based on the instructions of the manufacturer.

2.7.2.1 Test procedure

A sterilized swab stick which is inside the stool kit was used to take fecal specimen from the bottle. The specimen was introduced into the tube that has the assay diluents. The swab stick was swirled approximately 10 times inside the diluents until the specimen is totally liquefied. The specimen gathering tube was capped and left for approximately 5 minutes. Three droplets of prepared fecal specimen was placed in the specimen well of the test kit and the result was read in 15 minutes.

2.7.3 Interpretation of results

The test is positive when two red lines which depicts control (C) and test (T) is seen on the test device. It is negative when just a red line which depicts control is seen. Test is invalid when no line or only the T line is seen, in such situation, the test was repeated.

2.8 Data analysis

The information realized from this study was subjected to descriptive statistical investigation using Smith's Statistical Package (SSP version 2.80, Claremont, California-USA). Chi-square statistical analysis was used to decide associations. Data gotten were reflected statistically significant at $p \leq 0.05$.

3. Results

In this study, we investigated the epidemiology of *H. pylori* infection by serum antibody and stool antigen determination approaches among students of Nasarawa State University, Keffi. A total of 400 students consisting of 192(48.0%) males and 208 (52.0%) females were screened for both antibody and antigen to *H. pylori* using rapid diagnostic methods. Of the 400 students screened, 166 (41.5%) showed positivity to *H. pylori* IgG, and 128 (32.0%) showed positivity to *H. pylori* antigen (**Figure 1**).

There was no statistically significant difference between age and seromarkers of *H. pylori* ($P > 0.05$). However, the infection was highest both antibody (46.4%) and antigen (36.6) among those aged 21–30 years old. Also, students aged ≥40 years had the least prevalence of *H. pylori* infection (Ab = 25.0%, Ag = 16.7%) when compared to other age groups. There were more females (208) than males (192) and among the 192 males tested, 79 (41.2%) and 59 (30.7%) showed positivity for antibody and antigen of *H. pylori* respectively. Nevertheless, of the 208 females, 87 (41.8%) tested positive for *H. pylori* antibody while 69 (33.2%) were positive for stool antigen. However, this variation was not found to be statistically significant ($P > 0.05$). There was no statistical significant association between *H. pylori* seromarkers and marital status ($P > 0.05$). Of the 92 married students, 45 (48.9%) showed positivity for serum antibody while 35 (38.0%) showed positivity for stool antigen. Similarly, 121 (39.3%) of the unmarried reported positive for serum antibody and 93 (30.2%) tested positive for stool antigen. The rate of serum antibody was reported high among students from rural areas (44.4%) than those from urban areas (39.4%) ($P < 0.05$). But surprisingly, considering stool antigen as a marker for *H. pylori* infection, both those from rural (33.1%) and urban (33.2%) areas almost had equal infection burden (**Table 1**).

No statistically significant association was reported between the bacterial infections and socio-economic status ($P > 0.05$). Notwithstanding, students with poor socio-economic status had higher prevalence of both serum antibody (46.2%) and stool antigen (48.1%) while the least prevalence of the infections (Ab = 38.2%, Ag = 25.5%) was recorded among those with very good socio-economic status.

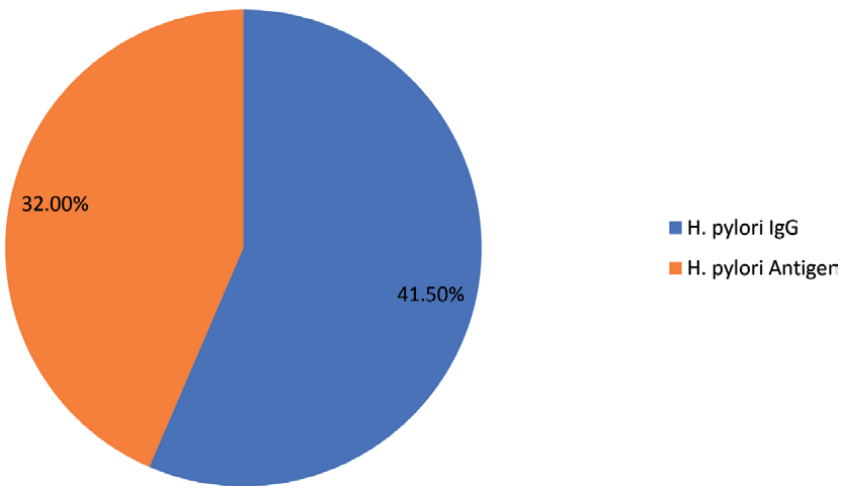

Figure 1.
Distribution of markers for H. pylori *infections among students of a tertiary institution in Central Nigeria.*

Socio-demographics	No. Examined	Prevalence (%)	
	(N = 400)	Serum IgG	Stool Ag
Age (Years)			
15–20	120	45(37.5)	35(29.2)
21–30	224	104(46.4)	82(36.6)
31–40	44	14(31.8)	9(20.5)
≥41	12	3(25.0)	2(16.7)
P-value		**0.1762**	**0.2417**
Gender			
Male	192	79(41.2)	59(30.7)
Female	208	87(41.8)	69(33.2)
P-value		**0.3010**	**0.2993**
Marital Status			
Married	92	45(48.9)	35(38.0)
Unmarried	308	121(39.3)	93(30.2)
P-value		**0.2811**	**0.1902**
Location			
Urban	231	91(39.4)	72(31.2)
Rural	169	75(44.4)	56(33.1)
P-value		**0.0814**	**0.0424***

**Statistically Significant.*

Table 1.
Distribution of markers of H. pylori *infections among students of a tertiary institution in Central Nigeria in relation to socio-demographic factors.*

Furthermore, *H. pylori* serum antibody was highest among students who source water from river/stream (54.2%) than those who source water from tap (43.1%), well (39.8%) and borehole (38.4%) ($P < 0.05$). However, those who source water from well (39.8%) had the highest prevalence of stool antigen when compared to other sources of water. Similarly, there was statistically significant association between type toilet and *H. pylori* stool antigen in this study ($P < 0.05$), as higher rate was recorded among students who use pit toilet (39.0%) than those who use toilet with water system (29.8%). On the other hand, higher rate of *H. pylori* serum antibody was recorded among those with toilets with water system (43.9%) compared to those with pit toilets (33.7%). Additionally, place of residence was also associated with *H. pylori* stool antigen ($P < 0.05$). Higher prevalence of both stool antigen (36.2%) and serum antibody (43.6%) To*h. pylori* were reported among students residing in the hostel as compared to those who reside at home/off-campus (Ab = 40.1%, Ag = 29.1%). Surprisingly, both smoking and alcoholism were not significantly associated with *H. pylori* infections in this study ($P > 0.05$). However, higher prevalence of the infections was recorded among smokers (Ab = 47.3%, Ag = 40.5%) than those who do not smoke (Ab = 40.2%, Ag = 30.4%). Nonetheless, the infections was higher among those who do not take alcohol (Ab = 42.4%, Ag = 33.1%) than those who take alcohol (Ab = 38.2%, Ag = 28.1%). Self-medication was also not significantly associated with *H. pylori* infections ($P > 0.05$). However, those who do not self-medicate were more infected (Ab = 42.7%, Ag = 33.7%) than those who self-medicates (Ab = 41.2%, 31.5%) (**Table 2**).

Risk factor	No. Examined	Prevalence (%)	
	(N = 400)	Serum IgG	Stool Ag
Socio-economic status			
Very good	55	21(38.2)	14(25.5)
Average	239	96(40.2)	63(26.4)
Poor	106	49(46.2)	51(48.1)
P-value		**0.0711**	**0.1002**
Water sources			
Tap	72	31(43.1)	24(33.3)
Borehole	172	66(38.4)	46(26.7)
Well	108	43(39.8)	43(39.8)
River/stream	48	26(54.2)	15(31.3)
P-value		**0.0432***	**0.6711**
Types of toilet			
Water system	305	134(43.9)	91(29.8)
Pit	95	32(33.7)	37(39.0)
P-value		**0.0991**	**0.0440***
Place of residence			
Home/off-campus	237	95(40.1)	69(29.1)
Hostel	163	71(43.6)	59(36.2)
P-value		**0.0672**	**0.0336***
Smoking Habit			
Yes	74	35(47.3)	29(40.5)
No	326	131(40.2)	99(30.4)
P-value		**0.1102**	**0.3447**
Alcohol intake			
Yes	89	34(38.2)	25(28.1)
No	311	132(42.4)	103(33.1)
P-value		**0.5048**	**0.6590**
Self-medication			
Yes	311	128(41.2)	98(31.5)
No	89	38(42.7)	30(33.7)
P-value		**0.0821**	**0.9115**

**Statistically Significant.*

Table 2.
Distribution of markers of H. pylori *infections among students of a tertiary institution in Central Nigeria in relation to risk factors.*

4. Discussion

Helicobacter pylorus is a ubiquitous bacterium which is found in about two-third of the world's population [15, 28]. This current study was conducted to investigate the seromarkers and predisposing factors of *H. pylori* infections among students of Nasarawa State University, Kefii, Nigeria. A total of 400 students participated in the

study and they were screened for both stool antigen and serum antibody (IgG) to *H. pylori* using rapid diagnostic approaches. Out of the 400 students screened, 166 (41.5%) showed positivity to serum antibody (IgG) and 128 (32.0%) were positive to stool antigen.

It is worthy of note that, presence of serum antibody (IgG) and absence of stool antigen in an individual is an indication of immunity to *H. pylori* due to past exposure to the bacterium. But when an individual is tested positive for both the antigen and the antibody at the same time, it means that there is an existing infection, while those that shows negative to both stool antigen and serum antibody are prone to *H. pylori* infections [22, 28, 29].

The 41.5% *H. pylori* IgG seroprevalence recorded in the study is higher than the 28.0% reported by Enitan *et al.* [22] among students of tertiary institution in Ogun, 35.0% by Ombugadu *et al.* [5] among dyspeptic patients in Jos and 15.4% by Moujaber *et al.* [30] in Australia. However, it is lower than the 57.9% found by Oti *et al.* [26] among patients in North-Central Nigeria, 51.9% by Gide *et al.* [6] among dyspeptic patients in Damaturu and 47.0% by Bastos *et al.* [20] among adult in Portugal.

On the other hand, the 32.0% stool antigen positivity recorded in this study is higher than the 22.8% reported by Adeniyi *et al.* [31] in malnourished children in Lagos and 23.5% by Enitan *et al.* [22] among students of tertiary institution in Ogun. Nevertheless, higher rates have also been reported. It was 38.8% among dyspeptic patients in Jos [5], 35.0% among malnourished children in Iraq [32] and 84.0% among African refugee children from resettlement in Australia [33]. The differences in the rates of *H. pylori* infection markers observed in different studies may be due to differences in population type and location with different peculiar risk factors.

Although, there is no statistically significant association between age and infections due to *H. pylori* ($p > 0.05$), students aged 21–30 years old were more infected than other age groups. This is indicated by high serum IgG (46.4%) and stool antigen (36.6%) among those in this age bracket. The results of this study were not in consonance with the reports of other previous studies [26, 34–38]. The higher prevalence of the infection among younger students in this current study might be because a good number of them reside in the hostel where there is overcrowding and poor hygienic conditions.

In this current study, gender was not statistically associated with infections due to *H. pylori* ($p > 0.05$) as both males (IgG = 41.2%, Ag = 30.7%) and females (IgG = 41.8%, Ag = 33.2%) were almost equally infected. This is consistent with the report of Ombugadu *et al.* [5] among dyspeptic patients in Jos but they however recorded higher prevalence of the infection among males (IgG = 43.2%, Ag = 45.5%) than females (IgG = 25.0%, Ag = 30.6%) which they attributed to higher exposure of males to possible environmental sources of infection such as smoking and alcohol intake.

In a related development, *H. pylori* infection was not associated with marital status ($p > 0.05$). This report is in consonance with the reports from other researchers [12, 22, 26]. However, the higher prevalence of the bacterial infection markers recorded among married (Ab = 48.9%, Ag = 38.0%) than unmarried (Ab = 39.3%, Ag = 30.2%) in this study might be unconnected to the engagement of married people particularly women in house chores such as washing of toilets, bathrooms and taking care of babies which may probably put them at high risk of the pathogenic agent through fecal-oral routes.

There is a statistically significant association between location and *H. pylori* stool antigen positivity in this study ($p < 0.05$). It was higher among rural settlers (IgG = 44.4%, Ag = 33.1%) than urban settlers (IgG = 39.4%, Ag = 31.2%). Most studies conducted in Nigeria also reported similar findings [5, 12, 26, 31]. This is

no surprise because most Nigerian rural communities are characterized by lack of basic social amenities, poor hygienic environment and low socio-economic status [31, 36, 39].

This study reported no significant association between *H. pylori* infection and socio-economic status of participants in this study ($p > 0.05$). However, infection with *H. pylori* increased progressively with a decrease in socio-economic status of the participants. This observation agrees with findings of other researchers [5, 6, 36, 38]. Living in overcrowded homes that possess ugly environmental hygiene is associated with poor financial status individuals [12, 31, 39], thus, the possible reason the bacterial infection was high among them. There was a statistically significant association between students' water sources and *H. pylori* IgG seropositivity ($p < 0.05$). The prevalence was highest among those that use river/stream as their source of water (54.2%). This is no surprise because open defecation is common in Nigeria and water from river/stream can be easily contaminated with pathogenic organisms including *H. pylori* which are a fecal-oral pathogen [9]. Nevertheless, students which source of water is well had the highest prevalence of *H. pylori* antigen (39.8%). Other studies have reported similar outcomes [5, 38, 40].

Type of toilet facilities of the students was statistically associated with *H. pylori* antigen positivity in this study ($p < 0.05$). It was high among those that use pit toilets (39.0%). On the other hand, *H. pylori* IgG seropositivity was high among participants that use water system toilets (43.9%) compared to those that use pit toilets (33.7%). This observation may be an indication that the hygienic nature of the toilet and not its types is the reason behind the spread of the bacterial infection [6, 17, 38].

There was a statistically significant difference between place of residence and *H. pylori* antigen positivity ($p < 0.05$). The infection was more for both antigen (36.2%) and antibody (43.6%) among students residing in the hostel. This is expected because most hostels in Nigerian public tertiary institution are characterized with overcrowding and poor sanitary conditions which have been noted previously to be risk factors for the transmission of the infection [12, 33]. In this current study, both smoking and alcoholism were not statistically associated with the bacterial infection ($p > 0.05$) but there were arithmetic differences. This observation agrees with the report of Eshraghian [41]. Most previous studies have documented smoking and alcoholism as potential risk factors for *H. pylori* infection [9, 11, 12, 22, 26]. The higher prevalence of the infection among smokers in this study (IgG = 47.3%, Ag = 40.5%) may be connected with the fact that smoking, taking alcohol and coffee have been reported to increase the volume and concentration of stomach ulcer which can worsen an existing ulcer [22, 37].

Self-medication was not statistically associated with *H. pylori* infections in this study ($p > 0.05$). However, ulcer development has been linked to non-steroidal anti-inflammatory drugs (NSAIDs) usage such as, aspirin, ibuprofen and prioxican [11, 42]. These drugs are commonly used self-medications for headache, tiredness and fever. But surprisingly, in this study, participants who do not engage in self-medication were more infected with *H. pylori* (IgG = 42.7%, Ag = 33.7%) than those who engage in self-medication (IgG = 41.2%, Ag = 31.7%). Students who do not indulge in self-medication may have contracted the bacterium by living in overcrowded environment with poor hygienic and sanitary conditions hence the possible higher prevalence among them.

5. Conclusion

This study confirmed the presence of anti-*H. pylori* IgG and Ag markers with 41.5% and 32.0% for past and current infections respectively. The antibody

seromarker was higher in female while the *H. pylori* antigen was higher in males. Those students aged 21–30 years old reported the highest prevalence of the seromarkers while those of more than 41 years old had the least prevalence. Location, sources of water, types of toilet facility and place of residence were statistically associated with the bacterial infection. This is the first public report that has successfully reported the prevalence of these seromarkers among students of a tertiary institution in Nasarawa state. Priority should be given to personal and environmental hygiene of students to mitigate the spread of the infections.

6. Limitations of the study

This study was limited to a population of undergraduate students of a tertiary institution and the results may not be a true representation of the general population. Additionally, the serum antibody and stool antigen to *H. pylori* were detected using rapid diagnostic methods. Culture and other invasive methods such as endoscopic biopsy were not employed. Thus, the prevalence rates of *H. pylori* infection is likely to be underestimated because most commercially produced rapid diagnostic test kits have sensitivity and specificity of less than 90%.

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Conflict of interest

The authors did not declare any competing interest.

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References

[1] Palamides P, Jolaiya T, Idowu A, Loell E, Onyekwere C, Ugiagbe R, *et al*. *Helicobacter pylori* patient isolates from South Africa and Nigeria differ in virulence factor pathogenicity profile and associated gastric disease outcome. *Scientific Reports.* 2020; *10*:11-19

[2] Alfarouk KO, Bashir AH, Aljarbou AN, Ramadan AM, Muddathir AK, Alhoufie ST, et al. *Helicobacter pylori* in gastric cancer and its management. *Frontiers in Oncology.* 2019; 9:75-81

[3] Oluwasola AO, Ola SO, Saliu L, Solanke TF. *Helicobacter pylori* infection in South Nigerians: a serological study of dyspeptic patients and healthy individuals. *West African Journal of Medicine.* 2012; 21(2):138-141.

[4] Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, et al. Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. *Gut.* 2017; 56(6):772-781.

[5] Ombugadu DO, Oladele OV, Onuoha SC, Omisope O, Ani A. Prevalence of *Helicobacter pylori* IgG and stool antigen detection from dyspeptic patients in Jos, Nigeria. *African Journal of Clinical and Experimental Microbiology.* 2018; 19 (3): 177-185

[6] Gide S, Ibrahim Y, Anas G, Alegbe SD. Prevalence of *Helicobacter pylori* Infection in patients with dyspeptic symptoms in Damaturu Metropolitan. *Journal of Advances in Microbiology.* 2019; 18(2): 1-8

[7] Chukwuma OM, Chukwuma GO, Manafa PO, Akulue JC, Jeremiah ZA. Prevalence and Possible Risk Factors for *Helicobacter pylori* Seropositivity among peptic ulcerative individuals in Nnewi Nigeria. *BioMed Research Journal*. 2020; 4(1): 165-171

[8] Brown LM. *Helicobacter pylori:* Epidemiology and routes of transmission. *Epidemiologic Reviews.* 2010; 22(2): 283-297.

[9] Mustafa M, Menon J, Muiandy RK, Fredie R, Sein MM, et al. Risks factors, diagnosis and management of peptic ulcer disease. *Journal of Dental and Medical Sciences.* 2015; 14(7): 40-46.

[10] Kusters JG, Arnoud HM, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clinical Microbiology Review.* 2006; 19(3): 449-490.

[11] Mbanisi BO, Effiong EB. Sero-prevalence of *Helicobacter pylori* antibodies and peptic ulcer patients in Aguata and Orumba Local Government Area of Anambra State, Nigeria. *Indian Journal of Applied Research*. 2015; 5(10):61-63

[12] Odigie AO, Adewole AJ, Ekunwe AA. Prevalence and factors associated with *Helicobacter pylori* infection among treatment naïve dyspeptic adults in University of Benin Teaching Hospital, Benin City, Nigeria. *African Journal Clinical and Experimental Microbiology.* 2020; 21 (2): 97-105

[13] Fashner J, Gitu CA. Diagnosis and treatment of peptic ulcer disease and *H. pylori* Infection. *American Family Physician*. 2015; 91(4): 236-242.

[14] Daniyan OW, Ibe CB, Ezeonu TC, Anyanwu OU, Ezeanosike OB, Omeje KN. Seroprevalence and risk factors of *Helicobacter pylori* infection among children in South-east Nigeria. *Journal of Gastroenterology and Hepatology Research*. 2020; 9(1): 3066-3070

[15] Santacrose L. *Helicobacter pylori* infectionhttps://emedicine.medscape.com/article/17 6938-overview.2017; Retrieved on November, 15, 2017.

[16] Hunt RH, Xiao SD, Megraud F, Leon-barau R, Bazzoli F. *Helicobacter pylori* in developing countries. *Journal of Clinical Gastroenterology,* 2011; 45:383-388

[17] Jemikalajah DJ, Okogun GRA. Health point prevalence of *Helicobacter pylori* in Central Hospital Warri, Nigeria. *African Journal of the Cellular Pathology.* 2014; 3(1): 57-60.

[18] Sonnenberg A, Turner KO, Genta RM. Low prevalence of *Helicobacter pylori*-positive peptic ulcers in private outpatient endoscopy centers in the United States. *American Journal of Gastroenterology*. 2020; 115(2):244-250.

[19] Epplein M, Signorello LB, Zheng W, Cai Q, Hargreaves MK, Michel A, *et al*. *Helicobacter pylori* prevalence and circulating micronutrient levels in a low-income United States population. *Cancer Prevention Research*. 2011; 4:871-878

[20] Bastos J, Peleteiro B, Barros R. Sociodemographic determinants of prevalence and incidence of *Helicobacter pylori* infection in Portuguese adults. *Helicobacter.* 2013; 18(6): 413-422.

[21] Olokoba AB, Gashau W, Bwala S, Adamu A, Salawu FK. *Helicobacter pylori* infection in Nigerians with dyspepsia. *Ghana Medical Journal,* 2013; 47(2):79-81

[22] Enitan SS, Ochei JO, Akele YR, Faloye TG, Adeniyi LO, Omotola D. Screening for *Helicobacter Pylori* infection among undergraduate students of a tertiary institution using serum antibody and stool antigen detection methods. *Biomedical Journal of Science and Technology Research*. 2018; 3(2): 3180-3189

[23] Akwa VL, Binbol NL, Samaila KL, Marcus ND. Geographical perspective of Nasarawa State. Onaivi Printing and Publishing Company Ltd, Keffi. 2007; 503.

[24] Yohanna J, Oti V, Amuta E, Philip A, Anizoba L. *Plasmodium falciparum* Infection among Febrile Patients Attending a Tertiary Healthcare Facility in Central Nigeria: Prevalence, Haematologic and Sociodemographic Factors. *International Journal of Tropical Diseases*. 2019; 2(019): 1-6.

[25] Naing NN. Determination of sample size. *Malays J Med Sci*. 2003; 10: 84-86.

[26] Oti VB, Pennap GR, Dennis O, Ajegena AS, Adoga MP. Prevalence and predictors of *Helicobacter pylori* infection among patients attending a healthcare facility in North-Central Nigeria. *Asian Pacific Journal of Tropical Disease.* 2017; 7(6): 352-355

[27] Cheesbrough M. District Laboratory practice in tropical countries. 2nd ed. Cambridge University Press, USA. 2006; 297.

[28] Smith S, Fowora M, Pellicano R. Infections with Helicobacter pylori and challenges encountered in Africa. *World J Gastroenterol*. 2019;25(25):3183-3195.

[29] Dube C, Nkosi TC, Clarke AM, Mkwetshana N, Green N, Ndip RN. (2009). *Helicobacter pylori* Antigenemia in an Asymptomatic Population of Eastern Cape Province, South Africa: Public Health Implications. *Reviews on Environmental Health*. 24(3):

[30] Moujaber T, Maclntyre CR, Backhouse J, Gidding H, Quinn H, *et al*. The seroepidemiology of *Helicobacter pylori* infection in Australia. *International Journal of Infectious Diseases.* 2008; 1(12): 500-504.

[31] Adeniyi OF, Fajolu IB, Temiye E, Esezobor CI, Mabogunje CA. *Helicobacter pylori* infection in malnourished children in Lagos. *Nigerian Medical Journal.* 2019; 60(4):205-210

[32] Najim HA, Habeeb SI. Frequency of *Helicobacter pylori* infection in

children admitted to the nutritional rehabilitation centre. *International Journal of Current Research*, 2014; 6:9440-9444

[33] Cherian S, Burgner DP, Cook AG, Sanfilippo FM, Forbes DA. Associations between *Helicobacter pylori* infection, co-morbid infections, gastrointestinal symptoms and circulating cytokines in African children. *Helicobacter.* 2018; 15(2): 88-97.

[34] Ayodele MBO, Aaron UU, Oluwatayo GA, Wariso KT. Prevalence of *Helicobacter pylori* infection among suspected peptic ulcer patients In Port Harcourt, South-South, Nigeria. *Gazette of Medicine.* 2017; 6 (1): 602-607.

[35] Duggal NA. Reversing the immune ageing clock: lifestyle modifications and pharmacological interventions. *Biogerontology.* 2018; 19(6):481-496

[36] Awuku YA, Simpong DL, Alhassan IK, *et al.* Prevalence of *Helicobacter pylori* infection among children living in a rural setting in sub–Saharan Africa. *BMC Public Health*. 2017;17(1):360.

[37] Etukudo OM, Ikpeme EE, Ekanem EE. Seroepidemiology of *Helicobacter pylori* infection among children seen in a tertiary hospital in Uyo, Southern Nigeria. *The Pan African Medical Journal*. 2012; 12: 39.

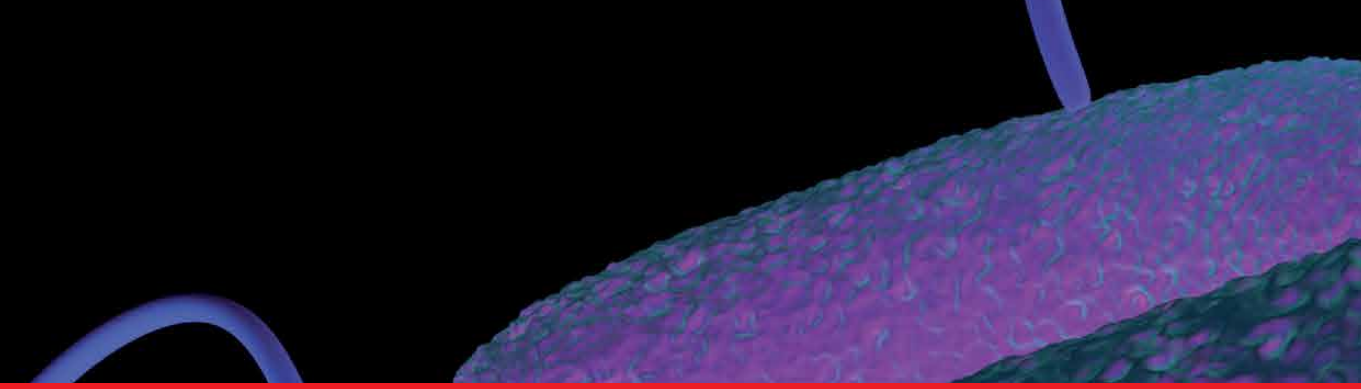
[38] Bello AK, Umar AB, Borodo MM. Prevalence and risk factors for *Helicobacter pylori* infection in gastroduodenal diseases in Kano, Nigeria. *African Journal of Medical and Health Sciences*. 2018; 17: 41-46.

[39] Hottez PJ, Kamath A. Neglected tropical diseases in Sub-Saharan Africa: Review of their prevalence, distribution and disease burden. *Plos Negleted Tropical Diseases.* 2009; 3(8):412-421.

[40] Kasmi H, Doukani K, Ali A, *et al*. Epidemiological Profile of *Helicobacter pylori* Infection in Patients with Digestive Symptoms in Algeria. *Journal of Epidemiology and Global Health*. 2020.

[41] Eshraghian A. Epidemiology of *Helicobacter pylori* infection among the healthy population in Iran and countries of the Eastern Mediterranean region: A systemic review of prevalence and risk factors. *World Journal of Gastroenterology.* 2014; 20(46): 17618-17625

[42] Gashau W, Adamu AS. Blind Helicobacter pylori treatment in dyspeptics in a high prevalence area. *Int J Cur Res Rev*. 2020;12(4):2-7.



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Helicobacter pylori is a universally distributed bacterium that affects more than half of the world population. *H. pylori* infection causes persistent inflammation with different clinical outcomes in humans, including chronic gastritis, peptic ulcer disease, and gastric cancer. The infection has also been associated with several extradigestive disorders. In this book, there is a comprehensive overview of contributors on *H. pylori* infection in diverse areas, including virulence factors of *H. pylori* and their importance for the clinical outcome of the diseases, discussions about the principal therapeutic regimens of bacterium eradication, also considering the antimicrobial resistance. *H. pylori* is clearly a very interesting bacterium and studies and discussions about its aspects are welcome to the medical and scientific communities.

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