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Hematological Extradigestive Manifestations of *Helicobacter pylori* Infection in Childhood

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Abstract

Helicobacter pylori infects more than 50% of the world population and is acquired in infancy. Higher prevalence is found in developing countries, and within geographic areas the predominance correlates inversely with socioeconomic status, especially with living conditions during childhood. Initially, in adults, *H. pylori* was only associated with gastric diseases, such as peptic ulcer, gastritis, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and in childhood, with chronic gastritis and duodenal ulcers in children. Recently, *H. pylori* has been related to non-gastric diseases, including hematological disorders such as iron deficiency anemia (IDA), chronic idiopathic thrombocytopenia (cITP), and vitamin B12 deficiency. *H. pylori* can trigger autoimmune atrophic gastritis and be responsible initially for an oral iron refractory anemia. Other hematological associations have been made, such as an increased risk of childhood leukemia in children of *H. pylori*-infected mothers and gastric bleeding in children with coagulation pathologies. *H. pylori* infection is important in the immune pathogenesis of chronic gastric inflammation and hematological diseases. The diagnostic methodology is based on non-invasive (serology, C-urea breath test, stool HP antigen) and invasive tests. The scientific community discussed and incorporated in international consensus for the investigation and management of these hematological extragastric pathologies (IDA, cITP, vitamin B12 deficiency, and MALT lymphoma). In children, a similar attitude was obtained in all of these pathologies except for cITP, in which the investigation for *H. pylori* is not indicated.

Keywords: *Helicobacter pylori*, iron deficiency anemia, immune thrombocytopenia, MALT lymphoma, vitamin B12 deficiency

1. Introduction

Helicobacter pylori is a gram-negative spiral bacterium, responsible for one of the most frequent gastrodigestive infections in the world. In 1883, Bizzozero reported spirochetes inhabiting the

gastric glands of dogs [1]. However, in 1954, after examining 1000 gastric biopsies, Palmer concluded that no microorganisms could survive in an acidic gastric environment [2]. This was contradicted by Marshall and Warren in 1982 [2]. They isolated the microorganism from the specimens of gastric mucosa in patients with gastritis and peptic ulcers, and for the first time it was isolated in vitro culture. As previously stated, at that time, it was thought that the acidic gastric environment was not compatible with the survival of this bacterium. In 1991, an association between *H. pylori* infection and gastric mucosa-associated lymphoid tissue (MALT) lymphoma was found [3]. Then, in 1994, The National Institute of Health consensus conference in the United States declared an association between *H. pylori* infection and peptic ulcer disease [4]. In that same year, *H. pylori* was identified with gastric adenocarcinoma and gastric non-Hodgkin lymphoma [3, 4].

After the discovery made by Marshall and Warren, histological features of chronic gastritis in upper gastrointestinal endoscopy were revealed. Moreover, a Canadian group also found *H. pylori* in the gastric mucosa of children with gastritis and duodenal ulcers [5].

Although over 80% of infected subjects remain asymptomatic, *H. pylori* chronic infection is associated with different clinical manifestations, including chronic gastritis, peptic ulcer disease, lymphoid tissue (MALT) lymphoma, and gastric adenocarcinoma. Furthermore, in 1994, *H. pylori* was classified as a type 1 carcinogen by the World Health Organization (WHO) [6-8]. In children, *H. pylori* has been considered a cause of chronic gastritis and duodenal ulcers. Recently, it has been related to diseases outside the stomach, namely those characterized by persistent and low-grade systemic inflammation: iron deficiency anemia (IDA), chronic idiopathic thrombocytopenia (cITP), vitamin B12 deficiency, growth retardation, and diabetes mellitus [9, 10]. Although a relationship between the *H. pylori* infection and autoimmune atrophic gastritis (AAG) is not possible, studies conducted by Hershko proved that *H. pylori* can be the trigger of AAG and be responsible in the initial phase of the disease for an oral refractory iron anemia [11-14]. More debated hematological associations have been made with monoclonal gammopathy, non-Hodgkin lymphomas of the stomach, myelodysplastic syndromes, increased risk of childhood leukemia in the children of *H. pylori*-infected mothers, and gastric bleeding in children with coagulation pathologies [15-17].

The *H. pylori* infection is acquired during childhood (before the age of 10) and in the absence of antibiotic therapy the host can carry it for life. In contrast to the great majority of bacterial infections, in which virulence is temporary due to an immune system mechanism, which results in the clearance of the pathogen, *H. pylori* can survive for many years [15-18]. *H. pylori* has mechanisms to escape the clearance of the innate and adaptive immune system, and the development of a symbiotic relationship between the bacteria and the human host is also a possibility [9-12]. Moreover, *H. pylori* has an important urease activity, leading to ammonia production in order to protect itself from gastric acidity. Also, *H. pylori* produces enzymes, such as phospholipase A2 and C as well as glycosulphatase, which leads to gastric mucosa damage [6].

2. The interaction between *H. pylori* and the immune system

Epithelial and myeloid cells form the initial line of the innate immune system response against *H. pylori* infection. They recognize the bacteria through a limited number of microbial pattern recognition receptors (PRRs), which include membrane associated Toll like receptors (TLRs), cytoplasmic Nod-like receptors (NLRs) and retinoic acid inducible gene I-like helicase receptor (RLRs). They recognize bacteria constituents termed pathogen-associated molecular patterns (PAMPs), present in the microbial proteins, nucleic acids, lipids and carbohydrates. These PAMPs contain molecules that act as ligands to trigger PRRs dependent intracellular signaling pathways, inducing the expression of pro-inflammatory molecules and cytokines as the first response to the bacteria infection. TLRs are the most studied of the larger family of the PRRs and participate in the first line of host defense against pathogens, with subsequent activation of adaptive immune response [17-21]. Ten TLRs have been identified in humans and can be identified by the type of ligand [12-14]. TLRs are type I transmembrane proteins characterized by an extracellular leucine-rich ectodomain involved in PAMP recognition from bacteria, fungi, parasites, and viruses, including lipid components of wall cell bacteria such as lipopolysaccharides (LPS) and lipopeptides, microbial components such as flagellin and nucleic acid components, as well as an intracytoplasmic domain that shows similarity to the IL1 receptor family termed a toll-IL1 receptor (TIR), which is involved in the activation of downstream signaling pathways. TLRs are expressed on the surface of cells or in intracellular vesicles like endosomes [18, 19, 22, 23]. Some TLRs recognize only one type of PAMP, while others such as TLR2 recognize bacterial lipoproteins, lipoteichoic acid and peptidoglycans [21]. The Goldberg Group revealed the role of TLRs during *H. pylori* infection, showing an overexpression of TLR2 and TLR5 in the gastric epithelial cells HEK293 of *H. pylori*-infected patients, as well as an increase of the activation of transcriptional factors: (1) nuclear factor- κ B (NF- κ B) and interleukin-8 (IL-8), (2) macrophage inflammatory protein 3 α (MI-3 α), and (3) the growth-regulated protein- α (GRO- α mRNA expression) [8]. The understanding of the role of TLRs in response to *H. pylori* infection requires an elucidation of cell-signaling events. After PAMP recognition, TLRs trigger the signaling pathways resulting in: (1) activation of the transcription of NF- κ B, protein-1 (AP-1), and of interferon regulatory factors (IRFs); (2) increased expression of inflammatory cytokines, antimicrobial peptides, and interferon type 1 (INF-1); and (3) recruitment of neutrophils, activation of macrophage, and dendritic cells. In epithelial cells, studies have found an increased induction of pro-inflammatory genes caused by the interaction between *H. pylori* and 4TLR [18, 19, 22, 23].

MyD88 (myeloid differentiation primary response protein 88) is an intracellular adaptor protein used by all TLRs except TLR3, and the signaling is done in two forms: MyD88 dependent, responsible for pro-inflammatory cytokine production as well as MyD88 independent, responsible for interferon type 1 production (ITF1). TLR4 induces both dependent and independent MyD88, so it induces both cytokines and ITF1. The expression of MyD88 in macrophage cells is essential for the induction of anti-inflammatory cytokines (IL-6, IL-1B, IL-10, and IL-12) [19]. Recently, a new group of innate immune molecules, including activating receptors involved in modulating the intensity of innate immune response in myeloid cells (TREM), has been described [24, 25]. TREM family receptors (TREM-1 to TREM 4), are cell

surface activating receptors with a transmembrane region containing charged lysine residues and a short cytoplasmic tail lacking signaling motifs. TREM-1, a 30-Kda glycoprotein of the Ig family is the most well-known member of the TREM family and is responsible for inflammation amplification and activation of antigen-presenting cells (APC). TREM-1 is exhibited in neutrophils and monocytes and linked to innate immunity by phagocyte secretion of pro-inflammatory chemokines and cytokines such as IL-8 and TNF- α . TREM-1 is involved in the amplification of TLR-dependent signals and in the improvement of NOD-like receptors (NLRs)-mediated responses, including NOD-1, involved in the protection against *H. pylori* infection [19]. An up-regulation of TREM-1 in gastric epithelial cells of *H. pylori* infected adults patients was found in a study by Schmausser et al. using immunohistochemistry in nine of twelve patients. No TREM-1 was found in non-inflamed gastric epithelium, in accordance with mRNA, TREM-1 also strongly expressed in *H. pylori* gastritis. [19, 26]. These results are not in accordance with Michalkiewics study done in 78 children with *H. pylori* infection. The gastric expression of TREM-1 and TREM-2 was not affected by *H. pylori* infection, reflecting the tolerance status of leucocytes present in gastric mucosa of *H. pylori* infected children [19]. TREM-2 is expressed in macrophage and dendritic cells, acts antagonistically to TREM-1 and its activation results in anti-inflammatory reactions. This receptor has not yet been observed in *H. pylori*-infected patients. M1 macrophage cells are linked to strong pro-inflammatory and cytotoxic responses induced by INF γ , tumor necrosis factor (TNF), and IL-6. M2 macrophage cells show markers of an anti-inflammatory process through CD163, a cell surface glycoprotein whose expression is induced by inflammatory mediators, such as glucocorticoides and IL-10. Also, they are inhibited by pro-inflammatory mediators, such as INF γ , TNF- α , as well as others [18, 19, 22, 23]. The CD14 receptor is a cell surface molecule expressed on monocytes and macrophages and linked to the recognition of *H. pylori* LPS, which leads to the production of many pro-inflammatory cytokines. The interaction of *H. pylori* and CD14 shows that its expression in gastric mucosa may indicate infiltration by monocytes/macrophages [27]. On the other hand, *H. pylori* has two major factors for virulence, vacuolating cytotoxin (VacA) and *H. pylori* neutrophil-activating protein (*H. pylori*-NAP), related to the stimulation of radical oxygen production by neutrophils. *H. pylori*-NAP is a 150-KDa oligomeric protein, a TLR2 agonist that exhibits chemotaxis for the neutrophils and monocytes, increasing the synthesis of tissue factor and type-2 plasminogen inhibitor, IL-23, and IL-12, a key cytokine for the differentiation of naïve T helper (TH) cells into the TH1 cytotoxic phenotype. Naïve T CD4+ helper TCD4+ cells can be called to differentiate towards TH1, TH2, TH17, and regulatory T cells (Tregs) phenotypes according to the cytokines. Classically, TH cells are divided into two functional subsets: Th1 and Th2, responsible for the production of a distinct pattern of cytokine secretion. The adaptive system produced by the host against *H. pylori* includes Th1 and Th17 components that are implicated in the control of infection. Despite these data, a number of reports suggest that this Th1-biased response is dysfunctional and can be considered as an important role in pathogenesis of *H. pylori*-related diseases [23, 27, 28]. Indeed the immunomodulatory properties of the pathogen can reprogram the host's immune tolerance and *H. pylori* escapes [19]. In a study published by Michalkiewics, 78 children, ranging in ages between 7 and 18 years, with a confirmed *H. pylori* infection in 40 patients (50% without symptoms), a Th1 profile of *H. pylori*-mediated inflammation was found [28]. *H. pylori* can modulate MyD88

expression. In other words, the lack of significant amounts of MyD88 can explain the persistence of the infection and the endotoxin tolerance [19]. Furthermore, the exact role of T Helper cells in *H. pylori* infection is not fully understood. Recent studies revealed that a deficiency in Th1 response may be responsible for the persistence of *H. pylori* infection [28, 29]. The role of *H. pylori* in pathogenesis of extra-gastrointestinal diseases may be based on the systemic effects of chronic gastric inflammation immune responses, which can induce lesions outside of the stomach and can be reversed after *H. pylori* eradication [10, 14-16].

3. Epidemiology and risk factors

More than 50% of the world's population is infected with *H. pylori* [7, 8, 30]. Although infection occurs worldwide, its prevalence shows a marked discordance within and between developed and developing countries. Overall prevalence oscillates between 30 and 50% in industrialized countries, in contrast with the ranges of 80–90% found in the developing countries. Within geographic areas, the predominance correlates inversely with socioeconomic status, especially those related with living conditions during childhood. The improvement in hygiene, sanitary conditions, and the active treatment of the *H. pylori* carrierships implemented in developed countries may be the responsible for the difference in these two worlds [6, 7, 17, 31-34]. Reports regarding racial and ethnic differences in the prevalence of *H. pylori* infection in children and adults have been carried out. Everhart et al. reported an overall seropositivity rate of *H. pylori* infection in 32.7% of 7465 adult participants in the United States. Higher prevalence was observed in non-Hispanic blacks and Mexican Americans (51.1 and 57.9%, respectively), compared with the values observed among non-Hispanic whites (26.9%) [29]. A study conducted by Nguyen et al. in two ethnic groups (Kinh and Khmer), with different cultures, involving 1596 individuals, 485 households, the seroprevalence was higher in adults than in children ≤ 18 years (40.2 and 32.1%, respectively). Variables related to promiscuity such as taking foods by hands, absence of good practice of hand washing after defecation, having mother or siblings infected by *H. pylori* were risk factors. There were no differences in *H. pylori* seropositivity based on sex ($P > 0.05$) or ethnicity ($P > 0.05$) [31]. In southern China, the prevalence of *H. pylori* infection in Chinese people was significantly higher than in Australians (44.2 vs. 21%). Comparing the prevalence of infection in children aged < 10 years, the Chinese showed 27% and the Australians 4%. In children over the age of 10, a similar rate of *H. pylori* acquisition was observed in both countries (1% per annum), according to epidemiological data [32]. Cherian conducted a cross-sectional study in 193 African refugee children (aged < 16 years) in Western Australia. The mean age was 7.9 years, and 18 children (9%, mean age of 11.3 months, and SD 5.2 months) were breastfeeding. There were 116 children living in refugee camps and the remaining lived in apartments or houses. The great majority came from Tanzania or Kenya, 96 of 97 (99%), lived in the camps, whereas 28 originating from Egypt lived in apartments and houses. *H. pylori* was present in 82% of children (63% aged < 2 years), rising to 95% for those older than 14 years, confirming a greater risk in Africa. Intrafamilial spread of *H. pylori*, particularly mother-to-child transmission or infected older siblings, was a potential mechanism of this acquisition. Concerning the breastfeeding children, a statistically significant correlation was not possible because the number of children was small. Curiously, those children who had done antimalarial treatment seemed to have eradicated *H. pylori*. It was

attributed to a probable effect of artemisinin on the iron dependence of the bacteria [33]. Differences in the prevalence of *H. pylori* infection among racial and ethnic groups have been described. Besides ethnicity, the difference in the prevalence between developed and developing countries seems to be linked to socioeconomic factors. Poverty and bad living conditions are the principle risk factors for acquiring *H. pylori* infection within industrialized countries [6-8, 34-36]. A high number of residents in the same home, sharing the same room or the same bed with *H. pylori*-infected children, as well as poor sanitary conditions were identified as major risk factors [6-8]. This infection is acquired in childhood and they are carrying the same strain as their parents and maintain this genotype even after moving to a different environment [37, 38]. In relation to the prevalence based on gender, differences have not been found between men and women, and no association with smoking or drinking habits has been made [6]. Nutritional status has been described as being associated to *H. pylori* infection. Goodman et al. found reduced odds of risk of *H. pylori* infection in adults and children with an increased ingestion of fruit, vegetables, and milk. The increased improvement of hygiene at home, as well as socioeconomic conditions, has resulted in a lower rate of *H. pylori* infection and an increase of gastroesophageal reflux disease (GERD) [8, 32, 35-37]. Studies revealed that gastric corpus inflammation induced by *H. pylori*, more pronounced with CagA+ strains, has an acid-suppressive effect, preventing the development of GERD. Also, asthma and allergy disorders are prevalent in developed countries and inversely associated with *H. pylori* [8, 14-16, 33, 34, 36]. Hygiene reduces the exposures to microorganism modifiers polarized Th1/Th2 responses leading to asthma. *H. pylori* can alter the polarized Th1/Th2 through dendritic cell-mediated T expression of IL-2, TNF- α , and INF- γ . Longitudinal studies revealed that asthma is a risk factor to GERD development and GERD can trigger asthma [36, 37]. The most well-known hematological pathology linked to *H. pylori* infection in children is IDA. Epidemiological studies have indicated that seropositivity is associated to low serum ferritin and hemoglobin levels compared with seronegative controls. Reports have been carried out concerning refractory iron deficiency anemia (refractory IDA), normalized after the *H. pylori* eradication therapy and also the improvement of anemia without iron supplementation [11-13].

3.1. Routes of transmission

A number of studies propose environmental sources, such as animal and water exposure, as potential forms of *H. pylori* infection. Also, a human-to-human transmission through oral-oral, fecal-oral, or both has been described. *H. pylori* has been detected in saliva, vomitus, gastric reflux, and feces [6, 8, 33, 34, 39-42].

3.1.1. Animals as potential source of *H. pylori*

Two epidemiological studies reported that the exposure to sheep was implicated in *H. pylori* infection. Goodman et al. [35] described a higher risk of *H. pylori* infection, in the Colombian Andes, in children who played with sheep. Dore et al. reported an association between a prevalence of infection in 98% of Sardinian shepherds and the contact with sheep and sheepdogs. In that study, the individuals had a significant increase of infection prevalence not observed in their family members, with no regular contact with sheep (73%) or sheepdogs (43%). A subsequent recovery of *H. pylori* in sheep milk led Dore to suggest that *H. pylori* may

be a commensal of sheep and thus the last ancestral host of *H. pylori*. The isolation of *H. pylori* in the stomach of cats led Handt et al. to suggest that cats may also represent a reservoir of the bacteria [27, 29, 43]. Rothenbacher et al. found that adults, who owned cats as children, had a higher prevalence of *H. pylori* infection. Contradicting these studies were El-Zaatari et al. who reported, in 25 stray cats, no infection by *H. pylori*. They found another strain, *Helicobacter heilmannii* to be responsible for chronic gastritis in cats. Two seroepidemiological studies in the United States, involving pet owners, failed to support the association of the relationship between pet owners and an increased prevalence of *H. pylori* [32]. One large population-based study in Canada, adjusted for social class, found no association between pet ownership and peptic ulcer disease. With these results, a relation between cats and *H. pylori* infection does not seem possible, and cats are not a health problem for cat owners [34].

3.1.2. Water transmission

H. pylori can survive for several days in milk and tap water in an infectious bacillary form. In rivers, it can survive in a coccoid form for several months. Under physical or chemical stress, *H. pylori* is capable of converting into a viable form. It is not known whether this coccoid form can revert to its infectious form. Several studies support this form of transmission [34, 39, 40, 44]. In 1993, Westbloom detected *H. pylori* in the sewage water in Peru [6]. Hulten et al. showed that in a population of Peruvian children, which consumed an internal source of water at home, the likelihood of having infection with *H. pylori* was tripled [39]. In Colombia, children who swam in rivers, streams, and pools and drank from a stream contaminated with sewage water had a higher tendency of acquiring the infection. In South America, Hopkins et al. found, in Chilean children who consumed uncooked vegetables contaminated with water containing raw sewage, an increased prevalence of the infection [33-35].

3.1.3. Fecal-oral transmission

Some studies support the evidence of the transmission of *H. pylori* through its elimination in feces after turnover of gastric mucosa. Thomas et al. in 1992 detected it in the feces of one adult and 9 of 23 children living in a Gambian village, using the culture method. The isolation of *H. pylori* from feces has been controversial. Parsonnet et al. [41] cultured the bacteria in feces, in 7 of 14 *H. pylori*-infected adults after cathartic-induced diarrhea. Mapstone et al. in 1993 detected *H. pylori* DNA by polymerase chain reaction (PCR). Some studies reported detection of *H. pylori* by DNA, in feces, in 25–90% of *H. pylori*-infected patients [34]. In 1993, Hopkins et al. observed that the consumption of fertilized vegetables with human feces was a risk factor for acquiring *H. pylori* infection. The spreading through feces is supported by the occurrence of infection in institutionalized people during gastroenteritis outbreaks [8, 34].

3.1.4. Oral-oral transmission

It is accepted that *H. pylori* infection acquisition occurs in early childhood by close interfamilial contact. Many scientists report that oral-oral transmission is the most common form in developed countries. This was supported by the high prevalence of *H. pylori* infection observed in institutionalized people, within families, and in dentists. Megraud found, in Western Africa,

a higher risk of *H. pylori* infection in children fed with pre-masticated foods by their parents [34]. Parsonnet et al. [41] isolated *H. pylori* from saliva, vomitus, and cathartic stools in 16 healthy volunteers, infected adults, using culture and PCR methods. Ferguson et al. also demonstrated by PCR, a low number of *H. pylori* from the saliva in one of the nine *H. pylori*-positive subjects. By using soluble electrophoresis on polyacrylamide slab gels, restriction endonuclease analysis, and Southern blot hybridization, they confirmed that the same strain was isolated from both saliva and gastric biopsy [42]. In 1993, Mapstone detected *H. Pylori* in the oral cavity, by culture, in one person. Majmudar et al. using PCR diagnosed *H. pylori* in dental plaque [6, 8]. Cellini et al. reported the isolation of *H. pylori* from the dental plaque of 1 in 20 *H. pylori*-positive patients. Comparing the protein patterns and the restriction endonuclease pattern of the bacteria, those isolated from gastric biopsy and from dental plaque showed to be identical [32]. Peach et al. found an association between *H. pylori* infection with a high plaque score. However, studies conducted by other investigators did not find a relationship between dentist visits, number of times that teeth were brushed, and periodontal status. Doré-Davin et al. found no correlation between *H. pylori* infection, determined by PCR, on saliva and dental plaque. They state that the transmission was done by gastro-esophageal reflux or regurgitation of gastric contents [34, 41, 42].

4. Pathogenesis of *H. pylori* infection in extradigestive pathologies

It is known that the immunological response triggered by the bacteria is responsible for gastric mucosa damage. Large amounts of pro-inflammatory substances, such as cytokines, eicosanoids, and acute phase proteins, are released after *H. pylori* colonization [14-19]. Also, cross mimicry between bacteria and host antigens may contribute to gastric mucosa damage [8, 22]. Although *H. pylori* gastric colonization induces histological changes in gastric mucosa, leading to chronic gastritis in all infected people, most have no symptoms. The risk of developing symptoms will depend on factors, such as the interaction between the host and the bacterium, host genetic and immune response, diet and level of gastric acid. Host immune gene polymorphisms and gastric acid secretion determine the bacterium's ability to colonize a specific gastric niche. Some bacteria strains are more virulent than others. The increase of pathogenicity is related to the induction of morphological changes, vacuolation, and successive degeneration of cells in vitro. The protein vacuolating cytotoxin VacA and the cytotoxin-associated gene pathogenicity island-encoded protein CagA seem to be essential for the colonization and for modulating the host's immune system. The pathogen effects of *H. pylori* are related to chronic inflammation, with the promotion of pro-inflammatory and anti-inflammatory mediators. Host genetics polymorphisms can affect the expression levels of these mediators [8].

4.1. Pathogenesis of *H. pylori* in extradigestive disorders

The role of *H. pylori* in the pathogenesis of extradigestive disorders is based on (1) local inflammation has systemic effects, (2) *H. pylori* infection is a chronic process that persists for several decades, and (3) the persistent infection induces a chronic inflammatory and immune response that can induce lesions locally and remote to the primary site of infection [14]. In

In addition to the above, several reports have revealed the role of *H. pylori* infection in hematological problems [10, 16, 45]. Unexplained ID, IDA, and cITP are the most common problems encountered in pediatric groups [11, 16]. Vitamin B12 (vit.B12) deficiency was the first hematological disease associated to *H. pylori* infection and considered an elderly age group's disease. A study conducted by Rogers et al. for the evaluation of risk factors contributing to low or marginal vitamin B12 concentration in Guatemalan school children revealed that malabsorption conditions can interfere with vitamin uptake of the diet. A normal vitamin B12 status is expected despite a low intake because of this efficient enterohepatic circulation. An additional hypo/achlorhydria produced by *H. pylori* infection can be the trigger to the development of cyanocobalamin deficiency in this young age group [46].

Gastric MALT lymphoma, also known as mucosa-associated lymphoid tissue (lymphoma), is a low grade B-cell lymphoma described by Isaac and Wright in 1993 as an adult disease and extremely rare in children. *H. pylori* stimulates the B and T lymphocytes of the stomach. B cells form clones, possibly by trisomia-3 mutations, and can become low-grade MALT lymphoma. The *H. pylori* dependence is by-passed to *H. pylori* independence with genetic mutations t(1:14) with a transformation to high-grade MALT lymphoma, not documented in children [9, 47, 48].

The Scientific community has discussed and incorporated international consensus guidelines for investigation and management of these hematological extragastric pathologies (IDA, cITP, vitamin B12 deficiency, and MALT lymphoma) [5, 49-51]. No international consensus was obtained for other hematological changes. However, autoimmune neutropenia was documented in 2002 by Gupta et al. [52] with the report of a patient with a neutropenia (400/ μ l), which normalized after *H. pylori* eradication. Papadaki et al. [53] in 2006, after evaluating 67 adult patients with chronic neutropenia and splenomegaly, found a higher prevalence of *H. pylori* infection compared to healthy controls.

According to literature, other hematologic problems may be associated to *H. pylori* infection. For example, leukemia in children, whose mothers are chronic carriers of *H. pylori*, increased risk of gastric bleeding in children with acute leukemia, *H. pylori* infected, and in patients with genetic diseases prone to hemorrhage (Hemophilia A and B and thrombasthenia) [15, 16].

5. Iron deficiency anemia

The World Health Organization (WHO) estimates that approximately 50% of all anemic patients have a diagnosis of IDA [1, 30, 31, 50, 51, 54]. In infancy, IDA is associated to abnormal mental and motor development. In developed countries, low intake, increased host requirements to supply the physiological needs for normal development, dietary errors, and blood loss are the most frequent causes of IDA in children [15, 45]. The evidence to support a causal association between *H. pylori* infection and ID or IDA is based on epidemiological studies and clinical trials. The first clinical case was described by Becker in 1991, in a 15-year-old girl, *H. pylori* positive, whose clinical presentation was a chronic active hemorrhagic gastritis, without gastrointestinal symptoms. In 1993, Dufour et al. reported a 7-year-old child without evidence

of hemorrhage or clinical symptoms, with refractory IDA to oral iron therapy. The investigation revealed an *H. pylori*-positive patient. High endoscopy showed chronic antral gastritis and *H. pylori* eradication reversed the anemia [55]. These case reports were followed by other studies that have documented an association between *H. pylori* infection and unexplained or refractory IDA with reversal of anemia and a normalization of iron stores after successful *H. pylori* eradication, with or without iron supplementation [10, 45]. A recurrent ID or an inadequate response to oral iron therapy implies searching for *H. pylori* infection. All guidelines and international consensus support that *H. pylori* should be sought and eradicated in ID cases [5, 49, 50, 56].

5.1. Pathophysiology of iron deficiency by *H. pylori*

The intrinsic biological mechanisms by which *H. pylori* infection induces ID, IDA, or refractory IDA are not fully understood. However, several pathways are involved: occult blood loss, changes in gastric physiology as the result of chronic gastric inflammation and of low gastric acidity and ascorbate, as well as mechanisms used by the bacteria for its growth [11-13, 55].

5.1.1. Occult blood loss

Chronic gastrointestinal blood loss is one of the supposed intrinsic mechanisms. However, most case series of *H. pylori* patients with anemia and chronic gastritis do not reveal gastric bleeding lesions at the time of endoscopy, and positive occult fecal blood tests are not always present [45, 57]. The occult fecal blood test is a qualitative and highly accurate (98%) form of detecting human fecal hemoglobin, based on a flow chromatographic immunoassay, it is a diagnostic approach for the evaluation of gastrodigestive pathology of detecting human fecal hemoglobin, based on a flow chromatographic immunoassay [58]. A literature review by Susan Owens et al. about gastrointestinal bleeding in children, considered the *H. pylori* infection in children with hemorrhagic hereditary disorders, such as hemophilia A and B as a risk factor for severe hemorrhage [16]. Also, Campuzano-Maya mentioned that an increased risk of hemorrhage is observed in patients with acute leukemia infected with *H. pylori*, which have a greater risk of gastrointestinal hemorrhage during treatment compared to uninfected patients. This risk is reduced if a screening and eradication therapy, is offered to all leucemia patients upon starting leucemia treatment if they are infected [16] Therefore, in patients with potentially hemorrhagic genetic diseases, such as hemophilias (A and B) and Von Willebrand disease, *H. pylori* infection should be considered as an important cause of upper gastrointestinal bleeding. The authors recommend a stool antigen test as a new and non-invasive screening test for diagnosing *H. pylori* infection in all patients with hereditary hemorrhagic disorders [16].

5.1.2. Changes in gastric physiology: gastric inflammation and chronic disease anemia

Gastric mucosal inflammation is an invariable finding in *H. pylori*-infected patients and represents the host's immune response to the bacteria [59]. Several mechanisms have been postulated: (a) directed mucosal injury enhancing permeability and the antigen exposure; (b) adherence of bacteria to the gastric mucosal, accompanied by microvilli loss, irregularity of luminal border, edema and vacuolation; (c) toxic effect of the bacteria on epithelial cells with

a gastric epithelial degeneration, access to underlying mucosa, stimulating host non-specific and specific responses involving several cytokines' liberation [59].

5.1.2.1. Directed mucosal injury

Two instances should be considered in the invasion of *H. pylori* in gastric mucosal. In the first, *H. pylori* colonizes, living in the mucus layer in proximity to the epithelial surface. This interaction leads to epithelial cell damage and liberation of pro-inflammatory cytokines [8, [59].

5.1.2.2. Adherence of bacteria to the gastric mucosal

After the initial colonization, *H. pylori* migrates to the mucosa with stimulation of immune responses. All *H. pylori* strains have the gene for vacuolation cytotoxin (VacA), but only 50% produce the VacA protein, capable of vacuolation eukaryotic cells and prone to insertion into the membranes of endosomal vesicles forming pores with chloride channel activity. This alters the anions' composition within the endosomes, which leads to osmotic swelling, cellular death and apoptosis, and a more severe degree of inflammation and peptic ulceration [59].

5.1.2.3. Toxic effect

Several enzymes produced by *H. pylori* may contribute to changes in gastric mucosa permeability. *H. pylori* is one of the most powerful urea-splitting organisms. Splitting urea leads to the release of nitrogen and ammonia, increasing the pH of the gastric antrum, one of the mechanisms that protects the bacteria from the gastric acidity and has a toxic effect on the mucosa. Bacterial phospholipases can destroy gastric mucosa phospholipids, and acetaldehyde dehydrogenase production may have also a toxic mucosal effect [59].

5.1.3. Recruitment of inflammatory chemokines and inflammatory cells

H. pylori is a non-invasive bacterium. However, after the initial phase of the colonization, a dense infiltration of granulocytes begins and it is followed, in the chronic phase, by a predominant infiltration of plasma cells and lymphocytes. It seems that signal transduction pathways must exist in order to induce the recruitment and immigration of inflammatory cells to the gastric mucosa. Additionally, soluble bacterial products may initiate the inflammatory cascade [17, 60]. Gastric *H. pylori* infection is associated to the epithelium release of several chemokines [60, 61]. Each chemokine is involved in the recruitment and activation of a specific immune cell. Potential chemoattractants of the CXC chemokine family, IL-8, GRO- α , and epithelial cell-derived neutrophil-activating protein 78 (ENA-78), are involved in the recruitment of neutrophils. Members of the C-C family (RANTES, MIP-1 α) have specificity for monocyte and lymphocyte recruitment. IL 8 expression has been detected in epithelial cells of antral biopsy and in the supernatant of antral biopsy samples in *H. pylori*-infected patients [59, 60]. *H. pylori* induces gastric epithelial cells to increase transcription and secretion of IL 8. TNF- α has an effect on leukocyte activation, and IL7 has a regulating role on lymphocytes B and T [59]. Studies state that cytokine levels, with a pro-inflammatory effect, such as IL-6, IL-8, and TNF- α are higher in *H. pylori*-positive patients than in *H. pylori* negative [49]. Among these,

IL-6 is the most important pro-inflammatory cytokine inducer of the hepcidin gene transcription through STAT3 (signal transducer and activator of transcription 3) [61, 62]. Hepcidin is a small peptide hormone primarily expressed in hepatocytes as a propeptide precursor. Prohepcidin is processed to a bioactive molecule of 25 amino acids, the hepcidin that is secreted into the blood stream and eliminated in urine. Hepcidin is the most important regulator of iron homeostasis [63, 64]. The expression of hepcidin is regulated on a transcriptional level by two principal pathways. The first is the activation of STAT 3. Upon activation, these proteins are transferred to the nucleus where they activate transcription of the hepcidin gene, binding to a sequence of DNA. The second is transcription control dependent on the BMP/Smad pathway, which involves Smad proteins and bone morphogenetic proteins (BMPs). Factors that can affect the expression of hepcidin are iron, anemia, hypoxia, and inflammation [61-68]. Hepcidin is a negative regulator of a transmembrane protein, ferroportin, which is expressed on the surface of all cells that release iron in circulation: enterocytes, macrophages, hepatocytes, and placental cells. Hepcidin regulates ferroportin at a translational level [67]. Under inflammatory conditions, hepcidin reacts as an acute reagent phase, increasing its expression, binding to ferroportin for tyrosine phosphorylation and also the internalization and ubiquitin-mediated degradation in lysosomes, resulting in hypoferremia and no iron availability for erythropoiesis, the hallmark of inflammatory anemia. In 1932, Locke et al. observed that infection was associated with low serum iron levels [63, 64]. This finding was corroborated by Cartwright and Wintrobe with the demonstration that anemia associated with infection was not different from anemia of inflammation, resulting in a pathology known as anemia of inflammation or chronic disease anemia [63, 68]. On the other hand, *H. pylori* can subvert human iron to benefit itself instead of the host, and recently hepcidin has been identified in the parietal cells of *H. pylori*-infected patients with an upregulated gastric hepcidin and a consequent downregulation of ferroportin [63, 67, 69].

5.1.4. Hypochlorhydria

Iron absorption takes place in the small intestine in accordance with the metabolic demands that reflect the amount of iron stored for erythropoiesis. Haem iron refers to the pool of iron incorporated in the protoporphyrin ring. Its absorption is more effective than non-haem iron and the mechanisms for absorption are different. HCP 1 (haem carrier protein) is a protein capable of the transmembrane transport of haem into the lumen of enterocytes to be catabolized by the microsomal enzyme haem oxygenase. The non-haem iron (Fe^{3+}) is not soluble and is the most prevalent alimentary iron. Its absorption is dependent on endogenous factors related to its reduction to a soluble iron form (Fe^{2+}), such as duodenal cytochrome *b* reductase, and the ascorbic acid released by gastric cells [70]. Gastric acidity increases the absorption of Fe^{3+} in two ways: (1) the high concentration of hydrogen ions at acidic pH ($\text{pH } 3 = 1 \text{ mM H}^+$) leads to an efficient competition for metal iron in bindings sites, on dietary components; (2) mechanisms related with the release of soluble ferric ions only in acidic pH ($\text{pH} < 4$) [71]. In 1905, John Edkins postulated gastrin as the hormone regulator for gastric acid secretion [72]. Sarker et al. [65] revealed that the basal and stimulated gastric acid was greatly reduced in *H. pylori*-infected children compared to non-infected [10, 16, 71]. The same author revealed, in a study with *H. pylori*-infected children of Bangladesh, that this infection was associated to impaired gastric

secretion. After *H. pylori* eradication, improved gastric secretion and hemoglobin concentration was observed but no influence on iron absorption was verified. Other possible mechanism such as competition for oral iron and inflammatory disease was considered a possibility. A study performed by Harris in 105 children, 33 *H. pylori* positive and 72 *H. pylori* negative, found a significantly higher percentage of *H. pylori*-infected children with a gastric juice pH above 4.0 (6/33, 18%) compared to *H. pylori*-negative children (6/10, 60%) [70]. While hypochlorhydria and acute *H. pylori* infection in adults is well documented, the mechanisms inducing hypochlorhydria in children are not well understood [8, 10, 16, 70]. *H. pylori* can induce hypochlorhydria through increased gastric IL-1 β and TNF- α , both in adults and in children, which inhibits acid gastric secretion, induces parietal apoptosis, and decreases enterochromafin-like cell histamine release [10, 70]. Capurso et al. [67] demonstrated that *H. pylori*-infected IDA patients, with involvement of corporal gastric mucosa, had an important decrease of gastric acidity (pH>4) with higher intragastric pH and high serum gastrin levels. Serum gastrin is an indirect marker of atrophic gastritis [11, 12, 73]. As described by Hartmann et al., iron malabsorption can be confirmed by an oral iron challenge. In fast, 2 mg of iron sulfate is administered, and the dosing of iron serum is done before ingestion and then after the first, second, and third hour. It is considered poor absorption when at the first hour the iron serum level is <100 μ g/ml (Figure 1) [74]. Recently, *H. pylori* has been implicated in unexplained refractory IDA to oral iron treatment. This refractoriness may be defined as a failure of the hemoglobin increase at least 1 g/dL after 2 months of treatment [13]. Celiac disease is the most known pathology related with malabsorption conditions. AAG, manifested as hypergastrinemia, with strongly positive antiparietal cell antibodies, was for many years considered a disease of older people and vitamin B12 deficiency the only possible presentation. The age, gender, and severity of the disease are determinants for the presentation in microcytic or macrocytic anemia [11-13]. Although atrophic gastritis may impair both vitamin B12 and iron, the increase in the iron demands for the observed growth in children and adolescents as well as the menstrual blood loss in pubescent females, make IDA easier to develop. *H. pylori* can trigger AAG by a molecular mimicry between the bacteria and H⁺ K⁺-ATPase. In children, a refractory IDA without evidence of gastrointestinal blood loss implies in first line, non-invasive tests for Celiac disease (anti-endomysial antibodies IgG and IgA), AAG (serum gastrin in fasting and antiparietal cells antibodies), and *H. pylori* infection (*H. pylori* IgG antibodies followed by urease breath test) [11-13]. IDA in the AAG context was described by Faber in 1909 and then completely ignored and forgotten. The role of *H. pylori* in AAG development is not consensual because of the high prevalence of this bacteria in the world population. However, eradication of *H. pylori* in this context is accompanied by a normalization of hemoglobin, iron stores, and gastrin levels [13, 16, 75].

5.1.5. Low ascorbic acid level

Vitamin C is an acidic molecule with strong reducing activity. Ascorbic acid (AA) is the reduced form of vitamin C. It is a potent antioxidant, a protector against gastric carcinogenesis, neutralizing nitrite-derived mutagens. Vitamin C is essentially absorbed and secreted in the antral mucosa. It has two major redox forms: AA and dehydroascorbic acid (DHA), the reduced

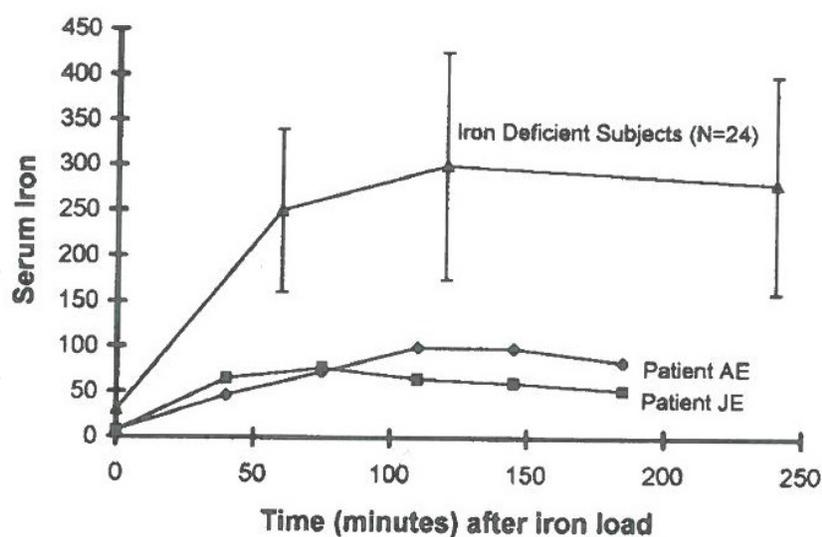


Figure 1. Oral iron challenge study. At the time, 0.2 mg/kg of elemental iron as ferrous sulfate was given. The expected normal response range, derived from 24 iron-deficient children but with normal absorption after given an oral dose of 1 mg/kg of elemental iron. Adapted with permission from Hartman and Barker [74]. Copyright ©1996.

and oxidized forms, respectively, and interconvertible. Within the cell, DHA is rapidly converted to AA by the specific enzyme systems, such as DHA reductase, glutaredoxins, and protein disulfide isomerase, in the presence of glutathione or other thiols as electron donors [76, 77]. Unlike AA, DHA is relatively unstable and undergoes rapid, spontaneous, and irreversible hydrolysis particularly at pH>4. Annibale et al. [73] demonstrated that gastric juice and ascorbate content are negatively affected by *H. pylori* infection and a normalization of ascorbate and gastric pH was obtained with eradication therapy [11-13, 78]. In *H. pylori*-infected children, a reduced gastric juice ascorbic acid concentration was found, and this effect was more evident in patients with CagA-positive strains [10, 30, 66, 71, 77]. A Korean study with the involvement of 452 patients (228 boys, 224 girls) aged 1–15 years revealed a negative correlation between gastric vitamin C level, *H. pylori* concentration, and the degree of active inflammation in antral mucosa. In this group, data showed that vitamin C levels in whole blood, plasma and gastric juice as well as the intragastric pH were closely related to the severity of the *H. pylori* infection and the histological changes in the stomach. These findings suggest that vitamin C may play a role in determining infection and progression [76, 79]. Studies have demonstrated that *H. pylori* produces gastric vitamin C reduction, promoting its degradation to DHA, a metabolite that may be oxidized at an irreversible form. This is unstable at high pH values, and thus hypochlorhydria reduces the bioavailability of this vitamin, essential for iron reduction to ferrous form, which forms an absorbable molecular complex with ferric iron, insoluble at pH>5 [10, 30, 45, 80]. Baysoy et al. documented a greater decrease in gastric juice ascorbate in *H. pylori*-infected patients with CagA-positive strains compared to those with CagA-negative strains [10, 45, 70, 71]. However, in this group, no association among CagA-positive strains and IDA has been found.

5.1.6. Utilization/sequestering iron by *H. pylori*

H. pylori, like other pathogenic bacteria, requires iron as a growth factor [8]. However, *H. pylori* does not produce siderophores capable of extracting iron from proteins. Potential iron sources for *H. pylori* are transferrin, lactoferrin, ferritin, hemosiderin, heme, and iron-containing enzymes [3, 81]. Lactoferrin is a source of iron for *H. pylori* in gastric mucosa. Choe et al. reported an abnormal elevated lactoferrin level in the stomachs of 101 adolescents with unexplained epigastric pain, ID, and *H. pylori* positive [10, 45, 82, 83]. A concordance was obtained with studies published by Dogan et al. [83] related to the gastric issues of 61 children with recurrent abdominal pain, 45 of which were *H. pylori* positive. It is known that lactoferrin captures iron from transferrin. *H. pylori* has a highly specific lactoferrin-binding protein. Thus, iron bound to lactoferrin is directed to *H. pylori*. The bacteria have a 19 KDa iron-binding protein that resembles ferritin, storing the iron, and making it unavailable for erythropoiesis [8, 67].

5.1.7. Inhibitor effect on duodenal mucosa cells

Duodenal mucosa cells are responsible for iron absorption. *H. pylori* can exert an inhibitor effect on the duodenal mucosa [8, 10].

5.2. Diagnosis

Symptoms of *H. pylori* infection in children are non-specific and can include: abdominal pain after meals, unexplained anorexia, nausea, recurrent vomiting, hematemesis, and IDA. These symptoms are considered to be of low predictive value. A meta-analysis of 45 studies concluded that *H. pylori* infection is not associated with abdominal pain. Therefore, an *H. pylori* infection diagnosing test for abdominal pain is not recommended as the first step of investigation [5, 49, 50, 56, 84]. In contrary, in a briefing for the European Gastroenterology Review, Mafertheiner et al. recommend that in children with a family history of peptic ulcer and gastric cancer, a recurrent abdominal pain should be subjected to testing for *H. pylori* after exclusion of other causes [49]. Based on the development of a chronic pangastritis with a consequent achlorhydria and reduced ascorbic acid, interference with iron absorption, occult blood loss by erosive gastritis or peptic ulcers, competition for the alimentary iron and sequestering by the bacteria, it seems reasonable to recommend testing for *H. pylori* infection on unexplained ID or IDA. Evidence-based guidelines from European Society for Pediatrics, Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the Group of the North American Society for Pediatric Gastroenterology (NASPGHAN) in 2011 for IDA in context of *H. pylori* infection in children are in accordance with the Canadian Group Consensus Conference in 1999 [5, 49, 50]. They recommend considering diagnostic tests in children with refractory IDA, after the exclusion of other causes and in children with first-degree relatives with gastric cancer. In 2007, the American College of Gastroenterology, in their guidelines for the management of *H. pylori* infection, questioned this conclusion. Large studies from North America have reported that *H. pylori* infection was an independent risk factor for IDA in 688 school-aged children from Alaska and 7462 children, adolescents, and adults, all from the United States. A recent unblinded study of 219 *H. pylori*-infected children, aged between 7 and 11 years, also from

Alaska, with pretreatment ID, found no difference in the likelihood of iron deficiency or anemia at 2 months or 14 months following a 6-week course of oral iron and antibiotics or no antibiotics. These data points support an association between *H. pylori* infection and IDA, but do not prove cause and effect. On the other hand, most studies refer to a relationship between *H. pylori* infection and IDA as well as a normalization with eradication therapy [11-13, 16, 49]. Antibody tests for *H. pylori* infection using whole blood, serum, and saliva are not recommended because of the low prevalence of *H. pylori*-related diseases in children and low specificity and sensitivity of the testes [51, 84].

6. Idiopathic thrombocytopenia purpura (ITP)

Immune thrombocytopenic purpura (ITP) is an autoimmune hemorrhagic disease, characterized by isolated thrombocytopenia, which affects nearly 1:25000 children per year [85-87]. ITP can present itself as an acute, self-limiting condition or a chronic process. Persistent thrombocytopenia, for longer than 6 months, may be present in about 20% of children [88-91]. A controversial issue in children is the designation "Chronic immune thrombocytopenia (cITP)," because about a third of children with ITP spontaneously cure from 6 months to 1 year later [87-89]. The International Childhood ITP Study Group recommends that 12 months must be the cutoff point for defining cITP [88, 90]. ITP is characterized by a premature destruction of platelets. Autoantibodies interact with the glycoprotein membrane on surface platelets (GIIb/IIIa or GIb), resulting in accelerated platelet destruction and clearance by mononuclear phagocytes [86, 91-93]. Increased megakaryocyte number in bone marrow is the marker of ITP in childhood [91]. However, studies performed in 1980 revealed that in two thirds of adults the expected increase of megakaryocyte production was not observed. Most of ITP had normal or depressed platelets turnover [87]. Chang et al. evaluated the effect of plasma, from childhood patients with ITP (44 with an acute form and 9 with chronic ITP), on induced thrombopoietin production of megakaryocytes in liquid culture. They reported that plasma from pediatric patients with ITP suppressed in vitro megakaryocyte production. They cultured cord blood cells as a source of CD34+ cells, which is a thrombopoietic growth factor, and plasma from control subjects or, children with ITP. After 8 days of culture, nearly 16% of cells were megakaryocytes. The effect of plasma in three groups was compared: control plasma, antibody ITP plasma (anti-GPIb, anti-GPIIIa, or both). They observed that in cultures with anti-GPIb antibodies the number of megakaryocytes were strongly reduced but this did not occur in ITP plasma negative antibody or in ITP plasma with only anti-GPIIb-IIIa antibody [93, 94]. Recently, thrombocyte kinetic studies have been carried out and autoantibodies affecting the megakaryocytes production in the bone marrow were observed [87, 91, 93, 95]. Also, studies using electronic microscopy showed 50–75% of ITP megakaryocytes damaged and in some cases attached by monocytes [96]. In most cases, ITP in childhood is triggered by a previous viral disease or a vaccination [87, 90]. HIV, hepatitis C, measles, cytomegalovirus, varicella, pertussis, and parvovirus can all be found in this context [95, 97]. After a benign viral infection, a predominant proinflammatory state may trigger ITP. Both proinflammatory cytokines and T-cells persist, creating a permissive environment for the emergence of autoantibodies that

bind to platelet membrane antigens [91, 93]. Zehnder et al. highlighted an increased expression of gamma interferon-dependent genes in early states of ITP, supporting a proinflammatory or TH1 profile [91, 95]. ITP affects both adults and children. However, the natural course of ITP in children is different from adults. Spontaneous recovery occurs in one third of the pediatric patients, months or years after the diagnosis. Several studies in adults reported improvement in platelet counts after *H. pylori* eradication therapy [91, 98, 99]. Few studies exist related to children and the ones that do exist have contradictory findings. In a study developed in Taiwan, Jaing et al. evaluated 22 cITP children *H. pylori* infected, after one or two courses of eradication therapy. They found that 55.6% of patients experienced total or partial remission after an average of 16 months. In the Netherlands, a study carried out by Niefjes et al. evaluated 47 children with cITP, 3 of them *H. pylori* infected. They were treated with a 2-week course of triple therapy and after 6 months all of them acquired complete remission. In an Iranian study by Hamidieh et al., 31 cITP children were evaluated. Four of them were *H. pylori* infected and treated with 2 weeks of triple therapy. None of them acquired partial or complete remission. In Italy, Bisogno et al. evaluated 25 children with cITP. Nine of them were *H. pylori* infected and treated with 1–2 courses of 2 weeks of triple therapy. After 6 months, three patients had an increase in platelet counts and one had remission. This author also reported complete remission in 16 *H. pylori*-negative patients. The follow-up of the last 10 *H. pylori*-negative patients presented a platelet count above 50×10^9 without any treatment [10, 45]. Several studies revealed a widely variable prevalence among different populations according to the countries' epidemiology. In Finnish populations, *H. pylori* was not found in any of the 17 pediatric patients [100]. Turkish children showed *H. pylori* infection in 11 of 35 children (31%). Japan reported a prevalence of *H. pylori* infection in 2 of 10 (2%). Chinese children from northern Taiwan showed the presence of infection in 9 of 22 (41%) children. The response to the eradication therapy was not uniform [101, 102]. *H. pylori* has been implicated in the pathogenesis of several autoimmune diseases [90, 95, 97]. ITP is a typical organ-specific autoimmune disease. In adult patients with ITP associated to *H. pylori* infection, eradication therapy has been associated to an increase in platelet count. The Maastricht Consensus in Florence in 2012 approved, in adults with ITP and *H. pylori* infection, a similar approach to the one used in those with IDA associated to *H. pylori* infection. In the pediatric age group with cITP, diagnosis tests for identification of *H. pylori* infection, such as ^{13}C -urea breath test, stool antigen test, or IgG serology, are not recommended. According to the guidelines described by Peter Mafertheiner, the recommendation for *H. pylori* eradication therapy in diseases like idiopathic thrombocytopenia was approved for adults [49].

Several mechanisms by which *H. pylori* may be associated to ITP have been proposed.

6.1. Possible mechanisms by which *H. pylori* may be related to ITP

6.1.1. Molecular mimicry

One theory is that cross-reactive antibodies produce reactions with *H. pylori* components and platelet glycoprotein through a molecular mimicry [90, 98]. Michael et al. tested platelet eluates derived from *H. pylori*-infected patients and found that platelet eluates with the capacity to react

with GPIIb/IIIa or GPIb failed to recognize any *H. pylori* antigens [90]. Recently, it was reported that monoclonal antibodies generated against *H. pylori* urease B react with GPIIb/IIIa, on platelet surface, suggesting that cross-reacting antibodies may be present in ITP patients [90].

6.1.2. Binding between *H. pylori* to von Willebrand factor/induction of platelet aggregation and apoptosis

Many diseases associated to platelet aggregation have been described as related to *H. pylori* infection [90, 101, 103]. Myocardial infarction, coronary heart disease, and stroke are examples of this prothrombotic condition. *H. pylori* may trigger both thrombotic thrombocytopenic purpura (TTP) and ITP by the induction of the interaction among platelets and von Willebrand factor (vWF). Studies suggested that vWF is essential for platelet aggregation induced by *H. pylori* [90, 103]. The mechanisms that lead to platelet aggregation in this infection are well not understood. Byrne et al. revealed that not all *H. pylori* strains can promote platelet aggregation [96]. *H. pylori* strain 60190 (ATCC49503) induces platelet aggregation through interaction between *H. pylori*, its antibody, platelet receptor Fc γ RIIA (CD32), and vWF and its glycoprotein receptor (GP)Ib/IX, produced in endothelium and in megakaryocytes (α 2 granules) [103]. *H. pylori* virulence factors, the Cag pathogenicity island (CagA), and the VacA are responsible for ITP, but are not related as a cause of platelet aggregation. Platelet apoptosis has also been described. *H. pylori* promotes platelet aggregates and platelet apoptosis, which may explain the decrease in platelet counts in TTP and ITP cases [101, 103]. This may also explain the remission observed with the eradication therapy in adults. In childhood, contradictory findings with discrepancy in the response to eradication therapy suggest that in most pediatric cases ITP is primary, not secondary to *H. pylori* infection [16, 89].

6.1.3. Modulation in the monocyte/macrophage function

Change in the balance of the Fc γ receptor, related to the activation of monocytes, leads to increased monocyte function with phagocytosis and autoreactivity of B and T lymphocytes [16]. *H. pylori* can be linked to autoantibodies produced by B lymphocytes and to an over reactivation of innate and acquired immune response against platelets [16, 85, 89]. CD4⁺ T helper cells regulate B cells, which release antithrombocyte antibodies [92, 95]. An upregulation of the genes involved in cell-related toxicity (granzyme, perforin) (CD3⁺ CD8⁺ T cell) is observed [92, 104].

6.1.4. Non-specific activation of immune system

The major antigenic component for antibody production against *H. pylori* is urease. Urease antibodies initiate autoimmune responses through autoantibodies produced by the activation of B1 cells [102].

6.2. Genetic characteristics of the patients

Recently, the role of genetic factors has emerged, indicating that *H. pylori* patients have a lower frequency of DRB1*03 and a higher frequency of DRB1*11, DRB1*14, and DQB1*03 compared to *H. pylori*-negative patients [1, 10, 90, 101].

Based on the previously mentioned studies and mechanisms described, which lead to ITP in *H. pylori* patients, as well as the discrepancy in the response to the triple therapy in pediatric cases, to treat or not to treat *H. pylori*-infected children continues to be an unsolved question [5, 49-51].

7. MALT lymphoma

For the first time, in 1983, Isaacson and Wright introduced the concept of mucosa-associated lymphoid tissue (MALT), a marginal, extranodal, indolent B-cell non-Hodgkin lymphoma [105, 106]. Predominantly found in females over the age of 50, it is quite rare in childhood. In prospective, multicenter NHL-BFM treatment studies performed since 1986, composed of 2703 children, only in 4 (0.1%) MALT lymphoma was found. *H. pylori* infection was documented in two out of four of these patients [106, 107]. The clinical pathological manifestation of extranodal B cell lymphoma is similar to that presented in the MALT. MALT is a system composed of small concentrations of lymphoid tissue, containing about half the lymphocytes of the immune system [106]. Situated along the surfaces of all mucosa tissues, its principal function is to produce IgA against specific antigens on the mucosal surfaces, TH2-dependent reactions, and TH1 cytotoxic T-cell-mediated reactions, thus resulting in immune tolerance. Gut-associated lymphoid tissue (GALT), nasopharynx lymphoid tissue (NALT), and bronchus-lymphoid tissue are the most well-known examples. Also described are conjunctiva-associated lymphoid tissue (CALT), lacrimal duct-associated lymphoid tissue (LDALT), larynx-associated lymphoid tissue (LALT), and salivary duct-associated lymphoid tissue (DALT) [105-109]. Although MALT sites are separate, they are functionally connected in what is known as the "common mucosal immune system". In other words, the antigen presentation and B-cell activation at one site can result in IgA secretion at another site in a different organ. MALT tissue contains B and T cells, as well as plasma cells and macrophages. The B-cell component (MALT) is found in Peyer's patches, plasma cells of the lamina propria, and in the B-cell compartment of the mesenteric ganglia. Gastric MALT lymphoma is a clear example of lymphoid malignancy associated to chronic inflammation [105]. It can occur in the context of chronic inflammation caused by some infectious agents, such as *H. pylori* (gastric lymphoma), *Chlamydia psittaci* (ocular adnexal lymphoma), and *Borrelia burgdorferi* (cutaneous lymphoma) [105]. MALT lymphomas arise in extranodal sites, frequently found in the stomach, lungs, ocular adnexa, and thyroid as well as a small percentage in the small intestine [105, 108, 110]. In 1991, Wotherspoon et al. demonstrated, for the first time, that primary gastric lymphoma was associated to *H. pylori* infection. Later, in 1993, the same author documented regression of low-grade B-cell gastric lymphoma of MALT in five of six *H. pylori*-infected patients after eradication [9, 110, 111]. Two types of MALT lymphoma have been identified: "native MALT type" corresponding to lymphoid tissue present in the gut and "acquired MALT type" developed in response to infectious events, such as *H. pylori* infection or autoimmune diseases (Sjogren's syndrome or Hashimoto thyroiditis). The histological feature of MALT lymphoma is an infiltration of the marginal zone and diffusion into surrounding tissues, as well as the presence of lymphoepithelial lesions formed by lymphoma cells in individual mucosal glands

or epithelial structures. MALT lymphoma cells have the same cytological and immunophenotype features (CD20+, CD21+ CD35+, IgM+, and IgD-) found in marginal zone B cells. Although gastric MALT lymphoma has an indolent course, rarely it can progress to aggressive high-grade tumors (extra-nodal large B cell lymphoma—eDLBCL) and, consequently, a drop in the survival rate from 90 to 40 %, respectively [105].

7.1. Pathogenesis and *H. pylori*

Gastric MALT lymphoma results from a multistage process that begins with the *H. pylori* infection and consequent recruitment of B and T cells [105, 112, 113]. The decrease in gastric acidity caused by the urease secretion triggers the lymphocyte infiltration and thus the establishment of MALT. B-cells are stimulated by *H. pylori* T-specific cells, acquires genetic abnormalities forming clones, possibly by trisomy 3, becoming *H. pylori*-dependent low-grade lymphoma [112, 114]. It has been demonstrated that lymphatic follicles, normally absent in the gastric mucosa, can appear, and consequently, configure the MALT after an inflammatory process [47]. *H. pylori* virulence factors seem to be crucial for the development of gastric lymphoma. CagA-positive strains are frequently related to a more aggressive lymphoma (eDLBCL) [115]. *H. pylori* can translocate the CagA protein into B-cells, inducing extracellular signal-regulated kinase activation and promoting upregulation of Bcl-2 expression, resulting in the inhibition of apoptosis. Otherwise, *H. pylori* can induce genetic mutation leading to the transformation of a normal B-cell to a malignant clone. The most frequent genetic mutations detected are t(11;18)(q21;q21), t(1;14)(p22;q32), and t(14;18)(q32;q21) involved in the activation of nuclear factor- κ B (NF- κ B), which plays a role in immunity, inflammation, and apoptosis [47, 113]. It has been found that positive CagA strains were higher in patients with t(11;18)(q21;q21) compared to those without such translocation (93.3 vs. 51%; P=0.01) [47, 114]. The transformation of low grade to high-grade lymphoma is rare in children, but it is documented in adults [107]. Normally, MALT lymphoma is a very indolent disease, localized for long periods of time. Systemic dissemination occurs in a few cases. In a large multicenter study of 93 patients with low-grade gastric MALT lymphoma in southern Switzerland and northern Italy, 49 *H. pylori*-infected patients with stage I of the disease. Eradication therapy was given in 97% of patients, and histological regression of MALT lymphoma was acquired in 67% of patients [114, 115]. The median time to achieve histological regression was 5 months (range 3–18 months). International guidelines recommend *H. pylori* eradication therapy in MALT lymphoma [5, 49, 50].

8. Diagnostic tests for *H. pylori*

Published consensus developed guidelines for the management of *H. pylori* infection in children. In 2000, ESPGHAN, and after NASPGHAN, published recommendations for *H. pylori* infection treatment [49, 51]. In 2004, the Canadian Helicobacter Study Group included consensus about how to approach *H. pylori* infection in children [5].

About testing *H. pylori* infection, questions must be asked in this age group:

Who should be tested? What tests should be used? Testing for *H. pylori* should be performed if a positive result is expected. A test and treat strategy is not recommended in children. The methods of diagnostic tests for *H. pylori* infection may be divided into two categories: invasive and non-invasive tests [5, 49-51].

8.1. Invasive tests

Invasive tests require a gastric endoscopy and a biopsy with culture for detecting *H. pylori* [116, 117]. They are the only tests considered to be 100% specific. The hematoxylin–eosin (HE) stain usually shows *H. pylori* strains [114, 118]. Nevertheless, when the results are inconclusive, there are at least two different stain techniques to be used on biopsied tissue: HE to evaluate inflammatory cells and Giemsa or Genta stain to detect *H. pylori*. Although the Genta stain due to a combination between a silver stain, HE and Alcian blue can visualize both inflammatory cells and *H. pylori* strain with higher accuracy, this formulation is technically complex. In contrast, the Giemsa stain is technically simple, highly sensitive, and inexpensive [117, 119].

8.1.1. Rapid urease test (RUT)

RUT identifies *H. pylori* through urease activity of the bacteria in gastric mucosa [120, 121]. Gastric biopsies are obtained and placed into an agar gel on a reaction contained urea, a buffer, and a pH-sensitive indicator. In presence of *H. pylori*, urea is metabolized to ammonia and bicarbonate leading to an increase of gastric pH, with changing in color [8, 117, 118]. The low cost, the simplicity, and rapid results make this test practical and effective, as long as the patient has not taken proton pump inhibitors or antibiotics. Medication that reduces the density of bacteria can decrease the sensitivity of the RUT up to 25%. Therefore, proton pumps and antibiotics must be not taken 1–2 weeks before the procedure [5, 49-51].

8.1.2. Histology

Histology is considered a standard method for detecting *H. pylori* infection. The sensitivity and specificity vary according to the site, number, and size of the biopsies, and also the experience of the pathologist [82, 117, 118]. False results can be obtained with a low density of bacteria. In these cases, multiple biopsies are needed. The majority of gastroenterologists obtained only biopsies of the antrum [122]. Recent studies revealed that the addition of corpus biopsies to antral biopsies increase the probability of *H. pylori* identification in all infected patients [50, 51, 117, 118].

8.1.3. Cultures

Cultures method has high specificity for the diagnosis of *H. pylori* infection, normally unnecessary for routine the diagnosis of *H. pylori*. The principal indication is testing for antimicrobial sensitivity in order to choose the appropriate antibiotic. Cultures are expensive and not as sensitive as RUT or histology and not available in many clinical laboratories [119, 120].

8.1.4. Polymerase chain reaction

PCR is a DNA amplification that uses the rapid production of a target DNA sequence to identify *H. pylori*. It is capable of identifying *H. pylori* strains in biopsies with chronic gastritis and non-identifiable bacteria. PCR can be performed on samples obtained by invasive and non-invasive methods using samples obtained of saliva, gastric juice, and stools [82, 119]. It is simple to perform and can provide additional genotypic information about the strain and antibiotic susceptibilities. PCR could be complete in 3–4 hours, and it is capable to detect the point mutations attributed to the development of clarithromycin resistance [119, 123].

8.2. Non-invasive tests

Although the gold standard method for diagnosis is gastric histology and rapid urea test, an important disadvantage is their invasiveness. Non-invasive tests, such as urea breath test (UBT), serology, and more recently, fecal *H. pylori* antigen, have been developed and all of them are feasible in children [50, 119].

8.2.1. Urea breath test (UBT)

UBT is one of the preferred tests to diagnose active *H. pylori* infection [5, 124]. It is based on hydrolyses of urea to ammonia and carbon dioxide, which diffuses into the blood and is excreted by the lungs. Since it requires the child's full cooperation, it is difficult to perform in children, especially toddlers and physically challenged [50]. This test is based on the ingestion of urea labeled with either non-radioactive isotope (C^{13}) or radioactive isotope (C^{12}). The first is preferred in children [5]. Labeled urea is dissolved in orange juice without sugar, given in fasting, and the primary objective is to validate C^{13} in children and adolescents. The ratio in exhaled air is measured at 0 and then 30 minutes after the intake, and the test is considered positive when the difference between these two ratios exceeds 4‰ (cut-off value) [124]. Antibiotics must be withheld at least 28 days and proton pump inhibitor (PPI) for 7–14 days before UBT [5, 82, 124].

8.2.2. Antibody test

The IgG-specific *H. pylori* antibodies are present in blood nearly 21 days after the infection and can persist for a long time after eradication therapy. With a sensitivity of 85% and specificity of 79%, they are not adequate for documenting *H. pylori* eradication [6, 50].

8.2.3. Fecal antigen test

The fecal antigen test (FAT) identifies *H. pylori* in stool by enzyme immunoassay (EIA) based on polyclonal or monoclonal antibodies, and immunochromatographic tests, so-called rapid or quick tests. The first commercial EIA test to detect *H. pylori* antigen in stool was the Premier Platinum HpSA based on polyclonal antibodies. These tests have a wide range of sensitivity and specificity both in pretreatment (86, 90–98%) and post-treatment (89, 91–92, 95%) [51]. The stool antigen tests using polyclonal antibodies demonstrated variable results and seem to have less accuracy than those using monoclonal antibodies. This diagnostic approach is convenient

for children in whom it is easy to collect feces [117]. Nguyen et al. evaluated the sensitivity and specificity of monoclonal enzyme stool antigen assay for diagnosis of *H. pylori* in 232 children age range 3–5 years *H. pylori* infection positive by culture from biopsies. They found a sensitivity of 96% and a specificity of 94.8% [125, 126]. FAT can be used to screen for infection before and after treatment [50, 82, 117]. It is considered as acceptable as UBT and is not dependent on the child's collaboration. Recent studies indicate that FAT can be done as early as 14 days after treatment, but should be done more than 4 weeks [50].

Note: PPI must be withheld two weeks before performing RUT, UBT, and FAT [49, 51]

9. Treatment

The goal of treatment is to obtain 90% eradication. The therapeutic regimens are variable and dependent on local resistance data. Several studies have documented high resistance to clarithromycin and metronidazole [127]. The first treatment commonly administered in children and adults is a triple therapy, for 14 days, that includes a PPI and two antibiotics, amoxicillin and metronidazole or clarithromycin [49-51, 127]. The first line of recommended eradication therapies are as follows: amoxicillin, 50 mg/kg twice a day for 14 days; clarithromycin, 15 mg/kg/twice daily for 14 days; and PPI, 1 mg/kg twice daily for 1 month. Alternatively, amoxicillin, 50 mg/kg twice a day for 14 days; metronidazole, 20 mg/kg twice daily for 14 days; and PPI, 1 mg/kg twice daily for 1 month or clarithromycin, 15 mg/kg/twice daily for 14 days; metronidazole, 20 mg/kg twice daily for 14 days; and PPI, 1 mg/kg twice daily for 1 month [50]. Several studies revealed that the use of probiotics improves the treatment tolerance, but there is no evidence of higher eradication [82]. Koletzko et al. [122] revealed a rate of resistance to clarithromycin of 20%, and a rate of resistance to metronidazole of 23% after a first treatment [82, 127]. In Portugal, a higher clarithromycin resistance rate was reported in children (44.8%) compared with adults (14.8%) [127]. The main factors for triple therapy failure are the low compliance and the bacteria's resistance to antibiotics. The risk for clarithromycin resistance is related with the previous consumption of macrolides, which is substantially prescribed in children for respiratory tract diseases. Double resistance strains were found in up to 50% of strains after failure of therapy using both clarithromycin and metronidazole [128]. The failure after the first therapeutic approach is predictive of resistance to other therapeutic approaches [50, 51, 128]. NASPHGAN recommend, in case of failure in the first line of treatment in children, and if possible, to perform culture with testing for antibiotic sensitivity to guide a second-line therapy. Other options may be taken if a *H. pylori* culture is not possible: quadruple therapy composed of PPI + metronidazole + amoxicillin + bismuth, triple therapy using fluoroquinolones, but of limited indication in children, or sequential therapy involving dual therapy with PPI and amoxicillin for 5 days followed sequentially by 5 days of triple therapy with PPI, clarithromycin, and metronidazole/tinidazole. In 2005, 74 children (randomized) received sequential therapy and triple therapy. Eradication was 97.3% in those with sequential therapy compared with 75.7% in children with triple therapy [51].

10. Conclusion and possibilities of new studies

Approximately 50% of the world's population is *H. pylori* infected. Although it has been a part of human microbiota, it is classified by The World Health Organization as a gastric carcinogenic. *H. pylori* is acquired essentially in childhood and through mechanisms of escape, it persists in gastric mucosa for life if left untreated. An association has been found between *H. pylori* infection and poverty, low socioeconomic conditions, poor hygiene, and nutritional deficiencies. With higher prevalence in developing countries, the predisposing factors are the high number of residents in the same home, sharing the same room or the same bed with *H. pylori*-infected children, as well as poor sanitary conditions. Hematological extragastric disorders have been described as being associated to *H. pylori* infection. Unexplained ID, IDA, and refractory IDA in childhood are the most well-known pathologies related to *H. pylori* infection. Two meta-analysis have supported the association between *H. pylori* infection and IDA, showing an increase of hemoglobin levels after *H. pylori* eradication [10, 45]. Many areas of the world have high prevalence of both *H. pylori* infection and IDA. ID in children leads to major consequences on children's health and neurodevelopment. Associations have been reported, in children, between IDA, lower intelligence scores or cognitive functioning in tests, and behavioral delays in children, showing that treatment can improve these outcomes [127, 129]. As a result, Update of US Preventive Services Task Force (USPSTF) and the Center for Disease Control and Prevention recommend screening for IDA in children aged 6–24 months, between 9 and 12 months, 6 months later, and then annually from the ages of 2 to 5 years, respectively, and to treat the ID by prescribing 3 mg/kg/day of iron drops between feedings [129-131]. Refractory to iron therapy implies, after the exclusion of ingestion deficiencies, hemorrhage, absorption problems, and (rarely) metabolic abnormalities, the search for *H. pylori* infection using in the first line non-invasive tests (UBT, FAT, and specific IgG *H. pylori* antibodies). All non-invasive tests are feasible in clinical use. C¹³UBT, with a good sensitivity for the diagnosis of *H. pylori* infection, shows limitations such as low specificity for very young children, being affected by previous ingestion of antibiotics or PPI and the requirement of patient cooperation [49-51]. Stool antigen test shows low sensitivity and good specificity in children of all ages, limited also by PPI and antibiotics. It is advisable to validate two non-invasive tests, the C¹³UBT and stool antigen for the diagnosis of *H. pylori* infection in pediatric ages. Serology is based on the immunological response to chronic *H. pylori* gastric inflammation and is not dependent on antibiotics or on the loading of gastric bacteria. International Guidelines are well defined regarding IDA in the context of *H. pylori* infection in childhood, in which eradication therapy is indicated as it is done in adults. Consensus about the approach of cTPI and *H. pylori* infection is well defined in adults, in which *H. pylori* eradication is followed by platelet recuperation. In childhood, there is no evidence to support a relationship between this infection and cTPI. Reports about prevalence of *H. pylori* and TPI seem to be according to the epidemiological data of the countries. Contradictory findings with discrepancy in the response to eradication therapy suggest that in most pediatric cases ITP is primary, with a spontaneous cure from 6 months to 1 year later, and not secondary to *H. pylori* infection [87-89]. Maastricht IV and other guidelines suggest not searching for or treating *H. pylori* in this context.

Gastric MALT lymphoma is a lymphoid malignancy that is quite rare in children. In these cases, eradication therapy must be performed regardless of the stage of lymphoma. The failure of *H. pylori* eradication is predictive of a resistant strain acquisition and also predictive of subsequent therapeutic failure. A primary *H. pylori* resistance in children of European countries has been estimated and ranges from 12.45 to 23.5% [128]. Therefore, clarithromycin-based triple therapy can be recommended after studies of antibiotic susceptibility or in countries with low resistance to clarithromycin. The high resistance of *H. pylori* to clarithromycin can justify the use, as first-line therapeutic approach in children, of the sequential therapy which was referred, resulting in eradication rates of 97.3% against 75.7% with triple therapy [49, 51]. In children, there are particular problems with alternative antibiotics, which are not approved for the age. Therefore, they are often treated multiple times without cure. The impact of this infection on public health implies applying active measures toward *H. pylori* eradication. A vaccination strategy should be implemented as the best option to eliminate *H. pylori* and therefore to improve public health.

11. New studies

Several new antibiotics are under investigation for the treatment of *H. pylori* in adults. (1) Rifabutin. Triple therapy for 10 days with rifabutin + pantoprazole + amoxicillin is showing good results in resistant *H. pylori* areas. Resulting in eradication rates of 50% [82, 132]; (2) Nitazoxanide that includes levofloxacin, nitazoxanide, doxycycline, and omeprazole is showing better efficacy than standard triple therapy [133].

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