

New diagnostic perspectives related to *Helicobacter pylori* infection in children

Noi perspective diagnostice în infecția cu *Helicobacter pylori* la copil

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Abstract

The Helicobacter pylori infection is one of the most widespread infections in the world, with the greatest incidence in developing and undeveloped countries (10%). It is approved today that Helicobacter pylori causes gastritis and peptic ulcer disease, and that the long-term infection is associated with a high risk for developing a gastric adenocarcinoma or malignant lymphoma. This infection is included by WHO in the 1st carcinogenetic group. The gold-standard in diagnosing the Helicobacter pylori infection is the gastric biopsy; the evaluation of treatments` efficacy involves urea breath test as well as the detection of stool antigens. The bases of the genetic predisposition depend on the genic susceptibility or on the DNA repairing mechanisms and carcinogenesis. The treatment response of the Helicobacter pylori infection has genic determinism. The polymorphism of the host, implying the genes CYP2C19 and MDRI, influences the pharmacokinetic and clinic efficacy of the treatment. The directions of fundamental research combine clinical aspects with endoscopic, histological and immunological examination. There are new innovative immunologic and genic methods to evaluate gastritis and ulcer associated with Helicobacter pylori in children. In order to elaborate new therapeutic protocols, it is important to make some correlations between the infection with Helicobacter pylori and the genic polymorphism. By means of molecular biology, scientists hope to identify the changes that determine resistance to antibiotics. The objective is to decrease the incidence in gastric malignancy in the adult period, diminishing the costs derived from malignancies, which could represent an important step forward in the practice of pediatric gastroenterology.

Keywords: *Helicobacter pylori, gastritis, child*

Rezumat

Infecția cu Helicobacter pylori este una dintre cele mai răspândite infecții în lume, incidența cea mai mare fiind în țările în curs de dezvoltare sau slab dezvoltate (10%). Este recunoscut astăzi că Helicobacter pylori cauzează gastrita și boala peptică ulceroasă, iar infecția pe termen lung se asociază cu un risc crescut de a dezvolta un adenocarcinom sau limfom malign gastric. Infecția cu Helicobacter pylori este încadrată după OMS în grupul I cancerigen. Standardul de aur în diagnosticul infecției cu Helicobacter pylori este biopsia gastrică; evaluarea eficacității tratamentului se face cu testul respirator marcat cu carbon radioactiv și prin determinarea antigenelor din scaun. Bazele predispoziției genice sunt dependente fie de susceptibilitatea genică pentru infecție, fie de mecanismele de reparare a ADN și carcinogeneza. Răspunsul la tratament al infecției cu Helicobacter

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*pylori are determinism genetic. Polimorfismul gazdei interesând genele CYP2C19 și MDR1 influențează farmacocinetica și eficacitatea clinică a tratamentului cu inhibitori de pompă protonică. Direcțiile cercetării fundamentale în infecția cu *Helicobacter pylori* combină aspectele clinice cu cele endoscopice, histologice și imunologice. Există metode imunologice și genetice inovatoare pentru evaluarea gastritei și ulcerului asociate infecției cu *Helicobacter pylori* la copil. Este importantă realizarea unor corelații între infecția cu *Helicobacter pylori* și polimorfismul genic pentru a crea noi protocoale terapeutice. Se speră identificarea prin biologie moleculară a modificărilor care determină rezistența la antibiotice. Se urmărește astfel scăderea incidenței malignității gastrice la vârsta adultă, diminuând și costurile aferente acestora, ceea ce ar putea reprezenta un important pas înainte în gastroenterologia practică pediatrică.*

Cuvinte-cheie: *Helicobacter pylori*, gastrita, copil

Gastritis is defined as an inflammatory process of the gastric mucosa, and the ulcer disease with gastric or duodenal localization represents the mucosal injury, clinically manifested as a dyspeptic syndrome with alimentary regularity and seasonal periodicity (1,2).

The present level of knowledge in digestive pathology has proven that *Helicobacter pylori* causes gastritis and peptic ulcer disease, and that long-term infection is correlated with a high risk for gastric adenocarcinoma or malignant lymphoma in adults (3,4). Since 1994 The World Health Organization (WHO) has included *Helicobacter pylori* in the first carcinogenic group, gastric cancer being the second cause of cancer death in the world (5). Usually, children are infected in the first decade of life, and gastric cancer develops in adulthood (50%) (3).

Helicobacter pylori infection is one of the most widespread infections in the world, the greatest incidence being found in the developing or undeveloped countries (Africa or South America - 84% at the age of 30 months), while countries in Europe or North America present a low incidence (6).

In the developed countries, the transverse serologic studies demonstrate that the rate of infection grows with 10% for every decade of life (6). Thus, in Italy, up to date cohort and epidemiological studies show that 11% of the persons between 6 and 18 years old – included in the study – have been infected, while the prevalence in persons of 50-60 years old was 50-60%.

There is a direct correlation between the low social and economic level and the *Helicobacter pylori* infection.

In chronic active gastritis, the prevalence of the infection exceeds 80%.

According to The International Statistics Center of Digestive Diseases (2007) the incidence of peptic ulcer disease is 4.4/10,000 in children, with a frequency of 3-6% in children who underwent a gastroscopy. The incidence of gastritis in children is 1% in the developed countries and up to 10% in the developing countries (7).

Clinically, the *Helicobacter pylori* infection is associated with acute or chronic gastritis, duodenal or gastric ulcer, gastric carcinoma, B lymphoma (Maltoma), as well as extra-digestive manifestations like iron deficiency anaemia, urticaria, low stature (6).

The following can be mentioned as results of gastritis in childhood: irritable stomach symptoms, symptoms due to gastric hypomotility (postprandial epigastric plenitude, decrease of appetite, prolonged plenitude, disinclination for food, nausea), symptoms of gastroesophageal reflux disease, symptoms of flatulence dyspepsia (distended abdomen, eructations); acid dyspeptic symptoms (undue postprandial epigastric pyrosis, hunger or nocturnal pains, relieving of pains after antacid drugs administration), non-specific symptoms (8).

The symptomatology in ulcer depends on: age - early infancy and later infancy - vomiting and/or digestive bleedings, school period - recurrent abdominal pain (RAP) and/or

vomiting. Epigastric pain and nocturnal pain, relieved after antacid drugs or eating, is typical, appears in teenagers and is called ulcer-like dyspepsia. Children with *Helicobacter pylori* gastritis without ulcer are almost every time non-symptomatic and they scarcely suffer from the same symptoms as children having ulcer disease. The complications of peptic ulcer are: digestive bleedings, perforation (10%), pyloric stenosis, malignant transformation of ulcer (exceptional in childhood), chronic diarrhea, malnutrition, iron deficiency anaemia, growth failure, sudden death (1,6).

***Helicobacter pylori* gastritis and ulcer diagnosis**

- Superior digestive endoscopy is the procedure of choice for the diagnosis of gastritis and peptic ulcer. From the endoscopic point of view, Dohil and co-workers (6) classify gastritis in erosive and non-erosive, and topographically in antral gastritis, gastritis of the corpus or pangastritis. The endoscopic aspect of *Helicobacter pylori* gastritis is characterized by a micronodular antral mucosa (over 50% of cases) or paving-stone appearance (98-100% of cases) (1). The classification of gastritis includes the

Sydney (endoscopic, histologic, etiologic) criteria. Following endoscopic criteria, there are: exsudative gastritis, erosive gastritis, atrophic gastritis, haemorrhagic gastritis, and reflux gastritis with hypertrophy of gastric mucosa folds. Following histologic criteria, there are: acute, chronic, special gastritis (eosinophilic, lymphocytic, granulomatous). In acute gastritis a neutrophilic infiltrate is present, while in chronic forms a lymphoid one is encountered. Using the Sydney classification, we have to perform two antral biopsies (2-3 cm from the pylor), two biopsies from the gastric body and one from the incisura. The active ulcer disease is round-shaped, oval, with a white base consisting of fibrin and cellular remains. Its border is red and raised. The duodenal ulcers are often associated with pyloric spasm or distortions of the pylor (1,8,9).

- Histology - the spiral-shaped *Helicobacter pylori* microorganism must be identified. The identification is possible using the Warthon - Starry silver staining (very sensitive and specific), the modified Giemsa, acridine orange, or cresyl violet coloration (sensitive and specific, easier to carry out

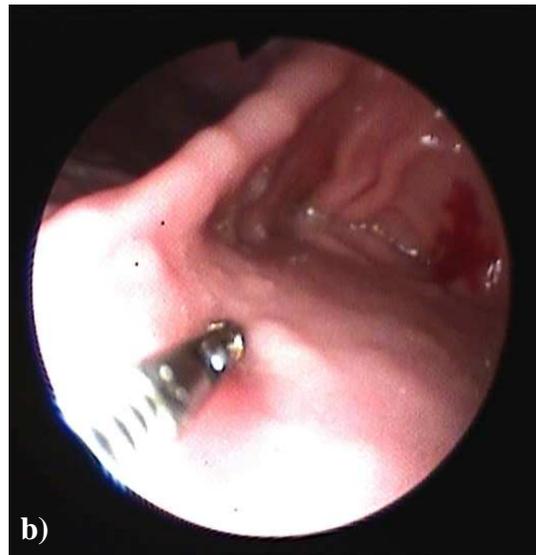
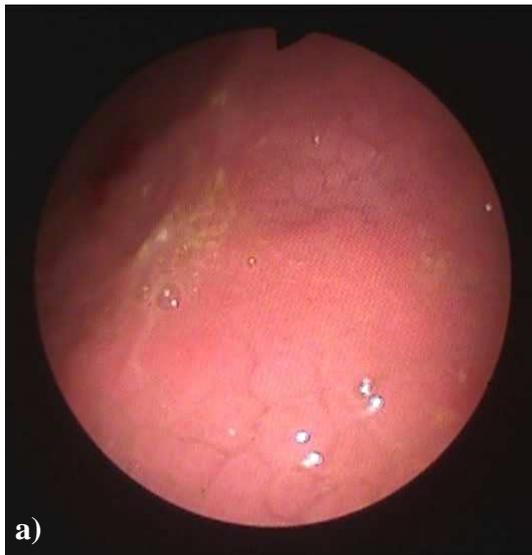


Figure 1. Endoscopic appearance of gastritis (a) and peptic ulcer (b) in *Helicobacter pylori* infection

than the silver staining). The Genta coloration marks out the bacteria and also the mucosal histological aspects. A new method includes the fixation of mucus film using the Carnoy solution associated with immunohistochemistry which decreases the positive false reactions as well as negative false reactions that appear in common histological stains (4,6).

- *Helicobacter pylori* culture is acquired by inoculating the biopsy fragments in agar - blood plate in microaerophilia - at 37°C.
- Rapid Urease test is used to detect *Helicobacter pylori* from gastric mucosa specimens contained in urea growth media. The color conversion is produced in 30 minutes and, in case of a small number of bacteria, after 24 hours(10).
- The respiratory test (urea breath test) marked with ¹⁴C represents a non-invasive diagnosis method. It has 100% sensitivity and 92% specificity. It is used as a method in evaluating the efficacy of *Helicobacter pylori*'s treatment (11).
- Serological tests detect antibodies. ELISA is the method used to detect the serum IgG or IgA. IgG serum level measurement has < 50% specificity in children under 7 years old, gaining a specificity similar to the one found in adults only after the age of 12. IgA antibody measurement in children is not a good indicator for gastric colonization, because only 45% from the *Helicobacter pylori* infected children have an increased level of specific serum IgA antibody (4,10).
- Salivary specific IgG detection has a specificity up to 82% and a 93% sensitivity, varying with the used reagents (10).
- The immunoenzymatic technique for the *Helicobacter pylori* antigens detection in human faeces (HpSA) is carried out with polyclonal and monoclonal antibodies; the method is a non-invasive one. The *Helicobacter pylori* stool antigen test - HpSA (Premier Platinum HpSA test, Meridian Diagnostics Inc, Cincinnati, OH) is using polyclonal antibodies and it has a 91-98% sensitivity in adults and a 83-100% specificity in diagnosing the infection. The development of monoclonal antibodies methods leads to a higher accuracy; the specificity and sensitivity reach up to 98-99% (12,13).
- Immunological tests PCR and DNA - enzyme. Although the most amplification PCR diagnoses are established by gel electrophoresis, the colorimetric detection method is preferred because it is faster and it can be automatized (4). There are three immunoenzymatic DNA tests developed to detect *Helicobacter pylori*:
 - GEN-ETI-K DEIA (Sorin, Italia); this test uses streptavidin coated microwell plates to which a biotinylated specific probe is added, based on the UreC gene of helicobacter. The amplified product is added to the plate and the duplex DNA detected with an enzyme linked antibody against double stranded DNA
 - Pylori-Prob (Biocode, Belgium) is a solid phase sandwich hybridisation assay.
 - PCR-ELISA (Boehringer Germany) uses a capture probe labelled with biotin (UreC) bound to the solid phase in a streptavidin coated microwell plate to which the amplified product labelled with digoxigenin is added and the hybridised product is detected with anti-digoxigenin peroxidase.

The actual hypothesis in the etiology of gastroduodenal diseases shows that beside environmental factors, the genic predisposition has an important role in the infection with *Helicobacter pylori* (the rate of seroprevalence was similar in identical twins). The familial studies proved that there is also a genic predisposition for gastric cancer (9, 14, 15).

Some authors highlighted that the basis of genic predisposition connect either with the

genic susceptibility for the infection, or with the DNA repairing mechanisms and carcinogenesis (6).

The mutations on the p53 level represent the most common genic injuries found in human cancer, including the gastric cancer. The inhibition of apoptosis via the adjustment of cyclooxygenase 2 by *Helicobacter pylori* generates inflammation and it can play an important role in the appearance of metaplasia. Microsatellite instability secondary to the germinal mutations of DNA has been found in a subgroup with gastric cancer as well as in intestinal metaplasia. (15,16).

The patients with low acidity are more predisposed to develop gastritis of the stomach body, gastric ulcer and gastric carcinoma. The IL-1 β polymorphism has been associated with the alteration of gastric acid secretion and is responsible for the subsequent premalignant histologic modifications. The severity of the host's response to the *Helicobacter pylori* infection has been reported in persons producing IL-1, which appears to be a possible gastric acid secretion inhibitor, like the pro-inflammatory cytokines (16).

Present day theories about the Cag A and Vac A positive *Helicobacter pylori* infection (known as type I) show that these are usually associated with severe gastro-duodenal disease and gastric cancer, compared to seronegative infections (type II). That is why the detection of these proteins and the proper treatment in childhood could diminish the risk of cancer development in adult period (1).

Thus, *Helicobacter pylori* produces inflammation in two ways: direct toxicity mediated by specific toxins and other aggressive factors, and immune-mediated toxicity.

In the second mechanism, the inflammatory response is produced by the bacterial adhesion to the epithelial cell, releasing neutrophils, macrophages, B and T lymphocytes, plasma cells. The high production of IL-8 interleukines leads to an activation of cytokines, in-

duced by NFK β activating through the Cag A positive bacterial type, more than those Cag A negative. IL-8 and other chemokines amplify the immune response, as well as the inflammatory response, generating an epithelial injury (1,17,18).

The immunological studies demonstrated that IL 18 generates an immune T1 response, not the expected Th 2 response, a fact that, associated with *Helicobacter pylori* specific T-cell clones Fas-mediated apoptosis, can help the persistence of infection. Some of the patients generate autoantibodies against the gastric parietal cells' H⁺/K⁺-ATP-ase which has an important role in generating gastric atrophy.

It is well known that gastritis and ulcer disease have a multifactorial etiology, generated by the imbalance between three pathophysiological pathways: the aggression factors (*Helicobacter pylori*, high serum levels of gastrin, parietal cell mass, high acid secretion, pepsinogen secretion, oxygen free radicals, nitrogen monoxide), protection factors (gastric mucus, cell barrier, bicarbonate secretion, growth factors and so on), genetic factors, environmental factors and other exogenous risk factors (2, 5).

Theories regarding response to gastroduodenal diseases and *Helicobacter* infection sustain the gene determinism. Thus, host polymorphism that implies the CYP2C19 and MDR1 genes influences the pharmacokinetic and clinic efficiency of the treatment with proton pump inhibitors (16,18,19).

Since 1983, Warren and Marshall have described *Helicobacter pylori* as a flagellated, spiral-shaped, Gram negative bacteria, of 2-3 microns in size, which survives within the mucus layer of the gastric mucosa epithelium, but also in metaplastic areas from the duodenum and esophagus (Baret's esophagus).

Helicobacter pylori has an abundant production of urease, as well as other enzymes with pathogenic implications, such as: catalase, phospholipase, protease, mucinase. Urease is

important for colonisation and an indirect marker of the presence of the microorganism; it is the base for the urease rapid test, urea breath test and serological tests (10,12).

It is well known that the treatment for classic *Helicobacter pylori* infections means a double antibiotic therapy (Amoxicillin+Clarithromycine or Amoxicillin+Metronidazole or Clarithromycine+Metronidazole for 2 weeks and proton pump inhibitors for 4 weeks).

Reports in USA and Europe have shown resistance rates to *Helicobacter pylori* in children of 21–41% for Clarithromycine, 43–46% for Metronidazole, 5% for Amoxicillin. The confirmation of *Helicobacter pylori* eradication can be obtained with either urea breath test or stool antigen test in 4 to 6 weeks after treatment. The follow-up in order to confirm healing and eradication of *Helicobacter* should be carried out 4 to 6 weeks after treatment using either the urea breath test or the stool antigen test. There have been a number of novel therapies proposed for treatment of *Helicobacter pylori* ranging from chewing gum to honey and green tea, berry juice and some milk proteins. The probiotics (*Lactobacillus casei*) have an important role not in eradicating the *Helicobacter pylori*, but in maintaining a lower density of pathogen in the stomach, and thus the combination with antibiotics may increase the eradication rate or decrease the adverse effects of the multidrug treatment regimens or both (20).

Other theories state that the virulence factors help the microorganism to affix itself to the gastric mucosa and to generate the disease into the host organism. These virulence factors are classified in colonisation factors (flagella, the enzyme urease, adherence factors) and factors responsible for tissue injury (lipopolysaccharides, leucocitary activating and leucocitary adhesion factors, cytotoxin Vac A and the cytotoxin associated antigen - Cag A).

The researchers showed that the correct treatment of gastritis and ulcer in children brings a regression of premalignant histologic

modifications and decreases the incidence of gastric cancer and gastric B cell lymphoma in adults. Antigen detection and treatment in *Helicobacter pylori* Vac A and Cag A would decrease the recurrence and malignancy rate. There is hope that antibiograms and even vaccinations against the different types of *Helicobacter pylori* will be discovered in the future. (1, 19).

Vaccination in childhood will prevent one of the most widespread infections, and will decrease the morbidity rate in adulthood by reducing the incidence of gastric cancer.

According to the present day hypothesis, *Helicobacter pylori* infection could be associated with the specific virulence of the bacterial genotypes. Often, the incriminated genes are: cag A and vac A with s1a/m1, s1a/m2, s1b/m2 subtypes and ice A, with two alleles, ice A1 and ice A2. These genes encode the synthesis of some enzymes / proteins with higher aggressiveness (Cag A, Vac A) (1,6).

One of Singh's studies revealed that S1m1 alleles of *Helicobacter pylori* were more frequently observed in children with CAP (in some opinions Cag PAI - pathogenicity island) than in children without CAP. It has been shown that in Cag A positive patients, Cag A associates with severe inflammatory injury and manifest disease, while Cag A negative patients are asymptomatic (3). Besides the microorganism's virulence, genetic and environmental factors (diet, alcohol, smoking) play an important role in the pathogenesis of ulcer and gastric cancer. This association can explain why the infection in childhood can generate disease in adulthood. Genetic factors (the polymorphism of interleukine 1L1 β gene, tumor necrosis factor TNF alpha, O blood group) cause the rise of *Helicobacter pylori* infection. The detection of Cag A and Vac A by using ELISA or immunodiffusion can reveal particular information with great clinical significance (1,4).

Conclusions

The directions of fundamental research in *Helicobacter pylori* infection combine clinical examination with endoscopic, histologic and immunological examination.

Recent theories and assumptions regarding the gene predisposition of the infection with *Helicobacter pylori*, respectively the genic polymorphism that influences pharmacokinetic and therapeutic efficiency, have determined the research team to perform some clinical, endoscopic, histologic, immunological and genetic correlations in the infection with *Helicobacter pylori* in children.

Cag A and Vac A determination by ELISA method or by immunodiffusion can bring new clinically relevant information. Compared to seronegative infections (type II), infections by Cag and Vac positive strains (type I strains) are usually associated with severe gastro-duodenal diseases and gastric cancer. A non-invasive assay based on *Helicobacter pylori* antigens detection in faeces is the immunoenzymatic stool test. Simple, useful and promising, cost-effective and fast, it provides with a reliable diagnosis and therefore, with the opportunity of an effective treatment evaluation. As a conclusion, the stool antigen test can predict the *Helicobacter pylori* status in the pretreatment stage and subsequently influence the evolution of this type of infection.

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Helicobacter pylori (H. pylori) is an important pathogen in human gastroenterology. H. pylori is commonly acquired during early childhood and it is more common among people in developing countries. The clinical consequences of H. pylori infection in children are yet to be fully understood. H. pylori infection in children differs from the same infection in adults, both in terms of the percentage of infected individuals, symptoms, degree of complication towards peptic ulcer disease or malignancy, the gastric areas affected, the degree of compliance to therapeutic management or bacterial resistance to treatment. 2. Context. The materialization of this risk depends on a summation of factors related to the genome of the bacterium, the host and environmental factors.[19].