



Review article

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DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION

SUMMARY

Helicobacter pylori induces persistent inflammation in the human stomach, yet only a minority of colonized persons develop peptic ulcer disease or gastric malignancy.

Results from recent investigations have demonstrated that *H. pylori* isolates possess substantial phenotypic and genotypic diversity which leads to different host inflammatory responses that influence clinical outcome.

Numerous studies are being published on diagnostic tests for *H. pylori*. The tendency is to favor noninvasive tests. The urea breath and stool antigen tests using monoclonal antibodies are applied in different types of patients, while serology is still a subject of interest. Invasive tests were the first to be applied to *H. pylori* detection and still remain the "gold standard". In patients having undergone upper endoscopy, in order to obtain gastric biopsy specimens, diagnosis of *H. pylori* infection is achieved using histological and cultivation methods and urease tests.

In order to get insight into virulence factors and macrolide susceptibility, the molecular methods (realtime PCR) have been developed.

Knowing the mechanisms of *H. pylori* pathogenesis and specific interaction between pathogen and the host, which are dependent upon strain-specific bacterial factors and induced host effectors, helps to define colonized persons bearing the highest risk for disease, and enable physicians to use the most appropriate diagnostic testing and eradication therapy.

Key words: *Helicobacter pylori*, virulence factors, diagnosis

INTRODUCTION

Helicobacter pylori is major representative of a new group of bacteria named *Epsilonproteobacteria*. It is a small, spiral-shaped microorganism, very motile, gram-negative bacillus that colonizes only the mucus of the human digestive tract. It can be found in half of the world population, hence, it is one of the most common pathogens. In contrast to the other *Helicobacter* species, it always leads to gastric inflammation. If not treated, this chronic gastritis persists as a long-life infection.

Recent studies have demonstrated that *H. pylori* isolates possess substantial phenotypic and genotypic diversity, which may engender differential host inflammatory responses that influence clinical outcome (1). It is likely that these polymorphisms play a central role in determining the outcome of the infection. *H. pylori* possesses numerous factors which allow its colonization: urease, adhesins (the Lewis b blood group antigen binding *adhesin-BabA*, and the sialic acid-binding *adhesin-SabA*), factors which induce epithelial cell damage (vacuolating cytotoxin A - VacA), as well as factors which trigger

inflammation (*cytotoxin-associated gen A - CagA* protein, *cag* pathogenicity island – *cag* PAI, outer inflammatory protein A - OipA). While most of the infected subjects are asymptomatic or have minimal symptoms, approximately 10% will suffer from severe diseases as peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma (2).

Disease risk involves specific interactions between pathogen and host, which, in turn, are dependent upon strain-specific bacterial factors and induced host effectors. The evolution of disease may be due to host factors, to environmental and bacterial factors, since *H. pylori* is a highly heterogeneous bacterium. Thus, blood group A (host factor), smoking cigarettes (environmental factor) and *cag* PAI (bacterial factor) are the main risk factors for peptic ulcer disease. Polymorphism of interleukin-1 β (host factor), vitamins-insufficient and diets rich in salt (environmental factor) and again *cag* PAI as well as VacA cytotoxin are risk factors which lead to gastric adenocarcinoma (3). As a cause of developing gastric MALT lymphoma no risk factors have been determined yet.

It is known that *cag* PAI is very important for *H. pylori*. This island encodes a type IV secretion system which enables the bacterium to inject molecules into host epithelial cells. Among these molecules are muramyl dipeptide which binds to nuclear oligomerization domain (NOD) receptors and leads to nuclear factor-kappa B (NF- κ B) activation and production of interleukin 8, a pro-inflammatory cytokine (4).

During *H. pylori* infection, the CagA protein is also introduced and phosphorylated by cell kinases of the Src family, and then interacts with the host cell causing morphological changes similar to that observed in cancer cells (5). In spite of sequencing of the whole genome of four strains, there are no other new pathogenic factors confirmed in this micro-organism.

Understanding the pathogen-host relationships in *Helicobacter* infection, and mechanisms responsible for the persistence of the infection as well as virulence factors ultimately helps to define the colonized persons bearing the highest risk for disease, and enable physicians to appropriately focus diagnostic testing and eradication therapy.

H. pylori infection in young patients (<45 years), without alarm symptoms such as evidence of gastrointestinal bleeding or weight loss (6), has to be diagnosed by non-invasive tests before medical treatment. Esophagogastroduodenal endoscopy permits gross visualization and localization of ulcerative lesions, mucosal nodularity associated with MALT lymphomas, and other malignant lesions which are not expected in this young population (7).

The recommended tests for initial diagnose of *H. pylori* infection, in these group of patients with uncomplicated dyspeptic disease, are urea breath test (UBT) or antigen stool test with monoclonal antibodies (8).

Detection of *H. pylori* using noninvasive ¹³C-urea breath test is based on principle that solution of ingested urea labeled with carbon ¹³C will be rapidly splitted to ammonia and ¹³CO₂ by influence of urease produced by *H. pylori*. The created CO₂ is absorbed across the gastric mucosa and through systemic circulation ends up excreted as ¹³CO₂ in the expired breath. The test reveals current infection and it is not radioactive. Due to its high sensitivity and specificity (≥ 95) it is considered as a referent method for setting up the initial diagnose of *H. pylori* infection, and for confirmation of eradication. However, disadvantages of UBT are expensive instrumentation and a specialized technicians required. In addition, the performance of the test has been associated with some disadvantages with infants and very young children as well as patients with certain neurological disorders (9).

Sensitivity and specificity of stool antigen test are close to that of the ¹³C-urea breath test (> 90%). Therefore, it is accurate both as a screening test and test for patient follow-up after treatment of eradication therapy. The principle of antigen stool test using monoclonal antibodies is enzyme immunoassay (EIA) for the determination of *H. pylori* antigens in stool specimens. This test offers the great advantage of a simpler collection specimens as well as inexpensive equipment for its performance (10).

H. pylori infection could be diagnosed by serological tests, though exclusively in those geographical areas with the low prevalence of *H. pylori* infection within population. Since use of proton pump inhibitors (PPI) significantly affects reliability of UBT and stool antigen test (11), the diagnosis of specific IgG antibody by serological methods presents the most reliable test in subjects who use anti-secretory drugs before consulting the physicians, which is very common in developed countries.

Serological tests are easy to use, less expensive than the other indirect tests and it can be performed on specimens' collection. Therefore, these methods are most suitable for epidemiological and retrospective studies for determination prevalence or incidence of *H. pylori* infection.

However, serology is not the best method for patient follow-up after treatment because decrease of antibody titre is very slow after successful eradication. Hence, the reduction of antibody titre should be monitoring instead of obtaining a positive

or negative test result. Still, serology has been developed in fashion of immunoblot techniques for detection individual serological response to different antigens, which enables not only to diagnose *H. pylori* status but also to detect infection with certain virulence factors as demonstrated by antibodies to antigens like CagA and VacA (12).

Though detection of *H. pylori* infection in patients with gastric cancer has no therapeutic value, it is important to confirm its association. In these cases, since low density of bacteria and nonuniform distribution of *H. pylori* in gastric mucosa, immunoblot method is significantly more reliable than direct detection tests (13, 14).

Current serological tests developed to detect specific IgG antibodies of *H. pylori* in samples of whole blood from patient finger simplify the diagnosis and enable initial screening in a primary care. The test results could be achieved in ten minutes only. Although, they are suitable for initial diagnosis, limitations of these tests are similar to other serological tests, for example, positive test result does not imply current infection since specific antibodies could persist for months after eradication of *H. pylori* (12).

In patients older than 45 years and youth population with alarm symptoms (6), it is necessary to perform endoscopy to diagnose *H. pylori* using histological and cultivation methods and urease tests (8). Rapid urease test is the simplest test to detect *H. pylori* infection in gastric biopsy specimens obtained by endoscopy. Sensitivity of rapid urease tests is usually higher than that in other biopsy-based techniques, because the whole specimens are placed into media, which reduce errors associated with sampling or processing of specimens (7).

The test is based on the fact that *H. pylori* produces large quantities of urease which hydrolyzes urea in the test media to ammonia and CO₂. Ammonia raises the pH which changes color of pH indicator from yellow to red. The first generations of these tests were the so-called agar-based tests as CLOtest. Today, we have liquid- or membrane-based tests with improved sensitivity allowing us to get faster results (in one hour). Advantages of these tests are reliability, simplicity and low-cost (7).

Disadvantages of biopsy-based methods for detection *H. pylori* could be sampling error, due to an irregular distribution of the bacteria among gastric mucosa, so called "patchy" infection. About 14% of infected patients do not have antral infection as *H. pylori* is present somewhere else in the stomach, especially in cases of gastric atrophy or intestinal metaplasia. In addition, after partial *H. pylori* eradication, it would be much harder to detect infection by endoscopic biopsy due to lowered number of bacteria. Use of PPI affects colonization

pattern of *H. pylori* in the stomach and makes biopsy-based detection methods less reliable. For that reason, obtaining multiple biopsies from the antrum and corpus for histology and one for culture or urease testing is recommended (7).

Along with detection of microorganism, histology has the important additional value of showing the status of the gastric mucosa, presence of gastritis or premalignant lesions as atrophy and intestinal metaplasia.

Although *H. pylori* could be recognized on sections stained with hematoxylin and eosin alone, some supplemental staining methods, such as Giemsa, Genta, Gimenez, Warthin-Starry silver, Creosyl violet, are necessary for detection of *H. pylori* due to its characteristic morphology. However, a small number or even single microorganism as well as coccoidal forms, though hardly detectable by traditional staining methods, could be easily identified with immunohistochemistry using anti *H. pylori* antibodies (15).

Cultivation is, theoretically, the gold standard in identification of any bacterial infection. For cultivating *H. pylori* selective medias, such as Skirrows agar (contains *vancomycin* 10 mg/L, *trimethoprim* 5 mg/L, and *polymixin B* 2 500 IU/L), and Wilkins-Chalgren agar with added supplement (*vancomycin* 10 mg/L, *trimethoprim* 5 mg/L, *cefsulodin* 5 mg/L, and *amphotericin B* 5 mg/L) are used. The plates should be incubated in microaerobic atmosphere (required condition: 37 °C, 10 % CO₂, and 95-100 % humidity) three to four days. Suspected *H. pylori* colonies are identified based on typical morphological appearance, Gram stain and positive biochemical test (oxidase, catalase and urease). The cultivation has high sensitivity and perfect specificity (12). However, its accuracy may be jeopardized by the bacterial overgrowth or contamination.

Only a few microbiological centers routinely offer microbiological isolation of *H. pylori*, since this bacterium is very fastidious. However, increasing prevalence of multiresistant strains required for culturing and antibiotic sensitivity testing of *H. pylori*, especially in patients with persistent infection after failure of initial or repeated therapy.

Antimicrobial susceptibility tests could be performed with different techniques, such as disc diffusion, the E-test method and agar dilution. Disc diffusion method is recommended for *H. pylori* susceptibility testing for metronidazole (16) and for macrolides (17).

Detection of *H. pylori* in biopsy specimens by molecular approach, polymerase chain reaction (PCR), is also possible but only for research, and so far, there are no commercially available tests. The important application of molecular techniques is the

direct detection of virulence factors *cagA*, *vacA* alleles, *iceA* allele (encodes CATG-recognizing restriction endonuclease) etc.

Performing realtime-PCR by fluorescence resonance energy transfer (FRET) assay and a melting curve analysis of the amplification products with the LightCycler apparatus allows specific detection of *H. pylori* as well as its susceptibility to clarithromycin within two hours. Using specific primers, it is possible to detect point mutations in 23S rRNA gene region which confers resistance to

clarithromycin (18, 19), or mutations in the *gyrA* gene of *H. pylori* responsible for quinolones resistance (20).

A large panel of tests is now available to detect *H. pylori* infection. Therefore, it is important to know which test to use in a certain clinical setting to get the most reliable result. Knowing the mechanisms of *H. pylori* pathogenesis may help to define the colonized persons bearing the highest risk for disease and enable physicians to appropriately focus diagnostic testing and eradication therapy.

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DIJAGNOZA INFEKCIJE IZAZVANE *HELICOBACTER PYLORI*

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SAŽETAK

Helicobacter pylori dovodi do perzistentne inflamacije u želucu ljudi. Ipak, samo mali broj inficiranih osoba podleže teškim oboljenjima kao što su peptična ulkusna bolest, gastrični MALT limfom ili želudačni adenokarcinom.

Brojne studije pokazale su fenotipsku i genotipsku raznovrsnost izolata *H. pylori*, što dovodi do različitih inflamatornih odgovora domaćina i različitog kliničkog ishoda. Poznavanjem mehanizama infekcije *H. pylori* moguće je odrediti povećani rizik za razvoj bolesti kod *H. pylori* kolonizovanih osoba.

Danas postoje različiti testovi za dokazivanje infekcija izazvanih *H. pylori*. Prednost se daje razvoju i usavršavanju neinvazivnih tehnika. Urea test disanja i test dokazivanja antigena *H. pylori* u stolici, primenom monoklonalnih antitela, najpouzdaniji su neinvazivni testovi, dok se pouzdanost seroloških testova kod različitih grupa bolesnika još uvek razmatra. Invazivni testovi su prvi primenjeni testovi za otkrivanje infekcije *H. pylori* i još uvek predstavljaju tzv. "zlatni standard". Kod bolesnika kod kojih je urađena endoskopija, radi prikupljanja biopsijskih uzoraka želuca, dokazivanje infekcije *H. pylori* postiže se primenom histoloških i kultivacijskih metoda i brzim ureaza testovima.

Da bi se direktno dokazali virulentni faktori *H. pylori*, kao i osetljivost na makrolide, razvijene su molekularne metode („realtime” PCR).

Danas postoje različiti testovi za otkrivanje infekcije *H.pylori*. Zbog toga je važno znati koji je test najpouzdaniji za određenu grupu inficiranih osoba. S obzirom da rizik za nastanak oboljenja zavisi od specifične interakcije između patogena i domaćina, poznavanje patogenih mehanizama *H. pylori* pomaže pri određivanju povećanog rizika za razvoj bolesti kod *H. pylori* kolonizovanih osoba i olakšava lekarima primenu najadekvatnijeg dijagnostičkog testa i primenu eradikacione terapije.

Ključne reči: *Helicobacter pylori*, faktori virulencije, dijagnoza