Dig Dis Sci (2016) 61:19–24
DOI 10.1007/s10620-015-3877-4

REVIEW

Helicobacter Pylori Infection: Diagnostic Strategies in Primary Diagnosis and After Therapy

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Received: 16 July 2015 / Accepted: 7 September 2015 / Published online: 21 September 2015

Abstract Accurate diagnosis of *Helicobacter pylori* infection pre- and post-treatment is mandatory in the current era of decreasing prevalence and increasing antibiotic resistance. The diagnostic performance of most tests is poorer in clinical situations with low bacterial density which is seen in conditions such as atrophic gastritis or intake of antisecretory and antibiotic medications. Noninvasive tests require less cost and resource but provide excellent accuracy; however, endoscopy with testing of gastric biopsy specimens is indicated where alarming symptoms are present or antibiotic susceptibility testing by culture is desired. Newer modalities such as polymerase chain reaction testing provide additional virulence and antibiotic sensitivity profiling. This article outlines new developments and the key parameters of each test, as careful selection of test modality within the clinical context is required for adequate management of infected symptomatic patients.

Keywords *Helicobacter pylori* infection · Endoscopy · Histology · Urea breath test · Stool antigen test · Rapid urease test · Serology · Polymerase chain reaction · Eradication

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Introduction

Although half of the world's population is infected with *Helicobacter pylori*, the prevalence in the Western world has decreased in the last decades [1]. Accurate detection of *H. pylori* infection is mandatory for adequate further management of infected symptomatic patients. Since the discovery of the gram-negative spiral-shaped bacterium in 1983 by Marshall and Warren, several diagnostic tests have been developed that base on its morphological (histology, culture), immunological (serology, stool antigen test, immunohistochemistry), genetic (PCR), or enzymatic (^{13}C-urea breath test, rapid urease test) characteristics. Generally, these methods can be grouped into non-invasive tests (serology, ^{13}C-urea breath test, stool antigen test) and invasive tests (histology, urease test, culture) requiring upper gastrointestinal endoscopy and gastric biopsies (Table 1). Depending on the clinical setting and question, each test has its advantages, disadvantages, and limitations.

Non-invasive Testing

Uncomplicated dyspepsia in young patients without alarming symptoms (weight loss, dysphagia, gastrointestinal bleeding, iron-deficient anemia, abdominal mass or persistent vomiting) can be managed using a "test-and-treat strategy" [2].

Acceptable tests in this setting are the ^{13}C-urea breath test and the stool antigen test, both providing sensitivity and specificity >90 %. Serologic tests detecting IgG antibodies against *H. pylori* should be used and interpreted with caution as the antibodies can persist sometimes for years and a positive test might only indicate past infection. The accuracy of available test kits is highly variable; only

Table 1 Comparison of diagnostic methods

	Primary diagnosis	Diagnosis after therapy	Sensitivity/ specificity	Costs	Advantages	Limitations
Noninvasive						
^{13}C-urea breath test	+++	+++	>95 %/ >95 %	$$	Reliable, simple, widely available, rapid	Special equipment required for analysis
Monoclonal stool antigen	+++	+++	>95 %/ >95 %	$$	Reliable, simple, rapid	Patient reluctance
IgG serology	(+)	–	75–85 %/ 79–90 %	$	Widely available, identifies virulence factors	Remains positive after eradication, low sensitivity
Endoscopic						
Histology	++	++	60–86 %/ >98 %	$$$	Good sensitivity, secondary diagnostic information	High inter-observer variability, time-consuming
Immunohistochemistry	(+)	(+)	>97 %/ 100 %	$$$$	Excellent sensitivity, secondary diagnostic information	Expensive, time-consuming
Rapid urease test	+++	(+)	80–95 %/ 97–99 %	$$$	Cheaper, simple, rapid	Requires high bacterial burden
Culture	–	+	60 %/100 %	$$$$	Antibiotic sensitivity profile	Limited availability, slow, technically challenging
PCR	(+++)	(+++)	Up to 100 %/up to 100 %	$$$$	Quick, antibiotic sensitivity profiling	Expensive, limited availability, contamination, presence of polymerase inhibitors

validated commercial tests with accuracy >90 % can be recommended [3].

^{13}C-Urea Breath Test

The urea breath test remains the most accurate diagnostic method with a sensitivity and specificity of about 95 % [1, 4, 5]. In children younger than 6 years, the diagnostic performance is slightly less but still >90 % [2, 6]. The test is simple to perform as it requires only two breath samples, before and 15–30 min after drinking the test solution which contains the ^{13}C-urea substrate. The test principle is based on the detection of the bacterial urease which—if the bacterium is present on the gastric mucosa—hydrolyzes the labeled urea to ammonia and labeled hydrogen carbonate. Finally, after absorption into the circulation, ^{13}C carbon dioxide is exhaled in breath. The increase in ^{13}C-labeled carbon dioxide compared with baseline reflects the bacterial urease activity present in the stomach [3]. Fasting conditions are recommended to optimize the contact of the test solution with the gastric mucosa.

Breath samples can be stored for more than a month [7] and sent for analysis to specially equipped laboratories. Thus, the breath test is ubiquitarily available. Mass spectrometry or non-dispersive isotope-selective infrared spectroscopy allows the ^{13}C-breath analysis [8]. Simplified, easy-to-operate instruments for direct ^{13}C-measurements have become available for the use in primary care settings [9].

The ^{14}C-breath test using the radioactive isotope should not play a role anymore as the ^{13}C-breath test provides an alternative without radiation exposure.

Stool Antigen Test

Stool antigen tests are relatively inexpensive and although some patients might be reluctant to collect a fecal specimen, stool sample collection is usually easy, even in young children. *Helicobacter*-specific antigen in stool samples can be detected using an enzyme immunoassay [10] or immunochromatography.

Immunochromatographic stool antigen tests do not require laboratory equipment and are rapid in-office tests, as simple as a pregnancy test but lacking accuracy [11, 12]. The sensitivity of monoclonal stool antigen test kits is higher than the polyclonal technique [13].

In patients with diarrhea, if the stool is unformed or watery, the sensitivity is reduced as the antigen concentration is diluted.

Serology

IgG-antibody-based tests are inexpensive, widely available, and often used for initial screening in the primary care setting or in epidemiological studies. As antibodies can persist even after successful eradication, serology cannot differentiate between acute and past infection, but the negative predictive value is high. Clearly, serology is not a method to check the success of eradication therapy. As the *H. pylori* strains differ worldwide, the performance of serological kits show high variation in populations from different geographical areas. Antibody tests evaluated in the East may not be appropriate for clinical diagnosis in the West [1, 14, 15]. Use of pooled antigen preparations might overcome this limitation.

In a population with low prevalence of *H. pylori* infection, the low specificity of the serology will cause false-positive results. Therefore, positive serology findings should ideally be confirmed by another method such as the ^{13}C-urea breath test or stool antigen test.

IgA testing is less reliable than IgG.

Furthermore, using specific antibodies serology can identify certain virulence factors in *H. pylori* strains such as CagA and VacA.

In patients on proton pump inhibitors, antibiotics, or in other clinical circumstances with low colonization density such as atrophic gastritis, extensive intestinal metaplasia or MALT-lymphoma, serologic tests do not produce false-negative results.

Helicobacter pylori antibody testing from urine or saliva seems attractive as samples are easily obtained. However, the antibody concentration in saliva and urine is lower than in serum, rendering the detection more difficult [2, 16].

Primary Diagnosis; Invasive Testing When Endoscopy Is Indicated

Endoscopy is indicated in patients with new-onset dyspeptic symptoms above a locally agreed age cutoff (older than 45 in the European guidelines [2, 3]), in patients with alarming symptoms or in patients not responding to therapy.

In most situations, biopsies from the antrum are sufficient. If the patient has taken proton pump inhibitors, biopsies from the body have a higher diagnostic yield.

Histology

Apart from the detection of *H. pylori* organisms, histology provides additional information on the degree of inflammation and complicating pathology such as atrophic gastritis, intestinal metaplasia, and malignancy. The bacteria can be identified on routine staining such as hematoxylin and eosin, but the diagnostic specificity can be improved using special stains such as Giemsa, Genta, or the more expensive Warthin–Starry silver stain, particularly in the presence of non-spiral coccoid forms. Histology is expensive as it is time-consuming and requires biopsy sample processing and trained personnel for the staining and interpretation.

Immunohistology

Immunohistochemistry increases the sensitivity and specificity of histological *H. pylori* detection but is not required for all biopsy samples [17]. It can be helpful if the degree of inflammation on the biopsies is suspicious for *H. pylori* infection but bacteria are not visible. The high specificity of the immunohistological diagnosis allows the exclusion of organisms with similar morphology.

Rapid Urease Test

The rapid urease test is also based on the characteristic urease reaction. The biopsy-based test is rapid, inexpensive and simple. The biopsy is placed into a gel containing the urea substrate. If the bacterial urease enzyme is present in the biopsy, it will hydrolyze the urea producing ammonia and carbon dioxide. The pH change is indicated by an added pH indicator.

The accuracy of various rapid urease tests as primary diagnostic tests is good, and the sensitivity varies between 80 and 100 %, and the specificity between 97 and 99 % [1, 4, 5, 18]. For the best diagnostic yield, a sample of the antrum and one from the body should be taken and can be placed into the same gel.

Other urease-positive bacteria (Proteus mirabilis, Citrobacter freundii, Klebsiella pneumonia, Staphylococcus aureus, Enterobacter cloacae) may produce false-positive results, but their presence is unlikely unless the patient has achlorhydria. The gel of most rapid urease tests contains an inhibitor to bacterial growth. Positive results after 24 h should be disregarded as they will be false-positive due to overgrowth by non-*Helicobacter* urease containing bacteria [2, 6, 19].

Determining Treatment Success

During the last decade, growing antimicrobial resistance resulted in declining effectiveness of current treatment strategies. Therefore, post-treatment testing for *H. pylori* infection has become increasingly important.

The urea breath test shows excellent accuracy in the assessment of *H. pylori* infection before and after treatment.

The sensitivity of monoclonal stool antigen tests is better than that of polyclonal tests also in the post-therapy setting [3, 20, 21]. The urea breath test and the stool antigen test are both suitable diagnostic tests for noninvasive therapy control.

The biopsy-based rapid urease test should be relied upon in the post-therapy setting only when the result is positive, as its sensitivity is not sufficient though the bacterial load is low.

Culture

Bacteriological culture from biopsies is difficult, time-consuming and lacks sensitivity, which limits its use in routine diagnosis of *H. pylori*. New microcapillary culturing methods and improved transport media might overcome some of these limitations [7, 22, 23].

On the other hand, the advantage of *H. pylori* isolation by culture is that it allows testing for antibiotic sensitivity to choose the most effective agents for treatment. The current Maastricht-IV guidelines recommend culture for antibiotic susceptibility testing after failure of second-line treatment or if the primary resistance to clarithromycin is expected to be more than 20 % [2, 8].

The culture of the organism from gastric biopsies requires incubation for several days. Recent reports show that *H. pylori* might be an capnophilic aerobe rather than a microaerophilic organism as previously assumed as its growth is promoted in an environment containing atmospheric oxygen level and 10 % carbon dioxide [9, 24].

Isolating *H. pylori* from stool or saliva, or dental plaque samples, is extremely difficult and does not play a role in diagnosis.

Special Situations

Atrophic Gastritis and Intestinal Metaplasia

Atrophic gastritis is associated with decreased bacterial burden in the antrum and at the lesser curve of the body, particularly in the presence of intestinal metaplasia. Detection of atrophic gastritis and intestinal metaplasia with standard white-light endoscopy is not satisfactory [10, 25], but is improved with targeted biopsies when advanced imaging techniques such as narrow-band imaging and magnifying endoscopy are used.

Further, reduced gastric acidity in atrophic gastritis is associated with reduced bacterial urease activity. The urea breath test, stool antigen test, and rapid urease tests are less reliable in atrophic gastritis, particularly when intestinal metaplasia is present. The sensitivity of histology of antral biopsies is also reduced, but corpus biopsies remain reliable irrespective of the presence of intestinal metaplasia [11, 12, 26]. Proximal biopsies should be taken for histology if atrophic gastritis with or without intestinal metaplasia is suspected.

Serology is the only test which is not affected by low bacterial load in atrophic gastritis.

Medication Causing Bacterial Suppression

The sensitivity of urea breath test, stool antigen test, rapid urease test, and histology is reduced in circumstances of bacterial suppression such as proton pump inhibitor therapy, H2 receptor antagonists, antibiotics, and bismuth use. Patients should be advised to stop antisecretory drugs 2 weeks and antibiotics 4 weeks before testing [2, 13, 27].

Acute Gastrointestinal Bleeding

The prevalence of *H. pylori* infection in bleeding peptic ulcer disease seems to be lower than in un-complicated ulcers, but this phenomenon might be explained by the diagnostic methodology. Studies that perform delayed testing 4 weeks after the bleeding event find similar prevalences to non-bleeding ulcers [28]. In an acute bleeding situation, the accuracy of diagnostic tests is reduced. The rapid urease test and culture seem to be unreliable in this situation, while histology is only mildly affected [29].

Research

Polymerase Chain Reaction (PCR)

Biopsy-based quantitative real-time polymerase chain reaction (qPCR) allows detection of low bacterial loads and also the identification of pathogenetic genes and specific mutations associated with antimicrobial resistance such as in clarithromycin- or fluorquinolon-resistant strains [30]. Nested and semi-nested PCR techniques targeting conserved genes such as Hsp60 increase the sensitivity and specificity to 100 % [31]. The high sensitivity enables also the detection of *Helicobacter* DNA in noninvasively obtained samples such as dental plaque, saliva, urine, stool samples, or gastric juice from string tests. The presence of polymerase inhibitors in the biological sample and possible contamination during sample collection present obstacles to overcome. Currently, the clinical use of PCR-based testing is limited by the expensive costs, but the high diagnostic performance in the pre- and post-treatment setting, with additional option of identifying clarithromycin-resistant strains, renders it an excellent future diagnostic method, particularly in children [32].

Novel Endoscopic Imaging Techniques

In the past, many attempts have been made to predict the presence of *H. pylori* infection directly during endoscopy. However, the correlation between described gastritis using conventional white-light endoscopy and histology has been poor.

High-resolution magnification endoscopes enable 115-time magnification and provide a resolution to 7.9 µm. This reveals the microvasculature in the body with a typical honeycomb-like subepithelial capillary network formed by polygonal loops of capillaries around the gastric pits and converging into collecting venules. This regular microvascular pattern is disturbed in chronic *H. pylori*-associated gastritis. The loss of collecting venules and the broadening of the pits indicate present *H. pylori* infection [33, 34].

The combination of narrow-band imaging and magnification endoscopy seems to allow reliable prediction of *H. pylori* infection with good interobserver agreement [35].

Novel endoscopic techniques such as confocal endomicroscopy aim on direct visualization of the bacteria [36]. Using 1400-fold magnification, the distinct shape and size of the bacteria including the flagella are identifiable.

Conclusion

In conclusion, the selection of the most appropriate test for diagnosing *H. pylori* infection depends on the clinical setting, the cost-effectiveness, the likelihood of positive tests, and the availability of the tests.

Compliance with ethical standards

Conflict of interest None.

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