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Diagnosis of *Helicobacter pylori* infection

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Abstract

A number of non-invasive and invasive tests are available for the diagnosis of Helicobacter pylori infection. These can be used to establish the initial diagnosis or to confirm the eradication of the bacteria after medical treatment and subsequent monitoring for re-infection or recrudescence. Non-invasive tests include antibody detection testing which use serum, whole blood or saliva to provide a rapid diagnosis but in general is less favorable in sensitivity and specificity. ^{13}C- or ^{14}C-urea breath test is non-invasive and is one of the most accurate tests available. Invasive tests rely on endoscopic biopsies of the gastric mucosa. Biopsy samples can be used in rapid urease test, histology examination, culture with sensitivity testing and polymerase chain reaction (PCR) technique. Choice of different tests depends on clinical setting, availability and technical support.

Key words: Diagnosis. Helicobacter pylori. Tests

Introduction

After the identification of *Helicobacter pylori* in human stomach in 1983[1,2], there were changes in management of gastrointestinal diseases such as peptic ulcer and gastric cancer. It is now clear that over ninety percent of the patients suffering from duodenal ulcer are infected with *Helicobacter pylori* and successful treatment of the infection in these patients reduced the relapse of ulcer from eighty percent to less than 5 percent. There is also a definite link between *Helicobacter pylori* and gastric cancer. The risk of gastric cancer in carriers is 2.8 to 6 times that of non-carriers. As more and more people, both general public and doctors, become aware of this infection, there is a great demand in testing for the presence of infection in various clinical settings, for example in outpatient clinics, in endoscopy suites and in hospitalized patients. Understanding the principles of various diagnostic tests is important not only to allow a rapid diagnosis but also to make an accurate diagnosis using the appropriate test. If a test is wrongly chosen for the clinical scenario, the diagnosis will be delayed or missed. It is therefore very important to choose the correct test once the test is considered necessary.

Non-invasive tests

The two main techniques for the diagnosis of *Helicobacter pylori* infection are serology and ^{13}C-urea breath test (Table 1).

Table 1. Techniques for the detection of Helicobacter pylori

Non-invasive	Invasive
Serological tests	Rapid urease test
^{13}C- or ^{14}C-urea breath test	Histology
	Bacterial culture
	Polymerase chain reaction

Serology

H. pylori infection provokes both local and systemic antibody responses. The response typically includes a transient rise in IgM, followed by a rise in IgA and IgG throughout the infection. The most commonly employed method is enzyme-linked immunosorbent assay (ELISA). Others include latex agglutination and Western blotting. A number of antigens have been used to date in a variety of ELISAs including highly purified antigens such as urease, glycine extracts, whole cell sonicates and filtrates of culture supernatants. Because of the high level heterogeneity of the immune response in *H. pylori* infected persons, the antigen preparation variety must be as high as possible. Antigen can be prepared from up to six strains of *H. pylori*. Serology tests can be applied to whole blood, saliva, urine or serum samples. Detection of salivary IgA is less sensitive.

Antibody responses to *H. pylori* vary considerably between individuals. Serological tests perform differently not only between individuals but between different population groups. Therefore it is necessary to pick several commercial kits, standardize them locally, including adjustment of the cut-off values, and use the one which performs best[3]. Once validated, they can be used in individual diagnosis or more applicable to epidemiological studies, as they allow a large number of people to be tested for *H. pylori* infection. The limitation of serology testing is the monitoring of treatment success. Antibody titres to *H. pylori* vary markedly between individuals and after treatment may take up to 1 or 2 years to return to the uninfected range. It is generally accepted that a 50% drop in titre at 6 months can reliably predict treatment success[4]. The paired sera of pre- and post-treatment should be tested together to avoid interplate variability. This adds to the cost of the test and also it may be difficult to find space to store the sera especially in general practice setting. Finally waiting for 6 months may not be acceptable to both the patient and his doctor.

The near patient tests(NPT) are antibody detection tests designed to be used in the doctor's office. It offers a rapid diagnosis to individuals and help in the patient management. These tests are individually packed and are therefore slightly more expensive than using the serum antibody test. However, it is still relatively cheap compared to UBT and endoscopy examination. The whole blood test requires a drop of blood from a finger prick and using a modified ELISA or latex agglutination method allowing the diagnosis in less than 5 minutes. These tests usually give a sensitivity and specificity of 80 to 90% but they must be validated in the local population to be used such that the sensitivity and specificity are within acceptable limits. NPTs are not quantitative and so cannot be used to monitor treatment success.

Serology testing has the following limitations: they are less suitable for monitoring treatment because the antibody level may take up to 6 months to decline; the tests must be validated in the population being studied; the sera collected before treatment must be tested at the same time as the post-treatment sera.

Urea breath test (UBT)

The test is one of the most accurate method available for the detection of *H. pylori* infection[5]. It is suitable for initial diagnosis as well as post-treatment evaluation and long term monitoring. UBT involves giving a test meal to delay gastric emptying first, and then to drink an oral dose of urea labelled with carbon-13 or carbon-14. If *H. pylori* is present, the carbon-labelled urea will be broken down by the urease produced by the organism to become labelled carbon dioxide that can be detected in the patient's breath (Figure 1). The breath samples were collected in pairs from pre-dose and 30 minutes after the oral dose of urea into vacutainers. These vacutainers are placed in an automated gas sampler (Figure 2a) and analyzed by the mass spectrometer (Figure 2b) when ^{13}C is used or using a ß scintillation counter when ^{14}C is used. The test is simple to perform and give a rapid and accurate diagnosis. Special collecting apparatus are available for use in small children and plastic bags instead of vacutainer has been used in some systems.

Comparing the non-radioactive ^{13}C and radioactive ^{14}C isotopes used in UBT, both isotopes give the same results. Therefore practical rather than theoretical considerations determine which test to use. As mentioned, ^{13}C UBT requires an expensive isotope ratio mass spectrometer. ß scintillation counters are more easily available to hospitals. The radiation exposure from a modern ^{14}C-UBT is less than 12 h background radiation. Some people are still concerned about the incorporation of ^{14}C into the bones and the US Food and Drug Administration (FDA) has not yet approved this test.

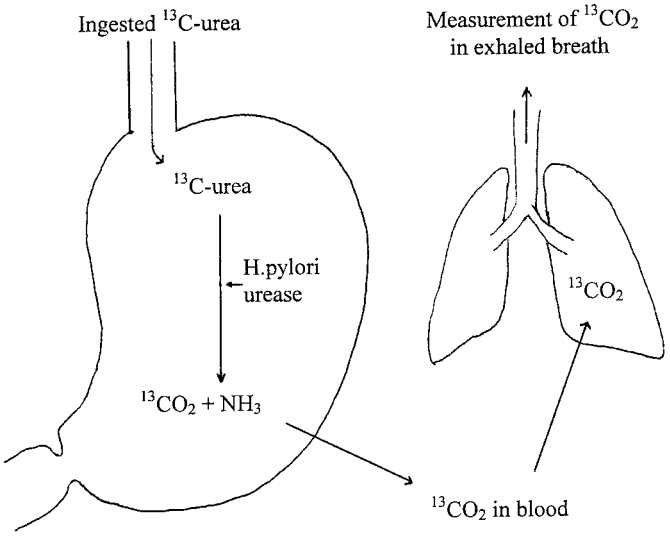

Figure 1. Principle of the ^{13}C-urea breath test.

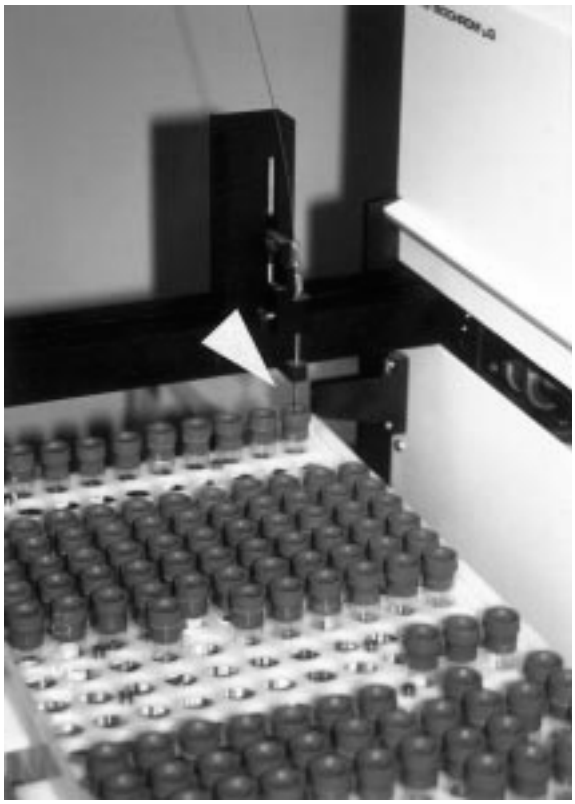

Figure 2. Mass spectrometer for ^{13}C-urea breath test
A. Vacutainers were placed in the automated gas sampler. A needle is being shown (arrow) aspirating breath sample from the vacutainer for analysis.

B. The mass spectrometer machine with 2 cylinders of reference gases on the right hand side.

Recent development includes smaller size machines to allow near patient testing in the doctor's office and using infra red in analysis to cut down the cost of the machine. UBT may become the standard diagnostic test in the near future.

UBT has the following limitations: the machine is expensive and therefore not widely available, false positive are found due to urea hydrolysis by oral bacteria; false negatives are found in patients with recent intake of proton pump inhibitors, bismuth, H_2-receptor antagonists or antibiotics days before the test, results are not reliable in patients with history of gastric surgery.

Invasive tests

Invasive tests include rapid urease test, histology, bacterial culture and polymerase chain reaction technique (Table 1).

Rapid urease test

This is a widely used test in endoscopy room for rapid detection of *H. pylori* infection[6]. A gastric biopsy from the antrum is placed in the urea broth (5% urea solution with phenol red). If there is preformed urease produced by *H. pylori* in the biopsy specimen, the urease will hydrolyze urea in the broth to ammonium ions. This raises the pH and can be detected by the indicator phenol red to produce a colour change from yellow to red (Figure 3). In those true positives, 75% of the broth will turn red within twenty minutes and 90% within three hours. Such self-made urea broth is very cheap and easy to use. Commercial rapid urease tests are available. The first commercial test was the CLOtest (devised by Barry Marshall before *H. pylori* was even named; i.e. CLO= Campylobacter Like **Organism**) (Figure 4). They are made into agar gel form with urea, phenol red and buffers in a sealed plastic slide. The biopsy sample is placed inside the yellow gel. Similarly a colour change from yellow to red indicates a positive test.. False positives may occur after 24 hours due to other urease-positive organisms in the gastric biopsy. Therefore test result should be read within 24 hours. The convenient times to read the results are 20 minutes, 1 hour, 3 hours and 24 hours.

Rapid urease test has the following limitations: the test has a limited sensitivity (70-90%); its sensitivity reduces further after eradication therapy; false negatives are seen in patients with recent intake of proton pump inhibitors, bismuth, H_2-receptor antagonists or antibiotics days before the test.

Figure 3. Rapid urease test. The yellow urea broth (right hand side) will turn magenta if urease (from *H. pylori*) is present in the gastric biopsy (left hand side).

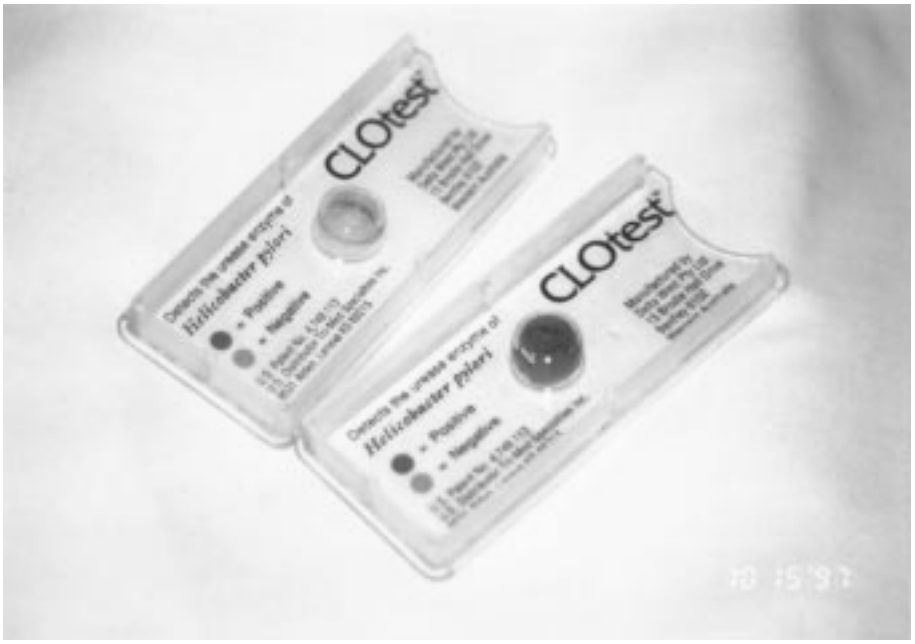

Figure 4. CLOtest®. A commercial modified rapid urease test which turns from yellow (upper left plate) indicating no infection, to magenta (lower right plate) when urease activity is present in the gastric biopsy (positive *H. pylori* infection).

Histology

Gastric biopsies from the antrum and body are immersed in formalin and sent to pathology department for embedding and section onto glass slides under routine preparations for microscopic examination. Using only the standard haematoxylin-eosin stain can only detect 66% of those considered positive using special stains[7]. There are a few staining methods aiming at improving the diagnostic accuracy. These include Warthin-Starry silver stain, Giemsa stain, cresyl fast violet stain and Genta triple stain. However, none of them are specific for *H. pylori*. Specificity can only be achieved by immunocytochemistry with commercial polyclonal or monoclonal preparations.

The organism appears as a curved, S-shaped bacilli in the epithelial cell surface, in the gastric pits and in the overlying mucous layer. The advantage of histology is that it allows simultaneous assessment of the presence and extent of gastritis and other histological changes. The test is widely available to most hospital settings and it allows storage of specimens for retrospective analysis.

The limitation of the test is the sampling error which may miss the scanty area of colonization, especially in post-treatment evaluation. This happens to all techniques using biopsy samples as well.

Bacterial Culture

H. pylori can be grown from biopsy samples[8]. This is the most specific test for the diagnosis of *H. pylori* and in experienced hands can also be very sensitive. Biopsy specimens are placed in Stuart's transport medium(STM) or saline. Bacteria will remain viable in saline for up to 6 hrs. *H. pylori* will survive for 6 hrs in STM at temperatures up to 15°C and for up to 48 hrs if maintained at 4°C. The homogenized broth is inoculated onto plates with Columbia agar with 10% horse blood and Hp selective supplement and incubated in a microaerobic atmosphere (5-6% O_2, 8-10%CO_2, 80-85% N_2, and a relative humidity of at least 95%). The plates should be inspected daily from 3 to 7 days in new cases and for 10 to 14 days in post-treatment cases. The colonies can be confirmed with Gram stain and tested for urease, catalase and oxidase activities. The biopsies or the isolates can be frozen in brain heart infusion (BHI) glyceral at -70°C for several months.

One of the advantage that none of the other tests has is the testing of the susceptibility of the infecting strain to antimicrobial agents, as well as typing of the strains. Increasing use of certain antibiotics creates a selective pressure for the development of drug resistance. For example, metronidazole resistance is common in many parts of the World including Hong Kong. Treatment of *H. pylori* infection can fail due to drug resistance of the organism. There is a need to assess the antimicrobial resistance either in the general community or in individual cases where treatment fails. There is however, no standard testing recommendations for sensitivity. Disc diffusion susceptibility test or epsilometer ε test on agar are common tests being used in this aspect.

Culture of clinical isolates allow the strain comparison by molecular typing methods, such as sequencing and random amplified polymorphic DNA (RAPD), which is useful in treatment and epidemiological studies. In case of treatment failure, it is essential to document for recrudescence of infection with the same strain or reinfection with a new strain. Transmission within family members or institutions can be assessed in the same way.

The disadvantage of bacterial culture as a diagnostic method includes the following: it is a demanding technique, requiring trained and motivated personnel; the facility is not widely available; it is less sensitive in post-treatment testing; and the diagnosis cannot be made rapidly.

Polymerase chain reaction (PCR) technique

Gastric biopsy samples are collected without the need of special transport medium. A specific segment of DNA from *H. pylori* can be amplified. The primers used routinely are derived from the 26 kDa antigen gene[9], or the urease C gene[10]. Others may use the urease A gene or the cagA gene for PCR. The PCR technique is no different from that in other situations. In addition, PCR allows the typing of bacterial strains by using Restriction Fragment Length Polymorphism (RFLP) on the amplified DNA fragments. False positive is a universal problem, due to contamination in general or due to cross reaction with other Helicobacter species or Campylobacter species. False negatives can be due to the following: gene being targeted is not present in the strain tested; gene detected is present but highly heterogenous; presence of inhibitors of Taq polymerase; and number of organisms is under the threshold of detection. The test result can be available within a day, depending on the manpower and the laboratory facilities.

PCR has the following disadvantages: the procedure demands meticulous handling of samples and materials, and a laboratory set up specially dedicated to PCR work; false positives and false negatives; and no information about the susceptibility of the infecting strains to antibiotics.

Choice of test for routine use

Initial diagnosis

In general practitioner setting, when endoscopy is not performed, ^{13}C-urea breath test is the choice for use. It is important to check that the patient is not taking any drugs mentioned above which will affect the breath test results. If this is not available, serology test can be used. In specialist clinics or hospital settings, usually endoscopy examination is performed. Routine testing should include rapid urease test and histology, the later give additional information on the extent of gastritis.

Post-treatment monitoring

Apart from gastric ulcer, other conditions do not require a follow-up endoscopy examination. Therefore , urea breath test is the choice of test to determine the success of eradication. If endoscopy is performed, histology from both antrum and body of the stomach is mandatory. Culture and PCR test can be done if facilities are available. All tests should be done 4 to 6 weeks after cessation of drugs. Rapid urease test has a low sensitivity and serology test is not accurate for reasons mentioned above.

Conclusion

Various tests for diagnosis of *H. pylori* are discussed. Because of the limitations in different clinical settings, a rational approach should be used in choosing the diagnostic tests. A better understanding of the principle and limitation of each test together with the patient's clinical history will allow an accurate choice of diagnostic test(s) to be used.

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