Diagnosis of *Helicobacter pylori*

Meltem Yalinay Cirak,* Yakut Akyön† and Francis Mégraud‡

*Department of Microbiology and Clinical Microbiology, Gazi University Faculty of Medicine, Besevler, Ankara, Turkey; †Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Hacettepe University, Sihhiye, 06100 Ankara, Turkey; ‡INSERM U853, Bordeaux, F 33076 France

**Abstract**

Although there are attempts to perform *Helicobacter pylori* diagnosis directly in vivo using magnification endoscopy, most articles on diagnosis this year concerned non-invasive tests and molecular methods. For urea breath tests, there are attempts to have a quicker and cheaper test and to evaluate its role in cases of premalignant lesions. For stool antigens tests, evaluation of kits using monoclonal antibodies was carried out. Molecular tests have been applied for typing and detection of resistant mutants.

**Endoscopy**

Among the new methods of magnifying endoscopy, a prototype of endocytoscopy developed by Olympus was used for ex vivo visualization of *Helicobacter pylori* on experimentally infected gastric biopsies. Moving bacteria were observed at 1100× magnification, giving hope for a possible direct detection during endoscopy [1]. Kim et al. also used magnifying endoscopy on 103 patients to classify the gastric surface according to four patterns: flat, irregular, papillary or nonstructured, which were then compared to the updated Sydney System for histologic gastritis. Histologic gastritis was found in 91% of the biopsy sections with a nonflat type, and among them, 96% were confirmed to harbor *H. pylori* infection [2]. In another study, the magnified endoscopic findings in the gastric body were classified into four patterns and then correlated with histology results. Type 1 pattern corresponded to normal gastric mucosa, types 2 and 3 to *H. pylori*-infected mucosa and type 4 to atrophy. The sensitivity and specificity for these endoscopic findings were 92.7% and 100% for type 1, and 100% and 92.7% for types 2 and 3 together, respectively [3].

**Histology**

Few studies concerned histology and led to the following conclusions: nodular gastritis increases with gastritis score [4], examination of antral biopsies is sufficient to screen for lymphoid follicles [5], mast cells do not appear to be related to other inflammatory parameters [6], and methylene blue staining can be substituted for Giemsa stain to visualize *H. pylori* [7].

**Keywords**

Endoscopy, urea breath test, stool antigen test, serology, molecular methods.

**Reprint requests to:** Francis Mégraud, INSERM U853, Bordeaux, F 33076 France.
E-mail: francis.megraud@chu-bordeaux.fr

The presence of *H. pylori* in neoplastic tissue is a matter of controversy. Using transmission electron microscopy, Necchi et al. were able to see cytochemically proven *H. pylori* in six of eight intestinal metaplasia samples and nine of 20 cancer samples [8].

Complementary techniques to histology include immunohistochemistry and fluorescence in situ hybridization. The former technique was used to detect East Asian type cagA present both in the cell nucleus and in the cytoplasm, and was in agreement with cagA gene sequence [9]. The latter was used to detect the presence of *H. pylori* [10] and its clarithromycin resistance [11].

**Rapid Urease Test**

A urease test based on an immunological detection of urease was proposed for the first time in Japan. Its sensitivity was 96% but its specificity only 90% [12]. Two new rapid urease tests (RUT) based on pH change were also tested. Unfortunately, the dry RUT (GUT test) was not reliable at a 15-minute reading time [13]. The motility indole urease test, in contrast, had a high sensitivity [14].

**Urea Breath Test**

The 13C-urea breath test (13C-UBT) has been recognized as an excellent test because of its accuracy as well as of its robustness: the specimens can be transported without special conditions, and the result is independent of human interpretation.

Mauro et al. compared the DOB values of 13C-UBT samples collected every 5 minutes up to 30 minutes from 67 patients. The value after 10 minutes showed 98.6%...
sensitivity and specificity compared to the test performed at 30 minutes [15]. Attempts were made to render the test cheaper by decreasing the dose of $^{13}$C-urea. Yong et al. compared various additives to $^{13}$C-urea in a capsule, and found that polyethylene glycol increased the initial dissolution rate of urea leading to an increased DOB and improved sensitivity of the test in volunteers [16].

The best cut-off for a positive test has been discussed at length. Based on 2232 patients explored for H. pylori infections with $^{13}$C-UBT in a Canadian community and a cluster analysis, a cut-off point of 3 $\delta$‰ was validated. There was a slight difference between the results of those submitted to a first diagnosis (3.09) and those tested post-treatment (2.88) [17]. However, the cut-off for children younger than 5 years is higher. A study carried out in a single center on 30 H. pylori-positive children during 7.5 years confirmed that the best specificity was obtained with a cut-off of 8 $\delta$‰ [18].

Interestingly, Shmuely et al. compared DOB values from a large series of tests (7373) and noted an age-adjusted difference of 7 (95% CI 6.4–7.9) between genders, with a higher DOB value for females. This result may indicate a difference of 7 (95% CI 6.4–7.9) between genders, with a large series of tests (7373) and noted an age-adjusted multivariate analysis and found that the only risk factor for a false negative UBT was corpus predominant gastritis [23].

Among the variations of $^{13}$C-UBT, $^{14}$C-UBT using a microdose of $^{14}$C has been proven to be accurate and economical [24,25]. A $^{13}$C-urea blood test was also found to be reliable and well tolerated in children [26].

**Stool Antigen Test**

The stool antigen test is considered as a valuable noninvasive alternative to diagnose H. pylori when UBT is not available. A second generation of kits, based on monoclonal antibodies, has already been used for several years.

Gisbert et al. carried out a systematic review and meta-analysis on the accuracy of these tests for diagnosis and for treatment follow up [27]. They analyzed 22 studies, including a total of 2499 patients where the tests were performed prior to eradication. Pooled sensitivity and specificity were 94% (95% CI: 93–95) and 97% (95% CI: 96–98), respectively. In 13 studies where polyclonal antibody-based stool antigen tests were compared to monoclonal antibody-based tests, a higher sensitivity was shown for the latter (95% vs. 83%). Twelve studies including a total of 957 patients assessed monoclonal antibody-based tests post-eradication, with pooled sensitivity and specificity of 93% (95% CI: 89–96) and 96% (95% CI: 94–97), respectively; again they showed a better sensitivity than polyclonal antibody-based tests (91% vs. 76%) in eight studies when both were performed.

In studies published this year, however, the results are not generally good. HpSTAR (Dako, Glostrup, Denmark) provided good results in some studies [28–30] but not in others. The pretreatment specificity was low in a study by Dominguez et al. [31] as was the post-treatment sensitivity as reported by Quesada et al. [32]: 70.7% and 73%, respectively.

**Antibody Detection**

The detection of multiple antibodies in serum by protein array has been used for H. pylori diagnosis. This array is comprised of three recombinant H. pylori antigens: UreB, VacA and CagA immobilized on nitrocellulose membranes. Bound IgG are detected using staphylococcus protein A labeled with colloidal gold. Sensitivity and specificity were above 90% compared to ELISA [34]. This rapid and reproducible test may be a future competitor to immunoblot.

Indeed, attempts to correlate a specific disease with antibodies directed toward specific H. pylori antigens are still being made. It has been known for many years that, antibodies against CagA are associated with peptic ulcer disease [35] but they are not specific enough to screen these patients among dyspeptic patients [36]. A specific immunoblot pattern indicating infection with a more virulent strain was associated with active inflammation as well as atrophy and intestinal metaplasia in the antrum [37]. A significant association (OR:19.5) was found between the presence of gastric cancer and the presence of IgG against three H. pylori antigens of 19.5, 33 and 136 kDa (CagA) [38]. CagA and VacA antibodies were valid markers of past H. pylori infection when standard H. pylori serology was negative following atrophic body gastritis [39]. Yang et al. also confirmed that immunoblot can detect H. pylori antibodies in gastric cancer patients when other tests are
negative [40]. However, the presence of antibodies to a VacA-m region-specific antigen was not able to predict the risk of gastric cancer development [41].

In the past, ELISA performed with antigens obtained from local strains led to better results than when kits were used. In a study carried out in Vietnam, Pyloriset EIA-GIII (Orion Diagnostics, Espoo, Finland) as well as Helicoblot 2–1 (GeneLabs, Singapore) performed equally well in this population [42]. However, in Thailand Pyloriset EIA-GIII had a low specificity (75.3%) [43].

The problem of distinguishing false positive tests from acute or transient infection when a single test is positive was addressed by measuring pepsinogen levels, and the conclusion was that most transient infections are indeed false positives [44].

**Molecular Methods**

Molecular methods are widely used for the diagnosis of *H. pylori* infection as well as analyses of diversity, virulence, persistence and resistance patterns of these bacteria. Minami et al. proposed a novel and quick identification system for *H. pylori* which is a combination of the endoscopic brushing technique and the loop-mediated isothermal amplification method (LAMP). Among the samples from 200 patients, 123 brushing samples were *H. pylori* positive using LAMP primers constructed for the *glmM* gene within a 90-minute detection time with 100% sensitivity and specificity, whereas 100 patients were positive when only biopsy samples were tested [45].

**Typing**

Genetic diversity of *H. pylori* in the same patient is a challenging dilemma considering the accuracy of diagnosis. For differentiation of mixed infections with *H. pylori* strains, enterobacterial repetitive intergenic consensus–polymerase chain reaction (PCR) has a high discriminatory power and is time-efficient compared to random amplified polymorphic DNA (RAPD) fingerprinting. Finger et al. detected the presence of more than one *H. pylori* strain in more than half of the 63 patients studied [46]. In another study where multiple single *H. pylori* colonies from different regions of the stomach of eight adult and four pediatric patients were analyzed, the presence of two distinct genomic profiles of *H. pylori* strains was demonstrated in a single adult patient, differing at 113 gene loci including the *cag* PAI virulence genes, by using RAPD, amplified fragment length polymorphism and comparative genomic hybridization microarray [47]. A study on 250 Jordan patients using PCR showed this genetic diversity with a predominance of *iceA2* (73.6%), a high frequency of the *vacAs2* allele, and a low proportion of *caga* genotype [48].

With regard to the importance of diagnosis in children, Oleastro et al. identified new candidate markers for childhood peptic ulcer disease by suppressive subtractive hybridization analysis [49]. Two *H. pylori* virulence genes, *jhp0870* and *jhp0562*, related to outer membrane protein and lipopolysaccharide biosynthesis, respectively, were shown to play a conspicuous role in the pathogenesis of peptic ulcer in children. The positivity rate for *jhp0870* was 80.0% in 15 ulcers versus 36.7% in the control group of 30 gastritis specimens and for *jhp0562* 80.0% versus 33.3%. A Brazilian study on *cagA* using both histology and PCR showed that 57 of 121 (47%) children were positive for *H. pylori*, of which *cagA* strains were found in 20 of 29 (69%) children with chronic gastritis and in 18 of 28 (64%) with normal mucosa, suggesting an initial infection with the bacteria [50].

The relationship between host gene polymorphisms and *H. pylori* genotypes has been emphasized in a certain number of studies as well. Among 302 *H. pylori*-infected cases in China, carriers of the proinflammatory IL-1B-511 T allele and *H. pylori vacA m1* genotype had an approximately fourfold higher risk of developing intestinal metaplasia [51]. Another study using oligonucleotide allele-specific PCR on samples from 233 patients detected a significant difference in the frequency of the IL-8-251 A/T polymorphism and *H. pylori vacA* gene polymorphisms among gastritis, peptic ulcer, and gastric cancer patients [52].

Multiplex PCR is also used for genotyping *H. pylori*. Bolek et al. found a correlation between *cagA*-positivity, *vacAs1m1* genotype, and peptic ulcers [53]. In another genotyping study, a significant association between *H. pylori vacAs1a, cagA*, and *cagE* genotypes and duodenal ulcer and gastric cancer as well as between *iceA1* and *babA2* and gastric cancer, was demonstrated in Turkish patients with dyspepsia [54].

Genetic diversity of the 3’ variable regions of the *cagA* gene and determination of the related EPIYA phosphorylation motifs were explored by one-step PCR in a Greek study of 75 adults and 60 children in order to identify closely related *H. pylori* subclones within the same patient; the results suggested that a prediction of the number of EPIYA repeats sheds light on the prognosis of infection [55]. In a Korean study on the diversity of the 3’ end of the *cagA* gene and the relationship between EPIYA motifs and clinical outcome among 79 patients suffering from gastritis, peptic ulcer and gastric cancer, 76 (96.2%) harbored the East Asian type without any significant difference [56].

**Antimicrobial Resistance**

Owing to the difficulties of culturing these bacteria, molecular methods are of great interest in the detection of
antimicrobial resistance. Nishizawa et al. developed an allele-specific PCR for the detection of gyrase A mutations in fluoroquinolone-resistant *H. pylori* strains [57]. The rate of *H. pylori* resistance to furazolidone was reported to be 8.7% in China, and six mutations in porA and oorD genes were identified in these resistant isolates [58].

Gerits et al. found that multiple mutational changes in the *ppbP* gene led to amoxicillin resistance in *H. pylori*, which renders the development of a molecular test difficult in contrast to cases of clarithromycin and tetracycline resistance [59]. At the same time, Kim et al. confirmed the association of *ppbP* gene mutations and amoxicillin resistance using sequence analysis [60].

A study employing TaqMan technology showed no association between clarithromycin resistance and cagA and vacA status in paraffin-embedded biopsy specimens by real-time PCR [61]. The same group from Italy also showed a twofold increase from 10.2 to 21.3% in primary clarithromycin resistance rate in *H. pylori* strains over 15 years and determined the most prevalent point mutation as A2143G [62].

Among the PCR-based methods, PCR-RFLP is an appropriate technique for detecting point mutations. Raymond et al. detected mutations in the 23S rRNA genes of *H. pylori*, the most prevalent being A2143G. Furthermore, two different mutations were identified in the same biopsy specimen and the rate of resistance increased from 18.6% during the period 1993–96 to 41.6% during 2001–04 [63].

A novel biprobe, the ClariRes real-time PCR assay, used for the detection of *H. pylori* infection and simultaneous clarithromycin susceptibility testing in stool samples was evaluated. It was less effective than expected with 63% sensitivity for an accurate diagnosis in children [64].

Further research concerning DNA biosensors was carried out for single-base polymorphism detection. A label-free electrochemical DNA hybridization detection method using peptide nucleic acid probes was developed for the evaluation of A2143G in the 23S rRNA gene of *H. pylori* [65].

A novel diagnostic microarray for the identification of a group of pathogenic bacteria including *H. pylori* using competitive oligonucleotide probes with a high detection sensitivity range of 0.1% was developed by Kostic et al. as a new approach [66].

DNA microarray analysis is currently used as well for the comparison of *H. pylori* genomes. In a Chinese study, 1636 genes including 522 strain-specific genes were tested for the identification of pathogenic strains. Results of this kind concerning genome evaluation highlight the virulence and pave the way for candidate vaccines for *H. pylori* [67].

In summary, there were no great breakthroughs this year in the diagnosis of *H. pylori* infection. However, serology, previously considered not specific enough for diagnosis, was recommended in the Maastricht III Conference Report because this method is not influenced by the consumption of proton-pump inhibitors which is currently a common treatment among patients seeking a specialized consultation [68].

**Conflicts of interest**

The authors have declared no conflicts of interest.

**References**


45. Minami M, Ohta M, Ohkura T, Ando T, Torii K, Hasegawa T, Goto H. Use of a combination of brushing technique and the
Loop-Mediated Isothermal Amplification Method as a novel, rapid, and safe system for detection of Helicobacter pylori. 
47 Salama NR, Gonzalez-Valencia G, Deatherage B, Aviles-Jimenez F.
Journal compilation © 2007 Blackwell Publishing Ltd, Helicobacter © 2007 The Authors
Cirak et al.