

# REVIEW

## Current consensus on the diagnosis and treatment of *H. pylori*-associated gastroduodenal disease

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(Received for publication on February 18, 2003)

**Abstract.** *Helicobacter pylori* (*H. pylori*) is a spiral shaped bacterium that resides in the stomach mucosa. Isolation of *H. pylori* from the stomach mucosa changed the erstwhile widely held belief that the stomach contains no bacteria and is actually sterile. Once *H. pylori* is safely ensconced in the mucus, it is able to neutralize the acid in the stomach by elaborating an enzyme called urease. Urease converts urea, of which there is an abundant supply in the stomach (derived from saliva and the gastric juice), into bicarbonate and ammonia, which are strong bases. These bases form a cloud of acid-neutralizing chemicals in the vicinity of the organisms, protecting them from the acid in the stomach. This urea hydrolysis reaction is utilized for the diagnosis of *H. pylori* infection in the urea breath test (UBT) and the rapid urease test (RUT). In Japan, both invasive tests, such as bacterial culture, histopathology and RUT, and non-invasive tests such as UBT and serology are conducted for the diagnosis of *H. pylori* infection. For confirming the results of eradication therapy, UBT is considered to be the most sensitive and specific. In order to treat *H. pylori* infection, a new one-week triple therapy regimen (lansoprazole or omeprazole + amoxicillin + clarithromycin) has been approved for use in patients with peptic ulcer disease in Japan. As for *H. pylori* eradication in the case of other diseases in which the bacterium has been implicated (e.g., chronic atrophic gastritis, gastric MALT lymphoma, gastric cancer, non-ulcer dyspepsia, chronic urticaria, idiopathic thrombocytopenic purpura (ITP)), further basic and clinical investigation is required. (Keio J Med 52 (3): 163–173, September 2003)

**Key words:** urease, apoptosis, rapid urease test, urea breath test, triple therapy

### Introduction

Twenty years ago in Australia, Marshall and Warren became the first in the world to succeed in culturing *Helicobacter pylori* (*H. pylori*) from the gastric mucosa of patients with gastritis.<sup>1</sup> Since Palmer<sup>2</sup> denied the existence of micro-organisms in human stomach, it had been thought that it is nearly impossible for any micro-organism to survive in the gastric acidic environment. However, some micro-organisms had already been discovered in the stomach in the 1890s.<sup>3</sup> In Japan also, Katsuya Kasai and Rokuzo Kobayashi of the Kitasato Institute reported isolating a spirochete-like organism, that might have been *Helicobacter felis*, from the stomach of dogs and cats, but not that of laboratory animals,

and demonstrated that when rabbits infected with the spirochetes isolated from dogs or cats were inoculated with the *virus fixe*, marked hemorrhagic inflammation occurred in the gastric mucosa.<sup>4</sup> They also demonstrated that spirochetes inoculated into the gastric mucosa of the mouse could be eradicated by the administration of arsaminol<sup>4</sup> (a modern equivalent of arsaminol is thought to be bismuth, a close relative of arsenic). Seventy years have elapsed before scientists are again attempting to treat gastritis associated with *Helicobacter* infection. To commemorate Kobayashi's achievements in the field of *Helicobacter* research, the "Rokuzo Kobayashi Memorial Symposium on *Helicobacter pylori*" was held on May 11, 2002 at the Kitakan Hall of the Keio University Mita Campus.<sup>5</sup> In this

Presented in part at the 2002 Keio Medical Science Prize Symposium, November 29, 2002.

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symposium, Dr. Thomas F. Meyer had a special lecture entitled “*Helicobacter pylori* – From molecular genetics and pathogenesis studies to vaccine design –”,<sup>6</sup> Dr. Barry J. Marshall had a historical special lecture entitled, “Kobayashi and *Helicobacter* pioneers”,<sup>7</sup> and Dr. Kenneth E.L. McColl gave a lecture entitled “How does *H. pylori* infection cause gastric cancer?”.<sup>8</sup>

*H. pylori* is a spiral-shaped gram-negative rod, measuring approximately 3–5 microns in length. The bacteria possess numerous long flagella, flailed movements of which allow them to advance vigorously with forceful screw-like movements, much like the spinning of the bit of a drill.<sup>9</sup> How does *H. pylori* survive in the strong acid environment of the stomach? The organism elaborates a large amount of urease, an enzyme that breaks down urea. When it releases urease, the urea present in the mucus layer of the stomach is converted into ammonia, which quickly neutralizes the acid in the immediate vicinity of the organism, thereby protecting it from the strong acidic environment of the stomach. The ability of the bacterium to elaborate urease, which neutralizes acid, together with its vigorous movements enables *H. pylori* to infect and colonize the gastric mucosa.

### Mongolian Gerbil Models for *H. pylori* Infection

A recently established animal model for *H. pylori*-associated gastric disease, produced by inoculating this bacterium to Mongolian gerbils,<sup>10</sup> is useful for *in vivo* observation of *H. pylori*-infected gastric mucosa. In this model, we previously reported the significant levels of gastric mucosal neutrophil accumulation, and the increase in the contents of lipid peroxides.<sup>11,12</sup> In the same model, Japanese investigators revealed the formation of gastric cancer by inoculating only *H. pylori*.<sup>13,14</sup> Although the exact mechanisms by which *H. pylori* infection results in gastric mucosal injury are unclear, it has been demonstrated that the extracts of the bacterium can induce a number of microvascular alterations within the rat gastric mucosa, including platelet aggregation and macromolecular leakage.

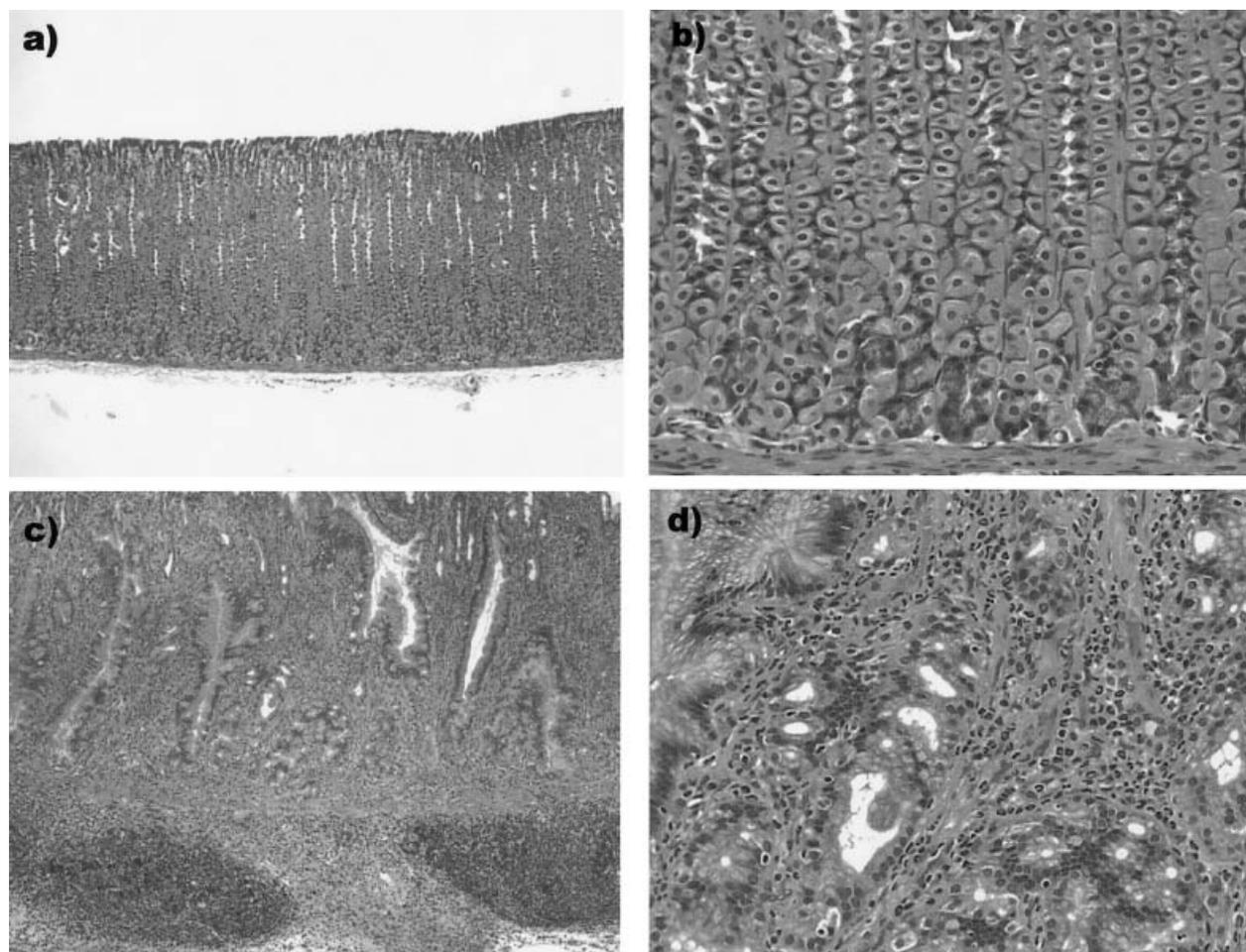
### Initial Gastric Mucosal Response to *H. pylori*

The Mongolian gerbil (MGS/Sea, 5–7 weeks) inoculated with *H. pylori*<sup>10</sup> is a suitable model for observing *H. pylori*-associated gastric mucosal response. In our laboratory, *in vivo* microcirculation in the gastric mucosa is visualized through an intravital microscope using a digital color 3CCD camera.<sup>15</sup> The bacterial body of *H. pylori* was detected in the mucus layer. The velocity of rolling leukocytes on the venular endothelium and the number of adherent leukocytes could be calculated by the playback image analysis.<sup>9</sup>

In the normal uninfected gastric mucosa of gerbils, the mesh of a capillary network is clearly seen between the large collecting venules. After the intragastric inoculation with *H. pylori*, the bacterial body moves in the mucus layer by the screw-like force of the flagella and adheres on the surface of epithelial cells of the pyloric antrum within several hours. The bacteria force their way through the mucus, and in large numbers work themselves closer to the epithelium. Once the tips of the bacterial body come into contact with the surface epithelium, the *H. pylori* in the mucous layer starts to establish colonies vigorously, and then firmly adheres to the surface epithelial cells. As a result of the energetic movement and the activity of urease, the bacteria break through the mucus layer and establish a colonization of the gastric mucosa.

Four weeks after the bacterial inoculation, no conspicuous changes in the gastric mucosa were seen macroscopically. However, when we observed the gastric mucosal microcirculation with the intravital microscope, some pathophysiological events occurred surrounding the collecting venules. Particularly, a phenomenon in which neutrophils are rolling along the luminal surface of the venular endothelium, or are adhering to it is documented. Eight weeks after the bacterial inoculation, macroscopic gastric mucosal damage had appeared in the pyloric antrum, near its border with the body of the stomach.

At the 12th week after the inoculation, the mucosal erosive lesion in the antrum was covered with necrotic tissue. Closer examination revealed considerable exfoliation of the epithelial cells and stagnation of the microvascular blood flow. There were great numbers of neutrophils adherent to the venular walls, and many neutrophils had extravasated. The velocity of rolling leukocytes in the mucosal and submucosal venules decreased in a time-dependent fashion in the *H. pylori* group.<sup>9</sup> Histological findings also showed a large number of leukocytes mainly in the submucosa in the stomach with *H. pylori* infection. Tissue myeloperoxidase (MPO) activity of the stomach started to increase significantly in the *H. pylori*-colonized gerbils at 12th week after the inoculation to a level eight fold that of the control.<sup>12</sup> Sixteen weeks after the infection, the area of mucosal injury had expanded and the mucosa itself had become noticeably thicker. Exfoliated epithelial cells in the mucus layer fragmented into small particles, suggesting they died as a result of apoptosis. Surrounding this portion, many leukocytes could be observed (Fig. 1). These leukocytes migrate from the venules to the deeper layers and then emerge on the mucosal surface. It is known that, as a result of leukocyte activation, large amounts of substances with deleterious effects on the gastric mucosal cells, such as reactive oxygen metabolites, are produced through the NADPH oxi-



**Fig. 1** Histological findings of the *H. pylori*-colonized-gastric mucosa of Mongolian gerbils at the 16th week after inoculation. a) Control, non-infected gastric corpus mucosa ( $\times 10$  objective lens). b) Control, non-infected gastric corpus mucosa ( $\times 40$  objective lens). c) *H. pylori*-colonized-gastric corpus mucosa ( $\times 10$  objective lens). d) *H. pylori*-colonized-gastric corpus mucosa ( $\times 40$  objective lens).

dase on the membrane of neutrophils. Of these substances, the monochloramine ( $\text{NH}_2\text{Cl}$ ) which is produced by the *H. pylori* urease-derived  $\text{NH}_3$  and neutrophil myeloperoxidase-derived  $\text{HOCl}$ , plays an important role on the gastric mucosal cell injury in the *H. pylori*-infected stomach.<sup>16,17</sup>

#### Gastric Mucosal Cell Turnover and *H. pylori* Infection

Gastric mucosal cell turnover is regulated by the balance between cell death and cell proliferation in the gastric mucosal epithelium. Such a regulated cell turnover is mandatory to keep healthy gastric mucosal integrity. Peek *et al.* investigated the level of gastric epithelial apoptosis and proliferation in *H. pylori*-colonized gastric mucosa of Mongolian gerbils at the earlier phase after the bacterial inoculation and de-

scribed an initial transient enhancement of cell apoptosis (2–4 weeks) and later increase in cell proliferation (16–20 weeks). We also previously reported that while the level of apoptosis increased with the extension of *H. pylori*-evoked gastric mucosal inflammation in C57BL/6 mice, the enhanced level of apoptosis was significantly attenuated in *H. pylori*-colonized gastric mucosa of Mongolian gerbils with an enhanced inflammation and epithelial proliferation in the gastric mucosa in the longer-term colonization (36 and 72 weeks after the bacterial inoculation)<sup>18</sup> and suggested the possible link of an attenuated gastric mucosal apoptosis with a specific carcinogenesis in gerbils after *H. pylori* colonization.<sup>13,14</sup>

One of the candidates for an apoptosis regulator might be *p53*. Mutations of the *p53* tumor suppressor gene constitute one of the most frequent molecular changes in a wide variety of human cancers. Fox *et al.*<sup>19</sup>

examined  $p53^{+/-}$  mice with *H. felis* inoculation and reported a significant enhancement of gastric mucosal cell proliferation at 48 weeks after the inoculation compared with the same strain-colonized stomach of wild-type  $p53^{+/+}$  mice. We also reported using the same  $p53^{+/-}$  mice as Fox *et al.* that no difference in the level of apoptosis and proliferation in the gastric mucosa was seen 24 weeks after *H. pylori* (not *H. felis*) inoculation.<sup>20</sup> Yamamoto *et al.*<sup>21</sup> investigated the susceptibility of  $p53$  nullizygote  $-/-$ , heterozygote  $+/-$  and wild-type  $+/+$  mice to N-methyl-N-nitrosourea (MNU) gastric carcinogenesis and reported that no adenocarcinoma in the stomach was observed in a  $p53^{+/-}$  mouse. These results suggest that the heterozygous mutation of  $p53$  may not be enough to induce mouse gastric pre-neoplastic and neoplastic lesions even by the administration of a chemical carcinogen. These previous studies suggest that the additional genetic change is necessary to evoke a dysregulation of gastric mucosal cell turnover in the *H. pylori*-colonized stomach.

Although the role of iNOS in the *H. pylori*-colonized stomach still remains unclarified, many reports showed the iNOS involvement in the extension of gastric mucosal inflammation with *H. pylori* infection. Kiss *et al.*<sup>22</sup> examined the actions of a purified *H. pylori* lipopolysaccharide (LPS) on rat gastric antral and duodenal microvascular integrity (determined as radiolabelled albumin leakage) and the expression of iNOS. According to their study, significant increases of albumin leakage and expression of iNOS in both antral and duodenal tissues were observed following LPS challenge. Recently Watanabe *et al.*<sup>23</sup> reported as *in vitro* study that chemical iNOS suppression by iNOS inhibitor (aminoguanidine) attenuated *H. pylori*-induced apoptosis of MKN45 cells, suggesting an important role of iNOS for evoking gastric cellular apoptosis. In  $iNOS^{-/-}$  mice, although gastric mucosal leukocyte accumulation in response to *H. pylori* was significantly increased, the level of gastric mucosal apoptosis was significantly attenuated, suggesting a key role of iNOS-derived NO for the induction of gastric epithelial apoptosis.<sup>24</sup>

The iNOS may be regulated by the oxidant-sensitive transcription factor, nuclear factor-kappaB (NF- $\kappa$ B). Lim *et al.*<sup>25</sup> reported that *H. pylori* induced cytotoxicity of gastric cells time- and dose-dependently, which occurred with induction in iNOS expression and nitrite production. In their study,<sup>25</sup> while a peroxyxynitrite donor and a nitric oxide donor induced dose-dependent cytotoxicity in gastric cells, catalase, an inhibitor of NF-kappaB activation; pyrrolidine dithiocarbamate (PDTC), iNOS inhibitors; N(G)-nitro-L-arginine-methyl ester (L-NAME), and 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT) prevented *H. pylori*-induced cytotoxicity and apoptosis, suggesting a possi-

ble link between iNOS and its derivatives with gastric cytotoxicity.

The levels of both oxidized and nitrated proteins were significantly higher in inflamed gastric tissue samples infected with *H. pylori*, especially  $cagA^+$  strains, and in those with expression of IL-8 and iNOS mRNAs than in those negative for these parameters,<sup>26</sup> indicating that infection with  $cagA^+$  *H. pylori* would induce significant oxidative stress and iNOS expression in the gastric mucosa, contributing to the pathogenesis of *H. pylori*-associated gastroduodenal diseases.

### Indications for *H. pylori* Eradication Therapy

There is worldwide consensus that *H. pylori* eradication prevents ulcer recurrence without any maintenance antisecretory therapy.<sup>27</sup> In Japan, similar results have also been obtained; a large-scale multicenter clinical trial of *H. pylori* eradication in patients with active ulcers using proton pump inhibitors plus an antibiotic demonstrated the inhibitory effect of eradication on ulcer recurrence. From the medico-economic point of view, it is also clear that *H. pylori* eradication reduces medical costs. Thus, the presence of *H. pylori*-positive gastric ulcers or duodenal ulcers is an indication for eradication therapy.

According to the recently revised guidelines published by the Japanese Society for *Helicobacter* Research on February 24th, 2003,<sup>28</sup> *H. pylori* eradication therapy is also recommended in patients with gastric low-grade MALT lymphoma. In patients after endoscopic mucosal resection of gastric cancer, those with atrophic gastritis and those with gastric hyperplastic polyps, *H. pylori* eradication therapy might be effective. In terms of patients with non-ulcer dyspepsia (NUD), gastroesophageal reflux disease (GERD) or extra-gastric diseases, the significance of *H. pylori* eradication therapy is still under evaluation. Considering the high prevalence of gastric cancer in Japan, a drive for eradication of *H. pylori* should be undertaken urgently to reduce the incidence of gastric cancer.

### Epidemiology of *H. pylori* Infection in Japan

*H. pylori* is believed to be transmitted via the oral route. Many researchers believe that *H. pylori* is transmitted by the orofecal route through the ingestion of food or water. In addition, it is also possible that *H. pylori* is transported from the stomach to the mouth in association with gastro-esophageal reflux (in which the contents of the stomach are refluxed in small amounts into the esophagus) or belching, both common symptoms of gastritis; the bacterium could then be transmitted via the oro-oral route.

The prevalence of *H. pylori* infection in Japan is extremely low in younger age groups, similar to the case in other developed countries, and subsequently the incidence increases rapidly until it reaches a plateau of about 70% in 50-year-old subjects.<sup>29</sup> This phenomenon is attributable to people around 50 years of age having been born soon after World War II and spent their childhood under poor hygienic conditions.

It has been reported that *H. pylori* infection may be associated with chronic gastritis regardless of the strain of the infecting organism, and that the course of the disease, however, does depend on such environmental factors as the diet, duration of infection, the age at the time of occurrence of *H. pylori* infection, the virulence of the *H. pylori* strains, and various host factors, including genetic make-up.

Little is known about factors, apart from poor living conditions during childhood, that affect either the acquisition or elimination of *H. pylori* infection.<sup>30</sup> Lifestyle factors operating during adulthood, such as smoking and alcohol consumption, may influence spontaneous eradication of the organism. Although studies investigating the relationship between antibody evidence of *H. pylori* infection and these factors have provided inconsistent findings,<sup>31–33</sup> it must be borne in mind that serological tests may misclassify as still infected those individuals in whom the organism has already been eradicated. Recent studies on subjects with evidence of active *H. pylori* infection, as measured by the <sup>13</sup>C-urea breath test, found no association between smoking and *H. pylori* infection, whereas coffee consumption was positively related to the infection.<sup>34,35</sup> A strong inverse association between *H. pylori* infection<sup>34</sup> and alcohol consumption (especially wine) was reported from one study, but a subsequent report indicated that the relationship was U-shaped and unrelated to the type of beverage consumed.<sup>36</sup>

Although *H. pylori* has been found throughout the stomach, *H. pylori*-associated inflammation is often mild, superficial or even absent in the gastric corpus.<sup>37</sup> In the natural history of gastritis associated with *H. pylori* infection, the inflammation progresses from the antrum to the adjacent corpus along a line of advancing injury, associated with a reduction of acid secretion and eventually, loss of parietal cells and the development of gastric mucosal atrophy.<sup>38–40</sup> This scenario, however, is not inevitable. In the general population, progression of gastric mucosal atrophy occurs at the rate of 1–2% per year.<sup>38</sup> Several studies have suggested an annual increase in the severity of gastritis affecting the corpus of approximately 6% amongst *H. pylori*-infected patients with GERD.<sup>41–46</sup> The rate of progression from active gastritis to gastric mucosal atrophy varies in different geographical regions in relation to other environmental factors, and, although diet is probably the most impor-

tant event causing a reduction of acid secretion, other factors, such as childhood infections, may also be very important.<sup>47–49</sup> The rate of development of the disease and the proportion of the population with atrophic gastritis are critical determinants of the risk of gastric cancer in the population.<sup>47</sup>

### Intervention Trial of *H. pylori* in Japan

To investigate whether or not a causal link exists between *H. pylori* infection and gastric cancer, an intervention study called JITHP (Japanese Intervention Trial of *H. pylori*) was planned in 1994 under the auspices of 2nd Term Comprehensive Strategies for Cancer Control in Japan, organized by the Ministry of Health and Welfare. Although the trial was performed by 145 affiliated institutions (including Keio University Hospital) from all over Japan, difficulties related to patient accrual were confronted during the trial, as a result of which only one of two factors taken into consideration initially could be evaluated in the study. That is, only the influence of *H. pylori* eradication in preventing the onset and progression of gastric mucosal atrophy could be studied, and its influence on the frequency of stomach cancer. Finally, a total of 682 patients, with 342 in the *H. pylori*-eradication group and 340 in the non-*H. pylori*-eradication group were enrolled in the study. The final outcome of the trial is expected to be reported in March 2004.

### Diagnosis of *H. pylori* Infection

Diagnostic methods for *H. pylori* infection are categorized into two groups as invasive and non-invasive tests. While invasive tests need endoscopic biopsy of gastric mucosal samples, the non-invasive test does not need an endoscopic procedure (Fig. 2).

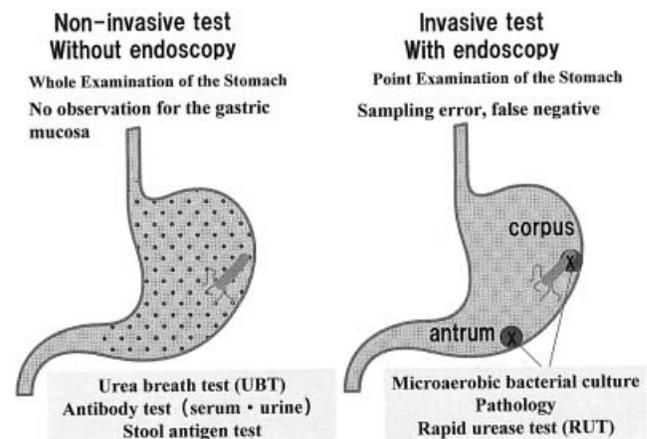


Fig. 2 Diagnostic methods for *H. pylori* infection. Left) non-invasive tests, Right) invasive tests.

### *Invasive tests*

**Histological diagnosis of *H. pylori* infection:** *H. pylori* infection is easily recognizable on histological examination;<sup>1</sup> both the organism itself, and the histopathological changes induced by it can be identified on histopathological examination of biopsy specimens. For a precise diagnosis, it is important to obtain biopsies from appropriate sites of the stomach (greater curvature of the antrum and corpus) and to recognize the characteristic pathological findings in the gastric mucosa associated with the infection (epithelial cell changes and neutrophilic and mononuclear cell infiltration), besides using special staining methods for identifying *H. pylori* (*i.e.* Giemsa staining, Gimenez staining, silver staining and immunohistochemistry, in addition to Hematoxylin-eosin staining) in the specimens.

**Rapid urease test:** The rapid urease test (RUT) is one of the invasive methods that is used for the detection of *H. pylori* infection.<sup>50</sup> *H. pylori*, if present in the biopsy specimen, splits the urea in the test container to yield ammonia. Elevation of the pH by ammonium hydroxide produced is detected by a color change of the pH indicator. The advantage of RUT is that it is an inexpensive, easy-to-use, and rapid diagnostic test. Its disadvantages, on the other hand, are that endoscopy is required and false-negative reactions are frequently encountered. Although RUT is useful for the diagnosis of *H. pylori* infection both before and after eradication treatment, the sensitivity of RUT is comparatively low during the first 6 months after completion of eradication therapy.

**Culture method for *H. pylori*:** Among the five diagnostic tests for *H. pylori* infection that are recommended by the Japanese guideline,<sup>51</sup> the microaerobic bacterial culture method may be the most difficult to use in the clinical setting, because of the requirement of 3 to 5 days for the organism to grow, the need for skill in performance of the test and elaborate equipment, and the high frequency of false-negative results (Fig. 2). On the other hand, it has the advantage of having a specificity of 100% (direct demonstration of the presence of *H. pylori*) and of allowing further characterization of the organism (determining its sensitivity to antibiotics, investigating its virulence factors, and typing of its DNA for epidemiological purposes). This method is especially useful for cases in whom the infecting strains' sensitivity to antibiotics needs to be determined. Considering the potential increase in the frequency of clarithromycin (CAM)-resistant *H. pylori* in the future, the microaerobic bacterial culture method may be the most suitable method for the diagnosis of *H. pylori* infection.

With treatment of *H. pylori* infection being con-

ducted frequently in Japan, drug resistance has emerged as a problem that requires attention. However, there are no standard methods for evaluating the antibiotic susceptibility of *H. pylori* in Japan. While in Europe and the U.S.A., the E-test is being used as the standard method for testing the drug susceptibility of *H. pylori*, the microplate method, which has been reported to have the same accuracy as the agar dilution method, is now becoming standard in Japan. In 2000, the Japanese Society of Chemotherapy proposed that the drug susceptibility testing of *H. pylori* be standardized and that the break point MIC of amoxicillin (AMOX) and CAM be listed.

### *Non-invasive Tests*

**<sup>13</sup>C-urea breath test:** Infection with *H. pylori* has been implicated as an important factor in the pathogenesis of gastritis, peptic ulcer and gastric cancer, and the <sup>13</sup>C-urea breath test (<sup>13</sup>C-UBT) is a convenient and non-invasive method for the detection of *H. pylori* in the stomach. The <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> ratio is measured by using infra-red spectroscopy (IR) and gas chromatography/mass spectrometry (GC-MS). The IR analyzer is considered to be particularly useful for the diagnosis of *H. pylori* infection by the <sup>13</sup>C-UBT.

The effect of the test meal used for the test was explored further. It was widely believed that citric acid was the best test meal for the use in the <sup>13</sup>C-UBT, hypothesized as being due to the efficient delay in gastric emptying induced by it. Shiotani *et al.*<sup>52</sup> compared a pudding test meal, ascorbic acid and two doses of citric acid in 11 volunteers, and clearly demonstrated that the increased intragastric urease activity could not be attributed to gastric emptying alone. They suggested that citric acid could have a direct effect on UreI, a proton gated urea channel, making urea more accessible to the intrabacterial urease.

The reasons for the discrepancies observed between the results of the <sup>13</sup>C-UBT and serology were assessed by Bode *et al.* in asymptomatic blood donors.<sup>53</sup> They did not find any case showing positive UBT among serology-negative subjects. The strongest predictive factor of a negative-UBT in serology-positive subjects was, not surprisingly, previous *H. pylori* treatment. Another significant factor was coffee consumption. A tendency towards increase in the frequency of negative UBT in subjects over the age 60 years was also noted, possibly due to increased mucosal atrophy and intestinal metaplasia.

**Antibody tests:** Serological detection of *H. pylori* infection by ELISA has been popularly used. Several ELISA kits are available in Japan, which, however, have varying sensitivity and specificity. The new version

of Pyloriset (EIA-G III) (Orion, Finland) has been shown to be reliable.<sup>54</sup> The immuno chromatographic flow-injection method, which allows the quantitation of specific IgG, has been developed as a new method for the serological diagnosis of *H. pylori* infection. This method is rapid, and is associated with minimal wastage of antigen, since the same immunosorbent reactor can be used for all tests.<sup>55</sup> It has been reported that the sensitivity and specificity of ELISA may be higher when the Japanese strain of *H. pylori* is used as the antigen.

CagA antibodies which persist for longer durations than *H. pylori* antibodies detected in a global test, can help in linking gastric carcinoma to *H. pylori* infection.<sup>56,57</sup> In a study of CagA, while the serological status as assessed by a commercial CagA immunoblot test was of no value for predicting the severity of the disease.<sup>58</sup>

Quantification of the IgG titers may be of interest. In one study, higher titers were reported to be associated with a lesser likelihood of the development of gastric carcinoma (GC) as compared to lower titers.<sup>59</sup> Similarly, lower UBT values corresponded to higher scores of atrophy, and therefore a higher risk of GC.<sup>60</sup>

Tests have been developed by Otsuka Pharmaceutical Co. to detect *H. pylori* antibodies in the urine, for example, the ‘doctor test’ (Rapirun) using an immuno chromatographic method using dried anti-human IgG-binding gold colloidal particles. This test was evaluated as being satisfactory in two studies,<sup>61,62</sup> in which the sensitivity and specificity were determined to be 94.6% and 95.3%, and 95.3% and 96.7%, respectively. These results are very promising and deserve to be confirmed, since this test could possibly become the most suitable noninvasive test for *H. pylori* infection.

### ***H. pylori* Detection in Stools**

The now well-known HpSA test (Meridian) has been evaluated in adults in three studies, 4–6 weeks after *H. pylori* eradication, using the UBT as reference. In the study of Manes *et al.* (106 cases, 16 failures), the sensitivity and specificity were 87.5%, and 95.7%, respectively.<sup>63</sup> In the study of Leodolter *et al.* (113 cases, 30 failures), the sensitivity and specificity were 76.2%, and 93.3%, respectively.<sup>64</sup> Based on the ROC curves, they showed that a slightly lower cut-off value increased the accuracy of the test. Vaira *et al.* found a sensitivity and specificity of 94% and 97%, respectively in 84 treated patients.<sup>65</sup> Gisbert and Pajares published a systematic analysis of 25 published studies including 2078 patients, in which HpSA was used for post-treatment follow-up. The sensitivity, specificity, NPV and PPV were 88%, 92%, 75% and 94%, respectively, *i.e.* not as good as the pretreatment values of 92%, 92%, 92% and 90%, respectively.<sup>66</sup>

A novel stool test (FemtoLab *H. pylori*, Connex, Germany) using monoclonal antibodies instead of polyclonal antibodies has been developed. There was good concordance between the two stool tests.<sup>67</sup> Stool tests can also be used in mice models of infection to facilitate basic research.<sup>68</sup> Monteiro *et al.* explored the stability of *H. pylori* DNA in stools for 3 days and the impact of a vegetable-free diet on the detection.<sup>69</sup>

### **Confirmation of *H. pylori* Eradication**

In Japan now, it is expected that general practitioners would test peptic ulcer disease patients with these tests before and after *H. pylori* eradication. To obtain highly accurate results after eradication, it is important to conduct the tests over 4 weeks (more desirably over 12 weeks) after cessation of eradication therapy with PPI and antibiotics. The UBT and the stool antigen test are the most suitable for confirmation of *H. pylori* infection after eradication, since these tests reflect all the changes in the stomach associated with *H. pylori* infection.

### **New Triple Therapy for *H. pylori* Infection in Japan**

In Japan, a phase III trial of one-week triple therapy with lansoprazole (LPZ), amoxicillin (AMOX), and clarithromycin (CAM) was completed in March, 2000.<sup>70</sup> Patients were randomized into three groups: LPZ 30 mg bid and placebos, and LPZ 30 mg bid, AMOX 750 mg bid and CAM 200 mg bid or 400 mg bid. The eradication rates were high in the LPZ+AMOX+CAM 200 (88–91%) and LPZ+AMOX+CAM 400 (84–89%) groups. Then, a phase III trial of one-week therapy with omeprazole (OPZ), AMOX and CAM was completed in 2001. The eradication rates were 78.8% in the OPZ 20 mg, AMOX 750 mg and CAM 400 mg bid, and 83.0% in the OPZ 20 mg, AMOX 1000 mg and CAM 500 mg bid groups. On the other hand, the eradication rate of 7-day therapy of rabeprazole (RPZ) 10 mg bid, AMOX 750 mg bid and CAM 400 mg bid (84%) was higher than the 5-day therapy with same regimens (66%).<sup>71</sup>

### **Second-line Therapy for *H. pylori* Eradication after Failure of First-line Therapy**

Even though the currently employed *H. pylori* eradication regimens in Japan fail to cure 10–20% of patients, an optimal re-treatment therapeutic regimen for eradication-failure has not been established, yet. Since patient compliance, bacterial resistance and genotypic differences in CYP2C19 influence the eradication rate, re-eradication therapy should be selected, taking these factors into consideration. In the West, meta-

analysis of second-line treatment of *H. pylori* infection showed that a regimen containing ranitidine bismuth and two antimicrobials was very effective as a re-treatment regimen, irrespective of the factors influencing failure of the first-line *H. pylori* eradication treatment. However, ranitidine bismuth is not available in Japan and a re-eradication regimen consisting of PPI, amoxicillin and metronidazole has often been used and a high eradication rate achieved, even in patients with metronidazole-resistant *H. pylori*. In our University hospital, second-line eradication protocol is decided by the results of antibiotic susceptibility test after the failure of first-line therapy with lansoprazole (LPZ), AMOX and CAM.<sup>72</sup>

### **Problems that May Occur after *H. pylori* Eradication and the Countermeasures**

Acute esophagogastroduodenal mucosal lesions have been demonstrated as an adverse event that may occur after *H. pylori* eradication therapy. According to Sakaki *et al.*, acute duodenal erosions were observed in 13.5% of patients who received eradication therapy. Duodenal erosions which occurred mainly in the early phase after eradication were transient and asymptomatic. Acute gastric erosions showed the same clinical characteristics. However, attention should be paid to the rare occurrence of acute ulcers with severe symptoms. On the other hand, mild but persistent reflux esophagitis occurred at a relatively late phase after eradication. The occurrence rates were 9.8% after 1 year and 11.4% after 3 years. The acute mucosal lesions which occurred are probably attributable to the increase in acid secretion associated with the resolution of gastric mucosal inflammation. Symptomatic treatment with antacids may be necessary on occasion for the treatment of acute ulcer formation and reflux esophagitis.

### **Tailor-made Medicine for *H. pylori* Eradication Therapy**

The pharmacokinetic profiles of omeprazole and lansoprazole have been reported to be correlated with the CYP2C19 genotype.<sup>73</sup> While the drugs are extensively metabolized in heterozygotes as compared to homozygotes, the difference between the two groups was not statistically significant. For rabeprazole, on the other hand, the pharmacokinetic profile was independent of the CYP2C19 genotype. Thus, CYP2C19 genotyping may serve as useful new strategy in the choice of an optimal regimen, and this genotype testing may be especially useful in Japan, as the frequency of poor metabolizers among the Japanese is five times greater than that among Caucasians.<sup>74</sup>

However, the recent increase in the emergence of antimicrobial-resistant strains of *H. pylori* may force us to examine the antimicrobial susceptibility in all patients in the near future, in order to achieve an eradication rate with first-line therapy of more than 80%. It would also be imperative to have proper knowledge of the influence of CYP2C19 genetic polymorphism on the treatment efficacy according to various PPIs used alone or in combination with other drugs.

### **The Present Status and Problems of *H. pylori* Eradication Therapy**

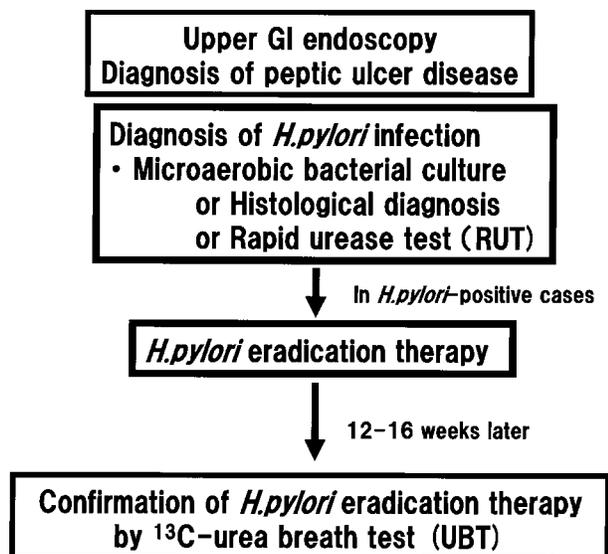
Since therapy for eradication of *H. pylori* in the treatment of peptic ulcer disease was approved for coverage by the Japanese Governmental Health Insurance System on November 1st in 2000, this therapy has been widely used in Japan. However, some problems of treatment have arisen, for example, the emergence of clarithromycin resistance. On the other hand, investigation of the relation between *H. pylori* infection and gastric cancer is making progress. In an elegant prospective study conducted in 2001, Uemura, *et al.* demonstrated that *H. pylori* infection plays an important role in gastric carcinogenesis.<sup>75</sup> Under such circumstances, the Japanese society of *H. pylori* Research has to reconsider the guidelines for eradication therapy of *H. pylori* infection.

### **New Prophylactic and Therapeutic Approaches against *H. pylori* Infection**

Probiotics have been shown to be effective against antibiotic-associated diarrhea in several randomized controlled studies. The rationale for their use lies mostly in their ability to restore the normal intestinal flora. Also, for *Lactobacillus* GG, inhibition of the prokinetic action of macrolides has been proposed. Many animal experiments indicate some degree of immune stimulation by probiotic species, and oral bacteriotherapy is a hot topic in the current experimental therapeutics of inflammatory bowel disease. To date, however, there is no definitive evidence to explain the mechanisms by which these biological agents influence the course of human disease.

### ***H. pylori* Diagnosis and Eradication Therapy at Keio University Hospital**

In the outpatient clinic (*Helicobacter* clinic; <http://web.sc.itc.keio.ac.jp/medicine/PYLORI/index.html>) of Keio University Hospital, patients are first subjected to upper GI endoscopy to diagnose peptic ulcer disease, and at the same time, biopsy is conducted for the diagnosis of *H. pylori* infection. Our method of choice



**Fig. 3** Flow-chart of *H. pylori* diagnosis and eradication therapy at Keio University Hospital.

among the three invasive diagnostic tests is the microaerobic bacterial culture method, because of its high specificity. However, this culture method also has a higher rate of false-negative results. To overcome this disadvantage, histopathological examination is performed in addition. In *H. pylori*-positive cases, one-week triple therapy is administered for *H. pylori* eradication. Twelve to 16 weeks after completion of the eradication protocol, disappearance of *H. pylori* from the stomach is usually confirmed by the UBT (Fig. 3). The *H. pylori* eradication rate of our outpatient clinic in 2002 was 72.4%. In case of eradication failure, an antibiotic susceptibility test is performed. Based on the results of this test, second-line therapy for eradication is administered.

Further investigations to establish an effective therapeutic regimen should be performed to reduce the emergence of antibiotic resistance and prevent eradication failure.

**Acknowledgments:** This study was supported by a Grant-in-Aid for Scientific Research C (2) from the Japan Society for the Promotion of Science (JSPS) (13670555, to H.S.), a grant from Keio University.

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