Molecular Approaches and Modern Clinical Strategies for the Management of *Helicobacter pylori* Infection in Japan

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Thirty years have passed since Warren and Marshall’s discovery of *Helicobacter pylori* (*H. pylori*). Since then, not only peptic ulcer diseases and chronic gastritis but also non-cardia gastric cancers have been recognized as diseases originating from *H. pylori* infection. Several combination therapies consisting of multiple antibiotics have been developed as first- or second-line regimens to eradicate *H. pylori* infection. Our extensive experience in the field of anti-*H. pylori* medicine suggests that clinicians should consider a possible role for unidentified, invisible pathogens to elucidate the pathogenesis and improve the treatment of refractory diseases of unknown etiology.


**Keywords:** *Helicobacter pylori*, CagA, indication, diagnosis, eradication

**Introduction**

Classical bacteriology techniques have demonstrated that *Helicobacter pylori* (*H. pylori*) is a gram-negative, flagellated bacterium that expresses catalase and urease, enzymes which help neutralize host responses and enable intragastric colonization. One of the early benefits of this basic research was the realization that virtually all strains of *H. pylori* produce urease. This finding led to the development of accurate diagnostic tests, including the rapid urease test and the urea breath test. A stool antigen test has also emerged as an informative, noninvasive means by which to diagnose infection via detection of bacterial antigens.

Before the discovery of intragastric bacteria 30 years ago, there were several reports of spiral gastric bacteria dating back to 1890 (Table 1).

The modern discovery of *H. pylori* may have been delayed by Palmer’s declaration in 1954 that there were no microorganisms in the human stomach. At that time, microorganisms were believed to be unable to survive in the acidic gastric environment, and intragastric spiral bacteria had been observed only post-mortem; however, the presence of organisms in the gastric mucosa had been described since the 1890s. In Japan, Kasai and Kobayashi of the Kitasato Institute reported propagation of a spirochete-like organism, probably modern *Helicobacter felis*, from the stomachs of dogs and cats but not from laboratory animals. They showed that when rabbits infected with these spirochetes were inoculated with *virus fixe*, marked hemorrhagic lesions were produced in the gastric mucosa. In addition, spirochetes inoculated into the mouse gastric mucosa could be eradicated by arsamolin (a classic equivalent of bismuth). Sixty five years later, scientists and doctors again attempted to treat gastric diseases associated with “spirochetes,” i.e., *Helicobacter pylori* infection. To commemorate the achievements of Kobayashi and coworkers in the Taisho era, the Rokuzo Kobayashi Memorial Symposium on *Helicobacter pylori* was held on May 11th, 2002, in Kita-kan Hall at Keio University Mita Campus, Tokyo, Japan.

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Pathogenesis

The most-investigated bacterial toxins of *H. pylori* are associated with a segment of bacterial DNA referred to as the cag pathogenicity island (cag PAI). Genes within the cag PAI encode proteins such as CagE, an ATPase that drives a type IV secretory apparatus enabling bacterial macromolecules, especially the toxin CagA, to translocate into the host cell. The intact cag PAI of *H. pylori* plays a significant role in the pathogenesis of chronic gastritis in humans because the cag PAI is associated with increased chemokine expression and greater inflammation in gastric mucosal specimens.\(^6\) After the CagA protein is injected into the host cell cytoplasm through the type IV secretory system, the EPIYA motif of CagA is tyrosine-phosphorylated by host Src kinases, and the phosphorylated protein subsequently alters the gastric epithelial morphology. Src homology-2 domain-containing phosphatase 2 (SHP2) is able to bind the EPIYA-B, EPIYA-C, and EPIYA-D motifs.\(^7\) Importantly, however, CagA with the EPIYA-D motif has a higher binding affinity for SHP2 than does CagA with the EPIYA-C motif.\(^8\) The sequence flanking the tyrosine phosphorylation site of the EPIYA-D motif (EPIYATIDF), but not that flanking the EPIYA-C motif (EPIYATIDD), perfectly matches the consensus high-affinity binding sequence for the SH2 domains of SHP2. The CagA proteins of strains from distinct geographic populations appear to be phosphorylated to different degrees, resulting in graded effects on intracellular signaling.\(^9\)

The biological half-life of intracellular CagA in gastric epithelial cells was recently reported to be approximately 200 min,\(^10\) and the activity of CagA as an epigenetic oncoprotein thus does not persist for a long period in any single cell. Therefore, the risk of gastric cancer may be determined by various factors that influence the stability of intracellular CagA. We recently reported that intracellular CagA is degraded by p53 degradation-induced autophagy;\(^11\) however, a specific mutation in p53 increases the intracellular stability of CagA by inhibiting its autophagic degradation. These findings suggest that disrupting the autophagic degradation of CagA increases the risk of developing *H. pylori*-associated gastric cancer.

VacA is the second-most extensively studied *H. pylori* virulence factor. In addition to inducing vacuolation, VacA also promotes several cellular activities, including membrane channel formation and the release of cytochrome c from mitochondria and consequent apoptosis. VacA can also specifically inhibit T-cell activation and proliferation.\(^12\) Further studies have confirmed that the risks for both cancer and peptic ulcers are associated strongly with the *cagA/vacA s1m1* genotype but rarely with the *vacA s2m2* polymorphism.\(^13\)

Oxygen-derived free radicals released from activated neutrophils extravasated from microvascular beds are considered to be potential toxic factors involved in *H. pylori*-induced gastric mucosal injury because *H. pylori* exhibits chemotactic activity for neutrophils.\(^1,14\) Neutrophil infiltration of the gastric mucosa leads to the development of the initial lesions of *H. pylori*-associated gastritis. Neutrophils express the enzyme myeloperoxidase, which, in the presence of Cl\(^-\), produces the potent oxidant hypochlorous anion (OCI\(^-\)) from H\(_2\)O\(_2\). This hypochlorous anion reacts with ammonia, produced from urea by *H. pylori*-associated urease, to yield the lipophilic cytotoxic oxidant monochloramine (NH\(_2\)Cl), which freely penetrates biological membranes to oxidize intracellular components (Fig. 1, left).\(^1\) 8-Hydroxy-2-deoxyguanosine (8-OHdG), which results from the attack of a single hydroxyl or oxygen radical on guanine, is one of the forms of DNA damage most commonly induced by reactive oxygen species. Patients with *cagA*-positive strains of *H. pylori* have higher 8-OHdG levels than do *cagA*-negative or *H. pylori*-negative patients.\(^15\) We previously reported greater enhancement of neutrophil-derived gastric mucosal luminol-dependent chemiluminescence levels in

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**Table 1** History of *Helicobacter* research

<table>
<thead>
<tr>
<th>Year</th>
<th>Name</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1892</td>
<td>Bizzozero</td>
<td>Discovery of snake-like bodies in a dog stomach</td>
</tr>
<tr>
<td>1906</td>
<td>Krienitz</td>
<td>Discovery of spirochete-like bodies in human stomach</td>
</tr>
<tr>
<td>1919</td>
<td>Kasai and Kobayashi</td>
<td>Establishment of an animal model for <em>Campylobacter</em> infection and first <em>Helicobacter</em> eradication experiment</td>
</tr>
<tr>
<td>1938</td>
<td>Doenges</td>
<td>Isolation of spirochete-like organism from a human stomach</td>
</tr>
<tr>
<td>1954</td>
<td>Palmer</td>
<td>Denied the existence of microorganisms in the stomach</td>
</tr>
<tr>
<td>1976</td>
<td>Lieber</td>
<td>Reduction of gastric NH(_3) by ampicillin</td>
</tr>
<tr>
<td>1982</td>
<td>Warren and Marshall</td>
<td>Detection and isolation of <em>Campylobacter</em>-like organism from biopsied gastric specimen (<em>Campylobacter pyloridis</em>)</td>
</tr>
<tr>
<td>1994</td>
<td>NIH Consensus Development Conference</td>
<td>Recommendation of <em>H. pylori</em> eradication therapy for all patients with peptic ulcer disease</td>
</tr>
<tr>
<td>2002</td>
<td>Marshall</td>
<td>Keio Medical Science Prize</td>
</tr>
<tr>
<td>2005</td>
<td>Warren and Marshall</td>
<td>Nobel Prize in Physiology and Medicine</td>
</tr>
</tbody>
</table>
cagA-positive patients than in cagA-negative patients.\textsuperscript{16} cagA-positive patients are characterized by greater oxidative DNA damage, both overall and at younger ages, in the presence of multifocal atrophy.

Infection of gastric epithelial cells with \textit{H. pylori} increases the accumulation of intracellular reactive oxygen species (ROS). Increased intracellular oxidative stress may play a role in the induction of programmed cell death.\textsuperscript{17} We recently detected increased mitochondrial ROS production in \textit{H. pylori}-infected gastric epithelial cells. This finding suggests that \textit{H. pylori} infection alters the mitochondria. In addition, the antioxidant N-acetyl cysteine inhibited induction of autophagy and induced the accumulation of intracellular CagA.\textsuperscript{11} These results suggest that intracellular oxidative stress is involved in the induction of autophagy required for CagA degradation (Fig. 1, lower right).\textsuperscript{11}

Metronidazole, a major component of the second-line \textit{H. pylori} eradication regimen used in Japan, enters cells by diffusion, and its antimicrobial toxicity depends on the reduction of its nitro group to nitro anion radicals and the subsequent generation of superoxide radicals (O$_2^-$).\textsuperscript{18,19} In detail, the NADPH nitroreductase of \textit{H. pylori} reduces the nitro group of metronidazole to anion radicals that induce oxidative stress and produce DNA strand breaks, causing rapid cell death.\textsuperscript{18} \textit{H. pylori} expresses only a single superoxide dismutase, the iron-cofactored enzyme SodB, which has 53.5% identity with the \textit{Escherichia coli} protein FeSod.\textsuperscript{20} SodB prevents the interaction between iron and superoxide and also catalyzes the dismutation of superoxide (O$_2^-$) into oxygen (O$_2$) and hydroperoxide (H$_2$O$_2$). The mRNA expression of sodB in \textit{H. pylori} is directly regulated by the ferric uptake regulator (Fur) protein.\textsuperscript{21} We recently found metronidazole-resistant strains of \textit{H. pylori} with amino acid mutations in Fur that significantly reduced its binding affinity for its operator sequence in the sodD promoter (Fur-Box), resulting in derepression of sodB mRNA expression.\textsuperscript{22} In other words, metronidazole resistance is due to enhanced scavenging activity of cytotoxic superoxide (O$_2^-$), a major bactericidal component of metronidazole, because of the derepression of \textit{H. pylori} SodB expression by mutant Fur (Fig. 1, left).\textsuperscript{22}

Ferrous iron (Fe$^{2+}$), an essential cofactor for many enzymes and metalloproteins, is necessary for the basal

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Molecular approaches to \textit{Helicobacter pylori} infection.}
\end{figure}
functions of all cells as well as for SodB activation. We recently demonstrated that the FecA1 protein, a Fe\(^{3+}\)-dicitrate transporter homolog, is essential for SodB activation but not for the bioactivity of \(H. pylori\).\(^{24}\) mRNA expression is derepressed in metronidazole-resistant \(H. pylori\) strains with mutations in Fur.\(^{24}\) Deletion of \(fecA1\) dramatically decreased the minimal inhibitory concentrations (MICs) of metronidazole for \(H. pylori\) strains with Fur mutations,\(^{24}\) suggesting that the activation of SodB by mutant Fur is supported by the FecA1-dependent Fe\(^{3+}\) supply system (Fig. 1, upper right).

Notably, in vitro study has shown that metronidazole-susceptible \(H. pylori\) became metronidazole resistant after several passages on agar plates containing sub-inhibitory concentrations of metronidazole.\(^{25}\) From these findings, it can be readily assumed that metronidazole-susceptible \(H. pylori\) may become metronidazole resistant through repeated exposure to sub-inhibitory concentrations of metronidazole. Therefore, it is important to understand the initial anti-metronidazole response of metronidazole-susceptible \(H. pylori\) to prevent the acquisition of resistance and consequent increased incidence of metronidazole-resistant \(H. pylori\).

Five families of multidrug efflux transporters have been described in bacteria.\(^{26}\) One of these five efflux systems, the RND family, has three components, namely, the inner membrane efflux proteins, a periplasmic efflux protein, and an outer membrane efflux protein (the ToC, or ToC homolog protein).\(^{27}\) Four RND families have been identified in \(H. pylori\) (HP0605 to HP0607; HefABC and HP0971 to HP0969; HefDEF and HP1327 to HP1329; HefGHI and HP1489 to HP1487), and these proteins are reportedly involved in the development of multidrug resistance.\(^{28}\) We recently explored the variations in transcription of the RND efflux pump systems in the initial phases of the development of metronidazole resistance in vitro. In this study, the MICs of metronidazole for 9 out of 10 metronidazole-susceptible strains cultured on plates containing sub-inhibitory concentrations of metronidazole increased to levels similar to those for metronidazole-resistant strains. In the newly metronidazole-resistant strains, exposure to metronidazole significantly increased the expression of the ToC efflux pump (\(hefA\)) with no decrease in the metronidazole-reduction activity, suggesting that overexpression of the ToC efflux pump \(hefA\) may be the first step in the acquisition of metronidazole resistance in \(H. pylori\).\(^{29}\)

\section*{H. pylori and gastric cancer – molecular approach}

Gastric epithelium changes during the progression from inflammation to cancer, with evidence of disruption of normal epithelial cell differentiation and recruitment of inflammatory cells.\(^{30}\) Coincident with the development of atrophic gastritis and intestinal metaplasia is the loss of expression of the gastric morphogen sonic hedgehog (Shh).\(^{31,32}\) Loss of Shh is reportedly an early change in carcinogenesis of the gastric mucosa, prior to neoplastic transformation. We have demonstrated in humans that the suppression of Shh expression in the gastric mucosa by \(H. pylori\) infection was restored after eradication of the infection and that earlier eradication more fully restored Shh expression in the gastric mucosa.\(^{33,34}\)

MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression that are involved in development, cell proliferation, and immune responses. Recent studies have shown that some miRNAs act as tumor suppressors or oncogenes in gastric cancer.\(^{35}\) Some miRNAs, including \(miR-146, miR-155, miR-21, miR-27a, miR-106–93-25, the miR-221–222\) clusters, and the \(miR-200\) family, are possibly involved in \(H. pylori\) infection and associated gastric cancers.\(^{36}\) miRNA expression profiling may be a powerful tool for clinical cancer diagnosis, and regulation of miRNA expression could be a novel strategy for the chemoprevention of human gastrointestinal cancers.\(^{37}\)

\section*{H. pylori and gastric mucosa-associated lymphoid tissue lymphoma – molecular approach}

\(H. pylori\) eradication has become widely accepted as an initial treatment strategy for stage I gastric marginal zone B cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type. \(H. pylori\)-positive low-grade gastric MALT lymphoma regresses both endoscopically and histopathologically after \(H. pylori\) eradication in 60%–80% of such cases.\(^{38}\) The presence of a t(11;18)(q21;q21) translocation appears to be a major predictor of failure to respond. This translocation is associated with an \(API2-MALT1\) fusion; the former is involved in the regulation of apoptosis, and the latter is a caspase-like protein with an as yet unknown biological function. The fusion causes suppression of apoptosis. Several studies have reported that MALT lymphomas with this translocation respond only rarely or not at all to \(H. pylori\) eradication.

Methylation of the \(p16/INK4a\) gene was observed in 60% of MALT lymphomas; however, \(p16\) gene methylation status did not correlate with the presence of \(API2-MALT1\) fusion or any other clinicopathological factor, suggesting that aberrant methylation of the \(p16\) gene might be an early event in MALT lymphomagenesis.\(^{39}\) Examination of the methylation profiles of eight CpG islands, namely \(p15, p16, p73, \text{hMLH1}, \text{DAPK, MINT1, MINT2, and MINT3}\), revealed that more than four genes were methylated in \(H. pylori\)-dependent MALT lymphomas while fewer than two genes were methylated in non-\(H. pylori\)-dependent cases, indicating that the pathogenesis of gastric MALT lymphomas, including the aberrant DNA methylation pattern, may differ between \(H. pylori\)-dependent and non-\(H. pylori\)-dependent cases.\(^{40}\) Aberrant DNA methylation thus plays critical roles in the
pathogenesis of gastric MALT lymphomas, and determination of the DNA methylation pattern may hold promise as a clinical tool for surveillance of gastric MALT lymphomas.

H. pylori and idiopathic thrombocytopenia purpura – molecular approach

Idiopathic thrombocytopenic purpura (ITP) is a bleeding disorder in which platelet-specific autoantibodies cause platelets to be lost. In a subset of H. pylori-infected patients with ITP, the number of platelets recovers after the eradication of H. pylori. According to our previous research into the role of H. pylori infection in the pathogenesis of ITP, a significant increase in platelet numbers was observed after H. pylori eradication in 61% of patients. In this study, at baseline, monocytes from H. pylori-positive patients exhibited an enhanced phagocytic capacity and low levels of the inhibitory Fcγ receptor IIB (FcγRIIB). This activated monocyte phenotype was suppressed 1 week after the start of the H. pylori eradication regimen, and this suppression was followed by improvements in other autoimmune and platelet kinetic parameters. H. pylori infection was also associated with an altered monocyte FcγR balance in individuals without ITP; a similar result was also found in mice. Our findings strongly suggest that the recovery in platelet numbers observed in ITP patients after H. pylori eradication is mediated through a shift of the FcγR balance toward inhibitory FcγRIIB.

Veneri et al. reported that the host factors HLADRBI11, HLA-DRB 114, and HLA-DQB 103 occurred at high frequencies in patients with H. pylori-positive ITP, while the frequency of the host factor HLA-DRB 103 was higher in the H. pylori-negative ITP group than in the H. pylori-positive ITP group. HLA-DQB 103 showed favorable platelet reactivity after H. pylori eradication therapy in H. pylori-positive ITP patients.

Functional dyspepsia and H. pylori infection

Functional dyspepsia (FD) is a condition in which upper abdominal symptoms occur in the absence of any explanatory organic disease. H. pylori infection is not an exclusion criterion for FD under the current Rome III classification. However, recent advances in basic and clinical research have revealed that H. pylori infection plays an important role in the development of gastroduodenal dysmotility and hypersensitivity and also in dyspeptic symptoms. For example, the authors revealed that in mice, chronic H. pylori infection downregulates expression of muscle-specific miRNAs (miR-1, miR-133a, and miR-133b) and upregulates expression of their targets, such as histone deacetylase 4 and serum response factor. These transcriptional changes cause hyperplasia of the muscular layer of the stomach and gastric emptying deregulation. In addition, H. pylori eradication therapy appears to have a small but statistically significant effect on FD. Therefore, H. pylori-associated dyspepsia (HpD) should be excluded from the umbrella category of FD to better elucidate the pathophysiological mechanisms of FD and to establish more precise diagnostic markers or criteria (Fig. 2). In Japan, for national insurance claim purposes, H. pylori-positive histological gastritis, NSAID-induced gastritis, and neurotic gastritis (or stress-induced gastritis) have all been combined under the diagnosis of “chronic gastritis” and have therefore been treated with the same drugs. Of these groups of diseases, FD would be categorized as classical stress-induced gastritis. However, as each type of gas-

\[\text{Fig. 2 New disease entity concept clarifying the difference between H. pylori-associated and non-H. pylori-associated dyspepsia.}\]
Gastroesophageal reflux disease (GERD) has a different etiology, only diseases produced by known pathogens, such as *H. pylori*, should be treated by targeting the pathogen directly. In addition, *H. pylori*-negative FD should not be categorized as so-called “chronic gastritis” because it is histologically negative for inflammation. As the pathophysiology underlying the disturbances of gastroduodenal motor and sensory functions and dyspepsia symptoms caused by *H. pylori* infection is gradually being elucidated, *H. pylori*-positive FD (i.e., HpD) should be considered an organic disease and dealt with as a disease entity distinct from FD *(Fig. 3)*. Separating HpD from FD may reconcile the conflicting results of previous studies on drug therapy for FD. The differences between the most effective therapeutic strategies against HpD and *H. pylori*-negative FD also require further investigation.

**Indications for *H. pylori* Eradication in Japan**

At present, *H. pylori* eradication is used not only for the treatment of peptic ulcer disease but also for prophylaxis and treatment of *H. pylori*-associated diseases such as gastric cancer, gastric MALT lymphoma, and ITP, as well as for inhibiting the spread of this bacterial infection.

*H. pylori* infection was approved as an official disease name in the 2003 edition of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). *H. pylori* infection itself is soon expected to be accepted by the Japanese national health scheme as a disease entity; this will make the diagnosis and treatment of *H. pylori*-associated diseases a part of routine medical practice, resulting in the successful prevention of gastric cancer. In addition, *H. pylori* eradication therapy could
prevent the spread of this infection, which would lead to dramatic savings in medical costs.


<table>
<thead>
<tr>
<th>Diseases</th>
<th>Minds recommendation grade</th>
<th>Level of evidence classification</th>
<th>Coverage by the Japanese national health insurance system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic ulcer (gastric/duodenal ulcer)</td>
<td>I</td>
<td>I</td>
<td>Yes</td>
</tr>
<tr>
<td>Gastric MALT lymphoma</td>
<td>III</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura (ITP)</td>
<td>I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Patients after endoscopic treatment of early gastric cancer</td>
<td>II</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

In contrast, early gastric cancer post-endoscopic treatment, gastric hyperplastic polypl, and reflux esophagitis were categorized as Level II (supported by at least one randomized controlled clinical trial), while gastric MALT lymphoma, iron-deficiency anemia, and chronic urticaria were categorized as Level III (supported by non-randomized controlled clinical studies) (Table 2).

**Diagnosis of H. pylori Infection**

H. pylori infection is investigated before and after eradication therapy using the following tests. Tests that require endoscopic biopsy include (i) the rapid urease test, (ii) histology, and (iii) culture, whereas tests not requiring endoscopic biopsy include (i) the 13C-urea breath test (UBT), (ii) detection of anti-H. pylori antibodies in the serum or urine, and (iii) measurement of H. pylori stool antigen. The national health insurance scheme now covers the use of two separate tests.

The rapid urease test has the advantages of low cost, ease of use, and speed, whereas the advantages of culture include 100% specificity (direct demonstration of the presence of H. pylori) and the ability to further characterize the organism (e.g., determination of its antibiotic susceptibility and investigation of its virulence factors). On the other hand, the UBT is a practical and readily available test with a diagnostic accuracy of >95%.

The detection of anti-H. pylori antibodies in the serum is a widely available and inexpensive test, but the diagnostic accuracy is low (80%–84%). Some highly accurate (90%) serology kits are recommended in validated settings. The anti-urease activity of proton pump inhibitor (PPI) treatment can result in false-negative results for invasive and noninvasive diagnostic tests; therefore, PPI treatment should be withheld for at least 2 weeks before testing. However, this consideration does not apply to serology.

A systematic review of 89 studies evaluating the stool antigen test found an aggregate sensitivity and specificity of 91% and 93%, respectively. Stool samples must be stored at −20°C prior to testing for this test to be accurate; the sensitivity of the stool antigen test decreases to 69% after storage of the samples at room temperature for 2–3 days. Stool antigen tests have low specificity in patients presenting with acute bleeding peptic ulcers, as there is cross-reactivity with blood products in the stool.

**H. pylori Eradication Therapy**

In Japan, 1 week of triple therapy using a PPI combined with amoxicillin (750 mg, b.i.d.) and clarithromycin (400 mg or 200 mg, b.i.d.) is recommended as the first-choice treatment for eradication of H. pylori. At the 2008 meeting of the JSHR, the mean national clarithromycin resistance rates from 2002 to 2006 were reported to be 18.9%, 21.2%, 27.7%, 29.0%, and 27.2%. The mean nationwide clarithromycin resistance rate determined by the Japanese Society of Chemotherapy in 2000 was 7.0%, so the resistance rate has increased by approxi-
mately 20% over several years. Given the recent surge in clarithromycin resistance, new strategies for first-line treatment of *H. pylori* infection should be considered.\(^5\)\(^8\) Recently, a European group performed a randomized, open-label, non-inferiority, phase 3 trial comparing the efficacy and safety of first-line quadruple therapy with omeprazole plus a single three-in-one capsule containing bismuth subcitrate potassium, metronidazole, and tetracycline (quadruple therapy) for 10 days versus omeprazole, amoxicillin, and clarithromycin for 7 days (standard therapy) in adults with known *H. pylori* infection.\(^5\)\(^9\) According to this study, the eradication rate in the intent-to-treat (ITT) population \(n = 440\) was 80% (174 of 218 participants) in the quadruple therapy group versus 55% (123 of 222) in the standard therapy group \(P < 0.0001\). This suggests that quadruple therapy would be considered for first-line treatment of *H. pylori* infection, especially in view of the rising prevalence of clarithromycin-resistant *H. pylori* and the fact that quadruple therapy provides superior eradication, with safety and tolerability similar to those of standard therapy, even though this study showed an extraordinarily low eradication rate in the standard therapy arm.\(^5\)\(^9\) However, several issues, including the optimal doses of bismuth and amoxicillin and the duration of treatment, must be also considered before quadruple therapy can be established as the standard first-line therapy for *H. pylori* eradication.\(^6\)\(^0\)

In Japan, the recommended second-choice treatment for *H. pylori* infection is 1 week of triple therapy using a PPI combined with amoxicillin (750 mg, b.i.d.) and metronidazole (250 mg, b.i.d.). The prevalence of resistance to metronidazole in *H. pylori* in Japan has been reported to range between 5% and 12%.\(^6\)\(^1\) The Tokyo *H. pylori* Study Group examined the rate of eradication in response to the second-line regimen consisting of PPI, amoxicillin, and metronidazole in a multicenter study in the Tokyo metropolitan area.\(^6\)\(^2\) ITT and per-protocol (PP) analyses revealed eradication rates of 87.6% and 90.6%, respectively. Murakami et al. reported that eradication rates following second-line treatment of *H. pylori* infection with the PPI + amoxicillin + metronidazole regimen were 97% for infections with metronidazole-sensitive strains and 82% for infections with metronidazole-resistant strains. Two explanations are available for the discrepancy between the MICs for metronidazole and the eradication rates for PPI-amoxicillin-metronidazole triple therapy: (i) metronidazole is relatively stable under anaerobic conditions, possibly making its antimicrobial activity stronger in the stomach than in vitro, and (ii) the mechanism by which *H. pylori* acquires resistance to metronidazole remains unclear (mutations in the nitroreductase *rdxA* chromosome have been implicated, but there are other possibilities), making it difficult to predict the effects of metronidazole-containing second-line treatment regimens from the MIC of metronidazole alone. To improve prediction of eradication success by second-line treatment, we developed and reported an eradication resistance index, calculated as:

\[
\text{Index} = \frac{[\text{Pre-treatment urea breath test result (‰)}] \times [\text{amoxicillin MIC (μg/mL)}] \times [\text{metronidazole MIC (μg/mL)}]}{\text{Pre-treatment urea breath test result (‰)}}
\]

When a cutoff value of 3 was used, the eradication resistance index predicted the response to therapy with a specificity of 81.8%, a sensitivity of 93.8%, and an accuracy of 92.5%. However, because the superiority of the first-line eradication regimen (PPI + amoxicillin + clarithromycin) to the second-line regimen (PPI + amoxicillin + metronidazole) in Japan has recently been called into question, we retrospectively confirmed that the metronidazole-containing second-line regimen could be a superior primary eradication regimen for *H. pylori* in Japan.\(^5\)\(^8\)

There is at present no standard third-line treatment regimen for eradication of *H. pylori*, and European guidelines recommend culture before the selection of a third-line treatment to tailor the regimen to the antibiotic sensitivity of the isolate. *H. pylori* isolated after the failure of first- and second-line eradication regimens is often resistant to both metronidazole and clarithromycin.\(^6\)\(^6\) Therefore, these two drugs are not recommended for inclusion in third-line regimens. In Japan, a PPI + amoxicillin + fluoroquinolone combination or high-dose PPI/amoxicillin therapy is recommended if second-line eradication therapy fails.\(^5\)\(^4\)\(^6\) However, we recently reported that even amoxicillin resistance (MIC \(\geq 0.5 \mu g/mL\)) is enhanced after multiple eradication failures (1st: 0%, 2nd: 0.9%, 3rd: 6.1%, and 4th: 18.2%) due to the accumulation of *PBPI* mutations.\(^6\)\(^6\) Clinicians should therefore be aware of the possibility of resistance to amoxicillin along with that to other antibiotics.

Novel fluoroquinolones have recently been developed, and sitafloxacin has been reported to have superior in vitro activity against *H. pylori* compared with levofloxacin.\(^6\)\(^5\)\(^6\)\(^6\) We investigated the efficacy of sitafloxacin-based triple therapy as a third-line treatment administered after assessment of the isolates for sitafloxacin susceptibility and the presence of *gyrA* mutations. Sitafloxacin-based triple therapy achieved 83.6% (PP) or 78.2% (ITT) success among 78 Japanese patients. Even among the 47 patients with *gyrA* mutation-positive *H. pylori*, the eradication rates were 74.4% (PP) and 68.1% (ITT).\(^6\)\(^7\) In that study, the position of the *gyrA* mutation (N87 or D91) was found to be superior to the MIC value for predicting the eradication outcome.\(^6\)\(^7\) A randomized, open-label, parallel study comparing two sitafloxacin-based triple therapies in patients with multiple eradication failures is now underway in Keio University Hospital (Tokyo, Japan) (UMIN000006483) (http://kompas.hosp.keio.ac.jp/contents/medical_info/presentation/201201.html).
Summary

H. pylori infection is now known to be the main cause of peptic ulcer disease, chronic atrophic gastritis, and gastric MALT lymphoma, as well as non-cardia gastric cancer. In addition, an association between H. pylori infection and some extra-gastrointestinal diseases, such as ITP, has been indicated. We summarized recent basic research and clinical data on the link between H. pylori infection and H. pylori-associated diseases. Accurate diagnostic methods for H. pylori detection are available, allowing ready detection of the infection. Although the recommended first-line therapy regimens are effective and well tolerated, their wide use throughout the country over the years has led to failure of these regimens in 20%–30% of patients. Treatment failure is due mainly to increasing antibiotic resistance, in particular clarithromycin resistance. Although novel therapeutic strategies should be considered for first-line treatment, local monitoring of antimicrobial resistance must be implemented as well. In 2009, the JSHR established a board certification system for doctors treating H. pylori infection, and 734 doctors have been registered as a board-certified doctor by the JSHR after four board certification examinations on March 2012 (http://www.jshr.jp/index.php?page=medic_list) (Fig. 3). The JSHR also reported the list of institutes performing H. pylori eradication not covered by national insurance in Japan (http://www.jshr.jp/index.php?page=medic_facility) (Fig. 3).

While medical qualifications, scientific knowledge, and techniques in the field of H. pylori infection all continue to improve, the total control of these silent organisms in the stomach remains a challenge.

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