Volume 11 Supplement 1 2006

Helicobacter

EDITOR: David Y. Graham, M.D.

The Year in Helicobacter 2006

Guest Editors: Peter Malfertheiner
Francis Mégraud
Pierre Michetti
Pentti Sipponen
on behalf of the European Helicobacter Study Group

Blackwell Publishing

VOLUME 11 SUPPLEMENT 1 OCTOBER 2006

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The publication of this supplement has been funded by unrestricted educational grants from AstraZeneca and INFAI, GmbH.

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1478-4041(200610)11:05+01;1-F

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Information for subscribers
Helicobacter is published in six issues per year. Subscription prices for 2006 are: Premium Institutional: £598 (Europe), US$1107 (The Americas), £659 (Rest of World); Personal: €188 (Europe, Euro zone), £125 (Europe, non-Euro zone), US$232 (The Americas), £138 (Rest of World). Customers in the UK should add VAT at 5%; customers in the EU should also add VAT at 5%, or provide a VAT registration number or evidence of entitlement to exemption. Customers in Canada should add 7% GST or provide evidence of entitlement to exception. The Premium institutional price includes online access to the current and all available previous year electronic issues. For other pricing options or more information about online access to Blackwell Publishing journals, including access information and terms and conditions, please visit www.blackwellpublishing.com/hel

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Helicobacter is indexed by: Current Contents/Clinical Medicine, Medical Documentation Service, Research Alert, Sci Search, Science Citation Index, Medline.

Publisher
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Journal Customer Services: For ordering information, claims and any enquiry concerning your journal subscription please contact your nearest office:
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Commercial reprints: Tracy James (email: tracy.james@oxon.blackwellpublishing.com).

ISSN 1083-4389 (Print)
ISSN 1523-5378 (Online)

For submission instructions, subscription and all other information visit: www.blackwellpublishing.com/hel

Periodical ID Statement C
Supplement to *HELICOBACTER*. *HELICOBACTER* (1083-4389) is published bimonthly. US mailing agent: G3 Worldwide (US) Inc., 8701 Bellanca Ave., Los Angeles, CA 90045. Periodical postage paid at Los Angeles, CA, and at additional mailing offices. POSTMASTER: Send all address changes to *HELICOBACTER*, Blackwell Publishing Inc., Journals Subscription Department, 350 Main St., Malden, MA 02148-5020.

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Typeset by Graphicraft Limited in Hong Kong, China, and printed and bound in Singapore by Markono Print Media Pte Ltd.

Helicobacter **VOLUME 11 SUPPLEMENT 1 OCTOBER 2006**

CONTENTS

ORIGINAL ARTICLES

Epidemiology of *Helicobacter pylori* Infection

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Keywords
epidemiology, *Helicobacter pylori*, prevalence, risk factors, transmission.

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Abstract

Differences may occur in the mode of transmission of *Helicobacter pylori* between developed and developing countries: direct human-to-human contacts have been suggested as the primary route in the former while the fecal–oral route, also, through contaminated water, in the latter. Data on intrafamilial transmission of *H. pylori* among children continue to be produced. The importance of low socioeconomic conditions on the acquisition of *H. pylori* infection has been confirmed in a number of population-based studies. Due to the improvement of living standards, the prevalence of the infection has fallen dramatically in many countries. It varies from 8.9 to 72.8% among children from developed and developing countries, respectively, the re-infection rate being also significantly higher in the latter. Conflicting data have been reported on the effect of breastfeeding against *H. pylori* colonization in infancy as well as on the occupational risk for acquiring *H. pylori*. This review summarizes recent results from the literature on these topics.

The Environmental Conditions in the Transmission of *Helicobacter pylori*

In a large epidemiologic study on the association between herpes simplex virus type 1 (HSV-1) infection and *Helicobacter pylori* conducted in 1090 subjects aged 12–19 years in the USA, it has been shown that the crude prevalence ratio (PR) of *H. pylori* in HSV-1 positive subjects was 2.2 (95% CI 1.69–2.85) (i.e., 6.9 versus 3.7% HSV-1 negative subjects). After adjustment for poverty level and race/ethnicity, the strength of the association varied according to household size and location: it was stronger in large urban households (PR 2.27, 95% CI 1.5–3.41) and negligible in small non-urban households (PR 1.15, 95% CI 0.79–1.67). Authors speculated that close interpersonal contact may favor the transmission of both *H. pylori* and HSV-1 and that direct human-to-human contact may be the primary route of transmission for both organisms [1]. Casting doubt on the contention that *H. pylori* and hepatitis A virus (HAV) share a common mode of transmission, none of 289 students (7–12 years) from a single primary school in Taiwan were seropositive for both *H. pylori* and HAV antibodies, whereas 223 (77.2%) were seronegative for both ($p = .294$). Furthermore, data have been produced indicating that sources of water supply (tap water, well water, bottled water) were not significantly associated with seropositivity for *H. pylori* [2]. Nevertheless, in a cross-sectional study in 121 children admitted in a pediatric hospital in Brazil, a fair agreement ($\kappa = 0.24$) was found between *H. pylori* (42.1%) and HAV (37.2%) seropositivity as well as an association between *H. pylori* seropositivity and the presence of *Giardia lamblia* in feces (15.7%). Of interest, no association was found between *H. pylori* and five other intestinal parasites in feces. The authors suggested that fecal–oral route may be relevant in the transmission of *H. pylori* among children in an urban setting of a developing country [3]. A high prevalence of *H. pylori* IgG seropositivity was found in 87 patients from Indonesia who had recently recovered from *Salmonella typhi* infection, a fecal–oral transmitted infection, than in 232 random healthy controls (67 versus 50%, $p = .008$, respectively). No association was found between elevated plasma gastrin concentration indicative of hypochlorhydria or achlorhydria and *S. typhi* infection. Authors concluded that *H. pylori* and *S. typhi* infection share low hygienic standards and common risk factors [4]. That water may have a role in the transmission of *H. pylori* in developing countries was pointed out in a study aimed to detect *H. pylori* in stools and water with different levels of fecal pollution, as measured by the presence of microbial indicators. Through a seminested polymerase chain reaction (PCR), *H. pylori* DNA was detected in 33% of 36 human fecal samples, in 66% of wastewater samples and 11% of river samples, but in none of spring water samples. A higher number of positive results were obtained in the more fecally polluted waters, so authors suggested that water may be a vector of *H. pylori* in its fecal–oral route [5]. The occurrence of *H. pylori*-specific genes, 16S rDNA and *cag*A, along with the presence of bacteria-like bodies was demonstrated by PCR and light microscopy, respectively, in yeasts taken from the oral cavity of 18 different dyspeptic patients. These findings indicate the possible symbiotic life of *H. pylori* inside the yeasts vacuole and hypothesize that yeasts might serve as reservoirs and vehicles of *H. pylori* [6].

Intrafamilial Transmission

There is increasing evidence supporting the intrafamilial transmission of *H. pylori* infection. Two seroepidemiologic studies, one from a developed country (Sweden) [7] and one from an underdeveloped country (Benin/Africa) [8], demonstrated a strong familial clustering of *H. pylori* infection. In both studies, an infected mother was a strong determinant for child infection. The presence of infected siblings was also an independent risk factor for the infection in children from Sweden.

One study in Ireland [9] and one in Taiwan [10] evaluating the children's *H. pylori* status by more accurate tests [culture, histology and urease testing on antral biopsies, ^{13}C-urea breath test (^{13}C-UBT) or stool antigen test (SAT)] also demonstrated that having an infected mother as well as infected siblings was predictor of infection.

Intrafamilial transmission from mother to her offspring was substantiated by the report of Konno et al. [11] from Japan. Fifty-one children from previously selected *H. pylori*-positive pregnant women were followed up during 5 years. Among them, four became positive by SAT in the first 2 years and one at 4 years and 4 months of age. Strains isolated from the gastric juice of these five children exhibited identical DNA fingerprint patterns to those taken from their corresponding mother.

Population-Based Studies from Developing and Developed Countries

In a sample of 2309 persons aged 5–100 years, representative of the general population in the Czech Republic, the age- and sex-standardized prevalence of current *H. pylori* infection was 41.7%, which is much lower than previously assumed upon limited data coming from selected groups in this geographic area (i.e., from 7.5 to 42% in children and from 45 to 83% in adults). Prevalence of *H. pylori* increased with age but was not related with gender. Mutually adjusted results indicated that low mother's education, sharing room with parents, and living in small town were the strongest risks for *H. pylori* positivity in children, whereas low education and heavy smoking were the risks in adults. The authors suggested that improved socio-economic conditions during recent years together with lower fertility rates has led to a decrease in the prevalence of *H. pylori* in the young cohorts in the Czech Republic [12]. Examination of *H. pylori* infection among several generations of Hispanics in the San Francisco Bay Area attempted to shed light on the respective contribution of household and environmental factors to *H. pylori* infection. Prevalence of *H. pylori* in immigrants and first- and second-generation US-born Hispanics was 31.4% (102/325), 9.1% (98/1076), and 3.1% (2/64), respectively. In the multivariate analysis, the trend of decreasing risk of *H. pylori* infection with increasing generations of Hispanics remained significant. Having an infected parent and low educational attainment in the household was independently associated with *H. pylori* infection. Authors suggested that household characteristics and birth-country environment may contribute to the risk of *H. pylori* infection among immigrants [13]. That the birthplace is a risk factor for the presence of *H. pylori* and that within industrialized countries the prevalence of *H. pylori* infection is higher in immigrants than among native-born residents was also confirmed in a point-prevalence survey conducted in a sample of 194 consecutive east Asian-born adults living in New York City. This study demonstrated that the seroprevalence of *H. pylori* in these immigrants was high (more than 70%) and inversely related with the years of residency in USA [14]. Suggesting that in poor population *H. pylori* infection is predominantly acquired in childhood, a study in a randomly selected population from a low-income community in north-eastern Brazil showed that the seroprevalence of the infection (165 of 204 adults, 80%) did not increase with age and that no risk factors were associated with infection. This lack of association may reflect that the population was homogeneous, with most of the enrolled subjects having a low educational level and low income [15]. In another population-based cross-sectional study that targeted the urban adult population in southern Brazil, the prevalence rate of *H. pylori* infection was 63.4% (231 out of 359 individuals) and socioeconomic conditions (low father's education, number of siblings, and attendance to day-care center in childhood), ethnicity (non-white skin colour), and dyspeptic symptoms were independently associated with the infection [16]. A recent published study from 610 adult Alaskan Eskimos showed that the seroprevalence of *H. pylori* was 80% in current samples, 75.3% in 15–20 years before archived samples, and that the seropositivity persisted in 67% of individuals. Data also showed that 72% of individuals had acquired *H. pylori* antibodies by the age of 24 years. Authors suggested that the low socioeconomic conditions among Alaskan Esquimos play an important role for high prevalence of *H. pylori* infection

[17]. In a population-based sample of 639 persons from Sisimiut community (5300 inhabitants), West Greenland, 280 persons were seropositive for *H. pylori* (43.8%). Seroprevalence for *H. pylori* increased with age but there was no overall significant difference in seropositivity among persons older or younger than 15 years. Multivariate analysis showed that male gender, number of children in household, number of older siblings, and narrow age gap to closest older sibling were associated with *H. pylori* seropositivity. There was no association between *H. pylori* seropositivity and socioeconomic status, possibly indicating that Sisimiut is a uniform community with less social diversity and relatively equal living conditions [18]. *H. pylori* seroprevalence in a New Zealand birth cohort at ages 21 and 26 was 4.2% (n = 795) and 6.3% (n = 871), respectively, with seroreversion rate lower than seroconversion rate (0.11 versus 0.53% per person-year) in contrast to the period from age 11–21 years when seroreversion rate exceeded seroconversion rate (0.35 versus 0.11% per person-year). The vast majority of study members (93.6%) remained stable with respect to serologic status from age 11–26 years [19].

Prevalence, Risk and Protective Factors, and Age of Acquisition of *H. pylori* Infection in Children

Seroepidemiologic studies evaluating the prevalence and risk factors associated with *H. pylori* infection in children demonstrated that prevalence rates varied among the countries. It was 8.9% in children aged 6 months to 10 years from Sana'a City, Yemen [20], 21.5% in school-aged children from a suburb near Taipei City, Taiwan [2], 55.5% in children 6–9 years old from Irbid, Jordan [21], and 72.8% in children 2–15 years of age from Benin/Africa [8]. By univariate analysis the infection was associated with the consumption of drinking water from re-used plastic jerry cans, poor mouth hygiene, and with coinfection by intestinal parasites in the study in Yemen [20]. By analyzing the data in logistic models, higher prevalence was associated with poor sanitation, overcrowding, low maternal educational level, low socioeconomic status, and rural areas in the Jordanian study [21] and with crowded sleeping accommodation and parental infection positive status in both rural and urban Beninese Communities [8]. The infection was also more prevalent in children from Taiwan whose parents did not know about *H. pylori* infection [2]. Although the sensitivity of serologic tests for *H. pylori* diagnosis in childhood especially in very young children has been considered low, the results of these studies regarding risk factors do not differ substantially from those that evaluated *H. pylori* status by more accurate tests such as SAT and ^{13}C-UBT. In a poor community in the north-eastern of Brazil, Rodrigues et al. [22] evaluated the prevalence of *H. pylori* infection in children living in crowded conditions without sewage systems by ^{13}C-UBT, and observed that the infection rate increased with age, being 47.5% in children 6 months to 10 years old and 73.3% in the group aged 11–20 years. By using a monoclonal SAT, Daugule et al. [23] evaluated the effect of previous antibiotic treatment for concomitant infections on the *H. pylori* infection rates in Sweden children aged 6 months to 5 years. A significantly lower *H. pylori* prevalence was observed in children who had been treated with antibiotics previously compared with those who had been never treated. Re-infection rate was investigated by ^{13}C-UBT in 52 children, from a high prevalence area in Italy, who had been successfully treated for *H. pylori* eradication. The re-infection rate was 12.8% per year and was associated with lower mean age at primary infection and the presence of an *H. pylori*-positive sibling. The rate is approximately four times higher than that of areas in which the prevalence of *H. pylori* is low, suggesting that the higher the prevalence of *H. pylori*, the greater the risk of children becoming re-infected [24]. In the study of Rowland et al. [9], who evaluated *H. pylori* status by ^{13}C-UBT, in addition of having infected mothers or infected siblings, having two or more bottles per day was also an independent risk factor for infection. According to the authors, a feeding bottle could potentially provide a vehicle for transmission of infection from mother to child or from sibling to sibling.

There are conflicting data regarding a protective effect of breastfeeding against *H. pylori* colonization in childhood. Some authors gathered data supporting an association between breastfeeding and prevention of the infection which were not confirmed by others. Furthermore, breastfeeding was associated with an increased risk of infection especially in children breastfed for more than 6 months. Conversely, Pearce et al. [25] demonstrated in the UK that the increased duration of exclusive breastfeeding in infancy was associated with a persistent *H. pylori*-negative status at the age of 50 years, which was restricted to men. Reinforcing a putative protective effect of breastfeeding, the presence of *H. pylori*-specific IgA in the colostrum, which theoretically can decrease *H. pylori* infection, was demonstrated in the majority of *H. pylori*-positive lactating mothers evaluated by Tanriverdi et al. [26]. In the study of Rowland et al. [9], however, there was no difference in the rates or duration of breastfeeding between infected and non-infected children.

The study of Rowland et al. [9] also examined prospectively the age-specific incidence of *H. pylori* in children > 24 months of age. They found that children in a developed country like Ireland become infected at a very young age, before the age of 5 years. According to the authors, because of the limitations of the ^{13}C-UBT in very young children, the incidence of infection could not be evaluated in those < 2 years old. They also found no evidence of spontaneous clearance of *H. pylori* infection during the period of the study. Conversely, two studies evaluating the acquisition and loss of the infection, one conducted in a US-Mexico cohort [27] and the other in Hispanics living in USA [28] by ^{13}C-UBT, corrected for age-dependent variation in CO_2 production, and SAT confirmed by a nested PCR detection of *H. pylori* DNA in stool samples, respectively, showed that the infection is acquired in early childhood, but does not persist in most of the children. However, differently from the study by Rowland et al. [9], the last authors studied children < 2 years old, which may explain the conflicting results. Nizami et al. [29], evaluating 148 infants aged 1–3 months, living in a periurban community from Karachi in Pakistan, observed that most 1-month-old infants were ^{13}C-UBT positive (80.0%). The authors also observed a lost of infection, but their results must be interpreted with caution because of the low specificity of ^{13}C-UBT without corrections in this age group.

Occupational Risk

Controversial data accumulate regarding an occupational risk for acquiring *H. pylori*. In a cross-sectional study, the seroprevalence of *H. pylori* in 74 nurses working in 10 gastroscopy units in the West of Scotland was compared to that in 148 nursing colleagues working in surgical specialties in the same hospitals. Gastroscopy exposure variables (i.e., job category; number of endoscopies per week; years of gastroscopy exposure; use of protective gloves, masks, and aprons; or reported hand-washing practice between cases) and socioeconomic factors, particularly those pertaining to childhood, were considered in a logistic regression model. Of the 222 participants, 32.4% of gastroscopy and 33% of comparators were seropositive for *H. pylori*. No association was found between gastroscopy exposure variables and *H. pylori* while significant association was found for age, childhood deprivation, and large number of siblings [30]. In a study involving 249 employees of an Italian hospital, 92 from gastrointestinal endoscopy units, 105 from other units with direct patient contacts, and 52 from laboratories and other units with no direct patient contact, the prevalence of current *H. pylori* infection was 37, 35.2, and 19.2% ($p < .01$), respectively, thus suggesting that hospital work involving direct patient contact is a risk factor for *H. pylori* infection. A not statistically significant difference between endoscopist and non-endoscopist physicians was observed. Nevertheless, multiple logistic regression analysis showed that, among the different health-care categories, only nurses had a significant higher prevalence of *H. pylori* infection. Unfortunately, no conventional socioeconomic parameters were considered in this study [31]. In the first report designed to assess the seroprevalence of *H. pylori* infection over a 6-year period in a cohort of 59 dental professionals (32 dentists and 27 dental nurses), four (6.78%) dental professionals (three dentists and one dental nurse) seroconverted compared to one (1.7%) among 59 age- and sex-matched randomly selected controls. The rate of acquiring *H. pylori* infection for dental professionals was 1.12% per year. The relative risk was 4.0 (95% CI 0.46–34.73) and the adjusted OR 2.68 (95% CI 0.55–19.67), with no statistical significance ($p = .36$ and $p = .256$, respectively) [32]. That white-collar workers had a lower risk of *H. pylori* infection has been reported in a study in 588 employees of the Subway Corporation in Seoul in which the proportion of white-collar workers was 44.6% in the *H. pylori*-positive group (n = 455) compared with that of 57.9% in the *H. pylori*-negative group (n = 133) ($p = .007$). This finding remained significant after adjustment for multiple variables (OR 0.62, 95% CI 0.41–0.92, $p = .018$) [33].

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Diagnosis of *Helicobacter pylori* Infection

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Keywords
Helicobacter pylori, diagnosis, urea breath test, stool antigen test, serology.

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Abstract

A growing interest in non-invasive tests for the detection of *Helicobacter pylori* has been observed recently, reflecting a large number of studies published this year. New tests have been validated, and the old ones have been used in different clinical situations or for different purposes. Stool antigen tests have been extensively evaluated in pre- and post-treatment settings both in adults and children, and the urea breath test has been studied as a predictor of bacterial load, severity of gastric inflammation, and response to eradication treatment. Several studies have also explored the usefulness of some serologic markers as indicators of the gastric mucosa status. With regard to invasive tests, molecular methods are being used more and more, but the breakthrough this year was the direct in vivo observation of *H. pylori* during endoscopy.

Although several diagnostic tests are available for the detection of *Helicobacter pylori* infection, all of them have both advantages and disadvantages, and none can be considered as a single gold standard. A combination of endoscopic biopsy-based methods (such as rapid urease testing, histologic examination, culture, and PCR) usually gives the most reliable diagnosis. However, these methods are invasive, expensive, and not always applicable. Therefore, there is an increasing interest in non-invasive tests for *H. pylori* detection. Several tests have been developed and introduced into clinical practice: urea breath tests, stool antigen tests, and serologic tests. In this article, we will first review the publications concerning non-invasive tests, then present an update on invasive tests.

Non-invasive tests

The potential indications for the use of non-invasive methods for *H. pylori* detection include: 1, screening of patients who do not require direct examination of gastric mucosa; 2, difficulties in obtaining biopsies (e.g., bleeding ulcers, anticoagulant therapy); 3, evaluation of eradication efficacy; and 4, epidemiologic studies [1].

Urea Breath Test

The urea breath test (UBT) is considered to be the most accurate non-invasive method to detect *H. pylori* infection. Although the original description of the ^{13}C-UBT was published nearly 20 years ago, and many modifications have been introduced since then, the test has not yet been definitely standardized. There are differences among protocols regarding methods of analyzing breath samples, types of test meal, doses of urea, time of breath sampling, and cut-off values. A Czech study conducted on healthy volunteers revealed that a citric acid solution as a test drink was superior to orange juice and water, and the optimal time intervals for breath sampling were 20 or 25 minutes after urea ingestion [2]. Another study demonstrated that, in contrast to ascorbic acid, citric acid and malic acid had the same enhancing effect on intragastric urease activity, so malic acid could replace citric acid as a test meal in the ^{13}C-UBT [3]. A study on the performance of the ^{14}C-urea breath test comparing citric acid, trisodium citrate, and drinking water as test meals showed that a small amount of citric acid (1.3 g) with only one breath sampling between 15 and 30 minutes after urea ingestion was sufficient to diagnose *H. pylori* infection accurately [4]. In the majority of settings, the carbon gas isotopic ratio is analyzed by mass spectrometry. However, improved infrared spectrometers show comparable performance and have the advantage of lower cost [5]. The cut-off point differentiating between positive and negative ^{13}C-UBT results is still a controversial issue. In a study analyzing $\delta^{13}CO_2$ values in *H. pylori*-eradicated patients up to 5 years after therapy, most of the positive and negative results were outside a 2 and 5‰ range [6]. The authors concluded that $\delta^{13}CO_2$ values < 2‰ precisely indicated *H. pylori* eradication, but borderline results could require verification either by repeated UBT or another diagnostic method. The post-treatment UBT is usually performed 4–6 weeks after completion of therapy. A study by Shirin et al. found that the use of a high dose (4 g) of citric acid as a test meal allowed the shortening of this period to 14 days after treatment (the rate of false-negative results was 3.2%, and the test sensitivity and specificity 80 and 100%, respectively) [7]. Some studies revealed that the urea blood test had a similar performance to UBT both for diagnosis and assessment of *H. pylori* eradication [8,9]. A gastroscopic real-time ^{13}C-UBT test was shown to be useful in patients undergoing gastroscopy with contraindications to biopsy [10]. The accuracy of UBT in patients after partial gastrectomy is controversial. A Japanese study revealed that under certain examination conditions (horizontal position on the left side, use of a film-coated ^{13}C-urea tablet, measurement after 30 minutes of urea ingestion, cut-off value 4.5‰), it was possible to obtain accurate UBT results in patients with a remnant stomach regardless of the method of anastomosis [11].

Some authors studied the δ^{13}C-UBT value as predictor of gastric lesions severity, bacterial load, and response to eradication therapy. Zagari et al. showed a correlation between UBT values, activity of gastritis, and *H. pylori* colonization density [12], whereas Tseng et al. did not find significant differences in UBT values between patients with gastritis, duodenal ulcer, gastric ulcer, and gastric cancer [13]. A weak correlation was observed between pretreatment UBT values and resistance to *H. pylori* treatment [14]. Several important issues associated with UBT have been systematically reviewed by Gisbert and Pajares [15].

Stool Antigen Tests

Several commercial stool antigen tests are currently available. A polyclonal antibody-based immunoenzyme assay (Premier Platinum HpSA, Meridian Inc., OH, USA), a monoclonal antibody-based immunoenzyme assay (Amplified IDEIA HpStAR, Dako, Glostrup, Denmark or Femtolab, Connex, Martinsried, Germany), and a rapid monoclonal immunochromatographic assay (ImmunoCard STAT! HpSA, Meridian Bioscience Europe, Milan, Italy) have been most thoroughly evaluated. The latter has the advantage of being fast (the results are available within 10 minutes), and easy to perform, so it can be carried out in the doctor's office. The sensitivity and specificity of stool antigen tests in various clinical settings are shown in Table 1. The studies comparing the three types of tests showed that the monoclonal EIA tests produced the most accurate results, both in pre- and post-treatment settings. However, the problem of stool antigen tests is their performance in populations with a low prevalence of *H. pylori* infection. One study [16] showed that all three tests had high positive predictive values (PPV) of 94.0–97.8% in untreated patients with a high rate of *H. pylori* infection (65.5%). The authors estimated that with an *H. pylori* prevalence of 12%, the PPV of HpSA, HpStAR, and ImmunoCard would be reduced to 76.6, 77.5, and 54.8%, respectively. Therefore, they suggested that in populations with a low *H. pylori* prevalence, it would be advisable to verify positive results of stool antigen tests with UBT or serology. Indeed, a combination of HpSA and UBT was shown to be useful for evaluation of eradication therapy [17].

Studies revealed that in some patient populations, in order to increase the sensitivity and the specificity of stool antigen tests, it may be necessary to adjust the cut-off values of EIA tests or to consider weakly positive results of the ImmunoCard test as negative for *H. pylori* diagnosis [18–20].

The stool antigen tests have been reported to give transiently positive results in children, frequently considered as false-positive findings [21]. In a study of 323 children who had stool samples taken twice at 3-month intervals, 26 were found to be transiently *H. pylori* positive by the HpSA test (first result positive and the second one negative) [22]. Using 16S rDNA primers and a nested PCR, *Helicobacter* sp. DNA was amplified from 15 of 26 (58%) stool samples originally positive in the antigen test. Taking into account the relatively low sensitivity of stool PCR (25–50%), the authors concluded that the vast majority of transiently positive stool HpSA tests represented transient infection with *Helicobacter* sp. (mainly with *H. pylori*, but also rarely with other *Helicobacter* species).

The performance of stool antigen tests in children has been shown to be associated with clinical manifestations and the patient's age. In symptomatic children with high *H. pylori* prevalence (> 60%), the sensitivity of polyclonal EIA tests ranged from 98 to 100%, and the specificity from 83.4 to 100% [23,24]. These values, however, were much lower in asymptomatic children [25]. In the latter group, with similarly high rates of *H. pylori* infection (52.6%), the sensitivity and specificity of the HpSA test were only 75.6 and 61%, respectively. The sensitivity and negative predictive values (NPV) of a rapid ImmunoCard test in children under 5 years of age were 75 and 93.3%, respectively, but they increased to 100% (both values) in patients older than 10 years [26].

A systematic review on the use of stool antigen tests and other non-invasive methods for *H. pylori* diagnosis in children was published by Koletzko [27].

The available studies indicate that stool antigen tests are usually inferior to the UBT, but they may represent an alternative option in patients with limited access to UBT who do not require endoscopy of the gastrointestinal tract.

Table 1 Evaluation of stool antigen tests in various clinical settings

Test	Patients	Clinical setting (No. of specimens)	Sensitivity (%)	Specificity (%)	Author (reference)
HpSA					
	Adults	Pretreatment (114)	79	92	Islam et al. [74]
		Post-treatment (18)	67	100	
	Adults	Pretreatment (282)	91.9	95.9	Veijola et al. [16]
		Post-treatment (182)	81.3	97	
	Adults	Pretreatment (82)	90.2	NA	Veijola et al. [75]
		Post-treatment (82)	75	95.5	
	Elderly	Untreated (56)	76	93	Inelmen et al. [76]
		On PPI (29)	82	83	
	Adults	Post-treatment (26)	25	91	Gisbert et al. [18]
	Adults	2-week post-treatment (48)	87	33	Erzin et al. [77]
		6-week post-treatment (48)	67	70	
	Adults	Post-treatment (70)	100	91	Aguemon et al. [78]
	Adults	Post-treatment (325)	73.4	97.8	Manes et al. [19]
	Children	Pretreatment (43)	94.4	100	Hauser et al. [20]
	Children	Pretreatment (80)	98	100	Gulcan et al. [23]
	Children	Pretreatment (86)	75.6	61	Shaikh et al. [25]
HpStAR					
	Adults	Pretreatment (282)	96.2	95.9	Veijola et al. [16]
		Post-treatment (182)	100	97.6	
	Adults	Pretreatment (82)	97.6	NA	Veijola et al. [75]
		Post-treatment (82)	93.8	98.5	
	Adults	Post-treatment (26)	100	46	Gisbert et al. [18]
		After cut-off adjustment	100	91	
	Adults	2-week post-treatment (48)	93	67	Erzin et al. [77]
		6-week post-treatment (48)	93	88	
	Adults	Post-treatment (325)	88.3	94.8	Manes et al. [19]
	Adults	Post-treatment (250)	100	97.4	Perri et al. [79]
ImmunoCard					
	Adults	Pretreatment (282)	93	88.7	Veijola et al. [16]
		Post-treatment (182)	93.8	97	
	Adults	Pretreatment (82)	96.3	NA	Veijola et al. [75]
		Post-treatment (82)	87.5	95.5	
	Adults	Pretreatment (187)	94.6	78.9	Wu et al. [80]
		Post-treatment (67)	100	92	
	Adults	Post-treatment (26)	75	90	Gisbert et al. [18]
	Children	Pretreatment (159)	88.1	88.1	Antos [81]
		Post-treatment (79)	88.9	93.9	
	Children	Pretreatment (43)	100	76	Hauser et al. [20]
		After adjustment[a]	100	96	
	Children	Pretreatment overall (128)	86.2	92.9	Kalach et al. [26]
		< 5 years of age (38)	75	93.9	
		5–10 years of age (47)	85.7	90.9	
		> 10 years of age (43)	100	94.4	

NA, not available; [a]weakly positive results treated as negative findings.

Antibody Tests

Numerous serologic tests are currently available for *H. pylori* testing, however, their application in a given population may require local adjustment of cut-off values [28]. Four rapid blood tests, i.e., BM-test (BM, Boehringer Mannheim, East Essex, UK), QuickVue (QV, Quidel, CA, USA), Pyloriset Screen (PS, Orion Diagnostica, Espoo, Finland), and Unigold (UG, Trinity, Biotech, NY, USA) were validated in Asian patients [29]. All tests showed a specificity above 95%, and a PPV exceeding 97%. However, their sensitivity varied from 43.3% (QV test) to 80.2%

(PS test), and the NPV ranged from 47.9% (QV test) to 73.1% (PS test). A study evaluating a rapid immunochromatographic (ICM) blood test (Assure *H. pylori* Rapid Test, Genlabs Diagnostics, Singapore) and immunoblotting (HelicoBlot 2.1, Genlabs Diagnostics) in Thai children found that both tests were reliable for diagnosis of *H. pylori* infection in this setting (sensitivity and specificity of ICM were 96.0 and 94.6%, respectively, versus 100 and 96.2% for immunoblotting, respectively) [30]. A study performed on *H. pylori*-eradicated patients showed that the current infection marker (CIM) in the HelicoBlot 2.1 test could be detected up to 4 years after successful treatment, so it was not a reliable marker of ongoing *H. pylori* infection [31]. The positive HelicoBlot 2.1 was more accurate than the CagA band (116 kDa) only in detecting past *H. pylori* infection [31]. Although there is usually a good correlation between CagA antibody detection by immunoblot and the detection of the *cag*A gene by PCR, in patients infected with strains with incomplete *cag*PAI, the serologic methods may fail to detect CagA antibodies [32]. Immunoblotting with total lysate of *H. pylori* proved to be superior to the commercial ELISA (Bio-Rad) in the diagnosis of past *H. pylori* infection in patients with atrophic gastritis [33].

A study of the urine ELISA test (URINELISA, Otsuka Pharmaceutical, Tokushima, Japan) for the detection of *H. pylori* IgG antibodies conducted in Taiwan showed a sensitivity and a specificity of 91.7 and 90.8%, respectively [34]. A rapid urinary immunochromatographic test (RAPIRUN, Otsuka Pharmaceutical) to detect *H. pylori* antibodies performed on patients with proteinuria had an accuracy of 95.0% as compared to serum ELISA [35]. The ELISA (Enzygnost Anti-*Helicobacter pylori* II/IgG, Dade Behring, Marburg, Germany) used for *H. pylori* IgG detection in saliva showed lower sensitivity and specificity than the serum test (87 versus 96%, and 73 versus 90%, respectively) [36].

Several studies investigated the usefulness of serologic markers as predictors of the gastric mucosa status. Serum anti-parietal cell antibodies were found to correlate with antral atrophy [37], and serum pepsinogen I/II ratio was inversely related to the grade of corpus atrophy [38]. One study showed that the combination of anti-*H. pylori* antibodies, serum pepsinogens I and II, and gastrin-17 could be used not only to identify patients with atrophic gastritis but also to localize the site of atrophy [39]. In other studies, anti-*H. pylori* antibodies combined with serum pepsinogens I and II [40] or the level of *H. pylori* IgG2 antibodies [41] appeared to be a predictive marker for the development of gastric carcinoma.

Invasive tests

A breakthrough this year was the publication of the in vivo observation of *H. pylori* during endoscopy. This new method, confocal endomicroscopy, allows a 1000 × magnification and is combined with topical acriflavine stain that stains the bacteria, and an IV fluorescein background, making it possible to observe the bacteria in their natural niche [42].

Culture remains a reference method that appears to be feasible in a clinical setting [43]. A new synthetic medium, replacing peptones and blood with hemoglobin, glutamine, and trace aminoacids in brucella medium, was proposed in Russia but no comparison with standard media was provided [44].

Because of the difficulty in obtaining biopsies, the string test has been further evaluated. When transport of the specimens is planned, gargling with a chlorhexidine mouthwash before swallowing the string test, in order to avoid oral contamination, improves the results [45]. In a study from Lima, Peru, *H. pylori* was cultured from 80% of the strings tested and detected by PCR from 91% in patients who were *H. pylori* positive by other invasive methods [46].

Gastric juice can also be used for biochemical measurements. Tucci et al. described a new device named Mt21–42 allowing an automatic measurement of pH and ammonium during endoscopy. They claimed that its sensitivity was similar to the UBT for *H. pylori* detection, and more sensitive than the currently available methods to detect atrophy [47].

As typical *H. pylori* gastritis may be less frequent, detection of the bacterium by molecular methods has become more valuable. Nested PCR has been successfully performed on biopsy samples exhibiting inflammatory changes [48] or on paraffin-embedded material [49]. In the latter study, PCR could detect *H. pylori* in 20% of histology negative biopsies, which increased positivity from 43 to 58% of the total samples. When real-time PCR is used, an assessment of *H. pylori* susceptibility to clarithromycin can be made. The TaqMan technology was used on 232 paraffin-embedded biopsies in Italy and detected 26.7% resistance, the A2143G mutation being the most frequent [50]. In another Italian study, PCR-based denaturing HPLC was performed on 101 biopsies and detected heteroduplex peaks in 25 of them. Culture confirmed the presence of clarithromycin resistance [51]. Resistance to tetracycline because of 16S rDNA mutations may also hamper the treatment success. A real-time PCR detection has been developed by Lawson et al. Eighteen strains with a decreased tetracycline susceptibility were studied, and 10 exhibited mutations in the 926–928 nucleotide triplet, but none of the 100 tetracycline-susceptible strains had similar mutations. The method was used on gastric biopsies successfully [52].

Another application is to study the *cag*A type. It was possible to distinguish between Western-type *cag*A and

East Asian-type *cag*A directly on gastric biopsies [53] as well as on paraffin-embedded material [54].

A comparison of *H. pylori* isolates cultured from the same patient was also carried out. Two isolates cultured 9 years apart were compared for 50 different parameters. The two isolates were found to be derived from the same strain [55].

The genome content of 21 strain pairs issued each from a different patient was also compared with DNA microarrays and multilocus sequence analysis and mathematical modeling. The great majority of genetic changes were because of homologous recombination [56].

A global study of the bacterial flora present in the human stomach was performed for the first time by molecular analysis. A small subunit 16S rDNA clone library approach was used, generating 1833 sequences from 23 cases. Surprisingly, a higher diversity than that previously described was found, including 10% of uncharacterized phylotypes out of 128. The presence of *H. pylori* did not affect the composition of the gastric flora, which was significantly different from mouth and esophagus flora [57]. Along the same lines, total bacterial counts and real-time PCR with 52 gene- and species-specific primer sets were performed on gastric mucosa and juice samples from 10 children and 10 adults in Japan. Bacteria other than *H. pylori* were cultured from all of the adults and only one child. The results were correlated to atrophy present in adults but not in children who had a lower pH [58].

With regard to the urease test, Pronto Dry (MIC, Brignais, France), a solid-phase rapid urease test, was evaluated in several studies. It had a better accuracy than both the CLOtest and a liquid-phase urease test. It also had a significantly faster reaction time. However, its performance like other tests was jeopardized by proton pump inhibitor use [59–61]. In another study, biopsies used for rapid urease tests were also employed for DNA extraction to determine CYP2C19 and *H. pylori* 23S rDNA polymorphism [62].

Histology is still preferred by many gastroenterologists for *H. pylori* diagnosis as it also allows the determination of the gastric mucosal status. The use of special stains in routine practice has been questioned by Wright and Kelly who favour their utilization only in special cases [63]. The main progress in this area is the use of fluorescence in situ hybridization, which allows the detection of both *H. pylori* and of its clarithromycin resistance directly on paraffin-embedded gastric biopsy sections with a good accuracy [64,65].

Nongastric Specimens

Molecular methods have also been used to look for *H. pylori* in liver diseases. Although no *Helicobacter* was grown from bile samples, *H. pylori* DNA (99% similar) was detected in bile from 96.7% of patients with hepatobiliary diseases versus 6.6% of controls in India [66]. In Southern Italy, bile and cholecystic tissue homogenate were positive by PCR in 51% of cases correlating with *H. pylori* stool antigen [67]. Gallstones were explored in Sweden using a multiple-displacement amplification (MDA) assay. Pyrosequencing analysis of 16S rDNA derived from MDA revealed 20 *H. pylori*-positive samples out of 33 [68].

Finally, *Helicobacter* was cultured from three liver samples with hepatocellular carcinoma in China (3/28) and observed by scanning electron microscopy in two, whereas 60% were positive by PCR [69].

Molecular tests also showed positive results in water samples from the environment [70–72]. However, the use of only one target gene casts doubts on the value of the results. Parallel staining of the filters with a species-specific fluorescent antibody in one study [72] was an interesting approach, as was a combination of direct viable counts and fluorescent in situ hybridization [73].

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Pathogenesis of *Helicobacter pylori* Infection

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Keywords
cag pathogenicity island, cyclooxygenase-2, nuclear factor-κB, proinflammatory cytokines, nonsteroidal antiflammatory drugs, ghrelin.

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Abstract

Much interest has been shown in the relationship between *Helicobacter pylori* infection and gastric carcinogenesis. It is becoming clearer that *H. pylori* strains carrying a functional *cag* pathogenicity island (*cag*PAI), which encodes the type IV secretion system (TFSS) and its effector CagA, play an important role in the development of gastric carcinoma. Furthermore, genetic polymorphism present in the *cag*A gene appears to influence the degree of an individual *cag*PAI-positive *H. pylori* to elicit gastric mucosal lesions, and this process is significantly affected by host genetic polymorphisms such as proinflammatory cytokine gene polymorphisms. Pathomechanism of gastric carcinogenesis associated with *H. pylori* includes bacteria–host interaction leading to morphologic alterations such as atrophic gastritis and gastrointestinal metaplasia mediated by COX-2 overexpression, cancer cell invasion, and neo-angiogenesis via TLR2/TLR9 system and transcription factors (e.g., NF-κB) activation. In addition, *H. pylori* infection triggers adhesion molecule expression and activity and produces an enhancement in oxidative stress interacting with gastric production of appetite hormone ghrelin and nonsteroidal anti-inflammatory drugs.

Helicobacter pylori–Host Cell Interaction

The lipopolysaccharide O-antigen is involved in the adherence of *Helicobacter pylori* to gastric epithelial cells. Fowler et al. showed that the O-antigen is specifically recognized by a β-galactoside-binding lectin, galectin-3 [1]. Infection with *H. pylori* increased the level of galectin-3 expression, which was mediated by the CagA-activated MAP kinase pathway. Backert et al. and Shibata et al. investigated changes in gene expression upon infection of gastric epithelial cells with *H. pylori* [2,3]. In accordance with previous reports, the majority of transcriptional changes induced by *H. pylori* infection were dependent on the *cag* pathogenicity island (*cag*PAI)-encoded type IV secretion system (TFSS), reinforcing the notion of a crucial role of *cag*PAI in *H. pylori*-mediated gastric pathogenesis. Backert et al. also performed proteome analysis and confirmed the impact of *cag*PAI on host cells at the protein level [2]. However, *H. pylori* targets identified by transcriptome and proteome approaches did not significantly correlate with one another. The importance of proteome analysis may be underscored by the results of a study showing that infection with *H. pylori* increases the level of c-Myc but decreases the level of p27 cyclin-dependent kinase inhibitor (CKI) expression in association with increased expression of the p27 ubiquitin ligase, Skp2. The fact that these changes were independent of transcription suggests that *H. pylori* can modify gastric epithelial cell growth through post-translational mechanisms [4]. A potentially interesting molecule upregulated by *H. pylori* infection is the prion protein [5]. This finding raises the possibility that *H. pylori* infection enhances propagation of pathogenic prion proteins in the gastric mucosa.

CagA and *cag*PAI Type IV Secretion System

*cag*PAI-encoded TFSS injects CagA and peptidoglycans into host epithelial cells. Injected CagA interacts with a number of host cell molecules in both tyrosine phosphorylation-dependent and -independent manners, whereas peptidoglycans are recognized by the bacterial sensor Nod1, which in turn activates nuclear factor (NF)-κB to elicit inflammatory responses such as interleukin (IL)-8 production. Perturbation of cell signaling by *cag*PAI-encoded molecules has been considered to cause cellular dysfunctions that lead to cell transformation. Zhu et al. provided in vitro evidence for the role of CagA as a potential oncoprotein. They showed that ectopic expression of CagA in immortalized gastric epithelial cells induces a transformed phenotype as demonstrated by foci formation in soft agar [6]. The most characterized cellular target of CagA is the SHP-2 phosphatase. Gain-of-function mutations in the *PTPN11* gene, which encodes SHP-2, have recently been found in a number of human malignancies, indicating a critical role of CagA-mediated SHP-2 deregulation in gastric carcinogenesis. Tsutsumi et al. reported that the focal adhesion kinase (FAK) is a substrate and downstream effector of CagA-activated SHP-2. Dephosphorylation of FAK by SHP-2 inhibited the FAK kinase activity. Since active FAK is required for the turnover of focal adhesion spots, cells expressing CagA showed a reduced focal adhesion, which was associated with elongated cell shape as well as elevated cell motility [7]. Goto et al. investigated the association of a single nucleotide polymorphism (SNP) in intron 3 (SNP; JST057927; G to A) in the SHP-2-encoding *PTPN11* gene with gastric atrophy and gastric cancer and found that lower risk of gastric atrophy was associated with the A allele of the *PTPN11* genotype. Their results indicated that G/A SNP in the *PTPN11* gene is a risk factor for gastric atrophy and gastric carcinoma [8]. In addition to SHP-2 and Csk, Suzuki et al. reported that CagA binds another SH2 domain-containing protein, Crk, in a tyrosine phosphorylation-dependent manner [9]. CagA also exhibits pathophysiologic activities that are independent of CagA tyrosine phosphorylation. Of particular interest is the ability of CagA to associate with and impair the function of the tight junctions in polarized epithelial cells. Bagnoli et al. demonstrated that cells expressing CagA lose apico-basal polarity and cell–cell adhesion, which was concomitantly associated with pseudopodia formation and degradation of the basement membrane [10]. Hence, CagA elicits cellular changes that highly resemble the epithelial-to-mesenchymal transition (EMT).

NF-κB transcription factor plays a crucial role in inflammation. *H. pylori* has been reported to activate NF-κB and thereby induce IL-8 and other proinflammatory cytokines by the translocation of peptidoglycans, but not CagA, via the *H. pylori* TFSS. However, Brandt et al. provided evidence that CagA per se is also capable of activating NF-κB via the Ras-MAP kinase pathway in a manner independent of CagA tyrosine phosphorylation [11]. Kim et al. also showed that transfected CagA in AGS gastric epithelial cells gives rise to the induction of IL-8, but not RANTES or IL-1β [12]. Hirata et al. reported that *H. pylori* stimulates IkB kinases (IKK) in gastric epithelial cells, which provides an additional pathway by which *H. pylori* activates NF-κB [13]. Collectively, these results indicate that *H. pylori* potentiates NF-κB transcriptional activity via multiple distinct mechanisms. As another phosphorylation-independent activity, Yokoyama et al. reported that CagA activates calcineurin, a Ca^{2+}-dependent serine/threonine phosphatase, which dephosphorylates cytoplasmic NFAT (nuclear factor of activated T-cells) and thereby causes nuclear translocation of NFAT [14]. Nuclear localized NFAT then transactivates NFAT-dependent genes. Intriguingly, *H. pylori* VacA cytotoxin counteracts the CagA activity to activate NFAT. Hence, NFAT is a common target for both CagA and VacA. Given a pleiotropic role of NFAT in cell growth and differentiation, deregulation of NFAT by these *H. pylori* virulence factors may contribute to the formation of diverged mucosal lesions caused by *cagA*-positive *H. pylori* infection.

Although the mechanisms as well as the involvement of CagA remain to be elucidated, *H. pylori cag*PAI appears to be responsible for several cellular responses. For instance, infection of gastric epithelial cells with *cag*PAI-positive *H. pylori* induces early growth response gene 1 (*Egr-1*) through activation of EGF receptor signaling [15]. Also, *H. pylori* infection strongly upregulates matrix metalloprotease-1 (MMP-1) expression in a manner that is dependent on *cag*PAI, but not CagA [16]. The MMP-1 induction involves activation of JNK and ERK MAP kinase pathways. Since MMPs degradate the extracellular matrix, elevated levels of MMPs in gastric mucosa infected with *cagA*-positive *H. pylori* may facilitate invasion and metastasis of transformed cells.

Rodent models have provided additional insights into the pathophysiologic role of the *H. pylori cag*PAI. Franco et al. demonstrated that *cagA*-positive *H. pylori* strains acquire the ability to induce gastric dysplasia and gastric adenocarcinoma in Mongolian gerbils upon in vivo adaptation [17]. The in vivo adapted, oncogenic *H. pylori* strains were capable of selectively activating β-catenin signal, which is dependent on CagA. Consistent with this observation, nuclear accumulation of β-catenin was increased in gastric mucosa from individuals infected with *cagA*-positive *H. pylori* compared with that in gastric mucosa from individuals infected with a *cag*A-negative strain. Although the mechanism by which CagA induces nuclear accumulation of the β-catenin remains to be elucidated, deregulation of β-catenin signal by *cagA*-positive *H. pylori* may play an important role in gastric carcinogenesis, as is the case with colorectal carcinogenesis. A role of *cag*PAI in selective colonization of *H. pylori* has also been reported. Corpus-dominant atrophic gastritis is a risk factor for gastric cancer. Reider et al. compared gastric lesions induced by chronic infection with intact *cag*PAI-positive *H. pylori*, a TFSS-defective isogenic mutant, and a *cag*A-disrupted isogenic mutant in Mongolian gerbils [18]. All infected gerbils developed strong antral inflammation with increased epithelial cell proliferation as well as elevated expression of proinflammatory cytokines such as IL-1β. In contrast, high-level colonization of the gastric corpus required the presence of an intact *cag*PAI in *H. pylori*. Thus, corpus colonization of *H. pylori*, which depends on the functional *cag*PAI, may be responsible for the development of corpus-dominant atrophic gastritis with lower gastric acidity, a precancerous condition from which gastric carcinoma develops.

The mechanism of CagA localization in the bacterium and its subsequent recognition by TFSS have also been investigated. Intrabacterial CagA translocates from the center to the peripheral portion of the cytoplasm in response to an extracellular decrease in pH [19]. The translocation depends on the presence of *Ure*I, a proton-dependent urea channel. The results indicate that *H. pylori* has a proton-dependent intracellular transport system that accelerates the interaction of CagA to the membrane-associated TFSS. At the inner bacterial membrane, CagA associates with CagF, another *cag*PAI-encoded molecule essential for the delivery of CagA into host gastric epithelial cells [20]. Translocation of CagA via the TFSS is dependent on the presence of the C-terminal 20-amino-acid stretch as well as the N-terminal region of CagA [21]. Upon injection into host gastric epithelial cells, CagA localizes to the inner surface of the plasma membrane, where it undergoes tyrosine phosphorylation by Src family kinases at EPIYA motifs. Higashi et al. revealed that membrane association of CagA in the host cell also requires the EPIYA motif but is independent of EPIYA tyrosine phosphorylation [22]. The presence of a single EPIYA motif is necessary and sufficient for the membrane localization of CagA. Accordingly, the EPIYA motif has a dual role, membrane association and tyrosine phosphorylation, both of which are crucial for CagA to deregulate host cell signaling. To investigate the role of cell-type specificity in CagA tyrosine phosphorylation, Bauer et al. infected 19 different mammalian cell lines with *cagA*-positive *H. pylori* [23]. The highest level of CagA tyrosine phosphorylation was observed in human gastric cell lines, with most nongastric human cells showing decreased CagA phosphorylation. CagA phosphorylation was hardly detectable in non-human cells. Infection with *H. pylori* that carries the functional TFSS stimulated IL-8 secretion in all human cell lines examined, whereas the AGS human gastric epithelial cell line was the only cell line that induced cell elongation known as the 'hummingbird' phenotype. The results indicate that host cell factors play an important role in the quality and quantity of cellular responses during *H. pylori* infection.

VacA Cytotoxin

VacA has been shown to assemble into an anion-selective channel upon integration into host cell membrane. Torres et al. reported that the p33 and p55 domains of VacA can interact with each other and that the intramolecular interaction contributes to the binding, internalization, and vacuolating cytotoxic activity of VacA [24]. Hennig et al. evidenced that VacA interacts with the arginine–glycine–aspartic acid (RGD)-containing region of fibronectin [25]. VacA–fibronectin interaction may therefore perturb integrin-mediated cellular functions. Gauthier et al. investigated the endocytic and intracellular trafficking pathways that were used by VacA in human epithelial cells [26]. VacA first binds to the plasma membrane domains localized above the F-actin structure that is regulated by Rac1 GTPase. VacA is then pinocytosed into early endocytic compartments, a process controlled by Cdc42 in a manner independent of clathrin, and subsequently sorted to late endosomes where it induces vacuolation. Yamasaki et al. showed that VacA induces cytochrome *c* release from mitochondria through activating pro-apoptotic proteins Bax and Bak and that VacA-induced apoptosis is independent of vacuolation of cells [27].

In addition to acting as a membrane channel, VacA has been shown to bind to receptor-like protein tyrosine phosphatases (RPTPs), RPTPα and RPTPβ. The interaction has been suspected to contribute to the induction of peptic ulcers. De Guzman et al. showed that both m1 and m2 forms of the VacA protein can bind RPTPα or RPTPβ [28]. The extracellular domains of the RPTP molecules undergo differential post-translational modification (glycosylation) in different cells, and this modification influences their VacA-binding activities. Thus, cellular responses to m1 or m2 VacA may be regulated at least in part by the post-translational modification of RPTPs. Through sequence analysis of *vacA*, *cagA*, and *cagE* genes in *H. pylori*, Yamazaki et al. found that Western *cagA* was genotypically associated with *s2* and *s1a/m1a vacA*, whereas East Asian *cagA* was associated with *s1c/m1b vacA* [29]. *H. pylori* strains possessing Western *cagA* and s2 or *s1a/m1a vacA* were found to be more closely associated with peptic ulcers.

COX-2 Expression as a Marker for *H. pylori*-Induced Carcinogenesis

Chronic gastritis induced by *H. pylori* is the strongest known risk factor for adenocarcinoma of the distal stomach and may also be a major cause of other malignancies that arise within the gastrointestinal tract [30,31]. Gastric intestinal metaplasia (IM) that is associated with *H. pylori* has been considered a premalignant condition in humans and Mongolian gerbils [32,33]. For instance, Kato et al. [34] studied whether *H. pylori* infection and inflammation leads to gastric atrophy in Japanese patients of a young age. They concluded that *H. pylori*-induced inflammation and gastritis could increase the risk of antral atrophy in children [34]. Using a colon epithelium-specific monoclonal antibody and anti-COX-2 antibody, Sun et al. [35] found that in about 60% of *H. pylori*-infected patients there was a development of colonic phenotype of gastric IM and that COX-2 expression was frequent in both cancer and gastric IM adjacent to the cancer. This finding suggests that COX-2 reactivity could help to identify the subgroup of patients at risk for gastric cancer with colonic phenotype of gastric IM. Moreover, Chan et al. [36] reported that *H. pylori*-induced COX-2 expression enhances cancer cell invasion and angiogenesis via TLR2/TLR9 to activate MAPKs, especially p38, and their downstream transcription factors (CREB-1, ATF-2, c-jun, c-fos) resulting in the activation of CRE and AP-1 on the COX-2 promotor. The effect of the cancer-cell invasion and angiogenesis mediated by TLR2/TLR9 were both inhibited by the specific COX-2 inhibitors, NS398 and celecoxib [36]. Expression of COX-2 and proinflammatory cytokines could trigger the transcriptional regulator NF-κB. Using *H. pylori*-infected mice, Kim et al. [37] showed that pretreatment of these mice with NF-κB (p65) antisense oligonucleotides inhibited the activation of NF-κB and the expression of tumor necrosis factor (TNF)-α and IL-1β in *H. pylori*-infected gastric mucosa. Various models of gastric carcinogenesis in nonhuman primates (Japanese monkeys) and rodent models without or with chemical carcinogens (e.g. nitrosourea or nitrosoguanidine) are useful in the assessment of the link between bacteria and carcinogenesis [33]. These changes in *H. pylori*-inoculated animals closely resemble those observed in *H. pylori*-infected humans and the development of gastric cancer in *H. pylori*-infected Mongolian gerbils interrupted by an eradication therapy [38]. Interestingly, probiotics containing *Lactobacillus rhamnosus* and *Lactobacillus casei*, attenuated hyperplasia of glandular structure and over-expression of COX-2 as well as functional changes such as enhancement of the apoptosis, impairment in gastric blood flow, and gastrin–somatostatin link induced by *H. pylori* in the Mongolian gerbil model [38]. These data indicate that probiotic treatment in addition to anti-*H. pylori* eradication therapy can effectively inhibit structural and functional consequences of *H. pylori* infection in the stomach.

Pathomechanism of *H. pylori* Infection

H. pylori pathology in the gastric mucosa includes a cascade of events [39]. Colonization of the gastric mucosa with the bacterium invariably results in the development of chronic gastritis, and for a subset of patients, progression of chronic gastritis to ulcers or cancer is observed [39]. *H. pylori* plays an important role in carcinogenesis by initiation, promotion, and progression of cancer cells in a multistep manner. A randomized controlled trial by Ito et al. [40] documented that anti-*H. pylori* eradication therapy attenuates the endoscopic and histologic lesions, suggesting its beneficial role in the prevention of gastric cancer. The mechanism of the pathologic changes induced by *H. pylori* involves an activation of NF-κB, proinflammatory cytokines, and an overexpression of growth factors such as vascular endothelial growth factor (VEGF) [41]. Neumann et al. [41] showed a direct interaction between p21-activated kinase 1(PAK1) and NF-κB-inducing kinase (NIK) in *H. pylori*-infected epithelial cells, identifying for the first time the role of PAK1 and NIK as a unique pathway involved in *H. pylori*-induced NF-κB activation. The pathogenic role of proinflammatory cytokines, especially TNF-α, was determined by Suganuma et al. [42], who proposed TNF-α as an endogenous tumor promotor. They identified the TNF-α-inducing protein (Tipalpha) gene family and confirmed that Tipalpha and HP-MP1 are new carcinogenic proteins of *H. pylori*. The Tipalpha gene family and protein seem to be unique for *H. pylori* and exhibit strong tumor-promoting activity in cooperation with the Ras protein and NF-κB activation [42]. Tuccillo et al. [43] found that VEGF is overexpressed in gastric mucosa of *H. pylori*-infected dyspeptic patients and that this effect paralleled the increase in a number of blood vessels suggesting the involvement of VEGF expression in the process of neo-angiogenesis. Moreover, O'Brien et al. [44] revealed that decay-accelerating factor (DAF), a protein that protects epithelial cells from complement, is an important factor relevant to pathogenesis because this protein is able to mediate the bacteria–host cell interaction in gastric inflammation. Maciorkowska et al. [45] evaluated the role of adhesion molecules ICAM-1, VACAM-1, and P-selectin levels in *H. pylori*-infected children showing that in these children with gastritis, the VCAM-1 reached the highest levels, whereas ICAM and P-selectin did not differ in the *H. pylori*-positive and *H. pylori*-negative groups. According to Chan [46], a family of adhesion glycoproteins such as cadherins, act not only as adhesive molecules but may be also responsible for the growth development and carcinogenesis. Taken together, adhesion molecules VCAM-1 and cadherins participate in the pathogenesis of gastric mucosal inflammation induced by *H. pylori*. Since nickel acquisition is necessary for urease activity, Wolfram et al. [47] examined the expression of nickel permease NixA, which was identified as a specific nickel uptake system in this organism. They found that the synthesis of the NixA nickel permease of *H. pylori* is an nickel-responsive regulation mediated by regulatory protein NikR to maintain the balance between effective nickel acquisition and a toxic overload [47]. However studying gastric carcinoma patients, Tsavaris et al. [48] showed that both retinol-binding protein (RBP) and acute phase reactants are increased in gastric cancer but only RBP is increased in *H. pylori*-infected individuals, suggesting a direct involvement of RBP in the pathogenesis of this disease.

Interaction between *H. pylori* and Physiologic and Pathophysiologic Factors in the Stomach

Two hormones, ghrelin and leptin, both originally discovered in the gastric mucosa, play an important role in the regulation of appetite but little is known about the influence of *H. pylori* on plasma levels of these hormones in *H. pylori*-infected subjects. Shiotani et al. [49] reported that plasma ghrelin but not plasma leptin is significantly lower in *H. pylori*-infected subjects than in respective controls. Moreover, this attenuation in plasma ghrelin levels by *H. pylori* was independent of gender and body mass index [49]. This indicates that some adverse appetite symptoms such as nausea and lack of appetite and possibly growth inhibition in *H. pylori*-infected children could be attributed to the suppression of ghrelin by this bacterium [50]. Mountain children living close to sheep, exhibit almost twice as high an *H. pylori* prevalence than children living in the neighboring city without contact with sheep. Early eradication of *H. pylori* in mountain children markedly improved their appetite and resulted in an increase in body mass index as well as an acceleration after delayed body growth, thus supporting the clinical relevance of endogenous ghrelin in maintaining gastric integrity, good appetite and proper body growth [50]. Fedwick et al. [51] revealed that *H. pylori* strain SS1 directly increased the paracellular permeability by activating myosin light-chain kinase to disrupt the tight-junctional proteins, occludin, claudin-4, and claudin-5, in confluent nontransformed cells and in cancer AGS cells. In another study, Farkas et al. [52] reported that SOD activity is elevated in gastric biopsies taken from the antrum of *H. pylori*-infected patients and that this effect is inhibited by successful anti-*H. pylori* eradication confirming that an enhancement in mucosal oxidative stress could be considered as an important mechanism in the pathology of *H. pylori* infection in the stomach.

H. pylori and aspirin are two independent factors in the development of peptic ulcer in humans but the possibility that they can influence the expression of the gastroprotective secretory leukocyte protease inhibitor (SLPI) has not been explored. Wex et al. [53] found that, in contrast to low dose aspirin, *H. pylori* downregulates the expression of antral SLPI, implicating that the fall in synthesis of this gastroprotective factor may predispose the gastric mucosa to inflammation. Schaeverbeke et al. [54] showed that the prevalence of gastrointestinal symptoms in rheumatoid patients infected with *H. pylori* and treated with a non-steroidal anti-inflammatory drug (NSAID) for 2 weeks exhibited similar gastrointestinal tolerance to NSAID irrespective of *H. pylori* infection; this suggests a lack of interaction between these two independent risk factors in the gastric mucosa. On the other hand, *H. pylori* increased the risk of duodenal ulcers in elderly individuals subjected to the treatment with vascular protective doses of aspirin [55]. Accumulated evidence indicates that the data on the interaction between the NSAID and *H. pylori* are still conflicting and that this issue needs to be addressed in further studies.

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Inflammation, Immunity, Vaccines for *Helicobacter* Infection

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Keywords
Helicobacter pylori, host response, inflammation, vaccines.

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Abstract

The reason why some individuals remain *Helicobacter pylori* infected for life but without any symptoms while others develop severe diseases is only partially clarified. Presumably, it depends on multifactorial interactions among host immunologic and physiologic factors, bacterial virulence determinants, and environmental influences modulating the host response. Much effort has been made to identify host genetic factors that may explain an individual susceptibility of the host to *H. pylori* infection. The identification of *H. pylori* determinants and the elucidation of their role in modifying the host immune responses were further delineated. The ability of *H. pylori* to overcome the defense mechanisms on mucosal surfaces as well as to modulate the immune response by interfering with host recognition and transduction systems has been shown. Also new bacterial anti-inflammatory defense systems have been described. Findings in experimental animal models and humans with natural *H. pylori* infection suggested a double role of regulatory T cells in the course of *H. pylori* infection: protecting the infected host against excessive gastric inflammation and, in contrast, promoting bacterial colonization.

In the last year, numerous publications were centered on the immunopathogenesis, inflammatory, and immune responses to *Helicobacter pylori* as well as on vaccine development against this pathogen. Several excellent reviews are available to provide in-depth analysis of these topics [1–3]. This review concentrates on the findings strictly associated with the host response. In this issue, another chapter is dedicated to bacterial pathogenesis, where some interactions between virulence factors and immune response are discussed.

Establishing the Infectious Niche

Bacterial motility and chemotaxis are important in establishing the infection. *Helicobacter pylori* mutants lacking *cheW*, *cheA*, or *cheY* genes encoding proteins responsible for chemotaxis were able to colonize only the corpus of the stomach of mice, while the wild-type bacteria occupied both the corpus and the antrum [4]. *CheY* is also crucial for gastric colonization in gerbils [5]. The polyphosphate kinase (PPK) has been found a new colonization factor of *H. pylori* [6]. The interactions between the adhesin BabA and the H-1-, Lewis b, and related fucose-containing antigens have been confirmed by overlay with fluorescence-labeled bacteria on immobilized (neo) glycoproteins [7]. However, the synthesis of BabA can be switched on or off, and thus provide the mechanism for adaptation to environmental conditions [6]. Several findings confirm the role of host factors mediating the binding of *H. pylori* to gastric epithelial cells. The correlation between the susceptibility to *H. pylori* infection and blood group O has been confirmed [8]. The topographic over- and underexpression of MUC5AC and MUC6 in *H. pylori*-associated gastritis disease suggests a role for such mucins in the pathogenesis of *H. pylori* infection [9].

The initial trigger for inflammatory response is mediated by epithelial cells. *H. pylori* does not invade gastric epithelial cells but adheres to the cell surface. This pathogen exhibits an unusually complex pattern of binding to various host glycoconjugates including interactions with sialylated, sulfated, and fucosylated sequences. Recently, it has been shown that repeating N-acetyllactosamine units of glycoconjugates may serve as bacterial attachment sites in the stomach [10]. Upon binding, *H. pylori* induces NF-κB activation, as recently confirmed in vivo by antisense oligonucleotide inhibition [11]. This pathway involves MyD88 and TNF receptor-associated factor 6 (TRAF6) [12]. In turn, expression of interleukin (IL)-8 by epithelial cells leads to overexpression of CD74, the major histocompatibility complex (MHC) class II invariant chain, a known receptor of *H. pylori*, thus favoring further colonization [13]. IL-8 may also upregulate the Reg protein, a potent regenerating gene product and a potent growth factor of gastric mucosal cells, associated with gastric inflammation [14]. In addition to previously described pro-inflammatory effects, NF-κB direct binding to the promoter of the syndecan-4 gene may contribute to the pro-inflammatory signalling induced by gastric epithelial cells [15]. CagA-positive strains are known for their ability to elicit IL-8 production, but strain-specific variants of CagA have been elegantly shown to have different IL-8 induction potential. Thus, CagA is a multifunctional virulence factor of *H. pylori* [16].

Overcoming the Immune Defense Mechanisms

The production and secretion of antimicrobial peptides (AMPs) from surface epithelia and circulating immune cells play a key role in host antimicrobial protection, including epithelial defensins [17]. There is evidence that *H. pylori* may modulate expression of genes encoding defensins, in a *cag*-dependent manner, through Nod1 activation [18].

Innate immune responses depend on host recognition of pathogen-associated molecular patterns (PAMPs) including lipopolysaccharide (LPS), lipoproteins, peptidoglycan, flagellin, double-stranded RNA, and zymosan by PAMPs receptors expressed on macrophages and dendritic cells (DCs) [19]. The recognition of PAMPs triggers a signaling pathway inducing pro-inflammatory cytokines, chemokines, type I interferons (IFN-α or -β), and DCs maturation that leads to activation of adaptive immunity. The best characterized PAMPs receptors are the Toll-like receptors (TLRs) and the Nod receptors displaying a high degree of PAMPs specificity [19,20]. The studies on immune response to *H. pylori* LPS in epithelial cells which were focused on TLR4 showed that TLR4 signalling does not mediate responses to this pathogen in gastric epithelial cells [21]. However, secreted *H. pylori* peptidyl- prolyl- *cis*-, and *trans*-isomerase HPO175 induced apoptosis of AGS cells in a TLR4-dependent pathway. This indicates that TLR4-signaling cell apoptosis contributes to the pathology of gastric epithelial cell damage during *H. pylori* infection [22]. Similar to TLR4, also a dominant role for TLR2, recognizing lipoproteins, and for TLR5, recognizing flagellin has been excluded in epithelial cell sensing of *H. pylori* bacteria [21]. *H. pylori* is also recognized by epithelial cells via Nod1 and detection depends on the delivery of peptidoglycan to the host cells by type IV secretion system.

The host factors such as Nod proteins, Toll-interacting protein (Tollip), and single immunoglobulin IL-1R related molecule (SIGIRR), which antagonize pattern recognition receptors (PRRs), may represent important mechanisms of immune suppression required to regulate the response to pathogens and to nonpathogenic flora [20]. On the other hand, pathogens may modulate the host immune response by interfering with host recognition and transduction systems. Both mechanisms have been considered for *H. pylori* since these bacteria appear to induce only a limited inflammatory reaction but also limit the efficacy of the adaptative immune response. It has been shown that lipid A of *H. pylori* has moieties that are poorly recognized by human TLR4 [21]. Moreover, *H. pylori* LPS with or without LewisXY determinants differ in the ability to induce inflammatory cytokines by peripheral blood mononuclear cells [23]. It has been suggested that not only an individual ability of the host cells to respond to LPS but also the structure of *H. pylori* LPS may both influence the course of infection [24].

Another anti-inflammatory mechanism of *H. pylori* is the scavenging activity of the reactive oxygen species (ROS) dependent on enzymatic activity of catalase, superoxide dismutase and alkyl hydroperoxidase, which is increased in cag-positive *H. pylori* strains [25]. Mutants defective in such enzymes were more sensitive to oxidative stress, accumulated more free (toxic) iron, and suffered more DNA fragmentation compared to wild type cells [26]. *H. pylori* cells are also protected from oxidative stress by a *mut*S homologue DNA mismatch repair system [27].

Inflammatory Response

Understanding of the IL-1β induction in monocytes/macrophages by *H. pylori* is of particular relevance because IL-1β gene polymorphisms are associated with increased risk of gastric cancer. Recently, the role of *H. pylori* LPS in IL-1β release has been established. Following *H. pylori* LPS stimulation, PI-3K/Rac1/p21-activated kinase (PAK1) regulates mature IL-1β production by inducing caspase 1 [28]. The *H. pylori* LPS-induced gastritis is also manifested by a marked upregulation of gastric mucosal endothelin-1 (ET-1), which influences local leptin release, a pleiotropic 16 kDa peptide hormone involved in the regulation of the immune responses by interaction with pro-inflammatory cytokines [29].

Antibody Response

Volunteer challenge studies permitted to determine the kinetics of IgM and IgG responses during acute *H. pylori* infection [30]. Specific IgM antibodies peaked at 4 weeks post-infection and fell within 2–4 months after eradication therapy. In contrast, the anti-*H. pylori* IgG response appeared about 4 weeks post-challenge and peaked 12–19 weeks post-infection. Four new low molecular weight proteins: hydrogenase expression/formation protein, alkyl hydroxyperoxide reductase, superoxide dismutase, and iron (III) ABC transporter periplasmic iron-binding protein, have been identified as targets for anti-*H. pylori* IgG antibodies [30].

Prior studies in B-cell-deficient mice have indicated a nonessential role of specific antibodies for host resistance against *H. pylori* infection. Recent studies in the same model suggest that antibodies could in fact impair both the elimination of bacteria and the development of gastritis. This effect appears to be IgA-dependent and is not a function of specific anti-*H. pylori* IgM or IgG antibodies [31]. Others found, however, that IgG monoclonal antibody recognizing the small Ure-A subunit enhanced urease enzymatic activity. Moreover, such antibody increased *H. pylori* colonization of BALB/c mice [32]. In the light of these findings, gastric colonization of *H. pylori*, which occurs in a very early age, could be enhanced by transplacentally transferred specific anti-*H. pylori* IgG antibodies [33].

T-lymphocyte Responses

Data from experimental *H. pylori* infection in humans showed that mucosal CD3-, CD4-, and CD8-positive T-cell numbers increase following infection. The CD4-positive T-helper cells expanded in the first 42–72 hours of infection. The major changes in T-cell subsets recruitment and expansion have occurred by 4 weeks post-infection [30]. *H. pylori*-specific CD4 T cells home to and accumulate in the infected stomach via homing receptor L-selectin and the chemokine receptor CCR4. Infected stomach mucosa contain increased levels of the CCR4 chemokine ligand MDC/CCL22 [34].

The effector functions of gastric *H. pylori*-specific T cells are different between patients with various disease outcomes. A predominant *H. pylori*-specific T-cell response characterized by high IFN-γ, TNF-α, and IL-12 production is associated with peptic ulcer, whereas secretion of both Th1 and Th2 cytokines appears in uncomplicated gastritis. The cause behind a particular type of T-helper response remains unclear. It has been suggested that bacterial proteins may possess Th1 and/or Th2 motifs (modulotopes) which may be involved in the modulation of the immune response. Amphipathic sequence motifs were identified in the N- and C-terminal domains of *H. pylori* catalase, which were linked to induction of Th1 or Th2 immune responses, respectively [35].

It has been shown that T cells from mucosa-associated lymphoid tissue lymphoma elicit abnormal help to autologous B cells and reduced perforin- and Fas-Fas ligand killing of B cells [36]. *H. pylori* infection can also trigger activation of cross-reactive cytotoxic, and pro-apoptotic T cells leading to gastric autoimmunity via molecular mimicry. Moreover, *H. pylori* may activate T cells reactive to self-glycosphingolipids (self-GSL) via $CD1^+$ antigen-presenting cells. This reaction may further predispose to autoimmune disease [37].

The role of CD8 T cells in the immune response to *H. pylori* in humans is less clear as compared to CD4 T lymphocytes. *H. pylori*-reactive CD8 T cells can be activated by B cells or DCs pulsed with *H. pylori* antigens. Peripheral blood memory cells of *H. pylori*-infected subjects were found to strongly proliferate in response to *H. pylori* urease, suggesting that CD8 T cells participate in the immune response to *H. pylori* [38].

Although known to play a role in the maintenance of self-tolerance, recent studies indicate that $CD4^+CD25^+$ regulatory T cells (Treg) can be activated and expanded against microbial antigens in vivo. Findings in both experimentally infected mice and humans with natural *H. pylori* infection indicate that Treg are important in protecting the *H. pylori*-infected host against excessive gastric inflammation but also may promote *H. pylori* colonization, increasing the risk for duodenal ulcers [39]. Treg cells, however, may not be the explanation for the lack of activation of T cells during this infection. An anergy mediated by CTLA-4 may rather be implicated [40].

Inhibition of cell-cycle arrest in lymphocytes may be of major significance for the persistence of bacteria in the human stomach. It has been shown that a secreted protein complex of *H. pylori* with molecular weight between 30 and 60 kDa, distinct from VacA, induced cell-cycle arrest of T cells. In these cells, antigen-specific cell activation remained intact but the entry in S phase was inhibited by a G1 cyclin-dependent kinase activity [41].

Vaccine Development and Probiotics

Vaccine development is an important goal but several challenges remain [42]. While protective immunity continues to be obtained in more animal models of *Helicobacter* infection following immunization [43], no clear mechanism of protection has been delineated, and thus no correlate of protection identified, hampering the development of a human vaccine. Progress in the understanding of the mechanisms of protection is thus needed. Meanwhile, the only available strategy to evaluate vaccine candidates is the use of an infectious challenge, a strategy also available for human use [44]. Using this strategy, a breakthrough has been achieved by showing for the first time protection in volunteers following oral immunization with a recombinant *Salmonella Ty21a* (attenuated vaccine strain approved for human use against *Salmonella* infection) expressing the urease A subunit [45]. This observation needs of course further validation but initiates renewed interest for vaccine development.

Recent data regarding IgA (see above) suggest that this antibody isotype response may in fact favor persistence of the infection. Upon immunization, the IL-10–/– and IgA–/– double-mutant mice showed an increased post-immunization gastritis and eliminated faster the infection than IL-10–/– IgA+/+ simple mutants or wild type animals, suggesting that it may not be ideal for a vaccine candidate to elicit exaggerated IgA response. However, this remains controversial, as other investigators successfully immunized animals while inducing strong IgA responses [46]. While the role of antibodies in protection remains controversial, in deficient mice models, Fas ligand was found crucial for protection, further indicating that cellular responses are involved [47]. Gastric IFN-γ also increase following immunization and seems essential protection [48,49]. Interestingly, gastric mucosa mast cells are increased early in the development of the protective response and needed for immunization-mediated protection [50]. Elevated beta defensin 1 is also correlated with protection [48]. These last two observations suggest that specific and innate responses collaborate in the clearance of gastric *Helicobacter* infection. Furthermore, immunization against *H. pylori* prevented the gastric mucosa colonization by lower gut bacteria in mice [51].

Numerous developments in vaccine vectors were reported during this review period. The efficacy of DNA vaccination in mice was confirmed [52]. Urease B subunit remains the antigen of reference for such experiments. It was successfully expressed in tobacco and in probiotic bacteria, where it conferred protection after oral administration in mice [53,54]. Inactive recombinant cholera toxin B subunit and nontoxic mutant of cholera toxins were shown effective in mice [55,56].

While probiotics have been shown beneficial in *Helicobacter* infection, their mechanisms of action remain unclear. One of the potent protective strains against *H. pylori*, *Lactobacillus johnsonii* La1, has been further studied. The heat-shock protein GroEL of La1 has been found to bind to mucins and to gastric epithelial cells as well as to be able to induce aggregation of *H. pylori*. The La1 rGroEL also stimulated IL-8 secretion in macrophages and HT29 epithelial cells in a CD14-dependent manner [57]. Thus the impact of this strain on gastrointestinal homeostasis may be associated with several mechanisms. Additional function of probiotics may be linked to the presence of immunostimulatory of secondary DNA structures. Stabilized six-base AT oligodesoxynucleotide structures were found abundant in the genome of *Lactobacillus gasseri*. These structures were shown to have a mitogenic effect on B cells and to augment Th1 response through TLR9 [58].

Conclusion

The immune response to *H. pylori* has been further delineated. These studies have implications not only in the understanding of the mechanisms by which this pathogen persists on the gastric mucosa but also on vaccine development. The first evidence of protection in humans following anti-*Helicobacter* immunization opens the way to further human trials, which are likely to occur in the near future.

The study of the host response to *H. pylori* also provided a novel understanding of mucosal immunity far beyond expectations. *H. pylori* gastric infection has become an established model for the study of host–pathogen interactions at mucosal surfaces. Recent links between *Helicobacter* infection and inflammatory bowel disease in animal models further support the study of the immune response to this expanding group of bacteria.

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Helicobacter pylori and Non-Malignant Diseases

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Keywords
GERD, NSAIDs, peptic ulcer disease, non-ulcer dyspepsia.

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Abstract

The prevalence of *Helicobacter pylori*-associated peptic ulcers, in particular duodenal ulcers, is decreasing following decreasing prevalence of *H. pylori* infection, while the frequency of non-steroidal anti-inflammatory drugs (NSAIDs)-induced and *H. pylori*-negative idiopathic ulcers is increasing. The incidence of bleeding ulcers has been stable during the last decades. Several putative *H. pylori* virulence genes, i.e., *cag*, *vacA*, *babA*, or *dupA*, as well as host-related genetic factors like IL-1β and TNFα-gene polymorphism, have been proposed as risk factors for duodenal ulcer. *H. pylori* eradication may prevent NSAID complications, in particular, when it is performed before introduction of NSAIDs. There is a complex association between *H. pylori* and gastroesophageal reflux disease (GERD), and the impact of *H. pylori* eradication on the appearance of GERD symptoms depends on various host- and bacteria-related factors. Eradication of *H. pylori* in GERD is recommended in patients before instauration of a long-term PPI treatment to prevent the development of gastric atrophy. A small proportion (10%) of non-ulcer dyspepsia cases may be attributed to *H. pylori* and may benefit from eradication treatment. A test-and-treat strategy is more cost-effective than prompt endoscopy in the initial management of dyspepsia.

Peptic Ulcer Disease

The prevalence of peptic ulcer disease (PUD) has been decreasing for decades. A 10-year retrospective study analyzed the changing demographics of PUD in a total of 2182 patients with PUD admitted to a predominantly immigrant public hospital in the USA, 1173 in the early period (1995–1999) and 1009 in the recent period (2000–2004). This study showed that incidence of PUD was only modestly decreasing, male predominance was disappearing, and gastric ulcer was more prevalent than duodenal ulcer [1]. Lassen et al. compared the incidence of PUD in four Danish counties between 1993 and 2002. The incidence of uncomplicated duodenal or gastric ulcer decreased by 37 and 29%, respectively. The incidence of perforated ulcer diminished by 43%. In contrast, the incidence of bleeding peptic ulcer was stable on this period [2]. A Swedish study explored the prevalence, symptomatology, and risk factors for PUD in a random sample (n = 3000) of a general adult population surveyed between December 1998 and June 2001. Smoking, aspirin use, and obesity were risk factors for gastric ulcer, whereas smoking, low-dose aspirin use, and *Helicobacter pylori* infection were risk factors for duodenal ulcer. Peptic ulcer often coexisted with atypical symptoms, and idiopathic duodenal ulcer was more common (19%) than anticipated [3]. Another retrospective survey, comparing PUD prevalence in patients referred for upper gastrointestinal endoscopy in two Italian hospitals in the pre-*Helicobacter* era (1986–1987) and 10 years after the progressive diffusion of eradication therapy (1995–1996), confirmed a significant reduction in PUD prevalence (from 8.8 to 4.8%, $p < .001$ for duodenal ulcer, and from 3.9 to 1.5%, $p < .001$, for gastric ulcer) which may be attributed to decreasing of *H. pylori* infection [4]. An increasing frequency of *H. pylori*-negative idiopathic ulcers was also confirmed in a prospective cohort study including consecutive patients with bleeding gastroduodenal ulcers from January to December 2000. In Hong Kong out of 638 patients with bleeding ulcers, 213 (33.4%) had *H. pylori*-positive ulcers and 120 (18.8%) had *H. pylori*-negative idiopathic ulcers. The corresponding figures for the time period 1997–1998 were 480 (50.3%) and 40 (4.2%), respectively ($p < .001$) [5]. Eradication of *H. pylori* reduces the relapse rate. Ford et al. analyzed 56 trials from the Cochrane Central Register of Controlled Trials to compare the effect of *H. pylori* eradication therapy to that of placebo or ulcer healing therapy on ulcer healing and relapse rate following successful healing. In duodenal ulcer healing, eradication therapy was superior to ulcer healing therapy and in preventing gastric ulcer recurrence, eradication therapy was superior to no treatment, confirming that eradication therapy is an effective treatment for *H. pylori*-positive PUD disease [6].

One of the challenges in *H. pylori* research is identification of disease-specific *H. pylori* virulence factors predictive of the outcome of infection. Although a number of putative *H. pylori* virulence genes such as the *cag* pathogenicity island (PAI), VacA, BabA, OipA, and HrgA have been associated with increased risks of PUD or gastric cancer, none could be clearly linked to a specific disease. Lu et al. examined 500 *H. pylori* strains from East Asia and South America associated with different gastric pathologies and found that a gene named *dup*A (duodenal ulcer-promoting gene) situated in the plasticity region of *H. pylori* genome was specifically associated with duodenal ulcer versus gastritis (42 versus 21%, respectively) [7]. The frequency and genotype of *bab*A2, a gene encoding a blood-group antigen-binding adhesin mediating attachment of *H. pylori* to human Lewis(b) antigens on gastric epithelial cells, and other virulence factors, were studied in *H. pylori* strains in Brazilian patients with PUD. Although *bab*A2 genotype was frequently found (70%), no significant correlation was observed between *bab*A2 and vacAs1 genotypes or between *bab*A2 and *cag*A status and intensity of gastritis in these patients [8]. Interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNFα) may play a role in the genetic predisposition to duodenal ulcer upon *H. pylori* infection by modulating the host immune response. Several studies evaluated a possible association between IL-1β and TNFα-gene polymorphism and risk of duodenal ulcer. In the Korean study including 1360 subjects, the frequencies of IL-1β-511 C/T and T carrier were lower in the *H. pylori*-positive patients with benign gastric ulcer as compared to controls, suggesting that the IL-1β-511T-carrier polymorphism has a preventive effect on the development of gastric ulcer [9]. In a case control study including 310 *H. pylori*-infected individuals from eastern India, analysis of genotype frequency revealed a significantly higher frequency of IL-1β-511TT and -31CC genotypes in individuals with duodenal ulcer compared to those with normal mucosa [10]. IL-1β gene cluster polymorphisms was also studied in 437 Brazilian children, 209 of whom were *H. pylori* positive and showed that in these infected children, the presence of ILRN*2 allele and *cag*A-positive status were independently associated with duodenal ulcer [11]. In contrast, Garcia et al. did not find an association between allelic variants of IL-1 and TNF gene and the susceptibility to duodenal ulcer [12].

Non-Steroidal Anti-Inflammatory Drug Consumption

An epidemiologic study from Hong Kong evaluated trends in the prevalence of PUD from 1997 to 2003 based on all upper endoscopies performed in a single endoscopy unit. A decreasing trend in PUD prevalence, mainly due to a decrease in duodenal ulcer, parallel to a decrease in the prevalence of *H. pylori* infection and non-steroidal anti-inflammatory drug (NSAID) use, was observed [13]. A case control study from the Netherlands evaluated NSAID use and the prevalence of *H. pylori* infection in patients hospitalized during the year 2000 for peptic ulcer bleeding. Among 361 cases collected from 14 hospitals deserving an area of 1.68 million inhabitants, the overall incidence of peptic ulcer bleeding was 21.5/100,000 persons. NSAID use was found in 52%, including 17% who were taking ulcer-preventing treatment. Among the 64% tested for *H. pylori*, 43% were positive. According to these results, half of the complicated ulcers could be related to NSAID use, and *H. pylori* may not be actually implicated in the majority of cases [14].

Ulcer prevalence was evaluated endoscopically in 187 patients taking low doses of aspirin for prevention of cardiovascular events. At inclusion, the ulcer prevalence was 11% and no association was found between dyspeptic symptoms and ulcers. At another endoscopy performed on 113 patients after 3 months, 7% had developed an ulcer. The factors associated with the risk of duodenal ulcer were *H. pylori* infection and age over 70 years [15]. Similar results were obtained in complicated ulcers in a retrospective study based on 128 patients who had surgery for PUD performed in a 5-year time period in a department of surgery in Dallas, Texas, USA. The mean age of the patients was 60 years and two-thirds of the total number of patients had comorbidity. Use of NSAID was found in 54% and 47% were *H. pylori* positive. In 81% of cases, surgery was performed in emergency for ulcer bleeding or perforation [16]. NSAID treatment in patient at high risk for peptic ulcer requires a preventive treatment, which has not been yet defined.

Lai et al. compared the incidence of complicated ulcers in patients treated either by COX-2 inhibitors monotherapy or by a non-selective NSAID treatment associated with proton pump inhibitors (PPIs). Patients who developed ulcer complication after NSAID treatment were recruited and received, if needed, *H. pylori* eradication treatment. Randomization defined a group of patients treated by celecoxib and another treated by naproxen associated with lansoprazole for 24 weeks. In the follow-up, complicated ulcer was observed in 3.7% in the celecoxib group and in 6.3% in the lansoprazole group, the difference being non-statistically significant. More patients experienced dyspepsia in the celecoxib group (15.0%) than in the lansoprazole group (5.7%) [17].

A meta-analysis evaluated the effect of *H. pylori* eradication on the prevention of peptic ulcer in patients taking NSAIDs. Analysis included 939 patients in five studies. Eradication of *H. pylori* was associated with a reduced risk of peptic ulcer (odds ratio (OR): 0.43). A significant reduction of risk was found by sub-analysis in patients not previously treated by NSAID (OR: 0.26) as compared to patients already treated. These results suggest that eradication prevents complicated ulcers especially in patients not previously treated by NSAID. In patients already treated, prevention by PPI is more effective than eradication alone [18]. The effects of *H. pylori* eradication on NSAID-related gastrointestinal symptoms were reported in a randomized, case-control study. *H. pylori*-positive patients who had NSAID prescription for at least 2 weeks were randomized to receive either eradication treatment or placebo. Non-infected patients recognized by a negative serology, were taken as the control group. The overall prevalence of gastrointestinal symptoms decreased from 43% at 2 weeks to 10% at 12 weeks. No difference in symptoms prevalence was seen between the patients who received eradication therapy and those who did not receive therapy or were *H. pylori* negative. However, at the 12 weeks evaluation, symptoms tended to be lower in the *H. pylori*-eradicated group, suggesting that eradication may provide a long-term beneficial effect on NSAID-related symptoms [19].

Gastroesophageal Reflux Disease

The association between *H. pylori* infection and gastroesophageal reflux disease (GERD) is controversial. Some studies suggested a protective role of *H. pylori* with respect to GERD and its complications, while the others did not confirm this finding. A trend in prevalence of reflux esophagitis parallel to a trend in lower prevalence of *H. pylori* infection was demonstrated in a large series of 16,375 patients referred for upper endoscopy in Singapore from 1992 to 2001 [20]. In this study, both increase in the prevalence of endoscopic esophagitis (RR 1.99, 95%CI, 1.18–3.36, $p < .009$) and its inverse relationship with urease test results (RR 0.991, 95%CI 0.983–0.999, $p < .04$) were significant. A Japanese study, in which the effect of *H. pylori* eradication on the development of reflux esophagitis was studied in a 5- to 6-year follow-up, showed that the frequency of reflux was higher in *H. pylori*-treated patients than in *H. pylori*-positive patients [21]. Some previous studies suggested that *H. pylori* eradication may reduce the efficacy of PPIs in GERD. This issue was revisited by Giral et al. who evaluated intragastric pH before and after administration of lanzoprazole for 8 days in 10 *H. pylori*-positive patients with reflux esophagitis before and after *H. pylori* eradication. Intragastric pH was significantly higher in the presence of *H. pylori*, whereas baseline pH remained unchanged after *H. pylori* eradication [22]. Similarly, Calleja et al. evaluated the effect of *H. pylori* infection on healing and symptoms relief in 227 patients with proven esophagitis treated for 8 weeks with pantoprazole. Comparison of healing and symptoms relief rates between *H. pylori*-positive and *H. pylori*-negative patients showed that at 8 weeks, patients with erosive esophagitis and *H. pylori* infection exhibited a significantly better response to pantoprazole through complete heartburn relief, although no difference in endoscopic healing rates between the groups was observed [23]. One of the potential mechanisms by which *H. pylori* could protect against development of GERD is induction of gastric atrophy since it is well recognized that GERD is associated with increased exposure to gastric acidity. Since proinflammatory IL-1β polymorphisms increase the risk of hypochlorhydria and gastric atrophy, the association between these polymorphisms, presence of gastric atrophy, and risk of GERD were studied in 320 *H. pylori*-positive and *H. pylori*-negative consecutive dyspeptic patients in Japan. A proinflammatory IL-1β genotype was associated with increased risk of atrophy and decreased risk of GERD in *H. pylori*-infected subjects confirming that in some genetically predisposed individuals, *H. pylori* infection may protect against GERD through induction of gastric atrophy [24]. A large amount of data recently accumulated pleads, however, against a protective effect of *H. pylori* against GERD in the majority of cases. In a randomized, placebo-controlled trial including 157 Japanese patients with dyspepsia, Ott et al. studied the risk of reflux esophagitis after treatment for *H. pylori* infection. No difference in terms of frequency of esophagitis or heartburn symptoms was found at 3 and 12 months between the antibiotic and placebo-treated group, indicating that *H. pylori* eradication does not cause reflux esophagitis [25]. Similarly, among 102 consecutive *H. pylori*-positive patients with peptic ulcers followed 1 year after eradication, no significant difference was found in the frequency of reflux esophagitis between *H. pylori*-positive and *H. pylori*-negative individuals and the only factor positively associated with the development of GERD was the presence of hiatal hernia before therapy [26]. In elderly patients on short- and long-term treatment with PPIs, eradication of *H. pylori* did not affect the clinical outcome of esophagitis, while it improved chronic gastritis and its activity, suggesting that *H. pylori* should be eradicated in elderly patients with esophagitis who need maintenance treatment with PPIs [27]. A physiological study by Moschos et al. showed no effect of *H. pylori* infection on esophageal peristalsis, the lower esophageal sphincter pressure, and the acidity of refluxates into the oesophageal lumen in 59 patients with established GERD [28]. Another controversial issue is a negative association between GERD and some particularly virulent strains of *H. pylori*. In 1622 patients submitted to routine upper endoscopy in Taiwan, reflux esophagitis was found in 21.2% of patients and occurred at a significantly lower rate among *H. pylori*-positive patients harboring triple-positive virulent genotype strains (*cag*A, *bab*A2, and *vac*As1-positive) [29]. However, another study performed in Spain, did not show an increased prevalence of Barret's esophagus, main complication of GERD, and *H. pylori* infection and in particular infection with CagA+ strains [30]. Altogether, the effect of *H. pylori* eradication on the development of GERD seems to depend on diverse individual genetic and/or environmental factors. However, because of the role of this infection in the development of distal gastric carcinoma, it seems reasonable to eradicate this bacterium in order to prevent gastric cancer, in particular in patients on a long-term PPI treatment.

Non-Ulcer Dyspepsia

Non-ulcer dyspepsia (NUD) is defined as chronic or recurrent pain/discomfort centered in the upper abdomen. Patients suffering from predominant heartburn or acid regurgitation should be considered to have GERD, until proven otherwise, according to recent guidelines [31]. Organic explanation for dyspeptic symptoms has not been found. Both Maastricht 2 and Maastricht 3 Consensus Reports advised eradication of *H. pylori* in patients with functional dyspepsia which was confirmed by the results of a meta-analysis [32]. Twenty-one randomized controlled trials were included in the systematic review. There was a 10% relative risk reduction in the *H. pylori* eradication group (95% CI 6 to 14%) compared to placebo, leading to the conclusion that eradication therapy has a small but statistically significant beneficial effect in *H. pylori*-positive functional dyspepsia [32]. The main result of the 7-year follow-up study of di Mario et al. is that dyspeptic symptoms improve after *H. pylori* eradication in 30–50% of patients over a long period of follow-up [33]. Mazzoleni et al. demonstrated, in a population with a high prevalence of *H. pylori* infection, the benefit of eradication in patients with normal endoscopy but not in those with erosive gastritis [34]. Ford et al. aimed to determine the effect of screening for *H. pylori* on dyspepsia and dyspepsia-related medical resource use over 10 years, including 2324 original participants, 1864 (80%) of whom were traced and contacted. A significant reduction in total dyspepsia-related health-care cost was found, the savings being more important than the initial cost of *H. pylori* screening and treatment [35]. Similarly, an economic analysis of a *H. pylori* test-and-treat strategy versus a prompt endoscopy approach in primary care setting performed in the Netherlands, suggested that the former was more cost-effective than the latter in the initial management of dyspepsia in general practice [36]. It is estimated that a gastric cancer is present in 1 to 2% of patients with dyspepsia. Liou et al. from Taiwan analyzed 17,894 endoscopy results in patients with uninvestigated dyspepsia. Gastric cancer was found in 225 patients (12.6 cases per 1000 endoscopies) who presented uninvestigated dyspepsia, among whom 114 (50.7%) did not have alarm symptoms but a simple dyspepsia. About 5.3% (12/225) of gastric cancer cases would have been missed if endoscopy had been omitted in patients without alarm symptoms and aged less than 45 years, indicating that 40 years old might be an optimal age threshold for screening endoscopy for uninvestigated dyspepsia in Taiwan [37].

Conclusion

In most of the nonmalignant diseases associated with *H. pylori*, bacterial eradication has a beneficial effect. It remains still a challenge for the clinicians and researchers to better identify the patients at high risk of development of serious *H. pylori*-related diseases. In patients with GERD, although a systematic search for *H. pylori* cannot be recommended, eradication treatment is indicated in *H. pylori*-positive patients before instauration of a long-term treatment by PPIs to prevent gastric atrophy. Along with disappearance of *H. pylori* in the developed world, the paysage of gastroduodenal pathologies is changing, with a decreasing incidence of *H. pylori*-induced ulcers and an increasing incidence of GERD and NSAIDs-induced and idiopathic ulcers.

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Helicobacter and Digestive Malignancies

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Keywords
Helicobacter, gastric malignancy, pathogenesis, risk factors, prevention.

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Abstract

Important new data were published during the past year on the relationship of *Helicobacter* infection and gastric neoplasias. In the pathogenesis of gastric cancer, a thrilling new hypothesis was put forward based on animal experiments. *Helicobacter* infection induces gastric mucosal damage and bone marrow-derived cells (mobilized into peripheral blood and attracted to the inflamed mucosa) replace the areas of damaged gastric tissue and turn into neoplastic proliferation. Several studies focused on mechanisms related to the development of gastric malignancy in infected individuals with particular attention to inflammatory cytokine gene polymorphisms.

Some new evidence is also reported to suggest that *Helicobacter* infection increases the risk of neoplasias outside the stomach in the liver and colon.

Gastric cancer

Mechanisms of *Helicobacter*-induced Carcinogenesis

Helicobacter pylori infection is a classical model to study cancer development as a consequence of chronic inflammation. The estimated total of infection-attributed malignancy per year is 1.9 million cases or 17.8% of the global cancer burden [1]. Among the principal cancerogenic agents, *H. pylori* is a leading factor responsible for 5.5% of all cancers.

There is accumulating evidence to suggest that malignancy is a stem cell disorder. Three possibilities are discussed as to how cancer stem cells (CSC) may originate in the affected tissues [2]: 1, mutations of differentiated cells occur locally; 2, CSC derive from a transformed local pool of normal stem cells (NSC); and 3, bone marrow-derived cells (BMDCs) can be mobilized into peripheral blood, migrate to and repopulate the tissue gradually transforming into CSC. This pathway was recently proven in an animal model of *Helicobacter*-induced gastric cancer [3].

In a superbly designed study, Houghton et al. demonstrated that infection of C57BL/6 mice with *Helicobacter felis* led to repopulation of the stomach with BMDCs. Subsequently, these cells progressed through the intermediate steps of metaplasia and dysplasia to cancer. To prove the bone marrow origin of these epithelial cells in the stomach, female animals were irradiated and underwent transplantation with bone marrow from male mice. The origin of these cells from bone marrow was confirmed by special labelling. A significant up-regulation of stromal-derived factor 1 (SDF-1), a factor responsible for mobilization and migration of marrow progenitor cells seems to play an important role in this mechanism. These observations suggest that *Helicobacter* infection is the initiator of the carcinogenetic process by creating an environment favorable for marrow stem cells recruitment.

An additional study from the same institution showed that bacterial eradication was effective in preventing gastric cancer development. Even if eradication was performed after cancer development, the progression of the malignant proliferation was significantly slowed down [4].

As a consequence of these findings, a new model for epithelial cancers is proposed [5]. The starting point is chronic inflammation, which through tissue injury leads to local (within gastric mucosa) stem cells failure. This event leads to recruitment and engraftment of BMDCs into the tissue stem cell niche, and they take over the function of the original tissue stem cells. In the abnormal environment because of the presence of inflammatory cytokines and a deregulated tissue architecture, BMDCs may fail to differentiate properly and progress to cancer. The authors further report that chemoattracted cells belong to the nonhematopoietic, adherent mesenchymal stem cells population [3].

Kucia et al. identified a population of very small embryonic-like (VSEL) cells in murine bone marrow, which in vitro responded strongly to SDF-1 [6]. They hypothesized that these cells are recruited early during development in bone marrow and may serve as a source of pluripotent stem cells for tissue regeneration as well as cancer formation.

The evidence that epithelial gastric tumors can be of BMDCs origin is a new concept in our understanding of cancerogenesis.

In addition, the link between chronic inflammation and malignancy, recognized since many years finds new support by the elucidation of basic mechanisms involved in this process. However, studies are needed now to investigate whether this concept is also applicable to humans.

Previous studies indicated that nuclear factor Kappa B (NF-κB) is essential for promoting inflammation-associated cancer [7]. Suganuma et al. complemented this finding by identifying the TNF-alpha-inducing protein (Tipalpha) gene family in *H. pylori* genome. They found that the Tipalpha proteins released from the bacteria act as carcinogenic factors through induction of TNF-α expression and NF-κB activation. The authors further suggested that NF-κB activation by Tipalpha may play a key role in stomach carcinogenesis [8,9].

Cell–cell interaction is essential for the maintenance of normal cell morphology, growth control, and differentiation. The cadherin–β-catenin complex represents an essential component of the tight junctions that link cadherin receptors with the actin cytoskeleton. In an animal model, "carcinogenic" *H. pylori* strains were shown to activate β-catenin through a CagA-related mechanism and thereby impair cell adherence. This phenomenon is likely to represent an early event that precedes malignant transformation in the stomach [10].

Factors Influencing the Risk of Gastric Cancer Development

Genetic polymorphisms in inflammatory cytokine genes, particularly interleukin 1 (IL-1) have emerged in recent years as an important determinant of gastric cancer susceptibility.

During the past year, this topic continued to receive much attention with research focused mainly on IL-1 (IL-1β and its receptor antagonist), TNF-α, interleukin 8 and 10 (IL-8, IL-10).

The studies on the association between IL-1β polymorphism and gastric cancer risk remained inconclusive, reporting both positive [11–15] and negative [16–18] associations. Studies presenting a positive link emphasized that the effect appears more modest than it was previously thought of [11,19]. Recent publications from Korea demonstrated even opposite results: Chang et al. described the positive relationship between increased gastric cancer risk and "wild IL-1β haplotype" in Korean patients [20].

This discrepancy may reflect mostly the genetic difference between populations. The reason for this may be also because of potential confounding variables, such as *H. pylori* status and *H. pylori*-related pathogenetic factors and a family history of malignancy. Studies from Italy demonstrated that IL-1β polymorphism may contribute to an increased gastric cancer risk (OR 6.5) only in *H. pylori*-negative subjects, whereas those carrying *H. pylori* have a similar risk as compared with controls [14,16]. In the study by Starzynska et al., IL-1 polymorphism analysis was helpful to identify individuals with an increased risk of gastric cancer development in the absence of a family history of gastric cancer. Analysis of IL-1β did not appear to be useful in gastric cancer family members [19]. The study also revealed an association between the IL-1β polymorphisms and gastric cancer risk with emphasis on the tumor stage.

The relationship between IL-1β polymorphism, gastric acid secretion, and *H. pylori*-associated gastric cancer, is now recognized to be more complex as initially recognized. A study from China investigated the effect of IL-1β genetic polymorphism on gastric acid secretion. IL-1β mRNA expression and gastric acid output were assessed in a large number of healthy volunteers [21]. In agreement with previous reports the positive association between IL-1β polymorphism and increased IL-1β expression was confirmed but contrary to the prior hypothesis an increased production of IL-1β did not inhibit gastric acid secretion. Authors suggested that the pathway, through which IL-1β may exert its effect in stomach carcinogenesis does not depend on acidity, but on other regulatory mechanisms. This study opens new questions related to IL-1β and gastric carcinogenesis. The suggestion that the role of IL-1β polymorphisms are not exclusively linked to gastric acid hyposecretion is strengthened by the observation in a subset of duodenal ulcer patients (32%), who do have increased acid secretion [19].

Also, other cytokine gene polymorphisms (TNF-α, IL-10, IL-8) and their relevance for *H. pylori*-induced gastric cancer risk have been reported with inconsistent results [16,17,22–25].

Recent studies from Asia on host factors and their impact on clinical outcome in *H. pylori* infection suggest the poor metabolizer genotype status to be a risk factor for developing gastric cancer, especially for the diffuse type (OR 3.4) [26].

In order to underline the differences in the carcinogenic potential of bacteria, Saganuma et al. proposed to divide *H. pylori* into four groups with respect to Tipalpha and *cag*PAI status [9].

In summary, the studies performed during last year support the view that polymorphism of inflammatory cytokines is not able to identify individuals at increased risk of gastric cancer, and those who might benefit from

H. pylori eradication. New markers indicating an increased risk from both, the host and the bacterial side have been identified. However, their role in clinical settings remains to be defined.

The basis for the development of gastric cancer in a small subset of *H. pylori*-infected individuals leaves may open questions.

A comprehensive review on all elements contributing to causality of *H. pylori* in gastric cancer development and the potential of *H. pylori* eradication for its prevention has been published in the American Journal of Gastroenterology [27].

Helicobacter and Extra-Gastric Malignancies

There is some evidence to suggest that *Helicobacter* infection may be associated with an increased risk of extra-gastric malignancies [28]. The latest research addressing the issue was related mostly to colorectal and hepatocellular carcinoma.

It has previously been shown that the risk of colon adenomas is increased in *H. pylori*-infected subjects. Two new studies from Japan based on a large number of patients added important evidence for the association between *H. pylori* infection and colorectal neoplasia [29,30]. The first study showed a positive relationship between *H. pylori* infection and the risk of adenoma and carcinoma, especially in women (OR 1.68 and 2.09, respectively). The second study found that *H. pylori* infection is connected with the presence of colon adenomatous polyps detected by high-resolution colonoscopy. A very significant increase in the incidence of adenomas was observed in the sero-positive group (44.3 versus 18.9%, $p < .0001$). Grahn et al. detected *Helicobacter* DNA in nearly 30% of colorectal cancer biopsy specimens and proposed 16S rDNA amplification and pyrosequencing analysis as a molecular tool to identify the bacteria [31].

The presence of *Helicobacter* species in the liver tissue from patients with different liver diseases including hepatic neoplasias has been reported by numerous authors. The most thrilling hypothesis is that these bacteria might play a role in the development of hepatocellular carcinoma [32].

The article of Rocha et al. demonstrated the association of *Helicobacter* species with hepatitis C cirrhosis, and hepatocellular carcinoma (HCC) [33]. The study included a large series of patients and was the first to examine both tumor and cirrhotic liver tissue samples from patients with HCV-positive HCC. *Helicobacter* DNA was found in a small percentage of liver biopsies from controls as well as from patients with chronic hepatitis C (4.2 and 3.5%, respectively). However, the prevalence was high in patients with HCV-positive cirrhosis and in those with cirrhosis and HCC (68 and 61%, respectively). In nearly all samples obtained from cancer tissue, *Helicobacter* DNA was detected and identified as *Helicobacter pullorum* or *H. pylori*-like organisms. The authors suggested the possible causal role of these bacteria in the progression of chronic hepatitis C and the development of HCC. However, further prospective studies are requested to prove this hypothesis.

To investigate the effect of *H. pylori* on human hepatic cells, comparative proteome analysis of untreated and *H. pylori*-treated hepatic cell line HepG2 was performed by Zhang et al. [34]. Seven proteins, which were up-regulated in *H. pylori*-treated cells, were identified including oncogene-encoded products, regulatory proteins of transcription and signal transduction. The above finding might be a basis for further studies on the role of *Helicobacter* spp. in liver diseases and in particular in HCC.

Research on *H. pylori* and other *Helicobacter* species will offer a great opportunity for further discoveries and the elucidation of mechanisms involved in carcinogenesis within the digestive system.

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Treatment of *Helicobacter pylori* Infection

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Keywords
antimicrobial resistance, eradication, first-line treatment.

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Abstract

In clinical practice the recommended treatment regimens achieve only an 80% *Helicobacter pylori* eradication rate and this rate is lower in patients who have failed first-line treatment. The increasing indications for *H. pylori* treatment (idiopathic thrombocytopenia and iron deficiency anemia) and an increasing trend of antibiotic resistance (especially in southern Europe) emphasize the need for more effective *H. pylori* eradication. Smoking and a short duration of treatment, especially in patients with functional dyspepsia, are predictors of eradication failure. In first line, the best option remains the clarithromycin-based regimens but an extended treatment duration is now indicated. Following first-line treatment failure, 14-day proton pump inhibitor triple therapy employing alternative antibiotics or quadruple therapy could be used. Levofloxacin-based 10-day triple therapy seems to be an encouraging strategy following one or more eradication failures.

Helicobacter pylori eradication remains an important public health challenge especially in light of broadening indications and increasing antimicrobial resistance. The recommended *H. pylori* eradication therapy continues to be triple therapy with a proton pump inhibitor (PPI) and two antibiotics of clarithromycin, amoxicillin, or metronidazole for 7 days. However, with these recommended regimens, at least 20% of patients do not achieve eradication in clinical practice (Table 1).This high rate of treatment failure reflects the need for continued revision and updating of treatment regimens for *H. pylori* eradication.

New Indications for *Helicobacter pylori* Eradication

At the United European Gastroenterology Week (UEGW), held in Copenhagen, October 15–19, 2005, the revised Maastricht Guidelines for *Helicobacter pylori* eradication, developed by the European *Helicobacter pylori* Study Group (EHSG), were presented [5]. All the previous indications for *H. pylori* treatment were endorsed [6]. These indications included peptic ulcer disease, investigated non-ulcer dyspepsia ("test and treat"), gastric mucosa-associated lymphoid tissue (MALT) lymphoma, atrophic gastritis, postgastric cancer resection, first-degree relatives of gastric cancer patients, and patient preference after consultation. A number of new indications for eradication were introduced: idiopathic thrombocytopenia purpura (ITP) and iron-deficiency anemia (IDA). Much consideration was also given to the role of *H. pylori* eradication in nonsteroidal anti-inflammatory drug (NSAID) users.

The association of IDA and *H. pylori* is now well established. Data from US National Health and Nutrition Examination Survey from 7462 participants confirmed the association between *H. pylori* infection and IDA prevalence (odds ratio: OR: 2.7) [7]. Similarly in a pediatric study conducted on 700 children from rural Alaska, *H. pylori* infection was associated with IDA [8], suggesting *H. pylori* eradication for the management of IDA in this region. Proposed mechanisms of IDA in patients with *H. pylori* include: chronic gastritis (causing occult blood loss or decreased iron absorption) or uptake and utilization of available iron by *H. pylori* [9]. An association between *H. pylori* infection and ITP has been established by a number of studies [10]. *H. pylori* eradication should be the first line of treatment in *H. pylori*-positive ITP patients [11]. *H. pylori* eradication is of use in preventing peptic ulcers and gastrointestinal bleeding in *H. pylori*-positive patients on NSAIDs [12].

Antimicrobial Resistance

Antimicrobial resistance is a major cause of treatment failure [13] and resistance rates vary considerably between regions. Reliable geographic data on the prevalence of antimicrobials resistance has a crucial role in choosing the best first- and second-line treatments for eradication.

Table 1 Triple therapy results

Regimen	Duration	Patients	Eradication rate (%)	Reference
PPI, CA	7	812	72%	[1]
PPI, CA	7	890	77%	[2]
PPI, CA	7	507	74%	[3]
PPI, CA	7 or 10	458	76%	[4]

In Germany, a satisfactory low resistance for clarithromycin (9.8%) and levofloxacin (3.2%) was observed [14]. In contrast in an Italian study evaluating 232 naïve *H. pylori*-positive patients by using a real-time polymerase chain reaction (PCR), a very high primary clarithromycin resistance (26.7%) was reported [15]. In a European multicenter study involving 1233 symptomatic *H. pylori*-infected children, a high primary resistance rate to clarithromycin (20%) and a reasonably low resistance rate to metronidazole (25%) were detected [16]. Regression analysis revealed a higher risk for clarithromycin resistance in children who were born in southern Europe, Asia, Africa or the Middle East. In a study of 111 adults in US-Mexico, only 3% had primary clarithromycin resistance by *E*-test methods [17]. In a Japanese study fluoroquinolone (gatifloxacin) resistance was extremely high (47%), therefore the potential use of fluoroquinolones as rescue therapy could be limited in Japan [18].

Culture for the management of *H. pylori* infection has been neglected for a long time, despite the fact that several studies have shown repeatedly that higher eradication rates are obtained when antibiotics are chosen based on susceptibility testing versus choosing empirically [19,20]. The high impact of clarithromycin resistance on eradication rates has suggested that culture and antimicrobial susceptibility testing is of benefit when the resistance rate reaches 15–20% in a region. Another condition where susceptibility testing is recommended is in the failure of second-line therapy. The monitoring of primary antibiotic resistance should be carried out in different areas in order to appreciate the risk of failure linked to antimicrobial resistance.

Other Factors Related to Eradication Failure

Other important factors related to eradication failure include compliance, duration of therapy, diagnosis of NUD, and smoking. In a randomized study of 458 *H. pylori*-positive patients into 7- or 10-day triple therapy with rabeprazole, clarithromycin, and amoxicillin, 10 days was superior to 7 days in patients with NUD; however, no difference was seen in patients with PUD [21]. In a meta-analysis of 22 studies involving 5530 patients smoking increased the risk of treatment failure (OR = 1.95) [22].

Table 2 Cure rates in alternative first-line treatments

First-line treatment	Eradication rate (%) (PP/ITT)	Reference
10 day quadruple therapy	95/91	[24]
Sequential therapy		
(5-day PPI triple after 5-day PPI dual therapy)	97/94	[25]
PPI–amoxicillin–levofloxacin[b]	93/87	[14]
PPI–clarithromycin–tinidazole–bLF[a]	93/89	[28]
PPI–clarithromycin–amoxicillin–bLF[a]	80/78	[29]

[a]bLF: bovine lactoferrin.
[b]36.7% of enrolled patients with one or more treatment failure.
PP, per protocol analysis; ITT, intention-to-treat analysis.

Table 3 Cure rates in second or third-line treatments

Second or third-line treatment	Eradication rate (%) (PP/ITT)	Reference
PPI–metronidazole–amoxicillin	90/74	[30]
PPI–metronidazole–tetracycline	92/83	[30]
PPI–minocycline–amoxicillin	85/85	[31]
Quadruple after 1 month of AB-yogurt	91/85	[32]
PPI–moxifloxacin–amoxicillin	84/76	[33]
10-day PPI–levofloxacin–amoxicillin	80[a]	[34]
4-day PPI–levofloxacin–tinidazole	83/83	[36]
PPI–rifabutin–amoxicillin	91/91	[37]

PP, per protocol analysis; ITT, intention-to-treat analysis.
[a]Overall cure rates of a meta-analysis.

First-Line Treatment

The usual first-line treatment regimen is clarithromycin, amoxicillin, and PPI twice daily for 7 days. Cure rates for eradication of *H. pylori* with conventional 7-day treatment appear to be decreasing. Other alternatives include longer duration of therapy, quadruple therapy, sequential therapy, adjuvant therapy, or new antimicrobials-based therapies which have been used with comparable or better success rate than the established regimens (Table 2). Extending the treatment duration of the traditional clarithromycin regimen to 14 days appears to significantly improve eradication rates [23]. The combination of PPI–clarithromycin–metronidazole leads to better eradication rates than the combination of PPI–clarithromycin–amoxicillin.

Dore et al. demonstrated good compliance and a satisfactory eradication rate with a twice a day quadruple regimen (PPI, bismuth, tetracycline, and metronidazole) in elderly *H. pylori*-positive dyspeptic patients [24]. Another Italian group confirmed the better success rate of the sequential treatment (5-day dual therapy with PPI–amoxicillin followed by 5-day triple therapy with PPI–clarithromycin and metronidazole) than 7- or 10-day PPI triple therapy with clarithromycin and amoxicillin [25]. This sequential regime may be an alternative in clarithromycin-resistant strains [26]. Several new antimicrobials have shown promising eradication rates. A study from Germany demonstrated a satisfactory eradication rate of 7-day PPI triple regimen with levofloxacin and amoxicillin, although it was not significantly better than the clarithromycin-based therapy.

Therapy with bovine lactoferrin (bLF) was effective to reduce colonization density of *H. pylori*; however, it did not lead to complete eradication [27]. Conflicting data from Italy showed that adjuvant therapy was beneficial in one study [28] but this was not confirmed in another [29]. This discrepancy could be explained by different clarithromycin resistance rates and the use of different antibiotics in the studies.

Treatments after One or More Eradication Failure

Quadruple therapy is the recommended option after treatment failure. However bismuth is not available in all countries. The schedule compliance, complexity, and side-effects of quadruple therapy have led to research for alternative antimicrobials. A number of treatment options are available after first-line treatment failure (Table 3).

Studies from Japan [30] with metronidazole in combination with tetracycline or amoxicillin have demonstrated satisfactory eradication rates and few side-effects. Another Japanese experience study with minocycline, amoxicillin, and rabeprazole treatment showed acceptable results as second-line treatment [31]. In Taiwan where bismuth is available, a study, giving 4 weeks AB-yogurt containing lactobacillus and bifidobacterium prior to 7-day quadruple therapy, achieved a higher eradication rate than simple quadruple therapy [32]. The yogurt pretreatment could reduce *H. pylori* colonization facilitating the bactericidal activities of the antimicrobials treatment.

Quinolone-based regimens, such as moxifloxacin 400 mg four times a day, amoxicillin 1 g twice a day, and esomeprazole 20 mg twice a day achieved a higher success rate with a better tolerability than quadruple therapy [33].

In a meta-analysis considering 977 *H. pylori*-positive patients who failed one or more eradication treatments, a high success rate (overall: 80%) of levofloxacin-based regimens for 10 days was evident. These regimens achieved a better eradication rate than classic quadruple therapy with fewer side-effects [34]. These results were comparable to another meta-analysis performed comparing once daily (500 mg) versus twice daily (250 mg) of levofloxacin, suggesting a more compliant use of the once daily dosage [35]. However, theoretically, a higher levofloxacin dosage daily could increase tissue concentrations. Another study showed a high-dose levofloxacin regimen over 4 days was as effective as the conventional treatment (7 days) with 250 mg twice a day [36]. A 12-day regimen of high-dose PPI and amoxicillin and low-dose rifabutin showed a very high eradication rate in 130 patients with clarithromycin-resistant strains [37]; however, a disappointing eradication rate was observed in a study using rifabutin 300 mg as third-line therapy [38]. Widespread use of rifabutin could induce resistance in mycobacteria and so it should be used with caution.

Conclusions

Although a trend towards an increasing primary clarithromycin resistance, clarithromycin-based regimens remain the best first-line option. An extended duration of treatment to 14 days treatment is increasingly indicated. Antimicrobial resistance should be monitored locally and directed treatment may increase eradication rates. Sequential regimen or adjuvant therapy may be of marginal benefit. In second line, 14-day treatment using metronidazole and tetracycline if not previously given is effective treatment. Levofloxacin-based 10-day triple therapy is an encouraging strategy as second-line treatment.

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Helicobacter pylori Infection in Pediatrics

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Keywords
Helicobacter pylori, pediatrics, recurrent abdominal pain, stool antigen test.

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Abstract

This review summarizes the literature on *Helicobacter pylori* infection in childhood between April 2005 and March 2006, and includes guidelines of the Canadian *Helicobacter* Study Group Consensus Conference, noninvasive tests, optimum therapy regimens and problems with resistance, and reviews on immune mechanisms in the gastric mucosa that may lead to the development of an effective vaccine.

In 2005 'the Nobel Prize in Medicine or Physiology' was awarded to Marshall and Warren for the discovery in 1982 that most peptic ulcers are caused by an infection with *Helicobacter pylori* [1–3]. It took a further 10 years before literature about *H. pylori* infection in children was published. It is now well accepted that the bacteria is usually acquired during childhood, mainly from the mother via vomitus or fecal oral route [4–9]. Great strides have been made since. In July 2005, the Canadian *Helicobacter* Study Group published an evidence-based Consensus Update on the approach to *H. pylori* infection in children and adolescents, adopted in 2006 by members of the *H. pylori* Pediatric Task Force in the USA [10]. We hope to reach a combined NASPGHAN–ESPGHAN consensus before the World Congress of Pediatric Gastroenterology Hepatology and Nutrition in Brazil in 2008.

Basic Research

Peeters published an excellent overview about the peptide ghrelin, related to motilin which has been suggested to have "saginary" effect in *H. pylori* infection. *H. pylori* infection could influence ghrelin-secreting cells, and impairs the secretion of histamine, pepsin, and gastric acid [11]. Eradicating *H. pylori* can lead to a rise in plasma ghrelin, which could promote the development of obesity, increasing the risk of reflux disease and subsequent risks of Barrett's esophagus and esophageal adenocarcinoma. The suggestion that *H. pylori*-eradication may contribute to the obesity epidemic in industrialized countries is an interesting hypothesis to ponder!

Ernst highlighted an article of Velin et al. about the essential role of mast cells as mediators in experimental *Helicobacter* clearance by vaccination because mast cells-deficient mice could not be protected by immunization; this could be changed by reconstituting them with bone marrow-derived mast cells [12,13].

Ceponis and Jones summarized in the Canadian *H. pylori* Update the current knowledge of specific *H. pylori* factors. *H. pylori* infection can modulate signal transduction pathways in multiple host cell types and specific bacterial factors can activate certain cascades in the mucosa. The bacterium can also lead to opposing effects: antiapoptotic by inducing NF-κB activity and can suppress interleukin (IL)-4-induced signal transduction [14].

Prevalence, Incidence, and Transmission

The prevalence of *H. pylori* infection is declining in developed countries. Nevertheless, immigrants and indigenous people continue to carry a high burden of *H. pylori* infection and disease in their children. In Canada, the immigrant population comprises 200,000 individuals per year. The prevalence in children undergoing gastrointestinal endoscopy from 1990 to 1994 was 26–43%: it has now decreased to about 5% [15]. Jacobson also highlights the study by Miller (2003), in which adopted children have a seroprevalence of 16, 20, and 49%, respectively. He suggests more studies in children with different geographic, socioeconomic, and ethnic backgrounds using validated screening tools to see whether eradication is associated with reduced *H. pylori*-related diseases or with significant benefit in adulthood [15].

New data on prevalence come from Asia: Nizami et al. found that early colonization in 148 children showed a decreasing trend with increasing age (80% at 1 month of age, 67% at 9 months). The researchers did not, however, study children over 1 year of age [16]. Singh et al. determined prospectively the prevalence of *H. pylori* in 58 children with upper abdominal pain (UAP) and 182 controls. In the UAP group, the prevalence of *H. pylori* was 53.4%, in the children without UAP, 28%. The overall prevalence increased with age; 82% of the children with UAP were *H. pylori* negative after eradication, with a further 18% after second eradication therapy. All treated children with UAP remained symptom-free for 2 years. They concluded to a strong link between *H. pylori* infection and UAP [17]. Alborzi et al. collected stool samples from children from different age groups in Shiraz and found prevalence rates of *H. pylori* of 82, 98, 88, 89, and 57% in age groups of 9 months, and 2, 6, 10, and 15 years, respectively. There was a significant decrease in the 15-year-old teenagers [18].

Wong et al. [19] studied the prevalence of *H. pylori* in symptomatic Chinese children retrospectively from 1997 to 2004 to assess the impact of an aggressive eradication program. From 159 patients undergoing gastroscopy, 119 had gastritis and 13 had peptic ulcer disease (overall rate of proven *H. pylori* infection was 25.6%). They did not find a significant decrease of overall prevalence (33% in 1997, 27.7% in 2004), but reported that increasing age was significantly associated with a higher risk of infection. Their hypothesis was that eradication efforts were unsuccessful, possibly due to eating habits in Chinese people (cross-infection from sharing chopsticks).

Recently, Rowland et al. published a prospective study of age-specific incidence of *H. pylori* infection in children between 24 and 48 months of age, by using ^{13}C-urea breath test (^{13}C-UBT). They found a positive test in 28 of 327 index children (8.6%) at baseline assessment; during the next 4 years, 20 children became infected. As in previous studies, the infection was acquired at a very young age with a declining risk after 5 years of age. Risk factors were having an infected mother, an infected older sibling, and delayed weaning from a feeding bottle [9].

Mother to Child Transmission

The role of infected mothers has been described in 2002 [4] and stated by Konno et al. [5] in a 5-year follow-up study of 44 children. They collected gastric juice samples for culture and DNA analysis from 69 *H. pylori*-positive mothers. None of the children acquired *H. pylori* during the first year of life. Five children were tested positive within 5 years by serology and stool antigen test. Strains of the five positive children exhibited DNA fingerprinting patterns identical to those of their mothers.

In a follow-up study of children up to 36 months old using monoclonal stool antigen test, infected mothers were the main source of *H. pylori* infection of their children, as the mother, being primary carrier, has closer contact with the infant than the father in the first year of life [8].

Symptoms

Abdominal Pain

The association between chronic abdominal pain and *H. pylori* infection is still hotly debated.

Tindberg et al. investigated the association between type-specific *H. pylori* infection and gastrointestinal symptoms in a cross-sectional study of Swedish schoolchildren. Infection was investigated by IgG antibodies in serum and confirmed by immunoblot and UBT. Abdominal pain was reported by 63% of the children and recurrent abdominal pain (RAP) by 13%; 16% were infected; 73% of these children had CagA antibodies and 59% VacA antibodies. The authors did not find a positive association between *H. pylori* status and occurrence of abdominal pain: RAP was unrelated to the infection (OR 1.0; 95% CI 0.5–2.1), when adjusted for sex, age, and family background. The prevalence of RAP was lower in children with CagA+ and VacA+ *H. pylori* infection than among uninfected children [20].

Yang et al. investigated 1271 children with questionnaires to define RAP or short-term RAP (SRAP) with pain duration from 2 weeks to 3 months. All children with RAP, SRAP, or a combination were tested for *H. pylori* by serology. Prevalence rates of RAP and SRAP were 9.8 and 5.5%, respectively. Children with SRAP were more frequently *H. pylori* seropositive than those with RAP and controls. One year later 71% of the seropositive children became symptom-free, regardless of persistence of *H. pylori*. Infection was more frequently found in children with SRAP [21].

The subcommittee on chronic abdominal pain reviewed the predictive value of laboratory tests. The authors conclude that the coexistence of abdominal pain and an abnormal test result for *H. pylori* infection does not necessarily indicate a causal relationship between the two [22].

Non-ulcer Dyspepsia (NUD)

Kalach et al. [23] investigated 100 children older than 6 years with NUD, by endoscopy for epigastric pain in a prospective double-blind study; 26 children were infected. No difference in age or symptom characteristics between infected and non-infected children was found, except for epigastric pain during meals, which was more frequent in non-infected children. They concluded that *H. pylori*-infected NUD children had no specific symptoms.

At the end of 2005, Talley et al. published a review on the evaluation of dyspepsia in adults. As is the case in adults, the test-and-treat strategy is not recommended in children with dypepsia; endoscopy is mandatory in this age group [24].

Gastroesophageal Reflux Disease

The question is whether testing for *H. pylori* is necessary in infants with gastroesophageal reflux disease (GERD). Moayyedi reviewed the literature about the association between GERD and *H. pylori* infection. His conclusion is that *H. pylori*-induced hypochlorhydria is frequent in adults, but rare in children and that therefore eradication is unlikely to have an important impact on GERD or proton pump inhibitor (PPI) efficacy in this age group. His advice is that children with GERD, diagnosed clinically or by pH studies, do not need to be tested for *H. pylori*, unless they have endoscopy and have proven infection [25].

As GERD has been suggested as a contributing factor to otitis media, Bitar et al. investigated the possibility of a role of *H. pylori* in middle-ear disease in children, however, all 28 middle-ear fluid cultures and polymerase chain reaction (PCR) were negative; also 13 adenoids were negative for *H. pylori* by PCR. Seven of 18 patients had symptoms suggestive of GERD preceding the study, but this had no impact on the results of the study [26].

Gastritis

Leung et al. emphasized the diagnostic role of macroscopic observation of the gastric mucosa at endoscopy [27]. Ricuarte et al. studied the prevalence of atrophic gastritis in childhood with this hypothesis. Of 173 children, atrophic mucosa near the antrum-corpus border was present in 16% of the positive children, primarily as pseudo pyloric metaplasia (median age 15 years). As gastric atrophy occurs in infected children living in countries with a high incidence of gastric cancer they recommend that biopsies be taken near the lesser and greater curvature just proximal to the anatomical antrum-corpus border as well [28].

Extradigestive Manifestations

In the pediatric age group the following have been reported: anemia, idiopathic thrombopenic purpura (ITP), short stature, diarrhea, food allergy, and sudden infant death syndrome, some without sufficient evidence [29].

Immune Thrombocytopenic Purpura

Although the same author in an earlier study in 2003 reported an increased incidence of *H. pylori* in patients with chronic ITP, in a prospective study, Jaing et al. did not find evidence of an association [30,31]. Sherman and Lin mentioned the potential for molecular mimicry with antiplatelet antibodies, recognizing the CagA protein of *H. pylori* as a possible explanation for an association. The Canadian group did not include ITP as extradigestive manifestation in the recent guidelines [29].

Anemia

The Canadian Consensus Group concluded that there is sufficient evidence available to consider unexplained sideropenic anemia as an extradigestive manifestation and to consider test-and-treat strategy in such cases.

One recent study from India reported reduced hematological response to iron supplementation in asymptomatic children with *H. pylori* compared to children without infection. The mean serum ferritin level was similar at admission and improved in both groups of children, but infection had a significant adverse effect on response to iron therapy [32].

Growth

In 2005, two studies have been published about the relationship between growth and *H. pylori* infection: Sood et al. compared height, weight, and body mass index of 97 positive children with dyspeptic symptoms to 160 children with dyspepsia without infection. They found no significant difference between mean weight and height SD score in the infected and not infected group [33]. Mera et al. investigated whether a newly acquired infection affects height and weight within 16 months by performing UBT and anthropometry every 2–4 months. Authors observed a significant decrease in growth velocity during the first 4 months after infection and the children showed no height catch-up. Infected children had a small decrease in weight, in comparison to non-infected children. Their conclusion is that *H. pylori* infection causes a nontransient negative effect on height and weight in Colombian children [34].

In the Canadian Consensus Report, Sherman and Lin did not find firm evidence for the role of *H. pylori* infection in growth [29].

Other reported manifestations such as food allergy, diarrhea, and sudden infant death syndrome were not added to the list of extra intestinal symptoms by the Canadian group.

Diagnostic Tools

None of the noninvasive tests are 100% specific and sensitive. ^{13}C-UBT is reliable for detecting infection in children older than 6 years of age but can give false-positive results in younger children [10]. There were a small number of very young *H. pylori*-positive patients in these studies, making it difficult to validate new diagnostic tests in this age group.

Mégraud et al. reported in a multinational study on four noninvasive tests that UBT had the best sensitivity in all age groups, followed by serology, stool antigen test, and antibody detection in urine. In all tests, except the stool test, better sensitivity was observed with increasing age. The urine office test exhibited a very low sensitivity [35].

The Canadian Consensus group concluded that UBT is currently the best available noninvasive diagnostic test in children and published a list of variables that may influence the results of the test [10,36].

Recently, Nugalieva et al. raised attention to the problem of false positive UBT and recommended confirmation of a single positive test in low-prevalence populations by using a test that measures a different parameter (UBT confirmed by stool test) [37].

The polyclonal antibody-based stool antigen test (HpSA) is not as reliable as UBT, neither pretreatment nor after eradication [35], but monoclonal stool antigen tests perform as well as UBT. The Canadian group judged that it was too premature to recommend stool antigen testing as an alternative to UBT, but in settings where the UBT is not available for children, the monoclonal test is an excellent alternative to assess *H. pylori* status pre- and post-treatment [36]. Raguza et al. evaluated 127 children to compare the accuracy of a modified polyclonal stool antigen test with the gold standard. However, there were no *H. pylori* positive infants and children below the age of 2 years in his study. Three patients showed false positive results and two false negative results for the HpSA. The sensitivity of the HpSA test was higher in children in the age group of 6–18 years (100%) than in 2–6 years (80%). The specificity was 95–100 and 96.4%, respectively [38].

Haggarty et al. also used the polyclonal HpSA in a study of stool samples from children at two time points, 3 months apart; PCR was performed on all 26 pairs reverting from positive to negative (transient positives), all four persistent positive pairs and 10 randomly selected persistent antigen-negative pairs. In 15 of 26 transient positive stools, *H. pylori* was sequenced and identified in 12 and other *Helicobacter* spp. were identified in three. He suggested that transient positive stool tests are common and represent *H. pylori* in majority of cases; however, some positive stools may represent other *Helicobacter* species [39].

Hauser et al. compared a multistep polyclonal versus one-step monoclonal enzyme immunoassay in stools with ^{13}C-UBT in 43 children; 18 children were positive (positive UBT). The polyclonal stool antigen test had a comparatively good sensitivity, but lower specificity compared to UBT. The one-step monoclonal rapid test also had a comparable sensitivity and specificity, when a weakly positive test (visual interpretation) was considered negative [40].

Kalach et al. tested the rapid monoclonal stool antigen test in 128 children and observed the highest performance of the test in children older than 10 years (sensitivity 100%), in contrast with 75% in those younger than 5 years; in this study he found 11 discordant results of the test compared with gold standard [41].

Antos et al. evaluated a novel rapid monoclonal one-step immunochromatographic assay for detection of stool antigen and concluded that this quick test shows a good interobserver agreement, but equivocal results in 5% [42].

More studies with quick tests in stools are needed; however, in settings without possibilities for UBT or enzyme immunoassay, the immunochromatographic test could become an alternative to assess *H. pylori* status pre- or post-treatment in children [36].

A study from Thailand evaluated the performance of a rapid office-based serologic test (Assure™, Genelabs Diagnostics, Singapore) and the immunoblotting for the diagnosis in symptomatic children. The sensitivity of the test was 96%, specificity 94.6% versus immunoblot, 100 and 96,2% respectively, so the test seems to be reliable for the diagnosis of infection in Thai children [43].

The above Assure test was also evaluated in 130 children by Pelerito et al. They found a lower sensitivity (75.7%) and a specificity of 95.0%, which increased to 98.6 and 95% when a longer reading time of 45 minutes was considered [44].

The use of serology-based tests could not be advocated by the Canadian Consensus group anymore because of their low accuracy in young children. In a study from Lithuania, a seroprevalence of 57% was found; following gold standard it was 79% [45].

Invasive diagnostic methods require endoscopic biopsies and include rapid urease testing, histology, and culture. When used in combination, these tests are still considered the "gold standard" for the diagnosis of *H. pylori* in children. The added advantage of this approach is the detection of upper gastrointestinal pathologies including complications of the infection, such as nodular gastritis, peptic ulcer disease, gastric cancer, and MALT lymphoma. Biopsies are necessary for determination of antibiotic resistance and virulence factors [46,47].

Treatment

Based on the data in pediatric literature and in adults, the Canadian consensus group decided that the recommended first-line eradication therapy should include a PPI and clarithromycin, combined with either amoxicillin or metronidazole. Higher eradication rates may be achieved by a longer treatment regimen (triple therapy 14 days instead of 1 week) [10].

A randomized clinical trial from Italy showed that a novel 10-day sequential treatment (omeprazole plus amoxicillin for 5 days, followed by omeprazole plus clarithromycin plus tinidazole for another 5 days) achieves a significantly higher eradication rate than standard triple therapy (omeprazole, amoxicillin, and metronidazole) for 1 week [48].

Khurana et al. summarized data from studies that have examined treatment efficacy, safety, drug resistance, and reinfection rates. Treatment efficacy was reduced in the presence of metronidazole and/or clarithromycin resistance. Resistance to metronidazole and/or clarithromycin is common and no therapy has yet been identified as safe and consistently effective to eradicate *H. pylori* infection [49].

Elitsur et al. assessed the resistance rate against clarithromycin in 16 positive children by the FISH technique in gastric biopsies; the primary resistance rate in this small group was very high (31–38%) [50]; Boyanova et al. found primary clarithromycin resistance in 12.5% and metronidazole resistance in 15% of Bulgarian children [51]. Recently, Koletzko et al. assessed the bacterial resistance in children from 14 European countries. She reports an overall resistance to clarithromycin of 24%, to metronidazole 25%, and to amoxicillin 0.6%, the last being very low [52].

A Russian group investigated failure of triple therapy; patients were randomized to receive a 2-week course of bismuth, amoxicillin with either nifuratel or furazolidone plus omeprazole. Both schemes produced good cure rates, but nifuratel is preferred because of lower frequency of side-effects. Antibiotics susceptibility tests have not been carried out in this study [53].

The Canadian group discussed whether treatment of *H. pylori* in childhood will alter the two- to sixfold increased risk of developing gastric cancer among infected patients. A population-based test-and-treat policy in children is not justified, except in groups with a high risk of developing gastric cancer (Japanese or those with a strong positive family history) regarding negative cost-benefit analysis [54].

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Extragastric Manifestations of *Helicobacter pylori* Infection – Other *Helicobacter* Species

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Keywords
cardiovascular diseases, idiopathic thrombocytopenic purpura, iron deficiency anemia, colorectal cancer, enterohepatic Helicobacters.

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Abstract

Recent studies have indicated a strong link between *Helicobacter pylori* and idiopathic thrombocytopenic purpura and iron deficiency anemia. Interesting results have also been obtained for ischemic heart disease, though most putative associations between *H. pylori* infection and extragastric disease remain speculative. With regard to other *Helicobacter* species, *Helicobacter felis* has been shown to play a role in gastric carcinogenesis in mouse models. An increased susceptibility to cholesterol gallstone formation has been described in animals fed a lithogenic diet and infected with *Helicobacter bilis*, or co-infected with *Helicobacter hepaticus* and *Helicobacter rodentium*. Finally, enterohepatic *Helicobacter* species have also been exploited to better understand inflammatory bowel disease.

The cultivation of *Helicobacter pylori* and the recognition of its clinical significance have had two important consequences beyond the identification of an infectious etiology of peptic ulcer disease and gastric cancer. First, it has stimulated a renewed interest in the bacteria associated with the enteric and hepatobiliary tracts of humans and other animals, many of which have now been identified as novel species of *Helicobacter*. Second, and perhaps more importantly from a medical perspective, it has prompted a reappraisal of whether some of these novel *Helicobacter* species might themselves be associated with human disease, or perhaps serve as models of human disease in other animals, and also whether *H. pylori* itself may be associated with diseases beyond the stomach. Here we review recent results concerning these other *Helicobacter* species and other diseases that may be associated with *H. pylori*.

Helicobacter pylori Infection

Cardiovascular and Cerebrovascular Diseases

This is the area in which the greatest number of studies have been published during the last year. Adiloglu et al. investigated 29 consecutive patients using polymerase chain reaction (PCR) to detect *Chlamydia pneumoniae* and *H. pylori* DNA in specimens of atherosclerotic plaque and left internal mammary artery. *C. pneumoniae* and *H. pylori* DNA was detected in two and four plaques, respectively. Moreover, the presence of both *C. pneumoniae* and *H. pylori* correlated with an increased titer of inflammatory markers and modification of the serum lipid profile in a way that may increase the risk of atherosclerosis [1]. Sung et al. evaluated the prevalence of *H. pylori* infection in 58,981 subjects who participated in a health-screening program in Korea. After adjusting for age and other confounding factors, infected subjects showed higher mean values for total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, and apolipoprotein B, and lower values for high-density lipoprotein (HDL)-cholesterol and apolipoprotein A1. In the univariate analysis, age, total cholesterol, LDL-cholesterol, and apolipoprotein B were positively correlated with *H. pylori* IgG titers, while HDL-cholesterol and apolipoprotein A1 were negatively correlated. The analysis of covariance for *H. pylori* infection showed a significant association between *H. pylori* infection and levels of triglycerides, HDL-cholesterol, and apolipoproteins. The authors concluded that *H. pylori* infection may be associated with these cardiovascular risk factors [2]. Another study investigated the effect of *H. pylori* on the pathogenesis of atherosclerosis and showed an association between *H. pylori* infection and arterial stiffness, especially in younger males, thus suggesting that inflammation following *H. pylori* infection may contribute to an early stage of atherosclerosis [3]. A case-control study by Masoud et al. provided evidence of an association between the immune response to *H. pylori* and noncardioembolic ischemic stroke [4]. In contrast, Kanbay et al. studied *H. pylori* infection in 151 patients with coronary artery diseases and 149 matched controls, and found no significant epidemiologic association [5].

Hematologic Diseases

The role of *H. pylori* in the pathogenesis of idiopathic thrombocytopenic purpura (ITP), which has been well described in the past, has been confirmed by other studies. Suzuki et al. reported a significant improvement in the platelet count in patients with ITP after *H. pylori* eradication. The titer of serum anti-CagA antibody was also found to be a good predictor of platelet recovery [6]. Hayashi et al. evaluated the effect of *H. pylori* eradication on platelets in children with ITP. They reported a significant increase in the platelet count after *H. pylori* treatment, even though the prevalence of *H. pylori* infection in those children was not higher than that observed in the general population [7]. Veneri et al. reported that ITP patients with *H. pylori* infection had HLA-DRB1*11, HLA-DRB1*14, and -DQB1*03 frequencies that were significantly higher than in *H. pylori*-negative patients, while the -DRB1*03 frequency was significantly lower. Moreover, an HLA-DQB1*03 pattern was associated with a higher probability of platelet response to eradication treatment [8]. Another study suggested that *H. pylori* could function as a triggering factor in ITP by inducing platelet aggregation through an interaction with Von Willebrand factor [9]. In contrast, Stasi et al. found that the prevalence of *H. pylori* infection in patients with ITP was similar to that of the general population. *H. pylori* eradication improved the platelet count in adults in whom ITP was of recent onset and in those with less severe degrees of thrombocytopenia, but it was not effective in patients with chronic severe ITP [10].

Studies also examined a possible association between *H. pylori* infection and iron deficiency anemia. Cardenas et al. screened 7462 subjects for *H. pylori* infection and serum ferritin levels and reported a 40% higher prevalence of iron deficiency among those infected, after controlling for relevant covariates, regardless of the presence or absence of peptic ulcer disease [11]. Kureki et al. reported that *H. pylori* eradication produced a significant increase in hemoglobin, ferritin, and mean corpuscular volume in infected and anemic children. The authors concluded that a complete recovery of iron deficiency anemia can be achieved in infected children after *H. pylori* eradication, without iron supplementation [12]. In a similar study, Mahalanabis et al. found no improvement in the ferritin levels in both *H. pylori*-positive and -negative children, though a significant association was found between seropositivity for *H. pylori* infection and an adverse response to iron therapy [13]. Finally, Papadaki et al. found no evidence of a role for *H. pylori* infection in the pathogenesis of monoclonal gammapathy of undetermined significance in patients with chronic idiopathic neutropenia, as previously reported [14].

Lung Diseases

Kanbay et al. compared 68 patients and 95 age- and sex-matched control subjects and found an association between *H. pylori* infection and chronic bronchitis [15]. A higher prevalence of *H. pylori* infection was also found by Gencer et al. in patients with chronic obstructive pulmonary disease (COPD). Moreover, a significant correlation was also found between the titer of *H. pylori* IgG and the severity of COPD [16]. Ece et al. found a higher prevalence of *H. pylori* in 43 patients with nonsmall cell lung cancer compared to 28 control subjects, particularly for VacA-positive strains [17]. In contrast, Jun et al. found no increase in *H. pylori* among patients with mild asthma [18], which emphasizes that the proposed relationship between *H. pylori* infection and lung diseases remains speculative.

Intestinal Diseases

Two studies showed a possible role of *H. pylori* in the pathogenesis of colorectal cancer. Fujimori et al. described an increased risk of colorectal cancer in female patients aged 40–80 years who were infected by *H. pylori* [19]. A similar study by Mizuno et al. examined 332 patients who underwent routine high-resolution total colonoscopy and serologic testing for IgG antibodies against *H. pylori*. They reported a significant increase in the incidence of adenomatous polyps and a decrease in normal colonoscopic findings in seropositive patients compared to seronegative, and proposed an etiologic link between *H. pylori* infection and colorectal neoplasia [20].

Head and Neck Diseases

Otasevic et al. evaluated *H. pylori* antibodies in aqueous humor in three of 15 patients with idiopathic anterior uveitis and in none of five matched healthy controls. The authors hypothesized a causative link between *H. pylori* infection and anterior uveitis, though the small sample size makes this conclusion tentative [21]. Sacca et al. reported a significantly higher prevalence of *H. pylori* infection in patients with blepharitis compared with healthy controls as well as an improvement after *H. pylori* eradication [22]. The presence of *H. pylori* in middle ear effusion of patients with otitis media has been reported by three recent studies [23–25]. However, as the detection of *H. pylori* was determined by indirect methods, such as CLO (*Campylobacter*-like organism) test, PCR without sequence confirmation, or immunohistochemistry, these findings require confirmation.

Other Diseases

A study by Baskan et al. showed no correlation between *H. pylori* infection and autologous serum skin test in chronic idiopathic urticaria [28]. Aydemir et al. postulated a possible link between *H. pylori* infection and insulin resistance in patients with diabetes mellitus [29]. Isomoto et al. evaluated the impact of *H. pylori* on ghrelin and other neuroendocrine hormones, demonstrating that *H. pylori* infection may influence plasma ghrelin dynamics and its interaction with diverse bioactive peptides involved in energy balance, growth, and neuroendocrine function [30]. Larizza et al. showed that the concomitant presence of *H. pylori* infection and human leukocyte antigen-DRB1*0301 allele may increase the risk of autoimmune thyroid disease in children [31]. Another study reported negative results concerning the presence of *H. pylori* in oral lesions of patients with recurrent aphthous stomatitis [32]. Adler et al. reported a significant association between *H. pylori* infection and the major symptoms of glossitis, such as burning, halitosis, and lingual hyperplasia [33]. Schulz et al. hypothesized that cholesterol glucosides arising from *H. pylori* infection may act as neurotoxins, promoting the degeneration of the dopaminergic neurons affected in parkinsonism [26]. A study by Weller et al. seemed to support such association [27].

Other *Helicobacter* Species

Systematics and Novel Species

Our current understanding of prokaryotic systematics is based largely on 16S rRNA gene sequence analysis. However, discrepancies have occasionally been noted between classification based on 16S rRNA analysis and the gold standard (albeit cumbersome) method, DNA–DNA hybridization, most notably with *Helicobacter cinaedi* [34]. In this context, it was reported that *Helicobacter* gene phylogeny based on 16S rRNA gene sequences is in fact discordant with gene trees based on 23S rRNA gene sequences and other data [35]. Although the convenience of 16S rRNA gene sequence analysis insures that it will likely remain the method of choice for classification of *Helicobacter* species, these data suggest that analysis of 23S rRNA or other informative gene sequence will sometimes be required for proper classification of novel *Helicobacter* species. A microarray-based method used a combination of 16S rRNA and *hsp60* sequences for specific detection of *Helicobacter* species [36].

No novel *Helicobacter* species have been validly published since this subject was last reviewed here in 2005. Sequence analysis of 16S rRNA genes was used to extend the host range of *Helicobacter* to new species of sea mammals and birds, polar bears, and rabbits [37–39]. Spiral organisms that morphologically resembled *Helicobacter* were seen by microscopy in an ocelot [40] and in captive marmosets [41], but identification was not performed. *Helicobacter*-like organisms were also shown to cause ulcers in gnotobiotic pigs [42,43], and to be associated with gastric adenocarcinoma [44] and typhlocolitis [45] in naturally infected Syrian hamsters. Direct visualization in tissue by fluorescence in situ hybridization (FISH) may be useful to identify the *Helicobacteriaceae* as well as the individual species [46].

Gastric *Helicobacter* Species

Although *H. pylori* in the mouse is now the most commonly used model of gastric *Helicobacter* pathogenesis, the *Helicobacter felis* model in mice is useful because animals reproducibly develop the progressive histologic changes leading to gastric cancer. Cai et al. showed that eradication of *H. felis* after 2 or 6 months of infection prevented development of gastric cancer in C57BL/6 mice [47]. Even in those cases in which gastric cancer was present before treatment, antimicrobial therapy substantially slowed progression of malignant lesions. The same group also used the *H. felis* model to demonstrate the role of Fas-mediated apoptosis in reducing the progression of gastric cancer [48]. Antibiotic therapy can also lead to histologic remission of mucosa-associated lymphoid tissue (MALT) lymphoma in *H. felis*-infected mice, though a transcriptional signature of tumor remains, and mice rapidly develop recurrent disease when reinfected [49]. Takaishi et al. inoculated *H. felis* into hypergastrinemic (INS-GAS) mice and showed that gastrin or histamine receptor blockade had a synergistic inhibitory effect on prevention of gastric atrophy and cancer, while omeprazole had no such effects [50]. These results support a role for the gastrin–histamine axis in *Helicobacter*-induced gastric cancer, and suggest the possibility that alternatives to proton pump inhibitors be reconsidered as agents for long-term acid suppression.

An important vaccine study also used the *H. felis* mouse model [51]. Immunization with urease and cholera toxin produced increase in mast cells ($CD3^{-}CD117^{+}$) and in expression of mast cell proteases. Immunization protected against *H. felis* challenge in wild type but not in mast cell-deficient mice (W/W^{v}), which could be protected after reconstitution with cultured bone marrow-derived mast cells. These results require replication with *H. pylori*, but suggest the unexpected finding that mast cells are important mediators of protection after *Helicobacter* vaccination.

An intriguing report of *H. felis* challenge in a mouse model of streptozotocin-induced diabetes suggested that infection was associated with increased HbA1c and increased mortality compared to uninfected mice [52], though the effects were of marginal statistical significance. Several studies also examined gastritis induced by *H. felis* and closely related *Helicobacter* species in mouse and gerbil models [53,54], and the antimicrobial susceptibility of these organisms in vitro [55] and in vivo [56]. It appears that *H. felis*-induced gastritis in the mouse is attenuated by short-term zinc supplementation [57] and by over-expression of the reactive oxygen species scavenger, thioredoxin-1 [58].

Enterohepatic *Helicobacter* Species

While the role of *Helicobacter* infection in human hepatobiliary disease remains controversial, accumulating evidence from animal studies supports its biological plausibility. C57L/J mice, which are highly susceptible to cholesterol gallstone formation when fed a lithogenic diet, developed gallstones at up to 80% prevalence when infected with *Helicobacter bilis* or co-infected with *Helicobacter hepaticus* and *Helicobacter rodentium*, compared with only 10% in uninfected controls [59]. However, follow-up experiments showed that *H. pylori* infection is not lithogenic in mice [60]. *H. hepaticus* strains that contain a putative genomic pathogenicity island appear to be associated with more severe hepatitis in A/JCr mice [61]. One can anticipate that these experiments will be verified by construction of isogenic mutants, which can now be made in *H. hepaticus*. For example, *H. hepaticus* hydrogenase mutants were shown to be deficient in hydrogen-supported amino acid uptake and to produce no histologic evidence of hepatic lobular inflammation or necrosis [62]. Isogenic deletion of the *H. hepaticus* cytolethal distending toxin plays a crucial role in its persistent colonization in Swiss Webster outbred mice [63].

Enterohepatic *Helicobacter* species have also been exploited to better understand inflammatory bowel disease using genetically susceptible mice. Natural infection with *H. hepaticus* in mice deficient in interleukin-10 (IL-10–/–) produces typhlocolitis, though more slowly than in experimentally infected mice [64]. Mdr1a–/– mice that lack the membrane efflux pump, p-glycoprotein, also develop spontaneous colitis that is accelerated and progresses to dysplasia when they are dually infected with *H. hepaticus* and *H. bilis* [65]. Development of *H. hepaticus*-induced colitis is associated with elevated IL-12, which is induced by *H. hepaticus* in macrophages from IL-10–/– and other susceptible mice, but not in those from wild-type mice. The elevation of IL-12 p40 after challenge with *H. hepaticus* results from defective activation of ERK in macrophages lacking the p50/p105 subunit of NF-κB. These results suggest that similar dysregulation of IL-12 could play an important role in inhibiting microflora-induced colitis [66]. Previous studies suggested that mice deficient in the TGF-β signaling molecule SMAD3 spontaneously develop colon cancer. However, it now appears that SMAD-3-deficient mice that are free of *Helicobacter* infection do not develop colon cancer when followed for up to 9 months, but then develop tumors in 2 to 7 months when challenged with *H. hepaticus* and *H. bilis* [67]. These results suggest that bacteria may be important in triggering colorectal cancer in the context of gene mutations in the TGF-β signaling pathway, which is one of the most commonly affected pathways in colorectal cancer in humans. *Helicobacter* infection may contribute directly to development of colitis or colorectal cancer, or perhaps it may exert an indirect effect by alteration of indigenous microbiota [68].

The observation that genetic susceptibility to disease in mice may be dependent on *Helicobacter* infection serves to emphasize the importance of knowing the *Helicobacter* infection status in all mouse experiments. Infection may spread easily among mice reared under specific pathogen-free conditions [69], but not by vertical transmission [70]. Neonatal fostering [71] and medicated feed [72] have both been used to eliminate colonization. Detection is now commonly done by genus-specific PCR on fecal samples [73].

Helicobacter pullorum has been linked with diarrhea, gastroenteritis, and liver disease in humans, and with enteritis and hepatitis in chickens. Although the gene for cytolethal distending toxin is common among *H. pullorum* strains, it appears that toxin activity is associated with human clinical isolates rather than isolates from chickens. Colonization among broilers in Belgium is common and probably spreads within flocks [74]. *H. pullorum* DNA was found by PCR to be present in 4.3% of 531 patients with gastroenteritis but also in 4.0% of healthy controls [75].

Conclusions

Several studies performed during the past year supported a possible role for *H. pylori* infection in the pathogenesis of ITP and iron deficiency anemia. Interest in a role for *H. pylori* in cardiovascular disease continues. For other extragastric diseases, the level of evidence is weak, and is supported only by small pilot studies or case reports. Regarding other *Helicobacter* species, a potential pathogenic role for *H. felis*, *H. hepaticus*, *H. bilis*, *H. rodentium*, and *H. pullorum* has been proposed in different hepatic and enteric diseases, though those observations are mostly supported by animal studies. Further work is needed to verify the role of these species in humans.

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Helicobacter pylori Eradication for the Prevention of Gastric Cancer

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Keywords
atrophic gastritis, cancer prevention, *Helicobacter pylori* eradication.

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Abstract

Even though *Helicobacter pylori* infection is an obvious cause of chronic non-atrophic and atrophic gastritis, as well as gastric dysplasia and cancer, several recent controlled intervention trials for the prevention of gastric cancer by *H. pylori* eradication have yielded disappointing results. They showed that cancer eradication may still appear in relatively high frequency after successful treatment of *H. pylori*. One explanation for this observation is that *H. pylori* treatment has less influence in development of gastric cancer in atrophic gastritis than in development of cancers in non-atrophic gastritis, and that eradication does not prevent the progression of precancerous lesions and small invisible early cancers to overt tumors. Noteworthy however, the available intervention trials show that gastric cancer can be prevented by *H. pylori* treatment in patients with non-atrophic gastritis. Trials with higher number of patients, with longer follow-up periods, and with more careful controlling of cancer type and underlying gastric mucosal conditions are needed.

The 2005 Nobel Prize of Medicine award to Marshall and Warren was a formal recognition of their discovery that many peptic ulcers were due to an infectious condition that could be cured with antibiotics. Already in their first publications these Nobel laureates did suggest that *Helicobacter pylori*, given its association with gastritis, might also be involved in the etiology of gastric cancer. Within several years, this hypothesis was corroborated by research data. It thus took only a relatively short period of 10 years before the World Health Organization (WHO) formally classified *H. pylori* as a class 1 carcinogen. At that time, the hepatitis B virus was the only other infectious organism with a similar classification. For *H. pylori*, the classification was in particular based on the very strong association between colonization of the gastric mucosa, and development of chronic active gastritis, atrophic gastritis, precursor lesions of gastric cancer, and overt distal gastric adenocarcinoma. The available evidence was convincing, but there were also several major areas that completely lacked any data. These included in particular 1, the absence of animal model confirming a relation between *H. pylori* infection and gastric carcinogenesis, 2, the absence of interventional data showing that *H. pylori* eradication could prevent gastric cancer, and 3, understanding of underlying pathways explaining how chronic bacterial colonization could lead to cancer formation. In all three areas, major advances have been made over the years since the International Agency for Research on Cancer (IARC)/WHO report. Animal data confirming the association between *H. pylori* infection and cancer development soon came in the years thereafter, in particular from the Mongolian gerbil model [1], but interventional data on the prevention of gastric cancer both in humans and in animals had to be awaited much longer. Recently, preliminary and final results of a number of intervention studies were however, published. The purpose of this review is to discuss the available evidence that *H. pylori* eradication may prevent gastric cancer.

H. pylori Eradication and Chronic Gastritis

Chronic Non-Atrophic Gastritis

Colonization of the human stomach with *H. pylori* virtually always leads to chronic active gastritis. This inflammation immediately occurs following colonization, and within a few weeks stabilizes, usually in an antral-predominant pattern. Remarkably, long-term cohort studies in persistently *H. pylori*-positive subjects have shown that the pattern and severity of gastritis in a particular individual show very little variation over time [2]. This usually occurs under persistent colonization with the same strain that, however, may show subtle genetic changes over time, potentially under the influence of changes in the host–bacterial interaction, for instance as a result of development of gland loss due to the chronic inflammatory process. In this field of research, various advances were made in the past few years, enabling identification of host factors that modulate the immune response. Furthermore, it has been shown that certain bacterial virulence factors may directly damage mucosal tissue and enhance the host immune response. Finally, environmental factors may modify growth-signaling pathways and thus induce changes in cell turnover. Taken together, these factors may all modify the risk for gastric cancer formation [3–5].

Various studies in the past have shown that *H. pylori* eradication without relevant exception leads to resolution of gastritis. The polymorph or "active" component of the inflammatory response usually resolves within weeks, whereas the mononuclear or "chronic" component may take months, even up to 2 years to completely disappear. The association between successful eradication and resolution of gastritis is so strong that demonstration of persistent gastritis indicates either failure of eradication therapy or presence of another underlying condition causing gastritis. Resolution of gastritis is associated with other improvements, such a reduction of mucosal cell turnover and increase of intraluminal ascorbic acid secretion.

Chronic Atrophic Gastritis

There is increasing evidence that *H. pylori* eradication not only leads to a resolution of mucosal inflammation, but may also, to a certain extent, lead to a regression of premalignant gastric conditions, like atrophic gastritis and intestinal metaplasia (IM). In experiments with C57BL/6 mice infected with *Helicobacter felis*, eradication did not only lead to regression of inflammation, but also to a restoration of parietal cell mass and normalization of the glandular architecture of the mucosa [6]. This was associated with changes in the expression patterns of cellular messenger proteins such as alpha- and beta-catenin. In humans, however, various studies of the effect of *H. pylori* eradication on premalignant conditions, such as IM, have yielded contradictory results. To a large extent, this may have been due to the fact that many studies only included small numbers of patients, had a limited follow-up, and often also had an insufficient study design, for instance, lack of a control group or lack of randomization. In addition, many studies used only limited numbers of biopsy samples per patient, which meant that small focal lesions like IM, which are liable to sampling errors dependent on the number of biopsies and on the extent of the lesion, may easily have been over- or underestimated, potentially leading to false conclusions. Only several studies had a randomized design and sufficient number of follow-up patients to yield reliable data (Table 1). These studies all consistently reported that *H. pylori* eradication can lead to a regression of atrophic gastritis,

Table 1 Randomized controlled trials into the effect of *Helicobacter pylori* eradication on premalignant gastric lesions

	Study population			Intervention			Outcome: cases vs. controls	
Reference	Baseline condition	N (cases/ controls)	N with premalignant lesions at baseline	Cases	Controls	Follow-up (years)	Effect on AG	Effect on IM
[23][a]	Pts with AG, IM, or DYS	120/117	NS	Bismuth triple therapy	No treatment	6	Regression	Regression
[18][a]		394/401	NS	Bismuth triple therapy ± antioxidants	No treatment/ antioxidants	12	Regression	Regression
[24][a]	*Hp*+ volunteers	295/292	AG: 231 IM: 228	PPI triple therapy	Placebo	1	Regression	No effect
[19][a]		220/215	AG: 198 IM: 194			5	Regression	NS
[25]	*Hp*+ NUD patients	45/45	NS	PPI triple therapy	Placebo	3	Regression	No effect
[26][a]	*Hp*+ volunteers	161/155	AG: 145 IM: 87 DYS: 30	PPI triple therapy	Placebo	1	Regression	Regression
[27]	*Hp*+ GERD patients	111/120	AG: 99 IM: 45	PPI triple therapy + PPI maintenance	PPI maintenance	2	Regression	No effect

N, number of patients; *Hp*+, *Helicobacter pylori positive*; AG, atrophic gastritis; IM, intestinal metaplasia; DYS, dysplasia; GERD, gastroesophageal reflux disease; PPI, proton pump inhibitor; NS, not stated. [a]Reference nos 15 and 19 are reports of the same study at different time points, this is also true for reference nos 16 and 20.

similar to the above-mentioned mouse data. A few studies also observed a regression of IM after *H. pylori* eradication, others could not confirm this. A further study on a molecular level showed by means of microarray experiments that these long-term improvements of the condition of the gastric mucosa after *H. pylori* eradication are associated with various changes in gene expression in mucosal cells [7].

H. pylori Eradication and Gastric Cancer

Epidemiologic and Animal Data

Premalignant conditions strongly increase the risk of gastric cancer. This knowledge originally came from various cohort studies in the 1960s and 1970s, and was later consistently confirmed. In a recent Japanese surveillance trial among 9293 individuals, the annual incidence of gastric cancer was low among both *H. pylori*-positive and -negative subjects with normal serum pepsinogen levels, but strongly increased among those with low pepsinogen levels as marker for the presence of atrophic gastritis [8]. In this study, the highest incidence of gastric cancer was noted among those subjects who at baseline had low pepsinogen levels and negative serology for *H. pylori*. Presumably, part of these subjects may have had such severe atrophic gastritis that they had lost evidence of previous *H. pylori* infection.

Because of the strong association of atrophic gastritis, IM, and dysplasia with gastric cancer, these lesions are used as surrogate markers for the prevention of gastric cancer. The regression of atrophic gastritis after *H. pylori* eradication suggested that such intervention could reduce the risk for adenocarcinoma of the distal stomach. This hypothesis was confirmed in the *H. felis*–C57BL/6 mouse model [6]. In these experiments, all persistently infected mice developed within 24 months gastric outlet obstruction due to distal cancer. These cancers were completely prevented in mice that had received eradication treatment within the first 12 months of infection, and only occurred in 30% of the mice that had received eradication treatment between 12 and 24 months after initial colonization. In humans, model studies predicted that *H. pylori* eradication could be cost-effective intervention for the prevention of gastric cancer if eradication treatment would reduce cancer incidence by at least 10–30% [9–12]. The predicted costs per life-year saved were remarkably similar with different models and roughly varied between €20,000 and €30,000. Unfortunately, actual human studies were less optimistic about the potential effect of *H. pylori* eradication on gastric cancer incidence, even though several observational studies first reported that antibiotic treatment during knee or hip replacement surgery reduced the incidence of gastric cancer in subsequent years [13–15]. This reduction amounted to 40% more than 10 years after surgery. However, these data could not be confirmed in recent study by the same investigators [16]. Analysis of the Swedish national hospital registration containing data on 501,757 patients admitted with an infectious disease between 1970 and 2003 showed that antibiotic treatment did not lead to any reduction of the gastric cancer incidence thereafter.

Intervention Studies

These conflicting results from epidemiologic studies requested for true intervention studies aiming at the prevention of gastric cancer. Such a study was first reported from China [17]. It had included 1630 individuals, half of whom were randomized to receive eradication therapy, the other half had received placebo treatment. Within a follow-up of 7.5 years, seven patients in the eradication group and 11 in the control group were diagnosed with gastric cancer ($p = .33$). A preventive effect of *H. pylori* eradication was only observed in a subgroup analysis of individuals without baseline signs of atrophy, or metaplasia. In this subgroup, no cancer occurred after eradication treatment, compared to six cancers in control subjects without atrophy or IM at baseline ($p = .02$) [17]. These results suggested that even though *H. pylori* eradication may lead to healing of atrophy, it does not reduce cancer risk. Part of this observation may have been explained by the hypothesis that *H. pylori* treatment does not prevent the progression of early invisible intramucosal cancer or precancerous lesions (adenoma/dysplasia), which occur in 2.5–5% of subjects with advanced atrophic gastritis, to overt gastric cancer. However, more generally, it may mean that subjects with atrophic gastritis and IM may have passed a point of no return, which would mean that *H. pylori* eradication can only reduce cancer incidence in subjects with non-atrophic gastritis.

A second randomized intervention study showed very similar results. In a 12-year follow-up study from Colombia, 394 subjects had received *H. pylori* eradication treatment, and 401 a placebo [18]. During follow-up, four and five subjects were diagnosed with gastric cancer, respectively. All these cancer cases had had IM and/or dysplasia at the time of inclusion into the study. In another study from China, 220 subjects were treated with eradication therapy and 215 with placebo and then all followed for 5 years [19]. During follow-up, four and six subjects were diagnosed with gastric cancer, respectively. All of them except for two patients in the placebo group had had IM and/or dysplasia at baseline. In 2005, the preliminary results of a fourth randomized intervention trial were also presented. This Japanese trial was started in 1994, but for

Table 2 Prospective, controlled trials into the effect of *Helicobacter pylori* eradication on the incidence of distal gastric cancer

Reference	Study population: Baseline condition	Study population: N (cases/ controls)	Study population: N with premalignant lesions at baseline	Design	Intervention: Cases	Intervention: Controls	Follow-up (years)	Gastric cancer: N (cases vs. controls	*P*-value
[17]	*Hp*+ volunteers	817/813	AG: 129 IM: 477 DYS: 9	Randomized	PPI triple therapy	Placebo	7.5	7 vs. 5	Not significant
[19]	*Hp*+ volunteers	220/215	AG: 198 IM: 194	Randomized	PPI triple therapy	Placebo	5	4 vs. 6	Not significant
[18]	Subjects with AG, IM, or DYS	394/401	NS	Randomized	Bismuth triple therapy with/without antioxidant treatment	No treatment/ antioxidant treatment	12	5 vs. 4	Not significant
[20]	*Hp*+ volunteers	379/313	NS	Randomized	PPI triple therapy	Placebo	> 4	2 vs. 3	Not significant
[21]	*Hp*+, peptic ulcer patients	944/176	NS	Nonrandomized (eradication successes vs. failures)	PPI dual/triple therapy	Not applicable	3.4	8 vs. 4	0.04

N, number of patients; *Hp*+, *Helicobacter pylori* positive; AG, atrophic gastritis; IM, intestinal metaplasia; DYS, dysplasia; NS, not stated.

long, suffered from a low inclusion rate. Eventually, 692 patients were included and followed for at least 4 years, 379 of them had received eradication treatment, the remainder placebo [20]. During follow-up, two subjects in the eradication group and three in the control group were diagnosed with gastric cancer, again no significant difference between both groups. No information was presented regarding the baseline condition of the subjects who developed cancer. Apart from these four randomized studies, another Japanese intervention trial reported on 1120 patients with peptic ulcer disease who received eradication treatment [21]. During a mean 3.4 years follow-up, eight gastric cancers occurred in 944 patients in whom *H. pylori* had been eradicated, compared to four cancers in 176 patients in whom eradication treatment had failed, compatible with a significantly increased risk of gastric cancer in persistently *H. pylori*-positive patients ($p = .04$). All 12 patients with gastric cancer had had gastric ulcer disease at baseline, and thus likely had had atrophic gastritis at baseline. Taken all together, these studies reported the development of 25 distal gastric cancers in 2754 patients who received eradication treatment, compared to 29 cancers in 1896 patients who remained *H. pylori* positive (Table 2).

Further Considerations

The results from the available intervention trials are valuable and have been obtained with considerable efforts, and without doubt, with high costs. Efforts to follow several hundreds or more than one thousand subjects for periods of time up to 12 years are remarkable. The available data show that *H. pylori* eradication does not completely prevent gastric cancer, despite the consistent reports on complete resolution of gastritis after eradication, and despite the convincing data that atrophic gastritis may be reversible. When pooling the available results from the randomized studies, the frequency of gastric cancers was identical in treatment vs. control groups (Table 2). Remarkably, the only significant effect was noted in the single nonrandomized intervention study that compared patients after successful vs. failed eradication treatment [21]. Three of the four randomized trials reported that eradication treatment failed to prevent gastric cancer in subjects with atrophic gastritis and IM at baseline [17–19]. This could imply that eradication treatment for gastric cancer prevention is useful only in patients with non-atrophic gastritis. This would contrast with the European guidelines for the management of *H. pylori*, which advise to consider eradication of *H. pylori* particularly in patients with atrophic gastritis [22]. Another explanation may lie in the knowledge that a small number of patients with atrophic gastritis (up to 5%) have precancerous lesions (adenoma/dysplasia), or early invisible intramucosal cancers. These lesions tend to progress to overt cancer within some 5–10 years. Treatment of *H. pylori* may not influence this process.

If *H. pylori* eradication for the purpose of gastric cancer prevention would only be of benefit for subjects with non-atrophic gastritis, this would strongly impair the potential usefulness of eradication treatment for cancer prevention for two important reasons. First, it would contradict significant benefits of preventive measures in patients at the highest risk, if preventive measures only help subjects with non-atrophic gastritis instead of on those with atrophic gastritis (for instance identified by biomarker screening). Second, specific treatment of non-atrophic gastritis for prevention of cancer would also imply that the effect of intervention on the reduction of gastric cancer incidence would only be noted after a considerable time interval in agreement with the usual process of progression from chronic gastritis to preneoplastic conditions to gastric cancer. This process is known to often take decades.

The observations that *H. pylori* eradication does not reduce gastric cancer incidence in subjects with atrophic gastritis contrast with the results of the Japanese gastric ulcer study [21]. Even though the authors of the Japanese study did not provide data on baseline histology, most patients presumably had atrophic gastritis when included in the study. Despite this, the authors observed a significantly lower incidence of gastric cancer in those in whom *H. pylori* was eradicated compared to those in whom eradication treatment had failed. This suggests that *H. pylori* eradication can reduce gastric cancer incidence even in patients with precancerous conditions, like atrophic gastritis. This strongly means that further studies are needed in this field. Some studies are ongoing, others may use the currently available data to tailor their design and optimize power calculations, and to prolong the follow-up time. The available experiences from present trials make clear that the conclusive studies on the influence of *H. pylori* eradication on gastric cancer incidence must be large. The follow-up periods must be considerable and the type of cancer and the grade and extent of atrophic gastritis in the stomach have to be carefully controlled. Unfortunately, it is questionable whether such studies will be feasible, or whether more definite answers may be more easily obtained from meta-analyses of many small trials.

Conclusions

Helicobacter pylori is the major risk factor for the development of adenocarcinoma of the distal stomach, a disease characterized by a dismal prognosis and a very high incidence worldwide. The process of *H. pylori*-induced gastric carcinogenesis may mostly take decades. Recent intervention studies have shown that the eradication of *H. pylori* invariably leads to a resolution of gastritis, and also to a restitution of normal mucosal architecture with regression of atrophic gastritis at least in some of the patients. Concerning gastric cancer, the studies have shown that *H. pylori* eradication alone does not completely halt the progression to cancer, particularly in patient with preneoplastic conditions, such as atrophic gastritis and IM. Results are, however, contradictory and incomplete, which means that the recently updated European guidelines for the management of *H. pylori* infection are justified. They advise to eradicate *H. pylori* in patients diagnosed with atrophic gastritis. These patients may have to be also identified and endoscoped to find the early neoplastic lesions, but do not respond to *H. pylori* eradication anymore.

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Tables should he typed double-spaced on separate sheets and numbered consecutively within the text. Tables and text should not duplicate each other. If possible, hold tables to one standard manuscript page; if the table continues past one page, repeat all heads and the stub (left-hand column). Explain all abbreviations in a footnote. Provide a number and title for each table.

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All illustrations should be able to be reduced to 50–66% of their original size with no loss of clarity or legibility. Note that an original and two copies of all illustrations must be submitted. Illustrations should be submitted unmounted. Do not trim or write on back; do not use paperclips. Attach a gummed label to the back of each illustration giving the figure number, author's name, and an arrow to indicate the top.

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For half-tones (photographs, photomicrographs), clear black and white prints must be submitted. For photographs of recognizable persons, submit a signed release form from the patient authorizing publication. Photomicrographs must have internal scale markers.

Indicate crop marks on tracing paper overlays. Arrows, indicators or letters can be applied using a professional graphic transfer product (such as Chartpak). Consider clarity after reduction.

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Nexium®
esomeprazole

ABBREVIATED PRESCRIBING INFORMATION: Nexium® (esomeprazole magnesium). See local prescribing information for full details. **PHARMACODYNAMIC PROPERTIES:** Nexium® reduces gastric acid secretion through a highly targeted mechanism of action by being a specific inhibitor of the acid pump in the parietal cell. **INDICATIONS AND DOSAGE: Treatment of erosive reflux esophagitis:** Nexium® 40 mg once daily for 4–8 weeks. **Long-term management of patients with healed esophagitis to prevent relapse:** Nexium® 20 mg once daily. **Symptomatic treatment of gastro-esophageal reflux disease:** Nexium® 20 mg once daily in patients without esophagitis. Once symptoms have resolved, an on demand regimen of 20 mg once daily can be used when needed, to control subsequent symptoms. ***Helicobacter pylori*-associated peptic ulcer disease:** Healing of *H pylori*-associated duodenal ulcer, prevention of relapse of peptic ulcers in patients with *H pylori*-associated ulcers: Nexium® 20 mg, amoxicillin 1 g and clarithromycin 500 mg, all bid for 1 week. USA – Nexium® 40 mg once daily, amoxicillin 1 g and clarithromycin 500 mg twice daily, all for 10 days **CONTRAINDICATIONS:** Known hypersensitivity to esomeprazole, substituted benzimidazoles or any other constituents of the formulation. **WARNINGS AND PRECAUTIONS:** In the presence of any alarm symptoms (e.g. significant unintentional weight loss, recurrent vomiting, dysphagia, haematemesis or melena) and when gastric ulcer is suspected or present, the possibility of gastric malignancy should be excluded before treatment is initiated. **INTERACTIONS:** Due to the decreased intragastric acidity, the absorption of ketoconazole and itraconazole can decrease during esomeprazole treatment. Concomitant administration of esomeprazole resulted in a 45% decrease in clearance of diazepam. Concomitant administration of esomeprazole resulted in a 13% increase in trough plasma levels of phenytoin in epileptic patients; but dose adjustments were not required in this study. In healthy volunteers, combined therapy with esomeprazole and cisapride resulted in a 32% increase in AUC and a 31% prolongation of elimination half-life but no significant increase in peak plasma levels of cisapride. Concomitant administration of 40 mg esomeprazole to warfarin-treated patients showed that, despite a slight elevation in the trough plasma concentration of the less potent R-isomer of warfarin, the coagulation times were within the accepted range. However, as with all patients receiving warfarin, monitoring is recommended during concomitant treatment with esomeprazole. **PREGNANCY AND LACTATION:** Caution should be exercised when prescribing Nexium® to pregnant women. Nexium® should not be used during breast-feeding. **UNDESIRABLE EFFECTS:** The following adverse drug reactions have been identified or suspected in the clinical trials programme. None was found to be dose related. Common: Nausea/vomiting, diarrhoea, constipation, abdominal pain, flatulence and headache. Uncommon: Dermatitis, pruritus, urticaria, dizziness and dry mouth. From marketed use, there have been rare reports of increased liver enzymes and of hypersensitivity reactions e.g. angioedema, anaphylactic reaction. For further information please contact AstraZeneca, SE-431 83 Mölndal or the local AstraZeneca subsidiary.

References: 1. Richter JE et al. Am J Gastroenterol 2001;96:656–65. **2.** Kahrilas PJ et al. Aliment Pharmacol Ther 2000;14:1249–58. **3.** Castell DO et al. Am J Gastroenterol 2002;97:575–83 **4.** Labenz J et al. Aliment Pharmacol Therap 2005;21:739–746 **5.** Miner P et al. Am J Gastroenterol 2003;98:2616–20.

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