From bench to bedside and back – Report on the European *Helicobacter pylori* Study Group Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*

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ABR Thomson, N Chiba, P Sinclair. From bench to bedside and back – Report on the European *Helicobacter pylori* Study Group Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*. Can J Gastroenterol 1998;12(6):437-446. The Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori* was held in Lisbon, Portugal, from September 12 to 14, 1997. State-of-the-art reviews and research findings were presented to over 2000 participants. This review focuses on important new developments and serves as a rapid communication of clinically relevant material.

Key Words: *Helicobacter pylori*, Review, Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*

Aller et retour entre labo et chevet du patient – Rapport du European *Helicobacter pylori* Study Group après le X^e Atelier international sur la pathologie gastroduodénale et *Helicobacter pylori*

RÉSUMÉ : Le X^e Atelier international sur la pathologie gastroduodénale et *Helicobacter pylori* a eu lieu à Lisbonne, au Portugal, du 12 au 14 septembre 1997. On y a alors présenté aux 2 000 participants des travaux de synthèses et les résultats de travaux de recherche de pointe. Le présent article insiste sur d'importants développements et se veut un moyen de communiquer rapidement certains renseignements pertinents sur le plan clinique.

Report on the European *Helicobacter pylori* Study Group Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*, September 12 to 14, 1997, Lisbon, Portugal

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The Xth International Workshop on Gastroduodenal Pathology and *Helicobacter pylori* was held in Lisbon, Portugal, from September 12 to 14, 1997. This annual meeting is organized by the European *Helicobacter pylori* Study Group (EHPSG). State-of-the-art reviews and research findings were presented to over 2000 participants. This review focuses on important new developments and serves as a rapid communication of clinically relevant material. References to specific presentations are annotated using the abstract numbers published in *Gut* 1997;41(Suppl 1). These are not relisted here, and the interested reader is encouraged to refer to this supplement. Information from presentations made at the plenary sessions of the Xth International Workshop are referenced by the speaker's name followed by EHPSG.

EPIDEMIOLOGY AND TRANSMISSION

An important new concept is that *H pylori* is predominantly a childhood infection, with generally lower rates of acquisition in adults. *H pylori* infection is more common in children of infected mothers (Reshetnikov, 131) or in families with infected parents or siblings (Sollano, 152).

There is a high association between maternal history of ulcer and breastfed children's *H pylori* infection (Brenner, 129). However, another study reached the opposite conclusion: that even a short duration of breastfeeding protected against infection (Murray, 237). This study also reported that the rate of acquisition of *H pylori* in childhood was three times that in adolescence. Paternal *H pylori* status does not appear to correlate with *H pylori* acquisition (Brenner, 129; Reshetnikov, 131).

Marital status may not be a risk factor because the risk of *H pylori* reinfection was not significantly greater when the spouse was infected (reinfection rate of 7.1%/year with an infected spouse versus 5.9%/year with a noninfected spouse) (Gisbert, 121). The infection may be cleared and then recur; reinfection occurs frequently among children younger than five years old and is rare in older children, even from lower socioeconomic groups with infected family members (Rowland, 118). In a cohort of Swedish children, 80% who were previously seropositive cleared their infection spontaneously by age 11 to 12 years (Tindberg, 235). However, after 12 years of age, seroreversion was rarely seen (Murray, 237).

H pylori infection may be by oral-oral, fecal-oral or gastro-oral routes (1) (Galal, 150). *H pylori* can be cultured in the gastric juice of about two-thirds of cases, and this finding supports the hypothesis that *H pylori* can be transmitted in vomitus (Galal, 150). The periodontal pocket may be a natural reservoir for *H pylori*, possibly because it can provide microaerophilic conditions. *H pylori* has been found in the oral cavity of dyspeptic persons (Young, 128). Gambian infants bottle-fed water appeared to become infected with *H pylori* more commonly than those not given water, suggesting that water was a source of infection (Bunn, 134).

The domestic housefly is unlikely to be an extragastric source for the transmission of *H pylori* (Osato, 146). Preva-

lence of infection is not associated with a child having a pet (Bode, 154). *H pylori* DNA was not detected by polymerase chain reaction (PCR) in gastric juice or gastric histology specimens from cats (Cutler, 140).

PATHOGENESIS AND PHYSIOPATHOLOGY OF *H PYLORI* INFECTION

An imbalance between epithelial cell proliferation and apoptosis (ie, programmed cell death) may determine the long term consequence of *H pylori* infection. *H pylori* directly causes apoptosis, rather than its being due to the *H pylori*-associated inflammation. Apoptosis is sensitive to regulation by proinflammatory stimuli such as tumour necrosis factor (TNF)-alpha, CD95 ligand and interferon (IFN)-gamma (Wagner, 53). This IFN γ -associated increase in apoptosis is associated with increased expression of surface class II major histocompatibility complex molecules and binding of *H pylori* (Fan, 34). This apoptosis is independent of *vacA*, *cagA* or *picB*, and inactivation of NF- κ B enhances apoptotic time course (Peek, 164).

H pylori colonizes the gastric mucosa. How then are granulocytes and lymphocytes signalled from this surface infection? The gastric epithelium releases the cytokines interleukin (IL)-8 and epithelial-derived neutrophil-activating protein (ENA-78), which are granulocyte chemoattractants that correlate with gastritis activity (Rieder, 41). *H pylori* binds specifically to stimulate human gastric epithelial cells to produce cytokines. *H pylori* membrane proteins alpA and alpB may act as adhesion molecules (Odenbreit, 391). The *vacA* gene product is involved in generating a signal leading to a tighter interaction between bacteria and cultured AGS cells (Su, 394). A 17 kDa surface adhesion on *H pylori* binds to mucin glycoproteins (Namavar, 398). Synthesis of catalytically active urease is required for colonization of the gastric mucosa by *H pylori*. Urease defective strains are unable to colonize the gastric mucosa, making this a candidate for vaccine development. Motility is needed for colonization, and the two flagellar proteins are essential (2). All *H pylori* strains express gammaglutamyl transpeptidase (GGT), which appears essential for *H pylori* colonization in a mouse model (Chevalier, 33). This property makes GGT another potentially suitable target for vaccine development.

Adherence of *vacA*-positive *H pylori* to the gastric mucosa induces reorganization of intracellular actin in vitro (Ashorn, 65). This may explain in part the increased permeability across the tight junctions when *H pylori* infection is present (Terres, 78) because the alterations in permeability were not due to cell death or altered secretory capacity of the T84 epithelial monolayers. There is a correlation between gastric autoantibodies directed to parietal cell canaliculi, gastric atrophy (GA) and hypergastrinemia (3). The *H pylori* lipopolysaccharide (LPS) may play a role in the pathogenesis of atrophic gastritis (Negrini, 51). Most *H pylori* LPS express the Le^x and Le^y blood group antigens that are also expressed by gastric epithelial cells. Patients with nonatrophic gastritis often do not express Lewis 4 antigens. The hypothesis that

antigenic mimicry leads to more severe gastritis and development of GA was not confirmed in one study that found that *H pylori*-infected subjects who did not express Le^x were at higher risk of developing atrophic gastritis (Kuipers, 191). The role of antigenic mimicry leading to atrophy still needs to be clarified.

PROPERTIES OF H PYLORI

Only a minority of *H pylori*-infected patients develop peptic ulcer disease (PUD), possibly due to differences in the host response or in bacterial strains. Cytotoxin-associated gene A (CagA) is a cryptic 128 kDa immunodominant antigen produced by *H pylori*. The gene *cagA* is a marker for a large region of DNA containing over 40 genes that has been termed the Cag pathogenicity island (4). While *cagA* may be associated with duodenal ulcer (DU) and gastric cancer (GCA), this is not found in all countries (5), and persons infected with CagA-negative strains are still at risk of PUD (6).

Because not all persons infected with *H pylori* develop a peptic ulcer, lymphoma or GCA, it would be helpful to determine which *H pylori* infection needs to be treated. The best characterized virulence factors include *vacA*, *cagA* and other genes in the pathogenicity island. The presence of CagA-positive strains in children is strongly associated with the occurrence of DU, as well as with the intensity and activity of gastritis (Queiroz, 47). In the elderly, CagA-positive *H pylori* infection is associated with gastric ulcer (GU) more often than with DU, GA and intestinal metaplasia. Infection with *H pylori* causes increased gastric mucosal production of IL-8. CagA-positive strains of *H pylori* induce greater IL-8 secretion than CagA-negative strains. The presence of the *cagA* gene in strains predominantly in the corpus is a risk factor for DU (Regula, 306) and may influence the severity and pattern of gastritis in patients with nonulcer dyspepsia (NUD) (Parente, 416). However, CagA seropositivity is not necessarily associated with a higher prevalence of dyspeptic symptoms (Pretolani, 125), so that one cannot base a decision to treat the dyspepsia with eradication therapy on the patient's CagA status.

The gene encoding *vacA* is present in nearly all cytotoxic and noncytotoxic strains of *H pylori*. Strains with *vacA* m1 alleles are more cytotoxic than those with m2 alleles, and within the m1 group those with s1a alleles are more cytotoxic than those with s1b (7). However, *vacA* genotype may correlate poorly with clinical presentation and does not reliably predict strain virulence or clinical outcome (Go, 404).

The *cagA*-positive phenotype may be a virulence marker for PUD, independent of the *vacA* genotype or the presence of *vacA*/vacuolating cytotoxin activity (Takata, 408). While the reasons for and significance of this are unknown, extensive genomic diversity can be identified via PCR-restriction fragment length polymorphism within one geographic region (Wolle, 147). The stomachs of *H pylori*-infected patients can be colonized by mixed populations; repetitive sequence-based PCR has shown that DU patients may har-

bour more than one *H pylori* genotype (Dore, 17). Similar genotypes can have identical antibiograms. There is no evidence that decreased intragastric acidity caused by dosing with omeprazole results in a markedly increased diversity of *H pylori* strain type (Owen, 18).

H pylori exists in an actively dividing spiral form as well as in a coccoid form (a form of growth arrest). Anaerobic stress, such as passing through the alimentary tract, may induce transformation from the spiral to the coccoid form (Shirai, 29). The early coccoidal forms maintain significant intracellular ATP levels and may represent the viable but nonculturable form of *H pylori* (Nilsson, 22). These coccoid forms may have increased cell wall resistance, leading to greater difficulties in detection when samples are prepared for PCR by boiling water (Song, 149). An electron microscopy study identified coccoid forms in 52% of patients when gastric mucosal biopsies were examined; however, of all bacteria observed, only 6% were coccoid. A minority of infiltrating bacteria (7%) were coccoid and the rest were spiral, and none of the coccoid forms showed adhesion pedestals (Neri, 399), which highlights the low pathogenic potential of coccoid forms.

HOST RESPONSE TO H PYLORI INFECTION

The binding of chemokines such as IL-8, secreted basolaterally by gastric epithelial cells to proteoglycans in the lamina propria, generates chemotactic gradients for directional neutrophil migration. *H pylori*-triggered secretion of IL-8 in cultured Kato III gastric epithelial cells is mediated by the NF- κ B signal transduction pathway and requires the presence of genes located in the Cag pathogenicity island (Pahl, 48). *H pylori*-infected mucosa is a major source of locally released IL-8, IL-1 β , TNF α and IL-12, and eradication decreases the luminal release of these cytokines (Konturek, 79).

IL-8 activity and somatostatin (SOM) levels in gastric antral mucosa are inversely related (Konagaya, 82). Mean tissue concentrations of IL-8 and IFN α are higher in those with *H pylori* infection, regardless of whether the patient has DU or NUD (Abbiati, 93). Serum TNF α levels are also increased with *H pylori* infection, and the levels are unrelated to the patient's sex, age or endoscopy findings (Porri, 108).

Strain heterogeneity may contribute to the severity of the gastric inflammatory response. Continued antigen exposure results in the generation of specific T and B cell responses. In chronic infection of adults with *H pylori*, a T helper type 1 (Th1) cell-mediated response predominates, with the IFN α produced by the Th1 cells contributing to the changes in epithelial phenotype and function. IFN α is a typical Th1 (proinflammatory) cytokine, and IL-4 is an important Th2 (anti-inflammatory) cytokine. A mixed Th1/Th2 type response may be required for effective immunization against *H pylori* (Radcliffe, 213).

H pylori infection affects the motility of the stomach; *H pylori* infection in DU is associated with increased gastrin release due to a deficiency of SOM release by endogenous

cholecystokinin (8). *H pylori* infection in DU patients is associated with accelerated gastric emptying, and the deficient release of SOM in *H pylori*-infected persons can be reversed by the eradication of *H pylori*, returning the accelerated transit to normal (Konturek, 49). On the other hand, in *H pylori*-infected NUD patients, eradication of *H pylori* increases the gastric emptying of solids, increases the antral postprandial motility index, increases the amplitude of postprandial electrical oscillations detected on electrogastrographic recordings, reduces postprandial plasma gastrin concentration and increases luminal release of SOM (Konturek, 50). The promotility benefit of eradicating *H pylori* infection may offer one explanation for the symptomatic benefit of eradication therapy in some persons with NUD infected with *H pylori*.

GASTROESOPHAGEAL REFLUX DISEASE AND *H PYLORI*

In a previously reported study, with over three-years' follow-up, 25.8% of *H pylori*-eradicated DU patients developed endoscopically proven esophagitis, compared with 12.9% who remained positive (9,10). At the reported meeting, little new data supported the hypothesis that gastroesophageal reflux disease (GERD) develops in ulcer patients following *H pylori* eradication therapy. In an Italian study that excluded patients with baseline esophagitis, only 4.9% of DU patients developed esophagitis after *H pylori* eradication in one year of follow-up (Di Mario, 358). In a study from the United States, half of *H pylori*-infected DU patients had heartburn before eradication therapy, and new symptoms of heartburn developed in 17% in six months (Vakil, 464), independent of *H pylori* status.

Although the role of *H pylori* in GERD remains controversial, there were more data presented at the meeting that showed no increased risk of developing GERD following *H pylori* eradication therapy. Overall *H pylori* prevalence was the same in GERD and NUD patients. However, in a subset of GERD patients with hiatus hernia only or with esophagitis, the prevalence was less than that in NUD patients (Pieramico, 83). In prospective studies, about one in five patients with *H pylori*-positive DU have baseline GERD. Thus, symptoms after *H pylori* eradication may reflect pre-existing disease as opposed to de novo development (O'Connor, 196).

In other studies in DU patients, fewer persons developed heartburn after successful than after failed *H pylori* eradication therapy when they were followed for four weeks (MACH 2), eight weeks (HOMER) or 24 weeks (DU-MACH) (Unge, EHPSG). Thus, *H pylori* eradication in these studies improved, not worsened, heartburn in six months of follow-up.

Since the study by Kuipers, in which patients on long term proton pump inhibitors (PPI) had greater progression of atrophy if *H pylori* was present, there has been considerable interest in whether *H pylori* should be eradicated in such patients. In one study, 66 patients were treated with 40 to

60 mg omeprazole/day over a mean of 83 months. Chronic gastritis improved and there was lack of progression of intestinal metaplasia in the antrum, ie, no change to support an increased risk of GCA was identified (Lamberts, 197), so this topic remains controversial.

H PYLORI-NEGATIVE ULCER DISEASE

While it is generally believed that *H pylori* is the cause of almost all peptic ulcers in patients not taking nonsteroidal anti-inflammatory drugs (NSAIDs), more recent evidence is accumulating that there may be an increasing prevalence of *H pylori*-negative ulcers. An Italian study of 411 consecutive patients found that 9% of DU and 12% of GU patients were negative for *H pylori*, and NSAID use accounted for the cause of the ulcer in only about 30% (Di Baptist, 138). In Chinese children three to 14 years of age, 45% were *H pylori*-negative, suggesting that in children, an alternative mechanism is responsible for ulcer formation (Mitchell, 244). In a Brazilian study, 17% of DU and 61% of GU patients were *H pylori*-negative (Carvalho, 247). Among *H pylori*-negative DU patients, portal vein thrombosis, cryptogenic cirrhosis, Zollinger-Ellison syndrome and sickle cell anemia were seen. In *H pylori*-negative GU, NSAID use and cryptogenic cirrhosis were seen.

NSAIDs AND *H PYLORI*

The degree of initial mucosal damage due to NSAIDs is not affected by the person's *H pylori* status (11). The adaptation associated with improvement in the mucosal lesions despite continued use of NSAIDs is not impaired by the presence of an *H pylori* infection (12). In contrast, gastric adaptation to acetylsalicylic acid (ASA) intake is impaired in *H pylori*-infected healthy volunteers, and *H pylori* eradication restores this adaptation to normal (Konturek, 195).

Gastric sucrose permeability is a marker of damage to the gastric epithelium, and gastric mucosal permeability to sucrose increases in association with mucosal damage. The presence of *H pylori* infection does not change the gastric mucosal sucrose permeability at baseline or after ASA ingestion (13).

A prospective study has shown that neither the rate of healing nor the rate of recurrence of ulcers by NSAID users is improved by *H pylori* eradication (14). An interesting study showed that NSAID ulcers were healed more effectively by omeprazole if the patient was *H pylori*-positive, but misoprostol was more effective in healing *H pylori*-negative erosions (Hawkey, 465).

GCA AND LYMPHOMA

In developed countries, GCA ranks as the fourth most common cancer, representing 8.5% of all cancers. Worldwide, GCA is the second most common cancer (after lung cancer), with over a million new cases diagnosed each year, largely in Asia (15). The highest rates (over 40 per 100,000) are re-

ported in Southeast Asia, where the rate is four times greater than that in countries such as Canada and the United States. The risk of GCA increases with age and is higher in men than in women, and in lower socioeconomic groups. Salty and preserved foods have been consistently incriminated as increasing the risk of GCA, with high consumption of fruit and vegetables deemed protective (16). *H pylori* infection is associated with an increased risk of distal GCA, including both the diffuse and intestinal types (Babiak-Vazquez, 183A). This risk varies from country to country, with odds ratios varying from 2.8 (Forman, 91) to 6.0 (Nomura, 91); for example, in Sweden the odds ratio is 5.0 (Simon, 163).

The risk of proximal GCA (cardia) is less than the risk of distal GCA in *H pylori*-infected Europeans (Hansen, 162). *H pylori* colonizing patients with GCA and DU exhibit a similar and increased pattern of virulence (*cagA*- and/or *vacA*-positive) compared with patients with esophageal cancer or with controls (Grimley, 184). It is not known whether GCA can be prevented by dietary supplementation with antioxidant vitamins or eradication of *H pylori*.

Precancer lesions carry a higher risk of malignant transformation, and include histological markers such as chronic atrophic gastritis, intestinal metaplasia and dysplasia. Biomarkers for malignant transformation include mucin, Lewis antigens, pepsinogen levels and polymorphism of antioxidant enzymes. There may be carcinogenic strains of *H pylori* based on genotypic characteristics.

Nitric oxide mediates endothelial relaxation and neurotransmission. At high concentrations it may be cytotoxic and cause DNA damage. *H pylori* infection induces inducible nitric oxide synthetase (iNOS) expression, especially in gastric biopsies from *H pylori*-infected persons with intestinal metaplasia and in the mucosa of stomach cancer patients (Riecher, 44). It remains to be established whether iNOS level may be used to assess the risk of GCA development.

Infection of the gastric mucosa in childhood may be an important risk factor for the development of GCA in adult life (17), and, therefore, prevention strategies must begin at a young age. It may be possible to immunize against *H pylori* via an attenuated live carrier in a mouse model (Corthesy-Theulaz, 209; Gomez-Duarte, 211). New *H pylori* vaccine candidate antigens have been identified (Radcliff, 212).

After *H pylori* eradication, the degree of GA improves but intestinal metaplasia remains unchanged (van der Hulst, 194). With long term use of PPI, chronic antral gastritis improves and intestinal metaplasia does not progress (Lamberts, 197). The presence of the CagA strain may be one of the risk factors (Perez-Perez, 181), but this is controversial. Also, the presence of the CagA strain may even be protective against esophageal and cardia cancers, while increasing the risk of noncardia GCA (Chow, 120). Individuals with the so-called small MUC1 genotype may be at risk of developing GCA (Carvalho, 141). Perhaps this genotype could become a marker to select patients at highest risk.

Neoplastic cells of mucosa-associated lymphoid tissue (MALT)-lymphoma arising in the gastrointestinal tract re-

veal a pattern of somatic mutations in the rearranged immunoglobulin (Ig) H V genes characteristic of antigen-driven affinity maturation. Primary large B cell lymphomas of the gut are likely related to MALT-lymphoma (Dirsson, 166). Although many (79%) of these lesions may be cured by *H pylori* eradication, long term follow-up is needed to ensure that relapse has not occurred (Morgner, 165; Driessen, 172) or that the lesions have not regressed into a high grade, diffuse large cell lymphoma.

PEDIATRIC ISSUES

The role of *H pylori* infection in recurrent abdominal pain (RAP) in childhood remains controversial (Tindberg, 235). Children with RAP may be infected more frequently with *H pylori* than healthy controls, but these *H pylori*-infected children have lower IgG and IgA CagA responses than healthy children (Torres, 230). Other studies have shown that there are nonspecific symptoms associated with *H pylori* infection and that infected children have fewer abdominal symptoms ($P=0.095$) than noninfected children (Bode, 231). Eradication of *H pylori* in children with RAP does not improve the child's symptoms after six months of follow-up (Wewer, 233).

H pylori infection is associated with chronic gastritis, but the inflammatory infiltrate is different in children from that in adults; at endoscopy in *H pylori*-infected children, a nodularity is often seen. The positive serological response in children to an ELISA may vary from that seen in adults and needs to be standardized using children's sera. Although simpler to administer, serological tests are less reliable than the ^{13}C -urea breath test (UBT) in children (Corvaglia, 246). The ^{13}C -UBT is 100% sensitive and 97.6% specific in the diagnosis and follow-up of *H pylori* infection in children (18). The methodology of performing a ^{13}C -UBT in children is becoming clearer (Klein, 239; Kindermann, 241), and a larger dose of ^{13}C -urea may be needed in young children to improve discriminatory power (Botems, 245).

DIAGNOSIS

There are advantages and disadvantages, with variations in sensitivity, specificity and cost, to each of the many methods available to diagnose *H pylori*. Serological tests are easy to perform but because they are sent to a laboratory, there are delays in diagnosis. Also, not all test kits perform equivalently (Marchildon, 316), and any test kit must be validated locally to ensure accuracy. Whole blood serological tests have been developed to permit rapid noninvasive diagnosis in an office setting. The QuickVue One-Step *H pylori* Test (Quidel Corporation, California) performed as well as laboratory-based serology, but false positive results were obtained in 15% (Williams, 293). Another study evaluating Pyloriset Screen (Orion Diagnostica, Finland) at 10 and 30 mins found low accuracy of about 60% (Leodolter, 297). PCR uses cumbersome gel electrophoresis, making it difficult to use in routine laboratories. PCR may result in false

positive results from contamination due to its excessive sensitivity. New DNA enzyme immunoassays with improved sensitivity, quick results and ease of sample processing and interpretation are being developed (Monteiro, 262; Glupczynski, 268).

While *H pylori* infection diagnosed by ELISA or UBT gave infection rates of greater than 90% in 68 bleeding ulcer patients, the rates were lower when biopsy-based tests were used (culture: 37%, CLO: 63%, biopsy: 71%) (Lee, 274). This finding is not due to the effects of admixture with whole blood (Perry, 307).

There have been attempts to simplify the UBT, and the use of citric acid to delay gastric emptying instead of a test meal is superior in terms of $^{13}\text{CO}_2$ recovery, time requirement and cost (Manes, 254). Acidification of the test meal administered with the UBT may increase its sensitivity in adults and children (Ali, 2771; Menegatti, 284). A capsulated minidose (38 mg) ^{13}C -UBT tended to avoid false positivity of the initial phase of breath sampling and did not require a test meal. Results were rapidly positive by 10 to 20 mins (Bielanski, 270). Ranitidine in doses of 150 or 300 mg bid did not adversely affect UBT results (Cutler, 310).

At issue is the high cost of the mass spectrometer needed to analyze ^{13}C -UBT samples. A more economical method of analyzing UBTs with a laser-assisted ratio analyzer was found to have sensitivity and specificity of 92%, even after two to three months of storage (van der Hust, 261).

After eradication treatment, the optimum method of *H pylori* detection remains unclear. A single ^{13}C -UBT at four to six weeks was 94% sensitive; if a second UBT was repeated at three months, the sensitivity rose to 97% and the specificity to 99% (Godfroid, 276). In another study in which patients were re-evaluated at four weeks with histology, culture and urease tests, both the ^{13}C -UBT test and the ^{14}C -UBT test were 98% sensitive and 100% specific, indicating the adequacy of a single UBT to define eradication (Menegatti, 284). If a patient has a UBT value close to the cut-off, the test should be repeated to avoid false negative results (Fraser, 260) or alternative endoscopic means should be used to determine whether there is an *H pylori* infection (Vazquez, 288).

ANTIBIOTIC RESISTANCE

Standardized testing methods to assess in vitro resistance of antibiotics to *H pylori* need to be established. The break point values of resistance need to take into account that multiple drug therapy is administered to treat *H pylori* infection and that the clinical bacterial correlates with in vitro sensitivity need to be established. While culture is necessary for antimicrobial susceptibility testing, routine culture of all biopsies is cumbersome. It is possible to culture *H pylori* successfully from the biopsies used in urease tests. Thus, only positive samples, ie, samples with persistent infection, will need to be processed (Rautelin, 319). A rapid new easy to perform flow cytometry method for assessing antibacterial

susceptibility gave susceptibility results after only 4 h of incubation (Best, 6).

Resistance to metronidazole is the major determinative factor in the failure of eradication therapies containing this antibiotic. The action of metronidazole in the metabolism of anaerobic bacteria is well established. In the microaerophilic *H pylori*, two genetic loci have been cloned that appear to be responsible for metronidazole resistance (Goodwin, 1). Metronidazole resistance may also be related to insufficient reduction of metronidazole to its active form (Jorgensen, 332). Mechanistically, it was proposed that the reduction in nicotinamide adenine dinucleotide oxidase activity results in increased levels of intracellular oxygen and, thus, increased futile cycling (Hughes, 3). These increases in intracellular oxygen reduce the activity of two oxidoreductases – pyruvate and oxoglutarate – (Hughes, 3) that may be essential enzymes for *H pylori* metabolism, permitting its survival in microaerophilic environments (Hughes, 8). However, another study concluded that futile cycling does not play a key role in *H pylori* killing (Elsberger, 26).

Macrolide (clarithromycin) resistance is due to adenine to guanine transition mutations in one or both of the 23S rRNA genes (Hulten, 20; Sevin, 25). One group showed that after treatment failure with PPI + amoxicillin + clarithromycin, clarithromycin resistance is frequently seen (69%), and that 42% are resistant to both clarithromycin and metronidazole; resistance is clearly a significant factor for deciding further therapy (Sevin, 25). Ten amoxicillin-resistant *H pylori* strains were isolated in Sardinia, Italy from patients treated with amoxicillin (Dore, 14). It is considered that amoxicillin resistance is rare.

DYSPEPSIA AND *H PYLORI* INFECTION

In perhaps no other area in 'Helicobacteriology' is there more controversy than in the discussion of whether *H pylori*-associated gastritis causes symptoms and whether there are any short or long term benefits of *H pylori* eradication. Dyspepsia is a very common problem with 40% prevalence in western society and high societal cost; more than half of all dyspepsia patients take medication, over 20% have visited their family physician during the previous year on a dyspepsia-related matter and 2% have been off work due to dyspepsia (19).

In managing patients with dyspepsia, it is important to differentiate those at first presentation who have undiagnosed dyspepsia from those who after investigation will have a diagnosis such as DU or NUD. A New Zealand family practice management study in which dyspeptic patients were provided with easy access to UBTs and in which patients with positive results were encouraged to have endoscopy, peptic ulcer was found in 47%. For those with negative results, fewer endoscopies were done, although some patients were dissatisfied and would have preferred to have had endoscopy (Fraser, 280).

The association of *H pylori* with dyspepsia subgroups is unclear. In a study of patients with functional dyspepsia,

H pylori positivity was associated with both ulcer- and reflux-like dyspepsia (Perri, 133). However, in another study, no association or discrimination of dyspepsia subgroups with *H pylori* infection was seen (Werdmuller, 153). In young patients with dyspepsia, symptom severity, quality of life scores and personality profiles were independent of *H pylori* status (Heaney, 473). Curiously, symptomatic improvement of NUD patients occurred with a bismuth-based therapy, independent of whether *H pylori* was eradicated (Phillips, 346).

The Maastricht Consensus Report (19) enunciated a summary of the indications for *H pylori* eradication therapy, the level of the recommendation, and the strength of the supporting evidence. A management strategy that considers the role of *H pylori* in dyspepsia offers symptomatic relief, aids in the selection of patients for further investigation and favours immediate logical empirical treatment (Greenaway, 474).

The algorithm for the management of dyspepsia patients is moving towards a strategy of recommending that the patient be tested for *H pylori* infection if they are younger than 45 years old and have no alarm symptoms. The usefulness of noninvasive *H pylori* testing instead of endoscopy depends on the prevalence of infection and the range of disorders causing dyspepsia in a given population. The frequency of significant non-*H pylori*-related abnormalities in the physician's practice needs to be known in order to appreciate better the threshold for performing endoscopy. If the prevalence of *H pylori* infection in dyspeptics is 25% and if 10% have PUD, then this 'test-and-treat' strategy is cost-effective (20,21). Treatment results in the community may be altered by compliance, but the results of effectiveness studies also support this approach (Vakil, 321; Johnston, 334).

In an open-access ¹³C-UBT service offered to dyspeptics younger than 40 years old who were not taking NSAIDs, *H pylori*-positive patients were offered omeprazole-based triple therapy, and only 4% were re-referred for endoscopy (Moayyedi, EHPG). This approach has translated into a 34% reduction in the number of patients younger than age 40 being referred for open-access endoscopy. In Ireland this approach reduced esophagogastro-duodenoscopies in this group of patients by 54%, and 90% of subsequent esophagogastro-duodenoscopies were normal (Heaney, 342). Thus, if a dyspeptic is *H pylori*-positive, then eradication therapy should be provided without first performing an endoscopy.

The risk of a missed GCA is always raised when any treatment algorithm is suggested, based on a nonendoscopic first approach. A Scottish retrospective study showed that upper gastrointestinal malignancy rarely presents as dyspepsia without alarm symptoms in people younger than 55 years old, but when this is the presentation the GCA is already too advanced for cure (22). Based on these data, the Canadian *H pylori* Consensus Conference concurred with this agreement (23).

In the United States, it is not worth using endoscopy and pretherapy antibiotic sensitivity testing over UBT as an initial diagnostic tool (Brewer, 461).

TREATMENT REGIMENS – GENERAL

A meta-analysis confirmed that prolonged acid suppression is unnecessary to achieve ulcer healing (Treiber, 336), and one-week PPI triple therapy is quite adequate (Gisbert, 325). Eradication of *H pylori* led to faster ulcer healing in patients with persistent *H pylori* infection (Gisbert, 325; Huang, 339). With omeprazole-based triple therapies, eradication efficacy did not vary as a function of age, or between patients with NUD versus peptic ulcer (Peramico, 333). A small randomized controlled trial of *H pylori* eradication versus placebo in patients with previous vagotomy and persistent symptoms found no improvement in symptoms after one year of follow-up (Lindsetmo, 460).

PPI triple therapies: PPI triple therapies include omeprazole + metronidazole + clarithromycin (OMC); omeprazole + amoxicillin + clarithromycin (OAC); omeprazole + amoxicillin + metronidazole (OAM); pantoprazole + clarithromycin + metronidazole (PCM); pantoprazole + clarithromycin + amoxicillin (PCA); etc. With OMC or OAC, high and consistent eradication rates were seen in the MACH 2 trial in patients with a history of DU (Lind, 324); in the GU-MACH trial of active GU patients (Malfertheiner, 356); and in the DU-MACH trial of active DU patients (van Zanten, 381). Similar results are obtained with PCM or PCA for one week, yielding eradication rates of over 90% (Frevel, 379). Using OAM reduces efficacy, despite increasing the omeprazole dose from 20 mg to 40 mg daily (Schotze, 361).

PPI + clarithromycin + metronidazole: In the MACH 2 study (Lind, 324), patients given metronidazole + clarithromycin alone achieved an *H pylori* eradication rate of only 72%, but when omeprazole was added, the rate improved to 91%; with the combination of amoxicillin + clarithromycin, the eradication rate was 25%, which improved dramatically to 95% when omeprazole was added. In the presence of metronidazole resistance, the success of the antibiotics alone, ie, metronidazole + clarithromycin, falls from 86% to 43%, but with OMC the eradication rates were 95% for metronidazole success and 76% for metronidazole resistance, indicating that omeprazole helps to overcome antibiotic resistance (24). Another study reported 90% eradication success in patients with primary metronidazole-resistant strains when treated with OMC (Jaup, 144). The addition of a PPI may be important because of its ability to affect gastric pH, as was shown in one in vitro study in which the susceptibility of clarithromycin-resistant *H pylori* was increased by omeprazole (Hyde, 115). In a double-blind, randomized, multicentre study of pantoprazole 40 mg bid with metronidazole + clarithromycin, seven- and 14-day treatment courses gave similar rates of eradication (Dammann, 349). After treatment failure, 13 of 13 failures developed metronidazole resistance and 12 of 13 developed clarithromycin resistance (Kist, 328).

PPI + amoxicillin + clarithromycin: In one report, OAC successfully eradicated metronidazole-resistant *H pylori* (Georgopoulos, 357) with no acquired clarithromycin resis-

TABLE 1
Diseases possibly linked to *Helicobacter pylori* infection

Disease	Reference(s)
Primary Raynaud's phenomenon	25,26
Headache	26
Autoimmune disease	
Schönlein-Henoch purpura	27
Thyroiditis	28
Skin diseases	
Rosacea	29
Chronic urticaria	30
Alopecia aerata	31

tance. Triple therapy using lansoprazole with amoxicillin + clarithromycin for longer durations of 10 and 14 days gave intention-to-treat (ITT) eradication rates of 86% to 88% (Krause, 373). For patients with chronic gastritis, OAC for 12 days was more effective than for treatment for six days (Hermida, 344). For OAC and OAM, longer 14-day treatment was more effective than that for seven or 10 days (Katicic, 367). Thus, unlike treatment with OMC where one week of treatment appears adequate, OAC administration may require a longer duration of therapy.

There is a higher eradication rate with higher doses of lansoprazole (ie, 30 mg bid versus 15 mg bid: ITT 71% and 83%, respectively) when combined with amoxicillin and clarithromycin, indicating that higher doses of acid suppression therapy are more efficacious (Buda, 335). When pantoprazole is used at the lower dose of 40 mg once daily, eradication efficacy is reduced compared to that of 40 mg bid used for one week in combination with clarithromycin and amoxicillin (Lamouliatte, 340). However, triple therapy for a further two weeks with lansoprazole 30 mg once daily and amoxicillin + clarithromycin can result in high eradication rates (Yang, 362). Thus, longer duration of therapy may be important with once-daily PPI dosing.

Other PPI triple therapies: The Bazzoli regimen of omeprazole, clarithromycin and tinidazole was compared with triple therapy comprising one week of lansoprazole in combination with azithromycin once daily and metronidazole for three days; eradication success with both was over 90% (Caselli, 376). However, another study using a similar three-day regimen had only 39% eradication (Seelis, 378).

PPI quadruple and quintuple therapies: Quadruple therapies have been proposed for first-line therapy. One such therapy using the PPI lansoprazole with bismuth + metronidazole + tetracycline (BMT) for four days achieved an eradication rate of 86%, including five of eight patients with metronidazole resistance (de Boer, 370). There were no drop-outs due to side effects. The same regimen given over a weekend was not sufficiently effective (de Boer, 355). These PPI-BMT

regimens for one week appear to overcome, to some extent, metronidazole-resistant *H pylori*.

A new quadruple therapy combining amoxicillin + metronidazole + omeprazole + clarithromycin for five days was as effective as seven-day OMC (Treiber, 252). A quintuple therapy was also reported! This combination used lansoprazole, colloidal bismuth subcitrate, achromycin V, metronidazole and roxithromycin to achieve 92% eradication (Daskalopoulos, 390). Side effects were not mentioned.

Ranitidine bismuth citrate-based double or triple therapy:

Ranitidine bismuth citrate (RBC) is the first new drug designed specifically for treating *H pylori* infection. RBC combines the antisecretory activity of ranitidine with the mucosal protective and anti-*H pylori* effect of bismuth. At this meeting, it was reported that, using RBC and clarithromycin (RBC-C) dual therapy, ITT eradication rates varied from 74% to 87% (Gudjonsson, 363; Cardelli, 350; Mégraud, 337). A drawback of RBC-C is that it is approved as a two-week treatment regimen. One study showed that *H pylori* eradication with RBC-C was as effective in seven days as in 14 days, although the ITT eradication success was only 75% to 80% (Cardelli, 350). In another study, patient results after using RBC-C for 14 days were comparable with those after using RBC-C + metronidazole for 7 days (ITT eradication of 74 to 79%), but withdrawals due to adverse events were high: 5% to 6% (Gudjonsson, 363). Thus RBC dual therapy may be as effective as triple therapies. Triple therapies with RBC + metronidazole + clarithromycin or with RBC + amoxicillin + clarithromycin for two weeks gave eradication rates of 82% and 92%, respectively (Laine, 382). In vitro studies show that the combination of RBC-C is effective against clarithromycin-resistant *H pylori* strains (Midolo, 31; Osato, 386). RBC-C may be able to overcome clarithromycin resistance; in one study, 11 of 12 'resistant' *H pylori* were successfully eradicated and only 3% acquired resistance after treatment (Mégraud, 337).

PPI-based triple therapy failures: When OAM fails for the first time, OAC is 83% effective in eradicating *H pylori* (Gisbert, 341). When OMC fails, a course of OAC is better than omeprazole with BMT, particularly if the *H pylori* is clarithromycin-sensitive and metronidazole-resistant (Peitz, 385). PPI plus BMT was recommended in the 1977 Maastricht report for treatment failures. Results are inconsistent and range from only 72% effective in one small study of 18 patients (Goddard, 353) to 93% effective in another study of 30 patients (Huelin Bénitez, 388). Rescue therapy with RBC, minocycline and amoxicillin for two weeks also shows promise (Cudia, 380).

Quadruple therapy with omeprazole, colloidal bismuth subcitrate, azithromycin and amoxicillin for two weeks gave good eradication rates (per protocol [PP]: 81.3%, ITT: 76.7%) in treatment failures (Coelho, 343). However, side effects were very frequent. Omeprazole + bismuth + metronidazole + amoxicillin, or RBC-C + tinidazole for two weeks are alternatives, although not highly effective (PP

eradication: 86%, ITT: only 60%) (Kusstatscher, 359). After treatment failure to OAC, 75% of isolates resistant to both metronidazole and clarithromycin were successfully treated with one week of omeprazole, amoxicillin and ciprofloxacin (Lamarque, 383).

New therapies: Oral hyperimmune bovine colostrum-derived IgG was not useful for *H pylori* eradication (Opekun, 366). NE-0080, an oligosaccharide postulated to disrupt *H pylori* adhesion, was also ineffective (Opekun, 375). Triple therapy of zinc citrate, amoxicillin and metronidazole was reported to be 75% effective (Phillips, 368). The new drugs nitazoxanide and tizoxanide (nitrothiazoles with similar properties to metronidazole) do not appear to be affected by pH or the development of resistance, and may be promising regimens for the future (Mégraud, 369).

MISCELLANEOUS H PYLORI ASSOCIATIONS

***H pylori* infection and ischemic heart disease:** *H pylori* infection may (Elizalde, 60) or may not (Vakil, 61) be associated with platelet activation, which plays a role in ischemic heart disease. Of the hemostatic parameters assessed, von Willebrand factor antigen was highly associated with *H pylori* infection (Cernuschi, 463). The prevalence of *cagA*-positive *H pylori* was higher in patients with coronary stenosis than in controls (Cammarota, 462).

There is a growing list of possible diseases associated with *H pylori* infections (Table 1). For example, primary Raynaud's phenomenon is associated with *H pylori* infection, and eradication resolves or improves symptoms in 90% of those patients (Gasbarrini, 70). Migraine-type headaches are associated with *H pylori* infections, independent of the presence of gastrointestinal symptoms (Gasbarrini, 157). *H pylori* eradication did not improve joint pain or swelling in patients with rheumatoid arthritis (Graff, 477).

***Helicobacter* other than *H pylori*:** While *H pylori* is clearly the most important human helicobacter pathogen, much work at the meeting was presented about other *Helicobacter* species that may infect humans. The first anaerobic helicobacter was identified in a mouse model (Robertson, 449). An interesting study demonstrated that *Helicobacter bilis* caused inflammatory bowel disease in severe combined immunodeficiency mice (Shomer, 450). Some Chilean patients with chronic cholecystitis were found to harbour a *Helicobacter* species, either *Helicobacter rappini* or *Helicobacter pullorum* (Fox, 451). *Helicobacter heilmannii* was identified in a man and his two cats (Dieterich, 453).

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