Review Article

Advances in the Diagnosis and Therapy of *Helicobacter pylori*

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Abstract

Background: *Helicobacter pylori* infection infects approximately 50% of the world's population, and is associated with significant morbidity and mortality. *H. pylori* infection is associated with large expenditures for diagnostic testing and prescription medication.

Summary: The C13-urea breath test remains the non-invasive test with the highest sensitivity and specificity, and is the test-of-choice to confirm eradication following treatment. Although the stool antigen test is an acceptable alternative, care should be taken to only use kits with a documented accuracy >90%. Due to the increasing resistance to clarithromycin, the efficacy of standard clarithromycin-based triple therapy is now below 75%. Current guidelines recommend that such treatment only be used in regions where *H. pylori* resistance to clarithromycin is below 20%. Concomitant therapy and bismuth-based quadruple therapy yield superior eradication rates compared to standard triple therapy and sequential therapy. First line therapy tailored to cytochrome P450 2C19 (CYP2C19) phenotype and clarithromycin susceptibility may increase treatment success, however large, randomized controlled trials are lacking. Salvage therapy with fluoroquinalones is effective in about three quarters of patients, and is superior to bismuth-based quadruple therapy in this setting.

Key messages: Clinicians must consider local antibiotic resistance patterns before choosing a particular regimen. In the absence of such information, clinicians should balance the need for a prolonged four-drug regimen against adverse effects and costs.

INTRODUCTION

H. pylori are a ubiquitous, gram negative, flagellated, spiral organism which survives in the mucous layer overlying the gastric mucosa. Chronic infection with *H. pylori* leads to degradation of the mucosal defense systems through several mechanisms: modulation of gastric mucus viscosity through the secretion of urease and the expression of BabA and SabA adhesins which bindmucin, direct epithelial damage caused by the pore-forming vacuolating cytotoxin A (VacA) and degradation of the stomach's acid secretory capacity [1]. Gastric hypochlorhydria is achieved byactivation of neutrophils and monocyte to produce IL-1β and TNF-α, both of which are potent inhibitors of gastric acid, and through the action of *cag* pathogenicity island gene products which suppress transcription of the H+,K+-ATPase αsubunit (HKα) [1]. *H. pylori* infection typically causes a superficial gastritis which is usually asymptomatic. However, in some patients, *H. pylori* may cause significant morbidity and mortality, including both benign and malignant disease. For this reason, there are ongoing efforts to increase the accuracy and acceptability of non-invasive diagnostic tests for *H. pylori*. At the same time, resistance of *H. pylori* to antibiotics has made treatment a challenge. Efforts to increase treatment efficacy, such as novel drug combinations

and prolonged treatment duration, must be balanced against treatment-related adverse effects.

EPIDEMIOLOGY

Prevalence of *H. pylori* infection

H. pylori is present in up to 50% of the world's population with the highest prevalence in developing countries. In Europe, asymptomatic *H. pylori* infection remains common, with 32% seroprevalence in a recent Dutch population-based study [2]. However, *H. pylori* seroprevalencehas decreased from 48% among subjects born between 1935 and 1946 to16% among those born between 1977 and 1987.Children born in the Netherlands have a seropositivity of only 10% and in Belgium, only 3.2% [3, 4]. *H. pylori* in Western and Northern Europe is less common than in the Southern and Eastern continent, where prevalence may exceed 80% in asymptomatic individuals [5].

Data from the National Health and Nutrition Examination Survey (1999-2000) estimate that the overall prevalence of asymptomatic *H. pylori* infection in the United States is 30.7% [6]. Recent data emerging from Texas found that asymptomatic *H. pylori* seropositivity is highest among 41-60 year olds (24%)

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and lowest among 0-20 year-olds (6%) [7]. Verma et al., reported an overall prevalence of 23.7% among subjects undergoing gastroscopy prior to bariatric surgery between 2001-2009 [8]. Interestingly, the risk of *H. pylori* in immigrants decreases with each successive generation born in the United States [9].

A recent study performed in the indigenous population of Canada found *H. pylori* in 37.9%of patients undergoing gastroscopy for dyspepsia. Although this is high compared to patients in urban settings, this rate of *H. pylori* infection is still lower than previous estimates 10.In a survey of 1852 healthy subjects in Latin America, *H. pylori* positivity by C13-urea breath test (UBT) exceeded 70% 11.

Risk factors for infection

Living in a rural area, a crowded home and having a contaminated source of drinking water have been consistently linked to *H. pylori* infection [5,12,13]. The National Health and Nutrition Examination Survey (NHANES) found that self-reported poor general health was associated with seropositivity [14]. Furthermore, the study found that in young patients infection is associated with having a well as the source of tap water (OR 1·7, 95% CI 1·1-2·6) and living in crowded housing conditions (OR 2·3, 95% CI 1·5-3·7).

The Southern Community Cohort Study performed in 12 southern US states, identified African ancestry, education, employment and house values as being associated with *H. pylori* CagAsero prevalence [15]. American adults in soil-related occupations had significantly higher rate of infection compared to those in non-soil-related occupations (OR 1·9, 95% CI 1·2-2·9) [14]. Studies in other geographical regions have also consistently linked low socioeconomic status with *H. pylori* infection [2,16]. Most studies do not identify smoking or alcohol consumption as risk factors for infection [16,17]. In New York, colonization with H. pylori was associated with a 50% reduction in the odds of being obese (adjusted OR = 0.5, 95% CI = 0.2-1.0) [18]. However, paradoxically, eradication of *H. pylori* may lead to weight gain, a process thought to be mediated by the hunger-inducing hormone, ghrelin [19]. Gender and age do not seem to be major risk factors for *de novo* infection. A higher prevalence of *H. pylori* in older age groups is likely a reflection of the declining *H. pylori* prevalence in many geographical regions.

Transmission

The most likely route of transmission for *H. pylori* is interpersonal, involving either fecal-oral or oral-oral spread. Intra familial transmission of *H. pylori* has been reported using multilocus sequence typing of DNA [20]. Mother-to-child transmission is the most likely route, and has been shown to occur more commonly than father-child or sibling-sibling transmission [21]. Urita et al., implicated grandmother-child transmission in a Japanese cohort [22]. A meta-analysis incorporating 16 studies found a moderate association between *H. pylori* infection and childcare attendance (OR 1.12; 95% CI 0.82-1.52; I=77.4%), although the studies included were heterogeneous [23].

Environmental exposure from contaminated water sources may also occur. *H. pylori* have been demonstrated in both still and running water sources [24].

Recurrence

Recurrence of *H. pylori* may represent either re-infection or recrudescence of the organism. While both are rare events in Western countries, studies discriminating between the two forms of recurrence, are lacking. Bacterial recrudescence is more likely within 4 weeks of eradication, and may be associated with a) premature assessment of eradication following treatment, b) sampling error in the presence of a low bacterial load (for example in the setting of intestinal metaplasia) or c) continued treatment with proton pump inhibitors (PPI) or bismuth salts.

A meta-analysis including 5085 patients in 17 studies followed for up to 60 months found that the annual recurrence of *H. pylori* in developed countries was only 2.67%. In developing countries, however, annual recurrence reached 13.0% [25]. Since the publication of this meta-analysis, additional studies have been published. A recent systematic review including16, 827 patients in 77 studies found a pooled recurrence rate of 2.8% per patient-year [26]. Low socioeconomic status was associated with an increased risk of recurrence, suggesting re-infection rather than bacterial recrudescence. In certain demographics, however, *H. pylori* recurrence may be significantly higher. In the indigenous population of Northwest Canada, *H. pylori* recurrence was 4.7% during follow-up [27], and in Latin America *H. pylori* recurrence was seen in 11.5% of cases followed for up to 1 year following successful eradication [28].

Burden of Disease

H. pylori is associated with various malignant and non-malignant diseases. A discussion of the role of *H. pylori* in the pathogenesis of these diseases is beyond the scope of this review. Malignant diseases associated with *H. pylori* include intestinal-type gastric adenocarcinoma and gastric MALT-lymphoma.

The non-malignant disease most strongly and frequently linked with *H. pylori* infection is peptic ulcer disease (PUD). The declining incidence of PUD has occurred in parallel to a decline in *H. pylori* infection rates. In areas of high *H. pylori* prevalence (for example, in Asia) *H. pylori* causes almost all uncomplicated duodenal ulcers and more than 80% of gastric ulcers, especially if the previous use of NSAIDs has been excluded. In low-prevalence areas such as the US, *H. pylori* accounts for a smaller proportion of PUD. Non ulcer dyspepsia may also be etiologically related to *H. pylori*. A recent systematic review identified 21 randomized controlled trials which have examined the efficacy of *H. pylori* eradication for the treatment of functional dyspepsia. Overall, *H. pylori* eradication is associated with a relative risk reduction of 10%, for dyspeptic symptoms, compared to placebo. The number needed to treat (NNT) to cure one case of dyspepsia is 14 [29]. Nevertheless, studies performed in Western populations have inconsistent results, with eradication having a variable impact on dyspeptic symptoms [30].

Gastroesophageal reflux disease is negatively associated with *H. pylori*. A systematic review including 20 studies and 4,134 patients found a lower prevalence of *H. pylori* in GERD subjects compared to controls (OR=0.60) [31]. Data from 1,611 African American patients with endoscopic evidence of esophagitis, found that the prevalence of *H. pylori* was 4%. After

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adjusting for age and gender, the odds ratio of *H. pylori* infection in erosive esophagitis was 0.06 [32]. A negative association is similarly observed between *H. pylori* and conditions which arise from GERD, including Barrett's esophagus and esophageal adenocarcinomas [33].

Patients with Crohn's disease (CD) have a disproportionately low prevalence of *H. pylori* [34]. The estimated relative risk of *H. pylori* infection in CD is 0.64. Subjects with *H. pylori* infection exhibit a blunted T_{h1}/T_{h17} immune response and have low tissue and serum levels of pro-inflammatory cytokines such as interferon-γ (IFN-γ). Similarly, *H. pylori* are able to influence the maturation and direction of host immune pathways. *H. pylori* infection can induce dendritic cells to generate regulatory T-cells (Tregs) which may subsequently protect against asthma. A meta-analysis of case-control and cross sectional studies by Zhou and co-workers including over 28,000 subjects across three continents, found a slightly lower rate of *H. pylori* infection in subjects with asthma (OR=0.84) [35].

H. pylori may have a role in the pathogenesis of idiopathic thrombocytopenic purpura (ITP). In a review of 16 studies including 1126 subjects with ITP, *H. pylori* prevalence was 64%. Following successful eradication, a platelet response was seen in 53%. However, the studies were heterogeneous, and mainly uncontrolled [36].

Although *H. pylori* have been associated with iron deficiency anemia in meta-analyses of observational studies, conclusive data from randomized controlled studies are lacking. There are no convincing data to support an association between *H. pylori* and halitosis, Alzheimer's disease, Parkinson's disease, Raynaud's phenomenon, scleroderma, idiopathic urticaria, acne rosacea, migraines, thyroiditis, and Guillain-Barré syndrome or coronary artery disease.

DIAGNOSIS OF *H. PYLORI*

Invasive tests (gastric biopsy required)

Endoscopic diagnosis: Endoscopic features of *H. pylori* infection may aid diagnosis and potentially obviate further diagnostic tests. A Japanese multicenter study of 275 cases found that the sensitivity and specificity of chromoendoscopy for the diagnosis of *H. pylori* infection in the gastric corpus, was 94.3% and 62.8%, respectively, compared to histology and serology [37]. Narrow band imaging (NBI) may predict *H. pylori* infection with sensitivity of 79% and specificity of 52% [38].

Rapid urease test (RUT): *H. pylori* has predilection to colonize the gastric antrum, but with time the organism may spread to the corpus, especially in patients receiving proton pump inhibitors. Combining antral and corporal specimens for RUT increases the diagnostic yield of the test by 5% [39].

Histology: After endoscopic mucosal resection of early gastric cancer it is important to test for *H. pylori* infection and to treat for eradication. In 91 patients, Lee et al., obtained 3 pairs of biopsies from the antrum, corpus lesser and greater curvature, and found a sensitivity of 30.3%, 47%, and 84.8% respectively [40]. They concluded that in cases with gastric atrophy the corpus greater curvature is the preferred site.

Culture: New blood culture bottles (BactectmPlus Anaerobic/F Medium, Beckton, Dickinson, Franklin Lakes, NJ, USA) may have a higher yield than agar plates [41]. The time to positive culture using this method was only 31.6h on average. Cryopreservation of *H. pylori* in gastric biopsies is possible for periods exceeding ten years, at -70⁰C 42.

Molecular methods: A peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) method has been applied to identify *H. pylori* infection in gastric biopsies with a sensitivity of 80% and a specificity of 93.8% [43]. A new fluorescence quantitative PCR was evaluated in 138 children and was found to be more sensitive than histology, RUT and UBT [44]. Nevertheless this test is resource-intensive and not widely available.

Non-invasive tests (gastric biopsy is not needed)

Serology: There have been recent efforts of French and German groups to improve the diagnostic yield of serological tests. Testing 29 commercial kits, only 3 were found to have excellent performance [45]. Although serology cannot reliably distinguish between current and past infection, it is the only diagnostic test whose sensitivity is unaffected by factors which reduce bacterial load, such as PPIs, antibiotics, bismuth, gastric bleeding and extensive intestinal metaplasia. Therefore, despite limited accuracy, serological assay remains and attractive option in various clinical scenarios. A sensitivity of 97.6% and specificity of 96.2% were demonstrated for serological testing of virulence factors such as CagA, VacA, GroEL, gGT, HcpC, UreA, and FliD [46,47].

C13-Urea breath test: The UBT is considered the non-invasive test with the highest sensitivity and specificity. In a study of 61,060 UBTs 21,767 were positive [48]. The male/female ratio for positive UBTs was 1:1·77. Delta over baseline results decreased as age increased from 38·6 ± 21 at age 3-5 years to 21·1 ± 12 at age 19-30 in females (P < 0·001) and from 30·0 ± 16 at age 6-10 years to 14·7 ± 8 at age 19-30 years in males (P < 0·0001). At this point, the values reached a nadir for both genders. In all age groups females had significantly higher UBT results than males.

Stool antigen test (SAT): The monoclonal SAT in considered accurate as UBT for primary diagnosis as well as for post-treatment evaluation of *H. pylori* infection, and also efficient in children [49, 50]. Unfortunately, several stool antigen tests that are available in the market, but not all of them have an acceptable diagnostic accuracy. Recently, only one out of 5 such kits had a sensitivity higher than 90% [51].

Stool DNA: DNA can be extracted from stool samples very efficiently using a new commercial kit (*H. pylori* ClariRes assay, Ingenetix, Vienna, Austria) and may then be tested using real-time PCR for *H. pylori* DNA and clarithromycin (CLA) susceptibility. The sensitivity and specificity were 69% and 100% and 83.3% and 100%, respectively [52]. The same method has been employed to tailor treatment in children, and was found to be as effective as traditional culture of gastric mucosa [53].

Clinical situations with indication for Helicobacter pylori eradication

Clarithromycin resistance: Molecular methods have been

recently used for the detection of *H. pylori* in the gastric mucosa. DNA is easily isolated from formalin-fixed, paraffin-embedded tissue, and PCR techniques applied for *H. pylori* identification and CLA resistance detection. CLA resistance was found in 50% of the cases by melting curve analysis by Oleastro et al., [54]. Interestingly, a heterogeneous population of non-mutated and mutated bacteria was detected in 21% of the cases. The most prevalent mutation is A2147G, but novel point mutations have been described [55]. In addition, lipopolysaccharide glycotyping found more α-1,6glucansin resistant strains [56].

Gastric cancer: A serology test (Helicoblot 2.1, Genelabs Diagnostics, Singapore) was able to identify VacA protein with a high risk of gastric cancer (OR = 2.7, 95CI 1-7.1) and 35KDa protein associated with a low cancer risk (OR = 0.4, 95CI 0.1-0.9) [57]. Detection of *H. pylori* infection in patients with gastric cancer by immunohistochemistry was associated with a 25% (9-month) decrease in survival [58]. Genotyping of the vacA intermediate gene region may be important for the prognosis of gastric cancer (OR=22) [59]. A novel primer for the amplification of smaller DNA fragments was successfully applied to paraffin-embedded tissue, and the diagnostic yield was similar to frozen gastric biopsies.

Bleeding ulcer: Free blood in the gastric lumen may interfere with accuracy of diagnostic tests that rely on biopsies. In 157 patients with active bleeding, histology had the highest sensitivity and specificity for *H. pylori* diagnosis (92.5% and 96%, respectively) [60]. Culture and serology had very low sensitivities for the detection of *H. pylori* (40% and 56%, respectively). RUT had a sensitivity of 85% and specificity of 92% in active bleeding, considerably lower than in patients without bleeding [60]. The sensitivity and specificity of UBT in the setting of active bleeding is only 86% and 66%, respectively [61]. Another study reported that the diagnostic modality with the highest sensitivity for *H. pylori* in patients with upper gastrointestinal bleeding treated with non-steroidal anti-inflammatory drugs, is culture [62]. Thus, the issue of *H. pylori* diagnosis in the setting of acute bleeding is still controversial.

Post gastric surgery: *H. pylori* diagnostic methods were evaluated by meta-analysis in patients with partial gastrectomy[63]. The pooled sensitivities and specificities were 79% and 94% for RUT, 77% and 89% for UBT and 93% and 85% for histology, respectively.

Post eradication therapy: UBT is considered the gold standard confirmatory test following treatment of *H. pylori*. It is necessary to discontinue PPIs, antibiotics and bismuth treatment for at least two weeks prior to UBT, and to perform the test no less than 4 weeks after the end of eradication therapy. Olafsson et al., evaluated 620 UBTs performed following treatment for *H. pylori*. 526 (84.8%) UBTs were negative. However, 23% of the negative results occurred in patients who did not discontinue one of the aforementioned drugs, and 45% underwent UBT less than 4 weeks before end of therapy [64]. Thus, protocol violation may have a significant impact on the reliability of UBTs and should be considered when interpreting these tests.

Guidelines recommendation for diagnostic tests: Several guidelines on the management of *H. pylori* infection have been published in the last decade, and most do not recommend a specific diagnostic test [49,65-69]. The Maastricht IV/ Florence Consensus report recommends UBT or SAT as the most accurate non invasive tests, especially for post treatment evaluation [49]. Serological tests should be used only if validated and not for post treatment evaluation. PPI should be stopped for at least 2 weeks before testing by UBT, SAT, RUT, histology or culture. In regions with high CLA resistance it is important to perform culture and susceptibility tests or molecular tests to detect resistance even before first-line treatment with CLA [49].

TREATMENT OF *H. PYLORI*

First line treatment

A first-line regimen for the treatment of *H. pylori* is considered acceptable if it is associated with at least 90% success [70]. Traditionally, first line treatment of *H. pylori* has incorporate damoxicillin (AMOX) and CLA together with a PPI, administered twice daily for 7-14 days. However, this so-called "triple therapy" (TT) is limited by emerging resistance to CLA. The Maastricht IV/ Florence Consensus report on the management of *H. pylori* infection recommends that CLA based TT may now be used only in regions with low (<20%) CLA resistance, or in instances where CLA susceptibility is tested prior to first line treatment [49]. Unfortunately, over the past decade *H. pylori* resistance to CLA has risen to over 20% in most of Western, Central and Southern Europe, as well as in the US [71, 72]. A meta-analysis of 104 Korean studies including 42,124 patients treated with first-line TT, found a pooled eradication rate of 74.6% in ITT analysis and 82.0% in PP analysis. In Korea, over the past 2 decades the efficacy of TT has significantly fallen [73].

In order to overcome the limitations of standard TT, anitroimidazole drug such as metronidazole (MET) may be added, in various combinations. Sequential therapy (ST) involves treatment with AMOX-PPI for 5-7 days, followed by CLA-MET-PPI for 5-7 days [49]. Concomitant therapy connotes treatment with AMOX-CLA-MET-PPI taken together for 10-14 days [49]. Hybrid therapy seeks the middle road, and involves administering AMOX-PPI for the entire duration of treatment, while adding CLA-MET for the second half alone [74].

Early studies assessing the efficacy of ST found eradication rates consistently higher than 90%. Gatta et al., found a pooled odds ratio of 2.99 for *H. pylori* eradication with ST compared to TT, and no increase in adverse events, in ten RCTs [75]. However most of these studies originated in Italy and such a high efficacy was not subsequently replicated in other geographical locations. In 2014, Kim et al., performed a meta-analysis of 9 Asian RCTs incorporating 3074 patients, comparing 7-14 day TT with 10 day ST [76]. The per protocol (PP) rate of eradication was 87.6% and 77.1% with ST and TT, respectively (OR 1.77, *p*<0.01).Here too, patients treated with ST did not report more adverse events that those receiving TT. Although ST clearly offers an advantage over TT, eradication rates remain sub-optimal [76].

A comprehensive meta-analysis of 46 RCTs incorporating 13,532 previously untreated patients compared ST with several other treatment protocols [77]. 22 studies compared ST with 7 day TT achieved eradication in 86.5% and 71.5%, respectively

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(OR=1.21). A benefit of ST was seen especially in studies emanating from China, Italy, Korea, and Morocco. In 14 studies comparing ST with TT given for 10 days, the ST was more likely to achieve eradication (84.3 *vs* 75.3%, OR=1.11). However, ST was not superior to TT given for 14 days, as examined in 7 studies (OR 1.00). Suffice it to say that comparisons of this kind are limited by high levels of bias and heterogeneity. ST was not superior to CT in6 studies comparing these two protocols, with a relative odds of eradication of0.99, favoring CT. The pooled eradication rate was 81.7% for ST and 81.3% for CT. In 5 studies ST was not superior to quadruple bismuth-based therapy given for 10-14 days. The pooled eradication rate was 84.9% for ST and 86.2% for CT, OR 1.01 [77].

Bismuth-based quadruple therapy (BQT), where available, is a valid alternative for first-line treatment. Treatment consists of PPI, bismuth salts, tetracycline and a nitroimidazole, all administered for 10-14 days.BQT may be particularly appealing in areas of high CLA-resistance and forpatients allergic to penicillin. In 12 RCTs comparing BQT and TT, eradication was achieved in 77.6% and 68.9%, respectively [78]. However when the duration of BQT and TT are equal, the eradication rates are similar between groups. Of significance is the finding that BQT is not adversely affected by MET resistance, whereas the effectiveness of ST is drastically reduced in cases of CLA resistance. Treatment-associated side effects are similar in both protocols [78]. The availability in Europe of a single three-in-one capsule containing potassium bismuth subcitrate, metronidazole, and tetracycline has rendered BQT an attractive option for first-line therapy [79].

The most recent treatment guidelines of the European Helicobacter Study Group recommend that in areas of low CLA resistance (<20%) TT or BQT be used for first line treatment. In areas of high CLA resistance, BQT or non-bismuth quadruple therapy (ST or CT) should be used [49]. Since the publication of these guidelines in 2012 many important papers have been published and the appropriateness of ST in areas of high CLA resistance may be questioned. New guidelines are expected in the coming year.

Due to the virtual absence of *H. pylori* resistance to AMOX in both naïve and treatment experienced subjects, an old-new regimen consisting of high dose AMOX and PPI has been proposed [80]. This so-called "high-dose dual therapy" achieved 95.3% eradication compared to 85.3% with ST. Although fluoroquinolones are usually reserved for salvage therapy, they have been found to be effective for first-line therapy, too. In 9 RCTs levofloxacin (LEV)-based therapy (LT) was superior to TT, regardless of treatment duration, with eradication achieved in 80.2% and 77.4%, respectively (OR=1.03). Interestingly, in Asian populations, TT remains superior to LT when each is given for 7 days [81].

Salvage treatment

A meta-analysis of 13 RCTs published up until 2009 found no significant difference between LT and BQT when given for persistent *H. pylori* infection [82]. However, 10 day-LT is superior to 7 day-BQT (OR=4.79) and is associated with fewer adverse effects (OR=0.41) and lower likelihood of discontinuation. This leads the authors to recommend LT as the treatment of choice

for persistent *H. pylori* infection. A later meta-analysis of studies using moxyfloxacin (but not LEV) found that the second-line eradication rate with moxifloxacin is higher than with BQT (73.3% *vs* 60.2%, OR=1.78) [83]. A recent study by Gisbert et al., found that following failure of ST or CT, second line treatment with LT may be successful in approximately three quarters of patients [84].

A recent single-arm open label study found that BQT is effective in up to 95% of patients following failure of TT [85]. A variation of BQT where MET is replaced with LEV also has over 95% success following failure of first-line CLA-based treatment [86]. BQT is also effective in about two thirds of patients when given as a third-line regimen following failure of both TT and LT [87]. High-dose dual therapy with AMOX and PPI alone may achieve eradication in 89.3%, compared to 78.6% with LT, when given as rescue therapy [80].

Rifabutin may be effective as fourth line therapy, following failure of TT, BQT and LT [88]. In this setting, rifabutin combined with AMOX and PPI for 10 days, achieves eradication of *H. pylori* in approximately 50%. Rifabutin has been used together with AMOX, PPI and ciprofloxacin in a modified sequential regimen, and may achieve eradication in over 90% of cases following at least one treatment failure [89].

Treatment duration

A 2013 Cochrane meta-analysis included 75 RCTs comparing treatment duration of 7, 10, or 14 days [90]. Most of the studies included involved first line triple therapy. Regardless of the antibiotic used, prolonging treatment duration from 7 to 14 days significantly improved eradication rates (72.9% *vs* 81.9%) and was associated with a RR for *H. pylori* persistence of 0.66 (95% CI 0.60 to 0.74). Prolonging treatment had a minimal effect on adverse events. This led the authors to conclude that triple therapy with PPI-AMOX-CLA or PPI-AMOX-MET should be administered for at least 14 days. There is insufficient data regarding treatment duration of other regimens [90].

Adjuncts to treatment: Lactobacillus and Bifidobacterium-containing probiotic compounds given together with antibiotics, may increase the likelihood of eradication two-fold, and decrease the incidence of total side effects by 70% [91]. Saccharomyces boulardii supplementation may also slightly increase eradication rates when given with TT (OR=1.13) and reduce treatment-associated side effects, particularly diarrhea [92]. These data suggest that probiotic compounds or their metabolites are bacteriostatic or bacteriocidal towards *H. pylori*. There is concern, however, that these properties are strain-specific in which case the applicability of the cited meta-analyses is limited. Antioxidants such as vitamin C and E have been studied as supplements to *H. pylori* treatment, however current data are insufficient to draw any conclusion regarding their effectiveness [93].

Proton pump inhibition: PPI is an essential component of every treatment protocol. Esomeprazole is more effective than first generation PPIs such as omeprazole, lansoprazole and pantoprazole (OR=1.32) [94]. This is likely to be related to more effective acid inhibition. Indeed, the benefit of esomeprazole is most profound in patients possessing polymorphisms in

S-mephenytoin 4' –hydroxylase (CYP2C19) associated with extensive PPI metabolism [94].

Individualized treatment: In recent years much effort has been directed towards identifying factors associated with treatment failure in order to individualize treatment protocols. Over 20 single nucleotide polymorphisms (SNPs) have been identified in the CYP2C19 gene, which affect gene function and lead to rapid PPI metabolism. Three or four loss-of-function variants are typically tested in commercial kits. In extensive metabolizers (EM), the success of *H. pylori* eradication may be improved by increasing PPI dosage and reducing intervals between doses [95]. In 16 RCTs, CYP2C19 phenotype was found to be a major determinant of the success of TT. However when newer generation PPIs are prescribed, CYP2C19 polymorphisms are less relevant [96]. Similarly, treatment may be individualized by testing antibiotic susceptibility prior to first line therapy. For example, 4 SNPs are responsible for most resistance to CLA, and may be tested in gastric or stool samples [97]. It remains to be seen, however, whether such an approach is cost effective, given the high efficacy of first-line CT and BQT.

Culture guided triple therapy yields superior eradication rates than TT for first-line treatment, and may save costs in some countries (OR 0.84). However the main disadvantage is the need for an endoscopic procedure to acquire a sample [98].

CONCLUSION

The high global burden of *H. pylori* necessitates ongoing efforts to develop rapid, accurate, non-invasive and acceptable diagnostic tests. In the absence of a "one-size-fits-all" treatment strategy, clinicians must consider local antibiotic resistance patterns before choosing a particular regimen. In the absence of such information, clinicians must balance the need for a prolonged four-drug regimen against adverse effects and costs. The implementation of "individualized" treatment tailored to CYP2C19 phenotype and antibiotic susceptibility based on rapid testing is promising, however large randomized controlled trials are needed.

Major diagnostic points

- When *H. pylori* infection is suspected a diagnostic test should be performed in accordance with the clinical situation. When atrophic gastropathy is present, the sensitivity of histology is enhanced by obtaining biopsies from the greater curvature.

- Cryopreservation of *H. pylori* within gastric biopsies at -70°C is successful for more than 10 years.

- Serology is not strongly recommended except when a validated kit is used, in which case sensitivity and specificity are high.

- Urea breath test and stool antigen test are by far the most recommended non invasive tests.

- Special attention is needed when diagnosing *H. pylori* infection in the setting of clarithromycin resistance, gastric cancer, bleeding ulcers, gastric surgery, and post eradication therapy. These situations may have a significant impact on test sensitivity and specificity.

Major treatment points

- A first-line regimen for the treatment of *H. pylori* is considered acceptable if it is associated with at least 90% success. In most geographical regions, standard triple therapy and even sequential therapy fall below this threshold.

- Concomitant therapy and bismuth-based quadruple therapy are more efficacious than first-line triple and sequential therapy.

- First line triple therapy with amoxicillin, clarithromycin and a proton pump inhibitor may be considered in regions where *H. pylori* resistance to clarithromycin is below 20%. Treatment should be administered for 14 days.

- Following treatment failure, salvage with a quinalone-based regimen may be more efficacious than bismuth-based quadruple therapy

- First line therapy tailored to CYP2C19 phenotype and clarithromycin susceptibility has the potential to increase treatment success, however the practicality and cost-effectiveness of this approach needs to be examined.

Clinical bottom line

- The C13-urea breath test is non-invasive test of choice to diagnose *H. pylori* infection and to confirm eradication following treatment. The stool antigen test is an acceptable alternative provided the assay used has an accuracy >90%.

- First-line therapy for *H. pylori* cannot be standardized, and must be considered in the constellation of regional epidemiology, microbial resistance, drug cost and availability, antibiotic allergy and side effects.

- New generation proton pump inhibitors such as esomeprazole and rabeprazole are more likely to achieve eradication compared to omeprazole, lansoprazole and pantoprazole.

- Lactobacillus, Bifidobacterium and Saccharomyces boulardii -containing probiotic compound may increase treatment efficacy and reduce treatment-associated adverse effects.

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