HELICOBACTER PYLORI: RESISTANCE AND TREATMENT RESULTS IN FINLAND

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Academic Dissertation

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ABSTRACT

Background: Helicobacter pylori infection is usually acquired in early childhood and is rarely resolved spontaneously. Eradication therapy is currently recommended virtually to all patients. Usually the first and second therapies are prescribed without knowing the antibiotic resistance of the bacteria. Thus, it is important to know the primary resistance in the population to choose a regimen with at least 80% efficacy, in preventing induction of resistant strains. Although dyspeptic symptoms are one of the main indications for therapy, the symptomatic gain from therapy has been only modest.

Aim: This study evaluates the primary resistance of H. pylori among patients in primary health care throughout Finland, the efficacy of three eradication regimens among these patients, the symptomatic response to successful therapy, and the effect of smoking on gastric histology and humoral response in H. pylori-positive patients. Based on this study, a first-line eradication therapy in Finland could be recommended.

Patients and methods: A total of 23 endoscopy referral centres located throughout Finland recruited 342 adult patients with positive rapid urease test results, who were referred to upper gastrointestinal endoscopy from primary health care. During endoscopy, one biopsy for culture and two for histology were taken from the gastric antrum and body. A blood sample for serology was taken. The patients were randomized to receive a seven-day regimen, comprising 1) lansoprazole 30 mg b.d., amoxicillin 1 g b.d. and metronidazole 400 mg t.d. (LAM), 2) lansoprazole 30 mg b.d., amoxicillin 1 g b.d. and clarithromycin 500 mg b.d. (LAC) or 3) ranitidine bismuth citrate 400 mg b.d., metronidazole 400 mg t.d. and tetracycline 500 mg q.d. (RMT). The eradication results were assessed, using the $^{13}$C-urea breath test 4 weeks after therapy. The negative breath test was confirmed with serology and the positive test with a new endoscopy with histological samples and H. pylori culture. The patients completed a symptom questionnaire before and a year after the therapy.

Main results and conclusions:
1. Resistance
Resistance in H. pylori was successfully assessed with the E-test in 292 cases. Primary
resistance to metronidazole was 38%, 48% among women and 25% among men. In women, metronidazole resistance correlated with previous use of antibiotics for gynaecologic infections and alcohol consumption. Resistance rate to clarithromycin was only 2% and was associated with previous antibiotics for dental and respiratory infections. Thus, metronidazole-based therapies cannot be recommended for women unless the sensitivity of _H. pylori_ is known.

2. Eradication results
The eradication result could be assessed in 329 cases. Eradication therapy was successful in 78% of intention-to-treat analysis in LAM, 81% in RMT and 91% in LAC. In metronidazole-sensitive cases, the cure rates with LAM, RMT and LAC were similar (93%, 91% and 95%), whereas in metronidazole resistance, LAM and RMT were inferior to LAC (53%, 67% and 84%). Clarithromycin resistance reduced the eradication rate from 84% to 43%. Previous antibiotic therapies reduced the efficacy of LAC, to the level of RMT. Thus, LAC is the best choice for first-line eradication therapy.

3. Symptoms
The symptomatic response to successful eradication of _H. pylori_ could be assessed in 216 patients. Dyspeptic symptoms in the Gastrointestinal Symptoms Rating Scale (GSRS) were analysed. All dyspeptic symptoms were decreased; the mean reduction was 30.5%. In logistic regression analysis, duodenal ulcer, gastric antral neutrophilic inflammation and age from 50 to 59 years independently predicted greater decrease in dyspeptic symptoms as a whole. The effect of eradication on dyspeptic symptoms was only modest and comparable to that in previously reported results with placebo.

4. Smoking
The effect of smoking on gastric histology and humoral response to _H. pylori_ was analysed in 318 patients. In the gastric body, smokers had milder neutrophilic and chronic inflammation and less atrophy and in the antrum denser _H. pylori_ load. Smokers also had lower IgG antibody titres against _H. pylori_ and a smaller proportional decrease in antibodies after successful eradication. Smoking slows the progression of atrophy in the gastric body and triples the risk of duodenal ulcers (32%) vs. 11% among nonsmokers.
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ASA</td>
<td>acetylsalicylic acid</td>
</tr>
<tr>
<td>b.d.</td>
<td>twice per day</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>DU</td>
<td>duodenal ulcer</td>
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<tr>
<td>GRSR</td>
<td>Gastrointestinal Symptoms Rating Scale</td>
</tr>
<tr>
<td>GU</td>
<td>gastric ulcer</td>
</tr>
<tr>
<td>LAC</td>
<td>lansoprazole 30 mg b.d., amoxicillin 1 g b.d. and clarithromycin 500 mg b.d.</td>
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<tr>
<td>LAM</td>
<td>lansoprazole 30 mg b.d., amoxicillin 1 g b.d. and metronidazole 400 mg t.d.</td>
</tr>
<tr>
<td>MALT</td>
<td>mucosa-associated lymphoid tissue</td>
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<tr>
<td>MIC</td>
<td>minimal inhibitory concentration</td>
</tr>
<tr>
<td>NNT</td>
<td>number needed to treat</td>
</tr>
<tr>
<td>NSAID</td>
<td>nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>NUD</td>
<td>nonulcer dyspepsia</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>q.d.</td>
<td>four times per day</td>
</tr>
<tr>
<td>PPI</td>
<td>proton pump inhibitor</td>
</tr>
<tr>
<td>PU</td>
<td>peptic ulcer</td>
</tr>
<tr>
<td>PUD</td>
<td>peptic ulcer disease</td>
</tr>
<tr>
<td>RBC</td>
<td>ranitidine bismuth citrate</td>
</tr>
<tr>
<td>RMT</td>
<td>ranitidine bismuth citrate 400 mg b.d., metronidazole 400 mg t.d., and tetracycline 500 mg q.d.</td>
</tr>
<tr>
<td>RUT</td>
<td>rapid urease test</td>
</tr>
<tr>
<td>t.d.</td>
<td>three times per day</td>
</tr>
<tr>
<td>UBT</td>
<td>$^{13}$C-urea breath test</td>
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LIST OF ORIGINAL PUBLICATIONS


III. Koivisto TT, Voutilainen ME, Färkkilä MA. Symptoms, endoscopy findings and histology predicting symptomatic benefit of Helicobacter pylori eradication. Scand J Gastroenterol, in press.

INTRODUCTION

It has been estimated that over half of mankind is infected by Helicobacter pylori. In the developing countries, 80% of the adults carry H. pylori. Along with improvements in hygiene, infections have become less frequent, and in the Western countries H. pylori has become an infection of middle-aged and elderly people. Helicobacter pylori is mostly acquired in early childhood, most probably from another family member. Very seldom will H. pylori be cleared spontaneously. After the primary infection, the patient develops symptomatic gastritis; thereafter, in most cases, the helicobacter leads a quiet life in the gastric mucosa for the rest of the host’s life. However, about 10–20% of people carrying H. pylori will develop gastrointestinal symptoms of infection. The infection can lead to a peptic ulcer (PU) and, in rare cases, to gastric malignancy.

Even though H. pylori is sensitive to many antibiotics, the niche it lives in makes eradication difficult. Only regimens consisting of at least two antibiotics and a proton pump inhibitor (PPI) or bismuth have achieved efficacies of over 90%. Amoxicillin, tetracycline, metronidazole and clarithromycin are the most used antibiotics. Antibiotic resistance is an important predictor for success of the eradication therapy. Usually the first eradication therapy is given without knowing the sensitivity of the bacteria. Thus, choosing the most effective first-line therapy requires knowledge of the primary antibiotic resistance of H. pylori in the population. While resistance of H. pylori to amoxicillin and tetracycline is very rare and very rarely develops after a failed therapy, resistance to metronidazole and clarithromycin is an important issue. Resistance to metronidazole varies widely in different parts of the world, from 20% in some Western countries to over 80% in the developing countries. Women have resistant strains more often than men. Resistance to clarithromycin is common, up to 20%, in countries with liberal antibiotic policies, such as those in Southern Europe, but less than 10% in Northern countries with more strict antibiotic policies. Recently, fluoroquinolones, such as levofloxacin, have been proven effective both in naïve cases and after eradication failures.

Since the first indications for H. pylori eradication therapy were peptic ulcer disease (PUD) and malignant and premalignant conditions, the Maastricht III consensus report now recommends eradication therapy for everyone infected and even test-and-treat strategy for
uninvestigated dyspepsia (Malfertheiner et al. 2007). Knowing that in nonulcer dyspepsia (NUD) about 10% of the dyspeptic symptoms are caused by \textit{H. pylori} infection, most of the symptomatic benefit comes from the placebo effect. PUD can in most cases be cured by \textit{H. pylori} eradication. For ulcers among users of nonsteroidal anti-inflammatory drugs (NSAIDs), \textit{H. pylori} has an additive effect and eradication is recommended.

In addition to \textit{H. pylori}, smoking predisposes to PU both by enhancing ulcerogenic factors and hampering restoration of mucosal lesions. It has also been associated with malignant changes induced by \textit{H. pylori}.

\textit{Helicobacter pylori} has coexisted with mankind since prehistoric times, thus one could also expect that it may be beneficial to its bearer. However, no proof of this is available, although \textit{H. pylori} has been suggested to protect from allergy.
REVIEW OF THE LITERATURE

HISTORY

In the year 1875 G. Bottcher in collaboration with M. Letulle, after finding bacteria colonizing gastric ulcers (GUs) and ulcer margins, regarded the bacteria responsible for the ulcer (Kidd and Modlin 1998). Then in 1889 W. Jarowski found spiral organisms in gastric washings, named them *Vibrio rugula* and suggested they were pathogenic in gastric diseases (Konturek 2003). In 1896 H Salomon could transfer the infection to mice, and 10 years later W. Krieniz associated the bacteria with gastric cancer. J.M. Luck discovered gastric mucosal urease as early as 1924. Investigations continued and in 1940 F.D. Gorham postulated gastric acidophilic bacteria as an aetiologic agent in PUD and treated PUs with bismuth (Kidd and Modlin 1998). However, E.D. Palmer later could not find these bacteria in vacuum biopsies in 1088 patients, and the issue was forgotten for 30 years (Palmer 1954). Finally, Robin Warren in 1979 rediscovered the bacterium, and he with Barry Marshall, over 100 years after the first description, cultured it and by Koch’s postulates proved it to be the cause of gastritis and subsequently of PU. This was the beginning of a new era in gastroduodenal diseases.

**HELIcobacter pylori InFECTION**

**Bacteriology**

*Helicobacter pylori* is a multiflagellated spiral bacterium in the Helicobacteraceae family, which in turn belongs to the Epsilonproteobacteria class (http://www.bacterio.cict.fr). The acid gastric mucus layer is its natural niche. It has been found in the duodenum with gastric metaplasia, and also in dental plaques and faeces. It is microaerophilic and capnophilic. In producing ammonia with its urease enzyme, it becomes adjusted to the acid milieu and thrives poorly in the nonacid mucosa of atrophic gastritis.

Epidemiology

*Helicobacter* infection is in most cases acquired in early childhood (Feldman et al. 1998). When the bacterium is found in the gastric epithelium, dental plaques, vomit and faeces, the mode of transmission can be oral-oral, gastro-oral and faecal-oral; the main route is not clear (Vaira et al. 2001). Overcrowding, large families, poverty and low hygienic standards during childhood predispose to the infection. When hygiene was improved along with rising living standards, the incidence declined accordingly (Ahmed et al. 2007). Clearance of the infection is a rare event: less than 1% per year (Kosunen et al. 1997). Thus, when the older people still have the infection and the younger ones seldom catch it, the cohort effect is seen in the developed countries. In Finland less than 10% of children harbour *H. pylori*, compared with 70-80% of those 70 years of age or over (Rehnberg-Laiho et al. 1998; Rautelin and Kosunen 2004). In Finland the rate of adult acquisition of new *H. pylori* infection is very low, app. 1% per year as in other Western countries (Seppälä et al. 1992; Sipponen et al. 1996; Gisbert 2005). *Helicobacter pylori* is still a major issue in the developing countries, but the prevalence will eventually decline as it has already done in the developed countries (Tkachenko et al. 2007).

ANTIBIOTIC RESISTANCE

The antimicrobial resistance of *H. pylori* varies throughout the world. The antibiotics most often used in eradication therapy are amoxicillin, metronidazole, tetracycline, clarithromycin and recently levofloxacin. Resistance is the most important factor impairing eradication therapy.

Metronidazole

Metronidazole is metabolized by oxygen-sensitive nitroreductase enzymes of anaerobic bacteria into toxic metronidazole radicals that react with proteins, DNA and RNA, resulting in the death of the bacteria. Mutations in these genes may block the effect of nitroimidazoles. In *H. pylori* the main enzyme is oxygen-insensitive nicotinamide adenine dinucleotide phosphate (NADPH) nitroreductase. The toxic nitrosoderivatives this enzyme produces are not reoxidized by existing molecular oxygen, and cell damage ensues. Metronidazole resistance is mostly associated with mutations in the rdxA gene that
encodes oxygen-insensitive NADPH nitroreductase. Mutations in the frxA gene, which encodes NAD(P)H-flavin oxidoreductase enhances the effect (van der Wouden et al. 2000; Bereswill et al. 2003). This complexity in nitroimidazole metabolism means that metronidazole resistance is not either/or. Furthermore, mutations in the rdxA or frxA genes alone do not explain the resistance (Chisholm and Owen 2003; Marais et al. 2003). As a result, metronidazole-based therapies can eradicate metronidazole-resistant strains, although not as effectively as sensitive strains (Lind et al. 1999).

Resistance to metronidazole in Europe and the USA is between 20% and 40%, but wide use of metronidazole for parasitic infections increases resistance in the developing countries up to 80% (Glupczynski 1998; Glupczynski et al. 2001; Osato et al. 2001). Local differences in Europe exist; e.g. in the southern part resistance is 40%, compared with 30% in the North. Immigrants bring their own helicobacters with them; thus people coming from the developing countries have more resistant strains (Banatvala et al. 1994; Glupczynski et al. 2001). In Japan, exceptionally low resistance rates are seen (12.4%), again with local differences (Kato et al. 2000). Women more often harbour resistant strains than men, most probably due to therapies for gynaecologic infections (Glupczynski et al. 2001; Bruce et al. 2006). In a large European study, metronidazole resistance among children was similar to that (25%) found among adults (Koletzko et al. 2006). Figure 1 shows the resistance frequencies found in various European countries.
Clarithromycin

Resistance to macrolides is in most cases caused by one of three single-point mutations in the peptidyltransferase region of the 23S rRNA gene, mostly mutation A2143G or A2142G, when the adenine residue is replaced by guanine and less commonly by a cytosine residue (A2142C) (Oleastro et al. 2003). These mutations decrease macrolide binding to the ribosomes.

Resistance to clarithromycin has been much less frequently observed than resistance to metronidazole. In a large European survey, resistance to clarithromycin was 9.9%. Again,
clear differences between Southern and Northern Europe were seen. Of those born in Southern Europe, 18.4% had resistant strains, compared with 9.3% in central Europe and 4.2% in Northern Europe (Glupczynski et al. 2001). In Norway and Sweden, even smaller figures were found (< 3%) (Lerang et al. 1997; Jaup et al. 1998; Storskrubb et al. 2006). Children show the same geographical distribution: the resistance frequency among Southern European children was 2.2 times that of Northern European children. Younger children, compared with older children, also more often had resistant helicobacters (Cabrita et al. 2000; Koletzko et al. 2006). In the USA, about 10% of helicobacters are clarithromycin-resistant (Osato et al. 2001; Meyer et al. 2002). Resistance can be seen as a direct consequence of increased macrolide consumption (McMahon et al. 2003). Clarithromycin resistance emerged in Estonia after clarithromycin became available, while the resistance frequencies in Japan correlated with macrolide sales (Loivukene et al. 2002; Perez Aldana et al. 2002). Thus, it is clear that clarithromycin resistance has increased and will continue to do so (Chisholm et al. 2007; De Francesco et al. 2007). Resistance after monotherapy with clarithromycin was 32% (Peterson et al. 1993). Figure 2 shows the resistance frequencies found in various European countries.
Amoxicillin

Fever than 1% of helicobacters are resistant to amoxicillin (Megraud 2004). The mechanism is based on mutations in the genes coding for penicillin-binding protein and, in consequence, decreased affinity for amoxicillin leads to decreased accumulation of the antibiotic (Co and Schiller 2006; Gerrits et al. 2006). Still, in a Japanese study H. pylori strains resistant to amoxicillin appeared after the year 1996, while insensitive strains also increased, the importance of this phenomenon remains to be seen (Watanabe et al. 2005).
**Tetracycline**

Resistance to tetracycline is also very low, less than 1% (Wolle et al. 2002; Megraud 2004). A triple mutation AGA 926-928→TTC in the 16S rRNA gene is responsible. Since one or two mutations are not capable of producing resistance, tetracycline resistance is very rare despite its wide use (Gerrits et al. 2003).

**Fluoroquinolones**

Of the fluoroquinolones on the Finnish market, levofloxacin has been tested in *H. pylori* eradication regimens. Resistance to fluoroquinolones is based on point mutations in the quinolone resistance-determining regions of gyrA. Due to the wide use of fluoroquinolones, resistance is already high in France (17%), Belgium (16.8%), and Portugal (up to 20.9%) and still higher among those with previous fluoroquinolone therapies (Cabrita et al. 2000; Bogaerts et al. 2006; Carothers et al. 2007; Cattoir et al. 2007). Of the *H. pylori* strains collected in Japan from 2001 to 2004, 15% were resistant (Miyachi et al. 2006). In Germany, resistance is currently increasing (primarily 9.5%) and is exceptionally high among patients who had a previous failed eradication therapy not including a fluoroquinolone (17.1%). Moreover, most fluoroquinolone-resistant strains were also resistant to metronidazole and clarithromycin (Glocker et al. 2007b). This makes rescue therapies with fluoroquinolones less favourable and at least will favour susceptibility testing before the attempt. Resistance is currently increasing so rapidly that rates found a few years earlier are no longer relevant in deciding on the use of fluoroquinolones.

**Rifabutin**

Rifabutin is used as a rescue therapy in metronidazole- and clarithromycin-resistant *H. pylori* cases. Resistance to rifabutin is extremely rare. In a German study only 1.4% of 1585 isolates were rifampicin-resistant (minimal inhibitory concentration (MIC) > 4 µg/ml) and most were also rifabutin-resistant (Glocker et al. 2007a). Mutations in the rpoB gene are responsible for the resistance (Heep et al. 2000b).
Dual resistance to both metronidazole and clarithromycin

Resistance to both the antibiotics most used in eradication therapy has become an important issue. In London, where immigrants make up a large proportion of the population, dual resistance is as high as 8% (Elviss et al. 2005). In a multicentre study in the USA, 5% of the strains collected from 1998 to 2002 were dual-resistant (Duck et al. 2004). Wong et al. (2003) in Hong Kong found a dual resistance of 8.7%. In the SHARP study, the dual resistance varied between 0% and 6.9% (2.4% in men and 6.9% in women) during 1993–1999 (Meyer et al. 2002). In a recent study, as many as 77.6% of the strains were dual-resistant after a failed therapy with clarithromycin and metronidazole (Branca et al. 2004). Finally, patients can harbour mixed *H. pylori* strains, and in these cases the risk for dual resistance is higher (Cellini et al. 2006).

Secondary resistance

After a failed therapy, the resistance rate is high; e.g. in an Italian study the rate was 69.8% for metronidazole and 82.3% for clarithromycin (Toracchio and Marzio 2003). In a large German study, 66–75% of the strains were resistant to metronidazole and 49–58% to clarithromycin, but none to amoxicillin after a failed therapy in 554 patients. The alarming aspect is that 85–89% of the metronidazole-resistant strains were also clarithromycin-resistant (Heep et al. 2000a). Figures in the study of Branca et al. (2004) were similar. In a Japanese study, secondary resistance to gatifloxacin was 47.9% (Nishizawa et al. 2006).

*Helicobacter pylori* and gastroduodenal diseases

Gastritis

After arriving in the stomach, *H. pylori* penetrates the mucous layer and adheres to the epithelial cells by adhesins. In some cases, the bacterium can penetrate the lamina propria; it also produces water-soluble antigens capable of penetrating the basement membrane. These and interleukins (e.g. interleukin-8) from the gastric epithelial cells attract polymorphonuclear leucocytes. Antigen-presenting cells activate B and T lymphocytes and plasma cells, and antibody production begins, at first the IgM type, thereafter the IgA and IgG types (Andersen 2007). After a recently acquired *H. pylori* infection, the gastric
Histology shows acute and chronic inflammatory cells. Organized lymphoid follicles have also been observed. Graham et al. (2004) showed that in 2 weeks the histology was very much like that seen in chronic infection. Acute infection produces achlorhydria for several months. Thereafter, the acid secretion recovers and, in patients prone to DU, will remain raised, while in those developing atrophic gastritis, it will diminish over decades.

Helicobacters with the cytotoxin associated gene (Cag) pathogenicity island (PaI) via increasing interleukin-8 production induce more intensive inflammation in the gastric mucosa. Also the outer inflammatory protein (OipA) and in East Asian countries, the RNA polymerase β-subunit variant of *H. pylori* promote interleukin-8 production. Further, the immune response of the host, which *H. pylori* tries to down-regulate, determines aggressiveness of the inflammation (Robinson et al. 2007).

In autoimmune gastritis, CD4+ T cells react with the adenosine triphosphatase of the gland cell. In some cases, molecular mimicry with *H. pylori* appears to trigger this autoimmune process (Amedei et al. 2003). Autoimmune gastritis in turn impairs acid production, which is needed in nonhaem dietary iron absorption. Vitamin B₁₂ deficiency will ensue after deficiency in intrinsic factor (Kaptan et al. 2000).

Smoking has been associated with increased atrophic change and intestinal metaplasia in *H. pylori*-positive patients (Nakamura et al. 2002). Also more dysplasia has been found among smokers (Kneller et al. 1992). Furthermore, smoking increases the risk for gastric carcinoma (Komoto et al. 1998). However, some studies found no connections between smoking and atrophy or intestinal metaplasia (Ohkuma et al. 2000; Ito et al. 2003). *Helicobacter pylori* density, assessed by the UBT, has been lower among smokers (Moshkowitz et al. 2000). The authors speculated that the reason was the thinner mucous layer in the smoker’s stomach. Smoking also promotes gastric lesions and ulceration by increasing duodenogastric reflux, decreasing production of protective prostaglandins and mucus, increasing acid secretion by stimulating H₂-receptors and increasing pepsinogen production (Bago et al. 2000; Parasher and Eastwood 2000; Tatemichi et al. 2001).
**Peptic ulcer**

*Helicobacter pylori* plays a major role in PUD. In a review of 21 studies with 10146 patients, the presence of *H. pylori* doubled the prevalence of a PU (odds ratio (OR) 1.81 among NSAID users and 2.17 among nonusers). In age-matched studies, the OR was as high as 4.03. *Helicobacter pylori*-positive NSAID users have an ulcer risk 17 times higher than that of *H. pylori* negative non-NSAID users and a bleeding ulcer risk 21 times higher (Papatheodoridis *et al.* 2006). The efficacy of the eradication therapy in ulcer healing and prevention of ulcer relapse also suggests the *H. pylori* aetiology in PUD (Ford *et al.* 2006). It was estimated that 10–20% of *H. pylori* positives will develop a PU (Rauws and Tytgat 1995). In a meta-analysis, 95% of PU-related risk was attributable to *H. pylori*, NSAIDs and smoking, *H. pylori* being the most important in the North American general population (Kurata and Nogawa 1997). Patients with antrum-predominant gastritis with high acid output and gastric metaplasia in the duodenal bulb are prone to develop a DU, whereas those with diffuse gastritis and perhaps atrophy are prone to develop a GU (Sipponen 2001). Still, it should be borne in mind that *H. pylori* is not the only cause of PU and that the ulcer can relapse after successful eradication (Hobsley *et al.* 2006).

**Malignancies**

*Helicobacter pylori* is a strong risk factor for gastric mucosa-associated lymphoid tissue (MALT) lymphoma and noncardiac adenocarcinoma. As early as 1994, the World Health Organization classified *H. pylori* as a definite carcinogen. The risk of gastric cancer among infected patients has been estimated to be 1–2%, at least eight times higher than that among noninfected patients, although lower figures were also stated (Kuipers 1999; Sipponen and Marshall 2000). In a large nested case-control study in Finland, *H. pylori*-positives showed increased risk for noncardiac gastric cancer (OR = 7.9) and a smaller risk for cardiac gastric carcinoma (OR = 0.31). In a Chinese study, *H. pylori* was a risk factor for both cardiac and noncardiac adenocarcinomas HR 1.64 and 1.60 (Kamangar *et al.* 2007). The pathogenic sequence of inflammation, atrophy, intestinal metaplasia, dysplasia and intestinal-type gastric cancer is known, but the exact mechanisms for carcinogenesis are still debatable (Genta and Rugge 2006). The properties of the *H. pylori* strain, such as CagA positivity, genetics of the host such as interleukin 1 gene cluster polymorphism and environmental factors, all appear to predispose to cancer.
Gastric MALT lymphomas are T-cell-dependent B-cell lymphomas (Lee et al. 2004). The precondition of MALT lymphoma, i.e. the presence of MALT, is always associated with \textit{H. pylori} infection, and in more than 90% of cases, MALT lymphoma is associated with \textit{H. pylori} (Stolte et al. 2002). Development of MALT lymphoma is still a rare event among \textit{H. pylori}-positive patients, e.g. 0.66% in a large German study (Stolte et al. 2002). Some factors predisposing to the malignant change were revealed: e.g. CagA can prevent lymphocyte apoptosis and \( \gamma \)-glutamyl transpeptidase inhibits regulatory T-cell proliferation (Moss and Malfertheiner 2007).

**Dyspepsia**

Dyspepsia is a combination of symptoms referring to the upper gastrointestinal (GI) tract, such as abdominal pain or discomfort, abdominal bloating, postprandial fullness, early satiety, heartburn and acid regurgitation and vomiting. Dyspepsia is further divided into organic dyspepsia with a distinct aetiology and functional dyspepsia, in which the aetiology is still obscure. Functional dyspepsia is difficult to define. The last attempt (Rome III criteria) is now available for scientific use. The functional gastroduodenal disorders are divided into three major categories: functional dyspepsia, belching disorders, and nausea and vomiting disorders. Rome III considers functional dyspepsia as too heterogeneous a term to be studied as an entity. Instead it recommends the use of postprandial stress syndrome and epigastric pain syndrome (Drossman 2006).

Acute \textit{H. pylori} infection induces transient symptoms. Graham \textit{et al.} (2004) successfully infected 18 healthy volunteers with a CagA-negative strain. All had mild to moderate symptoms, which began 3 days after inoculation, peaked between days 8 and 11 and resolved after 1 week. The symptoms were dyspepsia, abdominal pain, belching, halitosis, anorexia, chilly sensations and headache (Graham \textit{et al.} 2004). In the chronic phase, symptoms of \textit{H. pylori} infection can be assessed by symptom resolution in eradication studies. In several studies, the ulcer-like dyspepsia responded most favourably (Veldhuyzen van Zanten \textit{et al.} 2002; Treiber \textit{et al.} 2004; di Mario \textit{et al.} 2005). Several possible pathophysiological mechanisms for these symptoms were proposed: smooth muscle dysfunction, changes in secretion of gastric acid and peptides, changes in nociception, and even dysfunction in the brain-gut axis (Pieramico \textit{et al.} 1993; Mearin \textit{et al.} 1995; Mc Namara \textit{et al.} 2000; Lin \textit{et al.} 2001). However, the findings were not
consistent, and for now the mechanisms have remained hypothetical (Sarnelli et al. 2003). The properties of the H. pylori strains, such as cagA, were associated with symptoms (Treiber et al. 2004). Chronic H. pylori infection cannot be attributed to any specific dyspeptic symptoms, although statistically it is associated with increased abdominal pain, nocturnal pain and heartburn, called epigastric pain syndrome by Drossman (McNamara et al. 2000).

**DIAGNOSIS OF HELICOBACTER PYLORI INFECTION**

**Invasive tests**

Invasive tests were the first to be used in H. pylori diagnosis. The inconvenience of the upper GI endoscopy needed to obtain the biopsy samples for the tests led to the development of noninvasive tests. Other means to obtain H. pylori samples from the stomach have been introduced (nasogastric tube, string test and gastric brush), but they have not displaced endoscopy.

**Rapid urease test**

The rapid urease test (RUT) is based on the urease activity of H. pylori. Helicobacters in the gastric biopsy samples placed in the urea-containing media produce ammonia, raise the pH of the media and change the colour of the indicator. The RUT is the most rapid way to detect H. pylori infection. The most rapid modern tests give positive results in minutes, enabling endoscopists to begin eradication therapy immediately after endoscopy (Goh et al. 2007). The early tests were considered as true negatives after incubation for 24 hours. The biopsies for RUT are taken at the endoscopy, one from the gastric antrum and one from the body or fundus. Antrum biopsy alone is not enough (Bermejo et al. 2002; Abdul-Razzak et al. 2007). When interfering factors are excluded, the sensitivity and specificity are over 90% (Misra et al. 1999a). However, any condition reducing H. pylori density in the stomach reduces the sensitivity of the test.

Use of PPIs, by increasing the gastric pH, makes the environment less suitable for H. pylori and reduces the density of the bacteria. The change is most striking in the antrum and helicobacters may be found only in the fundus. The habit of taking the biopsy for the
RUT only in the antrum is inadequate in this situation. If the patient uses PPIs, the sensitivity of the RUT may even fall below 50% (Yakoob et al. 2005).

Bleeding from GUs or DUs or presence of blood in the stomach from any source is considered to decrease the sensitivity of the RUT to 60–70% (Gisbert and Abraira 2006). Still, the RUT was as sensitive as histology and culture in this situation. In contrast, Castro Fernandez et al. (2004) determined that the test was as accurate among bleeders, recent bleeders and nonbleeders. Use of PPIs before upper endoscopy may in part explain the inferiority of the results obtained with the RUT among these patients (Udd et al. 2003).

The longstanding infection can lead to atrophic change, especially in the gastric antrum, but also in the gastric body. Subsequently, an increase in gastric pH in the body atrophy reduces the density of H. pylori below the detection level of the RUT (Tucci et al. 2005). On the other side, the nonacidic ventricle may harbour other bacteria with urease activity, giving false-positive results (Brandi et al. 2006). Chronic renal failure, in which the prevalence of H. pylori infection is lower, may also decrease the sensitivity of the RUT (Misra et al. 1999b).

After a failed eradication therapy, H. pylori may require over 4 weeks for to recover to the level detected by the RUT (Laine et al. 1998).

**Histology**

Histology was the first means of recognizing H. pylori, preceding the other tools by over 100 years. It is still considered the gold standard for diagnosing H. pylori infection. The usual procedure of taking two biopsies from the gastric antrum and body is usually sufficient for the diagnosis (Dixon et al. 1996). The updated Sydney system was created for evaluation of the biopsy samples (Dixon et al. 1996). The value of histology is not only based on detecting H. pylori, but also on finding atrophic gastritis and premalignant and malignant changes in H. pylori gastritis, in addition to other diseases not associated with H. pylori. In spite of undetectable H. pylori, the presence of active gastritis indicates ongoing infection and suggests the need for further diagnostic tests. On the other hand, a normal histology practically excludes the infection (Megraud and Lehours 2007).
The sensitivity of histology is compromised under the same conditions as with RUT. In atrophic gastritis the density of *H. pylori* can be too low to be detected. The use of PPIs transfers the infection to the upper part of the stomach and reduces the amount of the bacteria (Nakshabendi *et al.* 1996). Antibiotic therapies can reduce the amount of *H. pylori*. Upper GI bleeding reduces the sensitivity (Gisbert and Abraira 2006). *Helicobacter pylori* infection can be patchy and more biopsies may be needed for detection. Under these conditions, Giemsa and silver staining make it easier to detect *H. pylori* (Dixon *et al.* 1996; Anim *et al.* 2000; Rotimi *et al.* 2000).

**Culture**

Culturing gastric biopsies is an almost 100% specific means to find *H. pylori* infection. However, the sensitivity is lower for several reasons. Culturing a slowly growing microorganism under microaerophilic conditions is a demanding process, and most endoscopy units in Finland do not have the necessary laboratories available. The specimen should be transported to the remote laboratory quickly and preferably in a cool package to keep the bacteria viable for culture. Unless frozen, the specimen should be cultured within 24 hours. In optimal circumstances the sensitivity can be as much as 95% (Perez-Perez 2000; Matsukura *et al.* 2004; Megraud and Lehours 2007). RUT tests, which also serve as a transportation medium for culture, make it more convenient to obtain cultures at the first endoscopy.

Culturing has the same problems in sensitivity as the RUT and histology. When the mucosal bacterial density is low, it may remain negative. In a bleeding PU the sensitivity was only 45% (Gisbert and Abraira 2006). After antibiotic therapies, it is recommended to wait 1 month before culturing. It may require 8 weeks before culturing succeeds after failed eradication therapy (Laine *et al.* 2000).

**Antibiotic Resistance**

Although culturing and determining antibiotic resistance are not recommended before the first attempt to eradicate *H. pylori*, it is valuable to know the primary resistance pattern in the area. Based on this knowledge, we can determine the first and second antibiotic combination. After the second failure, culturing is recommended (Malfertheiner *et al.*
Antimicrobial susceptibility is traditionally determined against metronidazole and clarithromycin, which are the compounds most frequently used as first-eradication therapy. Resistance against amoxicillin and tetracycline is very rare and does not need to be tested (Debets-Ossenkopp et al. 1999; Glupczynski et al. 2001; Lawson et al. 2005; Watanabe et al. 2005). The fluoroquinolones are emerging in _H. pylori_ therapy, and their widespread use in other diseases produces resistant strains, suggesting they should also be tested (Bogaerts et al. 2006). The MIC for metronidazole is 8 µg/ml, for clarithromycin 1 µg/ml, for ciprofloxacin 1 µg/ml and for rifabutin 4 µg/ml (Glupczynski et al. 2001). Because of discrepancy between in vitro and in vivo resistance, the Maastricht report does not recommend metronidazole resistance testing in routine clinical practice (Malfertheiner et al. 2007).

The disc diffusion test on an agar plate is an inexpensive, easy and reliable test in clinical practice. E-test is almost as inexpensive, gives an estimate of the MIC and is the test most used in trials. The gold standard is agar dilution, which is laborious and used in scientific research. Grignon et al. (2002) compared the E-test and disc diffusion in assessing the sensitivity of _H. pylori_ to macrolides and found a 100% concordance. Glupczynski et al. (2002) compared the E-test for clarithromycin with agar dilution and found the E-test reproducible and highly compatible with agar dilution (> 98% agreement within 2 log (2) dilution steps). In contrast, the E-test for metronidazole showed higher MICs in half of the strains tested. Moreover, both agar dilution and the E-test lacked reproducibility in assessing metronidazole sensitivity (Glupczynski et al. 2001). Mégraud et al. (1999) obtained similar results in the MACH 2 study.

The point mutations behind clarithromycin resistance can be found with polymerase chain reaction (PCR) assays (Grignon et al. 2002; Oleastro et al. 2003; Moder et al. 2007). The test is rapid and results can be achieved in 2 days, but a specialized laboratory is needed; in addition unknown mutations can also be responsible for the resistance. This test has thus not displaced the E-test and disc diffusion. The use of the PCR to test the point mutations behind fluoroquinolone resistance was also developed and showed good correlation with agar dilution (Nishizawa et al. 2007).
Noninvasive tests

Urea breath test

The urea breath test (UBT) exploits the ability of *H. pylori* to hydrolyse urea into carbon dioxide and ammonia ((NH$_2$)$_2$CO + H$_2$O → CO$_2$ + 2NH$_3$). Patients swallow urea, which is labelled either with $^{13}$C or radioactive $^{14}$C. After being hydrolysed in the stomach, the labelled carbon dioxide is absorbed into the bloodstream and exhaled. The concentration of carbon isotope is measured in the exhaled air by scintillation ($^{14}$C), isotope ratio mass spectrometry ($^{13}$C) or nondispersive isotope-selective infrared spectrometry ($^{13}$C). The original $^{14}$C method has given way to the use of nonradioactive $^{13}$C. Infrared spectrometry is a less expensive nonradioactive means to obtain results (Opekun *et al.* 2005). Citric acid given with urea improves the sensitivity of the UBT by increasing the acidity around *H. pylori*, thus increasing its urease activity (Pantoflickova *et al.* 2003). The UBT can be used to diagnose *H. pylori* infection without upper GI endoscopy as part of the ‘test-and-treat’ strategy, as well as to confirm the result of the eradication therapy after 1 month.

The test is easy to perform in clinical practice and the sensitivity and specificity of the UBT are over 95% (Gisbert and Pajares 2004). However, the same factors reduce its sensitivity, as with histology and culture. Antibiotics, H$_2$-blockers and PPIs can be withdrawn and GI bleeding can be found, but atrophic gastritis remains unnoticed unless specifically sought. In atrophic body gastritis, the UBT may miss more than half of the cases (Kokkola *et al.* 2000; Lahner *et al.* 2004). On the other hand, nonacid stomach may harbour bacteria with urease activity and lead to false-positive results, as in the RUT (Brandi *et al.* 2006). Oral bacteria having urease activity may also induce a false-positive UBT (Peng *et al.* 2001). Disturbances in gastric emptying, e.g. diabetic gastroparesis and gastric resection, can affect the results of the UBT. However, Togashi *et al.* (2006) obtained a 79% sensitivity in gastric resection patients by giving the urea in tablet form and having the patients lying in the left lateral position. $^{13}$C-urea can also be measured in the blood with comparable accuracy (Fry *et al.* 2005). The UBT is the recommended tool in testing eradication results (Malfertheiner *et al.* 2007).
Serology

The enzyme immunoassay (EIA) test is the most used serological test. In this method, antibodies in the serum of the patient reacting with antigens of \textit{H. pylori} (sonicated bacteria or specific parts of it, e.g. flagellin, urease, CagA) are labelled with enzyme-linked antibodies and the enzyme activity is measured. This enables quantification of specific anti-\textit{H. pylori} antibodies in the IgG and IgA classes. The reliability of the method is based on the \textit{H. pylori} antigens used as a substrate, thus genetic variations of the bacteria may reduce the sensitivity of the test (Megraud and Lehours 2007). On the other hand, cross-reaction with other bacteria may produce false-positive cases. This problem has been overcome by using more purified specific antigens. While both IgG- and IgA-type antibodies have been tested, the IgG type is more reliable (Laheij \textit{et al.} 1998; Herbrink and van Doorn 2000). The sensitivity and specificity can be similar to that of the UBT, around 95\% (Oksanen \textit{et al.} 1998). In a meta-analysis of commercial kits, the medians of the sensitivity and specificity were 92\% and 83\% (Laheij \textit{et al.} 1998). In a small study with 102 patients and 8 kits, the sensitivity was 93–98\% and specificity 95–98\% (Meijer \textit{et al.} 1997).

A major advantage of serology is that the former use of antibiotics or PPIs and upper GI bleeding do not affect the test, in contrast to invasive tests. In gastric body atrophy, serology is the most reliable means of discovering \textit{H. pylori} infection (Kokkola \textit{et al.} 2000). When histology shows body atrophy, serology is recommended to exclude \textit{H. pylori}.

Serology can also be used in confirming the result of \textit{H. pylori} eradication therapy. A decrease of at least 40\% in the IgG antibody level 4 months after therapy from the pretreatment level is diagnostic of treatment success (Kosunen \textit{et al.} 1992). Since in most cases IgG antibodies remain detectable for years after eradication, paired serum samples are always needed in this context (Kosunen \textit{et al.} 1992).

Serology also has some drawbacks. It is not recommended for use in populations where \textit{H. pylori} prevalence is lower than 30\%, due to false-positive results. Among young children, the accuracy is lower than among adults (Kolho \textit{et al.} 2002; Bonamico \textit{et al.} 2004; Sherman 2004). A single blood sample after the previous eradication therapy does not
indicate whether the infection is cured or not. In clinical practice, *H. pylori* serology is far too slow for confirming the eradication results.

**Stool antigen test**

Stool antigen tests are based on a polyclonal or monoclonal antibody-based EIA or a rapid monoclonal immunochromatographic assay. The last one can be performed in 10 minutes in the doctors’ office. Veijola *et al.* (2005) compared the Premier Platinum HpSA (Meridian Bioscience Inc., Cincinnati, OH, USA), Amplified IDEIA HpSTAR (Dako, Glostrup, Denmark) and ImmunoCard STAT! HpSA (Meridian Bioscience) tests, showing sensitivities of 91.9%, 96.2%, and 93.0% and specificities of 95.9%, 95.9% and 88.7% in the primary diagnosis. After eradication therapy the figures were for sensitivity 81.3%, 100% and 93.8% and for specificity 97.0%, 97.6% and 97.0%. However, in children younger than 5 years, the sensitivity was only 75% (Kalach *et al.* 2005). In children the stool antigen tests were also periodically positive, thus reducing the sensitivity of the test (Haggerty *et al.* 2005). Storing the sample at room temperature decreases the sensitivity after 2–3 days to 69%. A meta-analysis of 22 studies and 2499 patients showed that the overall sensitivity before therapy was 94% and specificity 97%. In direct comparison, monoclonal tests were more sensitive (95% vs. 83%). In a post eradication setting, the sensitivity of the monoclonal tests was 93% and specificity 96%. Again, in direct comparison the monoclonal test was more sensitive (91% vs. 76%) (Gisbert *et al.* 2006a)

**Clinical use of the tests**

The various tests have different drawbacks and none can be regarded as a gold standard (see Table 1). The UBT is easy to perform and accurate and can be used as a primary test when antibiotics, PPIs, upper GI bleeding, gastric resection or gastric atrophy are not interfering. In these situations, serology is an option. Invasive tests and stool antigen tests have the same sensitivity problems as the UBT. When the upper GI endoscopy is done, histology is the primary test, perhaps accompanied by the RUT, if immediate eradication therapy is planned. Culturing is recommended after the second eradication failure. The stool antigen test demands less time from the health care personnel and is currently replacing the UBT.
Table 1. Clinical use of diagnostic tests for *H. pylori*

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Aspects in clinical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology</td>
<td>&gt; 95%</td>
<td>100%</td>
<td>PPIs, bismuth and antibiotics reduce sensitivity</td>
</tr>
<tr>
<td>Rapid urea test</td>
<td>93–97%</td>
<td>&gt; 95%</td>
<td>PPIs, bismuth, antibiotics and bleeding reduce sensitivity.</td>
</tr>
<tr>
<td>Culture</td>
<td>70–80%</td>
<td>100%</td>
<td>Technically demanding</td>
</tr>
<tr>
<td>IgG serology</td>
<td>85%</td>
<td>79%</td>
<td>Not suitable for screening in populations with low <em>H. pylori</em> prevalence.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Recommended for use in diagnosis in bleeding ulcers, atrophic gastritis and MALT lymphoma or when PPI medication has not been stopped.</td>
</tr>
<tr>
<td>13C-urea breath test</td>
<td>95–100%</td>
<td>91–99%</td>
<td>Accurate and easy. Suitable for screening and confirmation of the eradication result.</td>
</tr>
<tr>
<td>Stool antigen test</td>
<td>91–98%</td>
<td>94–99%</td>
<td>Suitable for screening and for confirmation of the eradication result. Faecal sample must be stored at -20°C before analysis. In room temperature the activity decreases rapidly.</td>
</tr>
</tbody>
</table>


**HELICOBACTER PYLORI ERADICATION THERAPY**

**Challenges in the therapy**

*Helicobacter pylori* lives in an acid niche, covered with mucus, where antibiotics cannot easily reach. Thus, even though *H. pylori* is sensitive to several antibiotics, only two antibiotics combined with a PPI or bismuth salt give satisfactory results. Resistance of *H. pylori* against the antibiotics used is the most important factor impairing the eradication
results (Houben et al. 1999). Compliance is another main factor, in which the eradication rate was reduced from 96% to 69% when 60% of the medication was taken (Malfertheiner 1993; Wermeille et al. 2002). As a consequence, the next regimen is faced with more resistant bacteria. Some studies have suggested that \textit{H. pylori} is more easily eradicated in PUD than in NUD. However, studies with contrasting results have also been published, and a recent review of 22 studies could not confirm the difference between PUD and NUD (Huang et al. 2005).

**Indications for eradication therapy**

In 1996 the European Helicobacter Study Group organized in Maastricht a meeting at which recommendations were given for diagnosis and therapy of \textit{H. pylori}. This first meeting regarded only PUD and low-grade gastric MALT lymphoma as unequivocal indications for eradication. For gastritis with severe abnormalities and former gastric resection for gastric cancer, the evidence was only supportive, but therapy was still strongly recommended for these patients. Furthermore, eradication was advised in long-term PPI treatment of gastro-oesophageal reflux disease, functional dyspepsia, family history of gastric cancer, NSAID therapy, post gastric surgery for PU and if the patient so wishes (Malfertheiner et al. 1997). The second meeting, Maastricht 2-2000, further strongly recommended therapy in atrophic gastritis, for first-degree relatives of gastric cancer patients and if the patient so wishes (after a full consultation with their physician) (Malfertheiner et al. 2002). The third consensus in 2007 recommended eradication therapy also in investigated NUD and uninvestigated dyspepsia (Malfertheiner et al. 2007).

**Test-and-treat**

Upper GI endoscopy is an inconvenient procedure for patients and seldom reveals malignant diseases among patients younger than 45 years. An option for these patients is to test for the presence of \textit{H. pylori} and in positive cases to undergo eradication therapy. In comparison to endoscopy, this procedure was cost-effective when measured by quality-adjusted life-years with a follow-up at 1 year (Klok et al. 2005). In comparison to symptomatic therapy, test-and-treat was more successful with a follow-up at 6 months (Gisbert et al. 2004). In a study of 650 patients using long-term acid suppression therapy for physician-diagnosed PUD, test-and-treat patients showed less ulcer-like dyspepsia than
those with usual care. Still, after giving eradication therapy to \textit{H. pylori}-positive patients (38\% of the test-and-treat group), 75\% in the test-and-treat group continued using acid-reducing medication (Allison \textit{et al.} 2003). In a large Finnish study, testing for and eradicating \textit{H. pylori} reduced GI symptoms, but could not reduce the number of endoscopies (Färkkilä \textit{et al.} 2004). The Maastricht consensus report recommends test-and-treat strategy in dyspeptic adult patients under 45 years of age, although in population with a low \textit{H. pylori} prevalence, empirical acid suppression is an equivalent option (Malfertheiner \textit{et al.} 2007).

\textbf{First-line therapy}

To avoid development of resistance, the intention-to-treat eradication rate in the first eradication therapy should be at least 80\%, a level not as easily achieved in clinical practice (Malfertheiner \textit{et al.} 2007). Bismuth was the cornerstone in the first effective eradication regimen. Bismuth salts alone could eradicate \textit{H. pylori} in 20\% of cases and in dual therapy with amoxicillin in as many as 50\%. When bismuth was combined with metronidazole and tetracycline, results of over 90\% were achieved, but combination with metronidazole and amoxicillin was not as effective. However, the regimen required 2 weeks, was elaborate to follow, not suited for young children due to the tetracycline and was not effective in metronidazole resistant strains (Veldhuyzen van Zanten and Sherman 1994). The side effects and complicated regimen (over 10 tablets per day) in this triple therapy led to compliance problems and further to poorer eradication results (Malfertheiner 1993). Double therapy with omeprazole and amoxicillin appeared as an alternative regimen with fewer side effects, but the eradication rates remained unacceptable, around 65\%. Combination of a PPI with clarithromycin gave similar results, 76\% (Schmid \textit{et al.} 1999).

\textbf{Triple therapy with PPI and two antibiotics}

Seven-day triple therapy with a double-dose PPI and two antibiotics finally achieved acceptable eradication rates with tolerable amounts of side effects, and became the therapy of choice. PPI combined with amoxicillin and metronidazole achieved an efficacy of 80\% and became the first-choice remedy in Finland (Färkkilä \textit{et al.} 1996). However, metronidazole resistance decreased the eradication rate by 30–46\% (Dore \textit{et al.} 2000). In a large Finnish trial, the eradication rate was only 65.8\% (Färkkilä \textit{et al.} 2004). Still better
results were achieved when clarithromycin was substituted for metronidazole. Lengthening the therapy to 10–14 days slightly improved the result (Calvet et al. 2000). Clarithromycin resistance reduced effectiveness by 33–78% (Dore et al. 2000). In metronidazole resistance, PPI + metronidazole + clarithromycin was clearly better than PPI + amoxicillin + metronidazole (Katelaris et al. 2000). PPI + amoxicillin 1 g + clarithromycin 500 mg, all b.d. for 7 days, became the Finnish recommendation for the first-line therapy in 2002 (http://www.kaypahoito.fi). The Maastricht recommendation accepts substitution of metronidazole 500 mg b.d. for amoxicillin. Although in single trials over 90% results can be achieved with triple therapy, in clinical practice the results are much poorer: 60–80% (Moayyedi et al. 2000; Della Monica et al. 2002; Buzas and Jozan 2004). Different PPIs are equivalent in triple therapies (Vergara et al. 2003). Sequential therapy may achieve still better results. Zullo et al. (2005) showed that a 10-day sequential therapy with rabeprazole 20 mg b.d. plus amoxicillin 1 g b.d. for the first 5 days, followed by rabeprazole 20 mg, clarithromycin 500 mg and tinidazole 500 mg, all b.d., for the remaining 5 days, achieved better eradication rates than the standard 7-day triple regimen with rabeprazole 20 mg, clarithromycin 500 mg and amoxicillin 1 g, all b.d. (94% vs. 80%).

Ranitidine bismuth citrate
The only bismuth product (DeNol®) was withdrawn (now available under special licence) from the Finnish market after PPI-based triple therapies proved their efficacy. A combination of pH-raising ranitidine with the helicobacter-toxic bismuth, ranitidine bismuth citrate (RBC), was thereafter introduced as a substitute. Length of the therapy impacts eradication: 60% in 4-day, 84% in 7-day and 85% in 10-day treatments (Savarino et al. 1999). RBC 7-day triple therapies have been compared with the corresponding PPI triple therapies. The mean eradication for RBC-clarithromycin-amoxicillin, RBC-clarithromycin-metronidazole and RBC-amoxicillin-metronidazole was 83%, 86% and 71%. The results were comparable to those of PPI-based therapies, except that the combination of RBC with amoxicillin and metronidazole was better than the corresponding PPI combination, OR 1.65 (Gisbert et al. 2005). The reason is that in the presence of RBC, nitroimidazole resistance hampers less nitroimidazole-containing remedies (van der Wouden et al. 1999). RBC 400 mg + amoxicillin 1 g + clarithromycin 500 mg all b.d. for 7 days is the alternative for the first-line therapy in Finland (http://www.kaypahoito.fi).
**Impact of resistance**

Because antibiotic resistance is the most important risk factor for eradication failure, clarithromycin should be avoided when the primary resistance against clarithromycin in the area is at least 15-20%, unless the resistance is tested. Amoxicillin should be substituted for metronidazole in the clarithromycin-based therapy, if local nitroimidazole resistance reaches 40% (Malfertheiner et al. 2007).

**Rescue therapies after a failure**

In two large community-based studies, the eradication rates were only 61% and 73% (Moayyedi et al. 2000; Vakil et al. 2004). These are the levels most probably achieved in clinical practice. After a failed therapy, RBC 400 mg b.d. + metronidazole 400 mg t.d. + tetracycline 500 mg q.d. for 7 days is recommended in Finland. The Maastricht proposition is to use a quadruple therapy with bismuth or, if bismuth is not available, PPI combined with metronidazole and amoxicillin or tetracycline (Malfertheiner et al. 2007). The third therapy at least should be based on the resistance analysis.

Fluoroquinolones have proved their efficacy in rescue regimens and could be used in the third step. Levofloxacin was compared with rifabutin in a 10-day triple therapy after two failures with clearly better results (ITT 85% vs. 45%) (Gisbert et al. 2006b). In a meta-analysis, levofloxacin-based therapies were better than quadruple therapies (81% vs. 73%), with fewer adverse effects. A 10-day regimen was better than a 7-day; thus a 10-day levofloxacin-amoxicillin-PPI therapy could be recommended as a third-step therapy (Gisbert and Abraira 2006; Gisbert and Morena 2006). Widespread use of fluoroquinolones will increase resistance and may impair this regimen.

Strains resistant to both metronidazole and clarithromycin are a challenge. Still, effective choices are in use. Since metronidazole resistance is relative, a quadruple therapy with PPI, bismuth, metronidazole and amoxicillin or tetracycline for 14 days can achieve 80% results (Miehlke et al. 2003), but with numerous side effects and difficulties in compliance. Rifabutin 150 mg + amoxicillin 1 g + esomeprazole 30 mg b.d. for 7 days as well as omeprazole 40 mg + amoxicillin 1 g t.d. for 14 days achieved over 70% success in this situation (Miehlke et al. 2006). A 12-day therapy with rifabutin 150 mg t.d. + pantoprazole 80 mg and amoxicillin 1 g t.d. further increased eradication to 91%, regardless of
metronidazole and/or clarithromycin resistance. Increasing the amoxicillin dose to 1.5 g further improved the result to 97% (Borody et al. 2006). Treatment with levofloxacin 500 mg + lansoprazole 30 mg + amoxicillin 1 g b.d. for 7 days succeeded in 79% in dual-resistant but levofloxacin-sensitive cases (Wong et al. 2006). Remarkably, in a study of 500 patients with primary failure, eradication after up to four attempts finally succeeded in 99.5% of the patients (Gisbert et al. 2008).

Side effects of the eradication treatment

In the Cochrane analysis, 22% of patients undergoing eradication therapy experienced adverse effects, compared with 8% in the comparison regimen group (OR 2.2). The frequency of individual symptoms in the therapy and comparison regimen groups was: diarrhoea 8% vs. 2%, nausea and vomiting 5% vs. 0.5%, skin rash 2% vs. 1%, epigastric pain 5% vs. 0.6%, altered taste 7% vs. 0.5%, stomatitis 2.5% vs. 0.3% and headache 4% vs. 3%. The differences were significant, except for headache (Ford et al. 2006). The side effects, especially diarrhoea, can be reduced with probiotics and the eradication results may likewise improve (Tong et al. 2007).

Benefits from the eradication therapy

Peptic ulcer disease

The pros of H. pylori eradication are best shown in PUD. The Cochrane analysis of H. pylori eradication in PUD was published in 2006 (Ford et al. 2006). When H. pylori eradication therapies alone were compared with no treatment, eradication was better in DU healing, 76% vs. 41.5%, number needed to treat (NNT) = 2.5. In comparison of H. pylori eradication therapy and ulcer-healing therapy with ulcer-healing therapy alone in healing DU, only a small difference in favour of combination was found (NNT = 14). There was no difference with GU. Healed DU recurred in surveillance times of 2 months to 5 years in 14% after eradication therapy, but in 64% without treatment (NNT = 2); in GU, the NNT was 2.6. In this review, eradication therapy did not relieve symptoms better than no therapy and in combination with ulcer-healing therapy was not better than the ulcer-healing
remedy alone. However, the follow-up time in these studies was only 4–6 weeks. McColl et al. (1998b) clearly found fewer symptoms after successful eradication in 3 months.

**Nonulcer dyspepsia**

*Helicobacter pylori* eradication in NUD has long been controversial. The OCAY study found no symptomatic benefit from the eradication therapy (79% eradication success) over placebo (Blum et al. 1998), whereas McColl et al. (1998a) found a small but significant benefit (symptom resolution in 21% vs. 11%); in this study, the eradication rate was 88%. The debate continued until the Cochrane review was published in 2003 (Moayyedi et al. 2003), in which of 21 randomized controlled trials showed a small but significant 10% risk reduction in favour of eradication therapy (NNT = 14).

**Test-and-treat**

In assuming that the prevalence of *H. pylori* infection in Finland among adults under 45 years of age is 20%, that the test used to diagnose *H. pylori* had a 95% sensitivity and specificity, and that 100 patients with uninvestigated dyspepsia were tested, we treated 19 truly positive patients. If the true symptomatic benefit of eradication therapy over a placebo is 10%, only two patients of the 100 tested, derived any real symptomatic benefit from the therapy. On the other hand, with the 95% specificity we would treat four wrongly positive patients in vain. In clinical practice, the efficacy of the therapy is at best 80%; thus we also have four rescue therapies. We would thus have ordered 27 therapies and 127 tests to benefit two patients. Among older patients, *H. pylori* is more prevalent, but for these test-and-treat is not recommended. Of course, the subjective benefit will be much higher because the mean placebo effect in functional dyspepsia is around 40% (Musial et al. 2007).

**Gastric malignancies**

*Helicobacter pylori* is considered the most important risk factor for noncardiac gastric adenocarcinoma and eradication abolishes the cytological changes leading to cancer. Decisional models suggest that screening and eradication therapy could be cost-effective. Still, eradication therapy has not proven its efficacy in reducing gastric cancer (Fuccio et
al. 2007). However, in the rare MALT lymphomas, the benefit of the eradication therapy is proved when the lymphoma is confined to the mucosa and submucosa (Stolte et al. 2002). In a Japanese study with 74 patients, complete remission was achieved in 56, partial response in 14 and no response in only 4 patients (Terai et al. 2008). In another large study, 84 of 90 patients achieved complete remission, while during a follow-up of median 45 months, eight patients had a recurrence of MALT lymphoma; the presence of *H. pylori* infection was the risk factor for recurrence (Hong et al. 2006). Stolte et al. (2002) showed that in about 80% of cases of low-grade stage E1 lymphomas, complete remission is achieved with eradication therapy, with a 5% recurrence rate per year. Eradication therapy was effective even when *H. pylori* could not be found (Raderer et al. 2006).

**Iron deficiency anaemia**

*Helicobacter pylori* can cause iron-deficiency anaemia by blood loss from gastroduodenal lesions. Atrophic gastritis induced by *H. pylori* infection also impairs iron absorption by decreasing acid production. After *H. pylori* eradication, iron-deficiency anaemia was more successfully treated with iron supplementation (Chen and Luo 2007).
PRESENT STUDY

AIMS OF THE PRESENT STUDY

The aims of the present study were to determine the

1. primary antibiotic resistance of *H. pylori* in primary health care in Finland,
2. efficacy of three therapy regimens (amoxicillin + metronidazole + lansoprazole, amoxicillin + clarithromycin + lansoprazole and tetracycline + metronidazole and ranitidine bismuth citrate) in eradicating *H. pylori* among patients not previously receiving eradication therapy,
3. side effects of the therapy,
4. factors affecting the results of the eradication therapy and usefulness of the history of antibiotic use in deciding on the eradication regimen,
5. subjective benefit from the therapy, and
6. effect of smoking on symptoms and gastric histology in *H. pylori* gastritis.
PATIENTS

A total of 23 endoscopy referral centres recruited patients into the study. The centres were located in bigger and smaller towns and rural areas throughout Finland and chosen to receive representative samples of the Finnish population (Figure 3). The endoscopy centres recruited RUT-positive patients referred to upper GI endoscopy from primary health care.

Figure 3. Endoscopy units

Map of Finland. The endoscopy units are marked with spots. The population is concentrated in the southern part of the country.

The inclusion criteria were: upper GI endoscopy performed, positive RUT, indications for eradicating *H. pylori* according to endoscopists and ages between 18 and 75 years.
The exclusion criteria were:

1. former *H. pylori* eradication therapy,
2. PPIs or H₂-blockers used regularly within two weeks or antibiotic therapy within four weeks before endoscopy,
3. pregnancy, real or suspected and lactation,
4. hypersensitivity to any of the study medications for eradication therapy,
5. confirmed or suspected malignant disease,
6. gastric resection,
7. advanced kidney disease (s-creatinine > 200 µmol/l), severe liver disease, any serious illness with expected lifetime less than 2 years and
8. need for over 4 weeks of proton PPI or H₂-blocker therapy after the eradication therapy.

Initially, a total of 342 patients were recruited. At first 10 patients were excluded from analysis because the *H. pylori* infection diagnosed with the RUT could not be confirmed by histology or culture, leaving 332 patients for further analyses.

I. In the first study evaluating *H. pylori* resistance in Finland, the study population was comprised of 292 patients. In total, 40 *H. pylori*-positive cases were excluded in which the antibiotic sensitivity of *H. pylori* analysed in the E-test was not available.

II. In the second study analysing the different therapies for *H. pylori* eradication, the population was comprised of 329 patients, with three *H. pylori*-positive patients being excluded because the eradication result could not be assessed.

III. In the third study assessing the subjective gain of *H. pylori* eradication, the population was comprised of 216 *H. pylori*-positive patients, whose eradication therapy was successful with the first attempt at therapy and who answered all the questions in the Gastrointestinal Symptoms Rating Scale (GSRS) before and 1 year after therapy, and for whom the histology of all gastric antrum and body samples was reviewed.

IV. In the fourth study evaluating the effect of smoking on the immune response and on the degree of gastritis, 318 patients were included. Three *H. pylori*-positive patients were excluded, due to unconfirmed eradication results. Moreover, nine patients whose histological samples were not adequate for full analysis, and two patients whose serology
was not assessed, were also excluded from analysis. The patient selection as a whole is presented in Figure 4.

**Figure 4. Study flow chart.** *Helicobacter pylori* eradication was tested with the $^{13}$C-urea breath test 1 month after therapy. If patients had used antibiotics, $\text{H}_2$-blockers or proton pump inhibitors before the breath test, confirmation was also by serology or histology. Queries were answered before and 1 year after therapy. RUT: rapid urease test
METHODS

In the upper GI endoscopy, one biopsy sample was taken from the gastric antrum and body for the RUT (Pyloriset™, Orion Diagnostica, Espoo, Finland), in addition to the basic histologic samples (two biopsy samples from descending duodenum, gastric antrum and gastric body). If the RUT was positive and the investigator considered eradication therapy as indicated, the patient was asked to participate in the study and invited to give informed consent.

Patients with a GU and/or DU and the nonulcer patients were separately randomized into the three 7-day eradication regimens:

1. lansoprazole 30 mg b.d., amoxicillin 1 g b.d. and metronidazole 400 mg t.d. (LAM)
2. lansoprazole 30 mg b.d., amoxicillin 1 g b.d. and clarithromycin 500 mg b.d. (LAC)
3. ranitidine bismuth citrate 400 mg b.d., metronidazole 400 mg t.d. and tetracycline 500 mg q.d. (RMT)

If the medication was taken properly for at least 5 of the 7 days, it was considered to be carried out per protocol. The eradication results were assessed on an intention-to-treat basis. Success of the therapy was controlled 4 weeks after therapy by the UBT. If the UBT was negative, the patients were asked to give a blood sample for serology. If the UBT was positive, the patients were referred for a control endoscopy within 3 months after the first endoscopy with repeated histology, culture and RUT. A single pathologist (P. Sipponen) reviewed all the histologic samples according to the updated Sydney system. The specimens were stained with haematoxylin-eosin, Alcian blue periodic-acid Schiff and modified Giemsa stains.

Culture

The Pyloriset™ also serves as a medium for transport to the culture. The samples were mailed by special delivery in a cold package to Laboratory Diagnostics of Helsinki University Central Hospital (HUCH) for culture the following morning. Specimens in the RUT were cultured on Brucella agar plates (BBL; Becton Dickinson, Cockeysville, MD,
USA) supplemented with horse blood (7%). Selective Brucella agar plates containing Iso-Vitalex (1%), vancomycin (6 mg/l), amphotericin (2 mg/l) and nalidixic acid (20 mg/l) were used. The plates were incubated at 37 °C in a microaerobic atmosphere for a maximum of 12 days. The \textit{H. pylori} isolates were identified based on colony appearance, Gram staining, and positive reactions in the catalase, oxidase and urease tests (Mégraud 2007).

**Antibiotic resistance**

The MICs were determined for metronidazole and clarithromycin by the Etest™ method (AB Biodisk, Solna, Sweden). Colonies picked from agar plates were harvested in Müller-Hinton (BBL) broth. The bacterial suspensions were diluted to match the turbidity of a 4-McFarland standard. Mueller-Hinton agar plates (BBL) supplemented with 10% horse blood were inoculated with the suspension and the E-test strips were placed on agar plates. The plates were incubated at 37 °C in a microaerobic atmosphere for 72 h. The MIC values were determined according to manufacturer’s instructions. The breakpoint of resistance for clarithromycin (1 µg/ml) was determined according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS) and that for metronidazole (8 µg/ml) as earlier used and suggested by King (2001).

**Serum antibody determination**

Serum samples taken before and over 4 months after eradication therapy were stored at -20 °C and analysed in parallel. The \textit{H. pylori} IgG and IgA antibodies were analysed with an in-house EIA (Oksanen et al. 1998). If the \textit{H. pylori} IgG antibodies decreased at least 40%, eradication was considered successful (Rautelin and Kosunen 2004).

**Questionnaires**

At the endoscopy, the patients completed a query on previous antibiotic use, current medication, smoking, alcohol consumption and social situation. The GI symptoms were assessed, using the GSRS, which grades all symptoms during the previous week into seven categories (1–7) (Svedlund et al. 1988). The main concern in the analysis was in the symptoms that are categorized as dyspeptic symptoms in the original GSRS study: stomach
pain, heartburn, acid regurgitation, sucking sensation (hunger pain), and nausea and vomiting. These symptoms were combined to produce a total dyspeptic score. This questionnaire was repeated 1 year after therapy. During the therapy, the patients completed a diary on the use of eradication medication, side effects and other concurrent medication.

Four weeks after therapy, in the UBT, the patients completed a query on their opinion of the endoscopy, eradication medication, and use of the antibiotics and PPIs before the UBT. One year after therapy, the patients were sent a questionnaire containing GSRS and questions about medication, alcohol consumption and smoking and possible new therapies for *H. pylori* eradication after failure of the first therapy.

**Statistical analyses**

The dichotomic variables were analysed with the chi-squared test or Fisher’s exact test when appropriate and continuous variables with the Mann-Whitney U-test. The sign test was used to examine symptom changes as a whole. The correlations were analysed with canonical correlation. Logistic regression analysis (method: forward) was used to detect independent factors. The statistical analyses (I, II) were carried out with the software package SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA). NCSS 2000 software for Windows (NCSS Statistical Software, Kaysville, UT, USA) was used for statistical analyses (III, IV).

**Ethics**

The ethics committee of HUCH and local ethics committees serving the endoscopy centres approved the study. All study subjects gave their informed consent and the study was conducted according to the Declaration of Helsinki. All patients were given medical therapy already found effective in eradicating *H. pylori*. Only in case of therapy failure were patients asked to have a second upper GI endoscopy to reveal possible resistance of the bacteria and subsequently an effective second therapy.
RESULTS

Resistance

Of the 342 RUT-positive patients, both histology and culture were negative in 10 (3%) cases and these were considered as H. pylori-negative. Of the remaining 332 patients, H. pylori could not be cultured in 33 cases. Furthermore, in three culture-positive cases H. pylori was lost before resistance determination, and in four cases resistance was determined only with the disc diffusion test. Finally, the 292 cases (158 women, 134 men, mean age 56 years, 23 had gastric and 56 duodenal ulcers) with the E-test result formed the study population.

Resistance against metronidazole and clarithromycin was also tested with the disc diffusion test. The results of the disc diffusion and E-tests were 99.7% equivalent; only in one case did the disc diffusion show metronidazole resistance and E-test sensitivity.

The metronidazole resistant cases comprised 38% of the patients (110/292), 25% (34/134) of the men and 48% (76/158) of the women (p < 0.001). Figure 5 shows the risk factors for metronidazole resistance in males and females. Previous use of antibiotics for gynaecological infections (67% vs. 43%, p = 0.001) and alcohol consumption increased the risk of resistance among women. Marital status, education and occupation did not affect the resistance rate. The men had no significant risk factors. Resistance was more prevalent in towns with over 50 000 inhabitants (44% vs. 32% in smaller towns and rural areas, p = 0.03), but local use of nitroimidazoles, obtained from the statistics of the National Agency for Medicines, showed no significant correlations with resistance frequency.
Only seven patients (2%) had a clarithromycin-resistant *H. pylori* strain. Clarithromycin resistance was clearly associated with former antibiotic therapies: use of antibiotics for respiratory infections increased the resistance rate from 0% to 4% (p = 0.02) and for dental infections from 1% to 6% (p = 0.002) (Figure 6). Patients with known previous macrolide therapy also had resistant strains in 8% compared with 2% in those without. The difference was not significant in these low frequencies (p = 0.053).
Only four patients harboured strains resistant to both metronidazole and clarithromycin.

In multivariate logistic regression analysis, female gender (OR 2.2, 95% confidence interval (CI) 1.3–3.8) and previous use of antibiotics for gynaecological infections (OR 2.5, 95% CI 1.2–54) independently predicted metronidazole resistance. Previous use of antibiotics for dental infections was the only independent risk factor for clarithromycin resistance (OR 5.4, 95% CI 1.0–28.8).

Results of the eradication therapies
The study population comprised 329 patients, of which 106 had LAM, 110 LAC and 113 RMT therapy. The therapy groups did not differ in age, sex, PU, smoking, coffee consumption, NSAID or acetylsalicylic acid (ASA) use, macrolide or metronidazole resistance. Only alcohol consumption was significantly higher in the LAC group than in the others (p < 0.05). However, alcohol had no effect on the eradication results and cannot be regarded as a confounding factor.

The eradication rate in the LAC group was 91% (100/110) in the LAM group 78% (83/106), and in the RMT group 81% (92/113). The differences were statistically significant between LAM and LAC (p = 0.01) and between LAC and RMT (p = 0.04). Metronidazole resistance reduced the eradication rate in the LAM group from 93% (52/56) in sensitive cases to 53% (20/38), p < 0.001 and in the RMT group from 91% (58/64) to 67% (26/39), p = 0.002 (Figure 7). Reduction caused by metronidazole resistance in the LAC group was not significant (from 95% to 84%). LAC therapy failed in all three clarithromycin-resistant cases.

The history of macrolide therapy reduced the eradication rate from 86% to 67% (p = 0.002) and tetracycline therapy from 88% to 71% (p = 0.001). LAC therapy was most sensitive to former antibiotic therapies: if none of the antibiotics mentioned were used, the eradication rate was 98%, and if any were used, 81% (p = 0.002). Thus, when any of these antibiotics were used previously, RMT therapy was as effective as LAC. Antibiotic therapies against respiratory, dental, gynaecological and diarrhoeal infections did not predict failure; the only exception was dental diseases in the LAC group (79% vs. 96%, p = 0.01). Local consumption figures for metronidazole and clarithromycin did not predict therapy failure.
Smoking reduced the efficacy of treatment only in the LAC group: from 95% to 81%. As a result, all three remedies were equally efficient among smokers. Coffee consumption likewise impaired only the LAC therapy. Finally, all the LAC failures were persons who smoked and/or drank coffee. Age, gender and PUD did not affect eradication. The greater distribution volume of the drugs, measured by the body surface area, predicted therapy failure only in the LAM group. In the RMT group, 26% of the patients missed at least one tablet compared with 9% in the LAC and 12% LAM groups. Patients who missed at least one tablet in their therapy had a lower eradication rate than did those who took all the scheduled medication (73% vs. 86%, p = 0.04).

A multivariate analysis was performed to find independent factors for eradication failure. Only factors known at the endoscopy were included (age, present PU, previous PU, alcohol use, smoking and previous therapy with amoxicillin, macrolides or tetracycline and for females also previous use of nitroimidazoles). Among women, previous use of nitroimidazole antibiotics was an independent risk factor for failure of eradication therapy (OR 4.3, 95% CI 2.0–9.4). Among men, no independent risk factors for treatment failures were observed.
A total of 90% of patients experienced side effects; 40% at least moderate, interfering with daily activities. Although patients in the LAM group had mostly flatulence, in the LAC group taste disturbances and in the RMT group nausea and vomiting, the total side effect score was similar in the various treatment arms. Patients in the RMT group found it most difficult to take medication according to the instructions (p < 0.001 compared with the LAM and LAC groups).

**Symptomatic response to *Helicobacter pylori* eradication**

The success of the eradication therapy was confirmed in 275 patients. The histology was incomplete in nine cases and a further 50 patients did not return the query sent 1 year after therapy or the queries were incompletely filled. Thus, this study comprised 216 patients. One year after the therapy, all symptoms in the GSRS were reduced significantly. The mean severity of dyspeptic symptoms in the seven-category questionnaire before therapy was 2.64 and after 1 year 1.84 (31% reduction). The reduction was similar in each dyspeptic symptom. Those GSRS symptoms referring only to the lower GI tract (constipation, flatulence, diarrhoea, loose stools, hard stools, urgency and defective defecation) decreased 20%.

Before therapy, the mean total dyspeptic symptom score was higher among women than men (13.88, 95% CI 12.90–14.85, vs. 12.52, 95% CI 11.78–13.26, p = 0.046) and also decreased more (4.69 (95% CI 3.76–5.61) vs. 3.33 (95% CI 2.62–4.04), p = 0.03). Patients 50–59 years of age had slightly more severe symptoms than those younger and older (total dyspeptic score 14.24 (95% CI 13.21–15.27) vs. 12.63 (95% CI 11.45–13.82), p = 0.04 and 12.72 (95% CI 11.71–13.74), p = 0.03), and there was clearly a better improvement in the total dyspeptic score after therapy in this group, 5.32 (95% CI 4.37–6.28) vs. 3.37 (95% CI 2.37–4.37), p = 0.009, and 3.35 (95% CI 2.35–4.36), p = 0.003, among the younger and the older (Figure 8).
Duodenal ulcer was found in 28, gastric ulcer in 12, and both in 3 patients. Although dyspeptic symptoms did not differ significantly between duodenal ulcer and NUD patients, the DU patients had a more pronounced reduction in the dyspeptic symptom score (mean 5.93 (95% CI 4.45–7.41) vs. 3.65 (95% CI 3.04–4.26), p = 0.009). Differences in individual symptoms are presented in figures 9 and 10.

Figure 9. Mean severity of dyspeptic symptoms before the *H. pylori* eradication among duodenal ulcer and NUD patients
Although smoking did not affect the dyspeptic symptoms at baseline, the mean reduction in the total dyspeptic symptom score was greater among smokers (5.64 (95% CI 4.21–7.08) vs. 3.65 (95% CI 2.98–4.32), p = 0.008). Especially abdominal pain improved more in smokers than in nonsmokers (1.79 (95% CI 1.30–2.27) vs. 0.78 (95% CI 0.60–0.97), p < 0.001).

Changes in the number of medication the patients used did not significantly affect reduction in the total dyspeptic score (major medication classes analysed: vitamins and trace elements, cardiovascular, gastroenterologic, gynaecologic, endocrinologic and metabolic drugs, NSAIDs and ASA). Alcohol and coffee consumption likewise had no effect on symptom changes.

Gastric histology before therapy did not correlate with the total dyspeptic score, but patients with higher (Sydney 2-3, N = 82) antral neutrophilic inflammation had a clearly better symptom resolution than those with lower inflammation (Sydney 0-1, N = 134) (4.67 (95% CI 3.86–5.49) vs. 3.37 (95% CI 2.51–4.22), p = 0.008), while gastric body atrophy of any grade (N=35) had a contrasting effect (2.40 (95% CI 1.07–3.73) vs. 4.35 (95% CI 3.70–5.00) among those 181 patients without corpus atrophy).
In multivariate logistic regression analysis, DU (OR 3.2; 95% CI 1.3–7.8), age from 50 to 59 years (OR 2.2; 95% CI 1.2–3.9) and neutrophilic inflammation grade 2–3 in the gastric antrum (OR 1.9; 95% CI 1.1–3.3) predicted better response in the total dyspeptic score.

**Smoking, histology and serology**

The study population comprised 318 *H. pylori*-positive patients (73 smokers and 245 nonsmokers) with confirmed eradication results and complete gastric histology. Smokers had less often gastric body atrophy than nonsmokers (4.1% vs. 17.1%, p = 0.004) (Figure 11). Chronic inflammatory cell and neutrophilic inflammation were also milder in the gastric body in smokers. No differences were found in the gastric antrum. Compared to nonsmokers, smokers had a higher *H. pylori* load in the gastric antrum, but lower in the gastric body. Although body atrophy is more common in older age, and smokers were younger than nonsmokers, the difference was seen even when the population was divided into four age groups of equal size. Logistic regression analysis containing age, sex, and smoking, confirmed that smoking was independently associated with reduced body atrophy (OR=0.2, 95% CI 0.1-0.8). Smoking did not increase intestinal metaplasia in the gastric antrum or body.

**Figure 11. Smoking and atrophy in the gastric body**

![Graph showing smoking and atrophy in the gastric body](image)

Smoking reduced the IgG antibody response against *H. pylori*, with mean titres of 5587 vs. 8535, p = 0.002. The decrease in serum immunoglobulin levels after eradication therapy
was smaller among smokers (69% vs. 76%, p < 0.001). Likewise, serum IgA antibody levels decreased less after therapy among smokers (57% vs. 65%, p = 0.01).

Peptic ulcer

Of the 73 smokers, 23 (31.5%) had a DU, compared with 28 (11.4%) of the 245 nonsmokers. A GU was found in only 8.2% of smokers and 5.7% of nonsmokers; the difference was nonsignificant. None of the patients with any degree of atrophy in the gastric body had a PU. Use of NSAIDs, ASA, coffee or alcohol did not increase the prevalence of PUs.
DISCUSSION

Methodological aspects
To my knowledge, this is the first prospective nationwide population-based study of *Helicobacter pylori*. The endoscopy units were chosen in bigger and smaller towns and rural areas, so that the population they served could represent the Finnish population as a whole and the results could be generalized throughout the whole nation. However, some selection biases may still exist. The study population was a small portion of all patients referred for upper GI endoscopy in these centres during the time of recruitment. Younger patients were more prone to have undergone *H. pylori* eradication, based on noninvasive tests, thus excluding them from the study. The recruited patients were referred for upper GI endoscopy; thus, they probably had more severe and persistent dyspeptic symptoms. They also had an indication for eradication therapy, which could select more serious cases. Patient selection was based on the RUT; thus patients with severe gastric atrophy might not have been found. The symptomatic results of therapy could also have been biased if those patients who did not complete the query 1 year after therapy had outcomes different from those who did.

The UBT was used as a basic test to confirm the eradication result. The UBT can be unreliable in advanced atrophic gastritis (Kokkola et al. 2000). In our study, none had grade 3 atrophy, 6 had grade 2 atrophy and 29 grade 1 atrophy in the gastric body. Thus the effect on the UBT results was probably marginal.

Antibiotic Resistance
The sensitivity to both metronidazole and clarithromycin was polarized to the very sensitive and very resistant ends, leaving little room to speculate about the grey zone between sensitivity and resistance. In all metronidazole-sensitive cases the MICs were below 2 µg/ml, while among resistant cases only two had MICs near the breakpoint (12 and 16 µg/ml). The nearest clarithromycin MICs around the breakpoint 1 µg/ml were 0.125 and 6 µg/ml.
Currently when test-and-treat is recommended for dyspeptic patients under 45 years of age and without alarming symptoms, it is important to know the prevalence of *H. pylori* and the primary resistance pattern in the population. If primary metronidazole resistance is at least 40%, metronidazole-containing eradication remedies are not recommended as a primary therapy (Malfertheiner *et al.* 2007). If clarithromycin resistance rate is 15–20%, clarithromycin is not recommended without susceptibility testing (Malfertheiner *et al.* 2007). The overall resistance to metronidazole in our study was 38%, which is slightly higher than in Northern part of Europe. Resistance among women was 48%, too high for metronidazole-based therapies. The most logical explanation for higher resistance level is former nitroimidazole therapy. In this study, 69% of women remembering former nitroimidazole use had a resistant strain. Furthermore, in Finland, the metronidazole use in defined daily dose per 1000 inhabitants per day is 0.3, compared with 0.07 in Norway, where metronidazole resistance is 30%. Clarithromycin resistance was 2%, as low as that usually reported in Northern European countries (Lerang *et al.* 1997; Jaup *et al.* 1998). This low resistance level can be attributed to the restrictive use of macrolides in respiratory infections in Finland after the recommendation in 1991 (Huovinen and Klaukka 1991). In 2001, macrolide consumption in Finland was only one third of that in France, where clarithromycin resistance is 20% (Cars *et al.* 2001; de Korwin 2004). Macrolide resistance is exceptionally high in children, e.g. 24% in a large European study (Koletzko *et al.* 2006).

Metronidazole resistance among Finnish men was 25%. Men at least 60 years of age were born and thus had acquired their *H. pylori* infection before metronidazole was on the market. They also very seldom receive metronidazole therapies. Thus, it can be concluded that the inherent metronidazole resistance of *H. pylori* in Finland is 25% as found among these men. The life style among modern women apparently is associated with more resistant helicobacters (young, urban and alcohol-consuming women).

In the present analysis of *H. pylori* resistance, the patients were recruited from those referred for upper GI endoscopy; thus, the population was somewhat selected. However, all the patients with previous eradication therapy were excluded; thus, the results are representative of primary *H. pylori* resistance in Finland.
Based on resistance data, the Finnish recommendation to use clarithromycin and amoxicillin as the first-line eradication therapy is adequate. Fortunately, primary double resistance is very rare, only 1%, which makes it easier to find an effective metronidazole-based therapy if the first attempt with clarithromycin fails.

The results of the disc diffusion and E-tests for metronidazole and clarithromycin resistance were 99.7% equivalent. Thus disc diffusion can be recommended as a primary test in clinical practice.

**Eradication therapy**

For the eradication trial, two commonly used eradication therapies, LAM and LAC were chosen, in the dosages usually used (www.kaypahoito.fi/). The third, RMT, was an exception to that recommended. It was chosen to show, whether tetracycline would be effective when combined with RBC. If so, it would be an alternative therapy option for penicillin-allergic patients.

Eradication therapy with clarithromycin and amoxicillin, combined with a double dose of PPI, showed the best results in our study. The ITT success of 91% is very high and reflects not only a low resistance to clarithromycin but also a high level of commitment and compliance of Finnish patients to the therapy. Metronidazole resistance was the most important factor for eradication failure. In comparison to sensitive cases, metronidazole resistance reduced the efficacy of LAM from 93% to 53% and that of RMT from 91% to 67%. RBC is thought to be more effective than PPIs in eradicating metronidazole-resistant helicobacters. Our study also suggests this, although due to the small number of cases no statistically significant differences could be demonstrated. The high resistance level for metronidazole (48%) makes metronidazole combinations ineffective in treating *H. pylori* in Finnish women unless the sensitivity is tested. When the *H. pylori* strains were metronidazole-sensitive, all regimens were as effective (over 90%).

In asking the patient about his/her former antibiotic therapies, those that gave inferior eradication results following the LAC therapy could be found. However, even among these patients, LAC was equally as effective as the other two regimens in all patients (81%). Among patients without history of antibiotic use, LAC eradicated all but one *H. pylori*
case, a result better than the accuracy of any test to confirm eradication. The value of antibiotic history is diminished because patients do not seem to remember their former antibiotic use. In the LAC group, all patients with therapy failure were either smokers and/or coffee drinkers, which can be associated with increased gastric acid production induced by smoking or caffeine (Boekema et al. 1999; Maity et al. 2003). In acidic environments, the penetration of clarithromycin into the gastric mucosa is impaired (Endo et al. 2001). Lin et al. (2002) found that coffee drinking led to eradication failures in clarithromycin-based quadruple therapy. Studies on smoking as a risk factor in eradication therapy revealed conflicting results (Cutler and Schubert 1993; Treiber et al. 2002).

All three regimens caused many side effects; these effects, however, did not bring about compliance problems or reduce the efficacy of the therapy. RMT was a complex regimen and thus the most difficult to carry out, but the subjective difficulty had no impact on eradication results. As a whole, however, those who missed at least one tablet had poorer results, and this occurred most frequently in the RMT group.

The 7-day PPI-amoxicillin-clarithromycin therapy appears to be an effective first-choice therapy in Finland. The clarithromycin-metronidazole combination is unfavourable, due to high metronidazole resistance and the risk of biresistance after a failed therapy. This study emphasizes that RBC and metronidazole combined with tetracycline cannot overcome the metronidazole resistance.

**Effect of eradication on symptoms**

The dyspeptic symptoms (abdominal pain, heartburn, acid regurgitation, sucking sensation (hunger pain), nausea and vomiting) in the GSRS were assessed. Although hunger pain is frequently associated with DU disease, in our study the symptoms did not aid in picking out DU patients. The overall symptom reduction after eradication therapy was 31%, the level usually achieved with a placebo (Moayyedi et al. 2006). The GSRS symptoms referring only to the lower GI tract were decreased by 20%. It can be speculated that antibiotic therapies can reduce abdominal symptoms by modifying the colonic bacterial flora. However, the mostly bacterial dysbalance after antibiotic therapies is not beneficial (Beaugerie and Petit 2004). In our study the changes in symptoms after therapy did not
differ between the therapy arms with different antibiotics. Thus, the 20% decrease was probably the result of a placebo, not an antibiotic effect. Based on this assumption, the net benefit from \textit{H. pylori} eradication was 10%, as the Cochrane analysis showed among NUD patients. Usually, the placebo effect in functional gastroenterologic symptoms has been around 30% (Moayyedi \textit{et al.} 2006).

The study was planned to find factors that could better predict the symptomatic response to \textit{H. pylori} eradication. Gender was not a major factor. The age of the patient was surprising. Patients 50–59 years of age had slightly more symptoms in the beginning and the symptom resolution was clearly better than among the other age groups. In other studies, the age effect has been controversial. If this reversed U-shape is a universal phenomenon in this context, the discrepancy between studies comparing older with younger patients can be explained. The results of the study of Malfertheiner \textit{et al.} (2003) support our findings. The reason for the finding remains obscure; in the present study, associations with other factors predicting better response (smoking, DU, gastric histology) did not explain this. Duodenal ulcer also was an independent factor enhancing symptomatic response, especially stomach pain and sucking sensation. Effect of gastric ulcers was insignificant. Changes in gastric acid production could explain the difference.

\textbf{Smoking, symptoms and gastric histology}

Although smokers in some studies have had more dyspepsia and also poorer response to therapy (Nandurkar \textit{et al.} 1998; Delaney \textit{et al.} 2001; Wildner-Christensen \textit{et al.} 2006), this study showed better symptom resolution among them, possibly because smokers more often had PUs and less often gastric body atrophy. Patients with a PU had better results here, as well as those without body atrophy. In both cases, changes in acid production are potential explanations. Smoking, DU, gastric atrophy and gastric inflammation were strongly interrelated. Thus, strong factors in the univariate analyses were not independent risk factors in the logistic regression analysis. Only DU, ages of 50–59 years and antral neutrophilic inflammation grade 2–3 remained independent factors. Smoking may be a major factor in determining the symptomatic response after eradication therapy, although probably via decrease in gastric atrophy and increase in DU.
Among nonsmokers, antral neutrophilic inflammation was associated with higher *H. pylori* loads, which in turn led to higher levels of acid production and possibly to dyspeptic acid symptoms. After *H. pylori* eradication, acid production might be reduced, subsequently leading to alleviation of dyspepsia. Patients having more intensive neutrophilic inflammation had less body atrophy, and these together could lead to more intensive acid-related symptoms and later to better symptom resolution after therapy. On the other hand, increased acid production may make the environment in the antrum more suitable for *H. pylori* and increase the density of *H. pylori* in the antrum (Karttunen et al. 1991).

Smoking seemed to modify the antrum histology only by increasing the *H. pylori* load. In contrast, smoking was associated with the reduced acute and chronic inflammation, atrophy and *H. pylori* load in the gastric body. Reduced inflammation in the body can also lead to delayed atrophic changes among smokers. Thus, gastric inflammation and body atrophy do not explain the connection between smoking and gastric cancer.

Smoking also reduced serum anti-*H. pylori* IgG levels before therapy and also the relative decrease in the titre after successful eradication. This probably represents a drawback to the serologic diagnostic of *H. pylori* among smokers. However, in this study only two patients had eradication failure according to serology and success according to the UBT. The first patient had no gastric atrophy, whereas the second had grade 1 antrum atrophy only, but both were smokers. This means only a 1% discrepancy between serology and UBT (3% among smokers).

Smoking has been a known risk factor for PU before the *H. pylori* era. In *H. pylori* infection, smoking also increases the risk for DU (Parasher and Eastwood 2000). The mechanism by which smoking induces PUs is still debatable, although several possibilities have been introduced: reduced gastroduodenal motility and increased duodenogastric reflux with irritating bile salts, increased pepsinogen production, reduced mucus production and increased acid production leading to mucosal damage. Smoking also increases ulcerogenic reactive oxygen intermediates, endogenous vasopressin and platelet-activating factor, which increases the damage and reduces prostaglandin synthesis, as well as epidermal growth factor, which enhance ulcer healing. In our study, smoking also preserved the mucosa in the gastric body and increased the *H. pylori* load in the gastric antrum, thus further increasing acid and pepsinogen production (Parasher and Eastwood 2000).
2000). Thus, the almost three times higher risk for DU among smokers, found in our study, could be expected.
CONCLUSIONS

1. Metronidazole resistance of *H. pylori* in Finland and especially among women is high. Thus, metronidazole-based therapies cannot be recommended for women as a first-line treatment unless the sensitivity is tested beforehand. Clarithromycin resistance is extremely rare, but increased use of macrolide antibiotics may result in future change in this situation.

2. PPIs combined with amoxicillin and metronidazole, as well as RBC in combination with metronidazole and tetracycline, are inferior to the PPI-clarithromycin-amoxicillin combination, due to high metronidazole resistance.

3. Amount of side effects was on the same level in all therapy regimens.

4. Metronidazole resistance is the most important factor for eradication failure of metronidazole-based therapies, whereas macrolide resistance, smoking, and coffee drinking affected the clarithromycin-based therapy. History of antibiotic therapies has only limited use in deciding on the eradication regimen.

5. The symptomatic response to *H. pylori* eradication is only modest, and in most respects a placebo effect.

6. Smoking reduces the cellular and humoral responses to *H. pylori* infection, delays progression of atrophic gastritis in the gastric body, and almost triples the risk of duodenal ulcer. Smoking enhanced the subjective gain from the therapy.
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