HELICOBACTER PYLORI: RESISTANCE AND TREATMENT RESULTS IN FINLAND

Tarmo Koivisto

Division of Gastroenterology
Department of Medicine
Helsinki University Central Hospital
Helsinki, Finland

Academic Dissertation

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki in Auditorium XII, third floor of the main building of the University, Unioninkatu 34, Helsinki,
On June 6th, 2008, at 12 noon.

SUPERVISED BY

Docent Martti Färkkilä, MD, PhD
Division of Gastroenterology, Department of Medicine
Helsinki University Central Hospital
Helsinki, Finland

REVIEWED BY

Docent Pekka Collin
Department of Gastroenterology and Alimentary Tract Surgery
Tampere University Hospital
Tampere, Finland

Docent Tuomo Karttunen
Department of Pathology
Oulu University Hospital
Oulu, Finland

TO BE DISCUSSED WITH

Robert M. Genta
Clinical Professor of Pathology and Medicine (Gastroenterology)
University of Texas Southwestern Medical Center at Dallas
Chief for Academic Affairs, Caris Diagnostics
Dallas, Texas, USA

ISBN 978-952-92-3840-8 (nid.)
ISBN 978-952-10-4696-4 (PDF)
Helsinki University Print
Helsinki 2008

CONTENTS

Abstract 5
List of abbreviations 7
List of original publications 8
Introduction 9
Review of the literature 11
History 11
Helicobacter pylori infection 11
Bacteriology 11
Epidemiology 12
Antibiotic resistance 12
Metronidazole 12
Clarithromycin 14
Amoxicillin 16
Tetracycline 17
Fluoroquinolones 17
Rifabutin 17
Dual resistance to both metronidazole and clarithromycin 18
Secondary resistance 18
Helicobacter pylori and gastroduodenal diseases 18
Gastritis 18
Peptic ulcer 20
Malignancies 20
Dyspepsia 21
Diagnosis of *Helicobacter pylori* infection 22
Invasive tests 22
Noninvasive tests 26
Clinical use of the tests 28
Helicobacter pylori eradication therapy 29
Challenges in the therapy 29
Indications for eradication therapy 30
Test-and-treat 30
First-line therapy 31
Rescue therapies after a failure 33
Side effects of the eradication treatment 34
Benefits from the eradication therapy 34
Present study 37
Aims of the present study 37
Patients 38
Methods 41
Culture 41
Antibiotic Resistance 42
Serum Antibody determination 42
Questionnaires 42
Statistical analyses 43
Ethics 43
Results 44
Resistance 44
Results of the eradication therapies 46

Symptomatic response to *Helicobacter pylori* eradication 48
Smoking, histology and serology 51
Peptic ulcer 52
Discussion 53
Methodological aspects 53
Antibiotic Resistance 53
Eradication therapy 55
Effect of eradication on symptoms 56
Smoking, symptoms and gastric histology 57
Conclusions 60
Participating endoscopy units 61
Acknowledgements 62
References 64

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

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

LIST OF ORIGINAL PUBLICATIONS

I. Koivisto TT, Rautelin HI, Voutilainen ME, Niemelä SE, Heikkinen M, Sipponen PI, Färkkilä MA. Primary *Helicobacter pylori* resistance to metronidazole and clarithromycin in the Finnish population. Aliment Pharmacol Ther 2004;19(9):1009-17. Corrigenda: Aliment Pharmacol Ther 2004;20 (6): 701–701.

II. Koivisto TT, Rautelin HI, Voutilainen ME, Heikkinen MT, Koskenpato JP, Färkkilä MA. First-line eradication therapy for *Helicobacter pylori* in primary health care based on antibiotic resistance: results of three eradication regimens. Aliment Pharmacol Ther 2005;21(6):773-82.

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

IV. Koivisto TT, Voutilainen ME, Färkkilä MA. Effect of smoking on gastric histology in *Helicobacter pylori*-positive gastritis. 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 *H. pylori* infection, most of the symptomatic benefit comes from the placebo effect. PUD can in most cases be cured by *H. pylori* eradication. For ulcers among users of nonsteroidal anti-inflammatory drugs (NSAIDs), *H. pylori* has an additive effect and eradication is recommended.

In addition to *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 *H. pylori*.

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 *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.

Species: *H. pylori,* Genus: *Helicobacter*, Family: Helicobacteraceae, Order: Campylobacterales, Class: Epsilonproteobacteria, Phylum: Proteobacteria, Kingdom: Bacteria.

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.

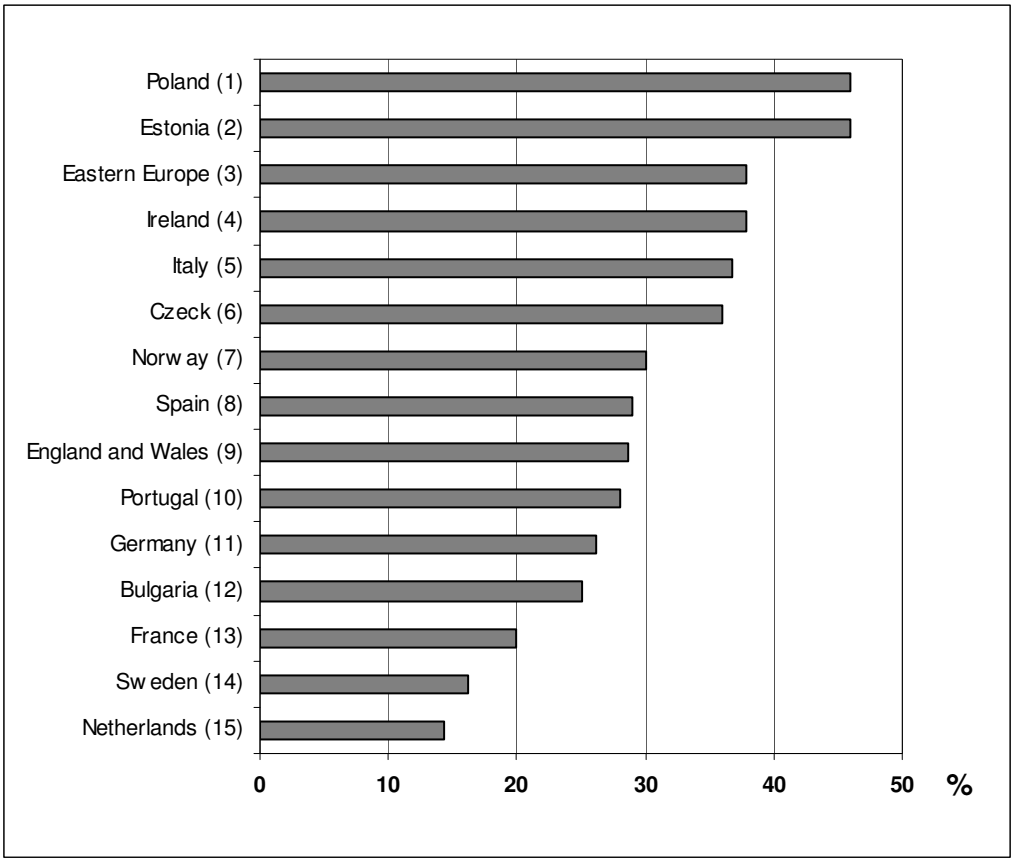
Metronidazole

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 nicotineamide 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.

Figure 1. Metronidazole resistance in Europe



1. (Dzierzanowska-Fangrat *et al.* 2005), 2. (Loivukene *et al.* 2002), 3. (Boyanova *et al.* 2002) 4. (Xia *et al.* 1996), 5. (Toracchio and Marzio 2003), 6. (Dite *et al.* 2002), 7. (Lerang *et al.* 1997) 8. (Gisbert and Pajares 2001), 9. (Chisholm *et al.* 2007), 10. (Cabrita *et al.* 2000), 11. (Wolle *et al.* 2002), 12. (Boyanova *et al.* 2008), 13. (de Korwin 2004), 14. (Storskrubb *et al.* 2006), 15. (Janssen *et al.* 2006)

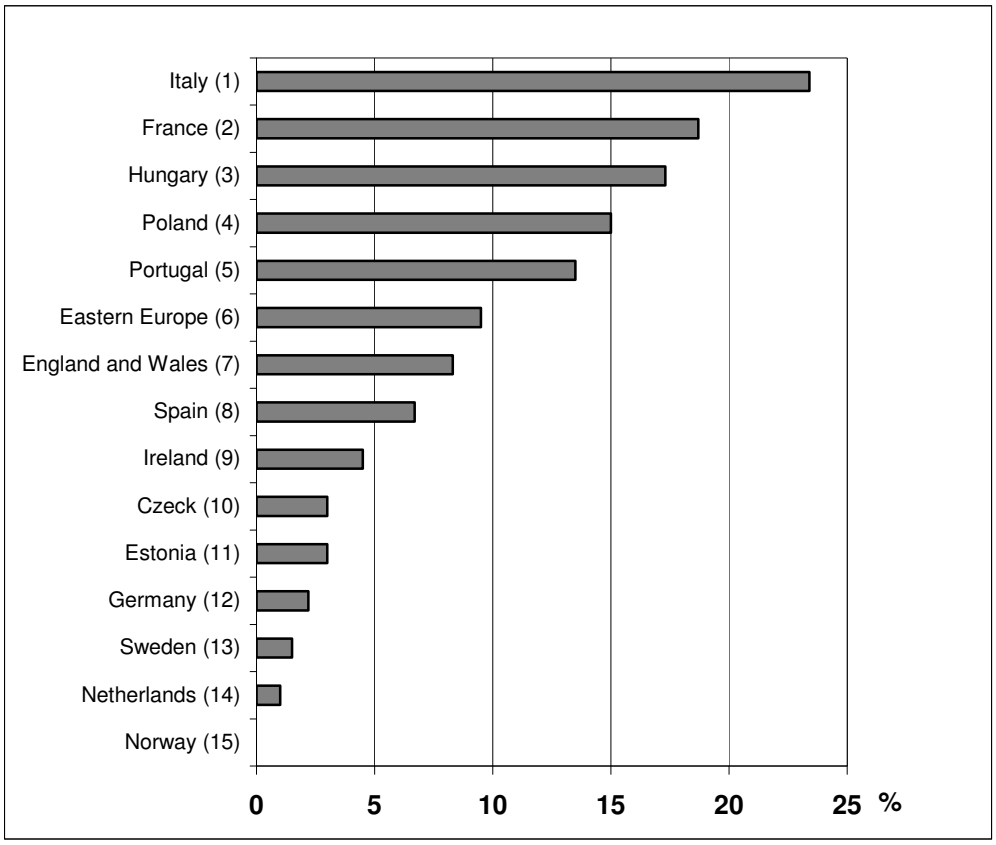
Clarithromycin

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.

Figure 2. Clarithromycin resistance in Europe



1. (Toracchio and Marzio 2003), 2. (Tankovic *et al.* 2001), 3. (Buzas *et al.* 2007), 4. (Dzierzanowska-Fangrat *et al.* 2005), 5. (Cabrita *et al.* 2000), 6. (Boyanova *et al.* 2002), 7. (Chisholm *et al.* 2007), 8. (Gisbert and Pajares 2001), 9. (Xia *et al.* 1996), 10. (Dite *et al.* 2002), 11. (Loivukene *et al.* 2002), 12. (Wolle *et al.* 2002), 13. (Storskrubb *et al.* 2006), 14. (Janssen *et al.* 2006), 15. (Lerang *et al.* 1997).

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 *gyr*A. 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 B_{12} 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 H_2-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 *H. pylori* infection, and in more then 90% of cases, MALT lymphoma is associated with *H. pylori* (Stolte *et al.* 2002). Development of MALT lymphoma is still a rare event among *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 γ-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 *H. pylori* infection induces transient symptoms. Graham *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 *et al.* 2004). In the chronic phase, symptoms of *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 *et al.* 2002; Treiber *et al.* 2004; di Mario *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 *et al.* 1993; Mearin *et al.* 1995; Mc Namara *et al.* 2000; Lin *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.*

2007). 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.* 2002). 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 ($(NH_2)_2CO + H_20 \rightarrow CO_2 + 2NH_3$). 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 *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-*H. pylori* antibodies in the IgG and IgA classes. The reliability of the method is based on the *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 *et al.* 1998; Herbrink and van Doorn 2000). The sensitivity and specificity can be similar to that of the UBT, around 95% (Oksanen *et al.* 1998). In a meta-analysis of commercial kits, the medians of the sensitivity and specificity were 92% and 83% (Laheij *et al.* 1998). In a small study with 102 patients and 8 kits, the sensitivity was 93–98% and specificity 95–98% (Meijer *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 *H. pylori* infection (Kokkola *et al.* 2000). When histology shows body atrophy, serology is recommended to exclude *H. pylori*.

Serology can also be used in confirming the result of *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 *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 *et al.* 1992)

Serology also has some drawbacks. It is not recommended for use in populations where *H. pylori* prevalence is lower than 30%, due to false-positive results. Among young children, the accuracy is lower than among adults (Kolho *et al.* 2002; Bonamico *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 posteradication 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*

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

Modified from Ables A, *et al.*: Update on *Helicobacter pylori* Treatment. Am Fam Physician. 2007 Feb 1;75(3):351-8.

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 *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 *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 *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 *H. pylori*-positive patients (38% of the test-and-treat group), 75% in the test-and-treat group continued using acid-reducing medication (Allison *et al.* 2003). In a large Finnish study, testing for and eradicating *H. pylori* reduced GI symptoms, but could not reduce the number of endoscopies (Färkkilä *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 *H. pylori* prevalence, empirical acid suppression is an equivalent option (Malfertheiner *et al.* 2007).

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 *et al.* 2007). Bismuth was the cornerstone in the first effective eradication regimen. Bismuth salts alone could eradicate *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 *et al.* 1999).

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 *et al.* 1996). However, metronidazole resistance decreased the eradication rate by 30–46% (Dore *et al.* 2000). In a large Finnish trial, the eradication rate was only 65.8% (Färkkilä *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 truely 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

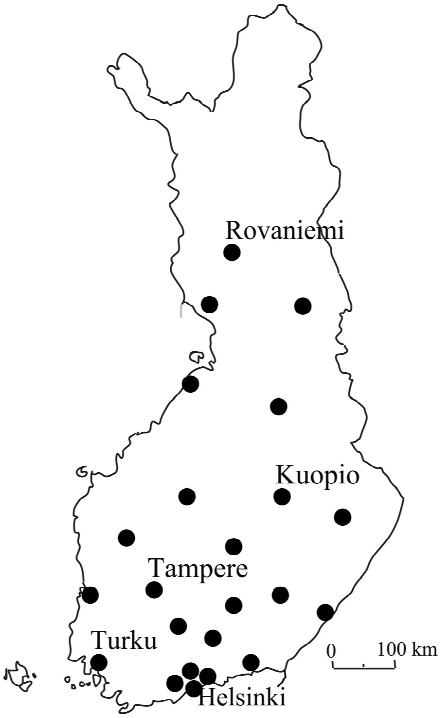
The aims of the present study were to determine the

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 H_2-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 H_2-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.

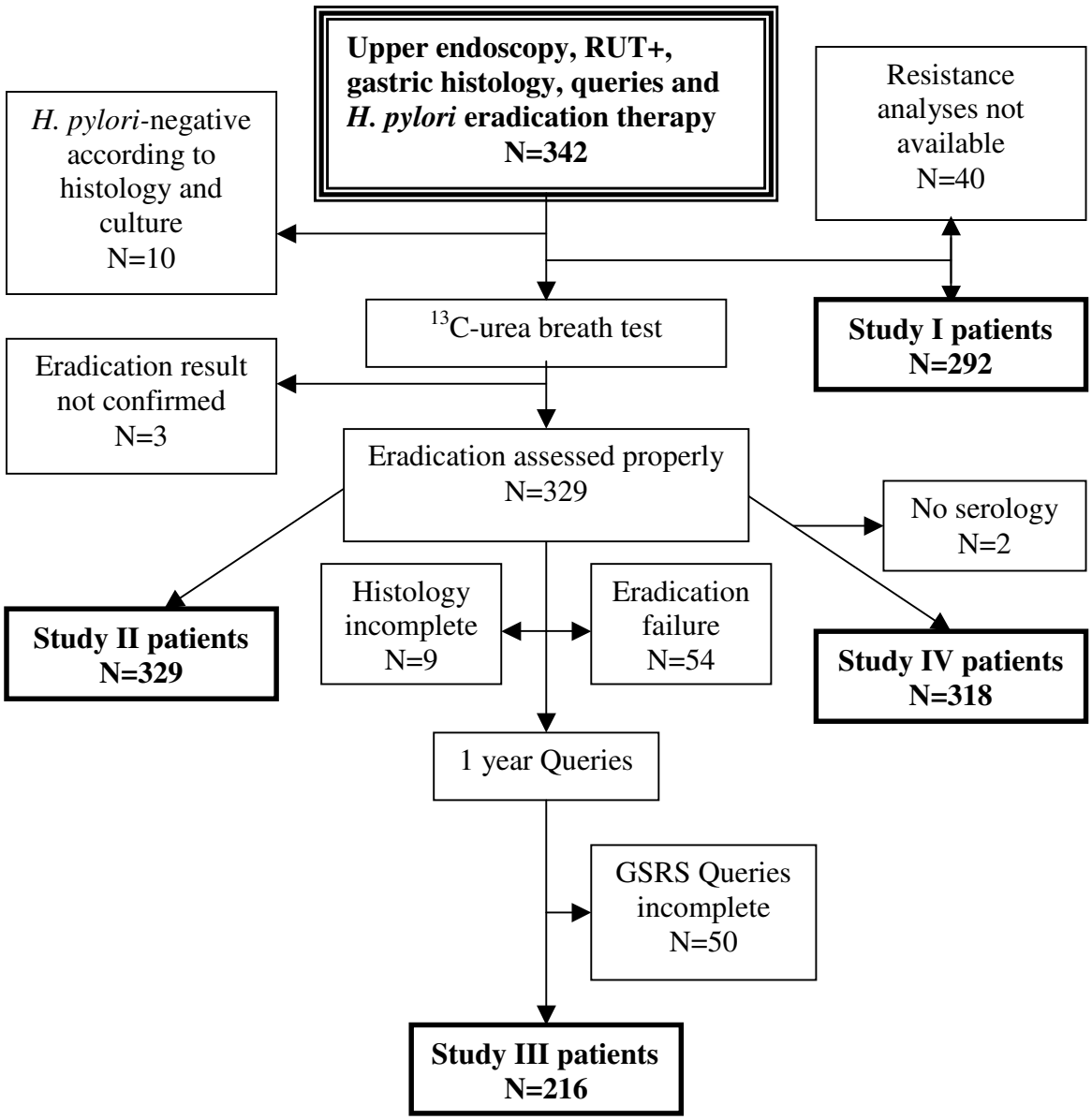
II. In the second study analysing the different therapies for *H. pylori* eradication, the population was comprised of 329 patients, with three *H. pylori*-positive patients being excluded because the eradication result could not be assessed.

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, 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 *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 *H. pylori* IgG and IgA antibodies were analysed with an in-house EIA (Oksanen *et al.* 1998). If the *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

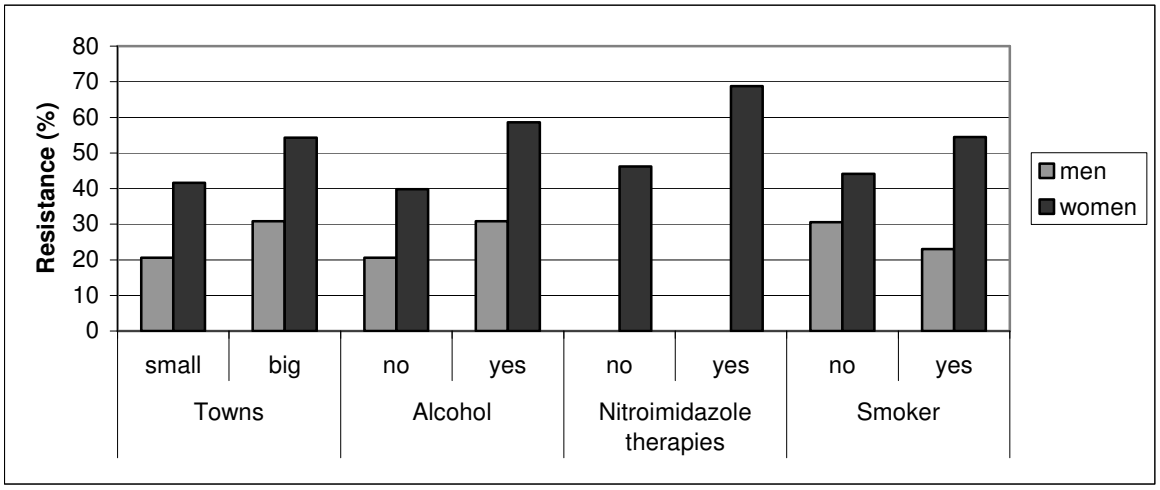
Resistance

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.

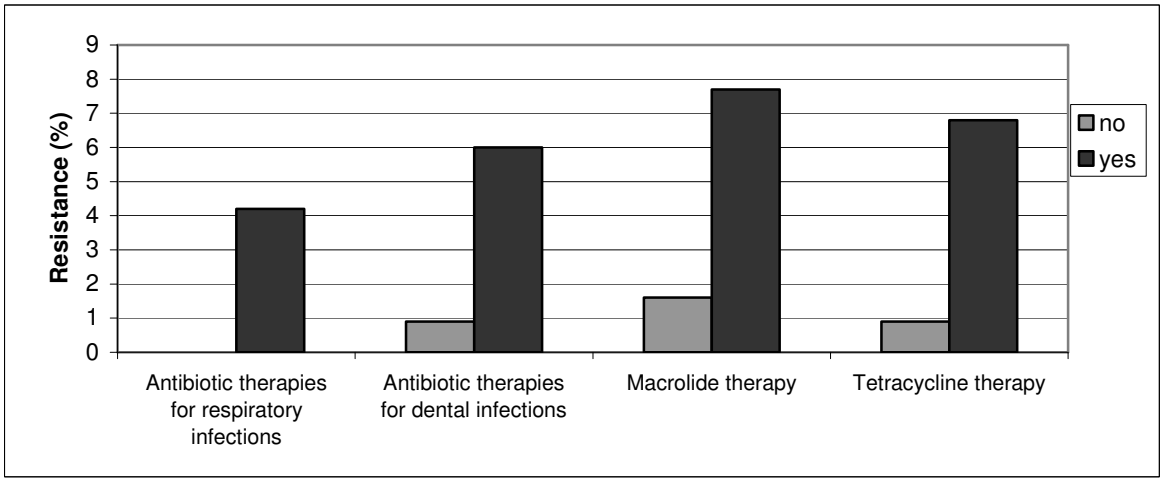
The metronidazole resistant cases comprised 38% of the patients (110/292), 25% (34/134) of the men and 48% (76/158) of the women ($p < 0.001$). Figure 5 shows the risk factors for metronidazole resistance in males and females. Previous use of antibiotics for gynaecological infections (67% vs. 43%, $p = 0.001$) 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.

Figure 5. Metronidazole resistance among men and women



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).

Figure 6. Clarithromycin resistance according to previous antibiotic therapies among all patients



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).

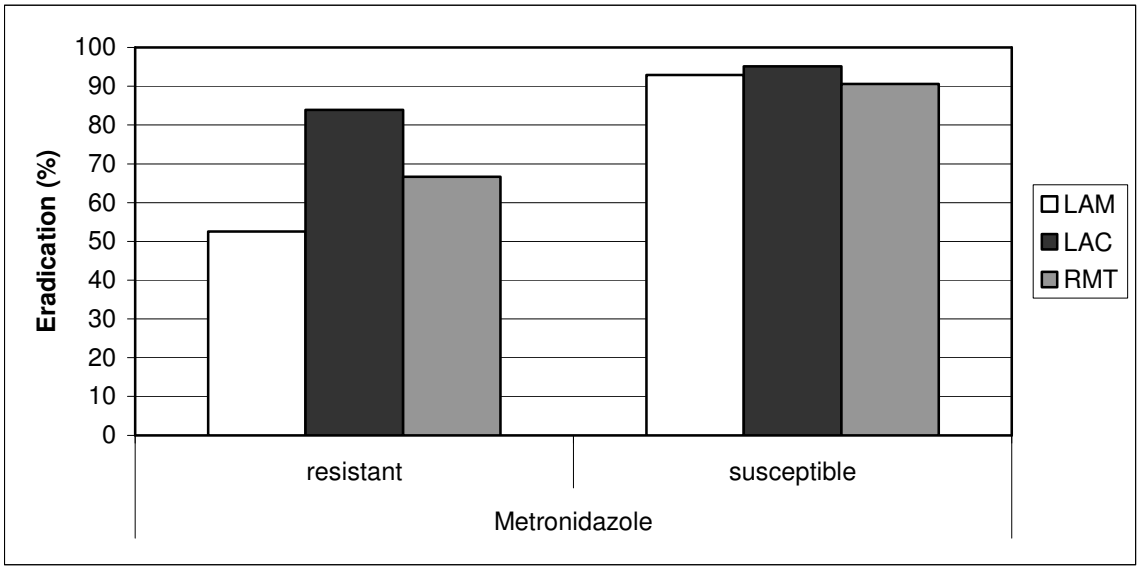
Results of the eradication therapies

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.

Figure 7. Eradication success between therapy groups according to metronidazole resistance



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.

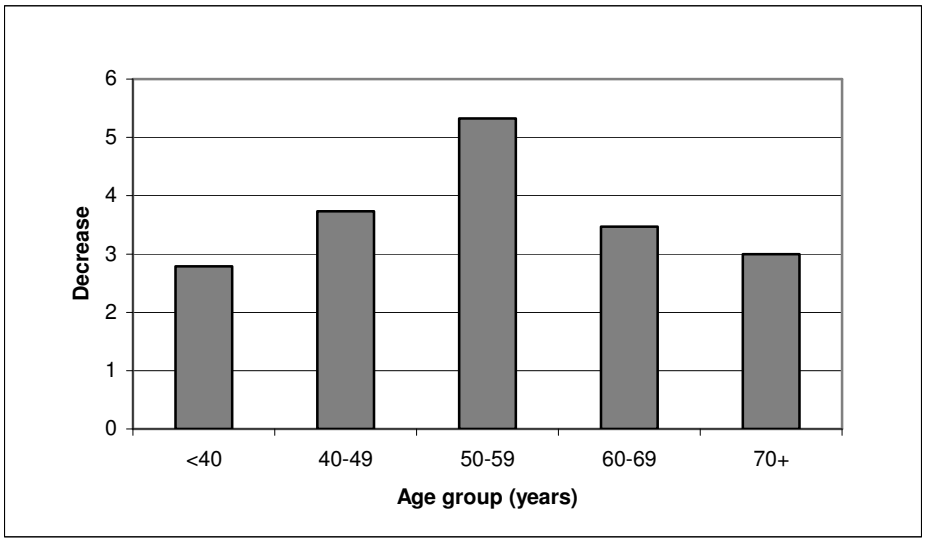
A total of 90% of patients experienced side effects; 40% at least moderate, interfering with daily activities. Although patients in the LAM group had mostly flatulence, in the LAC group taste disturbances and in the RMT group nausea and vomiting, the total side effect score was similar in the various 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).

Figure 8. Total dyspeptic score decrease in five age groups



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

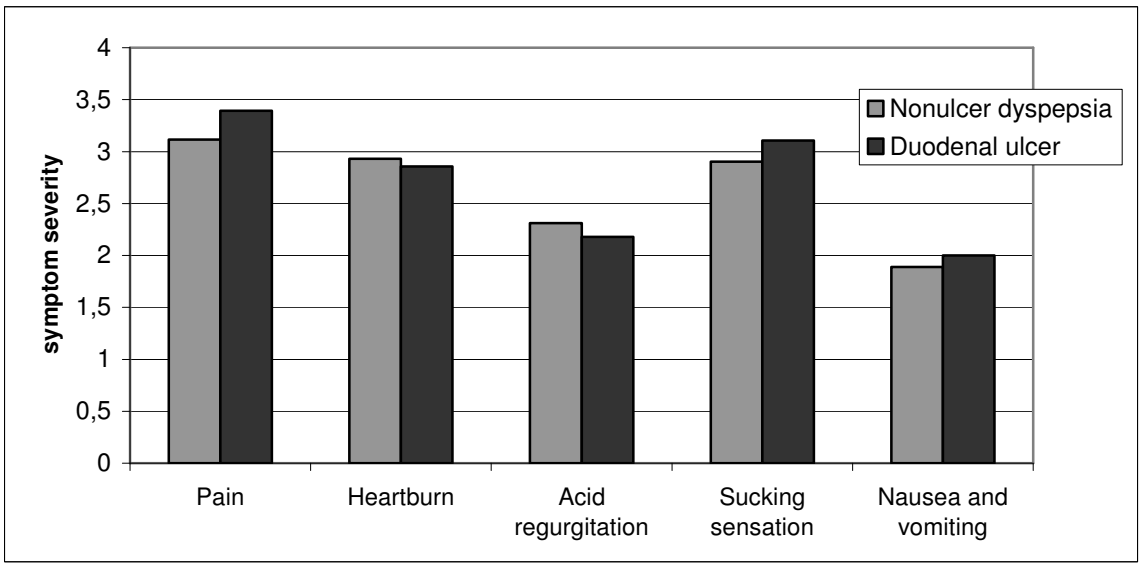
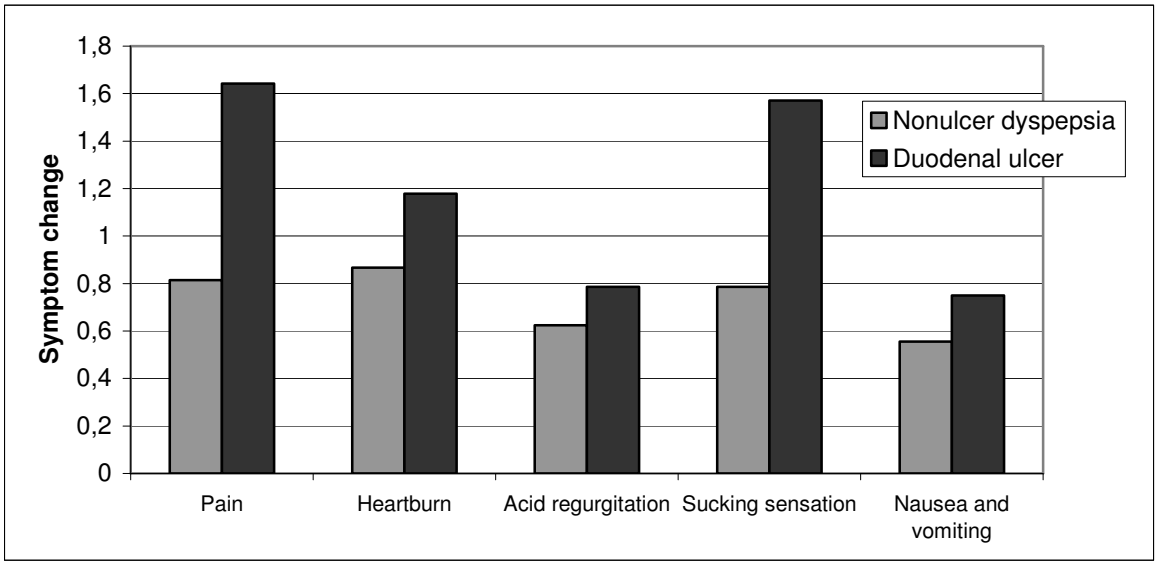


Figure 10. Symptom decrease after *H. pylori* eradication among duodenal ulcer and nonulcer dyspepsia patients



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.

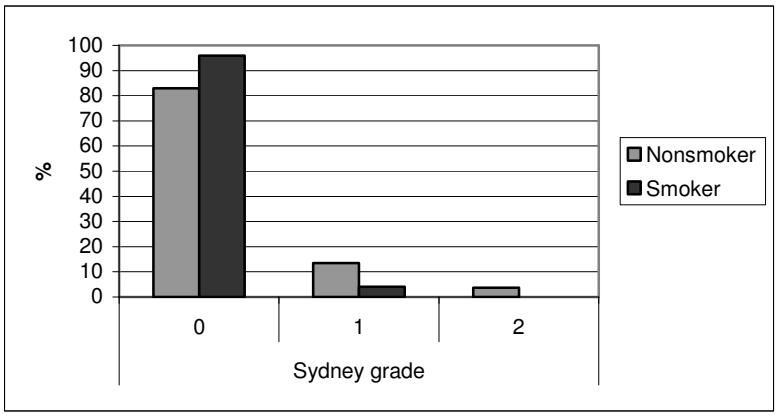
Gastric histology before therapy did not correlate with the total dyspeptic score, but patients with higher (Sydney 2-3, N = 82) antral neutrophilic inflammation had a clearly better symptom resolution than those with lower inflammation (Sydney 0-1, N = 134) (4.67 (95% CI 3.86–5.49) vs. 3.37 (95% CI 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



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 *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 *et al.* 2006).

The study was planned to find factors that could better predict the symptomatic response to *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 *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.

Smoking, symptoms and gastric histology

Although smokers in some studies have had more dyspepsia and also poorer response to therapy (Nandurkar *et al.* 1998; Delaney *et al.* 2001; Wildner-Christensen *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 acidsymptoms. 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). 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.

PARTICIPATING ENDOSCOPY UNITS

Heinolan terveyskeskus
Torikatu 13 18100 Heinola

Herttoniemen sairaala
Sisätautipoliklinikka
Kettutie 8 I, 00800 Helsinki

Honkaharjun sairaala
Honkaharju 4, 55800 Imatra

Idänpään terveysasema
Anttilankatu 4, 13210 Hämeenlinna

Joensuun terveyskeskus
Noljakantie 17, 80140 Joensuu

Kajaanin Lääkäripalvelu
Kauppakatu 27, 87100 Kajaani

Karhulan sairaala
Toivelinnankatu 2, 48600 Karhula

Koskelan sairaala
Sisätautiosasto
Käpyläntie 11, 00600 Helsinki

Kuusamon terveysasema
Raistakantie 1, 93600 Kuusamo

Lahden kaupunginsairaala
Harjukatu 48, 15100 Lahti

Länsi-Pohjan keskussairaala
Sisätautien osasto
Kauppakatu 25, 94100 Kemi

Lääkäriasema Hertta
Kiekkotie 2, 70200 Kuopio

Mikkelin keskussairaala
Sisätautien osasto
Porrassalmenkatu 35-37, 50100 Mikkeli

Palokan terveysasema
Ritopohjantie 25, 40270 Palokka

Porin kaupunginsairaala
Maantiekatu 31, 28120 Pori

Raahen sairaala
Sisätautiosasto,
Rantakatu 4, 92100 Raahe

Rovaniemen terveysasema
Sairaalakatu 1, 96100 Rovaniemi

Saarijärven terveysasema
Sairaalantie 2, 43100 Saarijärvi

Seinäjoen terveyskeskus
Huhtalantie 10, 60100 Seinäjoki

Tampereen kaupungin terveyskeskus
Hatanpään sairaala
Hatanpäänkatu 22, 33100 Tampere

Tikkurilan terveysasema
Kielotie 11, 01300 Vantaa

Turunmaan sairaala
Sisätautiosasto
Kaskenkatu 13, 20700 Turku

Vantaan lääkärikeskus
Jönsaksentie 6, 01600 Vantaa 60

ACKNOWLEDGEMENTS

This study was carried out in 23 endoscopy centres in Finland, from Helsinki up to Rovaniemi. Doctors and nurses there did a great job in recruiting and taking care of the study patients. It's been said that this kind of study could not be possible anywhere except in Finland. I thank Drs Reijo Grönfors, Markku Hartikka, Matti Honkala, Kalle Honkanen, Kari Humaloja, Antti Itkonen, Pekka Jauhonen, Hannu Jonsson, Tuomo Jääskeläinen, Hannele Karinen, Terttu Kauhanen, Veikko Korhonen, Hannu Kortesuo, Boriana Korudanova-Atanassova, Heimo Kovanen, Pauli Kulta, Pekka Lampela, Veikko Moilanen, Aino Oksanen, Ulla Palmu, Antti Rainio, Sakari Rajala, Arto Rantala, Martin Rasmussen, Sirpa Uusimäki, Jukka Viinikka, and Jorma Vuotila who recruited the patients and all the nurses who took care of the these patients.

I had the privilege to be supervised by the greatly honoured person in gastroenterology, docent Martti Färkkilä. He led me to the intriguing world of gastroenterologic science and helped me to handle the overwhelming bureaucracy before the fieldwork could begin. Without his connections, this kind of study could not have been carried out.

Docent Anna-Liisa Karvonen is the talented clinician who in 1986 convinced me that gastroenterology is the field of medicine for me. She is also an experienced scientist and helped me in writing my thesis. Special thanks to her!

I am grateful to Tuomo Karttunen and Pekka Collin, the official reviewers of this thesis, for the valuable advice on this work.

I'd like to thank docent Hilpi Rautelin, who besides taking care of serological testing and *H. pylori* culturing, helped me in writing the first articles.

I'd also like to thank professor Pentti Sipponen, who reviewed all the histologic samples and gave me the best possible basis for the fourth article.

Especially I want to express my gratitude to docent Markku Voutilainen who greatly helped me in the statistics, helped me to write the reports, and most of all, encouraged me in the tight corners of this work.

This work could not have been possible without the help of the nurses in the gastroenterological clinic in HUS. Especially I want to thank Virpi Pelkonen, who kept up contacts with endoscopy units. Mervi Ahokannas analysed all the UBTs. I will always remember them as joyful and efficient persons.

My colleagues in the gastroenterological clinic helped me many times by giving invaluable hints on different aspects of this work. They also encouraged me when I was pouring my scientific misery on their lap. I thank Hannu Nuutinen, Urpo Nieminen, Markku Hillilä, Juha Kuisma, Taina Sipponen, Henna Rautiainen, Perttu Arkkila, and especially Jari Koskenpato for lots of practical advice.

I must be grateful to my wife Tuula and daughters Johanna and Virpi for the patience, when my attention was directed to my computer instead of my family.

And last I'd like to thank my fellow workers in South Karelia Central Hospital for taking care of my tasks at the hospital, when I was doing this job, and most of all for the encouraging words: when will it be ready?

This work was supported by the grants from Glaxo-Wellcome, Wyeth-Lederle, Orion Pharma, Orion Diagnostica, Helsinki University EVO foundation, the Finnish Foundation for Gastroenterological Research and Viipuri Tuberculosis Foundation.

REFERENCES

Abdul-Razzak, K. K., A. M. Odeh and K. E. Bani-Hani. Fast agar-based urease test for detection of Helicobacter pylori infection in the stomach. Saudi Med J 2007; **28**(3): 379-81.

Ahmed, K. S., A. A. Khan, I. Ahmed, S. K. Tiwari, A. Habeeb, J. D. Ahi, Z. Abid, N. Ahmed and C. M. Habibullah. Impact of household hygiene and water source on the prevalence and transmission of Helicobacter pylori: a South Indian perspective. Singapore Med J 2007; **48**(6): 543-9.

Allison, J. E., L. B. Hurley, R. A. Hiatt, T. R. Levin, L. M. Ackerson and T. A. Lieu. A randomized controlled trial of test-and-treat strategy for Helicobacter pylori: clinical outcomes and health care costs in a managed care population receiving long-term acid suppression therapy for physician-diagnosed peptic ulcer disease. Arch Intern Med 2003; **163**(10): 1165-71.

Amedei, A., M. P. Bergman, B. J. Appelmelk, A. Azzurri, M. Benagiano, C. Tamburini, R. van der Zee, J. L. Telford, C. M. Vandenbroucke-Grauls, M. M. D'Elios and G. Del Prete. Molecular mimicry between Helicobacter pylori antigens and H+, K+ --adenosine triphosphatase in human gastric autoimmunity. J Exp Med 2003; **198**(8): 1147-56.

Andersen, L. P. Colonization and infection by Helicobacter pylori in humans. Helicobacter 2007; **12 Suppl 2**: 12-5.

Anim, J. T., N. Al-Sobkie, A. Prasad, B. John, P. N. Sharma and I. Al-Hamar. Assessment of different methods for staining Helicobacter pylori in endoscopic gastric biopsies. Acta Histochem 2000; **102**(2): 129-37.

Bago, J., D. Kranjcec, D. Strinic, Z. Petrovic, N. Kucisec, M. Bevanda, A. Bilic and D. Eljuga. Relationship of gastric metaplasia and age, sex, smoking and Helicobacter pylori infection in patients with duodenal ulcer and duodenitis. Coll Antropol 2000; **24**(1): 157-65.

Banatvala, N., G. R. Davies, Y. Abdi, L. Clements, D. S. Rampton, J. M. Hardie and R. A. Feldman. High prevalence of Helicobacter pylori metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastroduodenal disease. Gut 1994; **35**(11): 1562-6.

Beaugerie, L. and J. C. Petit. Microbial-gut interactions in health and disease. Antibiotic-associated diarrhoea. Best Pract Res Clin Gastroenterol 2004; **18**(2): 337-52.

Bereswill, S., C. Krainick, F. Stahler, L. Herrmann and M. Kist. Analysis of the rdxA gene in high-level metronidazole-resistant clinical isolates confirms a limited use of rdxA mutations as a marker for prediction of metronidazole resistance in Helicobacter pylori. FEMS Immunol Med Microbiol 2003; **36**(3): 193-8.

Bermejo, F., D. Boixeda, J. P. Gisbert, V. Defarges, J. M. Sanz, C. Redondo, C. Martini de Argila and A. Garcia Plaza. Rapid urease test utility for Helicobacter pylori infection diagnosis in gastric ulcer disease. Hepatogastroenterology 2002; **49**(44): 572-5.

Boekema, P. J., M. Samsom, G. P. van Berge Henegouwen and A. J. Smout. Coffee and gastrointestinal function: facts and fiction. A review. Scand J Gastroenterol Suppl 1999; **230**: 35-9.

Bogaerts, P., C. Berhin, H. Nizet and Y. Glupczynski. Prevalence and mechanisms of resistance to fluoroquinolones in Helicobacter pylori strains from patients living in Belgium. Helicobacter 2006; **11**(5): 441-5.

Bonamico, M., P. M. Strappini, E. Bonci, M. Ferri, M. Crisogianni, M. Guido, E. Thanasi, R. Nenna, S. Macchia, I. Luzzi, F. M. Magliocca and P. Mastromarino. Evaluation of stool antigen test, PCR on ORAL samples and serology for the noninvasive detection of Helicobacter pylori infection in children. Helicobacter 2004; **9**(1): 69-76.

Borody, T. J., G. Pang, A. R. Wettstein, R. Clancy, K. Herdman, R. Surace, R. Llorente and C. Ng. Efficacy and safety of rifabutin-containing 'rescue therapy' for resistant Helicobacter pylori infection. Aliment Pharmacol Ther 2006; **23**(4): 481-8.

Boyanova, L., G. Gergova, R. Nikolov, L. Davidkov, V. Kamburov, C. Jelev and I. Mitov. Prevalence and evolution of Helicobacter pylori resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. Diagn Microbiol Infect Dis 2008.

Boyanova, L., A. Mentis, M. Gubina, E. Rozynek, G. Gosciniak, S. Kalenic, V. Goral, L. Kupcinskas, B. Kantarceken, A. Aydin, A. Archimandritis, D. Dzierzanowska, A. Vcev, K. Ivanova, M. Marina, I. Mitov, P. Petrov, A. Ozden and M. Popova. The status of antimicrobial resistance of Helicobacter pylori in eastern Europe. Clin Microbiol Infect 2002; **8**(7): 388-96.

Branca, G., T. Spanu, G. Cammarota, A. M. Schito, A. Gasbarrini, G. B. Gasbarrini and G. Fadda. High levels of dual resistance to clarithromycin and metronidazole and in vitro activity of levofloxacin against Helicobacter pylori isolates from patients after failure of therapy. Int J Antimicrob Agents 2004; **24**(5): 433-8.

Brandi, G., B. Biavati, C. Calabrese, M. Granata, A. Nannetti, P. Mattarelli, G. Di Febo, G. Saccoccio and G. Biasco. Urease-positive bacteria other than Helicobacter pylori in human gastric juice and mucosa. Am J Gastroenterol 2006; **101**(8): 1756-61.

Bruce, M. G., D. L. Bruden, B. J. McMahon, T. W. Hennessy, A. Reasonover, J. Morris, D. A. Hurlburt, H. Peters, F. Sacco, P. Martinez, M. Swenson, D. E. Berg, D. Parks and A. J. Parkinson. Alaska sentinel surveillance for antimicrobial resistance in Helicobacter pylori isolates from Alaska native persons, 1999-2003. Helicobacter 2006; **11**(6): 581-8.

Buzas, G. M. and J. Jozan. [Eradication of Helicobacter pylori infection in Europe: a meta-analysis based on congress abstracts, 1997-2002]. Orv Hetil 2004; **145**(40): 2035-41.

Buzas, G. M., G. Lotz and A. Kiss. [The epidemiology of clarithromycin resistance of Helicobacter pylori infection in Hungary]. Orv Hetil 2007; **148**(31): 1461-7.

Cabrita, J., M. Oleastro, R. Matos, A. Manhente, J. Cabral, R. Barros, A. I. Lopes, P. Ramalho, B. C. Neves and A. S. Guerreiro. Features and trends in Helicobacter pylori antibiotic resistance in Lisbon area, Portugal (1990-1999). J Antimicrob Chemother 2000; **46**(6): 1029-31.

Calvet, X., N. Garcia, T. Lopez, J. P. Gisbert, E. Gene and M. Roque. A meta-analysis of short versus long therapy with a proton pump inhibitor, clarithromycin and either metronidazole or amoxycillin for treating Helicobacter pylori infection. Aliment Pharmacol Ther 2000; **14**(5): 603-9.

Carothers, J. J., M. G. Bruce, T. W. Hennessy, M. Bensler, J. M. Morris, A. L. Reasonover, D. A. Hurlburt, A. J. Parkinson, J. M. Coleman and B. J. McMahon. The relationship between previous fluoroquinolone use and levofloxacin resistance in Helicobacter pylori infection. Clin Infect Dis 2007; **44**(2): e5-8.

Cars, O., S. Molstad and A. Melander. Variation in antibiotic use in the European Union. Lancet 2001; **357**(9271): 1851-3.

Cattoir, V., J. Nectoux, C. Lascols, L. Deforges, J. C. Delchier, F. Megraud, C. J. Soussy and E. Cambau. Update on fluoroquinolone resistance in Helicobacter pylori: new mutations leading to resistance and first description of a gyrA polymorphism associated with hypersusceptibility. Int J Antimicrob Agents 2007; **29**(4): 389-96.

Cellini, L., R. Grande, E. Di Campli, S. Di Bartolomeo, S. Capodicasa and L. Marzio. Analysis of genetic variability, antimicrobial susceptibility and virulence markers in Helicobacter pylori identified in Central Italy. Scand J Gastroenterol 2006; **41**(3): 280-7.

Chen, L. H. and H. S. Luo. Effects of H pylori therapy on erythrocytic and iron parameters in iron deficiency anemia patients with H pylori-positive chronic gastristis. World J Gastroenterol 2007; **13**(40): 5380-3.

Chisholm, S. A. and R. J. Owen. Mutations in Helicobacter pylori rdxA gene sequences may not contribute to metronidazole resistance. J Antimicrob Chemother 2003; **51**(4): 995-9.

Chisholm, S. A., E. L. Teare, K. Davies and R. J. Owen. Surveillance of primary antibiotic resistance of Helicobacter pylori at centres in England and Wales over a six-year period (2000-2005). Euro Surveill 2007; **12**(7): E3-4.

Co, E. M. and N. L. Schiller. Resistance mechanisms in an in vitro-selected amoxicillin-resistant strain of Helicobacter pylori. Antimicrob Agents Chemother 2006; **50**(12): 4174-6.

Cutler, A. F. and T. T. Schubert. Patient factors affecting Helicobacter pylori eradication with triple therapy. Am J Gastroenterol 1993; **88**(4): 505-9.

De Francesco, V., M. Margiotta, A. Zullo, C. Hassan, F. Giorgio, O. Burattini, G. Stoppino, U. Cea, A. Pace, M. Zotti, S. Morini, C. Panella and E. Ierardi. Prevalence of primary clarithromycin resistance in Helicobacter pylori strains over a 15 year period in Italy. J Antimicrob Chemother 2007; **59**(4): 783-5.

de Korwin, J. D. [Helicobacter pylori infection and antimicrobial agents resistance]. Rev Med Interne 2004; **25**(1): 54-64.

Debets-Ossenkopp, Y. J., A. J. Herscheid, R. G. Pot, E. J. Kuipers, J. G. Kusters and C. M. Vandenbroucke-Grauls. Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxacin in The Netherlands. J Antimicrob Chemother 1999; **43**(4): 511-5.

Delaney, B. C., S. Wilson, A. Roalfe, L. Roberts, V. Redman, A. Wearn and F. D. Hobbs. Randomised controlled trial of Helicobacter pylori testing and endoscopy for dyspepsia in primary care. Bmj 2001; **322**(7291): 898-901.

Della Monica, P., A. Lavagna, G. Masoero, L. Lombardo, L. Crocella and A. Pera. Effectiveness of Helicobacter pylori eradication treatments in a primary care setting in Italy. Aliment Pharmacol Ther 2002; **16**(7): 1269-75.

di Mario, F., N. Stefani, N. D. Bo, M. Rugge, A. Pilotto, G. M. Cavestro, L. G. Cavallaro, A. Franze and G. Leandro. Natural course of functional dyspepsia after Helicobacter pylori eradication: a seven-year survey. Dig Dis Sci 2005; **50**(12): 2286-95.

Dite, P., M. Kunovska, D. Pulgretova, V. Woznica, J. Petrtyl, P. Hulek, J. Paskova, Z. Dostalik, I. Novotny, V. Prochazka, P. Hegyi, J. Zelenkova, M. Samek, Z. Kralova, P. Vyhnalek, F. Matejovic, J. Weinberg and J. Kyzekova. [Double blind randomized multicentre study of a seven-day eradication regime of Helicobacter pylori by omeprazole, clarithromycin and ornidazole vs. omeprazole, clarithromycin and metronidazole]. Vnitr Lek 2002; **48**(10): 976-80.

Dixon, M. F., R. M. Genta, J. H. Yardley and P. Correa. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996; **20**(10): 1161-81.

Dore, M. P., G. Leandro, G. Realdi, A. R. Sepulveda and D. Y. Graham. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome of Helicobacter pylori therapy: a meta-analytical approach. Dig Dis Sci 2000; **45**(1): 68-76.

Drossman, D. A. The functional gastrointestinal disorders and the Rome III process. Gastroenterology 2006; **130**(5): 1377-90.

Duck, W. M., J. Sobel, J. M. Pruckler, Q. Song, D. Swerdlow, C. Friedman, A. Sulka, B. Swaminathan, T. Taylor, M. Hoekstra, P. Griffin, D. Smoot, R. Peek, D. C. Metz, P. B. Bloom, S. Goldschmidt, J. Parsonnet, G. Triadafilopoulos, G. I. Perez-Perez, N. Vakil, P. Ernst, S. Czinn, D. Dunne and B. D. Gold. Antimicrobial resistance incidence and risk factors among Helicobacter pylori-infected persons, United States. Emerg Infect Dis 2004; **10**(6): 1088-94.

Dzierzanowska-Fangrat, K., E. Rozynek, D. Celinska-Cedro, M. Jarosz, J. Pawlowska, A. Szadkowski, A. Budzynska, J. Nowak, W. Romanczuk, R. Prosiecki, P. Jozwiak and D. Dzierzanowska. Antimicrobial resistance of Helicobacter pylori in Poland: a multicentre study. Int J Antimicrob Agents 2005; **26**(3): 230-4.

Elviss, N. C., R. J. Owen, A. Breathnach, C. Palmer and N. Shetty. Helicobacter pylori antibiotic-resistance patterns and risk factors in adult dyspeptic patients from ethnically diverse populations in central and south London during 2000. J Med Microbiol 2005; **54**(Pt 6): 567-74.

Endo, H., H. Yoshida, N. Ohmi and S. Higuchi. Effects of lansoprazole and amoxicillin on uptake of [(14)C]clarithromycin into gastric tissue in rats. Antimicrob Agents Chemother 2001; **45**(12): 3451-5.

Feldman, R. A., A. J. Eccersley and J. M. Hardie. Epidemiology of Helicobacter pylori: acquisition, transmission, population prevalence and disease-to-infection ratio. Br Med Bull 1998; **54**(1): 39-53.

Ford, A. C., B. C. Delaney, D. Forman and P. Moayyedi. Eradication therapy for peptic ulcer disease in Helicobacter pylori positive patients. Cochrane Database Syst Rev 2006; (2): CD003840.

Fry, L. C., W. H. Curioso, S. Rickes, G. Horton, B. I. Hirschowitz and K. Monkemuller. Comparison of 13C- urea blood test to 13C-breath test and rapid urease test for the diagnosis of Helicobacter pylori infection. Acta Gastroenterol Latinoam 2005; **35**(4): 225-9.

Fuccio, L., R. M. Zagari, M. E. Minardi and F. Bazzoli. Systematic review: Helicobacter pylori eradication for the prevention of gastric cancer. Aliment Pharmacol Ther 2007; **25**(2): 133-41.

Färkkilä, M., R. Julkunen, S. Niemelä, H. Nuutinen, T. Kosunen, A. Karvonen, I. Kunnamo and P. Pikkarainen. Suositus Helicobacter pylori -infektion diagnostiikasta, hoidosta ja seurannasta. Suomen Lääkärilehti 1996; **51**(1-2): 9.

Färkkilä, M., S. Sarna, V. Valtonen and P. Sipponen. Does the 'test-and-treat' strategy work in primary health care for management of uninvestigated dyspepsia? A prospective two-year follow-up study of 1552 patients. Scand J Gastroenterol 2004; **39**(4): 327-35.

Genta, R. M. and M. Rugge. Assessing risks for gastric cancer: new tools for pathologists. World J Gastroenterol 2006; **12**(35): 5622-7.

Gerrits, M. M., M. Berning, A. H. Van Vliet, E. J. Kuipers and J. G. Kusters. Effects of 16S rRNA gene mutations on tetracycline resistance in Helicobacter pylori. Antimicrob Agents Chemother 2003; **47**(9): 2984-6.

Gerrits, M. M., A. P. Godoy, E. J. Kuipers, M. L. Ribeiro, J. Stoof, S. Mendonca, A. H. van Vliet, J. Pedrazzoli, Jr. and J. G. Kusters. Multiple mutations in or adjacent to the conserved penicillin-binding protein motifs of the penicillin-binding protein 1A confer amoxicillin resistance to Helicobacter pylori. Helicobacter 2006; **11**(3): 181-7.

Gisbert, J. P. The recurrence of Helicobacter pylori infection: incidence and variables influencing it. A critical review. Am J Gastroenterol 2005; **100**(9): 2083-99.

Gisbert, J. P. and V. Abraira. Accuracy of Helicobacter pylori diagnostic tests in patients with bleeding peptic ulcer: a systematic review and meta-analysis. Am J Gastroenterol 2006; **101**(4): 848-63.

Gisbert, J. P., X. Badia, M. Roset and J. M. Pajares. The TETRA study: a prospective evaluation of Helicobacter pylori 'test-and-treat' strategy on 736 patients in clinical practice. Helicobacter 2004; **9**(1): 28-38.

Gisbert, J. P., F. de la Morena and V. Abraira. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: a systematic review and meta-analysis. Am J Gastroenterol 2006a; **101**(8): 1921-30.

Gisbert, J. P., J. L. Gisbert, S. Marcos, I. Jimenez-Alonso, R. Moreno-Otero and J. M. Pajares. Empirical rescue therapy after Helicobacter pylori treatment failure: a 10-year single-centre study of 500 patients. Aliment Pharmacol Ther 2008; **27**(4): 346-54.

Gisbert, J. P., J. L. Gisbert, S. Marcos, R. Moreno-Otero and J. M. Pajares. Third-line rescue therapy with levofloxacin is more effective than rifabutin rescue regimen after two Helicobacter pylori treatment failures. Aliment Pharmacol Ther 2006b; **24**(10): 1469-74.

Gisbert, J. P., L. Gonzalez and X. Calvet. Systematic review and meta-analysis: proton pump inhibitor vs. ranitidine bismuth citrate plus two antibiotics in Helicobacter pylori eradication. Helicobacter 2005; **10**(3): 157-71.

Gisbert, J. P. and F. Morena. Systematic review and meta-analysis: levofloxacin-based rescue regimens after Helicobacter pylori treatment failure. Aliment Pharmacol Ther 2006; **23**(1): 35-44.

Gisbert, J. P. and J. M. Pajares. Review article: C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. Aliment Pharmacol Ther 2004; **20**(10): 1001-17.

Gisbert, J. P. and M. J. Pajares. [Helicobacter pylori resistance to metronidazole and to clarithromycin in Spain. A systematic review]. Med Clin (Barc) 2001; **116**(3): 111-6.

Glocker, E., C. Bogdan and M. Kist. Characterization of rifampicin-resistant clinical Helicobacter pylori isolates from Germany. J Antimicrob Chemother 2007a; **59**(5): 874-9.

Glocker, E., H. P. Stueger and M. Kist. Quinolone resistance in Helicobacter pylori isolates in Germany. Antimicrob Agents Chemother 2007b; **51**(1): 346-9.

Glupczynski, Y. Antimicrobial resistance in Helicobacter pylori: a global overview. Acta Gastroenterol Belg 1998; **61**(3): 357-66.

Glupczynski, Y., N. Broutet, A. Cantagrel, L. P. Andersen, T. Alarcon, M. Lopez-Brea and F. Megraud. Comparison of the E test and agar dilution method for antimicrobial

suceptibility testing of Helicobacter pylori. Eur J Clin Microbiol Infect Dis 2002; **21**(7): 549-52.

Glupczynski, Y., F. Megraud, M. Lopez-Brea and L. P. Andersen. European multicentre survey of in vitro antimicrobial resistance in Helicobacter pylori. Eur J Clin Microbiol Infect Dis 2001; **20**(11): 820-3.

Goh, K. L., P. L. Cheah, P. Navaratnam, S. C. Chin and S. D. Xiao. HUITAI rapid urease test: a new ultra-rapid biopsy urease test for the diagnosis of Helicobacter pylori infection. J Dig Dis 2007; **8**(3): 139-42.

Graham, D. Y., A. R. Opekun, M. S. Osato, H. M. El-Zimaity, C. K. Lee, Y. Yamaoka, W. A. Qureshi, M. Cadoz and T. P. Monath. Challenge model for Helicobacter pylori infection in human volunteers. Gut 2004; **53**(9): 1235-43.

Grignon, B., J. Tankovic, F. Megraud, Y. Glupczynski, M. O. Husson, M. C. Conroy, J. P. Emond, J. Loulergue, J. Raymond and J. L. Fauchere. Validation of diffusion methods for macrolide susceptibility testing of Helicobacter pylori. Microb Drug Resist 2002; **8**(1): 61-6.

Haggerty, T. D., S. Perry, L. Sanchez, G. Perez-Perez and J. Parsonnet. Significance of transiently positive enzyme-linked immunosorbent assay results in detection of Helicobacter pylori in stool samples from children. J Clin Microbiol 2005; **43**(5): 2220-3.

Heep, M., M. Kist, S. Strobel, D. Beck and N. Lehn. Secondary resistance among 554 isolates of Helicobacter pylori after failure of therapy. Eur J Clin Microbiol Infect Dis 2000a; **19**(7): 538-41.

Heep, M., U. Rieger, D. Beck and N. Lehn. Mutations in the beginning of the rpoB gene can induce resistance to rifamycins in both Helicobacter pylori and Mycobacterium tuberculosis. Antimicrob Agents Chemother 2000b; **44**(4): 1075-7.

Herbrink, P. and L. J. van Doorn. Serological methods for diagnosis of Helicobacter pylori infection and monitoring of eradication therapy. Eur J Clin Microbiol Infect Dis 2000; **19**(3): 164-73.

Hobsley, M., F. I. Tovey and J. Holton. Precise role of H pylori in duodenal ulceration. World J Gastroenterol 2006; **12**(40): 6413-9.

Hong, S. S., H. Y. Jung, K. D. Choi, H. J. Song, G. H. Lee, T. H. Oh, J. Y. Jo, K. J. Kim, J. S. Byeon, S. J. Myung, S. K. Yang, W. S. Hong, J. H. Kim and Y. I. Min. A prospective analysis of low-grade gastric malt lymphoma after Helicobacter pylori eradication. Helicobacter 2006; **11**(6): 569-73.

Houben, M. H., D. van de Beek, E. F. Hensen, A. J. Craen, E. A. Rauws and G. N. Tytgat. A systematic review of Helicobacter pylori eradication therapy--the impact of antimicrobial resistance on eradication rates. Aliment Pharmacol Ther 1999; **13**(8): 1047-55.

Huang, J. Q., G. F. Zheng, R. H. Hunt, W. M. Wong, S. K. Lam, J. Karlberg and B. C. Wong. Do patients with non-ulcer dyspepsia respond differently to Helicobacter pylori eradication treatments from those with peptic ulcer disease? A systematic review. World J Gastroenterol 2005; **11**(18): 2726-32.

Huovinen, P. and T. Klaukka. Erytromysiinin käyttöä vähennettävä. 1991; **Suom Lääkäril**(46): 3241.

Ito, L. S., S. M. Oba-Shinjo, S. K. Marie, M. Uno, S. K. Shinjo, N. Hamajima, K. Tajima and S. Tominaga. Lifestyle factors associated with atrophic gastritis among Helicobacter pylori-seropositive Japanese-Brazilians in Sao Paulo. Int J Clin Oncol 2003; **8**(6): 362-8.

Janssen, M. J., L. Hendrikse, S. Y. de Boer, R. Bosboom, W. A. de Boer, R. J. Laheij and J. B. Jansen. Helicobacter pylori antibiotic resistance in a Dutch region: trends over time. Neth J Med 2006; **64**(6): 191-5.

Jaup, B. H., A. Brandberg, B. Stenquist and A. Norrby. [Antibiotic resistance among strains of Helicobacter pylori in Gothenburg. Bacteria resistant to metronidazole]. Lakartidningen 1998; **95**(4): 279-81.

Kalach, N., V. B. Nguyen, M. Bergeret, N. Boutros, C. Dupont and J. Raymond. Usefulness and influence of age of a novel rapid monoclonal enzyme immunoassay stool antigen for the diagnosis of Helicobacter pylori infection in children. Diagn Microbiol Infect Dis 2005; **52**(2): 157-60.

Kamangar, F., Y. L. Qiao, M. J. Blaser, X. D. Sun, H. Katki, J. H. Fan, G. I. Perez-Perez, C. C. Abnet, P. Zhao, S. D. Mark, P. R. Taylor and S. M. Dawsey. Helicobacter pylori and oesophageal and gastric cancers in a prospective study in China. Br J Cancer 2007; **96**(1): 172-6.

Kaptan, K., C. Beyan, A. U. Ural, T. Cetin, F. Avcu, M. Gulsen, R. Finci and A. Yalcin. Helicobacter pylori--is it a novel causative agent in Vitamin B12 deficiency? Arch Intern Med 2000; **160**(9): 1349-53.

Karttunen, T., S. Niemelä and J. Lehtola. Helicobacter pylori in dyspeptic patients: quantitative association with severity of gastritis, intragastric pH, and serum gastrin concentration. Scand J Gastroenterol Suppl 1991; **186**: 124-34.

Katelaris, P. H., D. Adamthwaite, P. Midolo, N. D. Yeomans, G. Davidson and J. Lambert. Randomized trial of omeprazole and metronidazole with amoxycillin or clarithromycin for Helicobacter pylori eradication, in a region of high primary metronidazole resistance: the HERO study. Aliment Pharmacol Ther 2000; **14**(6): 751-8.

Kato, M., Y. Yamaoka, J. J. Kim, R. Reddy, M. Asaka, K. Kashima, M. S. Osato, F. A. El-Zaatari, D. Y. Graham and D. H. Kwon. Regional differences in metronidazole resistance and increasing clarithromycin resistance among Helicobacter pylori isolates from Japan. Antimicrob Agents Chemother 2000; **44**(8): 2214-6.

Kidd, M. and I. M. Modlin. A century of Helicobacter pylori: paradigms lost-paradigms regained. Digestion 1998; **59**(1): 1-15.

King, A. Recommendations for susceptibility tests on fastidious organisms and those requiring special handling. J Antimicrob Chemother 2001; **48 Suppl 1**: 77-80.

Klok, R. M., N. L. Arents, R. de Vries, J. C. Thijs, J. R. Brouwers, J. H. Kleibeuker and M. J. Postma. Economic evaluation of a randomized trial comparing Helicobacter pylori test-and-treat and prompt endoscopy strategies for managing dyspepsia in a primary-care setting. Clin Ther 2005; **27**(10): 1647-57.

Kneller, R. W., W. C. You, Y. S. Chang, W. D. Liu, L. Zhang, L. Zhao, G. W. Xu, J. F. Fraumeni, Jr. and W. J. Blot. Cigarette smoking and other risk factors for progression of precancerous stomach lesions. J Natl Cancer Inst 1992; **84**(16): 1261-6.

Kokkola, A., H. Rautelin, P. Puolakkainen, P. Sipponen, M. Färkkilä, R. Haapiainen and T. U. Kosunen. Diagnosis of Helicobacter pylori infection in patients with atrophic gastritis: comparison of histology, 13C-urea breath test, and serology. Scand J Gastroenterol 2000; **35**(2): 138-41.

Koletzko, S., F. Richy, P. Bontems, J. Crone, N. Kalach, M. L. Monteiro, F. Gottrand, D. Celinska-Cedro, E. Roma-Giannikou, G. Orderda, S. Kolacek, P. Urruzuno, M. J. Martinez-Gomez, T. Casswall, M. Ashorn, H. Bodanszky and F. Megraud. Prospective multicentre study on antibiotic resistance of Helicobacter pylori strains obtained from children living in Europe. Gut 2006; **55**(12): 1711-6.

Kolho, K. L., J. Korhonen, M. Verkasalo, H. Lindahl, E. Savilahti and H. Rautelin. Helicobacter pylori serology at diagnosis and follow-up of biopsy-verified infection in children. Scand J Infect Dis 2002; **34**(3): 177-82.

Komoto, K., K. Haruma, T. Kamada, S. Tanaka, M. Yoshihara, K. Sumii, G. Kajiyama and N. J. Talley. Helicobacter pylori infection and gastric neoplasia: correlations with histological gastritis and tumor histology. Am J Gastroenterol 1998; **93**(8): 1271-6.

Konturek, J. W. Discovery by Jaworski of Helicobacter pylori and its pathogenetic role in peptic ulcer, gastritis and gastric cancer. J Physiol Pharmacol 2003; **54 Suppl 3**: 23-41.

Kosunen, T. U., A. Aromaa, P. Knekt, A. Salomaa, H. Rautelin, P. Lohi and O. P. Heinonen. Helicobacter antibodies in 1973 and 1994 in the adult population of Vammala, Finland. Epidemiol Infect 1997; **119**(1): 29-34.

Kosunen, T. U., K. Seppälä, S. Sarna and P. Sipponen. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of Helicobacter pylori. Lancet 1992; **339**(8798): 893-5.

Kuipers, E. J. Review article: exploring the link between Helicobacter pylori and gastric cancer. Aliment Pharmacol Ther 1999; **13 Suppl 1**: 3-11.

Kurata, J. H. and A. N. Nogawa. Meta-analysis of risk factors for peptic ulcer. Nonsteroidal antiinflammatory drugs, Helicobacter pylori, and smoking. J Clin Gastroenterol 1997; **24**(1): 2-17.

Laheij, R. J., H. Straatman, J. B. Jansen and A. L. Verbeek. Evaluation of commercially available Helicobacter pylori serology kits: a review. J Clin Microbiol 1998; **36**(10): 2803-9.

Lahner, E., D. Vaira, N. Figura, E. Pilozzi, A. Pasquali, C. Severi, F. Perna, G. Delle Fave and B. Annibale. Role of noninvasive tests (C-urea breath test and stool antigen test) as additional tools in diagnosis of Helicobacter pylori infection in patients with atrophic body gastritis. Helicobacter 2004; **9**(5): 436-42.

Laine, L., L. Suchower, E. Johnson, P. Ronca and G. Neil. Accuracy of CLOtest after Helicobacter pylori therapy. Gastrointest Endosc 1998; **47**(3): 250-3.

Laine, L., J. Sugg, L. Suchower and G. Neil. Endoscopic biopsy requirements for post-treatment diagnosis of Helicobacter pylori. Gastrointest Endosc 2000; **51**(6): 664-9.

Lawson, A. J., N. C. Elviss and R. J. Owen. Real-time PCR detection and frequency of 16S rDNA mutations associated with resistance and reduced susceptibility to tetracycline in Helicobacter pylori from England and Wales. J Antimicrob Chemother 2005; **56**(2): 282-6.

Lee, S. K., Y. C. Lee, J. B. Chung, C. Y. Chon, Y. M. Moon, J. K. Kang, I. S. Park, C. O. Suh and W. I. Yang. Low grade gastric mucosa associated lymphoid tissue lymphoma: treatment strategies based on 10 year follow-up. World J Gastroenterol 2004; **10**(2): 223-6.

Lerang, F., B. Moum, J. B. Haug, P. Tolas, O. Breder, E. Aubert, O. Hoie, T. Soberg, B. Flaaten, P. Farup and T. Berge. Highly effective twice-daily triple therapies for Helicobacter pylori infection and peptic ulcer disease: does in vitro metronidazole resistance have any clinical relevance? Am J Gastroenterol 1997; **92**(2): 248-53.

Lin, Z., J. D. Chen, S. Parolisi, J. Shifflett, D. A. Peura and R. W. McCallum. Prevalence of gastric myoelectrical abnormalities in patients with nonulcer dyspepsia and H. pylori infection: resolution after H. pylori eradication. Dig Dis Sci 2001; **46**(4): 739-45.

Lind, T., F. Megraud, P. Unge, E. Bayerdorffer, C. O'Morain, R. Spiller, S. Veldhuyzen Van Zanten, K. D. Bardhan, M. Hellblom, M. Wrangstadh, L. Zeijlon and C. Cederberg. The MACH2 study: role of omeprazole in eradication of Helicobacter pylori with 1-week triple therapies. Gastroenterology 1999; **116**(2): 248-53.

Loivukene, K., H. I. Maaroos, H. Kolk, I. Kull, K. Labotkin and M. Mikelsaar. Prevalence of antibiotic resistance of Helicobacter pylori isolates in Estonia during 1995-2000 in comparison to the consumption of antibiotics used in treatment regimens. Clin Microbiol Infect 2002; **8**(9): 598-603.

Maity, P., K. Biswas, S. Roy, R. K. Banerjee and U. Bandyopadhyay. Smoking and the pathogenesis of gastroduodenal ulcer--recent mechanistic update. Mol Cell Biochem 2003; **253**(1-2): 329-38.

Malfertheiner, P. Compliance, adverse events and antibiotic resistance in Helicobacter pylori treatment. Scand J Gastroenterol Suppl 1993; **196**: 34-7.

Malfertheiner, P., F. Megraud, C. O'Morain, F. Bazzoli, E. El-Omar, D. Graham, R. Hunt, T. Rokkas, N. Vakil and E. J. Kuipers. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 2007; **56**(6): 772-81.

Malfertheiner, P., F. Megraud, C. O'Morain, D. Bell, G. Bianchi Porro, M. Deltenre, D. Forman, G. Gasbarrini, B. Jaup, J. J. Misiewicz, J. Pajares, M. Quina and E. Rauws. Current European concepts in the management of Helicobacter pylori infection--the Maastricht Consensus Report. The European Helicobacter Pylori Study Group (EHPSG). Eur J Gastroenterol Hepatol 1997; **9**(1): 1-2.

Malfertheiner, P., F. Megraud, C. O'Morain, A. P. Hungin, R. Jones, A. Axon, D. Y. Graham and G. Tytgat. Current concepts in the management of Helicobacter pylori infection--the Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther 2002; **16**(2): 167-80.

Marais, A., C. Bilardi, F. Cantet, G. L. Mendz and F. Megraud. Characterization of the genes rdxA and frxA involved in metronidazole resistance in Helicobacter pylori. Res Microbiol 2003; **154**(2): 137-44.

Matsukura, N., T. Tajiri, S. Kato, A. Togashi, G. Masuda, A. Tokunaga and N. Yamada. Diagnostic value of culture, histology and PCR for Helicobacter pylori in the remnant stomach after surgery. Aliment Pharmacol Ther 2004; **20 Suppl 1**: 33-8.

Mc Namara, D. A., M. Buckley and C. A. O'Morain. Nonulcer dyspepsia. Current concepts and management. Gastroenterol Clin North Am 2000; **29**(4): 807-18.

McMahon, B. J., T. W. Hennessy, J. M. Bensler, D. L. Bruden, A. J. Parkinson, J. M. Morris, A. L. Reasonover, D. A. Hurlburt, M. G. Bruce, F. Sacco and J. C. Butler. The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for Helicobacter pylori infections. Ann Intern Med 2003; **139**(6): 463-9.

Mearin, F., X. de Ribot, A. Balboa, A. Salas, M. J. Varas, M. Cucala, R. Bartolome, J. R. Armengol and J. R. Malagelada. Does Helicobacter pylori infection increase gastric sensitivity in functional dyspepsia? Gut 1995; **37**(1): 47-51.

Megraud, F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut 2004; **53**(9): 1374-84.

Megraud, F. and P. Lehours. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev 2007; **20**(2): 280-322.

Meijer, B. C., J. C. Thijs, J. H. Kleibeuker, A. A. van Zwet and R. J. Berrelkamp. Evaluation of eight enzyme immunoassays for detection of immunoglobulin G against Helicobacter pylori. J Clin Microbiol 1997; **35**(1): 292-4.

Meyer, J. M., N. P. Silliman, W. Wang, N. Y. Siepman, J. E. Sugg, D. Morris, J. Zhang, H. Bhattacharyya, E. C. King and R. J. Hopkins. Risk factors for Helicobacter pylori resistance in the United States: the surveillance of H. pylori antimicrobial resistance partnership (SHARP) study, 1993-1999. Ann Intern Med 2002; **136**(1): 13-24.

Miehlke, S., K. Hansky, W. Schneider-Brachert, C. Kirsch, A. Morgner, A. Madisch, E. Kuhlisch, E. Bastlein, E. Jacobs, E. Bayerdorffer, N. Lehn and M. Stolte. Randomized trial of rifabutin-based triple therapy and high-dose dual therapy for rescue treatment of Helicobacter pylori resistant to both metronidazole and clarithromycin. Aliment Pharmacol Ther 2006; **24**(2): 395-403.

Miehlke, S., C. Kirsch, W. Schneider-Brachert, C. Haferland, M. Neumeyer, E. Bastlein, J. Papke, E. Jacobs, M. Vieth, M. Stolte, N. Lehn and E. Bayerdorffer. A prospective, randomized study of quadruple therapy and high-dose dual therapy for treatment of Helicobacter pylori resistant to both metronidazole and clarithromycin. Helicobacter 2003; **8**(4): 310-9.

Misra, S. P., V. Misra, M. Dwivedi, P. A. Singh, V. Bhargava and P. K. Jaiswal. Evaluation of the one-minute ultra-rapid urease test for diagnosing Helicobacter pylori. Postgrad Med J 1999a; **75**(881): 154-6.

Misra, V., S. P. Misra, M. Dwivedi, S. K. Shukla, R. Agarwal, P. K. Jaiswal and S. C. Gupta. Decreased sensitivity of the ultrarapid urease test for diagnosing Helicobacter pylori in patients with chronic renal failure. Pathology 1999b; **31**(1): 44-6.

Miyachi, H., I. Miki, N. Aoyama, D. Shirasaka, Y. Matsumoto, M. Toyoda, T. Mitani, Y. Morita, T. Tamura, S. Kinoshita, Y. Okano, S. Kumagai and M. Kasuga. Primary levofloxacin resistance and gyrA/B mutations among Helicobacter pylori in Japan. Helicobacter 2006; **11**(4): 243-9.

Moayyedi, P., R. Feltbower, W. Crocombe, S. Mason, P. Atha, J. Brown, A. C. Dowell, I. D. Richards and A. T. Axon. The effectiveness of omeprazole, clarithromycin and tinidazole in eradicating Helicobacter pylori in a community screen and treat programme. Leeds Help Study Group. Aliment Pharmacol Ther 2000; **14**(6): 719-28.

Moayyedi, P., S. Soo, J. Deeks, B. Delaney, A. Harris, M. Innes, R. Oakes, S. Wilson, A. Roalfe, C. Bennett and D. Forman. Eradication of Helicobacter pylori for non-ulcer dyspepsia. Cochrane Database Syst Rev 2003; (1): CD002096.

Moayyedi, P., S. Soo, J. Deeks, B. Delaney, A. Harris, M. Innes, R. Oakes, S. Wilson, A. Roalfe, C. Bennett and D. Forman. Eradication of Helicobacter pylori for non-ulcer dyspepsia. Cochrane Database Syst Rev 2006; (2): CD002096.

Moder, K. A., F. Layer, W. Konig and B. Konig. Rapid screening of clarithromycin resistance in Helicobacter pylori by pyrosequencing. J Med Microbiol 2007; **56**(Pt 10): 1370-6.

Moshkowitz, M., S. Brill, F. M. Konikoff, M. Averbuch, N. Arber and Z. Halpern. Additive deleterious effect of smoking on gastroduodenal pathology and clinical course in Helicobacter pylori-positive dyspeptic patients. Isr Med Assoc J 2000; **2**(12): 892-5.

Moss, S. F. and P. Malfertheiner. Helicobacter and gastric malignancies. Helicobacter 2007; **12 Suppl 1**: 23-30.

Musial, F., S. Klosterhalfen and P. Enck. Placebo responses in patients with gastrointestinal disorders. World J Gastroenterol 2007; **13**(25): 3425-9.

Nakshabendi, I. M., Q. B. Zhang, M. Mokhashi, C. G. Gemmell, F. D. Lee and R. I. Russell. Effect of omeprazole therapy on the survival of Helicobacter pylori, urease activity, and antral gastric histology in patients with duodenal ulcer. Helicobacter 1996; **1**(3): 155-8.

Nandurkar, S., N. J. Talley, H. Xia, H. Mitchell, S. Hazel and M. Jones. Dyspepsia in the community is linked to smoking and aspirin use but not to Helicobacter pylori infection. Arch Intern Med 1998; **158**(13): 1427-33.

Nishizawa, T., H. Suzuki, K. Kurabayashi, T. Masaoka, H. Muraoka, M. Mori, E. Iwasaki, I. Kobayashi and T. Hibi. Gatifloxacin resistance and mutations in gyra after unsuccessful Helicobacter pylori eradication in Japan. Antimicrob Agents Chemother 2006; **50**(4): 1538-40.

Nishizawa, T., H. Suzuki, A. Umezawa, H. Muraoka, E. Iwasaki, T. Masaoka, I. Kobayashi and T. Hibi. Rapid detection of point mutations conferring resistance to fluoroquinolone in gyrA of Helicobacter pylori by allele-specific PCR. J Clin Microbiol 2007; **45**(2): 303-5.

Ohkuma, K., M. Okada, H. Murayama, M. Seo, K. Maeda, M. Kanda and N. Okabe. Association of Helicobacter pylori infection with atrophic gastritis and intestinal metaplasia. J Gastroenterol Hepatol 2000; **15**(10): 1105-12.

Oksanen, A., L. Veijola, P. Sipponen, K. O. Schauman and H. Rautelin. Evaluation of Pyloriset Screen, a rapid whole-blood diagnostic test for Helicobacter pylori infection. J Clin Microbiol 1998; **36**(4): 955-7.

Oleastro, M., A. Menard, A. Santos, H. Lamouliatte, L. Monteiro, P. Barthelemy and F. Megraud. Real-time PCR assay for rapid and accurate detection of point mutations conferring resistance to clarithromycin in Helicobacter pylori. J Clin Microbiol 2003; **41**(1): 397-402.

Opekun, A. R., A. B. Gotschall, N. Abdalla, C. Agent, E. Torres, F. M. Sutton, D. Y. Graham and K. Tsuchiya. Improved infrared spectrophotometer for point-of-care patient 13C-urea breath testing in the primary care setting. Clin Biochem 2005; **38**(8): 731-4.

Osato, M. S., R. Reddy, S. G. Reddy, R. L. Penland, H. M. Malaty and D. Y. Graham. Pattern of primary resistance of Helicobacter pylori to metronidazole or clarithromycin in the United States. Arch Intern Med 2001; **161**(9): 1217-20.

Palmer, E. D. Investigation of the gastric mucosa spirochetes of the human. Gastroenterology 1954; **27**(2): 218-20.

Pantoflickova, D., D. R. Scott, G. Sachs, G. Dorta and A. L. Blum. 13C urea breath test (UBT) in the diagnosis of Helicobacter pylori: why does it work better with acid test meals? Gut 2003; **52**(7): 933-7.

Papatheodoridis, G. V., S. Sougioultzis and A. J. Archimandritis. Effects of Helicobacter pylori and nonsteroidal anti-inflammatory drugs on peptic ulcer disease: a systematic review. Clin Gastroenterol Hepatol 2006; **4**(2): 130-42.

Parasher, G. and G. L. Eastwood. Smoking and peptic ulcer in the Helicobacter pylori era. Eur J Gastroenterol Hepatol 2000; **12**(8): 843-53.

Peng, N. J., K. H. Lai, R. S. Liu, S. C. Lee, D. G. Tsay, C. C. Lo, H. H. Tseng, W. K. Huang, G. H. Lo and P. I. Hsu. Clinical significance of oral urease in diagnosis of Helicobacter pylori infection by [13C]urea breath test. Dig Dis Sci 2001; **46**(8): 1772-8.

Perez Aldana, L., M. Kato, S. Nakagawa, M. Kawarasaki, T. Nagasako, T. Mizushima, H. Oda, J. Kodaira, Y. Shimizu, Y. Komatsu, R. Zheng, H. Takeda, T. Sugiyama and M. Asaka. The relationship between consumption of antimicrobial agents and the prevalence of primary Helicobacter pylori resistance. Helicobacter 2002; **7**(5): 306-9.

Perez-Perez, G. I. Accurate diagnosis of Helicobacter pylori. Culture, including transport. Gastroenterol Clin North Am 2000; **29**(4): 879-84.

Peterson, W. L., D. Y. Graham, B. Marshall, M. J. Blaser, R. M. Genta, P. D. Klein, C. W. Stratton, J. Drnec, P. Prokocimer and N. Siepman. Clarithromycin as monotherapy for eradication of Helicobacter pylori: a randomized, double-blind trial. Am J Gastroenterol 1993; **88**(11): 1860-4.

Pieramico, O., H. Ditschuneit and P. Malfertheiner. Gastrointestinal motility in patients with non-ulcer dyspepsia: a role for Helicobacter pylori infection? Am J Gastroenterol 1993; **88**(3): 364-8.

Raderer, M., B. Streubel, S. Wohrer, M. Hafner and A. Chott. Successful antibiotic treatment of Helicobacter pylori negative gastric mucosa associated lymphoid tissue lymphomas. Gut 2006; **55**(5): 616-8.

Rautelin, H. and T. U. Kosunen. Helicobacter pylori infection in Finland. Ann Med 2004; **36**(2): 82-8.

Rauws, E. J. and G. N. Tytgat. Helicobacter pylori in duodenal and gastric ulcer disease. Baillieres Clin Gastroenterol 1995; **9**(3): 529-47.

Rehnberg-Laiho, L., H. Rautelin, M. Valle and T. U. Kosunen. Persisting Helicobacter antibodies in Finnish children and adolescents between two and twenty years of age. Pediatr Infect Dis J 1998; **17**(9): 796-9.

Robinson, K., R. H. Argent and J. C. Atherton. The inflammatory and immune response to Helicobacter pylori infection. Best Pract Res Clin Gastroenterol 2007; **21**(2): 237-59.

Rotimi, O., A. Cairns, S. Gray, P. Moayyedi and M. F. Dixon. Histological identification of Helicobacter pylori: comparison of staining methods. J Clin Pathol 2000; **53**(10): 756-9.

Sarnelli, G., R. Cuomo, J. Janssens and J. Tack. Symptom patterns and pathophysiological mechanisms in dyspeptic patients with and without Helicobacter pylori. Dig Dis Sci 2003; **48**(12): 2229-36.

Savarino, V., P. Zentilin, G. Bisso, M. Pivari, C. Bilardi, R. Biagini, M. R. Mele, C. Mansi, R. Termini, S. Vigneri and G. Celle. Optimal duration of therapy combining ranitidine bismuth citrate with clarithromycin and metronidazole in the eradication of Helicobacter pylori infection. Aliment Pharmacol Ther 1999; **13**(1): 43-7.

Schmid, C. H., G. Whiting, D. Cory, S. D. Ross and T. C. Chalmers. Omeprazole plus antibiotics in the eradication of Helicobacter pylori infection: a meta-regression analysis of randomized, controlled trials. Am J Ther 1999; **6**(1): 25-36.

Seppälä, K., M. Färkkilä, H. Nuutinen, K. Hakala, H. Väänänen, H. Rautelin and T. U. Kosunen. Triple therapy of Helicobacter pylori infection in peptic ulcer. A 12-month follow-up study of 93 patients. Scand J Gastroenterol 1992; **27**(11): 973-6.

Sherman, P. M. Appropriate strategies for testing and treating Helicobacter pylori in children: when and how? Am J Med 2004; **117 Suppl 5A**: 30S-35S.

Sipponen, P. Update on the pathologic approach to the diagnosis of gastritis, gastric atrophy, and Helicobacter pylori and its sequelae. J Clin Gastroenterol 2001; **32**(3): 196-202.

Sipponen, P., T. U. Kosunen, I. M. Samloff, O. P. Heinonen and M. Siurala. Rate of Helicobacter pylori acquisition among Finnish adults: a fifteen year follow-up. Scand J Gastroenterol 1996; **31**(3): 229-32.

Sipponen, P. and B. J. Marshall. Gastritis and gastric cancer. Western countries. Gastroenterol Clin North Am 2000; **29**(3): 579-92, v-vi.

Stolte, M., E. Bayerdorffer, A. Morgner, B. Alpen, T. Wundisch, C. Thiede and A. Neubauer. Helicobacter and gastric MALT lymphoma. Gut 2002; **50 Suppl 3**: III19-24.

Storskrubb, T., P. Aro, J. Ronkainen, K. Wreiber, H. Nyhlin, E. Bolling-Sternevald, N. J. Talley, L. Engstrand and L. Agreus. Antimicrobial susceptibility of Helicobacter pylori strains in a random adult Swedish population. Helicobacter 2006; **11**(4): 224-30.

Svedlund, J., I. Sjodin and G. Dotevall. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Dig Dis Sci 1988; **33**(2): 129-34.

Tankovic, J., D. Lamarque, C. Lascols, C. J. Soussy and J. C. Delchier. Clarithromycin resistance of Helicobacter pylori has a major impact on the efficacy of the omeprazole-amoxicillin-clarithromycin therapy. Pathol Biol (Paris) 2001; **49**(7): 528-33.

Tatemichi, M., M. Kabuto and S. Tsugane. Effect of smoking on serum pepsinogen I level depends on serological status of Helicobacter pylori. Jpn J Cancer Res 2001; **92**(3): 243-8.

Terai, S., K. Iijima, K. Kato, N. Dairaku, T. Suzuki, M. Yoshida, T. Koike, Y. Kitagawa, A. Imatani, H. Sekine, S. Ohara and T. Shimosegawa. Long-Term Outcomes of Gastric Mucosa-Associated Lymphoid Tissue Lymphomas after Helicobacter pylori Eradication Therapy. Tohoku J Exp Med 2008; **214**(1): 79-87.

Tkachenko, M. A., N. Z. Zhannat, L. V. Erman, E. L. Blashenkova, S. V. Isachenko, O. B. Isachenko, D. Y. Graham and H. M. Malaty. Dramatic changes in the prevalence of Helicobacter pylori infection during childhood: a 10-year follow-up study in Russia. J Pediatr Gastroenterol Nutr 2007; **45**(4): 428-32.

Tong, J. L., Z. H. Ran, J. Shen, C. X. Zhang and S. D. Xiao. Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during Helicobacter pylori eradication therapy. Aliment Pharmacol Ther 2007; **25**(2): 155-68.

Toracchio, S. and L. Marzio. Primary and secondary antibiotic resistance of Helicobacter pylori strains isolated in central Italy during the years 1998-2002. Dig Liver Dis 2003; **35**(8): 541-5.

Treiber, G., M. Schwabe, S. Ammon, S. Walker, U. Klotz and P. Malfertheiner. Dyspeptic symptoms associated with Helicobacter pylori infection are influenced by strain and host specific factors. Aliment Pharmacol Ther 2004; **19**(2): 219-31.

Treiber, G., J. Wittig, S. Ammon, S. Walker, L. J. van Doorn and U. Klotz. Clinical outcome and influencing factors of a new short-term quadruple therapy for Helicobacter pylori eradication: a randomized controlled trial (MACLOR study). Arch Intern Med 2002; **162**(2): 153-60.

Tucci, A., P. Tucci, M. Bisceglia, A. Marchegiani, G. Papadopoli, P. Fusaroli, A. Spada, M. O. Pistoletto, M. Cristino, L. Poli, A. Villani, M. Bucci, M. Marinelli and G. Caletti. Real-time detection of Helicobacter Pylori infection and atrophic gastritis: comparison between conventional methods and a novel device for gastric juice analysis during endoscopy. Endoscopy 2005; **37**(10): 966-76.

Udd, M., P. Miettinen, A. Palmu and R. Julkunen. Effect of short-term treatment with regular or high doses of omeprazole on the detection of Helicobacter pylori in bleeding peptic ulcer patients. Scand J Gastroenterol 2003; **38**(6): 588-93.

Vaira, D., J. Holton, C. Ricci, M. Menegatti, L. Gatta, S. Berardi, A. Tampieri and M. Miglioli. Review article: the transmission of Helicobacter pylori from stomach to stomach. Aliment Pharmacol Ther 2001; **15 Suppl 1**: 33-42.

Vakil, N., F. Lanza, H. Schwartz and J. Barth. Seven-day therapy for Helicobacter pylori in the United States. Aliment Pharmacol Ther 2004; **20**(1): 99-107.

Van Der Wouden, E. J., J. C. Thijs, A. A. Van Zwet and J. H. Kleibeuker. Review article: nitroimidazole resistance in Helicobacter pylori. Aliment Pharmacol Ther 2000; **14**(1): 7-14.

van der Wouden, E. J., J. C. Thijs, A. A. van Zwet, W. J. Sluiter and J. H. Kleibeuker. The influence of in vitro nitroimidazole resistance on the efficacy of nitroimidazole-containing

anti-Helicobacter pylori regimens: a meta-analysis. Am J Gastroenterol 1999; **94**(7): 1751-9.

Watanabe, K., A. Tanaka, K. Imase, K. Tokunaga, H. Sugano, A. Kai, H. Ishida, T. Itoh and S. Takahashi. Amoxicillin resistance in Helicobacter pylori: studies from Tokyo, Japan from 1985 to 2003. Helicobacter 2005; **10**(1): 4-11.

Veijola, L., E. Myllyluoma, R. Korpela and H. Rautelin. Stool antigen tests in the diagnosis of Helicobacter pylori infection before and after eradication therapy. World J Gastroenterol 2005; **11**(46): 7340-4.

Veldhuyzen van Zanten, S. J. and P. M. Sherman. Indications for treatment of Helicobacter pylori infection: a systematic overview. Cmaj 1994; **150**(2): 189-98.

Veldhuyzen van Zanten, S. J., N. J. Talley, A. L. Blum, E. Bolling-Sternevald, M. Sundin and O. Junghard. Combined analysis of the ORCHID and OCAY studies: does eradication of Helicobacter pylori lead to sustained improvement in functional dyspepsia symptoms? Gut 2002; **50 Suppl 4**: iv26-30; discussion iv31-2.

Vergara, M., M. Vallve, J. P. Gisbert and X. Calvet. Meta-analysis: comparative efficacy of different proton-pump inhibitors in triple therapy for Helicobacter pylori eradication. Aliment Pharmacol Ther 2003; **18**(6): 647-54.

Wermeille, J., M. Cunningham, J. P. Dederding, L. Girard, R. Baumann, G. Zelger, P. Buri, J. M. Metry, R. Sitavanc, L. Gallaz, H. Merki and N. Godin. Failure of Helicobacter pylori eradication: is poor compliance the main cause? Gastroenterol Clin Biol 2002; **26**(3): 216-9.

Wildner-Christensen, M., J. M. Hansen and O. B. De Muckadell. Risk factors for dyspepsia in a general population: non-steroidal anti-inflammatory drugs, cigarette smoking and unemployment are more important than Helicobacter pylori infection. Scand J Gastroenterol 2006; **41**(2): 149-54.

Wolle, K., A. Leodolter, P. Malfertheiner and W. Konig. Antibiotic susceptibility of Helicobacter pylori in Germany: stable primary resistance from 1995 to 2000. J Med Microbiol 2002; **51**(8): 705-9.

Wong, W. M., Q. Gu, K. M. Chu, Y. K. Yee, F. M. Fung, T. S. Tong, A. O. Chan, K. C. Lai, C. K. Chan and B. C. Wong. Lansoprazole, levofloxacin and amoxicillin triple therapy vs. quadruple therapy as second-line treatment of resistant Helicobacter pylori infection. Aliment Pharmacol Ther 2006; **23**(3): 421-7.

Xia, H. X., M. Buckley, C. T. Keane and C. A. O'Morain. Clarithromycin resistance in Helicobacter pylori: prevalence in untreated dyspeptic patients and stability in vitro. J Antimicrob Chemother 1996; **37**(3): 473-81.

Yakoob, J., W. Jafri, S. Abid, N. Jafri, Z. Abbas, S. Hamid, M. Islam, K. Anis, H. A. Shah and H. Shaikh. Role of rapid urease test and histopathology in the diagnosis of Helicobacter pylori infection in a developing country. BMC Gastroenterol 2005; **5**: 38.

Zullo, A., L. Gatta, V. De Francesco, C. Hassan, C. Ricci, V. Bernabucci, M. Cavina, E. Ierardi, S. Morini and D. Vaira. High rate of Helicobacter pylori eradication with sequential therapy in elderly patients with peptic ulcer: a prospective controlled study. Aliment Pharmacol Ther 2005; **21**(12): 1419-24.