NEW ASPECTS OF THE DIAGNOSIS OF
HELnCOBACTER PYLORI INFECTION

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Academic Dissertation

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This thesis is based on the following articles which are referred to in the text by their Roman numerals, and reprinted by the permission of the copyright holders.


ABBREVIATIONS

bid  bis in die (twice a day)
CI   confidence interval
DGM  duodenal gastric metaplasia
DNA  deoxyribonucleic acid
EIA  enzyme immunoassay
H. pylori  Helicobacter pylori
HSV  highly selective vagotomy
IgA  Immunoglobulin A
IgG  Immunoglobulin G
MALT mucosa associated lymphoid tissue
NSAID non-steroidal anti-inflammatory drug
OD  optical density
OR  odds ratio
PA  pernicious anaemia
PCA  parietal cell antibodies
PCR polymerase chain reaction
PG  pepsinogen
PPI  proton pump inhibitor
qid quater in die (four times a day)
RR  risk ratio
RNA  ribonucleic acid
RUT  rapid urease test
tid  ter in die (three times a day)
UBT  urea breath test
ABSTRACT

Aims: Helicobacter pylori infection, although the prevalence is declining in Western world, is still responsible for several clinically important diseases. None of the diagnostic tests is perfect and in this study, the performance of three stool antigen tests was assessed. In areas of high H. pylori prevalence, the definition of patients with the greatest benefit from eradication therapy may be a problem; the role of duodenal gastric metaplasia in categorizing patients at risk for duodenal ulcer was evaluated in this respect. Whether persistent chronic inflammation and elevated H. pylori antibodies after successful eradication are associated with each other or with atrophic gastritis, a long term sequelae of H. pylori infection, were also studied.

Patients and methods: The three stool antigen tests were assessed in pre- and post-eradication settings among 364 subjects in two studies as compared to the rapid urease test (RUT), histology, culture, the 13C-urea breath test (UBT) and enzyme immunoassay (EIA) based H. pylori serology. The association between duodenal gastric metaplasia with duodenal ulcer was evaluated in a retrospective study including 1054 patients gastroscopied due to clinical indications and 154 patients previously operated for duodenal ulcer. The extent of duodenal gastric metaplasia was assessed from histological specimens in different patient groups formed on the basis of gastroscopy findings and H. pylori infection. Chronic gastric inflammation (108 patients) and H. pylori antibodies and serum markers for atrophy (77 patients) were assessed in patients earlier treated for H. pylori.

Results: Of the stool antigen tests studied, the monoclonal antibody-based EIA-test showed the highest sensitivity and specificity both in the pre-treatment setting (96.9% and 95.9%) and after therapy (96.9% and 97.8%). The polyclonal stool antigen test and the in-office test had at baseline a sensitivity of 91% and 94%, and a specificity of 96% and 89%, respectively and in a post-treatment setting, a sensitivity of 78% and 91%, and a specificity of 97%, respectively. Duodenal gastric metaplasia was strongly associated with H. pylori positive duodenal ulcer (odds ratio 42). Although common still five years after eradication, persistent chronic gastric inflammation (21%) and
elevated *H. pylori* antibodies (33%) were neither associated with each other nor with atrophic gastritis.

**Conclusions:** Current *H. pylori* infection can feasibly be diagnosed by a monoclonal antibody-based EIA test with the accuracy comparable to that of reference methods. The performance of the polyclonal test as compared to the monoclonal test was inferior especially in the post-treatment setting. The in-office test had a low specificity for primary diagnosis and hence positive test results should probably be confirmed with another test before eradication therapy is prescribed. The presence of widespread duodenal gastric metaplasia showed promising results in detecting patients who should be treated for *H. pylori* due to an increased risk of duodenal ulcer. If serology is used later on in patients with earlier successfully treated for *H. pylori*, it should be taken into account that *H. pylori* antibodies may persist elevated for years for unknown reason. However, this phenomenon was not found to be associated with persistent chronic inflammation or atrophic changes.
INTRODUCTION

Since the discovery of *Helicobacter pylori* (*H. pylori*) in 1982 (Warren and Marshall, 1983) the associated diseases and the benefits of eradication therapy in many of them have been clarified. Recently, updated guidelines on several topics concerning the treatment indications and diagnosis have been published by the European Helicobacter Pylori Study Group as Maastricht III Consensus Report (Malfertheiner et al., 2007).

There are several methods available for the diagnosis of *H. pylori* infection, but none of them is ideal and therefore, in many clinical situations several tests are needed. Most of the tests originally developed for the diagnosis of *H. pylori* infection in patients with peptic ulcer disease are now used in different settings: peptic ulcer prevalence is rapidly declining and the prevention of gastric cancer at the precancerous stage has become relatively more important. Also, the prevalence of *H. pylori* infection is diminishing (Rehnberg-Laiho et al., 2001) and this has a great impact even on the performance of those tests with high sensitivity and specificity.

Gastroscopy has become a commonplace diagnostic investigation in western countries but at the same time the waiting list for this procedure has not necessarily shortened. On the other hand, the increasing use of proton pump inhibitors (PPIs), although efficiently alleviating abdominal symptoms hamper the endoscopic appearance and *H. pylori* diagnosis (Mégraud and Lehours, 2007). In areas with high *H. pylori* prevalence, the indications for eradication therapy must be allocated to patients with the greatest benefit, one such group being patients with duodenal ulcer. It is easy to detect an acute ulcer during gastroscopy, however, in patients with ulcer diathesis on PPI therapy the scars in the duodenal bulb might be minimal and the diagnosis may be overlooked. Clinically useful tools to identify patients at risk for duodenal ulcer are needed.

In a subgroup of patients after *H. pylori* eradication therapy, chronic inflammation of the gastric mucosa as well as elevated *H. pylori* antibodies prevail for long periods. The mechanism behind these phenomena is obscure as is their possible association
with each other or with clinical consequences e.g. atrophic gastritis, autoimmune phenomena induced by *H. pylori*, or even gastric cancer risk.

Although the past 25 years of the *H. pylori* era have dramatically changed our understanding and treatment of several gastric diseases, many questions still remain open and need to be answered.
REVIEW OF THE LITERATURE

HISTORY OF H. PYLORI

Spiral bacteria in gastric scrapings from dogs were described as early as in 1892 by Bizzozero at a meeting in Turin (Bizzozero, 1893, Egan and O'Morain, 2007). The first human report published was by Krienitz from a patient with gastric cancer in 1906 (Krienitz, 1906) and at that time spiral bacteria were also considered in the development of gastric and duodenal ulceration, sometimes patients were even treated with high doses of bismuth (Kusters et al., 2006). However, it was also suggested that those bacteria were only contaminants from the oral cavity (Egan and O'Morain, 2007). The modern era began when H. pylori was cultivated from gastric mucosa and it’s association with gastritis and later with peptic ulcer was demonstrated by Warren and Marshall, who were awarded with the Nobel Prize in Medicine 2005 (Warren and Marshall, 1983, Marshall and Warren, 1984).

BACTERIOLOGY AND EPIDEMIOLOGY OF H. PYLORI

1. Bacteriology

Helicobacter and Wolinella comprise the family Helicobacteraceae which in turn together with Campylobacteraceae belong to the Epsilonproteobacteria, class nov. (Mégraud and Lehours, 2007). Thirty different Helicobacter species have been formally named with a growing list of species waiting for formal recognition (List of Prokaryotic Names with Standing in Nomenclature [http://www.bacterio.cict.fr] Accessed 18\textsuperscript{th} May 2007).

H. pylori is a Gram negative spiral shaped multi-flagellated bacteria found almost exclusively on human gastric mucosa. Under unfavourable circumstances it can become coccoidal, a nonculturable form with debatable viability. The bacterium is a microaerophilic and capnophilic organism, slowly growing with rigorous culture demands (Mégraud and Lehours, 2007). The phenotypic identification is based on the growth of small, circular, smooth colonies observed after 3 to 4 days on selective
media plated with gastric biopsy specimens. The identification of the cultured bacteria is based on positive reactions for oxidase, catalase, and urease. So far, the complete genomes of three *H. pylori* strains have been published (Tomb *et al.*, 1997, Alm *et al.*, 1999, Oh *et al.*, 2006b, Josenhans *et al.*, 2007). *H. pylori* is genetically very heterogenous (Kusters *et al.*, 2006), and this genetic diversity of different *H. pylori* strains was even exploited in a study suggesting that humans harboured *H. pylori* as early as 58,000 years ago before migrations from Africa (Linz *et al.*, 2007).

### 1.1. Virulence factors

Several virulence factors of *H. pylori* have been identified. The most studied is CagA, which according to numerous studies, is associated with peptic ulcers, precancerous conditions and gastric cancer in the Western world (Kusters *et al.*, 2006). CagA protein is a highly immunogenic protein encoded by the *cag* A gene, present in approximately 50 to 70% of *H. pylori* strains. It is a marker for the presence of a genomic pathogenicity island PAI, encoding 27 to 31 proteins, 18 of which serve as building blocks of the type IV secretory apparatus, fundamental in the aberration of host immune responses (Odenbreit *et al.*, 2000, Kusters *et al.*, 2006, Robinson *et al.*, 2007). Another well-known virulence factor, cytotoxin VacA is associated with peptic ulcer and adenocarcinoma. It is encoded by the gene *vac*A which is present in all strains, but because of several alleles various amounts of toxin are produced and approximately 50% of *H. pylori* strains secrete the toxin VacA. The most virulent genotype s1/m1 of *vac*A is clearly associated with the *cag*A positive genotype (Kusters *et al.*, 2006). The gene *dup*A is associated with duodenal ulceration and has been associated with the decreased risk for gastric atrophy and cancer (Robinson *et al.*, 2007). Adhesin BabA is associated with peptic ulcers and gastric cancer (Kusters *et al.*, 2006), as is outer inflammatory protein OipA (Robinson *et al.*, 2007). SabA might be crucial in the control of the host immune response by *H. pylori* (Kusters *et al.*, 2006). Lipopolysaccharide contains oligosaccharide antigens structurally and immunologically closely related to human blood group antigens, and have been suggested to promote autoantibodies and to play a role in gastric autoimmunity (Kusters *et al.*, 2006). However, the significance of all these virulence factors is debatable since neither CagA nor VacA seropositivity is a better predictor of
increased peptic ulcer or stomach cancer risk than *H. pylori* antibodies (González *et al*., 2003, Mégraud and Lehours, 2007).

2. Epidemiology

It has been estimated that approximately 50% of the world’s population is infected with *H. pylori* (Horvitz and Gold, 2006). Transmission of the infection may occur via gastro-oral, oral-oral, and fecal-oral routes, but it is not clear which is the predominant mode of transmission (Delport and van der Merwe, 2007, Kusters *et al*., 2006, Parsonnet *et al*., 1999). In general the infection is acquired during childhood (Rehnberg-Laiho *et al*., 1998, Rowland *et al*., 2006, Malaty, 2007) and the source of the infection is most likely the family members (Konno *et al*., 2005, Malaty, 2007).

The incidence of new *H. pylori* infections among adults in the Western world is less than 0.5% per year (Kusters *et al*., 2006) and spontaneous disappearance of the infection is rare (Kosunen *et al*., 1997, Malaty, 2007). The prevalence of the infection is correlated with low socioeconomic status during childhood, high density of living, and low household income (Malaty, 2007). The infection is steadily declining in developed countries (Rehnberg-Laiho *et al*., 2001, Malaty, 2007, Kusters *et al*., 2006) and its prevalence in children under the age 20 in Finland is only 5.6% (Rehnberg-Laiho *et al*., 1998) as compared with 66% in Finnish centenerians (Rehnberg-Laiho *et al*., 1999). This decreasing *H. pylori* incidence in children leads to declining seropositivity rate in successive birth cohorts, so called cohort phenomenon.

*H. PYLORI ASSOCIATED DISEASES*

Since the discovery of *H. pylori* the associated diseases have been under intense research. It’s now well established that only a minority of *H. pylori* infected persons develop peptic ulcer or gastric cancer. The recent assumption of who develops disease is determined largely by the inflammatory response to infection, which in turn is determined by the virulence of the infecting strain, host genetic predisposition to disease and environmental co-factors (Robinson *et al*., 2007). The negative association with *H. pylori* and adenocarcinoma of the cardia has evoked concern of
the possible detrimental effect of widespread policy of *H. pylori* eradication (Blaser, 1999b). There is also a hypothesis of regarding the protective effect of *H. pylori* against orally ingested pathogens; and the long symbiosis between humans and *H. pylori* has evoked an opinion of *H. pylori* being a commensal and not causing an infection at all (Blaser, 1999b). Since atopy and autoimmunity have become more common in developed countries as the prevalence of *H. pylori* has decreased, it has been speculated that the immune response induced by *H. pylori* could play a general immunoregulatory role contributing to the hygiene hypothesis (Kosunen et al., 2002, Robinson et al., 2007).

1. *H. pylori* induced gastritis and atrophic gastritis

1.1. Gastritis

From the self inoculation studies it can be concluded that acute *H. pylori* infection in adults is thought to be symptomatic, and to provoke acute gastritis with polymorphonuclear infiltration in the gastric mucosa and transient hypochlorhydria (Sobala et al., 1991, Graham et al., 2004b, Robinson et al., 2007). Since the spontaneous clearance of the infection is rare (Valle et al., 1996, Kosunen et al., 1997), the infection proceeds in most subjects to chronic gastritis. This inflammatory response is mediated by cytokines, and the magnitude of the cytokine response is influenced by genetic polymorphism. An individual’s ultimate clinical outcome is dependent on the cytokine response and on the gastric acid secretion. Interleukin 1-beta is in this context one of the most important pro-inflammatory cytokines and the most powerful acid inhibitor known (El-Omar et al., 2000, Lochhead and El-Omar, 2007). An increase in stimulated acid production predisposes to duodenal ulceration and decreased acid production predisposes to corpus gastritis or pangastritis which in turn predisposes to gastric ulceration, atrophic gastritis, and gastric carcinoma (Lochhead and El-Omar, 2007, Robinson et al., 2007). The intragastric distribution of gastritis is thought to be dependent on host genetic factors, bacterial virulence factors and environmental factors including age at onset of infection (Robinson et al., 2007).
1.2. Atrophic gastritis

Atrophic gastritis is defined as loss of appropriate glands (Rugge et al., 2002). It is often accompanied by intestinal metaplasia, which is recognized morphologically by the presence of goblet and absorptive cells or cells resembling colonocytes or by its mucin content depending on the type of intestinal metaplasia (Dixon et al., 1996, Lauwers, 2003). It is estimated that about 50% of \textit{H. pylori} infected persons develop atrophic gastritis during their lifetime (Lauwers, 2003). Atrophic gastritis and intestinal metaplasia are considered to be precancerous conditions and there is a significant association between the extent of intestinal metaplasia, its phenotype (intestinal, incomplete, or colonic), and increased cancer risk (Lauwers, 2003). The cancer risk is 5 to 90-fold depending on the extent and severity of atrophy (Sipponen and Graham, 2007).

Chronic atrophic gastritis has been classically divided into type A, autoimmune corpus-dominated gastric atrophy, and type B \textit{H. pylori} associated multifocal atrophic gastritis affecting both antrum and corpus (Dixon et al., 1996, Pérez-Pérez, 1997). However, the division into two entities has now been impugned (see below). About 10 to 15% of patients with atrophic gastritis develop pernicious anaemia (PA), which is a malabsorption of vitamin B\textsubscript{12} leading to characteristic anaemia. There is a clear familial tendency to develop autoimmune gastritis (Baxter et al., 2005).

1.2.1. The role of \textit{H. pylori} in autoimmune gastritis

Accumulating data suggest that gastric corpus atrophy is caused by \textit{H. pylori} driven autoimmune process (Annibale et al., 2001, Bergman et al., 2005, Oh et al., 2006a), although the role of \textit{H. pylori} as a trigger in autoimmune gastritis is debatable (Faller et al., 1997, Pérez-Pérez, 1997, Hershko et al., 2005, Malfertheiner et al., 2007). A substantial proportion of patients with autoimmune gastritis has or has had \textit{H. pylori} infection (Bergman et al., 2005, Annibale et al., 2007). During long follow-up, patients with \textit{H. pylori} gastritis may develop autoimmune type gastritis with elevated parietal cell antibodies (PCA) and a normal antrum mucosa (Valle et al., 1996). In a recent population screening study, almost all patients with pre-autoimmune type, but
not yet atrophic, gastritis had active *H. pylori* infection (Storskrubb *et al*., 2005). With an increase in the grade of corpus atrophy, *H. pylori* seropositivity declines but the prevalence of PCA increases (Oksanen *et al*., 2000). In patients with atrophic corpus gastritis the eradication of *H. pylori* may restore the atrophic changes and function of the corpus mucosa (Annibale *et al*., 2002) the longer the follow-up period (Kokkola *et al*., 2002). Thus, atrophic corpus gastritis seems not to proceed at least after eradication therapy. Patients with positive *H. pylori* serology as the only marker of the infection had a higher grade of atrophy and more often pernicious anaemia as compared with patients with current infection detected by other methods (Kokkola *et al*., 2000). Seronegative patients had the highest grade of atrophy and PA, supporting the hypothesis that *H. pylori* indeed induces autoimmune gastritis but with increasing grade of atrophy, *H. pylori* disappears spontaneously (Karttunen *et al*., 1991, Kokkola *et al*., 2003) and PA is associated with a high grade of corpus atrophy (Bergman *et al*., 2005). However, among type 1 diabetics and patients with juvenile autoimmune thyroid disease, there are patients with high titres of PCA associated with gastric autoimmunity but no signs of *H. pylori* infection (Segni *et al*., 2004, De Block *et al*., 2002). These diabetics, as compared to the *H. pylori* infected patients with low titres of PCA, had an association with different HLA-DQ haplotypes (De Block *et al*., 2002, Bergman *et al*., 2005). Thus, at least among patients with diabetes or autoimmune thyroid disease, atrophic gastritis induced by *H. pylori* and autoimmune gastritis may be separate entities.

1.2.2. The mechanism behind *H. pylori* induced autoimmunity

*H. pylori* has been shown to induce antibodies reactive with gastric mucosa in approximately half of the infected individuals, and gastric H⁺,K⁺-ATPase has been identified as the single major autoantigen in chronic *H. pylori* gastritis with corpus atrophy (Claeys *et al*., 1998, Bergman *et al*., 2005). The autoantibodies correlate with the severity of gastritis, atrophy and apoptosis of the corpus mucosa. Possible mechanisms for *H. pylori* to induce autoimmune gastritis are the presentation of self antigens enhanced by inflammation, and the expression of epitopes structurally similar to those of autoantigens, so called molecular mimicry (D'Elios *et al*., 2004). At least 11 epitopes of human H⁺,K⁺-ATPase recognized by autoreactive T cells have been identified (D'Elios *et al*., 2005).
1.2.3. Histological assessment of gastritis and atrophy

The grade of gastritis and atrophy is assessed according to the updated Sydney classification (Dixon et al., 1996) with the help of the visual analogue scales, originally presented by Tuomo Karttunen (Smith and Genta, 2000). However, there is still some interobserver variability in these assessments among pathologists. The repeatability is poor concerning especially the estimation of chronic residual gastritis after H. pylori eradication therapy (Tepeš et al., 1999a) and antral atrophy (Rugge et al., 2002). A few mononuclear cells are always present in the lamina propria of the gastric mucosa but the criteria for mild chronic inflammation is fulfilled when more than two to five lymphocytes, plasma cells and macrophages are seen per highpower (×40 objective) microscopic field or two or three lymphocytes or plasma cells are seen between the foveolae (Dixon et al., 1996).

1.2.4. Serum tests in assessment of atrophy and autoimmunity

Pepsinogen (PG) I and II are two immunologically distinct hydrolyzing proteinases of the mucosal lining of the stomach. PG I consists of five isozymogens and PG II of two isozymogens. Both PG I and II are secreted from the chief and mucous neck cells of fundic glands, but PG II is also found in the antral and proximal duodenal mucosa. The predominant PG in atrophic corpus gastritis is PG II since, in parallel with the decline of fundic glands the secretion of PG I abates, and thus the low PG I and low PG I/II ratio are good predictors of atrophic corpus gastritis (Samloff and Taggart, 1987). These have been investigated in screening patients with increased risk for gastric cancer (Varis et al., 2000, Yoshida et al., 2006, Palli et al., 2007, Sipponen and Graham, 2007, Cao et al., 2007).

Gastrin is secreted by antral G cells and it regulates the gastric acid secretion. In blood several forms of gastrin exist, the most prevalent being gastrin-34 (this accounts for the basal secretion) and gastrin-17 (stimulated by food ingestion) (Kaneko et al., 2002). Low gastrin-17 is a sign of multifocal or antrum-limited atrophic gastritis in patients with H. pylori infection (Sipponen et al., 2002, Yoshida et al., 2006). In patients with atrophic corpus gastritis including patients with PA the serum gastrin levels are elevated (Bergman et al., 2005).
PCA were discovered in over 90% of patients with PA as early as in the 1960’s (Whittingham and Mackay, 2005). Later on the autoantigen was identified as H⁺/K⁺ adenosine triphosphatase, the proton pump of the gastric glands (Bergman et al., 2005). These antibodies are detected in some 60% of patients with atrophic gastritis. In 50 to 70% of patients with PA there are antibodies against gastric secreted intrinsic factor, a product of parietal cells, which enhances the absorption of vitamin B₁₂ (Whittingham and Mackay, 2005).

1.3. Dyspepsia

Dyspepsia or indigestion has several definitions. According to the Rome II criteria it is defined as upper abdominal pain or discomfort (Talley et al., 1999). The prevalence of dyspepsia in the community is about 40% during six months (Moayyedi et al., 2006). These patients constitute a significant burden to health care. Around 60% of them have negative investigations and are labelled as having non-ulcer or functional dyspepsia (Moayyedi et al., 2006), which remains a poorly defined entity and its association with H. pylori remains still unknown (Lai and Sung, 2007). In 10% of dyspepsia patients eradication therapy leads to long term symptom improvement according to the Cochrane review (Moayyedi et al., 2006) and in addition it reduces the risk of long term sequelae (atrophic gastritis and gastric cancer) of H. pylori infection (Malfertheiner et al., 2007). Dyspepsia is an accepted indication for H. pylori eradication (Malfertheiner et al., 2007).

2. Peptic ulcer

2.1. Epidemiology

H. pylori has apparently colonized human stomach since time immemorial (Linz et al., 2007), but duodenal ulcer disease has been present essentially only for 200 years (Blaser, 1998, Blaser, 1999a). The first description of gastric ulceration was made in 1506 by an Italian physician (Egan and O'Morain, 2007), but in mid nineteenth century physicians began to observe more often peptic ulceration, initially gastric
ulcers in young women and then duodenal ulcers in men (Blaser, 1998). The life-time risk of peptic ulcer in a person with *H. pylori* infection varies from 3% (USA) to 25% (Japan) (Suerbaum and Michetti, 2002). In Western countries duodenal ulcers are approximately four-fold more common than gastric ulcers; elsewhere gastric ulcers are more common (Kusters *et al*., 2006). It is widely accepted that the chronic active gastritis affecting mainly the antrum is associated with duodenal ulcer disease (Dixon, 2000).

### 2.2. Pathogenesis

The pathogenesis of peptic ulcer disease is not thoroughly understood. The localisation of the ulcer is determined by acid output; the higher the output, the more distal the ulcer (Johnson, 1957, Albillos *et al*., 1990, Savarino *et al*., 1995). The acid secretion however, varies widely in peptic ulcer patients (Savarino *et al*., 1995) and benign gastric ulcers in a persistently achlorhydric stomach have been described (Bynum, 1991). The peptic ulcer studies in animals indicate that pepsin in combination with acid produces much more severe mucosal damage than acid alone, but there is considerable overlap between ulcer patients and control subjects and no pepsinogen level is diagnostic for peptic ulcer (Samloff and Taggart, 1987). The “leaky roof” theory, linking *H. pylori* and increased acid secretion, presumes that acid injury in the duodenum promotes the development of gastric metaplasia, allowing the *H. pylori* to attach these areas and this leads to duodenal ulcer (Egan and O’Morain, 2007). However, attachment of *H. pylori* to gastric apical cells indeed induces proinflammatory chemokines and changes core protein expression leading to changes in the mucous gel layer, but neither these alterations in mucosal protection (Jass and Walsh, 2001) nor the studies on the host’s genetic polymorphism of inflammatory and immunoregulatory cytokines (Blanchard *et al*., 2004) account for the disease phenotype. The outcome of *H. pylori* gastritis on an individual basis cannot be predicted by virulence factors of the bacteria e.g. CagA either (González *et al*., 2003, Sgouros and Bergele, 2006). Venerably, we have to admit that we do not know why some people develop peptic ulcer, but the exogenous risk factors must be responsible for the rapid changes in the epidemiology of the disease (Kusters *et al*.,)
In areas of high prevalence of *H. pylori* infection it is a challenge to identify patients at risk of peptic ulcer.

### 2.3. Duodenal gastric metaplasia (DGM)

DGM indicates the presence of patches of mucus-secreting gastric-type cells along the villous surfaces in duodenum. It is differentiated from heterotopia, supposed to be a congenital abnormality, where the fully developed body-type gastric mucosa contains also parietal and chief cells (Wyatt and Rathbone, 1989). In 1935 artificial defects in the duodenal mucosa of cats were demonstrated to heal with a simple mucous epithelium identical with gastric surface epithelium, and in 1964 DGM was first described in a patient with suspected Zollinger-Ellison syndrome. DGM was shown to be associated with duodenal ulcer and high acid levels already before the discovery of *H. pylori* and it was suggested to be part of the healing process following tissue damage (Patrick *et al.*, 1974). Steer described the adherence of (at that time unknown) bacteria to the surface of these gastric type epithelial cells and he noticed that these bacteria were absent from the biopsies of the patients with normal gastric and duodenal mucosa (Steer, 1984). Active duodenitis was shown to be associated when the extension of DGM was more than 5% (Wyatt *et al.*, 1987). DGM was commonly found in asymptomatic volunteers and small foci were not associated with inflammation and therefore the clinical importance of gastric metaplasia was questioned (Fitzgibbons *et al.*, 1988). However, at the same time it was suggested that the gastric antrum was necessary for *H. pylori* colonization and the bacterial infection in the duodenal bulb was made possible by its mucosal change. This change was originally meant to protect the duodenal mucosa, but *H. pylori* infection in the bulb predisposed to ulcerative lesions (Caselli *et al.*, 1988).

After the strong association shown between DGM and duodenal ulcer, DGM if colonized by *H. pylori* turned out to be an even stronger risk factor for duodenal ulcer (Carrick *et al.*, 1989). The inflammatory injury of the duodenal mucosa caused by *H. pylori* was supposed to stimulate further gastric metaplasia and as part of the vicious circle the progressively increasing area led to ulceration (Wyatt *et al.*, 1990). Since then several studies on the association between DGM and duodenal ulcer have been published but the significance of DGM in duodenal ulcer pathogenesis is still...
questionable (Savarino et al., 1997, McColl, 1996, Jonkers et al., 1998). However, many studies have shown that in patients with duodenal ulcer, the prevalence and extension of gastric metaplasia in the duodenum are more widespread as compared to non-ulcer dyspepsia or gastric ulcer patients (Wyatt et al., 1987, Kreuning et al., 1989, Carrick et al., 1989, Wyatt et al., 1990, Andersen et al., 1991, Amarapurkar et al., 1993, Yang et al., 1995, Harris et al., 1996, Pan et al., 1996, Jonkers et al., 1998, Kim et al., 1998, Futami et al., 1999, Heikkinen et al., 2001, Chang et al., 2001).

Recently, in a prospective study positive H. pylori culture from the duodenal bulb was a strong risk factor for subsequent development of duodenal ulcer (Pietroiusti et al., 2005). In accordance with the association of DGM with hyperacidity, in atrophic gastritis DGM is non-existent (Wyatt et al., 1990). For reasons unknown, it has been shown to be common also in H. pylori negative non-ulcer dyspepsia (Heikkinen et al., 2001).

### 2.3.1. Regression of DGM

The effect of H. pylori eradication on the prevalence and extent of DGM is contradictory (Noach et al., 1993, Harris et al., 1996, Khulusi et al., 1995, Savarino et al., 2000, Bago et al., 2002). Healing of duodenal ulcer had no effect on the extent of gastric metaplasia but acid suppressive therapy and H. pylori eradication diminished the area significantly during a six month period (Khulusi et al., 1996), and a longer follow-up of four years also showed a declining tendency (Kim et al., 1998).

The prevalence of DGM in vagotomized patients was reported to be lower than in duodenal ulcer patients (Wyatt et al., 1987), although the 2 to 3 year follow-up of patients after vagotomy failed to show any change either in the prevalence or in the extent of DGM (Jönsson et al., 1988).

The mechanism behind DGM is suggested to be the high acid exposure which impairs the function of Cdx2, considered to be the master regulator of intestinal differentiation (Faller et al., 2004).
2.4. *H. pylori* eradication in peptic ulcer disease

Of the duodenal ulcers 95%, and of the gastric ulcers, 70% are associated with *H. pylori* (Ford et al., 2006). As far as ulcer healing is concerned in duodenal ulcer patients, *H. pylori* eradication is superior to ulcer healing drugs, but in gastric ulcers there is no difference between these two therapies. In both duodenal and gastric ulcers eradication therapy is clearly effective in preventing the recurrence of ulcers when compared to patients with no ulcer healing drug therapy: duodenal ulcers recurred in 6% of patients successfully treated for *H. pylori* compared with 67% of those who remained positive for *H. pylori*, whereas in gastric ulcers the response was 4% and 59%, respectively (Hopkins et al., 1996). In duodenal ulcer patients *H. pylori* eradication therapy and continuous therapy with modern ulcer healing drugs are equally effective in preventing recurrence. In patients with gastric ulcer there are no such studies (Ford et al., 2006). However, eradication therapy is cost effective and continuous PPI therapy is an option only for patients with contraindications for *H. pylori* eradication therapy or several unsuccessful eradication therapies. Patients with peptic ulcer disease benefit clearly from *H. pylori* eradication according to the recent Cochrane Database of Systematic Reviews (Ford et al., 2006) and *H. pylori* eradication is strongly recommended for these patients also in the Maastricht II and III Consensus reports (Malfertheiner et al., 2002) (Malfertheiner et al., 2007).

2.5. Non-steroidal anti-inflammatory drugs (NSAID) and peptic ulcer

NSAIDs or *H. pylori* alone increase the risk of peptic ulcer about 20-fold, but *H. pylori* infected NSAID users are 60 times more likely to develop a peptic ulcer than uninfected non-NSAID users (Lai and Sung, 2007). NSAIDs and *H. pylori* infection independently increase also the risk of peptic ulcer bleeding. In patients with chronic NSAID use, PPI maintenance treatment is better than *H. pylori* eradication therapy in preventing upper gastrointestinal bleeding. However, *H. pylori* eradication therapy is of value in chronic NSAID and low dose aspirin users although it is insufficient to prevent ulcer disease and bleeding completely. *H. pylori* eradication therapy is recommended to all naive NSAID users and to chronic NSAID users needing PPI therapy for NSAID related side-effects, the latter to prevent the development of
atrophic gastritis accelerated by PPIs (Malfertheiner et al., 2007). Eradication treatment is also recommended to patients on low-dose aspirin therapy with a history of peptic ulcer (Lai and Sung, 2007).

2.6. Vagotomy

Highly selective vagotomy (HSV) was the surgical treatment of choice for patients with chronic duodenal ulceration before the era of H. pylori eradication therapy. In the procedure the nerves controlling the gastric emptying were preserved while those controlling acid secretion were divided (Donahue, Griffith and Richter, 1996). The operation decreased the peak acid output by approximately 50% (Egan and O'Morain, 2007). The rate of ulcer recurrence in a study of 500 patients who had undergone HSV was 18.5% in 15 years (Macintyre and Millar, 1991).

3. Mucosa associated lymphoid tissue lymphoma (MALT)

Primary gastric lymphomas comprising 5% of gastric malignancies, are generally non-Hodgkin lymphomas (NHL) and the most common histological subtypes are diffuse large B-cell and marginal zone B-cell NHL of the MALT-type (Ferrucci and Zucca, 2007). In Finland according to the statistics of the Finnish Cancer Registry (www.cancerregistry.fi), there were 59 NHLs of the stomach in 2003. Less than 1% of H. pylori infected subjects develop MALT lymphoma (Kusters et al., 2006) but of the patients with MALT lymphoma 72-98% are infected with H. pylori (Suerbaum and Michetti, 2002). Twelve months after H. pylori eradication therapy 62% of patients with low grade gastric MALT lymphoma had complete remission and thus H. pylori eradication is first line therapy in patients in stage I disease and the indications for eradication therapy are strong in all patients with MALT lymphoma (Malfertheiner et al., 2007).
4. Gastric cancer

Gastric cancer has been known since 400 BC in writings of Hippocrates (Houghton and Wang, 2005). Environmental factors modulating the risk of gastric cancer include certain food items and cigarette smoking (Lochhead and El-Omar, 2007). Gastric cancer is divided according to the classification presented by Laurén into intestinal and the diffuse type (Laurén, 1965). The incidence of gastric cancer is declining in Western countries, especially the intestinal type of distal gastric cancer, but the diffuse type, although also associated with *H. pylori*, is not (Houghton and Wang, 2005). The association of *H. pylori* infection with both histologic types of gastric cancer has been equally strong (Lochhead and El-Omar, 2007) and CagA seropositivity has been associated with gastric cancer (Held *et al.*, 2004, Palli *et al.*, 2007). In most studies concerning the location of the cancer, the risk of *H. pylori* and CagA seropositivity was confined to non-cardia cancer (Lochhead and El-Omar, 2007, Palli *et al.*, 2007). Cardia cancer, formerly thought not to be associated with *H. pylori*, is now divided into type A being a consequence of atrophic gastritis due to *H. pylori* infection or autoimmune atrophic gastritis, and type B which resembles oesophageal adenocarcinoma and is likely to be a consequence of short segment gastro-oesophageal reflux disease (McColl, 2006, Palli *et al.*, 2007). *H. pylori* infection is negatively associated with gastro-oesophageal reflux disease, the reason for this being largely unclear, but *H. pylori* eradication does not exacerbate symptoms in these patients and reflux disease is not a contraindication for *H. pylori* eradication (Malferttheiner *et al.*, 2007).

The lifetime risk for gastric cancer in *H. pylori* infected persons is less than 2% (McColl, 2005). *H. pylori* has been classified as a class I carcinogen by the International Agency for Research on Cancer 1994 (Anonymous, 1994) and it is estimated that 60-90% of gastric cancers are associated with *H. pylori* (Malferttheiner *et al.*, 2005). In Finland the decreasing prevalence of *H. pylori* antibodies has been associated with subsequent decreasing incidence of gastric cancer after a median latency of 20 years (Rehnberg-Laiho *et al.*, 2001), and this result has also been confirmed in Japan (Kobayashi *et al.*, 2004).
4.1. Gastric carcinogenesis

The concept of the gastric carcinogenesis was introduced by Correa in 1988 as a sequence of consecutive events starting from chronic superficial gastritis, atrophy and intestinal metaplasia, dysplasia, and finally leading to gastric adenocarcinoma (Correa, 1988). However, some recent data suggest that intestinal metaplasia is a marker for atrophy, a paracancerous rather than precancerous condition, and that cancer arises from a different cell lineage in gastric glands (Graham and Shiotani, 2005, Tatematsu et al., 2003, Meining et al., 2001). Histogenetically numerous carcinomas of the stomach are primarily of the gastric type and may secondarily change into the intestinal type (Meining et al., 2001). In addition, not all gastric cancers rise from atrophic gastric mucosa (Sipponen et al., 1994), although in the totally normal stomach the cancer is extremely rare (Graham and Shiotani, 2005, Sipponen et al., 1992). At present gastric cancer is regarded as a paradigm for infection-induced chronic inflammation-mediated cancer (Lochhead and El-Omar, 2007). There is experimental evidence that the origin of these cancers are bone marrow derived stem cells (BMDC) and the role of inflammation is in creating a pro-carcinogenic environment for engraftment of circulating marrow-derived stem cells (Li et al., 2006, Cai et al., 2005, Houghton and Wang, 2005). It is suggested that the bone marrow derived cells take over the function of tissue stem cells and in the abnormal tissue environment with lack of glandular cells, the BMDC fail to differentiate properly and progress instead to metaplasia, dysplasia, and cancer (Houghton and Wang, 2005).

4.2. \textit{H. pylori} eradication to prevent gastric cancer

The studies on the effect of *H. pylori* eradication on gastric cancer risk have been somewhat disappointing (Kuipers and Sipponen, 2006, Malfertheiner *et al.*, 2006). In some studies, *H. pylori* eradication has been shown to be beneficial (Saito *et al.*, 2000, Uemura *et al.*, 2001, Take *et al.*, 2005, Zhou *et al.*, 2005, Nakagawa *et al.*, 2006), but in others there has been no statistical difference between treated and non-treated (Wong *et al.*, 2004, Leung *et al.*, 2004, Mera *et al.*, 2005). In a Chinese study including patients with no precancerous conditions at baseline, no gastric cancers were evident after eradication therapy (Wong *et al.*, 2004). Eradication therapy probably does not prevent gastric cancer in subjects with precancerous conditions (there is a point of no return) (Kuipers and Sipponen, 2006). The sustained chronic inflammation after successful eradication as a possible explanation for dismal results in these gastric cancer prevention studies is not thoroughly investigated.

The Maastricht III Consensus Report concluded by recommending *H. pylori* eradication to prevent gastric cancer because it has the potential to reduce non cardia gastric cancer risk and the optimal time to eradicate is before the pre-neoplastic changes appear. Eradication is strongly indicated in patients with gastric cancer resection, atrophic gastritis and first degree relatives of patients with gastric cancer (Malfertheiner *et al.*, 2007).

### 5. Extragastric diseases

*H. pylori* has been implicated in several studies as a cause of iron-deficiency anemia regardless of the presence or absence of peptic ulcer disease (Cardenas *et al.*, 2006). It is often refractory to oral iron treatment but responds favourably to *H. pylori* eradication (Hershko *et al.*, 2005). Unexplained iron deficiency anemia is an indication for *H. pylori* eradication (Malfertheiner *et al.*, 2007).

Idiopathic thrombocytopenic purpura (ITP) is also an extragastric disease associated with chronic *H. pylori* infection (Franceschi and Gasbarrini, 2007). In a recent study 58% of ITP patients had *H. pylori* infection and 62% of patients with successful eradication therapy showed increased platelet count (Kodama *et al.*, 2007). ITP is an indication for *H. pylori* eradication therapy according to the Maastricht III Consensus Report (Malfertheiner *et al.*, 2007).
Other extragastric diseases possibly associated with *H. pylori* infection are chronic ischemic heart disease and cerebrovascular disease but despite intensive studies on the field, the results are contradictory and the benefit of eradication therapy in these situations is controversial and not recommended for the present (Franceschi and Gasbarrini, 2007, Goodman *et al.*, 2006).

**DIAGNOSTIC METHODS OF H. PYLORI INFECTION**

The accuracy of all diagnostic tests described for the detection of *H. pylori* infection below depends on the clinical situation in which they are used. Especially in settings with a low prevalence of *H. pylori* infection even tests with high sensitivity and specificity may turn out to give low positive predictive values and this should be taken into account when these tests are used (Campbell and Machin, 1993). No single method can be considered to be ideal for the detection of *H. pylori* infection (Anonymous, 1997). On the other hand, the cost-effectiveness of the different diagnostic strategies is based on the prevalence of the infection and on the price and accuracy of different tests (Vakil *et al.*, 2000).

There are several both invasive and non-invasive methods for the diagnosis of *H. pylori* infection, see Table 1. The invasive methods need gastric biopsy material taken by gastroscopy and non-invasive methods are based on serum, stool, urine, or saliva specimens. In general, the choice between different methods is dependent on the clinical situation and if gastroscopy is needed (age over 45 or alarming symptoms) (Malfertheiner *et al.*, 2002, Pikkarainen *et al.*, 2002, Delaney *et al.*, 2007).
Table 1. Special characteristics of the diagnostic tests for the detection of *H. pylori* infection introduced into clinical practice.

<table>
<thead>
<tr>
<th>Diagnostic method</th>
<th>Special characteristics</th>
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<tbody>
<tr>
<td><strong>Invasive tests</strong></td>
<td></td>
</tr>
<tr>
<td>Gastroscopy needed</td>
<td></td>
</tr>
<tr>
<td>Rapid urease test</td>
<td>Quick, cheap, reduced sensitivity if low number of bacteria</td>
</tr>
<tr>
<td>Histology</td>
<td>Enables the assessment of gastric pathology, expensive</td>
</tr>
<tr>
<td>Culture from gastric biopsies</td>
<td>High specificity, enables the testing of antimicrobial susceptibility, needs sophisticated laboratory skills</td>
</tr>
<tr>
<td>PCR</td>
<td>Sophisticated method, enables the testing of antimicrobial susceptibility, detection of virulence factors, comparison of clinical isolates</td>
</tr>
<tr>
<td>FISH</td>
<td>Sophisticated method, enables the testing of antimicrobial susceptibility</td>
</tr>
<tr>
<td><strong>Non-invasive tests</strong></td>
<td></td>
</tr>
<tr>
<td>Gastroscopy not needed</td>
<td></td>
</tr>
<tr>
<td>EIA-based quantitative antibody tests</td>
<td>Accurate after validation, low cost, easy to perform, slow decline in antibody titers after eradication, need for paired serum samples in posteradication setting</td>
</tr>
<tr>
<td>Rapid blood-based tests</td>
<td>Low accuracy</td>
</tr>
<tr>
<td>Immunoblot assay</td>
<td>Detects antibodies to specific antigens e.g. CagA</td>
</tr>
<tr>
<td>Urea blood test</td>
<td>Promising, not well studied</td>
</tr>
<tr>
<td>Urea breath test</td>
<td>High accuracy, needs sophisticated laboratory equipment</td>
</tr>
<tr>
<td>Stool antigen tests</td>
<td>Monoclonal EIA-based test accurate, in-office tests have low accuracy, easy to perform, moderately expensive</td>
</tr>
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</table>
1. Invasive methods

1.1. Rapid urease test (RUT)

The RUT is based on the strong urease activity of *H. pylori*. Urea embedded in culture media generates ammonia when broken down by *H. pylori* urease and thus produces an increase in pH usually detected by a change in colour with an indicator. There are both non-commercial and commercial RUTs available. The sensitivity and specificity of these tests are dependent on the time elapsed between biopsy and reading of the result, and the number of biopsies taken (in general only one antral biopsy) (van IJzendoorn *et al.*, 2005). The reaction time for the newer tests is shorter, up to three hours compared with the 24 hours of older tests, without affecting their reliability (Tseng *et al.*, 2005, Malfertheiner *et al.*, 1996). RUTs are considered highly accurate with a sensitivity and specificity over 90% (van Keeken *et al.*, 2006, Kuo *et al.*, 2002, Laine *et al.*, 1996, Lin *et al.*, 1992). Gastric bleeding causes low accuracy and up to 54% of *H. pylori* cases may be missed (Archimandritis *et al.*, 2000b, Tu *et al.*, 1999, Gisbert and Abraira, 2006a, Güell *et al.*, 2006, Castro-Fernandez *et al.*, 2004, Peitz *et al.*, 2004, Chung *et al.*, 2001, Griñó *et al.*, 2001, Lee *et al.*, 2000). The low accuracy of the RUT in gastric bleeding is suggested to be partly due to technical reasons (insufficient number of good quality biopsies), and due to PPI therapy and naso-gastric lavage, but several studies seem to exclude the role of blood in the stomach as a direct cause of false-negative results (Gisbert and Abraira, 2006a). Other clinical situations with inferior performance of RUT include: post-surgical stomach (Archimandritis *et al.*, 2000a); gastric ulcer probably due to concomitant atrophic corpus gastritis and low numbers of *H. pylori* in antral biopsies (Bermejo *et al.*, 2002); use of H2-blockers (Koch *et al.*, 1990, Lerang *et al.*, 1998); and PPIs (Yakoob *et al.*, 2005, Griñó *et al.*, 2001). In addition to low sensitivity, hypochlorhydric patients harbour other urease-positive bacteria in the stomach possibly leading to false positive test results (Brandi *et al.*, 2006). The sensitivity of RUT is considered low also in the post-eradication setting and figures as low as 71% four weeks after therapy have been described (Laine *et al.*, 1998b). Therefore, in clinical situations when there is a low density of *H. pylori*, two biopsies, one from the antrum and one from the
1.2. Histology

Histology has long been considered the “gold standard” in *H. pylori* diagnosis (Kusters et al., 2006, Mégraud and Lehours, 2007). However, the results of histology are dependent on the experience of the pathologist (Metz et al., 1998, Maconi et al., 1999), the number of biopsies taken (van IJzendoorn et al., 2005), the use of special stains (Laine et al., 1997, Kassa et al., 1996) and the density of *H. pylori* in the gastric mucosa (Epple et al., 1997, Testoni et al., 2002). In clinical practice, two biopsies both from the antrum and the corpus are recommended (Dixon et al., 1996). However, the recommendation of the updated Sydney System to take one extra biopsy, from the incisura angularis gives very little additional information (Eriksson et al., 2005). The need for special stains is especially emphasized in cases with a low density of *H. pylori*; the specificity of hematoxylin-eosin stain is clearly lower (89%) when compared to Giemsa and Genta stains (98%) (Laine et al., 1997) and the use of these latter stains is strongly recommended (Dixon et al., 1996). In some studies immunohistochemistry has been superior to the standard stains for *H. pylori* (Marzio et al., 1998, Jonkers et al., 1997b, Ashton-Key et al., 1996, Mégraud and Lehours, 2007). In atrophic gastritis (Lahner et al., 2004, Kokkola et al., 2000, Testoni et al., 2002) and remnant stomach (Matsukura et al., 2004), histology is insensitive. The performance of histology in patients with gastrointestinal bleeding has been less consistent than expected with sensitivity between 70 to 86% and affected by medication, timing of endoscopy and the number of biopsies taken (often restricted to minimum in unstable bleeding patients) (Laine et al., 2005, Castro-Fernandez et al., 2004, Castillo-Rojas et al., 2002, Chung et al., 2001, Griñó et al., 2001). In a recent meta-analysis, the pooled sensitivity and specificity of histology in the diagnosis of patients with bleeding peptic ulcer was 70% and 90%, respectively (Gisbert and Abraira, 2006a). After *H. pylori* eradication therapy, the sensitivity of histology is lower compared to the primary diagnosis and moreover, increasing the number of biopsies is of limited value (Laine et al., 2000, Rollán et al., 1997). If histology is used to confirm the cure of *H. pylori* infection, the optimal time to test is 4 weeks
after antimicrobial therapy (de Boer, 1997). The absence of chronic antral inflammation is a very reliable parameter to exclude *H. pylori* infection (Cutler et al., 1995) and after eradication therapy, any persistent active gastritis may represent an indirect sign of eradication failure in apparently *H. pylori* negative biopsy samples (Anonymous, 1997, Mégraud and Lehours, 2007). PPI therapy with concomitant non-acidic stomach causes both false positive and negative results on histology (Jonkers et al., 1997a). However, although histology has its restrictions and is considered expensive (Hahn et al., 2000), the major advantage of using it is the assessment of morphological changes of the gastric mucosa (Dixon et al., 1996).

### 1.3. Culture and antimicrobial susceptibility

Culture of *H. pylori* from gastric biopsies is considered highly specific but tedious and insensitive even in experienced laboratories (Grove et al., 1998, Andersen et al., 1998, Ndip et al., 2003). The sensitivity is influenced by transport medium and time (Pérez-Pérez, 2000, Ndip et al., 2003). However, in favourable circumstances it can be very accurate, and sensitivity may reach up to 95%, and specificity is 100% (Rautelin et al., 1997, Matsukura et al., 2004, Monteiro et al., 2001, Laheij et al., 2000, Lerang et al., 1998, Thijs et al., 1996, Mégraud and Lehours, 2007). In general, culture is indicated after one or two failed eradication regimens, and one to two biopsies each from antrum and corpus are recommended for culture (Ndip et al., 2003, Mégraud and Lehours, 2007). The sensitivity of culture is affected by eradication therapy up to 8 weeks, and even more with triple than dual therapy (Laine et al., 2000). The sensitivity of culture performed from one antral biopsy was 22% lower in post-eradication setting when compared with sensitivity in primary diagnosis (Laine et al., 2000). In patients with bleeding peptic ulcer the sensitivity in a recent meta-analysis was 45%, but the specificity was high, 98% (Gisbert and Abraira, 2006a). Culture enables the possibility to study bacterial virulence factors (for scientific purposes) and the antimicrobial susceptibility of *H. pylori*, a debatable advantage in primary diagnosis (Zullo et al., 2003, Mégraud and Lehours, 2007). In areas with low prevalence of primary resistance to antimicrobials used in eradication therapy, culture gives little extra benefit. This is also true for geographical areas with high metronidazole resistance when its use as first line therapy should be avoided. In
Finland as reported in a recent study, the prevalence of metronidazole resistance of \textit{H. pylori} was high, 38% (45% in women) but clarithromycin resistance was only 2% (Koivisto \textit{et al.}, 2004).

1.4. Molecular methods

The polymerase chain reaction (PCR) is an \textit{in vitro} technique for exponentially amplifying a predetermined fragment of DNA. PCR tests for \textit{H. pylori} infection have been based on several genes of \textit{H. pylori} and all have shown good performance (Brooks \textit{et al.}, 2004, Monteiro \textit{et al.}, 2001, Lage \textit{et al.}, 1995, Chisholm \textit{et al.}, 2001, Pacheco \textit{et al.}, 2001, Peek \textit{et al.}, 1995, Andersen \textit{et al.}, 1998, Thijs \textit{et al.}, 1996, Fabre \textit{et al.}, 1994). PCR has had high accuracy also when applied to formalin-fixed paraffin-embedded specimens (Weiss \textit{et al.}, 1994). In patients with bleeding peptic ulcer, the sensitivity of PCR was shown to be comparable to histology and superior to RUT and serology (Castillo-Rojas \textit{et al.}, 2002). PCR-based methods also enable identification of antimicrobial resistance to clarithromycin (Marais \textit{et al.}, 1999, van Doorn \textit{et al.}, 2001, Oleastro \textit{et al.}, 2003, Chisholm \textit{et al.}, 2001). For scientific purposes, it is possible to determine virulence factors like \textit{cagA}, fingerprinting for epidemiologic surveys (Schwarz \textit{et al.}, 1997) and to differentiate between reinfection and recrudescence after failed eradication therapy (Jeen \textit{et al.}, 2001).

\textit{In situ} hybridization is a method that uses labelled complementary DNA or RNA strands to localize a specific DNA or RNA sequence in a tissue section. Fluorescent in situ hybridization (FISH) uses fluorescent probes. FISH is a new method for \textit{H. pylori} diagnosis (Trebesius \textit{et al.}, 2000, Rüssmann \textit{et al.}, 2001a). Fluorescent labelled oligonucleotide probes binding to 16S rRNA of \textit{H. pylori} provide detection of single bacteria by fluorescence microscopy and probes to 23S rRNA sequence enable to define clarithromycin resistance (Rüssmann \textit{et al.}, 2001a, Yilmaz \textit{et al.}, 2007). The method is also applicable to paraffin-blocks (Trebesius \textit{et al.}, 2000, Rüssmann \textit{et al.}, 2003) (Jüttner \textit{et al.}, 2004, Morris \textit{et al.}, 2005, Yilmaz \textit{et al.}, 2007). The correlation of this method with histology and conventional clarithromycin susceptibility testing has been excellent (Trebesius \textit{et al.}, 2000, Rüssmann \textit{et al.}, 2001b, Jüttner \textit{et al.}, 2004, Yilmaz and Demiray, 2007).
2. Noninvasive methods

2.1. Blood-based tests

2.1.1. Enzyme immunoassay (EIA) for *H. pylori* antibodies

In EIA-based tests the antibody of interest is captured by an antigen (or antigens of interest are captured by antibodies) usually on a solid-phase support. These captured antibodies (or antigens) are detected by an enzyme-labelled antibody, which catalyzes a color reaction when exposed to a substrate. The intensity of the color reaction enables the quantification of the material of interest. Several commercial tests for *H. pylori* antibodies are available (Wilcox et al., 1996, Meijer et al., 1997, Vaira et al., 1998). A comparison of several commercial EIA-kits testing the same sera in several laboratories in Europe indicated that Pyloriset EIA-G (Orion Diagnostics, Espoo, Finland), one of the most widely used serology kits in Finland, has the best accuracy (Feldman et al., 1995, Mégraud and Lehours, 2007). However, the wide range in sensitivities and specificities of the commercial EIA-kits prompted local validation and adjustment for cut-off values to ensure appropriate accuracy (Malfertheiner et al., 2002). The differences in the performances of diagnostic kits in different geographic regions are possibly due to the variation in antigenic properties of local *H. pylori* strains and those used to prepare antigen for the test. However, the diagnostic performance of properly evaluated serological assays is comparable to that of biopsy-based methods and UBT (Oksanen et al., 1998, Herbrink and van Doorn, 2000). In some clinical settings, serology can even be the best diagnostic modality. Recently, the role of EIA serology has been reappraised especially in certain clinical situations when other tests will give false negative results, e.g. bleeding ulcers, atrophic gastritis, MALT lymphoma, and recent use of PPIs and antimicrobials (Malfertheiner et al., 2007).

Patients with positive serology and both negative histology and culture are overrepresented among patients with atrophic gastritis (Storskrubb et al., 2005), and the seropositivity may actually represent individuals with an ongoing infection (Kokkola et al., 1998). In patients with atrophic gastritis, *H. pylori* EIA results formerly considered false positive, probably reflect the insensitivity of other tests.
when *H. pylori* density is low (Kokkola *et al*., 1998). The performance of new commercial EIA tests as compared with invasive tests has been excellent when patients in older age-groups with atrophic gastritis have been excluded from the analysis (Salomaa- Räsänen *et al*., 2004).

Serology is less affected by PPI and antibiotic therapy as compared with RUT, UBT or histology (Dickey *et al*., 1996). However, in very young children serology has low accuracy (Kindermann *et al*., 2001, Kolho *et al*., 2002). The disadvantage of serology is also that it does not necessarily indicate a current infection: one year after successful *H. pylori* eradication therapy antibodies decline below the cut-off level only in 28% of patients (Lerang *et al*., 1998) and 37% in 4 years (Cutler *et al*., 1998). In assessing the success of eradication therapy paired sera are needed (Kosunen *et al*., 1992), and the fall in antibody titre by 50% in six months (Kosunen *et al*., 1992, Fallone *et al*., 1998) or 40% in four months (Lerang *et al*., 1998) is considered a marker for successful eradication therapy. Locally validated serology with paired sera after eradication is highly accurate in confirming the cure of *H. pylori* infection, with sensitivity of about 95-97% and a specificity of 95-100% after six months of eradication therapy (Kosunen *et al*., 1992) (Hirschl and Rotter, 1996).

### 2.1.2. Near patient tests

These are mostly whole blood tests, but some require serum separation. The accuracy of these commercial tests is considered poor with a sensitivity as low as 59% (Talley *et al*., 1998) and a specificity as low as 54% (Sadowski *et al*., 1998) and they are not recommended in clinical practice (Malfertheiner *et al*., 2007).

### 2.1.3. Immunoblot assay

In immunoblot or the Western blot, antigens separated by electrophoresis according to their molecular weight and transferred onto nitrocellulose or other absorbent paper react with specific antibodies which are then detected by a conjugate (labelled antibody) and substrate as in EIA. In some studies the immunoblot assay has had better performance when compared to EIA serology and it enables the study of antibodies to specific antigens, for instance CagA (Nilsson *et al*., 1997, Andersen and Espersten, 1992). The commercial kit Helicoblot 2.1 (Genelabs Diagnostics,
Singapore, Singapore) is the most widely studied assay both among European and Asian populations with good sensitivity for the diagnosis of *H. pylori* infection (Hoang *et al.*, 2006). Immunoblot CagA positivity has been used to assess the present (Monteiro *et al.*, 2001, Yamaoka *et al.*, 1998) and past (Ekström *et al.*, 2001) *H. pylori* infection, the latter because these antibodies seem to stay elevated longer than EIA serology (Ekström *et al.*, 2001, Ekesbo *et al.*, 2006). In some studies, antibodies to CagA have been strongly associated with atrophic gastritis (Sande *et al.*, 2001) and with gastric cancer (Palli *et al.*, 2007, Ekesbo *et al.*, 2006, Rudi *et al.*, 1997, Vilaichone *et al.*, 2003). There are, however, also contradictory results (Sokic-Milutinovic *et al.*, 2004, Schilling *et al.*, 2000).

### 2.1.4. 13C-urea blood test

The 13C-urea blood test is based on the same 13C isotope as in UBT but the isotope ratio mass spectrometry measurement is done from blood instead of the exhalation, 30 minutes after the ingestion of 13C-urea. (Cutler and Toskes, 1999) The performance of the test in adults when compared with UBT or RUT (up to 97%) (Cutler and Toskes, 1999, Ahmed *et al.*, 2005) or histology (93%) (Chey *et al.*, 1999) has been excellent.

### 2.2. Urea breath test (UBT)

#### 2.2.1 Different UBT procedures

The UBT is based on the capacity of *H. pylori* on the gastric mucosa to hydrolyse orally administered isotope-labelled urea to produce isotopically labelled CO₂ which diffuses into the blood and is excreted by the lungs and can be measured in exhaled air. UBT has several modifications concerning the use of the radioactive 14C (its use is contraindicated in children and pregnant women) or non-radioactive 13C- isotope, the dose (100-15 mg) and the form (fluid or tablets) of the urea given, the test meal (no test meal, citric acid, commercial special drinks, or fruit juices), measurement of the isotope (13C can be measured by isotope ratio mass spectrometry IRMS, non-dispersive isotope-selective infrared spectrometry NDIRS or laser assisted ratio analyser LARA, which is based on an optogalvanic effect), and the optimisation of
the cut-off point after urea ingestion (affected by dose and form of the urea ingested and the use of test meal) (Gisbert and Pajares, 2004c).

2.2.2. The clinical usage of UBT

In general, the UBT is considered highly accurate both in primary diagnosis and after \textit{H. pylori} eradication therapy with almost all protocols having a sensitivity and specificity over 95% (Gisbert and Pajares, 2004c, Gomollón \textit{et al.}, 2003). Also the commercial form $^{13}$C UBT (50 mg of urea combined with 456 mg citric acid) with one to two tablets dosage has been studied in the primary diagnosis setting (Ohara \textit{et al.}, 2004, Gatta \textit{et al.}, 2003, Hamlet \textit{et al.}, 1999, Wong \textit{et al.}, 2003) and in the post-eradication setting (Gatta \textit{et al.}, 2003, Hamlet \textit{et al.}, 1999, Wong \textit{et al.}, 2003) with excellent concordance with invasive tests and the standard UBT. However, atrophic gastritis (Capurso \textit{et al.}, 2006, Lahner \textit{et al.}, 2004), gastric resection, fast gastric emptying, and antimicrobials and bismuth disturb the performance of UBT (Gisbert and Pajares, 2004c). Furthermore, PPI therapy decreases the accuracy of UBT substantially (Mana \textit{et al.}, 2005, Peitz \textit{et al.}, 2004, Gatta \textit{et al.}, 2004, Manes \textit{et al.}, 2001) and the effect lasts up to two weeks (Laine \textit{et al.}, 1998a, Graham \textit{et al.}, 2004a). To be fully confident with UBT results, PPI therapy should be withdrawn two weeks before testing, and although the effect of the H$_2$-receptor antagonists on UBT results is in most part remote, it would be safe to avoid them one day before testing (Gisbert and Pajares, 2005). The antacids and sucralfate have no deleterious effect on the performance of UBT (Murakami \textit{et al.}, 2003). In the oropharynx and in the hypochlorhydric stomach there might be other bacteria than \textit{H. pylori} with high urease activity responsible for false positive UBT results (Brandi \textit{et al.}, 2006) although the clinical relevance of this seems to be very limited (Gisbert and Pajares, 2004c). Actually, in case of apparently false positive UBT results the more probable explanation is in fact the insensitivity of the reference method used (Epple \textit{et al.}, 1997). The UBT has performed well in patients with upper gastrointestinal bleeding when performed in emergency room before endoscopy (Winiarski \textit{et al.}, 2003).
2.3. Stool tests

2.3.1. Stool culture

*H. pylori* culture of stool specimens has been reported, but it is tedious and not easily reproducible (Thomas *et al.*, 1992, Parsonnet *et al.*, 1999, Ndip *et al.*, 2003). It has neither clinical nor scientific use.

2.3.2. Stool antigen tests

These tests are based either on monoclonal or polyclonal antibodies. The laboratory tests are EIA tests (Apollo, HePy-stool, Hp Ag, Amplified IDEIA HpStAR and Premier Platinum HpSA) but also commercial near patient tests have been developed (ImmunoCard STAT! HpSA, MiniLab and Simple H. pyl, also marketed by the name Stick H. pylori). The most studied tests are the polyclonal antibody-based HpSA, the monoclonal antibody-based HpStAR, and the immunochromatographic ImmunoCard based on monoclonal antibodies.

HpSA test detects *H. pylori* antigens from stool specimens by an EIA technique where the capture antibody adsorbed to microwells and the antibody, as a component of the conjugate, are polyclonal. The HpStAR test is a monoclonal antibody-based EIA. The ImmunoCard is an immunoassay based on a lateral flow chromatography technique and monoclonal antibodies are used to detect *H. pylori* antigens. It is a rapid test with results available within five minutes.

The early studies of the HpSA test both in primary diagnosis and in post-eradication setting had inconsistent results. In a mini-review among 2879 patients in pre-treatment setting when compared to UBT or endoscopy with biopsies the HpSA test showed sensitivity of 93.1% and specificity of 92.8%, respectively and in a post-treatment setting among 614 patients the figures were 87.8% and 88.4%, respectively (Vaira and Vakil, 2001b). Another review article from that time included also studies published in abstract form and including 4769 patients in pre-treatment and 2078 patients in post-treatment analysis with comparable results (Gisbert and Pajares, 2001). However, despite good results as a whole, in individual studies even in pre-treatment setting the performance varied and sensitivity ranged from 80% to 100%
and specificity from 70% to 100% (Vaira and Vakil, 2001b). In addition, more information of the HpSA test especially in the post-treatment setting was demanded (Vaira and Vakil, 2001b, Gisbert and Pajares, 2001, Parente et al., 2002). More recently, a monoclonal antibody-based test HpStAR (also marketed under the name of FemtoLab) became available with promising results in children with a sensitivity and specificity of 98% and 96.7% in a pre-treatment setting and 100% and 96.9%, respectively four weeks after eradication therapy (Makristathis et al., 2000). Soon thereafter, two studies were published in adults one in a pre-treatment setting with a sensitivity of 88.5% and specificity of 96.3% with no statistical difference when compared with the polyclonal HpSA (Agha-Amiri et al., 2001), and another in a post-treatment setting where the sensitivity of the monoclonal antibody-based test after adjustment of the cut-off point was better than the sensitivity of the HpSA test with equal specificity (Leodolter et al., 2002).

2.3.3. PCR of stool specimens

PCR based methods to detect *H. pylori* and even clarithromycin resistance have been applied to stool specimens (Rimbara et al., 2005). The performance of the stool PCR has been variable (Sen et al., 2005, Kabir, 2004) and considered, so far, insufficient for clinical use (Wiśniewska et al., 2002, Watanabe et al., 1998).

2.4. Urine tests

The commercial tests to detect *H. pylori* antibodies in urine are based either on EIA or rapid immunochromatographic methods. The EIA based Urinelisa test (Kabir, 2003) has been evaluated in two European multicenter studies in children (Méraud et al., 2005) and in adults (Leodolter et al., 2003) with only modest results (sensitivity 63-89%, specificity 68-97%). The performance of the immunochromatographic Rapirun in adults showed a poor sensitivity of 82% and a specificity of 83% (Leodolter et al., 2003). The disadvantage of both tests is that the sensitivity is even lower when frozen urine samples are used (Adachi et al., 2002).
2.5. Vomit and saliva

*H. pylori* has been cultivated from vomitus in a clinical experimental study (Parsonnet *et al.*, 1999). The EIA based tests for detection of *H. pylori* antibodies in saliva have no current role in patient management because of less than optimal accuracy (Malfertheiner *et al.*, 2007, Kabir, 2003). A PCR method has been applied also to saliva (Tiwari *et al.*, 2005) but there has been considerable variation in the detection rate (Kabir, 2004).

3. Diagnosis of *H. pylori* in special clinical situations

3.1. Bleeding peptic ulcer

*H. pylori* eradication almost eliminates the rebleeding risk in *H. pylori* infected subjects; the risk is about 50% within the subsequent 10 years, if patients are left untreated after an ulcer has healed (Gisbert *et al.*, 2004a). The diagnosis of *H. pylori* in patients with a bleeding peptic ulcer is challenging. The number of biopsies that can be taken in clinically unstable patients is limited, and the regularly used antisecretory medication impairs the performance of invasive tests, UBT and stool antigen tests (Gisbert and Abraira, 2006a). Accordingly, during acute bleeding the sensitivity of RUT was shown to be 67%, histology 70% and culture 45% in a meta-analysis (Gisbert and Abraira, 2006a) and even the combination of RUT and histology was unreliable to rule out *H. pylori* infection (Güell *et al.*, 2006, Chung *et al.*, 2001). UBT is sensitive if performed during the first 24 hours of admission (Winiarski *et al.*, 2003, Chung *et al.*, 2001) with a sensitivity of 93% in a meta-analysis (Gisbert and Abraira, 2006a). PPI therapy, the standard treatment of patients presenting with upper gastrointestinal bleeding, impairs the UBT result enormously; 92% of patients with regular dose PPI therapy after one week (Peitz *et al.*, 2004) and 60% of patients with high dose PPI therapy after three days (Udd *et al.*, 2003) had negative RUT. However, serology has the advantage of not being affected by PPI therapy (Gisbert and Abraira, 2006a). The results of the polyclonal antibody-based stool antigen test have been inconsistent: some studies have shown 100% sensitivity with very low specificity (Griñó *et al.*, 2003, van Leerdom *et al.*, 2003) and in some studies the sensitivity has
been very poor, 74% (Gisbert et al., 2004d). After acute bleeding the combination of several tests and repeating them in negative cases, and with a sufficient interval after antisecretory medications diminishes the false negative results (Güell et al., 2006, Epple et al., 1997, Gisbert and Abraira, 2006a).

3.2. Children

In children presenting with abdominal symptoms, H. pylori-associated diseases such as ulcer disease, which might explain the symptoms, are uncommon and thus clinically appropriate diagnostic interventions, not only focusing on H. pylori infection, should be considered (Koletzko and Feydt-Schmidt, 2001, Jones et al., 2005). The H. pylori screening for gastric cancer in children is warranted only in families with a history of gastric cancer (Jones et al., 2005). The main H. pylori associated extragastric conditions in children are iron-deficiency anaemia and short stature (Horvitz and Gold, 2006). UBT is excellent in all age groups but false positive test results may occur in young children (Koletzko and Feydt-Schmidt, 2001). It has been suggested indeed that this may have led to the wrong conclusions in epidemiological studies which reported high spontaneous clearance rates of H. pylori during infancy (Koletzko and Feydt-Schmidt, 2001). Also the compliance in children less than three years of age may be a problem in UBT (Koletzko and Feydt-Schmidt, 2001). It remains to be shown if the urea blood test has less compliance problems; it has been very accurate in children 2 to 18 years of age (Jolley and Wagner, 2007). The sensitivity of serology based on IgG antibody detection is low in children under the age of six years (Kolho et al., 2002, Kindermann et al., 2001) and in a study from a developing country none of the children under the age of two infected with H. pylori had positive serology (Casswall et al., 1999). However, the decline in IgG antibody titre is useful in monitoring the eradication success also in children (Kolho et al., 2002, Kato et al., 1999). The evidence of stool antigen tests, although the monoclonal antibody-based test has performed well in recent studies in all age groups (Koletzko et al., 2003, Kolho et al., 2006), is currently regarded insufficient, and according to the last consensus conference of the Canadian Helicobacter Study Group in 2005 and the multicenter European Study by the Pediatric Task Force on Helicobacter pylori
infection, the UBT is the best non-invasive tool for \textit{H. pylori} diagnosis in children (Jones \textit{et al.}, 2005) (Méraud \textit{et al.}, 2005).

### 3.3. The elderly

\textit{H. pylori} diagnosis in the elderly has also some special characteristics. The diagnostic strategies of dyspepsia necessitate a full diagnostic approach which must include endoscopy so as not to ignore serious diseases more common in the old age. The diagnosis is more challenging because of the high prevalence of atrophic gastritis (the diagnostic difficulties are discussed below) and abundant use of antimicrobials (which impairs the performance of many diagnostic tests) for commonplace infections in old age (Salles-Montaudon \textit{et al.}, 2002). \textit{H. pylori} diagnosis and therapy is understated in the elderly hospitalized for peptic ulcer disease, at least in the United States (Pilotto and Salles, 2002). In Finland also, bleeding peptic ulcers are common in elderly women (Paimela \textit{et al.}, 2002) and the abundant use of NSAIDs in the elderly (Klaukka \textit{et al.}, 2005) may lead to overlook the role of \textit{H. pylori}. The specificity of serology as a diagnostic method in the elderly is confusing: as discussed above, in the absence of a real gold standard in \textit{H. pylori} diagnostics, the patients with positive \textit{H. pylori} serology alone may be false positives or the serology may be the only sensitive method to detect \textit{H. pylori} when it appears in low density (Salomaa-Räsänen \textit{et al.}, 2004).

### 3.4. Atrophic gastritis

The diagnosis of \textit{H. pylori} in atrophic gastritis is a challenge since all invasive and almost all non-invasive tests have been shown to have poor sensitivity in this connection (Ricci \textit{et al.}, 2007, Lahner \textit{et al.}, 2004, Testoni \textit{et al.}, 2002, Kokkola \textit{et al.}, 2000). Serology is sensitive, but has arguable specificity as it does not differentiate between current and past infection. When patients with positive \textit{H. pylori} serology as the only marker of \textit{H. pylori} infection received eradication therapy, they showed a fast decline in IgG antibodies corresponding to the decline known to be diagnostic for successful \textit{H. pylori} eradication therapy (Kokkola \textit{et al.}, 1998). In severe atrophic corpus gastritis the antibodies decline slowly also spontaneously and in almost one
fourth of these patients the antibodies decline below the cut-off level within 10 years (Kokkola et al., 2003). This discrepancy in positive serology is reflected in the use of serology as a diagnostic method in the elderly: serology has excellent accuracy in the elderly if the patients with atrophic corpus gastritis are excluded (Salomaa-Räsänen et al., 2004). In a population with positive *H. pylori* serology as the only sign of *H. pylori* infection, atrophic gastritis and intestinal metaplasia are much more common than in patients with current infection detectable by histology or culture. Negative serology indeed can be used to exclude precancerous conditions with a high probability in a screening situation (Storskrubb et al., 2005). Also in lymphocytic gastritis, characterized by a marked increase in the number of intraepithelial lymphocytes, histology has a low sensitivity in the diagnosis of *H. pylori* infection although patients are usually seropositive for *H. pylori* (Mäkinen et al., 2003). The need for a sensitive test to detect a still ongoing infection in atrophic gastritis is urgent, especially if *H. pylori* eradication therapy in the late stages of gastric atrophy is shown to prevent gastric cancer.

**H. PYLORI ERADICATION THERAPY**

According to the Maastricht III Consensus Report (Malfertheiner et al., 2007) the first choice in *H. pylori* eradication treatment is a standard dose of PPI combined with clarithromycin 500mg bid and amoxicillin 1000mg bid or metronidazole 400 to 500mg bid for seven days. If continued for 14 days instead of seven this treatment is 12% more effective. This treatment is preferable if the level of local clarithromycin resistance is less than 15-20%. Metronidazole instead of amoxicillin is recommended only if the resistance to it is less than 40%. Another first line therapy recommended is so called quadruple therapy consisting of a standard dose of PPI combined with tetracycline 500mg qid, metronidazole 400mg tid, and bismuth subsalicylate 120mg qid. Antimicrobial resistance is the main factor influencing the treatment outcome (Cavallaro et al., 2006, Mégraud and Lehours, 2007). The eradication rate following a seven day therapy regimen with clarithromycin, amoxicillin and PPI has been 72-77% (Cavallaro et al., 2006) but in Finland the same regimen had an eradication rate of 91% (Koivisto et al., 2005).
MUCOSAL RECOVERY AFTER *H. PYLORI* ERADICATION THERAPY

After *H. pylori* eradication therapy the acute inflammation disappears in one month, but chronic inflammation persists longer (Forbes *et al.*, 1996, Tepeš *et al.*, 1999b, Franceschi *et al.*, 2002, Hojo *et al.*, 2002, Kuipers and Sipponen, 2006): in a meta-analysis the chronic inflammation improved in almost all studies; the observation period in most studies, however, was one year or less (Hojo *et al.*, 2002). In one study with a four-year follow-up including only patients with a duodenal ulcer, the gastric antral mucosa recovered in half of the patients to normal (defined as a total gastritis score of 0 or 1) (Zerbib *et al.*, 2000), and in another study among 11 duodenal ulcer patients after four-year follow-up, the majority of patients had normal antrum and corpus mucosa (defined as chronic inflammation grade 0) (Tepeš *et al.*, 1999b). However, the interobserver agreement in the assessment of the reversibility of gastritis after eradication therapy has been poor (Tepeš *et al.*, 1999a).

The studies on resolution of atrophy after successful *H. pylori* eradication therapy have shown restitution of normal architecture of the mucosa with regression of atrophic changes at least in some patients (Kokkola *et al.*, 2002, Kuipers and Sipponen, 2006, Hojo *et al.*, 2002). In a few studies the regression of intestinal metaplasia has been observed (Kuipers and Sipponen, 2006, Hojo *et al.*, 2002). After spontaneous disappearance of *H. pylori* with increasing gastric mucosal atrophy, the normalization of antrum mucosa with disappearance of intestinal mucosa has been suggested (Valle *et al.*, 1996). However, in this long-term follow-up of 32 years biopsies of the antrum mucosa at the beginning of the follow-up were not taken.

HOST RESPONSE TO *H. PYLORI*

The humoral immune system has only marginal relevance for protective immunity in *H. pylori* infection. *H. pylori* induces a Th1-polarized response that unfortunately does not result in clearance of the infection. *H. pylori* is thought to manipulate the host immune response and inflammation (Kusters *et al.*, 2006). The key activator of the innate immune response is probably intracellular peptidoglycan (Kusters *et al.*, 2006).
H. pylori is capable of inhibiting phagocytosis by macrophages by an as-yet-unknown mechanism. IL-10-producing T cells seem to be crucial in the control of inflammation and they enable the bacteria to persist in gastric mucosa (Kusters et al., 2006). Several cytokine genes have stable polymorphisms which are known to affect the level of cytokine production in response to H. pylori infection (El-Omar, 2006, Kusters et al., 2006). The best known of these is interleukin 1-β, a potent proinflammatory cytokine and the most potent known inhibitor of acid secretion (Kusters et al., 2006). These cytokine polymorphisms may contribute to the risk of gastric adenocarcinoma, but their contribution to the risk of peptic ulceration is conflicting (Chakravorty et al., 2006, Robinson et al., 2007). Reflecting the importance of the innate immune response are the studies carried out in families with a high incidence of gastric cancer where the combination of certain cytokine polymorphisms confer a 27-fold increased risk of gastric cancer for those infected with H. pylori (Houghton and Wang, 2005). The combination of high risk bacterial genotypes elevated further the risk up to 87-fold (Houghton and Wang, 2005). The modulating role of the immune response is thought to be responsible for the large variation in the worldwide incidence rates of gastric cancer (Robinson et al., 2007).
PRESENT STUDY

AIMS OF THE PRESENT STUDY

1. To evaluate the performance of stool antigen tests in the diagnosis of *H. pylori* infection in the pre-treatment and post-treatment settings.

2. To study the association between the presence of widespread DGM and *H. pylori* positive peptic ulcer.

3. To determine the persistence of chronic gastritis and elevated *H. pylori* antibody levels after successful eradication and their possible association with each other and with atrophic gastritis.
PATIENTS

Study I
In five centres, 82 adult patients who had clinical indication for gastroscopy and who were positive for *H. pylori* with a positive RUT were enrolled. Patients with gastric surgery or history of malignancy, severe reflux oesophagitis, and PPI or antimicrobial therapy during the preceding 4 weeks were excluded. The age range was 24 to 79 years, median 53, and 46 patients were females. Patients were considered *H. pylori* infected if they had, in addition to the RUT, positive histology or culture. All patients received *H. pylori* eradication therapy and the treatment combination was chosen at the discretion of the endoscopist.

Study II
A total of 1574 volunteers without significant abdominal complaints were collected following a newspaper announcement and screened for *H. pylori* infection with a rapid whole-blood antibody test (Pyloriset Screen II, Orion Diagnostica, Espoo Finland, or Biocard *Helicobacter pylori* IgG, AniTech Ltd, Vantaa, Finland) and the positive screening results were confirmed by EIA-based serology and $^{13}$C UBT. Subjects with positive results in both of the confirmatory tests were enrolled. Exclusion criteria included antimicrobial treatment during the previous 2 months, use of H$_2$-blockers, PPIs or bismuth during the previous 2 weeks, *H. pylori* eradication therapy during the previous 5 years, gastric surgery, chronic gastrointestinal diseases, pregnancy and lactation, and contraindications to drugs comprising the *H. pylori* eradication regimen (allergy to penicillin and prolonged QT-interval and anti-fungal therapy for dermatophyte infection inhibiting the use of clarithromycin) or allergy to fruit juices containing the probiotics used in the subsidiary study (Myllyluoma et al., 2005). Of the 1574 subjects who were screened initially, 300 were positive for *H. pylori* using the screening tests, 196 were positive in both of the confirmatory tests, but 11 of them fulfilled one of the above exclusion criteria and thus, the *H. pylori* positive study population consisted of 185 subjects. From among the screened population who were negative in the *H. pylori* tests, 114 subjects were invited as a control group and of the 97 who accepted the invitation all were negative in both of the confirmatory tests. The final study population consisted of 185 *H. pylori* positive subjects, age range 25 to 71 years, median 55, and 97 *H. pylori* negative subjects, age
range 23 to 64 years, median 43 years. In total, there were 186 females and 96 males. All *H. pylori* positive subjects received the same eradication regimen viz: amoxicillin 1g bid, clarithromycin 500mg bid, and lanzoprazole 30mg bid for one week.

**Study III**
This study was retrospective and included 1241 mostly dyspeptic patients assessed by gastroscopy and an additional 167 patients who had previously undergone HSV. Patients with gastric malignancies were excluded. The HSV patients had been gastroscopied previously median 14 years (range 3 to 18 years) after vagotomy to assess the long-term efficacy of the surgical procedure. The endoscopy and histological records were reviewed. A total of 198 patients were excluded; duodenal biopsies were unavailable for 185 patients and 13 of the patients with vagotomy had undergone gastric resection. Thus, the final study population consisted of 1056 dyspeptics (age range 16 to 88 years, median 52) and 154 patients with vagotomy (age range 32 to 76 years, median 52 years).

Two duodenal bulb biopsies from two gastroscopies were available for 419 of the 1056 dyspeptic patients (median interval of gastroscopies five months) and for 102 of the 154 HSV patients (median interval 12.6 months). The patients were classified as *H. pylori* positive if they had positive *H. pylori* culture or serology and as *H. pylori* negative if all tests (culture, histology and serology) were negative.

**Study IV**
Of 345 consecutive patients with a clinical indication for gastroscopy, a subpopulation of 108 patients, who had at least one endoscopy performed earlier and successful *H. pylori* eradication therapy on a known date, comprised the study population. The age range was 28 to 87 years, median 65 years, and 83 were females. Seventy-seven of the patients gave a blood sample.

The success of *H. pylori* eradication therapy that had been verified as clinically appropriate was reviewed. In 54 patients verification had been made by $^{13}$C UBT, in 26 by a new gastroscopy including histology, in 11 with a significantly declining IgG antibody titre in paired sera, in three by the stool antigen test, and in six by culture and histology, and in eight by only histology following the gastroscopy performed in the present study.
ETHICAL CONSIDERATIONS

The Ethics Committee of the Hospital District of Pirkanmaa approved the study I, the Ethics Committee of the Hospital District of Helsinki and Uusimaa approved the studies II and IV, and the register-based study III was approved by the Hospital District of Helsinki and Uusimaa. All patients in studies I, II, and IV gave their written informed consent before the study and all studies were conducted in accordance with the Declaration of Helsinki.

METHODS

Gastroscopy and collection of biopsies
During the gastroscopies (I and IV) the evident macroscopical pathology was recorded and two biopsies were taken from the antrum and the corpus for histology. In addition, in the study I, one biopsy was taken from the antrum and the corpus for RUT, and the results were read within three hours according to the manufacturer’s instructions (Pyloriset Urease, Orion Diagnostica, Espoo, Finland) and the positive specimens were sent in the original test tubes for culture. In the study III, during the original gastroscopies most patients had had two biopsies taken from the duodenal bulb, antrum and corpus.

H. pylori culture
Gastric biopsies (I) were cultured on Brucella agar plates supplemented with 7% horse blood and also on selective Brucella agar plates containing 1% Iso-Vitalex, 6 mg/L vancomycin, 2 mg/L amphotericin B, and 20 mg/L nalidixic acid. Plates were incubated at 35 to 37 °C in a microaerobic atmosphere for a maximum of 12 days. Identification of H. pylori was based on colony appearance, Gram staining, and positive reactions for oxidase, urease, and catalase. The culture result was available for most patients in study III.

Histology
All specimens in studies I and IV were stained with haematoxylin-eosin, Alcian blue (pH 2.5)-periodic acid-Schiff and modified Giemsa stains and the samples were examined by one experienced pathologist unaware of the identity of the samples. The
assessment of gastric histology had been performed according to the updated Sydney System (Dixon et al., 1996). The retrospective specimens in study III had been stained with haematoxylin-eosin and Alcian blue (pH 2.5)-periodic acid-Schiff and examined by several pathologists in the Department of Pathology of the University Hospital (tertiary referral clinic). The assessment of DGM was graded as 0 (no gastric metaplasia was evident), 1 (less than 20%), 2 (20 to 50%), and 3 (more than half of the surface epithelium was covered with gastric epithelium). The duodenal biopsies with an indistinct grade of metaplasia were re-reviewed by the same pathologist. Of the biopsies available, the one with the most widespread DGM was included.

**Serum tests**

Serum samples at baseline (I, II, IV) and after therapy (I, II) were stored at -20 °C until analysed. To detect *H. pylori* antibodies the paired sera were analyzed in parallel. The measurements for both *H. pylori* antibodies of the immunoglobulin G (IgG) and IgA classes were performed with an in-house EIA as described earlier and demonstrated a sensitivity of 99% and specificity of 93% in detecting *H. pylori* infection as compared to histology (Oksanen et al., 1998). *H. pylori* eradication therapy was considered successful when antibody titres of at least the IgG class had fallen 40% or more from the pre-treatment level 4 to 6 months after therapy. Pepsinogen I and II, and fasting gastrin-17 levels (Gastropanel, Biohit PLC. Diagnostics, Helsinki, Finland), and PCA (Varelisa, Pharmacia Diagnostics, Freiburg, Germany) were investigated according to the manufacturers instructions. The normal ranges used were those recommended by the manufacturers: for PG I 30-120 μg/l; for PG II 3-10 μg/l; for PG I/PG II 3-20; for gastrin-17 2-10 pmol/l, and for PCA, below 10 U/ml.

**UBT**

UBTs (I, II) were performed after an overnight fast either with Diabact tablets (Diabact AB, Uppsala, Sweden) (collection of breath sample 10 min after ingestion) or liquid 75 mg urea-citric acid solution (collection of breath sample 30 min after ingestion). Both were validated 13C-labelled UBT methods and the results were measured either with isotope ratio mass spectrometry or with non-dispersive isotope-selective infrared spectroscopy. The cut-off values were determined as delta over
baseline (DOB) >2.2‰ in the tablet method and >4‰ positive and <2.5‰ negative in the liquid method.

**Stool antigen tests**

The stool specimens in studies I and II were collected at baseline and at least 4 weeks after eradication therapy. They were stored at -20 °C before analysis and were thawed twice at most. Stool samples before and after eradication therapy were run in parallel in all three antigen tests. All examinations were performed according to the manufacturers’ instructions.

In the polyclonal antibody-based Premier Platinum HpSA test (Meridian Inc, Cincinnati, Ohio, USA), the recommended sample size was diluted with buffer and added to the micro-titre wells with a peroxidase-conjugated polyclonal antibody and incubated for 1 hour at room temperature. After washing, substrate was added and after further incubation of 10 min the stop solution was added. Results were read at 450 nm by spectrophotometry (Titertek Multiscan analyser, Eflab Oy, Helsinki, Finland) and interpreted according to the manufacturer’s recommendations. An optical density (OD) value < 0.140 was regarded as negative, 0.140-0.159 as equivocal (grey zone), and ≥ 0.160 as positive. The grey zone results were retested using the same stool samples stored at -20 °C. In addition, in the study I, all samples were also examined after centrifuging the diluted stool specimens and using the supernatant in the further analysis.

For the Amplified IDEIA HpStAR monoclonal antibody-based test alternatively known as FemtoLab Cnx (Dako, Glostrup, Denmark) stool samples suspended in sample diluent were centrifuged for 5 min and the supernatant plus the monoclonal antibody solution were pipetted into the wells and incubated for 1 hour. After four washes followed by 10 min incubation with the substrate, the reaction was stopped. The OD values at 450 nm ≥ 0.190 were interpreted as positive and < 0.190 as negative.

For the ImmunoCard STAT!HpSA test (Meridian Bioscience Europe, Milan, Italy), which is based on monoclonal antibodies and a lateral flow chromatography, stool specimen with diluent was emulsified with an applicator stick and vortexed for 15 s and dispensed to the sample port of the test cassette. After incubation for 5 min at room temperature, the appearance of a pink-red line in the reading window was interpreted as a positive test result.
Statistical analysis
The comparisons between the different patient groups in study III were analysed using a multinomial logistic regression model. The results were expressed as odds ratios (OR), confidence intervals (CI) and \( P \) values. The Fisher’s Exact Test was used for categorical variables (III, IV). Two-sided \( P \) values less than 0.05 were considered statistically significant. Data were analysed using SPSS for Windows software version 12.0 (SPSS Inc, Chicago, IL). In study IV the decline in chronic gastritis and elevated antibodies were assessed by the Linear-By-Linear Association Test, the strata being the time. The comparisons between the patient groups in study IV were analysed with the Pearson Chi-Square test. These data were analysed using StatXact 7 (Cytel Inc, MA, USA, 2006).

RESULTS

The performance of stool antigen tests (Studies I and II)

Of the stool antigen tests studied, the monoclonal antibody-based EIA test HpStAR had the highest sensitivity, specificity, accuracy, and likelihood ratio both in primary diagnosis and in a post-treatment setting. The results of the different stool antigen tests at baseline and after eradication therapy investigated in studies I and II are combined and presented in Tables 2 and 3. The statistics of the performance of these tests before and after eradication therapy are presented in Tables 4 and 5.

In the study I, 18 patients did not submit a follow-up serum sample (five patients had a therapy failure and received new eradication regimen before the 4 months follow-up in the study protocol) and one patient was seronegative at baseline; the assessment of the eradication result in these patients was based on UBT only. In study II, the results of 182 patients were available after eradication therapy. Of the original 185 patients who received eradication therapy, one died before the follow-up serum sample was drawn, one interrupted the eradication therapy for severe headache suspected to be a side-effect of the medication (this turned out to be viral meningitis), and one had discrepant results in the confirmatory tests after eradication therapy and was thus excluded from the analysis.

In the HpSA test, the grey zone values were re-examined using the same stool specimens according to the manufacturer’s recommendations. Of the 628 test results,
19 (3.0%) were within the grey zone in the initial test, but after retesting only three remained in this category. When the HpSA test was repeated using centrifuged supernatant for 82 patients in study I the result changed for nine (11%) patients (six at baseline and three after eradication therapy). However, the results (at baseline a sensitivity of 96% and after eradication therapy a sensitivity of 75% and specificity of 92%) were only marginally affected. When the data was statistically analyzed the grey zone results, even after re-examination were considered neither true positive nor true negative.

Table 2. The results of the three stool antigen tests in 364 patients (82 in Study I and 282 in Study II) at baseline as compared to reference methods. *H. pylori* positivity was defined by RUT and culture or histology in patients studied with invasive methods and by both serology and $^{13}$C UBT in patients not endoscopied.

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>HpSA</th>
<th>HpStAR</th>
<th>ImmunoCard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive 267</td>
<td>Positive</td>
<td>244$^1$</td>
<td>258</td>
</tr>
<tr>
<td>Negative</td>
<td>21$^2$</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Grey zone</td>
<td>2$^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative 97</td>
<td>Negative</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>Positive</td>
<td>4$^4$</td>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>

$^1$One patient originally had a grey zone result  
$^2$Three patients originally had a grey zone result  
$^3$Grey zone results even in re-examination  
$^4$Three subjects originally had grey zone results
Table 3. The results of the three stool antigen tests in 264 patients (82 in Study I and 182 in Study II) after *H. pylori* eradication therapy as compared with the reference methods. The patients’ eradication success was classified according to the agreement of both UBT and serology\(^1\).

<table>
<thead>
<tr>
<th><em>H. pylori</em></th>
<th>HpSA</th>
<th>HpStAR</th>
<th>ImmunoCard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative 232</td>
<td>Negative</td>
<td>224(^2)</td>
<td>227</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Grey zone</td>
<td>1(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive 32</td>
<td>Positive</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Negative</td>
<td>7(^4)</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^1\)Serology unavailable for 18 patients and one was originally seronegative at baseline  
\(^2\)Seven patients originally had grey zone results  
\(^3\)Grey zone even in re-examination  
\(^4\)Two patients originally had grey zone results

Table 4. The results of the three stool antigen tests in the diagnosis of *H. pylori* infection of 364 patients (82 in Study I and 282 in Study II) at baseline.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td>Pos</td>
</tr>
<tr>
<td>HpSA</td>
<td>91.4</td>
<td>95.9</td>
<td>0.98</td>
<td>0.82</td>
<td>0.93</td>
<td>22.3</td>
</tr>
<tr>
<td>HpStAR</td>
<td>96.9</td>
<td>95.9</td>
<td>0.99</td>
<td>0.91</td>
<td>0.96</td>
<td>23.4</td>
</tr>
<tr>
<td>ImmunoCard</td>
<td>94.0</td>
<td>88.7</td>
<td>0.96</td>
<td>0.84</td>
<td>0.93</td>
<td>8.3</td>
</tr>
</tbody>
</table>

PPV = positive predictive value  
NPV = negative predictive value
Table 5. The results of the three stool antigen tests in the diagnosis of *H. pylori* infection of 264 patients (82 in Study I and 182 in Study II) after eradication therapy.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>Likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td>Pos</td>
</tr>
<tr>
<td>HpSA</td>
<td>78.1</td>
<td>96.6</td>
<td>0.78</td>
<td>0.97</td>
<td>0.94</td>
<td>23.0</td>
</tr>
<tr>
<td>HpStAR</td>
<td>96.9</td>
<td>97.8</td>
<td>0.86</td>
<td>1.00</td>
<td>0.98</td>
<td>44.0</td>
</tr>
<tr>
<td>ImmunoCard</td>
<td>90.6</td>
<td>96.6</td>
<td>0.78</td>
<td>0.99</td>
<td>0.96</td>
<td>26.6</td>
</tr>
</tbody>
</table>

PPV = positive predictive value

NPV = negative predictive value

The prevalence of DGM (Study III)

Among the 26 patients who had an ulcer but were *H. pylori* negative, widespread DGM was detected in four (15%) patients and there was no association with the location of the ulcer. The results of the remaining 1030 dyspeptic patients and 154 HSV patients, grouped according to the macroscopic findings in gastroscopy, *H. pylori* infection status and the graded extent of DGM are presented in Table 6. The statistical associations between grade 1 DGM and macroscopic findings were not significant (OR values between 1.44 and 3.57). The association between widespread (grade 2 and 3) DGM and endoscopy findings is presented in Table 7. Of the 256 *H. pylori* positive duodenal ulcer patients, with or without gastric ulcer, 55% had widespread (more than 20%) DGM (Table 6). In patients with duodenal ulcer only, the association with widespread DGM was very high, OR 42 (Table 7). In addition to duodenal ulcer, widespread DGM was also significantly associated with *H. pylori* positive distal gastric ulcer (Table 7). In vagotomy patients, widespread DGM occurred only in 8.4% (Table 6), although 32 (21%) of them had had recurrent ulcers after the primary operation; most recurrences had been gastric ulcers (19, of which 16 were prepyloric, and one further patient had both gastric and duodenal ulcer recurrences). The widespread DGM was an uncommon finding in patients with *H. pylori* gastritis and proximal gastric ulcer.
Table 6. Prevalence and extent of DGM in the duodenal bulb in 1030 patients grouped according to the gastroscopy findings (including a history of endoscopy-verified ulcer) and 154 patients who had had highly selective vagotomy (HSV).

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Grade 0 (%)</th>
<th>Grade 1 (%)</th>
<th>Grade 2 and 3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal ulcer, n=211</td>
<td>65 (30.8)</td>
<td>20 (9.5)</td>
<td>126 (59.7)</td>
</tr>
<tr>
<td>Duodenal &amp; gastric ulcer¹, n=45</td>
<td>22 (48.9)</td>
<td>8 (17.8)</td>
<td>15 (33.3)</td>
</tr>
<tr>
<td>Distal gastric ulcer², n=83</td>
<td>51 (61.4)</td>
<td>12 (14.5)</td>
<td>20 (24.1)</td>
</tr>
<tr>
<td>Proximal gastric ulcer², n=81</td>
<td>63 (77.8)</td>
<td>16 (19.8)</td>
<td>2 (2.5)³</td>
</tr>
<tr>
<td>Distal &amp; proximal gastric ulcer², n=17</td>
<td>13 (76.5)</td>
<td>2 (11.8)</td>
<td>2 (11.8)</td>
</tr>
<tr>
<td>Gastric ulcer, unknown location, n=4</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
<td>0</td>
</tr>
<tr>
<td>H. pylori positive gastritis, n=248</td>
<td>216 (87.1)</td>
<td>22 (8.9)</td>
<td>10 (4.0)</td>
</tr>
<tr>
<td>H. pylori negative, no ulcer, n=341</td>
<td>260 (76.2)</td>
<td>56 (16.4)</td>
<td>25 (7.3)</td>
</tr>
<tr>
<td>After HSV, n=154(H. pylori positive 142, 92%)</td>
<td>123 (79.9)</td>
<td>18 (11.7)</td>
<td>13 (8.4)</td>
</tr>
</tbody>
</table>

All the ulcer patients were *H. pylori* positive. Of the biopsies available, the one with the most widespread DGM was included.

¹The location of gastric ulcer was distal in 25, proximal in 15 and both proximal and distal in 4 patients.

²Distal gastric ulcer included ulcers at pylorus, prepylorus (less than 3 cm from pylorus), and antrum. Proximal gastric ulcer included ulcers in angulus, corpus, and cardia.

³One patient had ulcerative lymphoma.
Table 7. Odds Ratios (OR), Confidence Intervals (CI) and $P$ values in *H. pylori* positive ulcer patients, *H. pylori* negative non-ulcer patients and HSV patients according to the prevalence of widespread (grade 2 and 3) DGM. The group of patients with *H. pylori* gastritis and no ulcer disease was used as the reference category.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. patients</th>
<th>OR (CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal ulcer</td>
<td>126</td>
<td>41.87 (20.77 to 84.41)</td>
<td>0.001</td>
</tr>
<tr>
<td>Duodenal and gastric ulcer</td>
<td>15</td>
<td>14.73 (5.91 to 36.68)</td>
<td>0.001</td>
</tr>
<tr>
<td>Distal gastric ulcer$^1$</td>
<td>20</td>
<td>8.47 (3.74 to 19.20)</td>
<td>0.001</td>
</tr>
<tr>
<td>Proximal gastric ulcer$^1$</td>
<td>2</td>
<td>0.69 (0.15 to 3.21)</td>
<td>0.63</td>
</tr>
<tr>
<td>Distal &amp; proximal gastric ulcer</td>
<td>2</td>
<td>3.32 (0.66 to 16.76)</td>
<td>0.15</td>
</tr>
<tr>
<td><em>H. pylori</em> negative, no ulcer</td>
<td>25</td>
<td>2.08 (0.98-4.42)</td>
<td>0.06</td>
</tr>
<tr>
<td>After HSV</td>
<td>13</td>
<td>2.28 (0.97 to 5.36)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^1$Distal gastric ulcer included ulcers at pylorus, prepylorus (less than 3 cm from pylorus), and antrum. Proximal gastric ulcer included ulcers in angulus, corpus, and cardia.

**Persistence of chronic gastric inflammation and elevated *H. pylori* antibodies after successful eradication (Study IV)**

None of the patients had acute inflammation in the gastric mucosa in gastroscopy performed in the present study, median 6.4 years, range 0.1-15.4 years after successful *H. pylori* eradication therapy. However, the persistence of the chronic gastric inflammation and elevated *H. pylori* antibodies of the IgG class were quite common findings although the prevalence of both declined with time, as shown in Table 8. The decline in the prevalence of chronic gastritis ($P<0.0001$) and of the elevated *H. pylori* antibodies ($P<0.001$) was highly significant (Linear-By-Linear Association Test, strata is time).
Table 8. Proportion of patients (n=108) with chronic inflammation in gastric mucosa and proportion of patients (sera available n=77) with elevated *H. pylori* antibodies in different time cohorts after successful *H. pylori* eradication therapy.

<table>
<thead>
<tr>
<th>Time after <em>H. pylori</em> eradication (years)</th>
<th>Chronic gastric inflammation</th>
<th>Elevated <em>H. pylori</em> antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>12/13 (92)</td>
<td>9/11 (82)</td>
</tr>
<tr>
<td>1- 2.9</td>
<td>5/10 (50)</td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>3- 4.9</td>
<td>6/17 (35)</td>
<td>2/12 (17)</td>
</tr>
<tr>
<td>5- 9.9</td>
<td>12/51 (24)</td>
<td>14/37 (38)</td>
</tr>
<tr>
<td>≥10</td>
<td>2/17 (12)</td>
<td>3/14 (21)</td>
</tr>
</tbody>
</table>

To assess the long term sequelae, the patients were divided into two groups according to the time (< 5 years, ≥5 years) elapsed from successful eradication therapy. Persistent chronic gastritis, even in those patients who had less than five years from therapy, was mostly mild (Table 9). Five or more years after successful eradication therapy, there was no association between chronic inflammation and elevated *H. pylori* antibodies (Tables 10 and 11). The average age of patients at the time of eradication therapy was 56 years for those with chronic gastric inflammation and 57 years for patients not showing chronic inflammation at least five years after successful *H. pylori* eradication therapy.

The prevalence of atrophic corpus gastritis was rare throughout follow-up (Table 9). One of the study patients developed corpus atrophy suggestive for the autoimmune type. She had had no gastroscopy performed at the time of eradication therapy and thus the time point for the atrophy development is not known. In the assessment of serum markers of corpus atrophy, all patients with low PG I also had a low ratio of PG I/II and they all had elevated levels of gastrin-17. Although rare, atrophic corpus gastritis, whether assessed from histological specimens or by serum markers was not associated with chronic inflammation or elevated *H. pylori* antibodies after five years of eradication therapy, as shown in Tables 10 and 11. The prevalence of intestinal metaplasia in antrum clearly declined with time, however, the
proportion of patients with moderate to severe metaplasia remained the same (Table 9). Low gastrin-17 was found in four of the 32 patients with intestinal metaplasia in antrum. After five years of eradication therapy, intestinal metaplasia in the antrum was more common in patients with persistent chronic inflammation (Table 10) and in patients with no elevated *H. pylori* antibodies (Table 11), but the associations were not statistically significant.

Table 9. The findings in gastric histology of 108 patients presented according to the time elapsed from successful *H. pylori* eradication therapy.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Time elapsed from eradication therapy</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 5 y, n=40 (%)</td>
<td>≥ 5 y, n=68 (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic gastritis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antrum &amp; corpus</td>
<td>23 /40 (58)</td>
<td>14/68 (21)</td>
<td></td>
</tr>
<tr>
<td>antrum</td>
<td>8/23 (35)</td>
<td>5/14 (36)</td>
<td></td>
</tr>
<tr>
<td>corpus</td>
<td>1/23 (4)</td>
<td>1/14 (7)</td>
<td></td>
</tr>
<tr>
<td>grade 2 to 3 gastritis</td>
<td>4/23 (17)</td>
<td>1/14 (7)</td>
<td></td>
</tr>
<tr>
<td><strong>Antral intestinal metaplasia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade 2 to 3</td>
<td>7/19 (37)</td>
<td>5/13 (38)</td>
<td></td>
</tr>
<tr>
<td><strong>Atrophic corpus gastritis</strong></td>
<td>2/40³ (5)</td>
<td>1/68⁴ (1)</td>
<td></td>
</tr>
</tbody>
</table>

¹P<0.0001, Pearson Chi-Square
²P<0.0024, Pearson Chi-Square
³In both, atrophy was of grade 1
⁴Atrophy was of grade 3
Table 10. Histological findings in 68 patients with and without chronic inflammation in the gastric mucosa at least five years after successful *H. pylori* eradication therapy; serum markers were available for 51 patients.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chronic inflammation, n=14 (%)</th>
<th>No chronic inflammation, n=54 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic atrophic corpus gastritis</td>
<td>1 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Intestinal metaplasia in antrum(^1)</td>
<td>4(^2) (29)</td>
<td>9(^3) (17)</td>
</tr>
</tbody>
</table>

**Serum markers:**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Elevated IgG antibodies, n=17 (%)</th>
<th>Not elevated IgG antibodies, n=34 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated PCA</td>
<td>3/10 (30)</td>
<td>14/41 (34)</td>
</tr>
<tr>
<td>Elevated PCA</td>
<td>1/10 (10)</td>
<td>6/41 (15)</td>
</tr>
<tr>
<td>Low PG I</td>
<td>1/10 (10)</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\)The difference is not statistically significant, Fisher’s Exact Test

\(^2\) In two patients intestinal metaplasia was of grade 1

\(^3\) In six patients intestinal metaplasia was of grade 1

Table 11. Serum and histological findings in 51 patients with and without persistently elevated IgG antibodies five years or more after successful *H. pylori* eradication therapy.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Elevated IgG antibodies, n=17 (%)</th>
<th>Not elevated IgG antibodies, n=34 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated PCA</td>
<td>2 (12)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Low PG I</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Chronic inflammation in gastric mucosa</td>
<td>3 (18)</td>
<td>7 (21)</td>
</tr>
<tr>
<td>Atrophic corpus gastritis</td>
<td>0</td>
<td>1(^1) (3)</td>
</tr>
<tr>
<td>Intestinal metaplasia in antrum(^2)</td>
<td>2 (12)</td>
<td>9 (26)</td>
</tr>
</tbody>
</table>

\(^1\) Atrophy was of grade 3.

\(^2\) The difference is not statistically significant, Fisher’s Exact Test.
DISCUSSION

Evaluation of stool antigen tests in the diagnosis of \textit{H. pylori} infection (Studies I and II)

Stool antigen tests have been under intensive investigation and development during the last few years. In our study, where three stool antigen tests were compared, the monoclonal antibody-based stool antigen EIA test (HpStAR) showed the best overall performance. Our results are in accordance with a recent meta-analysis, in which the same test showed a pooled sensitivity of 94\% and a specificity of 97\% (our results, 97\% and 96\%, respectively) in pre-treatment setting and a sensitivity of 93\% and a specificity of 96\% (our results, 97\% and 98\%, respectively) in post-treatment setting (Gisbert \textit{et al.}, 2006b). In the same meta-analysis, the polyclonal antibody-based EIA test (HpSA) showed almost equal specificity but clearly lower sensitivity both in the pre-treatment and post-treatment settings. Especially in the post-treatment setting, the differences between the sensitivities of monoclonal and polyclonal antibody-based EIA tests were substantial (sensitivities 91\% and 76\%, respectively). This was also shown in our study, as the specificities of the tests were almost equal (96\% for both tests in pre-treatment, and 98\% and 97\% in post-treatment setting, respectively), whereas the sensitivities were for the monoclonal and polyclonal antibody-based tests at baseline 97\% and 92\%, respectively, and in the post-treatment setting 97\% and 78\%, respectively (Table 4 and 5). Furthermore, the differences between positive and negative test results obtained with the monoclonal antibody-based test are generally greater as compared with the polyclonal antibody-based test, allowing better distinction, reproducibility and no need for a grey zone.

In study I, at baseline, the reference methods used were invasive methods i.e. RUT, histology and culture; and in study I, after eradication therapy and in study II in the pre- and post-eradication settings, serology and UBT were used. This is in line with the recommendation that two reference methods should be used when new methods are being evaluated (Mégraud and Lehours, 2007) and also according to the fact that biopsy-based methods show low sensitivity in the post-treatment setting (Laine \textit{et al.}, 1998b, Laine \textit{et al.}, 2000). The different screening and confirmation methods used in Studies I (positive RUT in screening and confirmation with histology and serology) and II (positive whole blood serology in screening and confirmation by
EIA serology and UBT) had insignificant impact on the performance of the stool tests at baseline. Of the 300 \textit{H. pylori} positive subjects screened in Study II, only 196 had positive results in both confirmatory tests. The excluded patients were mostly positive in screening only, but 28 of them were also positive in EIA serology. These 28 UBT negative patients were not studied further with either the stool antigen tests or by any invasive tests.

Although the experience with rapid “in-office” stool antigen tests is still limited, including the ImmunoCard studied by us, they were assessed in a meta-analysis which showed that the results were inferior when compared to the EIA tests (Gisbert and Pajares, 2004b). As the results of the in-office tests are available within 10 minutes they have been suggested to be suitable for the use at the doctor’s office. No studies, however, exist of the patients’ ability to give a stool specimen at the office visit. In some studies, patients have shown an overall reluctance to collect stool samples (O’Connor \textit{et al.}, 2002). Subsequent to the latest meta-analysis, several other studies on the performance of the ImmunoCard test have been published with widely varying sensitivity and specificity values (Lu \textit{et al.}, 2006) as well as inferior results compared to EIA tests (Chisholm \textit{et al.}, 2004). Also in our study, the ImmunoCard had only a moderate specificity (89%) when used as a primary diagnostic test, although after eradication therapy the test had a better overall performance than the polyclonal antibody-based test.

Although not explored in our study, in some special clinical situations stool antigen tests have shown the same restrictions described for many other diagnostic methods. The accuracy of stool antigen tests is hampered by PPI therapy to the same magnitude as described for UBT (Gisbert and Pajares, 2004b). Despite the promising reports of the stool antigen tests among patients with a bleeding peptic ulcer (Peitz \textit{et al.}, 2003) (Gisbert \textit{et al.}, 2004d), none of the stool antigen tests can be recommended to diagnose \textit{H. pylori} infection in patients with upper gastrointestinal bleeding; performance of the polyclonal antibody-based test has been controversial. Moreover, the information on the monoclonal antibody-based test is limited (Gisbert and Abraira, 2006a). Partial gastrectomy (Sheu \textit{et al.}, 2002), cirrhosis (Gisbert \textit{et al.}, 2004b) and atrophic gastritis (Lahner \textit{et al.}, 2004) are other clinical settings when the performance of stool antigen tests has been suboptimal. The sensitivity of the HpSA test was only 40% in the study by Scheu and colleagues (Scheu \textit{et al.}, 2002) in gastrectomy patients after eradication therapy and in the study by Lahner and
The use of DGM in selecting patients for *H. pylori* eradication therapy (Study III)

Widespread DGM was a common finding in *H. pylori* positive patients with duodenal (60%) or distal gastric ulcer (24%), and it was rare in patients with proximal gastric ulcer (2.5%) and in patients with *H. pylori* gastritis but no ulcer (4%). The prevalence of *H. pylori* infection varies widely and in many developing countries 80% of adult population is infected (Malaty, 2007). As in such countries eradication of the infection
in the whole population is not possible, the Maastricht III Consensus Report (Malfertheiner et al., 2007) introduces guidelines for patient selection. Duodenal ulcer is a generally accepted indication for eradication therapy. However, at the time of gastroscopy, an ulcer may have healed with only minimal scar formation and the treatment of patients with bulbar deformity does not prevent new ulcers. The risk for a new peptic ulcer was 10% during a 10 year follow-up in patients with H. pylori gastritis and the highest risk was in patients with antral predominant gastritis (Sipponen et al., 1990). However, in that particular study, only 20% of patients with a new ulcer that had developed during the follow-up would have received eradication therapy on the basis of the scar formation seen in the initial gastroscopy. On the other hand, antral predominant gastritis, suggested as an indication for H. pylori eradication, occurs in half of the patients with chronic dyspepsia (Koskenpato et al., 2002) and during a 6-7 year follow-up, patients with antral predominant gastritis did not use more gastrointestinal medications or health services as compared to individuals with other topographic types of gastritis (Heikkinen et al., 2004). Thus, better diagnostic tools are needed to allocate the H. pylori eradication therapy properly.

Although the association between DGM and duodenal ulcer is recognised, the existence of DGM also in H. pylori negative subjects and the controversial results of its prevalence in different entities have led to an underestimation of the significance of DGM. As early as the late 1980’s, the presence of H. pylori in histological specimens of the duodenal bulb was found to be a very strong risk factor for a duodenal ulcer (RR 51), stronger than gastric metaplasia alone (RR 7.6). (Carrick et al., 1989) Since the colonization of the duodenal bulb is more probable in association with widespread DGM, these results are in line with our own study showing an OR of 42 for widespread DGM and duodenal ulcer. Recently, in a prospective study, positive H. pylori culture from the duodenal bulb was shown to predict the duodenal ulcer risk in a one-year follow-up (OR 6.29). (Pietroiusti et al., 2005) The sensitivity of culture is, however, prone to errors, and in order to find H. pylori in the duodenal bulb, histological specimens with special stains are needed. Instead, DGM and its extent are easily detected in histological specimens. However, despite the high OR values in the present study, widespread DGM only detected 55% of patients with duodenal ulcer but even so widespread DGM could be added to to the list of indications to eradicate H. pylori. On the other hand, the proportion of gastritis
patients with widespread DGM but no ulcer was 4% in our study. This is an acceptable number of patients receiving eradication therapy with no shown benefit (at least as duodenal ulcer is concerned) if widespread DGM is used to detect patients for treatment. Widespread DGM was rare or non-existent in patients with proximal gastric ulcers or with atrophic gastritis, both recommended indications for eradication therapy to prevent gastric cancer (Malferttheiner et al., 2007).

Based on previous studies (Jönsson et al., 1988) the low prevalence of widespread DGM (8.4%) in HSV patients was an unexpected finding in our study. Duodenal ulcer disease has been for all patients the indication for HSV and thus, it is conceivable that at the time of the operation the patients had had a high prevalence of widespread DGM which then vanished after operation probably in parallel with the decline in duodenal acidity. As 92% of the HSV patients were *H. pylori* infected many years after the operation, this opposes the hypothesis that DGM and duodenal *H. pylori* infection with resulting inflammation (and extension of DGM in a vicious circle) are crucial in the pathogenesis of duodenal ulceration. If anything, this finding supports the observation of the significance of acid output in determining the location of the ulcer since most of the ulcer recurrences of these HSV patients had occurred in the prepyloric area. This is also in accordance with the finding of the high acid exposure impairing the function of Cdx2, the master regulator of intestinal differentiation, the suggested mechanism behind the DGM (Faller et al., 2004).

The association between persistent chronic inflammation and elevated *H. pylori* antibodies after successful eradication therapy (Study IV)

The persistence of chronic inflammation in the gastric mucosa (21%) and elevated *H. pylori* antibodies (33%) were common findings after five years or more of successful *H. pylori* eradication, both are new findings as most of former follow-up studies have only lasted for a few years (Hojo et al. 2002). There was no association between these two phenomena and neither of them was associated with atrophic gastritis. Five years after eradication therapy, the persistent chronic inflammation was mild in all patients except one. For intestinal metaplasia in the antrum, although the prevalence decreased with time, the grade was stable. One patient developed an autoimmune type of total gastric atrophy between two consecutive gastroscopies, however, at the time of the
eradication therapy the gastroscopy was not performed and thus, the precise time for
the development of the atrophy can only be guessed.

Most of the studies assessing the reversibility of chronic gastritis after
eradication therapy have shown significant improvement (Hojo et al. 2002). However,
the follow-up time in these studies has been mostly one year or less. In one of these
studies with multiple biopsies taken both from the antrum and the corpus, chronic
inflammation was present in all patients after one year of successful eradication
therapy (Franceschi et al., 2002). However, studies with a longer follow-up (up to
four years) revealed conflicting results; one study showed normal antral mucosa in
only half of the patients (Zerbib et al. 2000) whereas in the other, normal antrum and
corpus mucosa was detected in the majority of the patients (Tepeš et al., 1999b).
Some of the discrepancies in the studies arise from the difficulties in the assessment
of mild chronic gastritis (Tepeš et al., 1999b). The advantage of our study is that one
experienced pathologist assessed all patient specimens unaware of the study protocol.
The possible role of the intensity of the inflammation before eradication therapy on
the persistence of the chronic inflammation after eradication has not been studied so
far.

Animal studies of experimental gastric cancer (testing the inflammatory
hypothesis) have shown that a longer duration of the infection increases the risk of
cancer, and there may be a point of no return when eradication therapy cannot
anymore prevent the cancer development (Cai et al., 2005). In our study, the average
age of the patients at the time of eradication therapy did not differ among those with
and those without chronic inflammation and therefore it is improbable that the
duration of H. pylori infection (presumably obtained in the childhood) would have
contributed to the persistence of chronic inflammation. Although we were not able to
show any association between the persistence of chronic inflammation and atrophic
gastritis, the clinical significance of the sustained inflammation in gastric
carcinogenesis remains to be shown.

Although a significant decline in H. pylori antibody titres 4 to 6 months after
eradication therapy indicates successful therapy (Kosunen et al., 1992, Lerang et al.,
1998), it may take several years before the antibodies decline below the cut-off level
(Cutler et al., 1998). The reason for the persistent positive serology is not known and
in our study it was not shown to be associated with persistent chronic inflammation
either. However, the persistent seropositivity emphasizes the importance of early
verification of the eradication success. If later on diagnostic methods of *H. pylori* infection are needed, tests capable of detecting a current infection should be used.

In our study, there was no association between persistent mostly mild chronic gastric inflammation and elevated *H. pylori* antibodies and the results do not support the hypothesis of *H. pylori* triggering an autoimmune process which could continue after eradication therapy. Neither was there any association between persistently elevated *H. pylori* antibodies or chronic inflammation and precancerous conditions e.g. atrophic gastritis and intestinal metaplasia. Considering the disappointing results of the human gastric cancer prevention studies (Wong *et al.*, 2004), the question as to whether sustained chronic inflammation has any role to play remains to be studied.
CONCLUSIONS

1. Of the stool antigen tests evaluated, the monoclonal antibody-based EIA demonstrated the best performance both in pretreatment and posttreatment settings. This is an accurate test for the diagnosis of *H. pylori* infection and the performance is comparable to other diagnostic tools. The polyclonal antibody-based EIA and the rapid test were less accurate.

2. The presence of widespread DGM was strongly associated with a *H. pylori* positive duodenal ulcer and could possibly be used to detect patients at a high risk for duodenal ulceration for *H. pylori* eradication therapy.

3. Chronic gastric inflammation and elevated *H. pylori* antibodies persist in some individuals, due to unknown reasons, for years after successful eradication therapy. These phenomena are not associated with each other or with atrophic changes in the gastric mucosa.
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